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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Analysis of Distillation Column in Cryogenic Air Separation Unit

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Received: 21 Jan 2022 Revised: 22 Feb 2022 Accepted: 23 Mar 2022

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ABSTRACT

In the present day scenario, separation of components from air is very much necessary either for medical or industrial use. Computer aided simulation and analysis pave an excellent way to analyze the working and performance assessment of components in the air separation units. In the present work, DWSIM is used to simulate and analyze the performance of distillation column in air separation unit. The effect of temperature, pressure, and mole fraction with varying stages is analyzed.

Keywords: Cryogenic air separation; Distillation column; DWSIM;

INTRODUCTION

The approximate composition of air is 78.08% Nitrogen, 20.95 % Oxygen and 0.93% Argon, 0.04% Carbon dioxide. Nitrogen, oxygen and argon can be separated by Liquefaction followed by Fractional distillation. The general procedure for production of oxygen is that the air is filtered to remove dust. It is then cooled in stages to 73K at which temperature, air is liquid. As it is cooled, water vapour condenses and is removed by absorbing filters. Carbon dioxide is then removed at 194K as it freezes. The oxygen liquefies at 90K, argon at 87K, and nitrogen liquefies at 77K. The liquid oxygen, nitrogen, and argon are then separated by fractional distillation. Rohit and Kanchan [1] have reconfigured the standard single column air separation unit to replace the auxiliary distillation column so as to produce pure and impure oxygen as when desired for the purpose. It was also observed that the power consumption has reduced by 15-25% with the use of reconfigured column. Armin and Masoud [2] have optimized the design and integrated an air separation unit for cryogenic LNG usage. It was observed that the cold energy recovered can be used for power generation or freezing of material. It was also observed from their investigation that there was 8.04% or power requirement reduction which also reduced capital cost. In an air separation unit, transition of product is

very challenging. Israel et al [3] have devised a dynamic model and used ASPEN dynamic simulation to explore the validity of the dynamic model. It was shown that the derivative free approach could be successfully applied to various oxygen production plants to handle transition issues.

Qian et al [4] have adapted self-heat recuperation technology in which the heat extracted from nitrogen gas is recirculated from top of single distillation column to bottom by exchanging heat with oxygen. It was shown that by adapting this technology in simulation, the energy requirement was reduced by almost 20%. Hengyang et al [5] have studied the characteristics of oxygen purity in an air separation process and compared this with argon recovery. A simulation model was established in HYSYS which incorporates a mixing column to analyze the process performance parameters such as oxygen recovery, power consumed and argon recovery. It was concluded that the triple column adaption has proved to reduce power consumption and recovery of oxygen. Quancong et al., [6] have simulated four configurations for air distillation column for steady state conditions. The exergy analysis revealed that the amount of oxygen produced varies with process of production. At higher rate of production of oxygen, it was also observed that the exergy efficiency is as well higher for single column distillation process. The aim of this paper is to simulate and analyze the performance of distillation column in air separation unit. The effect of temperature, pressure, and mole fraction with varying stages is studied and analyzed.

MATERIALS & METHODS

The scheme of design in the present work has an intake air at atmospheric pressure. It is considered that air comprises of nitrogen, oxygen and water vapour. The block diagram for air separation process is presented in Figure 1. Air is compressed to higher pressure using a multi stage compressor and cooled to retain the thermodynamic properties and removal of water particles and carbon dioxide. The air is passed through the heat exchanger where it is cooled. The liquid air formed is allowed to pass through the double separation column and secondary heat exchanger. In the double column rectification system, a pressure of 5 to 6 bar is exerted at bottom column and 1 bar at top column. Nitrogen has boiling point at 94.2K at 5 bar while oxygen has boiling point at 90.2K. Hence the upper part of bottom column is condensed by the bottom part of top column constituent. In this regard, the heat exchanger is coupled to boiler of the upper column and a condenser to the lower column. The energy needed for the separation of constituents is provided by the multi stage compressor installed at the inlet. This influences the efficiency of the plant and purity of constituents. Compressed air, as it passes through the absorber, split into two parts. One stream is allowed into the main exchanger while the other stream passes through the booster exchanger. Air passing through the booster exchanger is again split into two streams. One stream passes through the booster compressor and the other stream passes through the turbine booster compressor to get compressed to 64 bar pressure in 6 stages. Past this, the three streams of air are allowed to pass through heat exchanger and cooled using the streams of liquid oxygen, and nitrogen. The cooled air then passes through the turbine and fed into the upper part and lower part of the distillation column. The products from high pressure column, rich nitrogen and rich oxygen are fed into the sub cooler and JT valve for reflux. The streams from heat exchanger are recycled to achieve maximum purity of constituents. The separation process of air is simulated in DWSIM and the block diagram is presented in Fig. 1. The inclusive thermodynamics of physio-chemical properties of air and its constituents can be determined using DWSIM. Peng-Robinson model is used in the simulation.

RESULTS AND DISCUSSION

The steady state simulation is executed in DWSIM for the analysis of distillation column.

High pressure column

The temperature and pressure variations in high pressure column on the number of stages is presented in Fig. 2. It can be seen from the figure that the temperature exerted in the high pressure column reduces from bottom to top of the column. The profile of temperature increases slowly up to stage number 46 and further which increases rapidly.

It may be due to the boiling effect of the fluid in the distillation column. The pressure in the distillation column increases almost linearly from top to bottom. For the specified conditions when the compression pressure increases, the liquid fraction obtained as well increases. Subsequently, the production cost if desired liquid (oxygen or nitrogen) also increases. The composition variation from top to bottom stage of high pressure column is shown in Fig. 3. As we move from bottom to top of the column, there is increase in mole fraction of nitrogen. On the other hand, the mole fraction of oxygen increases from top to bottom of the column.

Low pressure column

The profile of temperature and pressure for different stages in the low pressure distillation column is presented in Fig. 4. It is observed form the figure that the temperature decreases from bottom to top and also it is observed that the temperature gradient is high past the stage 43. Whereas the pressure variation shows linear trend all across the column. The composition profile of low pressure column for mole fraction of components for different stages is presented in Fig. 5. It can be seen from the figure that the component separation is clear and well established as compared with the high pressure column. Such that the nitrogen component in high pressure column is only present without oxygen purity.

CONCLUSION

The simulation analysis of distillation column is executed using DWSIM and the following conclusions can be drawn: To increase the purity of the given product (oxygen or nitrogen), the gaseous withdrawal of that component shall be reduced. In high and low pressure column, with increase in number of stages, the temperature profile gradually increases whereas the pressure profile increases linearly.

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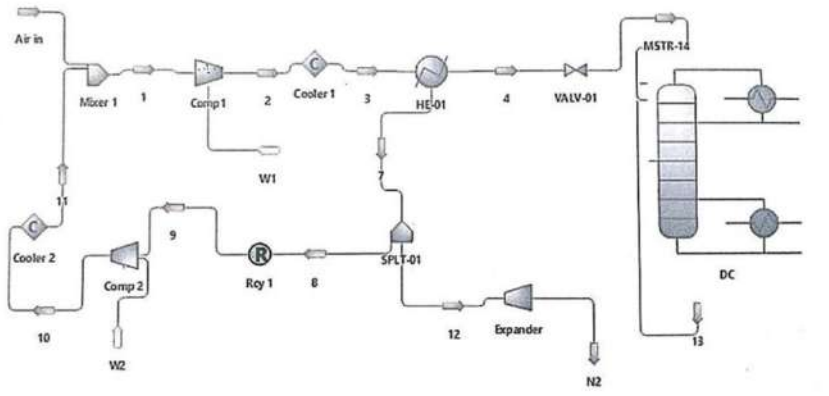

Fig 1: Block diagram of air separation process

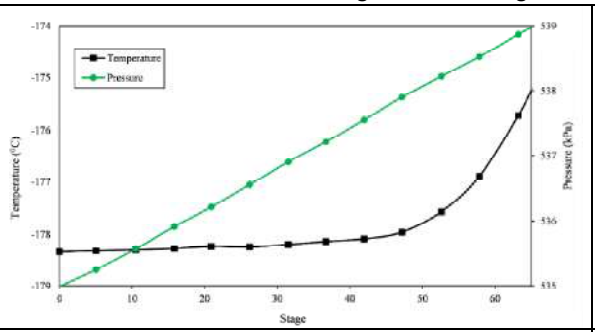

Fig 2: Variation of temperature and pressure with stages in high pressure column

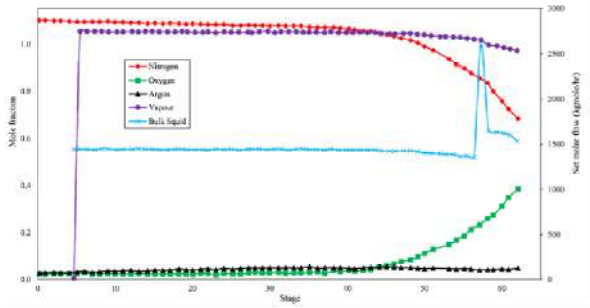

Fig 3: Composition variation of constituents in high pressure column

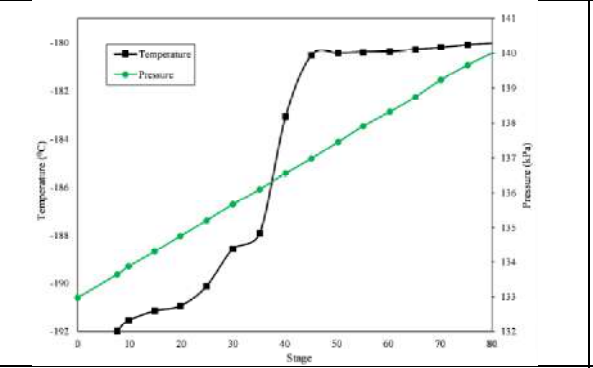

Fig 4: Variation of temperature and pressure with stages low pressure column

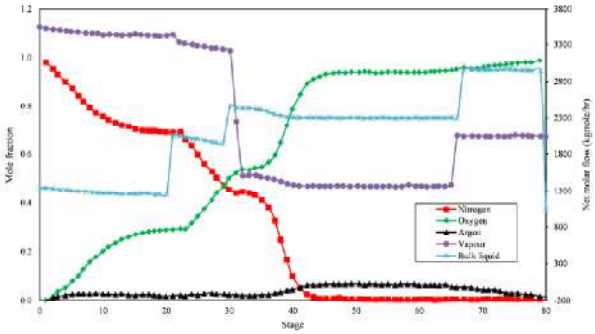

Fig 5: Composition variation of constituents in low pressure column

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

QbD Based Development of Two RP-HPLC Methods for Levofloxacin and Its Acid Degradation Product - Through Creation of Design Space for Critical Attributes and Application of ANOVA

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Received: 04 Jan 2022 Revised: 05 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

Fluoroquinolones are antibacterials that are widely used in many countries, however there are growing worries about their presence in soil and water. Analytical methods for these medications are continually needed not only in the pharmaceutical area, but also in other fields such as chemical engineering and environmental sciences. Quality by Design (QbD) is a concept that ensures the robustness and adaptability of an analytical method or manufacturing process to any instrument, laboratory, or application. The major goal was to develop some easy stability indicating analytical procedures for Levofloxacin, a fluoroquinolone member, in the presence of its acid degradation product. The goal was to create a Reversed Phase-High Performance Liquid Chromatographic technology that could be used in any business and simply changed to suit their needs. The RP-HPLC procedures provided here employ a C18 column with dimensions of 25 cm (length) 0.46 mm (i.d.) and a particle size of 5 m. The first approach employed a 43:57 ratio of methanol and phosphate buffer (pH 3.0) as the mobile phase, while the second method used a 15:85 ratio of acetonitrile and 0.1 percent triethylamine (pH 3.0). The analytes were detected using a PDA detector wavelength of 294nm in both procedures, which were performed at 0.8 ml/min flow. To validate parameters, ICH-recommended validation standards were used, as well as statistical methodologies such as ANOVA and the creation of a Design Space to assign limiting values for the essential variables impacting robustness. Two simple and reliable RP–HPLC techniques were devised, one of which could be modified for LC–MS compatibility.

Keywords: RP-HPLC, QbD, Critical Attributes, Design Space, LC-MS, Levofloxacin

INTRODUCTION

Antibacterials belonging to the fluoroquinolone class are commonly used to treat respiratory and urinary tract infections (FQs). According to the literature, the first quinolone was discovered as a by-product of the synthesis of chloroquine in the 1960s, and when this chemical was shown to have antibacterial action, it led to the development of nalidixic acid [1].Despite the passage of time and the introduction of newer members, older medications are still being utilised to treat a variety of infections [2-6].Quality by Design is a method of incorporating quality into a product or process from the beginning of the design or planning process. This approach is gaining traction in the pharmaceutical industry, owing to the fact that analytical techniques developed using QbD principles do not require redevelopment, revalidation, or reapproval after transfers, as long as the Design Space boundaries are not exceeded. This saves both time and money [7-11]. There are growing worries about these medications being found in soil and water as a result of their widespread and indiscriminate usage, stressing the need to find avenues to speed biodegradation. This highlights the need for novel and easy analytical methods that can be quickly adopted and implemented in a variety of sectors, including chemical engineering and environmental sciences. We attempted to develop a common HPLC approach for several fluoroquinolones previously, however the method failed to detect any degradants (12).Our efforts in developing and verifying two stability-indicating RP-HPLC techniques for the selected FQ, levofloxacin (LEV), while it is present alongside its acid degradant, LDA, acquired through forced degradation of LEV, are described in this paper. Figure 1 depicts the chemical structure of LEV.

A review of the literature revealed that there is no straightforward technique for this medicine. The majority of the described analytical approaches required simultaneous estimate of FQs, which made the method difficult [13-27].Some used gradient elution and fluorescence, as well as other advanced detection and quantification techniques [28-45].As a result, it was decided to design basic and quick stability signalling techniques that could be simply adapted for the bulk of the class. To design the techniques and validate the important parameters, ICH-specified guidelines and QbD principles were used [46-52].

MATERIALS AND PROCEDURES

Chemicals

Merck's methanol and acetonitrile (HPLC grade) were used. HPLC grade water, orthophosphoric acid and triethyl amine (HPLC grade of Merck), formic acid, and disodium hydrogen phosphate were all prepared in the lab using the BioAge Ultra Water Purification system or purchased from Merck (AR grade). Abaris Healthcare Pvt. Ltd., Ahmedabad, Gujarat, India, donated the pure medicine. The experiments were carried out at Goa College of Pharmacy's Central Instrument Laboratory in Panaji, Goa, India.

Instrumentation

The following equipment/instruments were used: a Wensar electronic weighing balance, a Citizen sonicator, a Labtronics pH metre, a Jasco HPLC with ChromNav software, and a Phenomenex C18 column.

Chromatographic Circumstances

Jasco CO-4061 HPLC system with Autosampler (AS-4050), PDA detector, and built-in degasser was employed. ChromNav software was utilised in the system .Phenomenex C18 column (25cm length, 0.46cm internal diameter, and 5 particle size of packing) was chosen after several testing. Varied solvents in various amounts were explored, with methanol with phosphate buffer and acetonitrile with triethylamine being the two compositions chosen. Method 1's mobile phase consisted of 43 parts methanol and 57 parts buffer. Dissolving disodium hydrogen phosphate in water and correcting the pH to 3.0 with orthophosphoric acid yielded phosphate buffer pH 3.0.
Method 2 utilised 15 parts acetonitrile and 85 parts 0.1 percent triethylamine (TEA) adjusted to pH 3.0 with formic acid as the mobile phase. Prior to use, the mobile phase components were sonicated for 15 minutes and filtered using

0.45 membrane filters. The flow rate was kept constant at 0.8mL/min for both procedures, and the detector was set to 294nm wavelength. The autosampler employed has a variable loop volume of 0-100L, and 20L was injected in this approach. Table 1 shows the optimal chromatographic settings for a certain fluoroquinolone.

Development of a Method

Mobile Phase Selection and Preparation

Mobile phases including methanol, acetonitrile, water, and buffers at various pH levels were tested in various quantities and flow rates[53-55].At flow rates of 0.8mL/min, satisfactory peaks were achieved using the mobile phases listed in Table 1 and setting the pH to 3.0 using a pH metre. Before being introduced into the system, both components of the mobile phase were vacuum filtered through 0.45m membrane filters and sonicated for 15 minutes.

Standard Stock Solution Preparation

Depending on the procedure, the standard solutions of the medicines were produced in methanol/acetonitrile. To make standard stock solutions of 1000 g/mL of each medication, a quantity of 25 mg of each drug was weighed and dissolved in methanol/acetonitrile in 25mL volumetric flasks. To produce the appropriate concentrations of each medication, the standard stock solutions were further diluted with methanol/acetonitrile.

Calibration Curve Preparation

Each drug's standard stock solution was transferred to a 10 mL volumetric flask and diluted to the desired concentration using methanol/acetonitrile. Aliquots were taken in such a way that the final concentrations were within the acceptable limit. Using optimal circumstances, three injections of 20 L of each concentration were examined. The average or mean of three readings was used to record each reading or peak area. For each medication, calibration curves were created by graphing peak areas recorded for each concentration on the y-axis and the drug concentration on the x-axis. For each drug's calibration curve, the coefficient of determination (R2) was computed.

Experiments on Forced Degradation

Stock solutions of the medication LEV were produced in methanol/acetonitrile at a concentration of 1000 g/mL (1 mg/mL).2mL aliquots were collected from the stock and used to make a 10mL volume of 5N HCl. For 6 hours, these acidic solutions were placed in a water bath at 65°C.The deteriorated solutions were then neutralised and diluted with water to make up to 25mL.From these damaged samples, 1mL was taken and built up to 10mL with methanol/acetonitrile to inject as a sample[56-61].

Validation of the Method

The method was validated using ICH guidelines, which included evaluating factors like accuracy, precision, linearity, robustness, ruggedness, detection, and quantification limits[62-71].When evaluating the results, acceptable limits for Relative Standard Deviation were set at less than 2%. (RSD).ANOVA was also utilised to prove the precision and robustness of the approach statistically.

Perfection

For each of the medications, the precision of the devised approach was confirmed. Actual investigation of six replicate injections of a standard concentration of each medication yielded the peak areas [72].The RSD was used to assess the method's precision in terms of intra- and inter-day fluctuation in the peak areas. ANOVA was also used to establish the method's precision.

Precision

For each of the drugs, the method's accuracy was tested by spiking a known concentration of each drug at three different concentration levels, namely 80%, 100%, and 1200%, and then comparing the difference between the expected/ theoretical value and the concentration actually determined by the method [73-75].

Linearity

For the medication LEV, stock solutions of 1000 g/mL in methanol/acetonitrile were produced. Working standard solutions in the appropriate range were generated from these stocks and injected into the HPLC apparatus. Each medicine has been shown to be linear in the specified range. The calibration graph (obtained by plotting the drug's peak regions versus its concentration) was made using replicate analyses at all concentration levels, and the linearity of the connection was determined using the Microsoft Excel® application.

Designing Critical Attributes with Robustness and a QbD Approach

Variation in crucial parameters, such as flow rate, pH, and fraction of mobile phase components, was used to confirm the robustness of the devised technique for chosen fluoroquinolones. ANOVA was used to verify robustness statistically [73,74,76].

Limits of Detection and Quantification

The linearity curve was used to calculate the limit of detection (LOD) and limit of quantification (LOQ).These values were calculated using the following formulas: LOD=3.3/s

LOQ=10/s, where s is the slope of the calibration curve and is the standard deviation of the y-intercept of the regression line.

LEV Calibration Curve

Table 3 shows the correlation coefficients (R2) and linearity equations for each of the methodologies under discussion.

Validation of the Method

The procedure was verified and applied to commercially available formulations.

Determining Critical Attributes and Their Scope

The important attributes determining the approach's effectiveness were identified and used to build a method that is both resilient and easy to transfer. These factors were then tested over a variety of ranges, as shown in Table 5.

The Use of ANOVA in the Design of Design Space

It was discovered that the approaches were both robust when using ANOVA as a statistical analysis tool. As shown in Table 6, this exercise also resulted in the creation of a Design Space for each essential variable The f-ratio demonstrates that there is no substantial difference in efficiency between the two methods in the event of any planned or unintentional alteration in any of the important parameters within the Design Space.

DISCUSSION

The new RP-HPLC techniques for the drug LEV in the presence of its acid degradant were developed and verified according to ICH guidelines in the study reported. It was demonstrated that any alteration within the examined Design Space had no effect on the validity and effectiveness of approaches using ANOVA as a form of statistical analysis for validating robustness. We accept the null hypothesis that there is no significant difference in the outcomes within this range because the result was not significant at p 0.05, indicating that modifications within the Design Space do not require revalidation of the method.

CONCLUSION

The developed RP–HPLC methods were proven to be suitable for analysing the fluoroquinolone LEV in both bulk and marketed forms such as tablets. Simple, rapid, sensitive, inexpensive, dependable, and precise procedures were discovered. Method 2 can also be altered for use with LC-MS. Simultaneously, a Design Space was constructed, which defined limitations for essential attributes determining robustness. Variations in parameters inside the Design Space have no effect on the method's performance, allowing it to be easily transferred without additional effort or expense. Both of these approaches can be used to estimate LEV in the presence of its acid breakdown product, whether in bulk or in medicinal dosage forms. Method 2 using acetonitrile is also compatible with LC-MS, which is a benefit over Method 1.

ACKNOWLEDGMENTS

The authors are appreciative to the Principal of Goa College of Pharmacy for giving working space and to Abaris Healthcare Pvt. Ltd., Ahmedabad, Gujarat, India, for donating clean medicine samples as gifts.

INTEREST CONFLICT: There is no interest conflict.

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Table 1: Chromatographic Conditions That Have Been Optimized

Parameters	Method 1	Method 2
Stationary phase (column)	C18 (250 mm×4.6 mm, 5 μm)	C18 (250 mm×4.6 mm, 5 μm)
Mobile phase	Methanol: 20mM phosphate buffer, pH 3.0 with Orthophosphoric acid	ACN: 0.1% TEA, pH 3.0 with Formic acid
Ratio (v/v)	43:57	15:85
Flow rate (mL/min)	0.8	0.8
Run time (min)	8.0	10.0

Injection Volume (µL)	20	20
Detection Wavelength (nm)	294	294

Table 2: Shows The Methods Used To Determine The Efficiency Of A System.

Sr No	Parameters	Acceptance Criteria	Method 1		Method 2	
			LEV	LDA	LEV	LDA
1	Plates of theorem	>1000	4517	4190	7642	7594
2	a restraining element	<2	1.296	1.362	1.243	1.522
3	RSD in the area	<2%	0.528	0.616	1.861	1.155
4	Retention Time RSD	<1%	0.488	0.356	0.255	0.093
5	Between-peak resolution	>2	____	2.169	____	12.000

Table 3: Shows the Correlation Coefficients (R2) and Linearity Equations for Each of the Methodologies under Discussion.

Sr.No.	Drug	R^2	Linearity equation
1	Method 1	0.999	y = 121479x + 6584.9
2	Method 2	0.999	y = 21142x – 49348

Table 4 : Summarises The Findings.

Parameter	Method 1	Method 2
Linearity range(µg/mL)	30-210	10-120
LOD(µg/mL)	5.92	1.29
LOQ(µg/mL)	19.75	4.29
Precision	Complies	Complies
Assay (Limits 90-110%) *	98.71%	98.71%
Accuracy	95-101%	100-101%
Robustness**	Complies	Complies

Table 5: Critical Attributes And Ranges Analyzed.

Sr.No.	Method 1			Method 2		
	Critical Attribute	Value	Range	Critical Attribute	Value	Range
1	% of Methanol	43%	41-45%	% of Acetonitrile	15%	13-17%
2	pH of Buffer	3.0	2.8-3.2	pH of Buffer	3.0	2.8-3.2
3	Flow Rate (mL/min)	0.8	0.6-1.0	Flow Rate (mL/min)	0.8	0.6-1.0

Table 6 : The Approaches Were Both Robust When Using ANOVA As A Statistical Analysis Tool

Method	CA	n	Mean Conc (µg/mL)	*F obtained*	Critical F-value (0.05 significance level)
1 (using concentration 60 µg/mL)	% of Methanol	9	59.984	2.278	5.14
	pH of Buffer	9	59.911	2.182	
	Flow Rate	9	59.696	1.915	
2 (using concentration 25 µg/mL)	% of Acetonitrile	9	24.836	0.529	5.14
	pH of Buffer	9	24.841	0.788	
	Flow Rate	9	24.873	0.383	

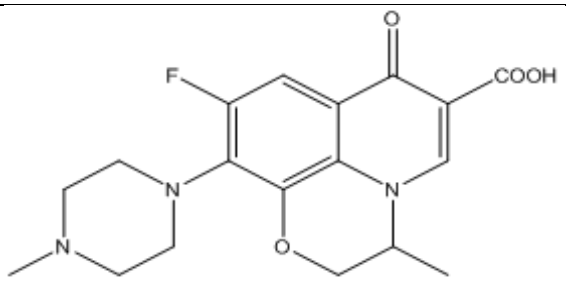

Figure 1: shows the LEV's structure.

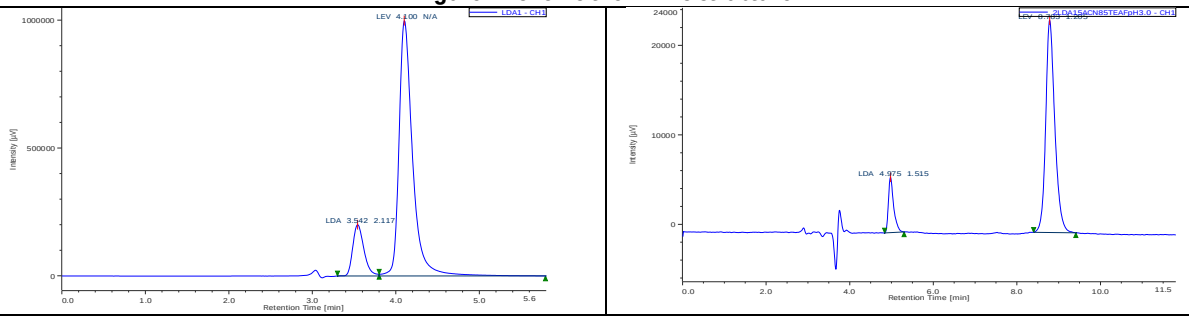

Fig. 2: Representative LEV and Acid Degradant LDA chromatograms obtained using (a) Method 1 and (b) Method 2 5.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Medical Infrastructure and Economy of Commercial Surrogacy

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Received: 04 Jan 2022 Revised: 14 Feb 2022 Accepted: 07 Mar 2022

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ABSTRACT

This paper deals with the concept of commercial surrogacy. Surrogacy is an arrangement to use the assisted reproductive techniques involving third person and this arrangement results in many legal, medical, moral and financial problems left for courts to resolve. Most of the issues relate to the contracts arising out of surrogacy and paying money to the surrogate for her services. There are thousands of the potential parents across the world with both the desire and the wherewithal to hire another woman to bear the children. India is marked as Global Hub in relation to commercial surrogacy. India is diverse and heterogeneous nation. Female infanticide, domestic violence, dowry deaths, honour killings and many other social evils show the position of women in the society. Surrogacy holds most attraction for the poorest women in India and though surrogates do tend to be of lower-income classes. Official statistics of who actually constitute surrogate mothers, however, are currently not available. Surrogacy in India expanded enough to meet economies of scale which leads to fertility business employing the services of thousands, even millions of women. Before commercial surrogacy was banned in India, it was a popular destination for surrogacy. The economic scale of surrogacy in India is unknown, but study backed by the United Nations in July 2012 estimated the business at more than $400 million a year, with over 3,000 fertility clinics across India.

Keywords: Commercialsurrogacy, poverty, surrogate mother

Global affairs in present time includefertility traveland childbearing. Scientific and technological development provides more and new ways to conceive beyond the limits of the body as well as national and cultural boundaries. India, a “global hub” of commercial gestational surrogacy, boasts more than two hundred fifty IVF (in vitro fertilisation) clinics and several agencies dedicated to commercial surrogacy, and the Indian Council for Medical

Research projects that profits will reach nearly Six billion dollars in the next few years (Deomampo 2013). The medical infrastructure in India attracts the clients from North America, Australia, Europe, Bangladesh, Pakistan and African Countries as well as clients from within India where ART (Assisted Reproductive Technology)infrastructure is underdeveloped. All the way, from far places infertile couple or intended parents seeking child through surrogacy travel to India due to availability of advance medical facilities and experienced IVF practitioners that are either unavailable or unaffordable in their own country.Many people imagined India as a place of origin of family, particularly highlighting the state of Punjab in India, district Jalandhar has emerged as the Asia's biggest Medicare hub with over 800 super speciality, multi-speciality centres, nursing homes and clinics. The Indian clinics charges $10000 and $28000 for the complete package of surrogacy. It becomes to be one third price of procedure followed in U.K. The surrogacy in India is low cost and laws were flexible before the passing of legislation to regulate the surrogacy. People relied upon the medical infrastructure of India for the journey of building a family through surrogacy.

Concept of Surrogacy can be traced back to Biblical times. In mid 1970s concept was developed with an advertisement and in the 1980s, surrogacy became a prominent practice. In 1986, first gestational surrogacy arrangement was done successfully. The Etymology for 'Surrogate' comes from Latin word Surrogatus, which is past participle of Surrogare or subrogare, which means to substitute. A combination of two words i.e. Sub which means in place of and rogare means to ask. The surrogate mother is one who gives away the child born to her to the intended parents. Surrogacy is a type of fertility treatment, which includes in vitro fertilization (IVF), helpful to made motherhood as well as fatherhood possible for women with infertility, gay couples and single man etc.

Surrogacy is a kind of technique, which helps intended parents to form their families with the help of assisted reproduction when they are unable to produce a natural born baby by themself. It is a long and complex phenomenon, which starts with searching and finding a womb for the child, goes through an arrangement, which includes social, medical and legal process. In general, Surrogacy means 'Rent a Womb' where a woman called Surrogate Mother agrees to give birth to a child for intended parents. Surrogacy is a complicated legal and medical process. Surrogacy is not same for all. It has different methods and types, which helps one to choose the best way of surrogacy.

Traditional Surrogacy is a method of surrogacy also known as artificial insemination surrogacy, partial, natural, or straight surrogacy. In this method, egg of surrogate mother and sperm of intended parent or donor is used. Insemination of the surrogate can occur naturally or through artificial insemination. Child is not genetically related to intended parents, but if sperm of intended father is used in the process then the child is genetically related to intended father and surrogate mother. Gestational Surrogacy is a method of surrogacy is also known as in vitro fertilization surrogacy, host or full surrogacy. In this method, an embryo is created, using intended father's sperm and intended mother's egg, by in vitro fertilization and implanted in surrogate mother. The resulting child is genetically related to the intended parents. If the donor sperm or donor eggs are used to create the embryo, the child is not genetically related to intended parents or surrogate mother in either way of gestational surrogacy.

Altruistic Surrogacy is a type of surrogacy in which a surrogate is provided with medical expenses or finances related to pregnancy. No additional compensation is given to surrogate. In these types of surrogacy arrangements mostly relatives helps the infertile couple to create their family. Commercial Surrogacy is one where the intended couple do not want involvement of relative; they may go for this type of surrogacy arrangement. In commercial surrogacy the womb is taken on rent and all the expenses incurred out of this arrangement is given to surrogate along with some compensation or the payment of the services given by her until the delivery of the baby.

In earlier times, the only way to produce child from surrogate mother is to have sexual relation with the intended father. However, AI (Artificial Insemination) made it possible to impregnate a surrogate without having physical contact. In economic terms, commercial AI increased the demand and supply of surrogate mother, increasing sperm banks and so on. Commercial potential for surrogacy first recognized by Noel Keane, by acting as a broker he placed

advertisements in Michigan papers offering surrogates a fee for surrogacy. Keane was successful in launching a market for the supply of mothers. However, under Michigan law commercial surrogacy was illegal. Therefore, Keane shifted to Altruistic model, offering surrogates the chance to give the gift of life. By the early 1980s, brokers had produced a handful of high profile babies: 100 by 1983, roughly 500 by 1987 (Spar, 2005).

Surrogacy continued and with new scientific development new technology emerged with IVF (in-vitro fertilisation). The first test tube baby "Louise Brown" made it famous in 1978. Initially IVF was used almost exclusively within the bonds of marriage (Spar 2005). United States, UK, and Australia approved IVF for the treatment of infertility with some restrictions.by the 1985; IVF was a commonly employed practice. The beauty of IVF was that it allowed some infertile women to by-pass the physical blockage that prevented pregnancy (Spar 2005). IVF splits the genetic mother from the surrogate mother; surrogate mother become pregnant with other woman's eggs and gives birth to genetically unrelated child. Commercially IVF was raising and many stories of egg sales or donation were on air. By 1998, the program's director reported that he was receiving 50 to 100 calls a week from potential donors and now had 500 donors on file (spar 2005). In economic terms, increased supply increased both price and demand. In 1999, an infamous ad in Ivy League newspaper offered $50,000 for the right kind of egg (Spar 2005). Women were willing to serve as Gestational Carriers, to provide eggs without womb attached and many infertile couples were interested in surrogacy arrangement with the advent of IVF. Legally and commercially, the market for surrogates becomes stable. The price of eggs raisedday-by-day giving relief to poverty of women.

Women in the world are deprived of the equal status and opportunities resulting in their low growth in the society. In the developing countries, women are burdened with many inequalities. In India situation is worse due to patriarchy and high traditional value system. The well-being of the nation is related to the well-being of the women to fully utilize the women's potential an every field. In past times, the role of women in the cottage industries contributed to economy. But at present their income related to commercial surrogacy is not contributing to the economy but helping their own poor families to at least earn livelihood and further raise their standards.

Experience of Surrogate Mothers

Surrogate Mother 1: Rayven Parkins (4 times surrogate) says, "we are not rich people….. but its' one way our family can give back a really big way." According to her, there are lot of sacrifices in this process. State laws vary and so the place to stay for surrogate mother varies. She even missed Christmas with her in laws as her doctor said she could not travel. Still she felt good as she has ability to help someone through this process.

Surrogate Mother 2: Robin Kaufer says "the hardest part was when they took the baby from me." However, in her case her family was also created from different means so her family members understand that families come to be in many different ways.

Surrogate Mother 3: Kymberli Barney says, "the hardest part was shouldering the burden of infertility." Her several cycles were resulted in miscarriage so she found it hard and decided to retire.

Surrogate Mother 4: Avani, 25 years old, wife of a watchman who could hardly earn US $ 40-60 per month. When she came to know that, she would earn US $ 4700 upon delivery of healthy baby she decided to become surrogate. First thing, she have to do was to leave her home and move to new accommodation where she remain indoors all the times with her mother in law and kids but she felt isolated.

Surrogate Mother 5: Urvashi wife of watchman who could earn US $ 150 per month but due to his poor health he left the job and family struggled a lot. Surrogacy helped them in the financial crisis. She left her place but not allowed to move around and work, which adds to her pain. She felt immobility as stressful and isolating.

Surrogate Mother 6: Meera wife of a man who was 10 years older than her. She was not satisfied from her marriage. Meera was under pressure due to her husband and in laws; she escaped to her boyfriend's home even during her surrogacy, as there was no fixed policy on housing so she seemed to move here and there. Her mobility ended on the request of intended parents.

Surrogate Mother 7: Najma Vohra, says "that local residents of her village would perceive her surrogacy to be dirty and that her family would be shunned if her neighbours knew." (Schanbacher 2014). This social stigma always let her in anxiety. She forcefully left her family and went to gestational dormitories.

From the above case studies, following reasons can be concluded in favour of surrogacy as employment and surrogates as labourers.

1. Primarily low-income families are indulged in surrogacy processes;
2. To solve the financial problems such as debt or lack of housing;
3. To raise family standards;
4. To prevent starvation;
5. Inadequacy of husband's income;
6. Husband's negligence in fulfilling family responsibilities;
7. To provide better facilities for children.

The employment in relation to surrogacy favours the poor world but the following problems are also related with the process of surrogacy.

1. Risk of post-partum depression and psychological harmful feeling of guilt or anger (Tehran et.al 2014).
2. Rejection by neighbour and friends;
3. Low level of closeness of surrogate mother with intended couple;
4. Less respected by intended couple;
5. Constrains not to feel the baby/ emotional detachment from baby;
6. Fear of husband's reaction in marital relationship;
7. Complication of pregnancy/ risks in process of surrogacy;
8. No enough payment for expenses involved in surrogacy;
9. Family disruption due to sexual relationship issue during surrogacy;
10. Lack of social support due to negative attitude of people;
11. Spatial imprisonment/ restricted mobility;
12. Isolation;
13. Instability.

The medical procedure involved in the process of surrogacy must follow the medical ethics. This again in India when not followed further leads to many problems for surrogates' mother. There is a principle of non-maleficence, which says that no needless harm or injury should be involved in the medical procedure, which affects the patient. In the U.S Surrogates are given no more than two embryos for their safety but in India up to five embryos were given to increase the chance of pregnancy (Schanbacher 2014). India's gestational surrogacy market exploits the poor, as there are gender disparities; female infanticide is in practice in every corner of India. Good nutrition, education and health care is only for sons and daughters are heavy burden on Indian parents due to dowry or domestic violence. Next is principle of beneficence, which says that benefits should of those who are undergoing medical procedures. Most Indian surrogates are paid US $ 3000- $ 6000 for each surrogacy (Schanbacher 2014); which might be equal to fifteen years of wages. Therefore, this might be called as fastest route to money, but this is pay on production if failed to produce a baby then they are not paid.

Medical autonomy principle requires that for every person there is right to make his or her own choices and develop their own life plan.it is also the basis for informed consent. Due to starvation or poor condition, surrogates do not refuse the offer of high payments for surrogacy.Even the surrogacy clinics also pressurise women to become surrogates for outside or foreign clients. Many surrogates are illiterate or uneducated they even do not know the meaning of IVF (in vitro fertilisation) even then they give consent only for money. Sometimes, doctors control the funds of surrogates. The contract between surrogate mother and intended parents bounds the surrogate mother not to terminate the pregnancy if she wishes to. This again violates the autonomy. The justice is also denied. The Indian surrogates are treated as inferior than western surrogates. American surrogates are paid $ 20,000 to gestate a foetus while in India; a sum paid is between $ 3000 to $ 6000 with the fee often influenced by the women social status (Schanbacher 2014).

CONCLUSION

The qualitative analysisis done for the secondary data collected through various article, books and websites. The paper highlighted the exploitation of poor in the current medical infrastructure and the economy of commercial surrogacy has the positive as well negative consequences on the surrogate mothers as labourer. Through this process baby is commercialised, but they should be the product of love not of the money and for the money. Still in India, the surrogacy market provides a baby for the infertile couple and economic benefits to the poor surrogates. Several parties are involved in the process of surrogacy so regulatory measure is must for this process. India has recently passed a legislation to control the surrogacy practices including a ban on commercial surrogacy but still many foreigners are attracted by the medical facilities in India and they want Indian element in their babies born out of this process.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Pilot Study to Assess the Effectiveness of Interventions on Problems Related to Puberty among Adolescent Girls in Selected Rural Areas

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Received: 06 Jan 2022 Revised: 29 Jan 2022 Accepted: 21 Feb 2022

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ABSTRACT

A Quasi-experimental study, pre-test and post-test with control group interrupted time series design with quantitative approach was undertaken to assess the effectiveness of interventions on problems related to puberty among adolescent girls in selected rural areas, Salem, Tamilnadu. 30 adolescent girls (15 Control group and 15 Experimental group) were selected by using cluster sampling technique and data were collected by Semi-structured Interview schedule, Observational checklist, Self-Administered rating scale and Numerical rating pain scale. Finding revealed that highest percentage (60%) of adolescent girls were in the age group of 16-17 years in experimental group and (46.7%) of them 18-19 years in control group, 53.3% of the adolescent girls had primary school in experimental group and 46.7% of them high school in control group. 53.3% of the adolescent girls belongs to Hindu in experimental group and 40% of them Muslim in control group, 73.3% of the adolescent girls belongs to nuclear family in experimental group and 53.3% of them in control group,53.3% of the adolescent girls had family monthly income Rs.10001-15000 in experimental group and 40% of them had Rs.5000-10000 in control group. More or less similar (86.7% & 80%) percentage of the adolescent girls had non-vegetarian in control and experimental group, 53.3% of the adolescent girls had sources of information from television in experimental group and 40% of them in control group, 66.7% of the adolescent girls had attained age of menarche 10-12 years in control group and 46.7% of them in experimental group, more or less similar (66.7% & 60%) percentage of the adolescent girls had 28-35 days duration of menstrual cycle in

experimental and control group, similar (60%) percentage of the adolescent girls had 3-5 days and 5-7 days duration of menstrual flow in control and experimental group, 60% of the adolescent girls had excess menstrual flow in experimental group and 46.7% of them in control group. 53.3% of the adolescent girls had excess menstrual flow day1 of menstruation period in experimental group and 46.7% of them day2 of menstruation period in control group, 86.7% of the adolescent girls had pain during menstruation in control group and 66.7% of them in experimental group, more or less similar (66.7% & 60%) percentage of the adolescent girls had pain after day1 menstruation in control and experimental group. Similar (53.3%) percentage of the adolescent girls used 4-6 vaginal pads per day in control group and 7-9 in experimental group, similar (60%) percentage of the adolescent girls changed 4-6 hours once vaginal pads per day in control and experimental group, 60% of the adolescent girls used in disposable commercial vaginal napkins in control group and 46.6% of them in experimental group. 40% of the adolescent girls slightly satisfied vaginal napkin was used in control group and similar 33.3% of them satisfied in control & experimental group and not satisfied in experimental group. Similar (40%) percentage of the adolescent girls had onset of puberty problems 6 months in control and 12 months in experimental group, 80% of the adolescent girls had disturbances of daily activities in control group and 66.7% of them in experimental group. Similar (53.3%) percentage of the adolescent girls had ability to daily activities extend in control and experimental group, 66.7% of the adolescent girls had sleeping pattern disturbance in experimental group and 53.3% of them in control group, 53.3% of the adolescent girls had sleeping per day 8 hours in control group and 40% of them in experimental group 73.3% of the adolescent girls had family history of puberty problems in experimental group and 60% of them not had family history of puberty problems in control group. 73.3% of the adolescent girls had BMI 18.5 - 24.9 in experimental group and 60% of them in control group. Highest percentage of the adolescent girls had moderate problems in experimental group pre-test. Highest percentage of the adolescent girls had mild pain in experimental group 2nd post-test. Highest percentage of the adolescent girls had good development in experimental group 2nd post-test. Hence, it shows that effectiveness of interventions on problems related to puberty among adolescent girls.

Keywords: television, vaginal, Numerical, group.

INTRODUCTION

Puberty is the process of physical changes through which a child's body matures into an adult body capable of sexual reproduction. It is initiated by hormonal signals from the brain to the gonads: ovary in a girl. In the response to the signals, the gonads produce hormones that stimulate libido and the growth, function and transformation of the brain, bones, muscle, blood, skin, hair, breasts and sex organs. Physical growth-height and weight-accelerates in the first half of puberty and is completed when an adult body has been developed. The maturation of the reproductive capabilities average girls begin puberty around ages 10-11 and end puberty around 15-17, the major landmark of the puberty for females is menarche. The first onset of menstruation which occurs on average age group between 12 and 13 years. Puberty which starts earlier than usual is known as precocious puberty and puberty which starts later than usual is known as delayed puberty.

Statement of the Problem

A pilot study to assess the effectiveness of interventions on problems related to puberty among adolescent girls in selected rural areas, Salem, Tamilnadu.

Objectives

- To assess the problems related to puberty before administering interventions among adolescent girls in experimental group and control group.
- To assess the problems related to puberty after administering interventions among adolescent girls in experimental group.
- To compare the effectiveness of interventions on problems related to puberty among adolescent girls with demographic variables in experimental group.
- To find out the association between pre-test score of interventions on problems related to puberty among adolescent girls with demographic variables in experimental group and control group.
- To find out the association between post-test score of interventions on problems related to puberty among adolescent girls with demographic variables in experimental group and control group

Research Design and Approach

A Quasi-experimental study, pre-test and post-test with control group interrupted time series design with quantitative approach

Study Setting

The study was conducted in Chenraya Perumalmalai and Kanchamalai, Salem district.

Population

The study population comprised of the entire individual with the adolescent girls living in Chenraya Perumalmalai and Kanchamalai, Salem.

Sampling

The study samples were adolescent girls living in Chenraya Perumalmalai and Kanchamalai, Salem who fulfilled the inclusive criteria.

Sampling Technique

Cluster sampling technique was used as a sampling technique for the present study.

Sampling Size

30 adolescent girls living in Chenraya Perumalmalai and Kanchamalai(15 control group and 15 experimental group)Salem.

Tool used

Semi-structured Interview schedule, Observational checklist, Self-Administered rating scale and Numerical rating pain scale was used to collect the data regarding the effectiveness of interventions on problems related to puberty among adolescent girls.

RESULT AND DISCUSSION

30 adolescent girls were selected by cluster sampling technique and data were collected by using Semi-structured Interview schedule, Observational checklist, Self-Administered rating scale and Numerical rating pain scale. The collected data was analysis by inferential statistics. Demographic characteristics reveals that highest percentage (60%) of adolescent girls were in the age group of 16-17 years in experimental group and (46.7%) of them 18-19 years in control group, 53.3% of the adolescent girls had primary school in experimental group and 46.7% of them high school in control group. 53.3% of the adolescent girls belongs to Hindu in experimental group and 40% of them Muslim in control group, 73.3% of the adolescent girls belongs to nuclear family in experimental group and 53.3% of

them in control group, 53.3% of the adolescent girls had family monthly income Rs.10001-15000 in experimental group and 40% of them had Rs.5000-10000 in control group, more or less similar (86.7% & 80%) percentage of the adolescent girls had non-vegetarian in control and experimental group, 53.3% of the adolescent girls had sources of information from television in experimental group and 40% of them in control group.

66.7% of the adolescent girls had attained age of menarche 10-12 years in control group and 46.7% of them in experimental group, more or less similar (66.7% & 60%) percentage of the adolescent girls had 28-35 days duration of menstrual cycle in experimental and control group, similar (60%) percentage of the adolescent girls had 3-5 days and 5-7 days duration of menstrual flow in control and experimental group, 60% of the adolescent girls had excess menstrual flow in experimental group and 46.7% of them in control group, 53.3% of the adolescent girls had excess menstrual flow day1 of menstruation period in experimental group and 46.7% of them day2 of menstruation period in control group. 86.7% of the adolescent girls had pain during menstruation in control group and 66.7% of them in experimental group. More or less similar (66.7% & 60%) percentage of the adolescent girls had pain after day1 menstruation in control and experimental group, similar (53.3%) percentage of the adolescent girls used 4-6 vaginal pads per day in control group and 7-9 in experimental group, similar (60%) percentage of the adolescent girls changed 4-6 hours once vaginal pads per day in control and experimental group, 60% of the adolescent girls used in disposable commercial vaginal napkins in control group and 46.6% of them in experimental group,

40% of the adolescent girls slightly satisfied vaginal napkin was used in control group and similar 33.3% of them satisfied in control & experimental group and not satisfied in experimental group, similar (40%) percentage of the adolescent girls had onset of puberty problems 6 months in control and 12 months in experimental group, 80% of the adolescent girls had disturbances of daily activities in control group and 66.7% of them in experimental group, similar (53.3%) percentage of the adolescent girls had ability to daily activities extend in control and experimental group, 66.7% of the adolescent girls had sleeping pattern disturbance in experimental group and 53.3% of them in control group. 53.3% of the adolescent girls had sleeping per day 8 hours in control group and 40% of them in experimental group, 73.3% of the adolescent girls had family history of puberty problems in experimental group and 60% of them not had family history of puberty problems in control group, 73.3% of the adolescent girls had BMI 18.5 - 24.9 in experimental group and 60% of them in control group. Highest percentage of the adolescent girls had moderate problems in experimental group pre-test. Highest percentage of the adolescent girls had mild pain in experimental group 2nd post-test. Highest percentage of the adolescent girls had good development in experimental group 2nd post-test. Hence, it shows that effectiveness of interventions on problems related to puberty among adolescent girls.

Percentage wise distribution to assess the effectiveness of interventions on problems related to puberty among adolescent girls reported that highest percentage (86.7%) of the adolescent girls had moderate problems in experimental group pre-test and lowest percentage (13.3%) of them in experimental group 3rd post-test. 40% of the adolescent girls had severe problems in control group 3rd post-test and only 6.6% of them in experimental group 2nd post-test. Further, 60%of the adolescent girls had mild problems in experimental group 3rd post-test and 20% of them in experimental group 1st post-test. Whereas, 26.7% of the adolescent girls had no problems in experimental group 3rd post-test. None of them had no problems in control group pre-test, 1st, 2nd& 3rd post-test, experimental group pre-test, 1st& 2nd post-test, Mild problems in control group pre-test, 1st, 2nd& 3rd post-test and experimental group pre-test and severe problems in experimental group 3rd post-test. Hence, it can be interpreted that highest percentage of the adolescent girls had moderate problems in experimental group pre-test it shows that effectiveness of interventions on problems related to puberty among adolescent girls.

Percentage wise distribution to assess the effectiveness of interventions on problems related to puberty among adolescent girls reported that highest percentage (93.3%) of the adolescent girls had mild pain in experimental group 2nd post-test and similar lowest percentage (20%) of them in control group pre-test & 1st post-test. Similar highest percentage (73.3%) of the adolescent girls had moderate pain in control group 2nd& 3rd post-test and similar lowest percentage (6.7%) of them in experimental group 2nd&3rd post-test. Similar highest percentage (26.7%) of the

adolescent girls had severe pain in control group pre-test, 1st, 2nd&3rd post-test and only 6.6% of them in experimental group pre-test. Whereas, 26.7% of the adolescent girls had no pain in experimental group 3rd post-test. None of them had no pain in control group pre-test, 1st, 2nd& 3rd post-test, experimental group pre-test, 1st& 2nd post-test, Mild pain in control group2nd& 3rd post-test and severe pain in experimental group 1st, 2nd&3rd post-test. Hence, it can be interpreted that highest percentage of the adolescent girls had mild pain in experimental group 2nd post-test it shows that effectiveness of interventions on problems related to puberty among adolescent girls.

Percentage wise distribution to assess the effectiveness of interventions on problems related to puberty among adolescent girls reported that highest percentage (80%) of the adolescent girls had good development in experimental group 2nd post-test and lowest percentage (46.7%) of them in experimental group 1st post-test. Similar (40%) highest percentage of the adolescent girls had fair development in control group pre-test,1st, 2nd& 3rd post-test and lowest percentage (26.7%) of them in experimental group pre-test. Further, 73.3%of the adolescent girls had average development in experimental group pre-test and 20% of them in experimental group 2ndpost-test. None of them had poor development in control and experimental group,Fair development in experimental group 1st, 2nd& 3rd post-test and good development in experimental group pre-test, 1st, 2nd& 3rd post-test and experimental group pre-test. Hence, it can be interpreted that highest percentage of the adolescent girls had good development in experimental group 2ndpost-test it shows that effectiveness of interventions on problems related to puberty among adolescent girls.

CONCLUSION

In the present study it can be concluded that effectiveness of interventions on problems related to puberty among adolescent girls. Hence, it can be interpreted that the investigator needs to conduct experimental study to assess the adolescent girls had average effectiveness of interventions on problems related to puberty.

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TableNo.1: Frequency and percentage wise distribution to assess the effectiveness of interventions on problems related to puberty among adolescent girls.

Level of Problems related to Puberty	Control Group								Experimental Group							
	Pre test		1st Post test		2ndPost test		3rdPost test		Pre test		1stPost test		2ndPost test		3rdPost test	
	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
No Problems	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	**26.7**
Mild Problems	0	0	0	0	0	0	0	0	0	0	3	**20**	7	46.7	9	**60**
Moderate Problems	10	66.7	10	66.7	10	66.7	9	60	13	**86.7**	10	66.7	7	46.7	2	**13.3**

Severe Problems	5	33.3	5	33.3	5	33.3	6	**40**	2	13.3	2	13.3	1	**6.6**	0	0
Overall	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**

Table No..2: Frequency and percentage wise distribution to assess the effectiveness of interventions on problems related to puberty among adolescent girls.

Level of Pain	Control Group								Experimental Group							
	Pre test		1st Post test		2ndPost test		3rdPost test		Pre test		1stPost test		2ndPost test		3rdPost test	
	F	%	f	%	f	%	F	%	F	%	F	%	F	%	F	%
No Pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	**26.7**
Mild Pain	3	**20**	3	**20**	0	0	0	0	4	26.7	6	40	14	**93.3**	10	66.6
Moderate Pain	8	53.3	8	53.3	11	73.3	11	**73.3**	10	66.7	9	60	1	**6.7**	1	**6.7**
Severe Pain	4	**26.7**	4	**26.7**	4	**26.7**	4	**26.7**	1	**6.6**	0	0	0	0	0	0
Overall	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**

Table No. 3: Frequency and percentage wise distribution to assess the effectiveness of interventions on problems related to pubertal development among adolescent girls

Level of Pubertal development	Control Group								Experimental Group							
	Pre test		1st Post test		2ndPost test		3rdPost test		Pre test		1stPost test		2ndPost test		3rdPost test	
	F	%	F	%	f	%	F	%	F	%	F	%	f	%	F	%
Poor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fair	6	40	6	40	6	40	6	40	4	26.7	0	0	0	0	0	0
Average	9	60	9	60	9	60	9	60	11	73.3	8	53.3	3	20	5	33.3
Good	0	0	0	0	0	0	0	0	0	0	7	46.7	12	80	10	66.7
Overall	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**	**15**	**100**

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prospect of Online Education in Coimbatore District

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Received: 07 Feb 2022 Revised: 18 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

Online learning is educations that take place over internet. It is often referred to as e-Learning among other terms. Online learning has gained popularities in recent times. However student satisfaction plays an important work with all the education related works. In this concern the study is confined to Coimbatore district, by taking various factors like app preferred by students for e-learning, fees paid for e-learning, problems experienced during e-learning, etc. The main objective of the study is to find out prospect of towards online learning, problems faced during e-learning in Coimbatore district. Online education is a form of education where students use their home computers or their personal computers through the internet. For many non-traditional students, among them all those who want to continue working full time or raising families, online graduations and courses have become popular in the past decade. Online education is also known as E-learning. E-learning typically refers to the online interaction between you as a student and the teacher; basically, you receive the training through an online medium, even though your teacher may be in the same building. E-learning can be used in a classroom or an online setting.

Keywords: e-Learning, Student Satisfaction, Online learning, Online education.

INTRODUCTION

In the last 20 years, the Internet has grown from being nearly non-existent into the largest, most accessible database of information ever created. It has changed the way people communicate, socialize, do business and think about knowledge and learning. Much more than just a new twist on distance learning, online schooling is changing the face of traditional classrooms and making education more accessible than ever before. Online education is a form of education where students use their home computers or electronic gadgets that supports through the internet to

continue their education. For many non-traditional students, among them all those who want to continue working full time or raising families, online graduations and courses have become popular in the past decade. Often online graduation and course programmes, some of which are conducted using digital technologies, are provided via the online learning portal of the host university. The Boston-based consulting firm Edu-ventures, Inc (2006) accepts more than 60 percent of employers generally with the high quality of online learning, but students' perceptions differ. Only about 33 percent of prospective online students said that they perceive the quality of online education to be "as good as or better than" face-to-face education. At the same time, 36 percent of prospective students surveyed cited concern about employers' acceptance of online education as a reason for their reluctance to enroll in online courses.

Operational Definition

Cent per cent Online Education: Fully-online degrees are earned from the comfort of your own home with no required visits to your college or university campus.

Hybrid Education: Hybrid education allows students to pursue a combination of online and on-campus courses.

Online Courses: While online courses may be part of a degree program, they can also be taken on their own in order to master a certain subject or learn a specific skill.

MOOCs: MOOCs, or massive open online courses, are usually delivered in lecture form to online "classrooms" with as many as 10,000 people.

Objectives of the study

- To portray the current trend of online education in Coimbatore District
- To identify the profile of stakeholders undergoing online education
- To make know the society on the strengths and weakness of online education
- To suggest optimum statements for uplifting the online education

METHODOLOGY STATEMENT

Until now online education was a choice, due to this unprecedented situation like Covid, it has become the new norm. In order to study the actual situation of the same, this study is carried out through convenience sampling method from the selected student population (60) located in the Coimbatore District. The Period of study is two months (April & May 2021). To conclude with the analysis part, Simple Percentage Analysis and Ranking Methodology were carried out. As a coin has both sides, there are equal numbers of Strengths and Weakness to this online education. It is been discussed as below

Statistical Analysis on Online Education

From the above table it is seen that 51.8% of the respondent (31) are PG Students, 26.6% of the respondents (16) belong to students of UG and 21.6% of the respondent (13) are School Students. Majority of the respondents are undergoing their Post Graduation. The table 4.2 shows that 45% of the respondents (27) are above 21 years, 31.6% of the respondents (19) are belonging to age group 18 to 20 and the remaining 23.4% of the respondent (14) are below 17 years of age. Most of the respondents (31.6%) are above 21 years. The table 4.3 exhibits that 11.6% of the respondents (7) are earning above Rs. 50001, 31.6%of the respondents (19) earn between Rs. 30001 and Rs. 50000, 41.6% of the respondent (25) earn above Rs.15001 and Rs. 30000 and the remaining 15.2% of the respondent (9) are earning below Rs. 15000 as their family income. Table 4.4 reveals that 81.6% of the respondent (49) says offline classes as the best method of teaching by giving YES opinion and 18.3% of the respondents (11) says its second to online teaching by opting the option NO. Table 4.5depicts that 85% of the respondent (51) has regularly faced the problem 15% of the respondents (9) have frequently faced the problem. From the above table, six strength parameters of online education is studied using ranking method. The results are as shown. The Review lectures instantly is Ranked as I, followed by Group communication as II, Cost as III, Work from anywhere, at any time as IV, Diversity as V and Flexible learning schedule as VI rank Respectively.

Suggestions through the analysis on online education

When it is seen, the strengths deemed to be there always and it sounds great. But when the weaknesses are addressed, it requires the following suggestions. If they have been fine-tuned, we can take online education to the next level in the higher education.

- The students in the online classes must be allowed to give feedback. Although it cannot be asked from everyone, at-least it must be heard optimum
- It is seen from majority of the students that E-Learning can cause social Isolation, it has to be prevented. Although the classes are conducted online, some practical works are to be given using the society and it has to be monitored.
- Every student must require strong self-motivation and time management skills. It has to be embedded by the course teacher through their strong endurance
- Online classes urge heavy lack in communicational skill development. It is true and it has to be taken care by giving seminar, just a minute talk and also through snap talk through the same online mode itself.
- Teachers must follow strategic move in-order to prevent cheating during online assessments. Although it is complicated to eradicate but if assessment is done through some specific moves, it can be curtailed.
- Online instructors can also tend to focus on practice so that the students may not feel boredom during their online classes
- The students and teachers must in majority times, should focus on video recorded classes. This will help the teachers to focus on the eye contact or the attention of the students in the classes
- The management and the parents are equally responsible to provide computer literacy to their students so that the overall population is moving towards uplifting the quality standards and this may not lead to lack of accreditation & quality assurance in online education

CONCLUSION

The facts seen in this study are the strengths and weakness of online education and it is similar to every type of learning environment. Students have to analyze both the pros and cons factors which contribute them greatly on the direction of his/her career path in the current situation. Students have to decide, how they are going to accomplish their goals: online, in the classroom or a combination of both. It is also true that learning is highly dependent on the individual's motivation to learn. So the bottom line is that the efforts that a student puts into the education will eventually determine the beneficial experience to shape the future career.

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Table 1: Education level

Description	Frequency	Percentage
Schooling	13	21.6%
UG	16	26.6%
PG	31	51.8%
Total	**60**	**100%**

Source: Primary data

Table 2: Age composition

Description (in Years)	Frequency	Percentage
Above 21	27	45%
18-20	19	31.6%
Below17	14	23.4%
Total	**60**	**100**

Source: Primary data

Table 3: Family Income

Description (in Rupees)	Frequency	Percentage
ABOVE 50001	7	11.6%
30001-50000	19	31.6%
15001-30000	25	41.6%
BELOW 15000	9	15.2%
Total	**60**	**100%**

Source: Primary data

Table 4: Offline classes is best teaching

Description	Frequency	Percentage
Yes	49	81.6%
No	11	18.3%
Total	60	100%

Source: Primary data

Table 5: Frequency of problem faced in online

Description	Frequency	Percentage
Regularly	51	85%
Rarely	0	0%
Frequently	9	15%
Total	60	100%

Source: Primary data

Table 6: Ranking on the strengths of online classes

S.no	Strengths	6	5	4	3	2	1	Total	Arithmetic mean	Rank
1.	Work from anywhere, at any time	8	16	7	16	6	7	60	3.71	IV
		48	80	28	48	12	7	223		
2	Review lectures instantly	21	9	13	7	5	5	60	4.31	I
		126	45	52	21	10	5	259		
3	Group communication	11	19	9	7	7	7	60	3.98	II
		66	95	36	21	14	7	239		
4	Cost	8	7	22	8	8	7	60	3.63	III
		48	35	88	24	16	7	218		
5	Diversity	6	9	6	11	19	9	60	3.80	V
		36	45	24	33	38	9	185		
6.	Flexible learning schedule	2	4	5	9	19	21	60	2.30	VI
		12	20	20	27	38	21	138		

Source: Primary data

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Synthesis and Characterizations of Polymeric Film Prepared from Orange Peels

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Received: 10 Dec 2021 Revised: 22 Jan 2022 Accepted: 23 Feb 2022

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ABSTRACT

Bio degradable polymeric film is made out of orange peels taking starch along with glycerol as a plasticizer and turmeric as an antioxidant so as to prevent any antimicrobial activity by blending all together. The mechanical properties such as tensile strength, young's modulus, break point elongation of the dried film was analyzed by tensile tester (Colorcon).Biodegradability of the prepared film was also tested by soil burial method and the morphology of the degradable film was examined by SEM. Also the porosity of the film was measured by water dipping method. The antibacterial test result against *staphylococcus aureus*, a bacterial generally found in oral environment revealed the development of antimicrobial characteristic due to presence of curcumin in the prepared film. The developed film is biodegradable and a suitable alternative for packaging as well as in reducing the pollution caused by plastics.

Keywords: Biodegradable, Orange Peel, Tensile Strength, porosity, *staphylococcus aureus* packaging.

INTRODUCTION

In modern civilization packaging materials like polyethylene carry bags and pouches, PET bottles have gained high importance for their easy processing methods and low cost. But they are regarded as solid waste after use and their improper disposal can cause serious negative impact on marine and terrestrial ecosystem. According to the report of Central Pollution Control Board (CPCB), the total plastic waste generation in India is around 3.3 million metric tonnes per year and contribution of packaging materials is significant in this regard. Due to their non biodegradability they can remain as such for long years and enters into marine as well as terrestrial ecosystem by wind or human activities. Due to heavy demand it is also not possible to stop the manufacturing of these materials and the municipality of different metros across the country has playing a vital role in managing this waste. That's why now-days a number of researches are going on in developing biodegradable packaging materials out of

agricultural, vegetable and fruit wastes. In the literature it was reported that banana and orange peels can be suitably converted into biodegradable plastics in presence of cellulose or starch [1]. Antimicrobial properties of the biodegradable films for food packaging [2] also a major area of focus. Curcumin has been used as an antibacterial agent in many researches and subsequently non toxic [3][4][5]. In this research films were developed out of orange peels by blending with a developer like starch along with glycerol and turmeric using as plasticizer and antimicrobial agent respectively. The properties like biodegradability, tensile strength, break elongation of the film were also analyzed.

Experimental

Materials

Orange peels were collected from domestic waste. Cleaned and washed with water prior to use. Turmeric powder was purchased from local market. Glycerol, Ethanol and acetic acid are reagent grade chemicals and were used as such.

Preparation of film

The orange peels were cut into small pieces with a knife. Then they were boiled in ethanol along with turmeric powder in an reflux condenser for 2 hrs, filtered and dried in an over at 60 ^{0}C.After that made into powder by the help of a blender. To 2.5 g of orange peel powder 4mL of glycerol [6] and 4 mL of acetic acid were added. The mixture was stirred with an electrical stirrer for 45 mins in order to get a uniform paste. The pastes so obtained is spread on a Petridis and dried in over at 60 ^{0}C for 24 hrs.

Characterizations

Measurement of Hardness

The hardness of the film was measured by Rockwell hardness Tester A300(HR320S), Mitutoya, Brazil at temperature(23±5)^{0}C and relative humidity (50±*10*)% with specimen size 30mm x 30mm x 1mm in HRB Hardness scale. The range of force was 98.07N with minor load 60Kgf and the type of Indenter used was Ball 1/16

Measurement of Mechanical Properties

Mechanical properties of the of the film were determined by tensile tester (Colorcon) specimen size was 4 x 3 cm^2 in area and 0.08 ± 0.015mm in thickness and the test speed of the machine was 5mm per minute. Experiments were performed at 22°C and 30-40% RH. Load and displacement values were recorded. Typical test parameters like maximum stress, modulus of elasticity, work done, extension at break and strain at maximum load were automatically computed by software

Porosity

The porosity of the film is determined by dipping in water in a Petridis for 24 hrs and weighing both dry and wet film. Then the films were again weighted by digital weight balance machine and the porosity was determined by the given formula,

Porosity = W-w /qAl

Where W and w are the weights of the wet and dry films respectively.

q = density of water = 1g cm^{-3}

A = Area of the film

l = thickness of the film

Biodegradability test

The biodegradability of the films were tested by burring the films under soil in ½ feet distance from the ground surface for 10 to 30 days and the films were taken out , cleaned properly with dry cotton cloth, weighed and kept for image analysis by Scanning Electron Microscopy.

SEM

The surface structure of the films was viewed through Scanning Electron Microscope (JEOL 6510 LV, Japan).The membranes were rubbed with tissue paper and then dipped in liquid nitrogen for freezing before analysis.

RESULT AND DISCUSSION

Mechanical Properties Analysis

The mechanical properties like hardness, tensile strength, break point elongation as well as young's modulus of elasticity has been calculated and the data were recorded in table-1. It was observed that all the properties improved with increase in glycerol concentration as shown in figure-2.The weight of the film also increased with addition of glycerol to the reaction mixture as evidenced from figure-1.

Porosity and Biodegradability Analysis

From the porosity measurement it was confirmed that there is a decrease in porosity of the film with increase in glycerol content as mentioned in table-2 which may be attributed to the fact glycerol binds firmly to the orange peel surface forming a good and compact structure. The biodegradability of the prepared film was tested by soil burial method and the data has been tabulated in the table-2. It has been found that the percentage of decomposition increased from 10 to 30 days simultaneously .Also the biodegradability increased with increase in conc. of glycerol as shown in the figure-3.

SEM Analysis

The SEM analysis of the film has been done and it also was observed that it's degradability percentage increased with time as evidence from the figure- 4. From Image 4(b) is it clear that the degradation of the film has been started though the percentage is less but from image 4(c) and (d) it is confirmed that the film has been degraded a lot upto 30% as mentioned in the table-3.

Antibacterial Properties

The antibacterial study of the biodegradable film was done by using a natural and nontoxic additive like turmeric. The antibacterial action of turmeric has been widely reported in the literature [7-12].Here we tested the antibacterial properties of the film against *staphylococcus aureus* that is a common type of bacteria found in oral environment .It was observed that the bacterial growth was less around turmeric treated films as compared to the non turmeric treated film as shown in figure-5. Hence it can be concluded that addition of turmeric to polymeric films is an effective way to improve the antibacterial properties of the films.

CONCLUSION

Ecofriendly polymeric film was successfully developed from orange peels that are easily available. The method of development or preparation is also cost effective and can easily prepared without need of any sophisticated instrument in the laboratory. The tensile strength and young's modulus value says that the film keeps a load bearing capacity and can withstand small loads. From the porosity result it was confirmed that there are less pore formation with increase in glycerol concentration in the structure. Moreover the biodegradability test was impressive as percentage of decomposition under soil has been increased with time as well as glycerol concentration, confirming the ecofriendly used so as to have a positive impact on soil and water pollution. Also using turmeric as an additive improved the antibacterial properties of the film. Hence the developed biodegradable film may be suitable for packaging applications and environmental friendly in near future.

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Table 1: Mechanical Properties of the film

Amount of glycerol (mL)	Wt. of the film (g)	Hardness(HRB)	Tensile strength (N/mm^2)	Break elongation (%)	Young's modulus ($N-mm^2$)
18	2.62	33.4	36.12	436.32	762.13
22	2.69	42.6	39.31	547.21	825.34
26	2.73	46.1	43.58	558.23	852.45

Table 2: Biodegradability test of the film

Amount of Glycerol added(mL)	Wt. of dry film (g)	Porosity	% of Decomposition		
			10days	20 days	30 days
18	2.62	0.054	10.23	19.33	28.95
22	2.69	0.036	11.84	21.53	30.34
26	2.73	0.027	13.65	22.93	34.20

Preparation of film

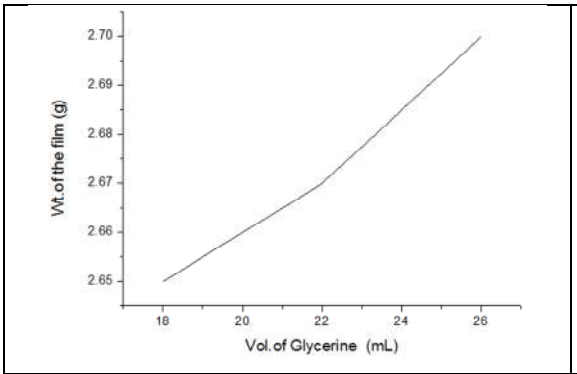

Figure 1: Weight gain against Vol. of Glycerol added

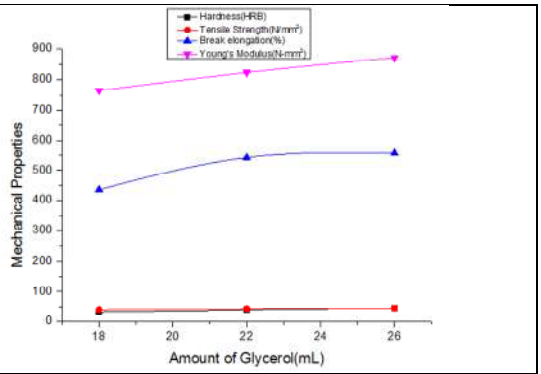

Figure 2: Mechanical Property analysis of the film

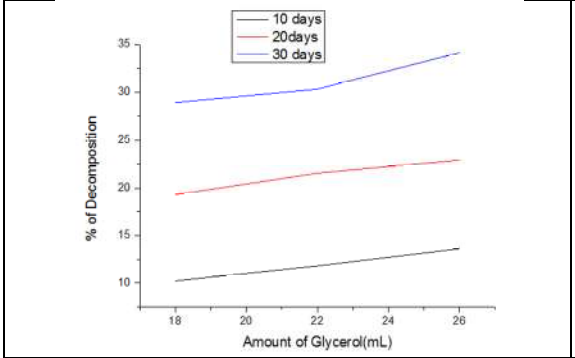

Figure 3: Biodegradability comparison of the film

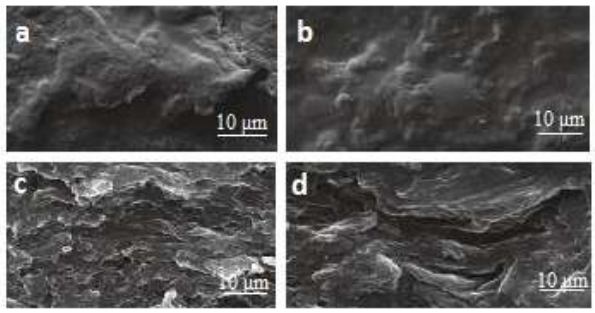

Figure 4: SEM images of the soil buried films after (a)0 (b)10 (c)20 (d)30days

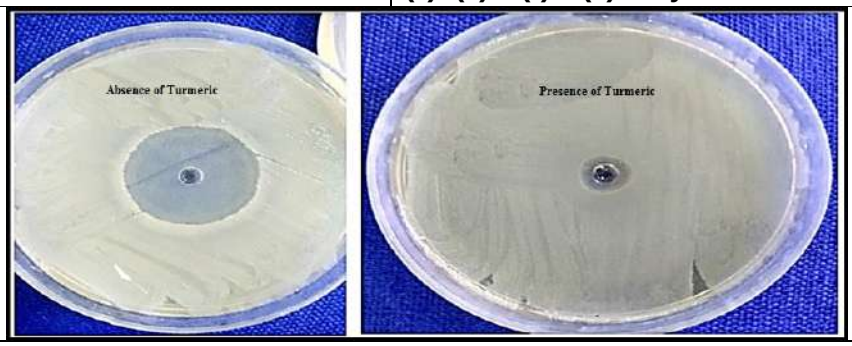

Figure 5: Antibacterial Properties

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study to Evaluate the Effectiveness of Comprehensive Nursing Interventions on Skin Health among School Children at Selected School Madurai

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Received: 21 Feb 2021 Revised: 01 Mar 2022 Accepted: 10 Mar 2022

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ABSTRACT

The main objective of the study was to evaluate the effectiveness of comprehensive nursing interventions on skin health among school children at selected schools Madurai. This study was employed at true experimental research design. Totally 60 male school children were included in the study. The study result showed that Pretest Level of skin health among control group students was 86.7 % mild skin disease and 13.3% moderate skin disease, in post test 73.3 % mild skin disease and 26.7 % moderate skin disease. Pretest level of skin health among experimental group was 73 % mild skin disease, 26.7 % moderate level but in post test 66.7 % were normal skin and only 33% were mild skin disease. The control group pretest mean was 26.3 and standard deviation was 3.82. Post test mean 26.7 and standard deviation 3.95. Difference in mean percentage was 1. Among experimental group pretest mean was 26.9 and standard deviation was 1.54. Post test mean was 15.9 and standard deviation was 4.69. Difference in mean percentage was 16. p value of the study was less than 0.001. The comprehensive nursing interventions had highly significant on improvement of skin health and control of diseases among school children.

Keywords: skin health, school children, comprehensive nursing interventions.

INTRODUCTION

School children are vital part of our society. They are active and playful during this stage. So they are closely contact with soil and dust environment. Personal hygiene, especially care of skin is important for the children. Skin is the major organ of the human body. It is doing excretory and protective functions. Sweat glands are active during play and accumulate metabolic waste on skin. The proper skin care is essential to prevent skin infections among school children. The children and their parents should educate about importance of skin health. This research study is aimed to protect and improve the skin health among school children.

Statement of the Problem

A study to evaluate the effectiveness of comprehensive nursing interventions on skin health among school children at selected schools Madurai

Objectives

1. To assess the pretest level of demographic variables among male adolescents at Madurai in experimental and control group
2. To evaluate the effectiveness of comprehensive nursing interventions on skin health among school children at selected schools Madurai
3. To find the association between the skin health and selected demographical variables among school children at selected schools Madurai

Hypotheses

H1: There is a statically significant difference between pre and post test of skin health among school children in experimental group

H2: There is a statically significant association between skin health and selected socio demographic variables among children in experimental and control group

METHODOLOGY

The research study was employed with quantitative research approach. The research design was true experimental research design. The pretest-posttest control group design was adopted for conducting this study. Totally 60 male adolescents aged between 11-17 years were participating in this study. Random sampling technique was used and school children who satisfied the inclusion criteria were selected for this study.

Selection and Development of Study Instrument

The instruments used in the study were demographic variable proforma, and dermatological assessment tool. Demographic variables proforma consist of age, religion, occupation of parent, educational status of parent, family income and residential area of school children.

Data Collection

The data were collected from the students for the period of six weeks. Rapport was established with adolescents after a brief introduction about the study and its purpose. The written consent was obtained from the school children after fully explaining the procedure of the study. Based on the criteria for sample selection, Totally 60 school children with poor skin health were selected using lot method of random sampling and assigned as experimental and control group. The Pretest screening of dermatological assessment was done for both group students.

Comprehensive nursing interventions like care of skin by regular bath and injury preventions were demonstrated with laptop, pamphlet and power point presentation and implemented to the experimental group for six weeks. The

control group was allowed to stay as routine. Post test screening of dermatological assessment was done after six weeks among both groups. Data were analyzed for the findings.

RESULTS AND DISCUSSION

The findings of the study revealed that Pretest level of skin health among control group students was 86.7 % mild skin disease and 13.3% moderate skin disease but in post test 73.3 % mild skin disease and 26.7 % moderate skin disease. The Pretest level of skin health among experimental group was 73 % mild skin disease, 26.7 % moderate level but in post test 66.7 % were normal skin and only 33% were mild skin disease. The control group pretest mean was 26.3 and standard deviation was 3.82. Post test mean 26.7 and standard deviation 3.95. Difference in mean percentage was 1. Among experimental group pretest mean was 26.9 and standard deviation was 1.54. Post test mean was 15.9 and standard deviation was 4.69. Difference in mean percentage was 16. The t value of the study 8.88 was higher than table value means the study was effective. The p value of the study was less than 0.001 which was highly significant. Hence the Hypothesis (H1) There is a statically significant difference between pre and post test of skin health among school children in experimental group was accepted. There was no significant association between skin health and demographical variables in both groups. Hence the Hypothesis (H2) There is a statically significant association between skin health and selected socio demographic variables among children in experimental and control group were rejected.

CONCLUSION

Comprehensive nursing interventions had highly significant on control of skin diseases and improve the skin health among school children. It is non invasive and non pharmacological interventions which is highly feasible. The authors were recommended to implement health awareness programme regarding skin health among school children to promote child health.

Dermatalogical Assessment Tool

This tool is designed to assess the level of skin health of the participants. This is developed by the investigator with experts opinion (Dermatologist and pediatrician) to assess the skin health among students. This is a 4 point rating scale ranging from 0-4 (None, Very Mild, Mild, Moderate, Severe).

Ethical Clearance

Ethical clearance obtained from Institutional human ethical committee of Arupadai Veedu Medical College and Hospital, Puducherry. (**AVMC/IEC2019/95).**

Source of Funding- Self

Conflict of Interest – I am doing my PhD in nursing at Vinayaka mission research foundation, Salem, India. As per curriculum I have to publish my original research at reputed journal. My specialty is M.Sc., Pediatric nursing. During my clinical posting I have witnessing the majority of the school children with skin disease which is preventable. Review of literatures also evidenced the prevalence of skin infection among school children. Keeping this mind the investigator conducted this research.

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Table- 1 Frequency And Percentage Wise Distribution Skin Health Among School Children

Level of skin assessment	Control group				Experimental group			
	Pre test		Post test		Pre test		Post test	
	f	%	f	%	f	%	f	%
Normal	0	0	0	0	0	0	20	66.7
Mild	26	86.7	22	73.33	22	73.3	10	33.3
Moderate	4	13.3	8	26.67	8	26.7	0	0
Severe	0	0	0	0	0	0	0	0
Overall	30	100	30	100	30	100	30	100

Table 2 : Comparison Of Pretest And Post Test Skin Health Mean Score Of Control Group And Paired T Test Score To Evaluate The Effectiveness Of Nursing Interventions.

SKIN assessment	Control pre test		Control post test		Difference in mean	't'-value	p-value
	Mean	SD	Mean	SD			
Overall total	**26.3**	**3.82**	**26.7**	**3.95**	**0.4**	**1.57**	**0.138(NS)**

Table 3 : Comparison Of Pretest And Post Test Skin Health Mean Score Of Experimental Group And Paired T Test Score To Evaluate The Effectiveness Of Nursing Interventions

SKIN assessment	Experimental pre test		Experimental post test		Difference in mean	't'-value	p-value
	Mean	SD	Mean	SD			
Overall total	**26.9**	**5.14**	**15.9**	**4.69**	11	8.88	P<0.001***(HS)

Table 4 : point rating scale ranging from 0-4 (None, Very Mild, Mild, Moderate, Severe).

Skin conditions	NONE 0	Very Mild 1	Mild 2	Moderate 3	Severe 4
Dryness					
Erythema					
Itching					
Swelling					
Rash					
Wound					
Hair loss					
Wart					

Scaly					
Bleeding					
Dermatitis					
Infections					
sun burn					
Cellulitis					
Pigmentation					

Table 5. Skin Assessment Score

Skin Assessment Score Total Score= 60	Interpretation	
0- 15	Normal skin	Healthy
16- 30	Mild skin disorders	Unhealthy
31-45	Moderate skin disease	
46-60	Severe skin disorders	

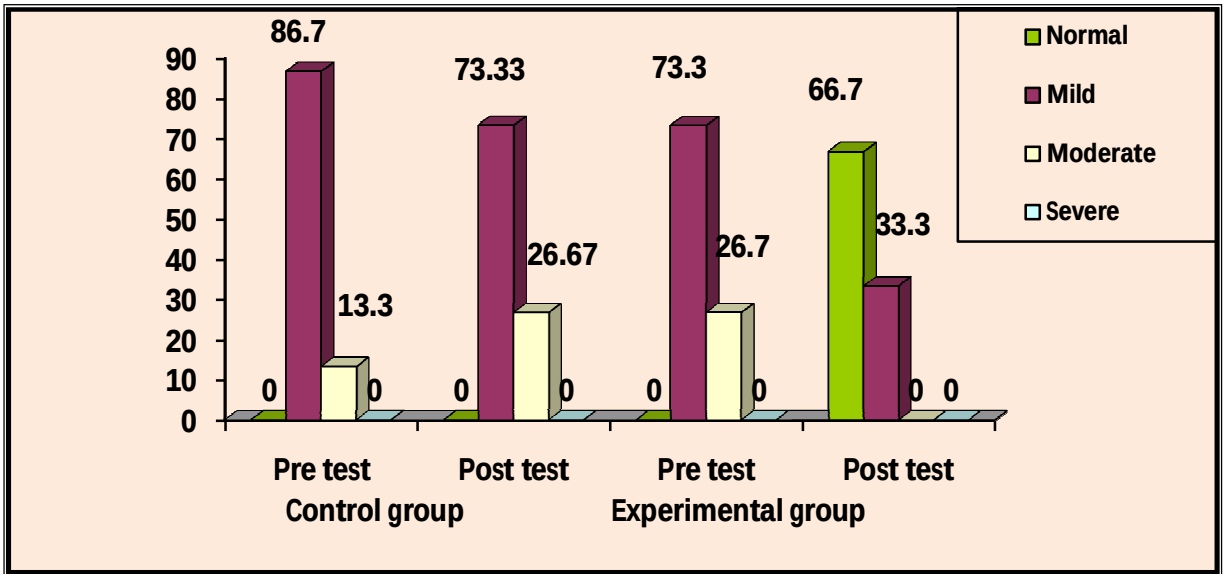

Fig 1. Percentage Wise Distribution Of Skin Health Among School Children

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

COVID-19 Crisis – Impact on Managing Projects

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Received: 31 Jan 2022 Revised: 24 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

Since the outbreak of coronavirus in Oct 2019 and further declaration of same by world health organisation (WHO), almost all countries have been affected by some or large extent. This has resulted in implementation of lockdowns as the cases shot up beyond control. The lockdown was implemented to largely restrict the movement of people, however this had a cascading effect of a big impact on industries, business and eventually the economy of the countries. This article aims to capture on how the covid pandemic affected the projects – that were already running, were about to start and the sequence of events that unfolded that the project managers of organisations were not prepared for. The impact was not just on the phases of project but also resulted in thought process on options to bring in the shift in the way projects are managed or delivered. "Fast digitalization of our services helped us not losing or stopping all services but low IT literacy and habits of market to work and study online is an obstacle." "This global crisis clearly indicated that project management are more than just processes, methods and tools". "We have learned working in virtual teams, which wasn't part of the company culture and I believe this is a positive change in future."

INTRODUCTION

COVID-19 Pandemic: Its Impact on Project Management

Since the inception of the Covid-19 pandemic, the effect of that is felt across all industries and it has changed the way people live, socialise and work. The corporate white collared jobs have switched to remote working or teleworking, while a lot many have faced job loss or prolonged lay-off's or cut in pay. In some way or the other almost all of us have felt the devastating effect of pandemic where business have shuttered from economic instability. The Impact of Covid, for project managers has been catastrophic. With little or no option, project managers have resorted to remote working, this has brought in paradigm shift in the otherwise collaborative approaches that were often seen within a traditional team environment. Lockdowns/border closures/ curfews have caused a major disruption to supply chain

network, this has resulted in heavy risk of operating a business and managing projects. The pandemic has given rise to the work from home model breaking all the technological & cultural barriers, setting in motion for a structural shift where the work continues at least for some people. Despite these major shifts, in the world beyond the COVID-19 pandemic, project management continues to be key to success for many businesses. We can look the impact of pandemic in three specific areas: (1) remote working, (2) Up skilling, and (3) the residual effects (positive & negative) of the pandemic on our industries, economy and country.

Remote Working in Project Management

With almost all countries now in the processes of vaccinating their population, with some going in for booster shots too, the question that looms now is: remote work will persist to what extent ? Are people willing to go back to traditional work environment? Some research and analysis finds that there is potential for remote work or the hybrid model only among the highly skilled and for educated employee workforce. But this is again restricted to certain type of industries and geographies and demographics of the country. From facing issues like network connectivity, power failure, challenges around remote collaboration, communication to surrounding environmental challenges and cultural adaptability – everything is getting onto digital platform. With the onset of pandemic, the future of project management has been revealed — like many other industries — is remote working. However, the implementation of remote working for project management has been easy at all. Remote working in project management environment is an adjustment that is made by all the stakeholders, however there are also some benefits to managing a team of remote team members, like potential to hire the best suited team members for the open positions from any part of the country or world or as required. To manage a virtual team to best, the project managers need to repurpose their focus on clear lines of communication, clear expectations and goals, and direct feedback, which might have got diluted in traditional working space.

Remote working has to be executed properly, which will result in many benefits to an organization, project managers, and to the team members

- Better employee productivity
- Access to best talent across geographies
- Reduced employee turnover
- Reduced stress levels
- Better work/life balance and wellbeing

Adopting to remote working or hybrid work environment is not easy, it requires a through act of balancing. If executed properly, project managers can gain the advantage of establishing a highly skilled team members that yield outstanding results. Although India is globally known for its high-tech software and financial services industries, still, a vast majority of its active workforce of 464 million is engaged in occupations like retail services and agriculture that cannot be done remotely in any circumstances. Among all the industries, majority of employee workforce cannot work remotely, however, in advanced economies, it is said that upto one quarter can do so three to five days a week. Some of the sectors like management, financial services, information services, software development services have larger potential for remote or hybrid model of working when compared to mechanical or other labour intense work environment. One of the research that was conducted in United States shares the below details on the sectors that have higher potential for remote working.

Upskilling of employee workforce in wake of the COVID-19 Pandemic

The traditional way the project managers used to work has now changed- for better or for worse. With this shift comes the growing need to up skill and optimise the operational procedures and to successfully achieve the project results. Some of the areas that needs an attention both at individual and organisational level are- people, technology, Quality management, communications, time management among others. Project management practices need to evolve not just by upskilling in similar lines, but also helping organizations to achieve this vision. Details, in context of project managers, around the same are stated below.

People Management: Working remotely results in entire new scheme of set-up and their challenges in managing the projects. Developing talent and encouraging innovation is not easy, there has to be well thoughtful and laid out plan. Working remotely comes with its inherent challenges. Upskilling of project team members to learn new collaborative approaches and manage in a virtual set-up can empower remote workforce towards better results.

Technology: Innovation is the key driver for businesses into the future which is driven by the Project managers, Hence, upskilling everyone's knowledge to constantly stay ahead of the curve in emerging technology is crucial to long-term success. This can include:

- Cloud-based systems
- Artificial Intelligence and automation
- Data analytics tools & platforms
- Project management softwares

Quality & Continuous Improvement: Limited budgets leave little to no room for mistakes in managing a project. Besides the standard project management practices, newer practices like Agile, lean and using scrum methodologies largely help in continuous improvement. Implementing quality monitoring framework is an effective procedure in optimising internal processes and better the project efficiencies through continuous improvement. One such program is Lean six sigma practice. In recent years, the usage of the term 'Project Management' has increased manifold. Project management is most commonly understood and misunderstood term, that is used as a tool to define, plan and execute the series of steps & processes to create something that can be of some help in the future. However, the benefit of project management which serves as a platform for overall organisational growth and for personal development is often ignored.

Communication: Project managers will need to connect and communicate with their team members on both scheduled or ad-hoc basis, it could be through meetings, calls, video conferencing, emails. Project managers communications ability and skills has a heavy dependency on the successful execution of project. Effective interaction among project team members and the project managers are very important for the execution and to monitor the progress of the project. Effective communication is very important at the start when gathering requirements from client, and then there on among the internal and external stakeholders too. Setting up and maintaining of the most suitable and effective channels of communication is very critical to ensure proper flow of information across stakeholders. This will also help to manage if there are any changes that could be required during the course of the project execution. A patient and active listening is also an important and a crucial part of communication. Sometimes not being able to listen entirely of a conversation could lead to unexpected consequences. Although technology today has opened up new options of communication like video calling, conference calling etc., it is advisable to have in-person conversation with your colleagues and people around you.

Time management: Time management is one of the triple constraint and the crucial part of the project from start through the closure. Project lifecycle has multiple phases and has multiple tasks that are time bound. There is a constant check on the activities and their respective timelines. If the task exceeds their allocated time, it will have a cascading effect on other constraint of the projects like budget and resource management. Time management has always been of top priority in the list of skills for managing the projects. The project manager should avoid the things that are not necessary and extends the timelines of the projects, like having too many meetings (that could sometimes be unnecessary), micro-managing the teams, lack of priorities and overloading the work beyond what could be managed. The effective project manager should be very cognisant of time on productive and unproductive activities.
In our personal life too, we face situations when are left with no time or very little time to complete critical tasks. Time management is very important is not just in managing projects timelines but in our life too. Creating priority to do list, concentrating one task at a time, avoiding urgency in the work and maintaining time discipline and such things will help us to balance and manage the time efficiently and effectively.

Organize: We humans always think that we can manage multiple time bound tasks by multi-tasking, but largely we fail in doing so, as we tend to lose focus on tasks – their deliverables, timelines, quality etc. This is where prioritising the work becomes all the more important, which helps us in organising our work and time effectively. On the personal front too, it becomes difficult to complete the tasks if we are not organised. In order to organize our work, our thoughts need to be organized. We need to have a routine set in your mind and follow it accordingly. A project manager has to deal with multiple parallel tasks at given point of time. Each task will have an owner, timeline and budget to work within. The efficient project manager has to list down the critical and important tasks that sensitive and have higher impact. Breaking down the bigger tasks into smaller one would and complete them on priority. Also project manager has to identify the tasks that can be delegated to others and keep a check on the progress and completion of same.

Problem Solving: There is no project that has no challenges, hence is the importance of project manager to deal and fix the challenges. A project manager has to develop a problem-solving approach in managing the project related challenges. Being that single point of contact to identify the problems proactively and solve them before they become bigger challenge is an advantageous attribute. For an escalation or a problem during the course of the project, there is a root-cause analysis that is done. A detailed analysis on the cause of the problem, the solution that was implemented, the rational to the solution and the preventive steps that are going to implemented in future to avoid reoccurrence of such problem. The root cause analysis is commonly known as RCA report for records. In our personal life as well, we will face problems that have a deep effect. We need to find, practical, time sensitive and critical thought solutions for the problems that we have faced.. There is no right or wrong way of solving a problem. It is just a matter of making things right. Problem solving skills will help you develop composure, self-esteem, and self-confidence.

Teamwork: Project happen with team, with group of people with individual role and responsibility. Every team member of a project is equally important irrespective of the role that is aligned to one. In long run, a project manager should have good team interactions. Working a team will help the project and the project manager to achieve the targets smoothly as per the timelines and any delay. Delegation of work to team members will work much efficiently if there is comfortable bonding and understanding among team members. Work will not seem like burden or tough when we cohesively work as a team. Work among the team members is shared and balanced among the Team members. In our personal life, events like parties, weddings or even informal even get-togethers, when we divide and share the work, the job gets done quickly and helps to build the lasting and trusting relationships.

Budgeting: Among the various parameters and knowledge areas in project management, project budget is one the key constraint the project has to be delivered. Project budgeting is the one of the triple constraints in project management, so it very important and critical to get the budgeting numbers, approvals, sign-offs in time and as per the expectations. Under the project management, there is function called PMO (Project management Office), whose primary task if to keep a close monitor on budgets, timelines and reporting of same. As part of the project management office (PMO), there is a through monitoring that happens to keep a track of the budget through the lifecycle of the project. There are work breakdown structures (WBS) that are designed at the start of the project, budgets are allocated to respective WBS's and the same are monitored through regular status checks and reports. In our personal life too, we all do budgets for a task, for a year, to keep track of the expense, income, if the expenses are going over or under. This helps us to utilise the available resources in a optimal way and help in saving in budgets.

The Trickling Effects of the Pandemic in Project Management

Here are a few ways COVID-19 continues to shape the future of project management

Working with contract workforce: Hiring the right contract workforce is not an easy task, considering the specific requirements the project will have. Some of the considerations that are to be kept in mind while hiring right contract workforce will be right fitment, skillset, sustaining stability, the contract terms and considerations. The project manager should weigh all options in hiring the right contractors, the associated risk especially with critical skillset and critical contract workforce to avoid any disruptions in project delivery.

Supply Chain management: Supply chain is the back bone for essential commodities. For productions industries, especially for essential commodities industries, the covid related lockdowns and border restrictions has been a huge challenge. This has indeed contributed to increase in cost and longer lead times for products and parts. It is very evident that the project management has to be forward-thinking, and take a proactive approach to deal with any potential threat of disruptions to entire supply chain cycle. Project managers have to have alternate plans to manage any such disruptions in future that could be sourcing from locals or stockpiling critical materials.

Risk Mitigation plan: Risk mitigation is one of the knowledge areas in project management. Mitigation of risk is all the more important, in the forward looking project management. Identification of risk and issues is not a one time or static tasks. During the course of project management, risk are ever evolving. In the Covid era, the risk could be sudden spike of cases, newer variants of virus, lockdowns, and border restrictions among others. A project manager should proactively plan and have contingency and alternative plans to deal with any current or potential risks. And these mitigation plans are also not static, they are also to be regularly reviewed and necessary actions taken.

Cost Implications: The pandemic has had the cost implications in some way or the other. With budget and cost management being one of the triple constraints in project management, project managers through their forward thinking and proactive-ness have to manage the project cost and budget very meticulously Managing cost, not only affects the project's risk mitigation but also the consequential implication of. The number and kind of covid virus variants and rapid spread of may affect the employee workforce with respect to the safety and wellbeing. There could be partial closure or complete shutdown of the offices or manufacturing premises for cleaning and sanitization purpose, and measures like this would have a major cost pressure and implications for companies. The burden of an unstable economy may result in limited cash flow for project managers in a post-COVID world.

CONCLUSIONS

People personally and organisations are getting used-to the new normal way of life. It will not be an understatement to say that the pandemic has had and will continue to have a significant impact in the way organisation and business operate across various industries. Preparation will be key in a post-coronavirus world and businesses are already taking steps to integrate initiatives, such as better work-from-home capabilities, and measures to reduce the negative impacts of major pandemics. While it is important to note that Preventing, mitigating and addressing the damage being done to human rights will be key to maintaining trust – with clients and consumers, your employees, your shareholders and your communities. It will be key to building a world of greater resilience. However, there is a light at the tunnel as leaders and respondents are optimistic that once the dust settles on COVID-19, businesses and consumers will be quick to return to normal.

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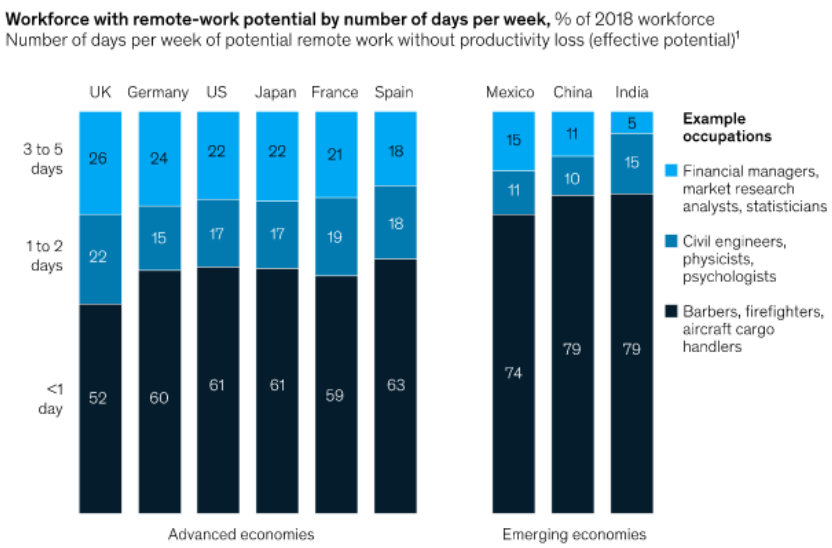
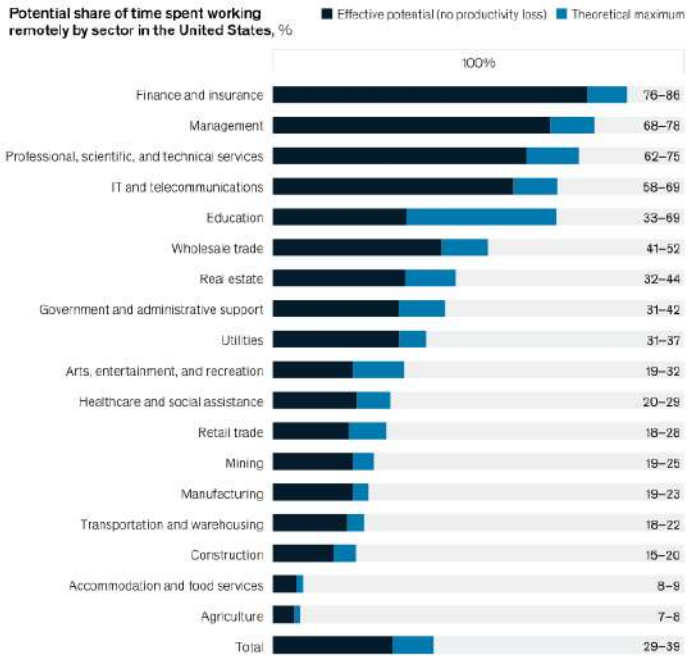


Source: McKinsey Global institute analysis



Source: McKinsey Global institute analysis

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Urbanization and Nesting Behaviour of Heronry Birds of the Order Ciconiiformes at PCBL Colony, Durgapur, West Bengal, India

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Received: 11 Feb 2022 Revised: 21 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

The heronry is the assemblage of single or multiple species of water bird that make colonial nests near water bodies. The present study is an attempt to understand the nesting behaviour of different species of egrets, herons, cormorants belonging to Order: Ciconiiformes and try to understand the population trends, selection of nesting plants by water birds. This present study also aims to investigate the impact of urbanization on birds in the study area. In Despite being a residential colony, the study area has low chances of human disturbances and contains a large number of trees that provide nesting and breeding places for birds like herons and cormorants in the rainy season. A total number of five species were recorded among which four species of Family: Ardeidae and one species of Family: Phalacrocoracidae. Water birds and nest count in the PCBL residential complex were much more than its adjacent forest habitat. Statistical analysis showed a significant difference between the means of total nest count of two habitat types as indicated by a one way ANOVA ($F=5.90, P<0.05$) followed by a Tukey's HSD test ($P<0.05$).The result of the analysis showed a significant difference between the total nest counts between forest and PCBL colony in 2021.For the implementation of proper avifaunal conservation strategy of any bird species, knowledge of its ecological requirements, population size, threats are very much needed.

Keywords: breeding; Heronry, Ciconiiformes, Waterbirds, anthropogenic impact, conservation

INTRODUCTION

The heronry is the assemblage of single or multiple species of water bird-like storks, herons, cormorants and egrets, which make colonial nests near water bodies [1,2] . Heronries play an important role in the conservation of various water birds in general and threatened species in particular. Nest preparation and assemblage of water bird depends upon many factors like availability of food material, presence of nesting trees, adequate supply of nesting materials, minimum pollution, safety and overall undisturbed ambience [3, 4]. In India about 26 species of water birds have recorded to nest colonially among them Purple heron, Grey heron, Black-crowned night heron, Indian Pond heron and Cattle Egrets, Little Egret Greater Egret were commonly reported. In India over 553 heronries were recorded and most of them are found in wetlands or very near to water bodies [5, 6, 7]. Various internal and external factors directly control the breeding success of animals. It also depends upon inter-species and intra-species relationships in nesting colonies, access to food resources. Predation is one of the major selective forces that regulate the shape of communities in almost all types of ecosystems [8,9]. To mitigate the predation pressure, birds adopt various anti-predatory strategies like colonial nesting, nesting tree selection, through parental care. Group nesting in heronry also minimizes the predation pressure in nests and nestlings [10, 11, 12]. Several species of water birds have adapted to novel urban ecosystems and used them for both breeding and feeding habitats.

For the determination of a successful conservation strategy of any bird species, knowledge of its ecological requirements is very much needed [13]. Climatic conditions especially the arrival of monsoon season directly affects nest making and breeding behaviour of birds [14]. Ali & Ripley (1987) [15] also reported that the breeding season of storks is very much dependent on the monsoon and related water conditions, which directly regulate the abundance of food. So the knowledge of the climatic condition of a particular habitat, arrival dates and breeding season of birds is very much important for studying the long term effects on breeding in the ongoing climate changes [14, 16]. Water birds generally prepare a nest at the upper canopy level of large trees and they select the tree after careful evaluation of the prevailing safety conditions [17]. Water birds usually construct nests set up simply exposed nests at the upper canopy level of large trees. Nest building at the canopy is also common in cormorants and egrets but herons prefer lower branches for nest building purposes[18,19].
Near water bodies,

Water birds generally prepare nest on indian banyan (*Ficus benghalensis*) trees , mango trees (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*) and copper pod (*Peltophorum pterocarpum*) trees in urban but they also make nest in forest area also [19,20]. To overcome the predation pressure birds select new unconventional habitats which have less number and diversity of predatory species. Human influenced urban ecosystem is such a habitat that has less diversity and density of enemy as well as the source for easily available food [21, 22,23]. So several species of water birds now select novel urban ecosystems as breeding and feeding grounds. These types of wetland-dependent breeding sites are also found within highly crowded towns and human settlements in Kerala [22] and different places [19]. More than 45% of all heronries in India are located in parks and gardens in urban areas [6] and nests are found in human-inhabited areas such as residential areas, towns, along roads sides, and non-residential areas [24,25].

A similar type of nesting behaviour is also observed in many Indian cities like National Zoological Park in New Delhi [26] , Piele Gardens in Bhavnagar city[27], Karanji Tank in Mysore[28]. However, only a few studies were carried out to understand the urban stress of water birds on nesting and breeding. So the purpose of this study was also to access the population trends, anthropogenic impacts, preference of water bird nesting in Philips carbon black limited residential colony of Durgapur in comparison to its surrounding forest. This present study also aims to investigate the impact of urbanization on birds in these areas.

MATERIALS AND METHODS

Study area

The present study was conducted in Philips carbon black limited (PCBL) residential complex and forest surrounding this residential complex situated in Durgapur, West Bengal, India beside NH-2, which falls between 23°30'28'' N, 87°21'11'' E (Figure 1 and figure 2). There is a forest area adjacent to this residential colony and a water body nearby. The colony has a thick canopy of deciduous trees which provides the nesting place for residential and migratory birds including heronry birds of the order Ciconiformes.

Field observation

Regular observation and all counts were made during early hours (6.00 am to 8.00 am) of the day during monsoon months of June 2020 to September2020 and June 2020 to September 2021. During this study point surveys and line transects surveys were used for counting birds and nests [25, 29]. The count was done by walking inside the residential complex and its adjacent forest and bird or nest count were done with the help of a binocular (Olympus). Digital documentation was done with the help of a digital camera (Canon EOS 1200D DSLR) from time to time. The field trips were carried out at an interval of seven days for proper counting of the bird and its nest in the study site. Identification of birds in this study area was done using "The Book of INDIAN BIRDS" by Salim Ali [30]. Similarly secondary information about heronries was collected from people residing in the residential complex and other local people. No birds were killed or captured during the study period.

Statistical analysis

Statistical analysis and graphical representation of data were done using Microsoft Excel, 2007.To compare bird count and nesting preference between two habitats i.e residential complex and adjacent jungle, one-way analysis of variance (ANOVA) was done setting Alpha to 0.05.At the same time, Tukey's HSD post hoc analysis between pairs or subgroups were done using Tukey HSD calculators (astatsa.com) to identify specific variables that differ significantly.

RESULT

Bird survey

The spread of urbanization rapidly decreases the habitat of flora and fauna. But sometimes it creates alternative habitats and support nesting as well as the breeding populations of various animals including heronry birds. In this present study altogether five (5) nesting populations were documented out of 26 water bird species normally found in India[6] . Five heronry birds were recorded in the study area which were Black-crowned Night Heron (*Nycticorax nycticorax*), Cattle egret (*Bubulcus coromandus*), Great white egret (*Ardea alba*), Little egret (*Egretta garzetta*), Little Cormorant (*Phalacrocorax niger*). Among these five species, cattle egrets are highest in number, little egrets and great white egret are moderately found but the number of cormorants and black-crowned night heron population is less in number (Figure 3) in both the studied areas. As per local people information, these water birds prepare nest for last 20-25 years. Water birds found in Philips carbon black limited (PCBL) residential complex are much more than in its adjacent forest (Figure 4).

Statistical analysis showed a significant difference between the means of total nest count of two habitat types as indicated by a one way ANOVA ($F=5.90, P<0.05$) followed by a Tukey's HSD test ($P<0.05$).The result of the analysis showed a significant difference between the total nest counts of two different habitats like Forest 2020 and PCBL colony in 2021 as well as between forest and PCBL colony in 2021(Figure 4 and figure 5).

Nesting behaviour

The residential complex is covered with different types of trees, but the main trees are mango, Indian fig, eucalyptus, coconut and jack fruit. *Ardea alba*, *Egretta garzetta* and *Bubulcus coromandus* usually prefer mango trees here for making whereas *Phalacrocorax niger* and *Nycticorax nycticorax* select eucalyptus tree for the same purpose. The total number of a nest found in mango trees is higher than in other trees. Birds select a plant in the core area of the colony than the periphery for making a nest in the study site. A mixed population of three species of the egrets are observed in the same mango tree but sharing of the same eucalyptus tree by herons and cormorants is less frequent. Egrets and cormorants generally prepare a nest in the canopy layer but herons less used the canopy layer. An interesting difference in nest number was observed between two habitats (Figure 4). Nest numbers are much more in residential complex than adjacent forest and number of bird population increases in 2021 in comparison to the bird population of 2020 in both the habitats. In residential complex nest count of Cattle egret (34.53%), little egret (25.9%), Great white egret (20.1%), little cormorant (10.7%) and Black-crowned Night heron was (8.6%) were found in 2020(Figure 5 & 6). The percentage was more or less the same in 2021 with a slight decrease in nest preparation by Great white egret (18.4%) in 2021. Nest number is much lower in the adjacent forest near residential colony than inside the residential colony.

Anthropogenic Impact on bird population

During our survey in the PCBL residential colony, we observed the number of birds and nest is much more than the adjacent forest area. Restricted entry of a vehicle and local people in residential colonies triggers the nesting of heronry birds. National Highway (NH-2) is passing very close to adjacent forest which creates noise and air pollution. High- speed dust storms and predatory animals sometimes damage nests and chicks more in forest areas than a residential area. In the close vicinity of the forest area there lies a slum, people of that area belong to below poverty line (BPL) category. Children of that area throw stones as a fun game and with a special wooden device catapult kill the birds for flesh in the adjacent forest so the number of rejected nest in forest areas is much more than in the residential colony. Birds present in residential colonies gather food material easily in comparison to birds colonize in the adjacent forest.

DISCUSSION

In the Indian subcontinent approximately 1300 species of birds are commonly seen. The main reason for high avifaunal diversity in this region is its diverse landscape like hills, plain land, Grassland, wetlands and marine environment with unique vegetation as well as unique diversified climatic conditions which attract and give suitable habitat for many bird species around the year [31]. Migratory birds are the key mobile links that contribute to the function of a diverse eco-system. This makes migratory bird's prime witnesses to global climate change. Environmental factors like vegetation, food availability, rail fall, presence of prey species and other factors also determine the population size of a bird population in any particular area [14]. Nesting behaviour and nest design of any bird is regulated by natural selection, risk of predation and sexual selection [32]. Nest design is also regulated by the habitats and habits of specific bird species so a tremendous amount of convergent, recurrent, and parallel evolution of different nest types is observed in birds which make it difficult to delineate particular phylogenies [33]. The rhythmic patterns of the arrival of birds in the PCBL colony are phenomenal as well as very much seasonal. This area provides a suitable habitat for the distribution of the above- mentioned bird species. The study area is covered by different types of trees, but the main trees are mango, eucalyptus, Indian fig, coconut and jack fruits but water birds select different vegetation strata to minimize competition for resources. Previous study showed that three types of egrets, one type of cormorant and heron commonly found in this study area [19]. In the residential complex maximum number of the nest were found from Cattle egret and the number of the nest counts was less among Black-crowned Night herons. A previous study suggests that Cattle egret, herons and cormorants prefer low water level areas for foraging. The study site provides a large number of trees for their habitat and a small water body nearby this residential complex provide food for water birds. The selection of particular trees by bird species must play

some evolutionary role. Nests on the tree might give the species a proper niche where it can breed or camouflage from the surrounding, thus can increase reproductive success.

In the residential complex nest of Cattle egret is much more than other bird species. The total number of nest is also more in residential complex than adjacent forest near residential colony. The reason behind this is that birds collect food material easily from the domestic waste of residential colony. Normally human settlement near heronry and repeated visit near nesting tree sometimes produce a detrimental effect on water birds as they leave the nest at various stages of breeding which directly decreases the bird population [34,35]. But people who reside in the study site of residential complex don't disturb the birds so a natural harmony was made between various bird species and residents which trigger the population of birds. A similar type of natural harmony between bird species and tribal people were reported by Dey *et al*., 2021[29]. Residential complex study sites follow the strict rules for the to and fro movement of local people or poachers so they do not easily enter the residential area and birds receive extra protection. But adjacent forest is open type so many people including children enter the forest for various purposes and annoying birds. Sometimes they kill the bird or destroy the nest. So birds prefer residential complexes as safe habitats for nesting rather than the adjacent forest. The selection of nesting plants very near to human habitation gives a better chance to avoid their predator and colonial nest formation behaviours also provides better chance to survive [14]. Horns of the heavy vehicle passing through the jungle and pollution also disturb the birds in the adjacent jungle so birds shift their habitat in the residential complex which is a less noisy and calm environment suitable for bird nesting.

In recent times , bird population decreases very rapidly.. There are many factors that accelerate this decline are felling of nesting trees, indiscriminate use of pesticides, drainage and conversion of wetland feeding habitats, collection of eggs and chicks and conversion of coastal mangroves. Wetlands are also an important part of the ecosystem. It plays a vital role to regulate the hydrological cycle. It is an ideal habitat for different types of migratory and resident habitat birds like herons, cormorant, lesser whistling duck, cotton teals, egret and many water birds [36]. Rapid urbanization also destroys the habitats of many organisms and destroys local biodiversity. Now a day's urban habitat are suitably used by the colonial water birds both for roosting and breeding. Urban ecosystems have less diversity and density of predators, but supply of year-round food resources and nesting locations with a stable environmental condition [21, 22]. Although the selection of habitat by a bird species is mostly genetically controlled learning and experience also play a great role. Water birds construct nests at the upper canopy level of large trees. They choose nesting sites after careful evaluation of the prevailing safety conditions [17]. In our study; we found the colonial nest of heronry birds in two different habitats like wild habitats (forests) and urban sites (PCBL Colony).Birds select nesting tree very near to human habitation which provide them better chance to avoid their predator, as well as colonial nest formation behaviors, also give them a better chance to survive[14]. Urbanization helps the water birds for getting food from waste material, protects against predators and poachers so bird count and nest number are more in urban study sites than wild habitat. There are various hypotheses for nesting plant selection by birds in human-influenced habitats. Among them enemy exclusion hypothesis one of the principle hypothesis [23]. Urban environment protect birds against predators. Urban heronries are also exposed to man-made stresses like the occurrence of close high-rise apartments, noisy streets and light pollution from high mast lighting towers, vehicular emissions, effluent discharge and direct human access to nesting locations. But as this is a protected residential complex so this type of manmade stress is very less in this study site which triggers the increase of bird population and created urban habitat of this study site is no longer a lost habitat for birds and various wild birds. They successfully acclimated with their new habitat and population size increases regularly. This type of survey work may attract government/non-government avian faunal conservation organization(s) to implement proper conservation strategy in the PCBL colony. Reporting of colonial nest selection by birds in cities for nesting and foraging habitat also support the findings of this study [6, 26, 27,28].

CONCLUSION

Predation is the main selective pressure in nature that regulates the population size of various including birds. Nesting preference, habitat selection, and nest architecture directly correlate with the predation rate, and birds prefer to prepare nests in an area where predation pressure is low. Throughout the globe, the rapid growth of urbanization exerts a detrimental effect on biodiversity. Recent studies suggest that minimum water level is an important source to attract egrets, herons and cormorants since most of the species prefer to forage in low-level water areas. So awareness campaign for local diversity conversation is very much needed in the study site to accelerate the process of avifaunal conservation and this type act should promote other urban people to come forward and only then we can successfully implement any conservation strategy to protect our biodiversity.

Ethical approval

No birds were captured or killed or subjected to any experimental treatment during the study period.

ACKNOWLEDGEMENTS

This research received no specific grant from any government or non-government agencies. The authors want to acknowledge Principal, Bankura Sammilani College, Bankura and Vice-Principal, Asansol Girls' College, Asansol for their kind help and support in conducting the research work. Author also wants to acknowledge the help received from Dr Ananya Nayak and Muktadar Hossain, faculty member of Bankura Sammilani College for statistical analysis and preparation of map using QGIS software.

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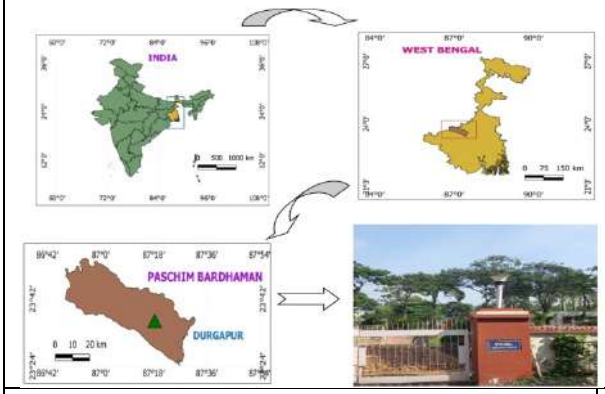

Figure 1: Geographical Location of the study area (India, West Bengal and Paschim Bardhaman District map was prepared using QGIS software and Study site of Philips carbon black Limited (PCBL) residential complex

Figure 2. (2a) Urban habitat of Philips carbon black limited (PCBL) residential complex (2b) Adjacent forest habitat for Heronry birds

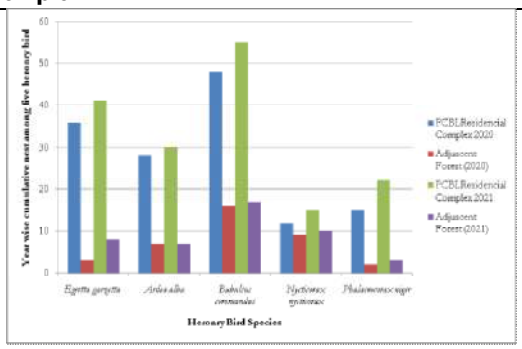

Figure 3: Column representing habitat wise total number of heronry birds of five species

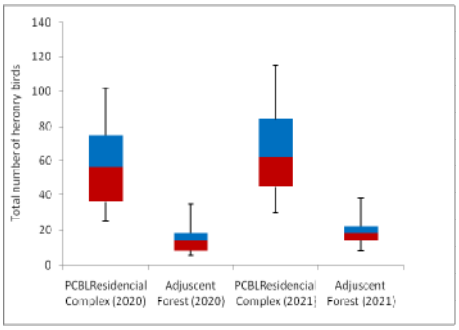

Figure 4: Box plots representing habitat wise total heronry birds of five species

Figure 5: Cattle egret nest in the study area

Figure 6: Cormorant nest in the study area

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Comparative Study on the Characterization and Its Properties of Isolated Starches from Native and Industrial Turmeric Spent

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Received: 08 Feb 2022 Revised: 03 Mar 2022 Accepted: 22 Mar 2022

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ABSTRACT

In Asia, the plant turmeric has been used for centuries with a long historical medicinal use. The turmeric cultivated in India is very fine quality in the world for its high content and its inherent qualities of the curcumin a significant phytoconstituents. Separation of curcumin from turmeric makes the turmeric (spent flour) as a waste and used as a fuel in boilers. Thus the present study was aimed to extraction of curcumin from turmeric and isolation of starch from native turmeric spent and industrial turmeric spent and analysed for its physical, functional, anti nutritional and chemical properties to make it suitable in the application of food industry. This isolated starch was subjected to nutrient analysis, mineral composition, microbial analysis, functional properties and anti nutritional properties. The results revealed that industrial turmeric spent starch were observed to have good qualities with reference to high nutritive value, functional properties and low amount of anti nutritional parameters with low microbial activity when compared to native turmeric spent starch. Thus the industrial turmeric spent starch should not be wasted and can be used as an ingredient in the food product development, nutraceutical and pharmaceutical formulations.

Keywords: Turmeric, Turmeric Spent, Starch, phytoconstituents, Nutraceutical and Pharmaceutical.

INTRODUCTION

The spice, turmeric is widely used for its medicinal properties and as a colouring agent [1]. In Asia, the plant turmeric has been used for centuries with a long historical medicinal use. In the Southern Asia, the turmeric is a principal spice and it is also used as a component in many ceremonies. The turmeric is called as "Indian saffron", because of its brilliant yellow colour [2]. In the South Asia, the turmeric – Curcuma longa is the Zingiberaceae ginger family which is a herbaceous rhizomatous plant. The turmeric cultivated in India is the very good quality in the world for its high content and its inherent qualities of the curcumin a significant phytoconstituents. The Erode city, a state of South Indian Tamil Nadu, the most important trading center and the turmeric world's largest producer. The turmeric is called as "Turmeric City," "Yellow City," or "Textile City." A Maharashtra city, Sangli is next to Erode in significant as a productive and in size and exporting for turmeric. Within the last 25 years and with over 3000 publications, the modern medicine has recognized turmeric importance. They differ in the functional properties, chemical structures and physico-chemical characteristics. The various health benefits of curcumin made it as one of the most selling and useful nutraceuticals in pharmaceutical industries. So, the component curcumin is scientifically removed from the fresh turmeric flour and is used as nutraceutical. The spice industries while processing have to give importance to utilize the by- products and its waste produced during spices processing that turns into value added products. The additional effect on textural and nutritional quality of the product, dosage of addition and incorporation of turmeric spent starch in selected food products needs to be standardized [3]

The world's turmeric crop is produced in India and 80% of turmeric is consumed. The raw flavour from the rhizomes are removed by steamed or boiled, the starch is gelatinized and to produce a colored product. From turmeric, the 100 components have been isolated. From the *C. longa and C.zedoaria* has extracted starch and there is no differences significantly between these two species in size, granule shape and amylose content were observed [4]. The turmeric rhizomes has a 40% (w/w) isolated starch, after oil extraction from turmeric by using supercritical fluid extraction have observed [5]. The turmeric worldwide production is 1.115million tons and in India is for approximately 80%. From India annually, turmeric oleoresin of 200 tons are traded. The turmeric liquor of 140 tons as Removed Curcumin Turmeric Oleoresin and spent 18000 tons is manufactured by oleoresin turmeric industries and the spent from turmeric oleoresin industries does not have any uses commercially [6]. This industrial turmeric spent could be used in manufacture of food products such as baked products and other value added food products. Thus the present study was focused to extraction of curcumin from turmeric and isolation of starch from native and industrial turmeric spent and analysed for its physical, functional, anti nutritional and chemical properties to make it suitable application in food industries.

MATERIALS AND METHODS

Sample Collection

Commercial industrial turmeric spent flour was bought from RKS Healthcare. For the preparation of native turmeric spent flour, Turmeric was purchased from a Erode traditional market and the chemical reagents were also purchased.

Extraction of Curcumin

The turmeric rhizome was rinsed and washed in tap water and dried at room temperature. The turmeric rhizome was pulverized and grinded up to it was powdered. With soxhlation method, turmeric powder of 500mg were extracted and dichloromethane and ethanol solvents were used. The extraction was stopped when, the solvent has not turned orange, and the results were measured. By column chromatography, the isolation was completed with (97:3) % dichloromethane-methanol as mobile phase on silica gel. [7]

Isolation of starch from turmeric spent flour

The 1000grams of turmeric spent flour was dried in air then dispensed in five times its pure water weight and allowed to settle for two hours and with a muslin cloth this was purified. Then the residue was washed with water till it was clear. Then the impured milk of starch was centrifuged at 5000 rpm for 30 minutes and the supernatant was filtered. By centrifuging, the developed starch sediment was washed with water for several times until a pure white starch appeared. By employing a hot air oven, it absolutely was dried at 50-58^{o}C for two hours to permit the 200 gms of starch and it was kept in impermeable container [8]

Nutrient analysis

The nutrient composition like carbohydrate, pH, moisture, protein, ash, crude fibre, total titrable acidity, fat, energy, dietary fibre, amylose content and total starch were determined for the selected two native turmeric spent starch and industrial turmeric spent starch flours using standard procedures.

Mineral Composition

The mineral composition such as zinc, sodium, magnesium, potassium, calcium, iron and phosphorus for native turmeric spent starch and industrial turmeric spent starch by Atomic Absorption Spectrometry (AAS) were determined [9]

Microbial Analysis

The microorganisms pure culture was obtained from the Microbiology Department, DKM College for Women (Autonomous), Vellore. The fungi and bacterial stock cultures were sub - cultured in nutrient agar (NA) slants while that of yeast and mould on Sabour and Dextrose Agar (SDA) slants and stored at 40^{o}C [10]

Functional Properties

The functional properties such as bulk density, oil absorption capacity, water absorption capacity, hydration capacity, hydration index, swelling power, solubility, swelling capacity, swelling index and solid loss were determined. The bulk density of 2 different samples was determined [11] The oil absorption capacity was employed to determine native turmeric spent starch and industrial turmeric spent starch [12]. By the method of Takashi and Sieb (1988), the solubility and swelling power was determined[13]. By the method of Sosulski (1962), the water absorption capacity was determined [14]

Anti-Nutritional parameters

The natural or synthetic compounds are anti-nutrients, it reduces the availability of the nutrients when present in foods. By Sadasivam and Manickam (2005), the antinutritional parameters such as trypsin inhibitor, tannin and total phenolics using standard procedures was determined [15]

Statistical Analysis

By the statistical methods, the final data was assembled together and examined. The Descriptive statistics results such as one way ANOVA, standard deviation, mean were represented. A p-values <0.05 were mentioned as important. The Analaysis of Variance (ANOVA) among the native turmeric spent starch and industrial turmeric spent starch was used to test the differences.

RESULTS AND DISCUSSION

The data on nutrient analysis of native and industrial turmeric spent starch were presented in table1. It shows that pH (7.33), ash (8.56gm) , total titrable acidity (0.56gm) , crude fibre (5.13gm) , carbohydrates (74.53gm), proteins (9.86gm), energy (324.13kcals), total starch (12.46gm) and dietary fibre (4.16 gm) of the Industrial turmeric spent starch is greater than that of native turmeric spent starch. This indicates the good quality of the industrial turmeric spent starch with respect to its chemical composition. Similar observations were reported by Sowbhagya et al., 2015

[16] The figure 1 shows the nutrient analysis of native and industrial turmeric spent starch. The mineral composition of native and industrial turmeric spent starch were presented in table 2. The mineral composition of industrial spent starch shows a higher content of sodium **(**25.3mg), potassium (550mg), Iron (63.56mg), Calcium (97.6mg), Phosphorus (290mg), Zinc (7.95mg) and Magnesium (21.66mg). Kumar et al (2005) reported the high mineral composition of turmeric spent starch indicates its anti cancer, anti diabetic, antioxidant, and anti inflammatory activity [17]**.** Figure2 shows the mineral composition of native and industrial turmeric spent starch. The antinutritional properties of native and industrial turmeric spent starch were presented in table 3. It was found that antinutritional properties such as tannins (2.15mg), total phenolic content (9.4mg) and trypsin inhibitors (2.09mg) are lower in industrial turmeric spent starch when compared to native turmeric spent starch. This indicates the high antioxidant capacity of industrial turmeric spent starch. This low anti nutritional values shows the efficacy of industrial turmeric spent starch in scavenging the superoxide free radical effectively thereby improving the body's defence mechanism [18]. The antinutritional properties of native turmeric spent starch and industrial turmeric spent starch is showed in figure 3.

The functional properties of native and industrial turmeric spent starch were presented in table 4. It was observed that bulk density (1.0g/ml), oil absorption capacity (1.21g/g), water absorption capacity (7.44g/g), swelling power (0.72g/g), solubility (0.82%), solid loss (37.49%), swelling capacity (0.82) and its index (5.86%) hydration capacity (3.87) and its index (80.03%) is higher in industrial turmeric spent starch than native turmeric spent starch. These higher functional properties of industrial turmeric spent starch indicates it good baking property and its suitability in the application of food industry [19]. The greater bulk densities are advantageous for lowering the paste thickness and better ease of dispersibility by Udensi et.al, 2000 [20]. An important factor of oil absorption in formulation of food is because of fat increases the mouth feel of foods and it is a flavour retainer [21]. The figure 4 shows the functional properties of native turmeric spent starch and industrial turmeric spent starch. The results from the table 5 revealed that growth of fungi named Rhizopus species was found in native turmeric spent starch and not in industrial turmeric spent starch. The growth of gram-negative bacteria such as Bacillus and gram-positive bacteria Staphylococcus Aurius were found in both native and industrial turmeric spent starch.

CONCLUSION

The nutrient analysis, mineral composition, anti-nutritional parameter, functional properties and microbial analysis of industrial turmeric spent starch showed a good result than native turmeric spent starch. The best way to enrich the food production is to use industrial wastes is very important. The turmeric spents, a spice industry by-product, is a source of functional ingredients also phytoconstituents such as dietary fibre, protein along with mineral. The foods nutritional aspect is increased by adding the turmeric spents as a important origin of functional ingredients. There is much of potential for the turmeric spent usage, since India is the major producers of turmeric also turmeric spent starch products. Thus the industrial turmeric spent starch should not be wasted and can be used as an value addition in the food product development, nutraceutical and pharmaceutical formulations.

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Table 1 Nutrient analysis of Native and industrial turmeric spent starch

S:NO:	Parameters	Native turmeric spent starch	Industrial turmeric spent starch
1.	pH	6.13±0.75[a]	7.33±0.97[a]
2.	Ash (g)	7.1±0.91[a]	8.56±0.50[a]
3.	Total titrable Acidity (g)	0.33±0.10[a]	0.56±0.16[a]
4.	Moisture (g)	10.73±0.80[a]	9.4±0.75[a]
5.	Crude Fibre (g)	4.83±0.75[a]	5.13±1.00[a]
6.	Carbohydrates (g)	68.53±0.75[a]	74.53±0.94[a]
7.	Proteins (g)	9.56±0.65[a]	9.86±0.35[a]

8.	Fat (g)	7.16±0.70[a]	6.83±0.70[a]
9.	Energy (Kcals)	313.33±0.61[a]	324.13±1.05[a]
10.	Total Starch (g)	12.23±0.80[a]	12.46±0.60[a]
11.	Amylose content (%)	12.26±0.70[a]	11.6±0.7[a]
12.	Dietary Fibre (gm)	3.6±0.5[a]	4.16±0.70[a]

The values are expressed as the mean of two samples ± SD. Values in a row do not differ significantly (p>0.05).

Table 2 Mineral composition of Native and industrial turmeric spent starch

S:NO:	Minerals	Native turmeric spent starch	Industrial turmeric spent starch
1.	Sodium (mg)	19.73±0.47[a]	25.3±0.81[a]
2.	Potassium (mg)	487.5±0.45[a]	550±0.85[a]
3.	Iron (mg)	54.2±0.65[a]	63.56±0.45[a]
4.	Calcium (mg)	84.4±0.55[a]	97.6±0.55[a]
5.	Phosphorus (mg)	247.6±0.41[a]	290±0.7[a]
6.	Zinc (mg)	7.18±0.54[a]	7.95±0.60[a]
7.	Magnesium (mg)	18.33±0.98[a]	21.66±0.49[a]

The values are expressed as the mean of two samples ± SD. Values in a row do not differ significantly (p>0.05).

Table 3 Anti nutritional parameters of native and industrial turmeric spent starch

S:NO:	Parameters	Native turmeric spent starch	Industrial turmeric spent starch
1.	Tannin (mg/g)	17.32±0.53[a]	2.15±0.68[b]
2.	Total Phenolic content (mg/g)	32.3±1.01[a]	9.4±0.81[b]
3.	Trypsin inhibitor (TIU/mg)	13.59±1.16[a]	2.09±0.92[b]

The values are expressed as the mean of two samples ± SD. Values with different superscripts in a row differ significantly (p<0.05).

Table 4: Functional properties of native and industrial turmeric spent starch

S.No:	Functional properties	Native turmeric spent starch	Industrial turmeric spent starch
1.	Bulk Density (g/ml)	0.88±0.14[a]	1.0±0.17[a]
2.	Water Absorption Capacity (g/g)	7.12±0.11[a]	7.44±0.08[a]
3.	Oil Absorption Capacity (g/g)	1.15±0.05[a]	1.21±0.10[a]
4.	Swelling Power (g/g)	0.52±0.43[a]	0.72±0.16[a]
5.	Solubility (%)	0.69±0.20[a]	0.82±0.23[a]
6.	Solid loss (%)	32.30±0.84[a]	37.49±0.81[a]
7.	Swelling Capacity (g/1000seed)	0.77±0.55[a]	0.82±0.47[a]
8.	Swelling Index (%)	5.6±0.45[a]	5.86±0.45[a]
9.	Hydration Capacity (ml/1000seed)	3.04±0.09[a]	3.87±0.26[a]
10.	Hydration Index (%)	76.07±0.87[a]	80.03±0.20[a]

The values are expressed as the mean of two samples ± SD. Values in a row do not differ significantly (p>0.05).

Table 5: Microbial Analysis of Native and industrial turmeric spent starch

S.No:	Microbial parameters	Dilutions	Organisms Present in Native turmeric spent starch	Organisms Present in Industrial turmeric spent starch
1.	Fungi culture	10^{-2}	*Rhizopus* Sps	No growth
		10^{-3}	*Rhizopus* Sps	No growth
		10^{-4}	*Rhizopus* Sps	No growth
2.	Bacteriology Culture	10^{-4}	Gram positive cocci in clusters and rods Gram negative in rods	Gram negative in rods
		10^{-5}	Gram positive cocci in clusters and rods Gram negative in rods	Gram negative in rods
		10^{-6}	Gram positive cocci in clusters and rods Gram negative in rods	Gram negative in rods

(*Staphyloccus aurius* – clusters and Bacillus – rods)

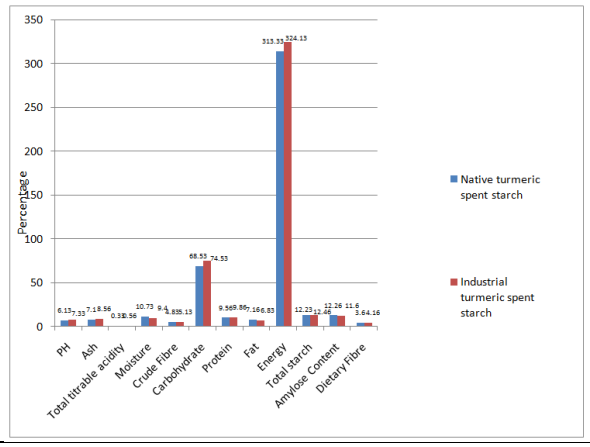

Figure 1. Nutrient analysis of Native and industrial turmeric spent starch

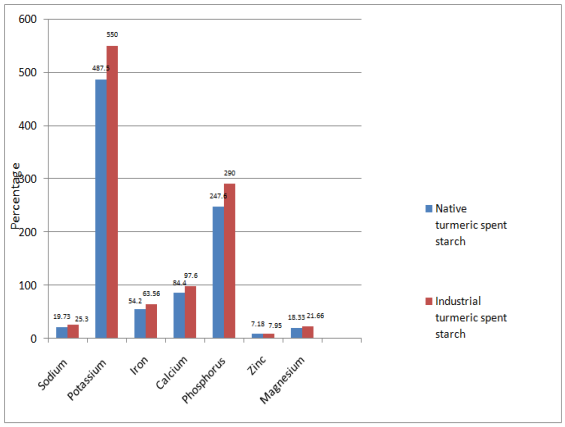

Figure 2. Mineral composition of Native and industrial turmeric spent starch

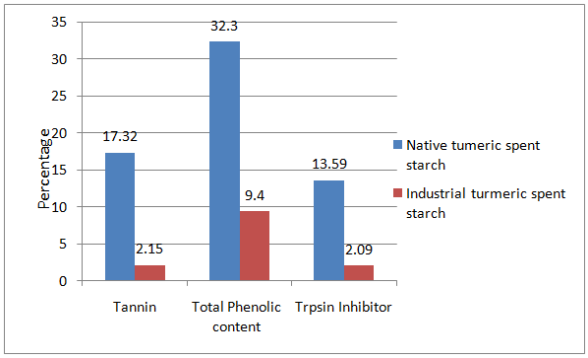

Figure 3. Anti nutritional parameters of native and industrial turmeric spent starch

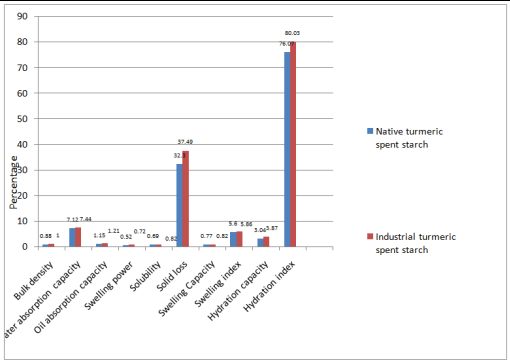

Figure 4. Functional properties of native and industrial turmeric spent starch

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

On Solving Narayana Puzzle

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Received: 31 Jan 2022 Revised: 18 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

Indian mathematician Narayana of 14th century CE posed several interesting problems in mathematics. In this paper, I will introduce one such puzzle and determine its smallest solution in positive integers through continued fraction method. In doing so, I will also derive a general way to generate infinitely many solutions to the given problem. The way adopted in this paper using continued fraction provides infinitely many solutions to two equations simultaneously,

Keywords: Pell's Equation, Smallest Solution, Continued Fraction, Convergents, Infinitely many solutions

INTRODUCTION

Around 1356 CE, Indian mathematician Narayana Pandita wrote a wonderful mathematical treatise titled Ganitha Kaumudi. This work had several interesting puzzles and mathematical ideas. Most notable among them were Narayana Cows, a puzzle resembling Fibonacci sequence pattern generation, but Narayana considered cow population instead of Fibonacci's rabbits. In this paper, however, I will introduce another interesting and important puzzle posed by Narayana and derive its smallest solution in positive integers as well as infinitely many solutions using continued fraction method.

Description of the Puzzle

Narayana posed the following puzzle:

Find the smallest solution in positive integers to the equation $x^2 - 103y^2 = 1$ (1)

Using continued fraction of $\sqrt{103}$ we shall obtain the smallest solution of (1). However, in this paper, I shall discuss the way to obtain the smallest positive solution as well as generate infinitely many solutions using a novel method.

Solution to the Puzzle

First, we notice that

$(477-47\sqrt{103})\times(477+47\sqrt{103})=2$

From this, we get the following computations

$$477-47\sqrt{103}=\frac{2}{477+47\sqrt{103}}=\frac{2}{954-(477-47\sqrt{103})}$$

$$=\frac{2}{954-\frac{2}{954-(477-47\sqrt{103})}}=\frac{2}{954-\frac{2}{954-\frac{2}{954-(477-47\sqrt{103})}}}$$

$$=\frac{2}{954-\frac{2}{954-\frac{2}{954-\frac{2}{954-(477-47\sqrt{103})}}}}=\frac{2}{954-\frac{2}{954-\frac{2}{954-\frac{2}{954-\frac{2}{954-\cdots}}}}}$$

$$47\sqrt{103}=477-\frac{2}{954-\frac{2}{954-\frac{2}{954-\frac{2}{954-\frac{2}{954-\cdots}}}}} \quad (2)$$

If we now compute successive convergents of the continued fraction represented by (2), then

$$c_0=\frac{477}{1}, c_1=477-\frac{2}{954}=\frac{227528}{477}, c_2=477-\frac{2}{954-\frac{2}{954}}=477-\frac{954}{455057}=\frac{217061235}{455057},$$

$$c_3=477-\frac{2}{954-\frac{2}{954-\frac{2}{954}}}=477-\frac{455057}{217061712}=\frac{103537981567}{217061712},\ldots$$

In each of the above convergents, if we consider the numerators as *x* and 47 (the coefficient of $\sqrt{103}$ in left hand side of (2)) times the denominators as *y*, then we see that the convergents $c_0, c_2, c_4, \ldots$ provides infinitely many solutions to $x^2-103y^2=2$ whereas the convergents $c_1, c_3, c_5, \ldots$ provides infinitely many solutions to $x^2-103y^2=1.$

In particular, we find that $(x, y) = (477, 47); (217061235, 21387679); \ldots$ are solutions to the equation $x^2 - 103y^2 = 2$ and $(x, y) = (227528, 22419); (103537981567, 10201900464); \ldots$ are solutions to $x^2 - 103y^2 = 1$.

Thus the smallest solution in positive integers for $x^2 - 103y^2 = 1$ is given by $(227528, 22419)$. The second smallest solution is given by $(103537981567, 10201900464)$. In this way using (2), computing $c_5, c_7, c_9, \ldots$ we get infinitely many solutions to $x^2 - 103y^2 = 1$. Similarly from (2), computing $c_4, c_6, c_8, \ldots$ we get infinitely many solutions to $x^2 - 103y^2 = 2$.

CONCLUSION

In this short paper, I had introduced the amusing puzzle introduced by Indian mathematician Narayana. Using the recursive method proposed by Brahmagupta or by computing the continued fraction expansion for $\sqrt{103}$ we can determine the smallest solution in positive integers to the puzzle posed as in (1) of this paper. But I had provided the same solution through a novel way by constructing a continued fraction expression as presented in (2) and considering its alternate convergents.

In particular, by extracting the convergents $c_1, c_3, c_5, \ldots$ and considering the numerators as *x* and 47 times the denominators as *y*, we can generate infinitely many solutions to the given puzzle $x^2 - 103y^2 = 1$. Doing the same procedure for the convergents $c_0, c_2, c_4, \ldots$ we can generate infinitely many solutions to the equation $x^2 - 103y^2 = 2$. Thus not only the continued fraction expansion in (2) generates infinitely many solutions to the puzzle posed by Narayana but it also has generated infinitely many solutions to the equation $x^2 - 103y^2 = 2$.

From the calculations performed in this paper, the two smallest solutions to the equation $x^2 - 103y^2 = 1$ were given by $(x, y) = (227528, 22419); (103537981567, 10201900464)$. Thus the novel method of first solving the equation $x^2 - 103y^2 = 2$ and then developing the continued fraction for $47\sqrt{103}$ as provided in (2), we have generated infinitely many solutions to two equations in most comprehensive way. This is the main feature of this paper.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Vacuum Based Paddy Harvesting System using Wifi Module

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Received: 08 Jan 2022 Revised: 19 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

Reaping is the method of removing the mature rice crop from the field at the right time. Reaping will be done by manually with help of sickles and knives or by mechanically combined harvesters or thresher. Reaping, threshing, sweeping, hauling, field drying, stacking, and bagging are among them. Now a day, reaper or combined harvesters are mostly used in harvesting process. The harvester machine is used to pick paddies where only a small percentage of the paddies have fallen due to the harvesting machine's air and vibration. Many people struggle to pick the paddies from the ground as a result of the process and the majority of their time is spent in the process. In harvesting processes, the machine wastes about 20% of the paddies. However, the proposed model was able to solve the problem by collecting the wasted paddies via vacuum inhalation processes and it will be done automatically. Farmers will get benefited through our proposed model and the paddies loss has been minimized.

Keywords: Harvesting Machine, Vacuum, Node MCU.

INTRODUCTION

After air and water, food is mostly needed for living beings to provide energy for doing the physical activities, for sustain the life and to stimulate the growth of human beings. Grains, pulses, fruits, vegetables, oils, and other foods are examples. About half of the world's population eats rice as a staple meal. Paddy farming is an intricate process that involves number of steps to produce the final outcome [1]. Selection of seed, cultivation land concoction, establishment of crop, water level management, crop nutrient management and health management, harvesting and

post-harvesting are the basic stages of reaping. In the olden days, harvesting method is effective if the crop has lodged or fallen over, but this method is a labour intensive [2,3]. Reaping nearly takes 45 to 80 hours per hectare if it is done by hand. Manual collecting and hauling the harvested crop takes even more time [4]. A real harvesting method will help to maximize the grain output and minimize the grain damage and decay [5,6].

Literature Review

Dr.S.Saravanan et al. (2020) proposed about the importance of paddy harvesting. They have proposed Vacuum based suction of paddy harvesting methodology. This idea is better suited for harvesting the unused paddies from the land, which is beneficial to our country's economic growth and the project's implementation would benefit our farmers [1]. Jeihoon Back et al (2014) proposed about the electrical power transfer to robot vacuum cleaner through high frequency wireless method [2]. P.B.Jarande et al (2018) proposed an idea to provide solution for manufacturing the robotic cleaner by utilizing the resources available in the local. They also proposed to design the robotic vacuum with Node MCU [3].

METHODOLOGY

Air and vibration from the harvesting machine causes nearly 20% of the paddies to fall in the field. The farmers are facing several difficulties to collect the paddies from the ground during this process. Also collecting the fallen paddies from the field took a long time. Figure 1 shoes the manual collection of wasted grain from the field is difficult and the cost needed to collect is huge. This method reduces the amount of food production and also causes huge financial losses to the farmers.

Proposed System

While drinking upon the water there are most microorganisms that are mixed not visible to our eyes and due to the impurities that have been mixed the PH level of the water gets lesser, making more likely to be contaminated with pollutants and making it unsafe to drink. Fig.2 shows the picture of contaminated water. In the proposed method, Vacuum inhalation methodology and a special mechanical setup is used to collect the dropped paddies. The Figure 2 Block diagram of the model is shown in figure. The electrical setup consists of relay, DC Geared motor, NODEMCU-ESP8266, IR sensor, buck boost converter. Relay is used as a driver circuit for DC Geared motor and vacuum ON&OFF Mechanism. Buck-Boost converter is used for converting high voltage to low voltage. It's mainly for Wi-Fi module which operates at low voltage. IR sensor is used for detect the object which is closer to machine nearly equal to 3cm. NODEMCU is an open source firmware, used to control the entire system. LCD display is used for displaying the vacuum ON/OFF state and wheeling position like right or left. The proposed setup will travel freely anywhere in the field and it can also build its own path and obstacles will be avoided with the help of sensors.

Hardware Setup and Working

A cage wheel is designed for the wheel motion and a structured frame for carrying the vacuum mechanism and storage tank shown in the Figure 3. The cage wheel is chosen instead of a regular wheel to drive the machine in wet as well as dry land. Geared DC motor is fitted to the wheel shaft. The storage tank is built with a capacity of 5 liters. Designing of the Vacuum Suction mechanism is a biggest challenge of this proposed methodology. The paddies are sucked from the field by a vacuum pressure created by the DC series motor. During this process, the soil is also sucked. To avoid the entry of soil into paddies, a filter is included in this storage tank to isolate the soil from the paddies. The proposed model is a hybrid one which consists of electrical and mechanical components. In reality, the paddy field is not completely dry, it is a little wet (puddle) in nature. So, in the design of wheel and framework, the regular rubber wheel can't be used at this place. In order to get rotation in field conditions, a cage wheel is designed. The cage wheel is made of steel and can be used in any situation. A special structured frame is designed to support the vacuum, storage tank, and other required components such as a battery, relay and so on. The vacuum architecture is the most important aspect in this model. For designing the vacuum, vacuum outline pressure, density and volume were taken into account. DC series motor is used for its rotation the table 1 shows the specification of the cage wheel.

The sucked paddies and soils were fallen into storage tank through pump shown in the figure 3. A filter is designed in the storage tank. Soil was deposited on the bottom of the storage tank because of less density when compared to paddies and paddies were deposited on the top using this filter. Table 2 shows the specification selected for performing simulation. The output from the proteus software shown in the figure 4. The electrical circuit is designed in the software to meet the desired specifications. The microcontroller family Node MCU has a feature of WIFI module as well as controlling operation. Blynk app is configured with Node MCU for ON/OFF of vacuum setup and wheel direction. The figure 5 and 6 shows the final structure of the developed model to collect the waste paddies from the field.

CONCLUSION

This proposed method of paddy collection is more suitable for collecting the wasted paddies from the ground which is very much useful for the economic growth for our country and the implementation of this idea will be very useful for the farmers in for collecting the paddies form ground without human work. The developed model can travel freely anywhere in the field and it can also build its own path and obstacles will be avoided with the help of sensors. Since the model will collect the paddies in the field automatically, the food production will increase and also the labor cost will be reduced

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Table 1: Wheel Design Dimension

Wheel Diameter	Material Used
20 CM	Heavy Duty Steel

Table 2: Specification of Vacuum Pump

Pressure	5 psi
Capacity	5 litre
Voltage	12V
Current	500mA
RPM	30 RPM

Fig 1. Existing Method (Source: Internet)

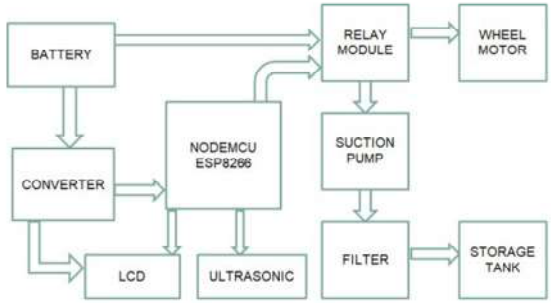

Fig 2. Block Diagram of Proposed Method

Fig 3. Cage Wheel Design

Fig 4. Suction Cylinder

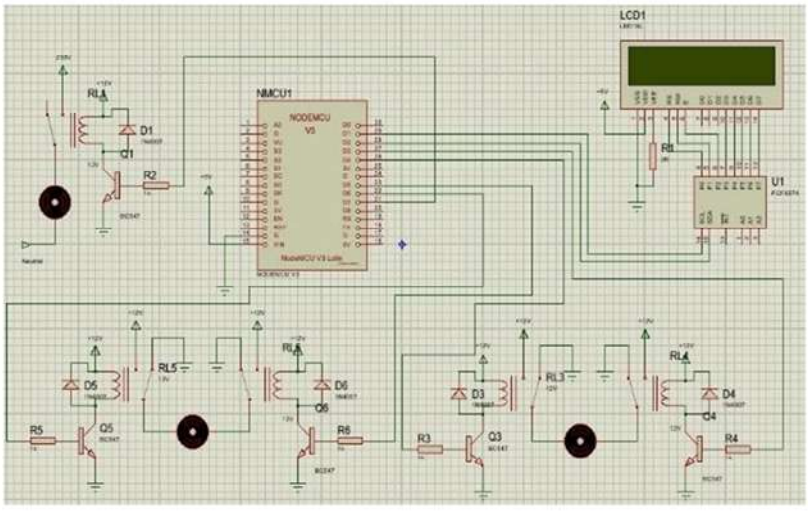

Fig 5. Spice Model of the proposed circuit

Fig 6. Front View and Back View

Fig 7. Harvesting Machine

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study to Assess the Knowledge Regarding Selected First Aid Measures among Adolescent at TBML College, Poraiyar

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Received: 01 Feb 2022 Revised: 03 Mar 2022 Accepted: 22 Mar 2022

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ABSTRACT

A study to assess the knowledge regarding selected first aid measures among adolescent at TBML college, poraiyar. The objectives of the study to assess the knowledge regarding selected first aid measures among adolescent students, to associate the knowledge regarding selected first aid measures among adolescent with selected demographic variables and to prepare the booklet regarding selected first aid measures. Descriptive research design was used to assess the knowledge regarding selected first aid measures among adolescent at TBML college, poraiyar. A total of 100 students were selected by simple random sampling technique. Data was collected by closed questionnaire method. A booklet on awareness on first aid measures and skill was given to each student. This study was concluded that among the 100 student (1) 1% of the student had adequate knowledge. The present study assessed the knowledge regarding first aid management. Most of the student had moderate knowledge.

INTRODUCTION

Life is characterized by precarious Taoists and turns risky conditions that emerge all the time. All throughout the globe, people suffer from heart attack, accidents, organ failure along with this hypovolemic shock, chocking, bleeding incidences are more common among the children's and adolescent. The highly mortality associated with them can be easily prevented most of the times by some very simple maneuvers and skills kike fluid resuscitation immediate oxygen supply, prevent further fluid loss, abdominal thrust and also further blood loss. First aid is the immediate action taken to save a life and reduce the effects of injury and illness medical help is obtained. First aid provider should be able to handle the situation without panicking. Long go, henry sigerist, the medical historian stated that "The people health ought to be the concern of the people themselves. They must struggle for it and plan for it. The war against disease and for health cannot be fought by physician alone. It is a people's war in which the entire population must be mobilized permanently" many times, death results because of delay in reaching the

casualty as soon as possible following the accident or injury, a life could be saved. This helps lower mortality and morbidity rates, complications due to injury or delay in the treatment and a lesser monetary burden on the causuality. Administration of first aid must not delay activation of the emergency medical services system or other medical measures when required. First aid is the name implies is the first care given to a victim of an accident, injury or sudden illness, before the provision of advanced medical care. First aid should be aimed to preserve life, promote recovery and prevent worsening of the victim's condition. Every year 1,50,000 people may die in the situations, where first aid could have given them a chance to live. Almost 35% of death occurs within 5 minutes of an accident while another 54% would die within 80 minutes.

However, by giving first aid at the site of the injury deaths can be decreased in 20% (METIN AND MUTHU, 2010). According to the reports injuries are the leading cause of death, hospitalization and disability throughput the world accounting for 9% of all deaths and 16% burden of disability annually. First aid is an important responsibility of all the person, because in remote areas, there is very limited access to fully fledged hospitals and dispensaries in nearby vicinity. Therefore we should be able to provide care of those who are injured. The aims of first aid are to preserve life. Promote recovery and prevent worsening of the victim's general conditions and quickly transport of the casualty to the nearest medical aid is required. Hypovolemic shock or hemorrhage shock is a dangerous condition that occurs if an individual's loss more than 20% of the blood or fluid supply in the body. The significant loss of fluid makes it hard for the heart pumps enough blood to the body. Remember that hypovolemic shock can result to organ failure and necessitates immediate emergency care. A certain percentage of children to adolescent between the age group of 5-16 years old end up wounded while playing games. As a result many considered a hard games such as the football often face a greater risk for sustaining a hard injury cause heavy bleeding. According to the injury facts 2017, chocking is the fourth leading causes of unintentional injury death. Of the 5, 051 people who died from chocking in 2015. Then 2,848 were older than 74. Food is often responsible for chocking incidents in elderly. Living alone and having dentures or difficulty or swallowing can increases at risk.

Statement of the Problem

A Study to assess the knowledge regarding selected first aid measures among adolescent at TBML college, poraiyar.

Objectives

- To assess the knowledge regarding selected first aid measures among adolescent students.
- To associate the knowledge regarding selected first aid measures among adolescent with selected demographic variables.
- To prepare the booklet regarding selected first aid measures.

Research Approach

Research approach used for this study was quantitative approach.

Research Design

Research design is a blue print for conducting the study that maximizes control over factors that could interfere with the validity of the findings. The research design adopted for this study was non-experimental (descriptive) research design.

Setting

The study was conducted at tranquebar bishop manickam Lutheran college, poraiyar.

Population

Population is all elements (individuals, objects or substance) that meet certain criteria for inclusion in a study. The population is a well-defined set that has certain specified properties. The population was this study was adolescent (17-19) years.

Sample Size

A subset of population selected to participate on study. In this study sample size was 100.

Sample Technique

Sampling is the process of selecting representing units of a population for the study in a research. In this study simple random sampling technique was used

Sample Criteria

Sample where selected with the following pre-determined set criteria during the period of the study.

Inclusive Criteria

- Both boys and girls
- Age group between 17-19years
- Present during data collection
- Students who were able to understand English/ Tamil.

Exclusive Criteria

Students who are not willing to participate in the study.

Data Collection Procedure

Each day 25 students selected using simple random sampling technique. Data collection was conducted by using structured questionnaire method. After data collection, a booklet were prepared for a awareness of first aid management was given to each student to enrich and reinforce the knowledge and attitude regarding first aid management.

Data Analysis

This chapter deals with the analysis and interpretation of the data collected from regarding first aid management. This study results were tabulated, analyzed and presented on the data collected from the adolescent student. The data were collected regarding the level of the knowledge about first aid management.

RESULTS

Table 1: Shows that put of 100 students 76(76%) of them belongs to 17 – 19 years, 13 (15%) of them belongs to 20-22 years, 9 (9%) of them belongs to 23-25 years. Regarding sex, 45 (45%) of them were male and 55 (55%) of them female. The student fathers income 41 (41%) were earned below Rs. 5000 per month, 35 (35%) earned Rs. 5000-10,000 per month, 12 (12%) were earned 10,000-15,000 per month, 12 (12%) were earned >15,000 per month. Regarding residence 46(46%) of them students were from urban area 43 (43%) of them were from rural area and 11 (11%) were from municipality. Regarding the source of getting information 26 (36%) were gained knowledge from family members 64(64%) were gained from college students. Regarding religion 41(41%) of them students are Hindu, 40(40%) of them students are Muslims and 19 (19%) of them students are Christian. Regarding family type 36 (36%) of them students had nuclear family, 47 (47%) of them students had joint family. Table 2Shows that 1(1%) of students had adequate knowledge, 60 (60%) of students had moderately inadequate knowledge and 39 (39%) of students had adequate knowledge.

CONCLUSION

The study demonstrated that the structured teaching programme regarding knowledge on hypertension and its prevention among adolescents residing in rural areas was effective to improve the knowledge of adolescents.

Recommendation

A similar study may be replicated on a larger scale. A comparative study may be conducted to find out the effectiveness of using different teaching strategies regarding the same topic. The study can be replicated with control

group. A study can be conducted to assess the knowledge and practices of other health practices like exercise, relaxation techniques and Yoga etc.

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Table 1: Frequency and Percentage Distribution of Demographic Variables among Adolescent

S.NO	DEMOGRAPHIC VARIABLES	FREQUENCY	PERCENTAGE %
1.	Age		
	17-19 years	76	76%
	20 -22 years	15	15%
	23 – 25 years	9	9%
2.	Sex		
	Male	45%	45%
	Female	55%	55%
3.	Religion		
	Hindu	41	41%
	Muslim	40	40%
	Christian	19	19%
4.	Monthly income		
	<5000	41	41%
	5000 -10,000	35	35%
	10,000- 15,000	12	12%
	>15,000	12	12%
5.	Family type		
	Nuclear family	46	46%
	Joint family	54	54%
6.	Area of living		
	Urban	46	46%
	Rural	43	43%
	Municipality	11	11%
7.	Source of getting information		
	Yes	36	36%
	No	64	64%

Table 2: Distribution of Level of Knowledge among Adolescent

S.NO	LEVEL OF KNOWLEDGE	PERCENTAGE	FREQUENCY
1.	Inadequate (Above 50%)	39	39%
2.	Moderate adequate (51-75%)	60	60%
3.	Adequate (below 76%)	1	1%
TOTAL		**100**	**100%**

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Reliable Method for Monitoring Distribution Transformer

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Received: 28 Dec 2021 Revised: 24 Jan 2022 Accepted: 19 Feb 2022

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ABSTRACT

Distribution Transformers plays a vital role in the distribution of power from the secondary transmission to the distribution systems. The distribution transformers have to be maintained periodically for its reliable operation. This paper presents an online monitoring system to monitor the parameters like level of the oil, values of voltage and current, temperature, level of oil and temperature of winding, loading parameters and harmonics. This system consists of node Microcontroller unit, ESP 8266 unit interfaced with the sensors and the communication unit. If any abnormalities occur in the transformer, then the microcontroller unit will send the information to the web server and alert will be given to the attending person by sending message or by automatic phone call.

Keywords: Online Monitoring, ESP 8266, Temperature, oil level, Distribution Transformer

INTRODUCTION

In developed countries, the failure rate of the transformers is less than 1% which is about 15% in developing countries like India. The major failure occurs in the distribution transformer and it occurs due to the theft, loading effects due to overloads and unbalanced loading. Overloading occurs in a transformer due to the summer seasons, holidays and due to the lifestyle of the people. Due to these overloading phenomena, the voltage of the distribution transformer drastically changes which increases the temperature of the motor and the insulation of the transformer

worn out [1]. The increase in temperature of the transformer also affects the dielectric strength of the insulation oil in the transformers by forming sludge in the oil. The two main reasons for forming sludge in the transformer is due to the moisture and oxidation in oil. The windings of the transformer made up of copper coil also get damaged due to the increase in the temperature. Power theft is also occurring in the transformer by using metal hooks in the transmission system. Distribution Transformers are maintained periodically by a person appointed by the electricity board but this method will not be effective as it involves time period and also discontinuity in power supplies. There is a need for frequent monitoring of Transformer through web server in order to reduce the issues [2]. We proposed a system for the automatic monitoring of transformer parameters such s temperature of the windings, current, voltage and oil level. The main objective of this paper is to propose a user friendly IoT based online Monitoring system for a distribution transformer.

Faults in Transformer and methods to detect faults

Faults in Transformer: The fault in the transformer can be classified as external and Internal Faults. The Faults occurred due to the overheating, Design procedures; loss of insulation across the windings, contamination in the insulating oil is called internal faults. The faults which occur due to the overloading, switching operations and due to lightning strikes are known as External Faults.

Methods to detect faults: There are several techniques available to monitor the working of transformer they are partial discharge analysis method, thermographs method, vibration analyzers and Dissolved gas analyzer method. The partial Discharge technique is used to find the insulation strength of the windings of the transformers [3]. The partial discharge is the discharge of the dielectric material subjected to the high voltage and it affects the insulation of the windings. Partial discharge analysis involves sensors such as optic senor or wavelet transformation techniques for the detection of partial discharge in a transformer. Dissolved Gas Analyzer method is used to analyze the degradation of the insulation material based on the type of gas dissolved in the transformer oil. The gases evolved due to the deterioration of Insulation material are CO, CO_2, CH_4, C_2H_4, H_2 etc. The following tabulation shows the faults based on the gases in the insulation oil [4]. Thermograph is the famous method used to detect the hotspots in the external surface of the transformer due to the raise in the temperature by using the infra red imaging process. This process is effective in finding the temperature raise of any electrical device. The above methods are effective in finding the faults in the transformer by means of the periodical evaluation and maintenance of the transformer. Distribution transformer can be monitored effectively by means of online monitoring of the devices by using the IoT mechanism and we can able to detect fault by using the above technique.

Proposed Method

The following block diagram shows the online monitoring of the distribution transformer. The parameters such as voltage, current, temperature of the windings and the level of the oil in the distribution transformer are sensed by the suitable sensors and it is converted to the digital value by the analog to digital converter and it is compared with the predetermined set values in the microcontroller. The microcontroller process the input parameters and it is sending to the ESP 8266 wifi module to display the data in the web server and also alert the maintenance person if there is any deviation by sending SMS and automated calls. The workflow of the project starts from the data collection unit which consist of sensors used to measure the physical parameters of the Transformer which is processed data processing unit consist of driver circuit for the micro controller circuit and microcontroller unit and the communication device used to display the parameters and alerts if there is any fault [5]. Rectifier based voltage sensor is used to detect the overvoltage and under voltage phenomena in the transformers. The capacitor is used a filter circuit. The variation of the voltage causes the variation in the transformer flux leads to the Iron losses. This can be detected by the voltage sensors and alert the maintenance engineers if there is any overvoltage or low voltage. LM 35 is used as a temperature sensor used to detect the temperature changes in the windings of the transformer [6]. The detection of variation of winding temperature is important parameter in decision of assessing the transformer life. The sensor detects the changes of the temperature and it is given to the analog to digital converter unit of the transformer.

The temperature changes in the winding are due to the overvoltage and over current of the transformers. The sensor output changes 10 millivolt for every degree centigrade. So we can able to read the temperature by the following equation

$$Output\ Temperature = output\ voltage\ of\ the \frac{Sensor}{0.01}$$

The current sensor ACS 712 senses the changes in the current by using the principle of Hall Effect. The output current measured by this sensor module is the instantaneous currentvalue which can be converted to RMS value by suitable calculations [7]. The main feature of this sensor is that it can able to measure both AC and DC currents and it is compatible with any type of microcontroller unit. Ultrasonic sensors are used as level sensors for detecting the level of oil in the transformer tank. An ultrasonic sensor uses the ultrasonic wave for measuring the distance of the object or to detect a level of the liquid [8]. The GSM module is used to communicate the user when there is an emergency or fault occurs in the transformer. The GSM module will make a call to the number stored in the module in case of faults [9]. The ESP 8266 module is used to send the messages and display the measured parameters in the web server. The ESP 8266 module is having wifi module which will act as a transceiver to send the messages in the web server [10].

Implementation in Algorithm

The steps of the algorithm is shown below

1. Start
2. Initialize proposed system with button switch.
3. All sensors such as current sensor, temperature sensor, oil level sensor, vibration sensor and humidity sensor take the reading from the transformer [11].
4. All analog values which are collected in converted into digital value with the help of program.
5. Digital values are converted in the PIC microcontroller.
6. PIC microcontroller shows these values on LCD.
7. Microcontroller sends these values on webpage having Thingspeak where we have created a UI to display the data.
8. If any emergency condition occurs then immediately SMS is sent to engineers present over there through GSM.
9. Webpage Valued box will blow red to alert online.
10. All the data values are saved in database periodically.
11. End.

Software Design: The software program is written in C programming which is responsible for controlling the microcontroller operation. Database is created first for remote monitoring and storing of transformer data and the database is designed by using MYSQL Database and the complete data of the transformer is stored in IOT software Thing speak. By using this software, the data can be retrieved and analyzed in the future. The software implementation of the project is done by using proteus software and the diagram is shown below.

The health index of the transformer is calculated by taking the monitoring parameters of the Transformer such as oil level, temperature, loading parameters and noise etc. The formula used to calculate the health index of the transformer is calculated as

$$HI = \frac{\sum_{j=1}^{n} KjHIF_j}{\sum_{j=1}^{n} 4K_j}$$

$K_j$is the weightage of the i^{th} parameter

HIF_j is the health index of the i^{th} parameter

Based on the parameter, the health index values and status are shown in the following table 2.

Hardware implementation of the Project: The Hardware implementation of the proposed system is shown in the figure. The output of the sensor is given to the microcontroller and the microcontroller is driven by a driver circuit. The sensor is connected to measure the various parameters of the Transformer. The predetermined cut off level for

various parameters are set up in the microcontroller by the use of programming. The output parameters such as temperature, oil level, voltage and current will be displayed in LCD and the same will be stored in the IOT software Things speak. The microcontroller used in the hardware implementation is PIC controller.

ANALYSIS OF RESULTS

The output of the hardware is shown in the below figure. In the figure the value of oil level is indicated as r. If the value of the r is 1, the oil level is normal. If r is equal to 0 , then the oil level is low and hence the transformers needs maintenance and the oil should be replaced. The value of temperature is indicated as 30 and it is in the normal state. If the temperature raises suddenly increases then the alert will be sent to the mobile of the respective authority. The figure shows the sudden rise in the temperature, since the set value for the temperature is 40 degrees. The temperature value shown in the below figure is 41 degrees, so the alert message will be sent to the respective person. The following figure shows the oil level is low and temperature is normal. Displayed values r: 0 and temp:0030, when oil level is low then r: 0.

Outputs in ThingSpeak Platform

The measure output parameters are stored in the IOT Software thing speak are shown in the following figure. The figure shows the temperature measurement of the sensors in the webpage. We can able to retrieve and analyses the data. The status of the oil level, moisture level, voltage and current values are recorded in the ThingSpeak software. The following graph clearly shows the normal and abnormal conditions of the transformers. The First graph shows the output of measurement of temperature over a time period. The continuous monitoring of temperature level of the distribution transformer is important since, it affects the operating life period, maintenance and reliability. For analysis purpose we have set the dates as years. The second graph shows the measurement of oil level in the transformer, A value of 0 represents the low value and the value of 1 represents the normal value. The level of oil has to be maintained at proper level, which is a important parameter for the operation of distribution transformer. If the level of oil is low, the temperature of the transformer will be increased and overheat the transformer. If the level of the oil is high, then it leads to decrease of dielectric strength of the oil and oil has to be replaced. The third graph shows the measurement of moisture content due to rainy Seasons. Since the moisture content will affect the dielectric strength of the insulation. If the transformer is loaded at peak levels, this moisture will create gas bubbles and it leads to contribute significant damage to the strength of the dielectric material present in the insulation. The following graphs shows the measurement of voltage and current monitoring in the Distribution transformer.Monitoring of current and voltage is important since the increase or decrease of these parameters will drastically affect the consumer ends

CONCLUSION

This paper presents a reliable system for monitoring various parameters of transformers such as monitoring of oil level, rise of temperature, voltage and current in a single platform. Furthermore, this system proposes the effective online monitoring and easier preventive maintenance in the transformer. The user can easily rectify the faults. This system provides higher reliability in the remote maintenance of the distribution transformer. If a fault occurs in a distribution transformer, the responsible person can easily rectify the fault in remote operation itself by giving commands to the service authorities.

FUTURE ENHANCEMENTS

In future, we can able to monitor the transformer by implementing fuzzy logic algorithms and machine learning algorithms for calculating health index of the transformer. Since this algorithm provide accurate and reliable results. We can also implement higher IoT techniques like raspberry pi to improve the quality of the system.

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Table 1. Types of Faults in the Transformer

S.No	Type of Faults	Gases in the oil
1	Arcing in the winding	C_2H_2
2	Corona discharge	H_2
3	Oil breakdown to high temperature	C_2H_4
4	Oil breakdown to low temperature	CH_4 and C_2H_6

Table 2. The health index values and status

S.No	Health Index	Health Status
1	100 % < HI > 80 %	Excellent
2	80 % < HI> 70 %	Good
3	70 % < HI >60 %	Alarm State.
4	60 % < HI> 50 %	Needs maintenance
5	HI <50 %	POOR

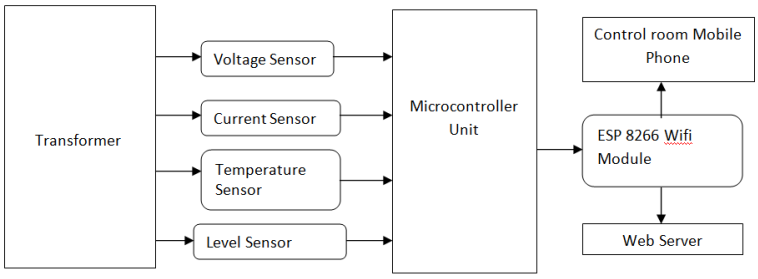

Figure 1: Proposed Method

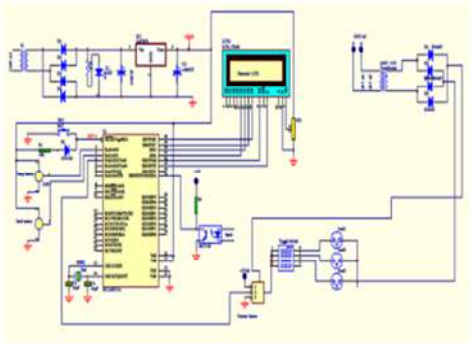

Figure 2: Software Design

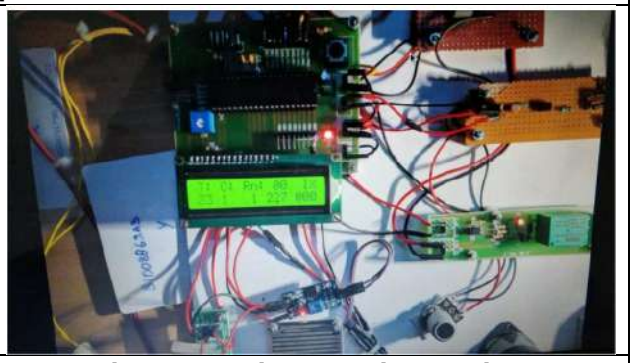

Figure 3: Hardware Implementation

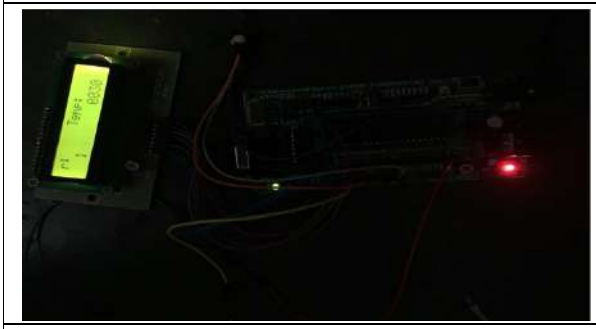

Figure 4: Oil level and Temperature in Normal state

Figure 5: Oil Level is normal and temperature is high

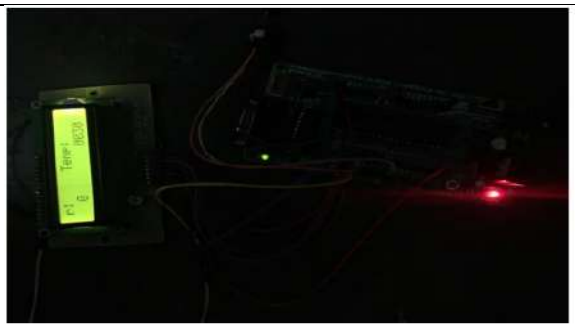

Figure 6: Oil Level is low and temperature is normal

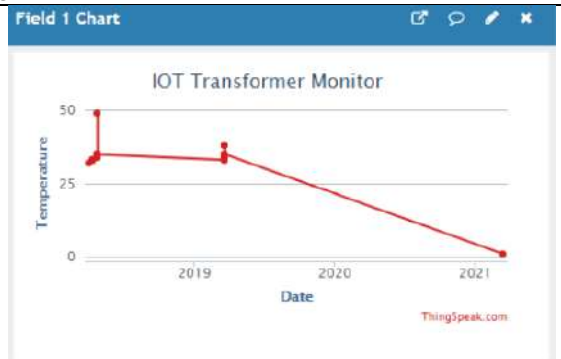

Figure 7: Measurement of Temperature

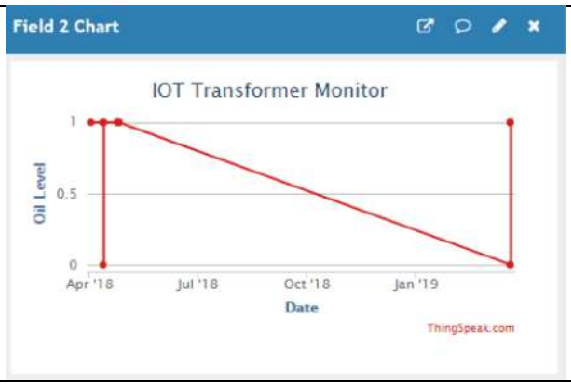

Figure 8: Measurement of oil level

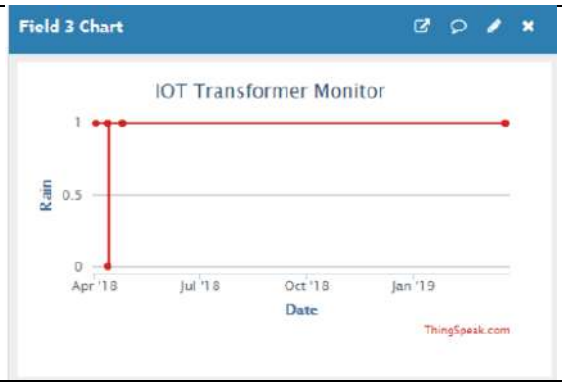

Figure 9: Measurement of Moisture content

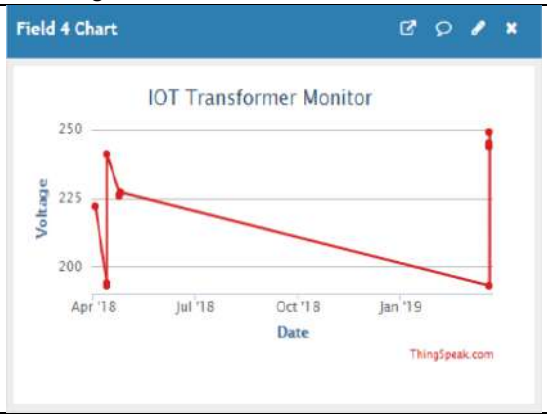

Figure 10: Measurement of voltage

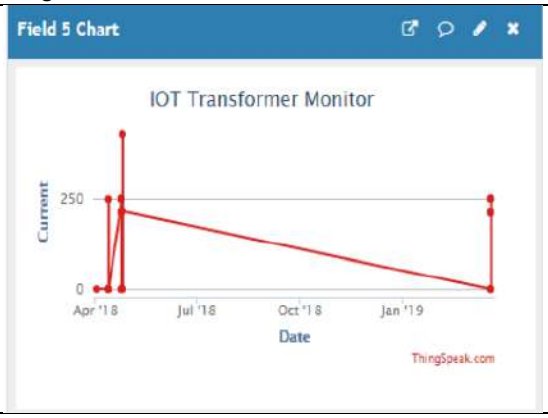

Figure 11: Measurement of current

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Assess the Knowledge on Breast Self Examination and Identify the Common Obstacles among Working Women

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Received: 26 Dec 2021 Revised: 24 Jan 2022 Accepted: 25 Feb 2022

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ABSTRACT

A cross sectional study with quantitative approach was used to assess the knowledge on Breast Self Examination and identify the obstacles among 100 working women coming to Sacred Heart Hospital, Thoothukudi for treatment were selected by convenient sampling technique and self administered questionnaire was used to assess the knowledge and identify the common obstacles to practice breast self examination .The result showed that most of working women had moderate knowledge and it revealed that the common obstacles to do breast self examination were not prioritizing Breast self Examination ,not believing in Breast Self Examination ,lack of knowledge about how to perform Breast Self Examination, being worried to findout breast cancer ,not having enough privacy and time to perform Breast Self Examination were the common obstacle to practice Breast Self Examination.

Keywords: Breast Self Examination

INTRODUCTION

Breast cancer is the most common invasive cancer in females. Breast cancer is the second most common cancer in women after skin cancer. It is also a leading cause of cancer deaths among females. With being the most common type of cancer in women, breast cancer accounts for 14% of cancers in Indian women. It is reported that with every four minutes, an Indian woman is diagnosed with breast cancer. In 2020, there were 2.3 million women diagnosed with breast cancer and 6, 85, 000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer. There are more lost disability-adjusted life years (DALYs) by women to breast cancer globally than any other type of cancer. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life.

The incidence rates in India begin to rise in the early thirties and peak at ages 50 – 64 years. Overall, 1 in 28 women is likely to develop breast cancer during her lifetime. The objective of the WHO Global Breast Cancer Initiative (GBCI) is to reduce global breast cancer mortality by 2.5% per year, thereby averting 2.5 million breast cancer deaths globally between 2020 and 2040. Reducing global breast cancer mortality by 2.5% per year would avert 25% of breast cancer deaths by 2030 and 40% by 2040 among women under 70 years of age. The three pillars toward achieving these objectives are: health promotion for early detection; timely diagnosis; and comprehensive breast cancer management. Breast cancer treatment can be highly effective, especially when the disease is identified early. Providing health education to public improves awareness among women regarding breast cancer and helpful to the family members about the importance of early detection and treatment. Due to late screening and diagnosis the survival rate of breast cancer is becoming low. Women can very well identify the breast cancer by doing Breast Self Examination and treat cancer outgrowths. So practising regular Breast Self Examination will help to detect breast cancer early and increases quality of life and survival and prevent the complications and disability. Lack of knowledge about the symptoms of breast cancer and the importance of doing Breast Self-examination are the main obstacles for not practicing BSE. Therefore the investigator was interested to do this study aimed to assess the knowledge on Breast Self Examination and identify the obstacles among working women

Background

Breast cancer is the most common cancer worldwide surpassing lung cancer for the first time in 2020.It is considered to be a progressive disease with a poor prognosis if detected late. It is considered to be a progressive disease with a poor prognosis if detected late..Breast self examination is an important prevention method of breast cancer. This study was aimed to assess the knowledge and barrier factors practising breast self-examination among working women. Coming to Sacred w=c Heart Hospital, Tuticorin. Methods. A cross-sectional study was conducted in 2021 among 100 working women using self-administrated questionnaire. Convenient sampling technique was used to select the study participants. Descriptive and Inferential analysis were done. Results. Majority of the study participants were between 35 to 40 years old. Seventy six (76%) of the participants had heard about BSE and 62% had moderate knowledge about BSE. None of them had good knowledge about BSE. Mass media was the most common source of information about breast cancer. Few of the participants (13%) had performed BSE. It revealed that not prioritizing Breast Self Examination, not believing in Breast self examination, lack of knowledge about how to perform Breast self examination. Being worried to find out breast cancer, not having enough privacy and time to perform BSE were the obstacles to perform BSE.

Objectives of the study

1. To assess the knowledge on Breast Self Examination among working women
2. To identify the common obstacles of performing Breast Self Examination
3. To find out the association between the level of knowledge and selected demographic variables.

METHODOLOGY

Research design

Cross sectional research design with quantitative approach was used to assess the knowledge of BSE and identify the common obstacles of performing BSE.

Setting of the study

Study was conducted in Sacred Heart Hospital, Thoothukudi which is 250 bedded hospital. It is the parent hospital of St.Ann's College of Nursing. It has the facilities of outpatient department, Medical, surgical, pediatric, ICU, IMCU, pharmacy, lab, CSSD, OT and physiotherapy.

Samples

100 working women who fulfill the inclusion criteria and coming to Sacred Heart Hospital for treatment were selected by convenient sampling technique to participate in this study.

Description of tool

Structured questionnaire to assess the knowledge and checklist to assess the common obstacles for performing Breast Self Examination.

Protection of human rights

Written permission was obtained from the Administrator, Sacred Heart Hospital, Tuticorin. The data was collected after clear discussion with the study participants about the purpose and procedures of the study and after obtaining informed consent form each participant.

Findings

The demographic variables reveal that (28%) majority of the study participants were between 35 to 40 years old. Seventysix (76%) of the participants had heard about BSE and 62% had moderate knowledge about BSE. None of them had good knowledge about BSE. Mass media was the most common source of information about breast cancer. Few of the participants (13%) had performed BSE. It revealed that not prioritizing Breast Self Examination, least importance to perform Breast self examination, not understanding the importance of performing BSE, having disturbance to perform were the obstacles to perfom BSE.

CONCLUSION

The present study assessed the knowledge on Breast Self-Examination and identify the Obstacles to perform BSE among working women coming for treatment in a selected hospital, Tuticorin. The findings revealed that most of the participants had moderate knowledge and having Obstacles for practising Breast Self Examination. There was no significant association between knowledge and selected demographic variables .In this study, though most of the women had moderate knowledge about breast self-examination they had obstacles to perform BSE. So it is essential to educate and improve knowledge among the public about BSE to prevent breast cancer.

ACKNOWLEDGEMENT

I extent my deep sense of gratitude and respect to the Guide Dr.S.Lakshmi Prabha, Ph.D.(N),Vinayaka Mission Annapoorana College of Nursing, Salem for her constant guidance, highly instructive suggestion, precious advice, as well as providing all possible facilities for successfully carrying out this study. I extend my deep sense of gratitude to Sr. Liz John, Administrator, Sacred Heart Hospital, Thoothukudi for given me permission to conduct the study in the hospital. I express my heartfelt gratitude to all the patients who have participated in the study. Without their co-operation, the study would not have been possible.

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Table 1: Knowledge of Breast Self-Examination among working women

S.No	Knowledge level	No	Percentage
1.	Adequate(76 -100)	-	-
2.	Moderate(50 -75)	62	62%
3.	Inadequate(0 – 50)	38	38%

Table 2: Obstacles to perform Breast Self Examination

Obstacles	No	Percentage
Least importance to perform Breast Self Examination	40	80%
Not understanding the importance of performing Breast self examination,	40	80%
Don't know how to perform Breast self examination	30	60%
afraid	10	20%
Not having symptoms of breast cancer.	30	60%
Having disturbance to perform Breast self Examination	30	60%

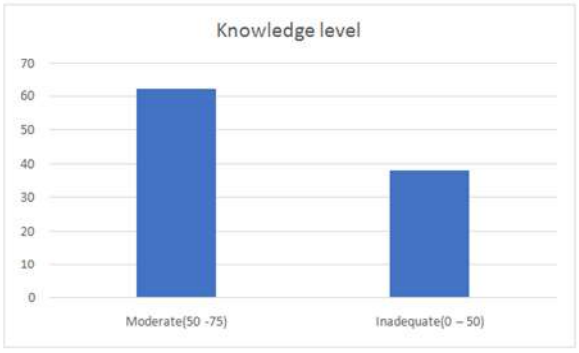

Figure 1: Knowledge of Breast Self Examination among working women

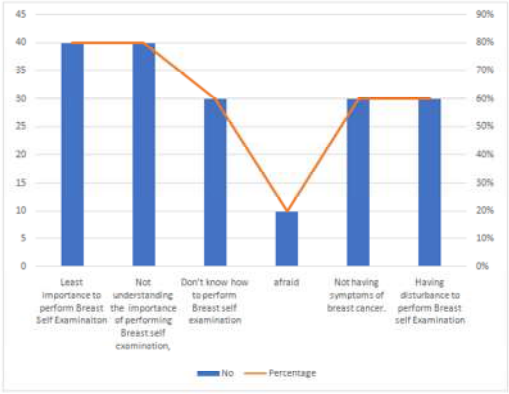

Figure 2: Obstacles to perform Breast Self Examination

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Artificial Bee Colony Algorithm Based Speed Control of BLDC Motor Tuned with Spider Web Based Controller

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Received: 04 Feb 202 Revised: 27 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

We present a Spider web-based controller based on the ABC algorithm to help improve the performance of a BLDC motor. Brushless DC motors must be able to function at a variety of speeds and loads while maintaining a positive variable speed. The BLDC motor drive's prolonged settling time, varying power variation, set point inaccuracy, and nonlinear features lead to lower adaptability in practice. To address the issues, a revised Spider controller is proposed, which employs to lessen the following optimal solution, should use Proposed method in a self-tuned regulator structure. while meeting imbalance restrictions. Component failure, low reliability, the necessity for specialized mechanical mounting, and electrical noise concerns are just a few of the disadvantages of Hall Effect sensors. ABC's suggested tuning Spider, the results of the controller are compared to those of a traditional controller and enhanced artificial bee colony algorithmtuned spider controllers.When it comes to time-domain characteristics, control commitment as well as performance objectives then planned ABC tuned controller is preferred, according to the results.

Keywords : Spider Web, BLDC, ABC

INTRODUCTION

BLDC motors are widely employed used in electric vehicles for a variety of industrial and domestic applications, as well as as computer peripherals. This motor's advantages include increased power intensity, greater power factor, increased performance, higher consistency, higher torque, small size, longer life, low noise, less maintenance, and simplified control techniques. The conversion procedure is complete wirelessly because the BLDC motor is brushless. In this sort of commutation, feature enables are conducted to analyze the rotor relative position to the stator winding. A three-phase BLDC motor requires details concerning the operating point every 60 electrical degrees.As a result, even when a rectangular current is applied, torque ripple exists. Furthermore, due to the inductive nature of the motor windings and the finite dc bus supply voltage, the control system usually fails to generate the required waveform during the commutation process. As a result, an output waveform known as transmission output waveform is created. This torque ripple increases in proportion to the speed, reaching a maximum of 50% of the average torque. In sensor less motor drives, they also cause noise, vibrations, and major failures. As a result, in order to use a BLDC motor for household applications, this torque must be reduced. The commutation torque ripple in the BLDC motor can be decreased by employing separate current sensors because each phase's current management is accomplished with different current sensors. Smooth torque is crucial for enhanced performance in industrial applications, hence obtaining ripple-free instantaneous torque is critical.A brushless DC motor is one that is devoid of brushes. As the name implies, there are no brushes or commutators. Commutation in a BLDC motor is achieved through the use of an electronic circuit, which reduces mechanical losses and increases efficiency. Significant energy savings can be realised by replacing inefficient motors with more efficient BLDC motors. A BLDC provides a number of advantages over other machine types. They require less maintenance because the mechanical commutator has been removed. It also has a high-power density. In comparison to induction motors, BLDC motors have reduced inertia, allowing for faster dynamic response to reference commands. In terms of speed-torque characteristics, BLDC motors can surpass AC motors in the classic sense. However, there are substantial drawbacks to using a mechanical commutator, such as brush sparks and friction losses. As a result, the motor's performance must be improved, which is done with the help of an electronic commutator. The progress of brushless DC motors is depicted in the Fig.1.

MATHEMATICAL MODEL OF BLDC MOTORS

A three-phase stator winding is being used in the BLDC motor variable equation model. The stator is connected in a star pattern, and the permanent magnet rotor contains non-salient poles. The minimum and maximum values are A1, A2, B1, B2, C1, C2 of inverter feet, respectively, only with constants A1, A2, B1, B2, C1, C2. The signals produced by the Hall effect are represented by the letters Ha, Hb, and Hb. Every winding's voltage output, which includes voltage loss, resistance, and created EMF, is as follows:

$$U_y = R_y\, i_{y^+} e_{\varphi y} \text{----------} \rightarrow 1$$

The change of rate of the flux is equal to the winding generated EMF.

It can be given as,

$$e_{\varphi y} = \frac{d\varphi y}{dy} \text{----------} \rightarrow 2$$

We take the Phase A as an example, the flux is,

$$\text{Phase P} = L_p i_y + M_{PQ} i_Q + M_{PR} i_R + \varphi_{PM} \text{----------} \rightarrow 3$$

PM flux of Phase P, $\varphi_{PM}(\propto) = N\emptyset_{PM}(\propto)$----→4

$$\emptyset_{PM}(\propto) = \int_{-\frac{\pi}{2}+\propto}^{\frac{\pi}{2}+\propto} B(\theta) S d\theta \text{ ----------} \rightarrow 5$$

Sub (2) & (5) in (1)

$$U_p = R_{ip} + \frac{d}{dt} + (L_p i_p + M_{PQ} i_Q + M_{PR} i_R + \varphi_{PM})$$

$$= R_{ip} + \frac{d}{dt} + (L_p i_p + M_{PQ} i_Q + M_{PR} i_R + \varphi_{PM}) + \int_{-\frac{\pi}{2}+\propto}^{\frac{\pi}{2}+\propto} B(x) S dx$$

$$= R_{ip} + \frac{d}{dt} + (L_p i_p + M_{PQ} i_Q + M_{PR} i_R + \varphi_{PM}) + e_p \text{----------} \rightarrow 6$$

$L_P = N^{2\wedge P}$ ----------→7

$M_{PQ} = N^{2\wedge PQ}$ ----------→8

$L_P = L_Q = L_R = L$, $M_{PQ} = M_{QR} = M_{RP} = M_{RQ} = M_{QP}$=M

substitute in equation 6,

$$U_p = R_{ip} + L\frac{diP}{dt} + M\frac{diQ}{dt} + M\frac{diP}{dt} + e_p \text{----------} \rightarrow 9$$

Were,

$$e_p = \frac{d}{dt}\left[NS\int_{-\frac{\pi}{2}+\theta}^{\frac{\pi}{2}+\theta} B(x)dx\right]$$

$$= NS\left[B\left(\frac{\pi}{2}+\theta\right) - B\left(-\frac{\pi}{2}+\theta\right)\right]\frac{d\theta}{dt}$$

$$= NS_\omega\left[B\left(\frac{\pi}{2}+\theta\right) - B\left(-\frac{\pi}{2}+\theta\right)\right] \text{----------} \rightarrow 10$$

$$e_A = NS_\omega\left[B\left(\frac{\pi}{2}+\theta\right) - B\left(-\frac{\pi}{2}+\theta\right)\right]$$

$$= NS_\omega\left[B\left(\frac{\pi}{2}+\theta\right) - B\left(-\frac{\pi}{2}+\theta+\pi-2\pi\right)\right]$$

$$= 2NS_{\omega B}\left(\frac{\pi}{2}+\theta\right) \text{----------} \rightarrow 11$$

$e_A = 2NS_{\omega BM}, f_A(\theta) = W_{\omega m f A}(\theta)$ ----------→12

The rotor position causes the $f_A(\theta)$ to have a trapezoidal distribution, with maximum and lowest values of 1 and -1, respectively. There are also three-phase symmetrical windings available,

fB (θ) = fA ($\theta - \frac{2\pi}{3}$)

f (θ) = fA ($\theta + \frac{2\pi}{3}$)

The winding flux connection formed by the revolving rotor produces ex, which is a rotating back-EMF, as shown in Equation (10) The currents in all three stages meet the requirement.

$i_p + i_Q + i_R = 0$ ----------→13

9---------→ $U_y = R_{ip} + (L - M)\frac{diP}{dt} + e_p$

MATRIX FORM

$$\begin{bmatrix} U_A \\ U_B \\ U_C \end{bmatrix} = \begin{bmatrix} R & 0 & 0 \\ 0 & R & 0 \\ 0 & 0 & R \end{bmatrix} \begin{bmatrix} i_P \\ i_Q \\ i_R \end{bmatrix} + \begin{bmatrix} L-M & 0 & 0 \\ 0 & L-M & 0 \\ 0 & 0 & L-M \end{bmatrix} \frac{d}{dt} \begin{bmatrix} i_P \\ i_Q \\ i_R \end{bmatrix} \begin{bmatrix} e_P \\ e_B \\ e_C \end{bmatrix}$$

$$\begin{bmatrix} U_{AB} \\ U_{BC} \\ U_{CA} \end{bmatrix} = \begin{bmatrix} R & -R & 0 \\ 0 & R & -R \\ -R & 0 & R \end{bmatrix} \begin{bmatrix} i_P \\ i_Q \\ i_R \end{bmatrix} + \begin{bmatrix} L-M & M-L & 0 \\ 0 & L-M & M-L \\ M-L & 0 & L-M \end{bmatrix} \frac{d}{dt} \begin{bmatrix} i_P \\ i_Q \\ i_R \end{bmatrix} + \begin{bmatrix} e_{P-} e_P \\ e_B - e_B \\ e_C - e_C \end{bmatrix} \text{------}\rightarrow 16$$

$P_e = e_P i_P + e_Q i_Q + e_R i_R$ ----------→17

$P_e = T_e \Omega$ ----------→18

(17) & (18)

$T_e = \frac{e_P i_P + e_Q i_Q + e_R i_R}{\Omega}$ ----------→19

(12) & (19)

$T_e = P\left[\varphi_m f_P(\theta) i_P + \varphi_m f_Q(\theta) i_{Q+} \varphi_m f_R(\theta) i_R\right]$----→20

Simplify (20)

$T_e = 2P\varphi_m i_P = K_T i$----------→21

$T_e = T_i = \frac{d\Omega}{dt} + B_V \Omega$----------→22

In a 6-step inverter, there are frequently only two switches, one on each good aspect belongs to the positive group, while the negative side belongs to the treated samples. The negative group energizes any 2 phases at a time while remaining neutral. The other phase switches have been turned off. A technique is utilized because a small capacitor is required rather than a large DC connection capacitor. In this case, a duty ratio is used to drive only one switch. For a specific value of output from a hall sensor, keep the other switch on. The following diagram depicts the switching sequence.

HALL SENSOR SIGNALS

At a 120° angle, three Hall Effect sensors are used. The differential equation for the BLDC motor is also defined by the following assumptions. Eddy current and hysteresis losses, as well as core saturation, are not considered. A trapezoidal wave with a flat top width of 120° electrical angle is used to illustrate the armature reaction and magnetic field dispersion in the air gap. Two of the three electrical windings are energized at a time in three-phase BLDC motors (any two phases). Three Hall Effect sensors are utilised as a consequence. A trapezoidal back-EMF waveform is the back-EMF waveform of a perfect BLDC motor. When a rectangular current waveform is applied to this type, the torque ripple is zero. Leading to absence of the magnetic material and limitations, the ideal trapezoidal wave is not obtained in practise.

SPIDER-BASED CONTROLLER

The spider produces an attain particular aims fiber from its mouth as part of its web-building operation. To imitate this action, the controller circuit is fed with rectified voltage and hall sensor output. The controller can be used in two different modes.

Mode 1

1. The controller only reads the value of the input voltage once every second in this mode..
2. The spider approaches the input voltage's zero-crossing point.
3. Spider explores for and travels to a new site.

4. Changing between old and new locations (by destroying previously produced silky like fiber). The SDC (controlling switch) is demonstrated in this activity.

Mode 2

1. A hall sensor output is obtained by the spider. As a result, it pauses and looks for an optimal position for the silk to also be connected.
2. After selecting an appropriate position, such as the switch that corresponds to the positive terminal of the input voltage, the silky fiber is fixed, indicating that the inverter switch is ON.
3. It then searches for a suitable location to connect the fiber.
4. And that's where the silky fiber is secured by the switch, which is connected to the negative pole of the input voltage and does not correspond to the phase previously specified.
5. For forward and backward movement between newly discovered and previously identified sites, the inverter switch must be controlled with a duty ratio 'D' as indicated by the switching time.

BLDC MOTOR WITH SPIDER BASED CONTROLLER SIMULATION CIRCUIT DIAGRAM FOR MATHEMATICAL MODELLING

ABC ALGORITHM

The bee behaviour is the natureof ABC algorithm which is based on find the best solution to limited situations. ABC algorithm's features.

(i) Small control parameters,
(ii) Quick computation time and convergence ratio
(iii) Bothexploration and exploitation, and
(iv) Good adaptability.

ABC ALGORITHM

Starting phase :$x_i^j = x_{min}^j$ + rand(0,1)$\left(x_{maxi}^j - x_{min}^j\right)$, j=1,2,…., D

Bees phase(employed) : $v_{ij} = x_{ij} + \emptyset_{ij}\left(x_{ij} - x_{kj}\right)$

Bees phase (Unemployed):$p_i = \frac{fit_i}{\sum_{i=1}^{SN} fit_i}$

CONCLUSION

This paper proposes the use of a multi-model MPC technique to control a low-cost BLDC motor. The multi model MPC, as intended, enables the drive to provide a superior motor kinetic than a single model approach. This is especially true when using a Hall Effect sensor to determine velocity. At low speeds, the controls on the modelling framework had a lot more problems dealing with the drive non-linearity. The multi-model strategy, on the other hand, provides for the use of well-known local talent as well as enhanced local control tunings. When opposed to a single-model approach, this flexibility is a significant benefit. A method for measuring the current rating in the controller, as well as a direct rotation speed, were also proposed. In addition to being sufficient for a good prediction of the current momentary peak, the proposed method demonstrates its conservatism. In future work, it could be improved. Comparing waveforms with and without the application of a fuzzy based control algorithm, including with and without a capacitor, demonstrates the effectiveness of the spider web construction method in generating the necessary switching control pulses. The control pulses are generated using a spider web-based control algorithm, which improves the system's speed stability. Despite the addition of a switch and a tiny capacitor to the design, the total cost of the drive is reduced. Likewise, the switch control approach is simple and does not require additional components.

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Table 1 Six-step Switches of inverter's phases A, B and C.

Sequence of Switches	Signals of Hall Sensor			ACTIVE mode	Control
	Ha	Hb	Hc		
1	1	0	0	A2	C1
2	1	1	0	C1	B2
3	0	1	0	B2	A1
4	0	1	1	A1	C2
5	0	0	1	C2	B1
6	1	0	1	B1	A2

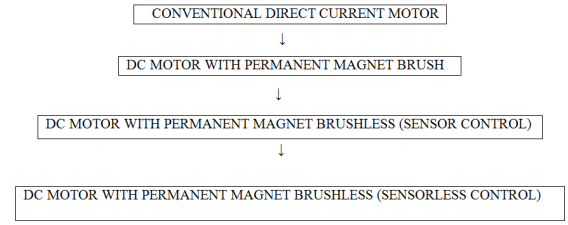

Fig.1 - BLDC Motor Progress Flow

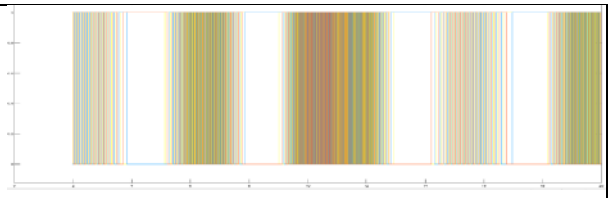

Fig.2 Hall Sensor Signals

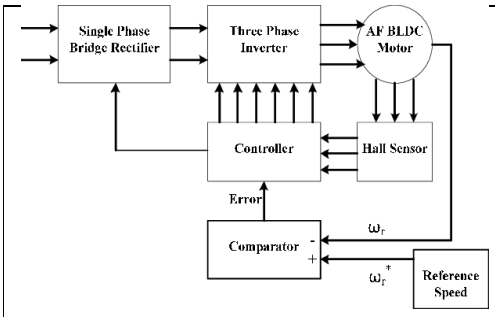

Fig.3 Block Diagram

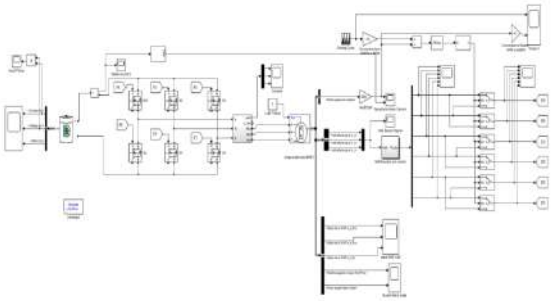

Fig.4 Simulink Model

Fig.5 BLDC motor back emf in 3 phases and phase current

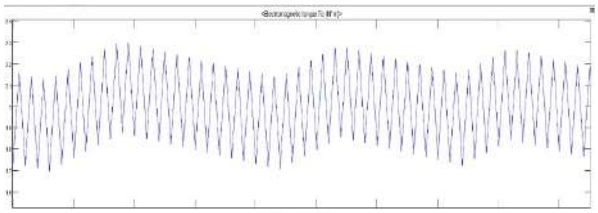

Fig.6 Electromagnetic torque

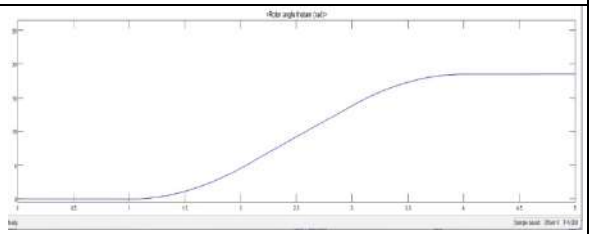

Fig.7 Rotor angle waveform

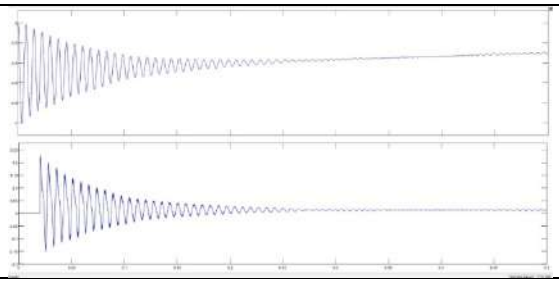

Fig.8 BLDC motor speed and mean current waveform with spider-based controller

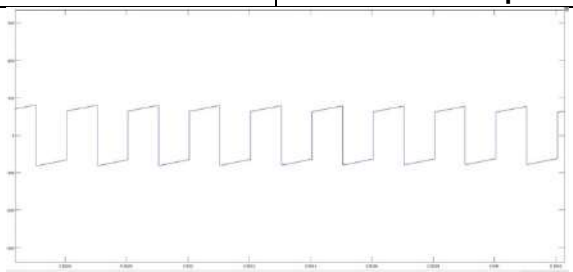

Fig.9 Spider-based controller -battery current

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

EEG based Classification of Posed and Spontaneous Emotion using Temporal Characteristics

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Received: 30 Dec 2021 Revised: 13 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

Spontaneous emotions are diverse from posed ones in appearance, timing, face and body movements, as well as in neural gestures. In this paper, we focus to provide novel system architecture for distinguishing paired emotions (Posed versus Spontaneous) using EEG signals. We attempt to analyze the performance of different classifiers for discrimination of paired emotions using sub_ band_ energy of EEG signals as a feature. Five performance parameters True Positive & Negative Rate (TPR & TNR), Positive & Negative Predictive value (PPV & NPV), Accuracy (ACC) and F-Score (FSC) are utilized to measure the effect of eight classification techniques Ensemble, k-Nearest Neighbor (k-NN), Linear Discriminant, Logistic Regression, Naïve Bayes, Neural Network, Support Vector Machine (SVM), and Tree. As a result Ensemble, SVM and Tree classifiers shows the effective outcome for classifying paired emotions.

Keywords: Spontaneous, Accuracy, energy, emotions.

INTRODUCTION

Human-human communication is governed by emotions; an innate attribute to every human. Spontaneous emotions diverge from posed ones in appearance, timing, head movement, body gestures, as well in neural signature. Most of the work is focused on distinguishing spontaneous and posed emotions using facial and speech modality [1][2]. Further, it is proven that the brain signals, being the source of emotional exhibits, serve as the most authoritative modality for emotion recognition [3]. Temporal dynamics of EEG signals such as; amplitude, total duration, trajectory and speed of onset and offset are highly relevant parameters for EEG based emotion recognition [4]. Rhythmic and temporal memory characteristic of EEG signals are used to recognize emotion based on Long–Short-Term Memory (LSTM) neural network. The performance of model evaluated against benchmark dataset DEAP achieving classification accuracy of 69.5% and 62.6% for valance and arousal respectively [5]. Spatial and temporal

characteristics of EEG signals associated with Event Related Potentials (ERPs) are used to classify brain activities. Temporal features are extracted using Global Field Power (GFP) and weighted SVM (sw-SVM) spatial filter is employed to optimize classification accuracy with respect to each temporal feature. The inclusion of temporal feature attributed to improved classification results and physiological understanding [6]. However, scope persists in analyzing temporal dynamics of posed and spontaneous emotions using EEG signals.

The latent developments in EEG signal analysis using emerging technology allow exploration of EEG signals in numerous fields. In this paper we propose the system architecture Temporal Characteristics based Classification of Posed and Spontaneous Emotion (*TC²PSE*) using *sub_band_energy* feature. The efficiency of the proposed *TC²PSE*is outlined using experimental results. Section 2 presents the proposed *TC²PSE* system with its architecture and systematic description of each component. In section 3 the experimental setup and execution specifications are described along with the performance parameters such as True Positive & Negative Rate (TPR & TNR), Positive & Negative Predictive value (PPV & NPV), Accuracy (ACC) and F-Score (FSC)to analyze the efficiency of classifiers. The performance of classification techniques is compared using aforesaid performance parameters using experimental results in Section 4. The comparison reveals the effectiveness of classification techniques for paired emotion discrimination. Finally, in section V we conclude with the future direction of the work.

System Architecture for Analyzing Variation in Paired Emotions

Neural patterns in time-frequency domain provide more objective measures for assessing emotional states of subjects. In this work, we intend to analyze the variation in brain signature and activation patterns of brain signals for paired neutral, happy and sad emotions. System architecture namely Temporal Characteristics based Classification of Posed and Spontaneous Emotion (*TC²PSE*) is proposed to classify and analyze variation in paired emotion (posed and spontaneous) and their activation patterns in time-frequency domain. The four components of system architecture namely; data acquisition, data preprocessing, feature extraction, and classification of paired emotions are as shown in Fig. 1 and described as follows:

Data Acquisition

EEG data acquisition has turn out to be an exigent task due to non-stationary nature of EEG signals and subjective dependence of emotions. The EEG signal acquisition from various brain locations (*bl*) for paired (posed and spontaneous) emotions is conceded in data acquisition phase. Spontaneous emotional experience persists for a changeable length of time depending on the subjects and the emotion-eliciting perspective. However, the length of posed emotional excerpt is normally set by the posing instructions. Identification of apposite subjects capable to articulate target emotion is a decisive part of effective posed emotion evocation. Similarly to elicit spontaneous emotions, selection of efficient and consistent emotion elicitation stimuli is vital. In posed emotion (directed emotional action task) dataset acquisition is carried out against deliberate elicitation by subjects. In spontaneous emotional EEG dataset acquisition subjects are exposed to stimuli (film clip viewing context) and signals from specific regions of the brain are captured using meticulous EEG signal acquisition device. The captured multichannel EEG signal is characterized as:

$$X(t) = [x^{C_1}(t),\ x^{C_2}(t), \dots, x^{C_{bl}}(t)] \in R^{bl \times N} \quad \dots \quad (1)$$

where $X(t)$is the multichannel raw EEG signal, *t* is the time indicator, *bl*, *N* are the number of *brain locations* and *sample points* respectively, and $x^{Ch_i}(t)$ is the EEG signal corresponding to *I* th channel and *R* is bl×*N* dimensional real space.

Preprocessing

The EEG signals are subject to filtering after data collection step. The raw EEG signals are contaminated with noise and require preprocessing to eliminate it and realize the relevant signals. Artifacts such as eye blinking or muscular movement may taint the data and deform the neural activity. Numerous preprocessing methods such as Butterworth filter, Notch filter, and Band-pass filters pre-treats EEG signal and remove noise and artifacts. The EEG signal may be

decomposed into numerous frequency bands : δ (0.5–4 Hz, generally appears when infants or adults are in a state of quietness, lethargy, fatigue, etc.), θ (4–8 Hz, generally appears when the person gradually becomes sleepy from the awake state, or the emotion gradually becomes calmer), α (8–13 Hz, generally appears when people are awake, relaxed, or closed eyes), β (14–30 Hz, generally appears when people are alert or focused), γ (> 30 Hz, generally appears in short-term memory process, multisensory information integration process, etc.) [7]. Frequency band extraction is performed on the preprocessed EEG signal to obtain a frequency band of interest:

$$Fb_k(t) = \left[{f_k}^{C\square_1}(t),\ {f_k}^{C\square_2}(t), \ldots, {f_k}^{C\square_{bl}}(t)\right] \in R^{bl\times N} \quad \ldots \quad (2)$$

Where*k* represents the emotion-related rhythm obtained from the filtering. In addition, blind source separation based on Independent Component Analysis (ICA) technique efficiently contribute in mining out noise free components of EEG signals and transform raw EEG signal into preprocessed signal. The components $Fb_i$of the observed random vector $Fb = (Fb_1, \ldots, Fb_m)t$ are generated as a sum of the independent componentsc_k, k = 1,..., N:

$$Fb_i = \left[{f_k}^{C\square_1}(t),\ {f_k}^{C\square_2}(t), \ldots, {f_k}^{C\square_{bl}}(t)\right] \in R^{bl\times N} \quad \ldots (3)$$

weighted by the mixing weights.

Feature Extraction

Temporal feature extraction pretends a central role in dragging out significant features from pretreated noise free EEG signals for further analysis. The extraction and usage of emotion specific channels based on specific time-frequency domain features add in efficient analysis of paired emotions. The temporal feature namely; sub band energy against preprocessed EEG signals is mined for analyzing variations in paired emotions. The sub band energy is square of time-amplitude values of the signals with respect to specific brain location. The Feature Vector FV is constructed based on the filtered dataset using the temporal feature extraction component of the *TC²PSE*. The sub-band energy parameter represents the signal intensity over a time sliced period, the variation of a time function as shown in Eq. (4):

$$Sub\_band\_energy = \left(\sum_{t=1}^{ts*sr}({f_k}^{C\square_{bl}}(t))^2\right)/ts * sr \quad \ldots \quad (4)$$

Where t is time in seconds, ts is time slice in seconds, sr is sampling rate, $k$is frequency rhythm, bl is brain location with respect to Ch channel.

Classification

Classification component is responsible for identifying the emotion class where new EEG signal resides. Here, *sub_band_energy* of EEG signals are the key inputs for predicting the equivalent emotion class for EEG signal. A trained classifier forms the association between predefined emotion classes and corresponding EEG features and categorizes new EEG instances in an unseen testing dataset. The classification component of *TC²PSE*architecture involves eight classifiers namely; Ensemble, k-Nearest Neighbor (k-NN), Linear Discriminant, Logistic Regression, Naïve Bayes, Neural Network, Support Vector Machine (SVM), and Tree for discriminating paired emotions in eight regions namely; frontal, parietal, temporal, combined, reference electrodes, left hemisphere, right hemisphere and whole brain region using EEG signals.

Ensembles are collection of predictive models highly useful when performance of predictive models is most important outcome. The contributing models are referred to as ensemble members, may be the same type or different types. The predictions made by the ensemble members may be combined using statistics, such as the mode or mean. An ensemble can make better predictions and achieve better performance than any single contributing model. An ensemble reduces the spread or dispersion of the predictions and model performance. Ensembles are used to achieve better predictive performance on a predictive modeling problem than a single predictive model [8].

k-Nearest Neighbor (k-NN) is one of the simplest Machine Learning algorithms based on Supervised Learning technique. It assumes the similarity between the new case/data and available cases and put the new case into the category that is most similar to the available categories.k-NN classifier depends on the emotional class labels of closest training patterns in the feature space where the nearest neighbors of a given feature vector are defined in terms of the standard Euclidean distance. An EEG signal is classified by a plurality vote of its neighbors such that EEG signal assigned to the emotion class which is the most common among its k nearest neighbors. The best choice of k depends upon the nature of the data to be classified [9].

Linear Discriminant Analysis (LDA)is a dimensionality reduction technique used as a preprocessing step in Machine Learning and pattern classification applications. The main goal of dimensionality reduction techniques is to reduce the dimensions by removing the redundant and dependent features by transforming the features from higher dimensional space to a space with lower dimensions. Some popular extensions include, Quadratic Discriminant Analysis (QDA), Flexible Discriminant Analysis (FDA),and Regularized Discriminant Analysis (RDA). LDA is a simple model in both preparation and application. The representation of LDA is straight forward.It consists of statistical properties of your data, calculated for each class [10].

Logistic regression (LR) is a statistical analysis method used to predict a data value based on prior observations of a data set. The approach allows an algorithm being used in a machine learning application to classify incoming data based on historical data. As more relevant data comes in, the algorithm should get better at predicting classifications within data sets. A LR model predicts a dependent data variable by analyzing the relationship between one or more existing independent variables. It is a process of modeling the probability of a discrete outcome given an input variable. The most common logistic regression models a binary outcome; something that can take two values such as true/false, yes/no, and so on. Logistic regression is a useful analysis method for classification problems, where we are trying to determine if a new sample fits best into a category [11].

Naïve Bayes' algorithm is a supervised learning algorithm, which is based on Bayes' theorem and used for solving classification problems. It is mainly used in text classification that includes a high-dimensional training dataset. Naive Bayes classifier is one of the simple and most effective classification algorithms which help in building the fast machine learning models that can make quick predictions. It is a probabilistic classifier, which means it predicts on the basis of the probability of an object. The Naïve Bayes' algorithm is comprised of two words Naïve and Bayes', can be described as; Naïve because it assumes that the occurrence of a certain feature is independent of the occurrence of other features andBayes, because it is contingent on the principle of Bayes' Theorem [12].

A neural network consists of units (neurons), arranged in layers, which convert an input vector into some output. Each unit takes an input, applies a (often nonlinear) function to it and then passes the output on to the next layer. Generally, the networks are defined to be feed-forward: a unit feeds its output to all the units on the next layer, but there is no feedback to the previous layer. Weightings are applied to the signals passing from one unit to another, and it is these weightings which are tuned in the training phase to adapt a neural network to the particular problem at hand. Neural nets take inspiration from the learning process occurring in human brains. They consist of an artificial network of functions, called parameters, which allows the computer to learn, and to fine tune itself, by analyzing new data [13].

SVM is a supervised machine learning algorithm for solving classification or regression problems that are linearly inseparable. The basic idea in SVM is to transform data using kernel trick to estimate a hyper plane in order to categorize EEG features from the predefined emotional classes in the most optimal way. The hyper plane is linear in nature and several solutions exist to categorize EEG data. SVM works on two approaches One-vs-One (OvO) and One-vs-All (OvA) to deal with the multiclass classification problems. It does complex data transformations, then figures out how to separate data based on the labels or outputs defined [14]. Tree-based classification models are a type of supervised machine learning algorithm that uses a series of conditional statements to partition training data into subsets. Each successive split adds some complexity to the model, which can be used to make predictions. Tree

is constructed by two entities; branches and nodes. Tree-based ML methods are built by recursively splitting a training sample, using different features from a dataset at each node that splits the data most effectively. The splitting is based on learning simple decision rules inferred from the training data. To predict a class label or value, it starts from the root and, using branches, go to the nodes by comparing features on the basis of which will provide the best split [15].

Experimental Study

This section outlines the hardware and software required for implementation of *TC²PSE*architecture. The *sub_band_energy* is extracted as feature from filtered EEG signal with respect to paired neutral, happy and sad emotions. Here, some recognition issues along with four performance considerations used for analyzing depiction of various EEG classification techniques are discussed in detail.

Experimental Setup and Execution

The Emotiv Epoc+ 14 channel high resolution, moveable, wireless EEG data capturing device is used to acquire raw EEG data for *TC²PSE*. The sampling rate of device is 128 / 256 samples per second coupled with single analog to digital converter (16 bit). The channel positioning is as per 10-20 international system [16]. The *TC²PSE* architecture is implemented using MATLAB 2021a software on a laptop with Intel(R) Core (TM) i5-4200U CPU @ 1.60GHz and Windows 10 operating system.

The native EEG signals are captured for paired*neutral,happy,* and*sad* emotions from 8 fine fettle subjects'in19-45 years of age group. The posed emotions are intentionally enacted by subjects based on past experience. The spontaneous emotions are evoked using emotion inducing movie clips. The neutral emotion is conjured usingserene scenes and light instrumental music whereas happy emotion is elicited using movie clips in subject's mother tongue (*Hindi*). The raw EEG signals are assimilated in European Data Format (.edf) and stored in paired native EEG datasets. The duration of recording is fixed as for60 second.The pretreatment phase deals with the filtering of paired raw EEG signals using band pass filters in order to preserve the frequencies amid 0-50Hz. The independent components are separated using ICA for eliminating the artifacts ascended due to eye blinking, eye movement and muscular movement. The P3 and P4 electrodes (Common Mode Sensitivity (CMS) / Driven Right Leg (DRL)) are set as reference electrode. The outcome of pre-treatment phase is binary .mat files that are generated corresponding to filtered EEG signal using EEGLAB toolbox.

The time sliced *sub_band_energy* is extracted as feature from preprocessed EEG signal. The time slice of 60 seconds is considered and hence *sub_band_energy* with respect to 60 second is computed for each subject. Feature vectors, *PNFV*, *SNFV* are constructed corresponding to paired neutral emotion, *PHFV*, *SHFV* and *PSFV*, *SSFV*for paired happy and paired sad emotions respectively. In case of neutral emotion, alpha rhythm corresponding to frontal, parietal, temporal, combined, reference electrodes, left hemisphere, right hemisphere and whole regions is considered for classification [17]. Similarly, beta and gamma rhythms with respect to aforementioned eight regionsare considered for happy and sad emotions [18]. For posed and spontaneous neutral emotion alpha rhythm corresponding to 14 channels, 8 subjects and three sets of paired emotions each of size [14*8*6] is computed using Eq. (4).The averaged values of feature vectors of paired neutral, happy and sad emotions ofall subjects for 14 channels are depicted in Table 1.Accordingly, the training dataset is formed based on calculated values of *Sub_Band_Energy* feature of all 8 subjects and 3 paired emotions.

Emotions are discriminated against above mentioned eight brain regions andfor an extensive evaluation of extracted feature, eight classifiers namely; Ensemble, k-Nearest Neighbor (k-NN), Linear Discriminant, Logistic Regression, Naïve Bayes, Neural Network, Support Vector Machine (SVM), and Treeare utilized. The confusion matrix generated by all three classifiers in each region is shown in Table 2 (for Neutral), Table 3, Table 4 (for Happy) and Table 5, Table 6 (for Sad). In all cases70% data is used as training set and 30% data is used as testing set.

Performance Parameters

The performance parameters such as, True Positive& Negative Rate (TPR& TNR), Positive & Negative Predictive value (PPV& NPV), Accuracy (ACC) and F-Score (FSC)are useful to determine accurateness of EEG classification. Above mentioned performance parameters provide effective measure to analyze the extent of inarticulateness/ disjointedness that subsists in different classification methods of EEG signal. TPR or Sensitivity exemplifies the ability of classifier for accurately classifying proportion of true positive samples and TNR or Specificity determines the ratio of number of accurate negative predictions to the total number of negatives and are stated as

$$TPR = \frac{TP}{TP+FN} \quad \ldots. \quad (5)$$

$$TNR = \frac{TN}{TN+FP} \quad \ldots. \quad (6)$$

PPV or precision and NPV yields the proportion of identified positive samples which are true positive and true negative [19] and are defined as

$$PPV = \frac{TP}{TP+FP} \quad \ldots. \quad (7)$$

$$NPV = \frac{TN}{TN+FN} \quad \ldots. \quad (8)$$

Accuracy represents the fraction of number of all accuracy predictions to the total number of the samples in the dataset and is defined as

$$ACC = \frac{(TP+TN)}{TP+TN+FP+FN} \quad \ldots. \quad (9)$$

Where True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN) specify accurate positive prediction, accurate negative prediction, inaccurate positive prediction and inaccurate negative prediction respectively and are computed using confusion matrix of classifiers.

F-score or F-measure is a measure of a test's accuracy. The F-score, also called the F1-score, is a measure of a model's accuracy on a dataset. It is used to evaluate binary classification systems, which classify examples into 'positive' or 'negative'.The F-score is a way of combining the precision and recall of the model, and it is defined as the harmonic mean of the model's precision and recall.

$$F_1\text{Score} = 2 * \frac{2\text{TP}}{2\text{TP}+\text{FP}+\text{FN}} \quad \ldots. \quad (10)$$

It is apparent that the best value for all the five measures is 1.0 whereas worst value is 0.0. Thus, classifier with a higher value for all four parameters is capable to recognize emotion more efficiently. The performance measures are estimated for each classifier and each emotion in all eight regions of brain using*sub_band_energy*. The computed values of these measures for each paired emotion in each region are depicted in Table 7 (a)-(b), Table 8 (a)-(b), Table 9 (a)-(b), Table 10 (a)-(b) and Table 11 (a)-(b) respectively.

EXPERIMENTAL RESULTS

The EEG signal classification based on *TC²PSE*architecture is carried out against eight different regions of the brain using eight classifiers namely, Ensemble, k-Nearest Neighbor (k-NN), Linear Discriminant, Logistic Regression, Naïve Bayes, Neural Network, Support Vector Machine (SVM), and Tree. The performance of these classifiers

considerably depends on EEG signal contents as well as features extracted. Four performance measures namely; True Positive & Negative Rate (TPR & TNR), Positive & Negative Predictive value (PPV & NPV), Accuracy (ACC) and F-Score (FSC) are applied for evaluating the effectiveness of classifiers depending on kind of paired emotion evoked. Numerous results are evaluated for three paired emotions namely Neutral, Happy and Sad in eight regions of brain as Frontal, Parietal, Temporal, Combined, Reference, Left Hemisphere, Right Hemisphere and Whole Region. In case of paired neutral emotion features are extracted using alpha band where as for paired happy and sad emotions features are extracted for beta and gamma bands as depicted in Table 7 (a)-(b), Table 8 (a)-(b), Table 9 (a)-(b), Table 10 (a)-(b) and Table 11 (a)-(b) respectively. The observations drawn from these regions are deliberated as follows:

Case A: Neutral Emotion (alpha band)

The Ensemble classifier exhibited the maximum TPR, PPV, accuracy, and F-score values as 1.00, 1.00, 1.00 and 1.00 respectively for posed and spontaneous *Neutral*e motions in all the regions of the brain. Similarly tree classifier shows the best performance in frontal, temporal parietal, combined and reference electrode regions as 1.00, 1.00, 1.00 and 1.00 respectively. From results it is evident that reference electrodes region is showing best performance (1.00, 1.00, 1.00 and 1.00) for all the classifiers except Logistic regression. Similarly, in case of parietal region all the classifiers are reflecting best performance except Linear Discriminant and Logistic Regression classifiers. The worst performance (TPR 0.38, ACC 0.56) is reflected by logistic regression classifier in case of combined regions for spontaneous neutral emotion.

CASE B: Happy Emotion (beta band)

Under this scenario, the best performance (1.0, 1.0, 1.0, and 1.0) is logged for ensemble and k-NN classifiers for combined and whole brain region for posed and spontaneous happy emotions whereas for left hemisphere region best performance (1.0, 1.0, 1.0, and 1.0) is shown for ensemble, k-NN, Naïve Bayes, Neural network and SVM classifiers for paired emotions. The worst performance (TPR 0.38) is shown for ensemble classifier in temporal region whereas in case of temporal and whole brain region it is recorded as (TPR 0.38) for linear discriminant and logistic regression classifiers. The average best performance (TPR) of eight regions is encountered as 0.90 and 0.97 for neural network and ensemble classifiers respectively whereas worst performance as 0.69 and 0.75 using logistic regression classifier for paired happy emotion.

CASE C: Happy Emotion (gamma band)

In this set-up, the best performance for posed happy emotion is observed in case of SVM classifier (averaged PPV 0.96) whereas it is recorded as (averaged PPV 0.93) in case of spontaneous happy emotion for all the regions combined. The worst performance is reported as (averaged PPV 0.74 & 0.77) using linear discriminant classifier for paired happy emotion. Further, FSC is logged as 0.92 using k-NN and SVM classifier. Under this scenario, the best performance (1.0, 1.0, 1.0, and 1.0) is noted for ensemble and k-NN classifiers for whole brain region for paired happy emotion whereas worst performance is shown as (0.5, 0.67, 0.63, and 0.57) for spontaneous happy emotion using logistic regression classifier.

CASE D: Sad Emotion (beta band)

In this case, the best performance (1.0, 1.0, 1.0, and 1.0) is witnessed for ensemble and neural network classifier for whole region and tree classifier for left hemisphere area. The worst performance (TPR 0.38) is shown for ensemble classifier in temporal region whereas in case of temporal and whole brain region it is recorded as (TPR 0.38) for linear discriminant and logistic regression classifiers. The average best performance (TPR) of eight regions is encountered as 0.90 and 0.97 for neural network and ensemble classifiers respectively whereas worst performance as 0.69 and 0.75 using logistic regression classifier for paired happy emotion.

CASE E: Sad Emotion (gamma band)

In this set-up, the best performance for posed happy emotion is observed in case of SVM classifier (averaged PPV 0.96) whereas it is recorded as (averaged PPV 0.93) in case of spontaneous happy emotion for all the regions

combined. The worst performance is reported as (averaged PPV 0.74 & 0.77) using linear discriminant classifier for paired happy emotion. Further, FSC is logged as 0.92 using k-NN and SVM classifier.

CONCLUSION

We have presented innovative system architecture namely *TC²PSE*using extracted features for EEG based paired emotion recognition. In particular, time-intensity approach for feature extraction has been used. Moreover, the performance of computed features has been evaluated against Ensemble, k-Nearest Neighbor (k-NN), Linear Discriminant, Logistic Regression, Naïve Bayes, Neural Network, Support Vector Machine (SVM), and Tree classifiers in eight different regions of the brain using performance parameters. Ensemble, k-NN and SVM classifier reports the best results for emotional classification in all regions of brain for extracted features. Further, we have observed that emotional activity of brain is spread all over the entire region of the brain. The presented architecture will provide a new way using features for solving the EEG based multiclass paired emotion recognition problems.

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Table 1 Averaged Values of *Sub_Band_Energy* for Paired Emotions

Channels	Neutral		Happy (Beta)		Happy (Gamma)		Sad (Beta)		Sad (Gamma)	
	P	S	P	S	P	S	P	S	P	S
AF3	40.29	430.96	29.14	60.95	11.79	30.45	42.25	17.54	23.40	7.87
F7	32.57	694.83	24.67	51.83	10.89	27.98	36.60	20.10	21.01	7.16
F3	83.13	509.32	28.09	65.75	14.41	49.99	40.11	18.26	23.98	6.00
FC5	20.72	286.43	13.20	68.44	7.00	35.46	41.24	16.09	22.97	5.46
T7	21.45	532.15	67.36	114.49	34.07	79.31	37.67	21.67	31.11	8.36
P7	18.85	354.25	20.57	74.78	15.06	67.00	31.00	12.94	19.12	6.55
O1	20.46	215.67	16.17	43.31	7.21	25.60	33.36	20.05	19.71	4.88
O2	65.86	360.07	39.38	57.03	11.80	36.11	48.29	17.60	25.12	8.36
P8	20.12	341.74	18.53	80.06	7.68	54.12	31.79	17.52	18.69	6.32
T8	34.57	411.72	71.10	124.13	22.27	69.40	40.12	28.55	31.05	9.94
FC6	30.22	432.56	30.79	83.20	17.46	55.23	35.66	17.15	24.91	9.95
F4	55.15	386.77	31.40	68.03	12.54	47.06	40.07	16.35	25.76	6.93
F8	65.69	423.89	33.07	65.34	15.45	50.86	45.94	27.09	22.30	8.85
AF4	44.86	509.93	33.83	81.31	11.72	32.61	51.25	21.05	26.24	5.57

Table 2 Confusion Matrix of Classifiers in Each Region for Paired Emotion (Neutral) using Alpha Band

Region	Emotion	Ensemble		kNN		Linear Discrim.		Logistic Regres.		Naïve Bayes		Neural Network		SVM		Tree	
		P	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
F	P	7	1	6	2	6	2	5	3	7	1	8	0	7	1	7	1
	S	0	8	0	8	1	7	0	8	0	8	0	8	0	8	1	7
P	P	8	0	8	0	8	0	5	3	7	1	7	1	8	0	7	1
	S	1	7	1	7	1	7	1	7	0	8	1	7	1	7	2	6
T	P	3	5	4	4	6	2	6	2	6	2	7	1	6	2	7	1
	S	1	7	2	6	5	3	4	4	2	6	3	5	2	6	1	7
C	P	8	0	8	0	5	3	5	3	6	2	7	1	7	1	7	1
	S	0	8	0	8	0	8	1	7	0	8	0	8	0	8	1	7
R	P	7	1	7	1	7	1	6	2	7	1	7	1	7	1	8	0
	S	0	8	1	7	0	8	1	7	0	8	0	8	0	8	1	7
LH	P	8	0	8	0	7	1	6	2	8	0	8	0	8	0	6	2
	S	0	8	0	8	2	6	2	6	0	8	0	8	0	8	1	7
RH	P	6	2	7	1	6	2	6	2	6	2	6	2	6	2	8	0
	S	0	8	0	8	1	7	2	6	1	7	0	8	0	8	1	7
WR	P	8	0	8	0	7	1	5	3	6	2	8	0	7	1	6	2
	S	0	8	0	8	1	7	5	3	0	8	0	8	0	8	1	7

Table 3 Confusion Matrix of Classifiers in Each Region for Paired Emotion (Happy) using Beta Band

Region	Emotion	Ensemble		kNN		Linear Discrim.		Logistic Regres.		Naïve Bayes		Neural Network		SVM		Tree	
		P	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
F	P	7	1	6	2	6	2	5	3	7	1	8	0	7	1	7	1
	S	0	8	0	8	1	7	0	8	0	8	0	8	0	8	1	7
P	P	8	0	8	0	8	0	5	3	7	1	7	1	8	0	7	1
	S	1	7	1	7	1	7	1	7	0	8	1	7	1	7	2	6
T	P	3	5	4	4	6	2	6	2	6	2	7	1	6	2	7	1
	S	1	7	2	6	5	3	4	4	2	6	3	5	2	6	1	7
C	P	8	0	8	0	5	3	5	3	6	2	7	1	7	1	7	1
	S	0	8	0	8	0	8	1	7	0	8	0	8	0	8	1	7
R	P	7	1	7	1	7	1	6	2	7	1	7	1	7	1	8	0
	S	0	8	1	7	0	8	1	7	0	8	0	8	0	8	1	7
LH	P	8	0	8	0	7	1	6	2	8	0	8	0	8	0	6	2
	S	0	8	0	8	2	6	2	6	0	8	0	8	0	8	1	7
RH	P	6	2	7	1	6	2	6	2	6	2	6	2	6	2	8	0
	S	0	8	0	8	1	7	2	6	1	7	0	8	0	8	1	7
WR	P	8	0	8	0	7	1	5	3	6	2	8	0	7	1	6	2
	S	0	8	0	8	1	7	5	3	0	8	0	8	0	8	1	7

Table 4 Confusion Matrix of Classifiers in Each Region for Paired Emotion (Happy) using Gamma Band

Region	Emotion	Ensemble		kNN		Linear Discrim.		Logistic Regres.		Naïve Bayes		Neural Network		SVM		Tree	
		P	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
F	P	7	1	7	1	6	2	7	1	6	2	7	1	7	1	7	1
	S	2	6	2	6	0	8	2	6	0	8	2	6	1	7	2	6
P	P	8	0	7	1	7	1	7	1	7	1	7	1	8	0	7	1
	S	1	7	1	7	1	7	1	7	1	7	1	7	0	8	1	7
T	P	7	1	8	0	7	1	6	2	6	2	5	3	7	1	5	3
	S	2	6	3	5	2	6	2	6	2	6	1	7	2	6	1	7
C	P	7	1	7	1	7	1	7	1	6	2	7	1	7	1	7	1
	S	0	8	0	8	3	5	2	6	0	8	0	8	0	8	1	7
R	P	7	1	7	1	7	1	7	1	7	1	7	1	7	1	8	0
	S	0	8	0	8	3	5	1	7	0	8	1	7	0	8	1	7
LH	P	7	1	7	1	6	2	6	2	6	2	7	1	7	1	6	2
	S	0	8	0	8	4	4	4	4	0	8	0	8	0	8	1	7
RH	P	8	0	8	0	5	3	6	2	5	3	7	1	6	2	8	0
	S	1	7	0	8	3	5	2	6	0	8	0	8	0	8	1	7
WR	P	8	0	8	0	5	3	5	3	7	1	7	1	7	1	7	1
	S	0	8	0	8	3	5	3	5	0	8	0	8	0	8	1	7

Table 5 Confusion Matrix of Classifiers in Each Region for Paired Emotion (Sad) using Beta Band

Region	Emotion	Ensemble		kNN		Linear Discrim.		Logistic Regres.		Naïve Bayes		Neural Network		SVM		Tree	
		P	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
F	P	6	2	5	3	5	3	6	2	5	3	7	1	5	3	7	1
	S	2	6	0	8	1	7	1	7	0	8	2	6	0	8	2	6
P	P	6	2	5	3	5	3	5	3	5	3	5	3	5	3	6	2
	S	2	6	1	7	1	7	1	7	1	7	2	6	0	8	1	7
T	P	5	3	6	2	7	1	3	5	5	3	6	2	4	4	3	5
	S	1	7	2	6	1	7	2	6	1	7	3	5	0	8	2	6
C	P	4	4	5	3	4	4	5	3	5	3	6	2	5	3	6	2
	S	1	7	0	8	2	6	2	6	2	6	1	7	0	8	3	5
R	P	6	2	7	1	6	2	7	1	5	3	7	1	5	3	7	1
	S	1	7	2	6	0	8	0	8	1	7	1	7	0	8	1	7
LH	P	6	2	5	3	5	3	4	4	5	3	6	2	5	3	7	1
	S	1	7	0	8	1	7	0	8	2	6	1	7	1	7	1	7
RH	P	7	1	7	1	4	4	5	3	8	0	7	1	7	1	8	0
	S	2	6	2	6	1	7	2	6	2	6	1	7	2	6	0	8
WR	P	8	0	7	1	2	6	2	6	7	1	8	0	8	0	7	1
	S	0	8	0	8	2	6	0	8	2	6	0	8	1	7	1	7

Table 6 Confusion Matrix of Classifiers in Each Region for Paired Emotion (Sad) using Gamma Band

Region	Emotion	Ensemble		kNN		Linear Discrim.		Logistic Regres.		Naïve Bayes		Neural Network		SVM		Tree	
		P	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
F	P	8	0	8	0	7	1	7	1	8	0	8	0	8	0	6	2
	S	0	8	0	8	0	8	0	8	0	8	0	8	0	8	1	7
P	P	6	2	6	2	7	1	7	1	7	1	7	1	7	1	6	2
	S	0	8	0	8	1	7	1	7	1	7	0	8	1	7	1	7
T	P	8	0	8	0	6	2	8	0	8	0	8	0	8	0	8	0
	S	0	8	0	8	0	8	1	7	1	7	0	8	0	8	0	8
C	P	8	0	8	0	4	4	3	5	8	0	8	0	8	0	7	1
	S	0	8	0	8	0	8	0	8	1	7	0	8	0	8	0	8
R	P	8	0	8	0	8	0	7	1	8	0	8	0	8	0	8	0
	S	0	8	0	8	1	7	0	8	1	7	0	8	0	8	1	7
LH	P	7	1	7	1	5	3	6	2	8	0	8	0	7	1	8	0
	S	0	8	0	8	1	7	1	7	0	8	0	8	0	8	1	7
RH	P	8	0	8	0	4	4	4	4	8	0	8	0	8	0	8	0
	S	0	8	0	8	2	6	2	6	2	6	0	8	0	8	1	7
WR	P	8	0	8	0	6	2	5	3	8	0	8	0	8	0	8	0
	S	0	8	0	8	0	8	4	4	1	7	0	8	0	8	1	7

Table 7(a): Performance Analysis of Classifiers in Each Region for Paired Neutral Emotion (Alpha Band)

Emotion	Region	Ensemble				k-NN				Linear Discriminant				Logistic Regression			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	0.75	1.00	0.88	0.86
	P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.88	0.89	1.00	0.80	0.88	0.89
	T	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	0.88	0.86	1.00	0.89	0.94	0.94
	C	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.75	0.55	0.56	0.63
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.88	0.89
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.73	0.81	0.84	1.00	0.73	0.81	0.84
	RH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	0.64	0.69	0.74
	WR	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	1.00	0.73	0.81	0.84	0.63	0.56	0.56	0.59
S	F	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	1.00	0.80	0.88	0.89
	P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	0.88	0.86	0.75	1.00	0.88	0.86
	T	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.88	0.89	0.88	1.00	0.94	0.93
	C	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.38	0.60	0.56	0.63
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	0.88	0.86
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	0.81	0.77	0.63	1.00	0.81	0.77
	RH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.50	0.80	0.69	0.62
	WR	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.63	1.00	0.81	0.77	0.50	0.57	0.56	0.53

Table 7(b): Performance Analysis of Classifiers in Each Region for Paired Neutral Emotion (Alpha Band)

Emotion	Region	Naïve Bayes				Neural Network				SVM				Tree			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.63	1.00	0.81	0.77	0.88	1.00	0.94	0.93	1.00	0.80	0.88	0.89	1.00	1.00	1.00	1.00
	P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	T	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	0.63	1.00	0.81	0.77	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	LH	0.75	1.00	0.88	0.86	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88
	RH	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94
	WR	0.88	1.00	0.94	0.93	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88
S	F	1.00	0.73	0.81	0.84	1.00	0.89	0.94	0.94	0.75	1.00	0.88	0.86	1.00	1.00	1.00	1.00
	P	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	T	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	1.00	0.73	0.81	0.84	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	LH	1.00	0.80	0.88	0.89	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88
	RH	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93
	WR	1.00	0.89	0.94	0.94	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88

Table 8(a): Performance Analysis of Classifiers in Each Region for Paired Happy Emotion (Beta Band)

Emotion	Region	Ensemble				k-NN				Linear Discriminant				Logistic Regression			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.88	1.00	0.94	0.93	0.75	1.00	0.88	0.86	0.75	0.86	0.81	0.80	0.63	1.00	0.81	0.77
	P	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.63	0.83	0.75	0.71
	T	0.38	0.75	0.63	0.50	0.50	0.67	0.63	0.57	0.75	0.55	0.56	0.63	0.75	0.60	0.63	0.67
	C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	0.81	0.77	0.63	0.83	0.75	0.71
	R	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	0.88	1.00	0.94	0.93	0.75	0.86	0.81	0.80
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.78	0.81	0.82	0.75	0.75	0.75	0.75
	RH	0.75	1.00	0.88	0.86	0.88	1.00	0.94	0.93	0.75	0.86	0.81	0.80	0.75	0.75	0.75	0.75
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88	0.63	0.50	0.50	0.56
S	F	1.00	0.89	0.94	0.94	1.00	0.80	0.88	0.89	0.88	0.78	0.81	0.82	1.00	0.73	0.81	0.84
	P	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.70	0.75	0.78
	T	0.88	0.58	0.63	0.70	0.75	0.60	0.63	0.67	0.38	0.60	0.56	0.46	0.50	0.67	0.63	0.57
	C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.73	0.81	0.84	0.88	0.70	0.75	0.78
	R	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	1.00	0.89	0.94	0.94	0.88	0.78	0.81	0.82
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.86	0.81	0.80	0.75	0.75	0.75	0.75
	RH	1.00	0.80	0.88	0.89	1.00	0.89	0.94	0.94	0.88	0.78	0.81	0.82	0.75	0.75	0.75	0.75
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88	0.38	0.50	0.50	0.43

Table 8(b): Performance Analysis of Classifiers in Each Region for Paired Happy Emotion (Beta Band)

Emotion	Region	Naïve Bayes				Neural Network				SVM				Tree			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88
	P	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	1.00	0.89	0.94	0.94	0.88	0.78	0.81	0.82
	T	0.75	0.75	0.75	0.75	0.88	0.70	0.75	0.78	0.75	0.75	0.75	0.75	0.88	0.88	0.88	0.88
	C	0.75	1.00	0.88	0.86	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88
	R	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	1.00	0.89	0.94	0.94
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.86	0.81	0.80
	RH	0.75	0.86	0.81	0.80	0.75	1.00	0.88	0.86	0.75	1.00	0.88	0.86	1.00	0.89	0.94	0.94
	WR	0.75	1.00	0.88	0.86	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.75	0.86	0.81	0.80
S	F	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88
	P	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	0.88	1.00	0.94	0.93	0.75	0.86	0.81	0.80
	T	0.75	0.75	0.75	0.75	0.63	0.83	0.75	0.71	0.75	0.75	0.75	0.75	0.88	0.88	0.88	0.88
	C	1.00	0.80	0.88	0.89	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88
	R	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	1.00	0.94	0.93
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.78	0.81	0.82
	RH	0.88	0.78	0.81	0.82	1.00	0.80	0.88	0.89	1.00	0.80	0.88	0.89	0.88	1.00	0.94	0.93
	WR	1.00	0.80	0.88	0.89	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	0.78	0.81	0.82

Table 9(a): Performance Analysis of Classifiers in Each Region for Paired Happy Emotion (Gamma Band)

Emotion	Region	Ensemble				k-NN				Linear Discriminant				Logistic Regression			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.88	0.78	0.81	0.82	0.88	0.78	0.81	0.82	0.75	1.00	0.88	0.86	0.88	0.78	0.81	0.82
	P	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
	T	0.88	0.78	0.81	0.82	1.00	0.73	0.81	0.84	0.88	0.78	0.81	0.82	0.75	0.75	0.75	0.75
	C	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.70	0.75	0.78	0.88	0.78	0.81	0.82
	R	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.70	0.75	0.78	0.88	0.88	0.88	0.88
	LH	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.75	0.60	0.63	0.67	0.75	0.60	0.63	0.67
	RH	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	0.63	0.63	0.63	0.63	0.75	0.75	0.75	0.75
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
S	F	0.75	0.86	0.81	0.80	0.75	0.86	0.81	0.80	1.00	0.80	0.88	0.89	0.75	0.86	0.81	0.80
	P	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
	T	0.75	0.86	0.81	0.80	0.63	1.00	0.81	0.77	0.75	0.86	0.81	0.80	0.75	0.75	0.75	0.75
	C	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.63	0.83	0.75	0.71	0.75	0.86	0.81	0.80
	R	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.63	0.83	0.75	0.71	0.88	0.88	0.88	0.88
	LH	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.50	0.67	0.63	0.57	0.50	0.67	0.63	0.57
	RH	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	0.63	0.63	0.63	0.63	0.75	0.75	0.75	0.75
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63

Table 9(b): Performance Analysis of Classifiers in Each Region for Paired Happy Emotion (Gamma Band)

Emotion	Region	Naïve Bayes				Neural Network				SVM				Tree			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.75	1.00	0.88	0.86	0.88	0.78	0.81	0.82	0.88	0.88	0.88	0.88	0.88	0.78	0.81	0.82
	P	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88
	T	0.75	0.75	0.75	0.75	0.63	0.83	0.75	0.71	0.88	0.78	0.81	0.82	0.63	0.83	0.75	0.71
	C	0.75	1.00	0.88	0.86	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88
	R	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	0.88	1.00	0.94	0.93	1.00	0.89	0.94	0.94
	LH	0.75	1.00	0.88	0.86	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.75	0.86	0.81	0.80
	RH	0.63	1.00	0.81	0.77	0.88	1.00	0.94	0.93	0.75	1.00	0.88	0.86	1.00	0.89	0.94	0.94
	WR	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88
S	F	1.00	0.80	0.88	0.89	0.75	0.86	0.81	0.80	0.88	0.88	0.88	0.88	0.75	0.86	0.81	0.80
	P	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	1.00	1.00	1.00	1.00	0.88	0.88	0.88	0.88
	T	0.75	0.75	0.75	0.75	0.88	0.70	0.75	0.78	0.75	0.86	0.81	0.80	0.88	0.70	0.75	0.78
	C	1.00	0.80	0.88	0.89	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88
	R	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	1.00	0.89	0.94	0.94	0.88	1.00	0.94	0.93
	LH	1.00	0.80	0.88	0.89	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.78	0.81	0.82
	RH	1.00	0.73	0.81	0.84	1.00	0.89	0.94	0.94	1.00	0.80	0.88	0.89	0.88	1.00	0.94	0.93
	WR	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88

Table 10(a): Performance Analysis of Classifiers in Each Region for Paired Sad Emotion (Beta Band)

Emotion	Region	Ensemble				k-NN				Linear Discriminant				Logistic Regression			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.75	0.75	0.75	0.75	0.63	1.00	0.81	0.77	0.63	0.83	0.75	0.71	0.75	0.86	0.81	0.80
	P	0.75	0.75	0.75	0.75	0.63	0.83	0.75	0.71	0.63	0.83	0.75	0.71	0.63	0.83	0.75	0.71
	T	0.63	0.83	0.75	0.71	0.75	0.75	0.75	0.75	0.88	0.88	0.88	0.88	0.38	0.60	0.56	0.46
	C	0.50	0.80	0.69	0.62	0.63	1.00	0.81	0.77	0.50	0.67	0.63	0.57	0.63	0.71	0.69	0.67
	R	0.75	0.86	0.81	0.80	0.88	0.78	0.81	0.82	0.75	1.00	0.88	0.86	0.88	1.00	0.94	0.93
	LH	0.75	0.86	0.81	0.80	0.63	1.00	0.81	0.77	0.63	0.83	0.75	0.71	0.50	1.00	0.75	0.67
	RH	0.88	0.78	0.81	0.82	0.88	0.78	0.81	0.82	0.50	0.80	0.69	0.62	0.63	0.71	0.69	0.67
	WR	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.25	0.50	0.50	0.33	0.25	1.00	0.63	0.40
S	F	0.75	0.75	0.75	0.75	1.00	0.73	0.81	0.84	0.88	0.70	0.75	0.78	0.88	0.78	0.81	0.82
	P	0.75	0.75	0.75	0.75	0.88	0.70	0.75	0.78	0.88	0.70	0.75	0.78	0.88	0.70	0.75	0.78
	T	0.88	0.70	0.75	0.78	0.75	0.75	0.75	0.75	0.88	0.88	0.88	0.88	0.75	0.55	0.56	0.63
	C	0.88	0.64	0.69	0.74	1.00	0.73	0.81	0.84	0.75	0.60	0.63	0.67	0.75	0.67	0.69	0.71
	R	0.88	0.78	0.81	0.82	0.75	0.86	0.81	0.80	1.00	0.80	0.88	0.89	1.00	0.89	0.94	0.94
	LH	0.88	0.78	0.81	0.82	1.00	0.73	0.81	0.84	0.88	0.70	0.75	0.78	1.00	0.67	0.75	0.80
	RH	0.75	0.86	0.81	0.80	0.75	0.86	0.81	0.80	0.88	0.64	0.69	0.74	0.75	0.67	0.69	0.71
	WR	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.75	0.50	0.50	0.60	1.00	0.57	0.63	0.73

Table 10(b): Performance Analysis of Classifiers in Each Region for Paired Sad Emotion (Beta Band)

Emotion	Region	Naïve Bayes				Neural Network				SVM				Tree			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	0.63	1.00	0.81	0.77	0.88	0.78	0.81	0.82	0.63	1.00	0.81	0.77	0.88	0.78	0.81	0.82
	P	0.63	0.83	0.75	0.71	0.63	0.71	0.69	0.67	0.63	1.00	0.81	0.77	0.75	0.86	0.81	0.80
	T	0.63	0.83	0.75	0.71	0.75	0.67	0.69	0.71	0.50	1.00	0.75	0.67	0.38	0.60	0.56	0.46
	C	0.63	0.71	0.69	0.67	0.75	0.86	0.81	0.80	0.63	1.00	0.81	0.77	0.75	0.67	0.69	0.71
	R	0.63	0.83	0.75	0.71	0.88	0.88	0.88	0.88	0.63	1.00	0.81	0.77	0.88	0.88	0.88	0.88
	LH	0.63	0.71	0.69	0.67	0.75	0.86	0.81	0.80	0.63	0.83	0.75	0.71	0.88	0.88	0.88	0.88
	RH	1.00	0.80	0.88	0.89	0.88	0.88	0.88	0.88	0.88	0.78	0.81	0.82	1.00	1.00	1.00	1.00
	WR	0.88	0.78	0.81	0.82	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88
S	F	1.00	0.73	0.81	0.84	0.75	0.86	0.81	0.80	1.00	0.73	0.81	0.84	0.75	0.86	0.81	0.80
	P	0.88	0.70	0.75	0.78	0.75	0.67	0.69	0.71	1.00	0.73	0.81	0.84	0.88	0.78	0.81	0.82
	T	0.88	0.70	0.75	0.78	0.63	0.71	0.69	0.67	1.00	0.67	0.75	0.80	0.75	0.55	0.56	0.63
	C	0.75	0.67	0.69	0.71	0.88	0.78	0.81	0.82	1.00	0.73	0.81	0.84	0.63	0.71	0.69	0.67
	R	0.88	0.70	0.75	0.78	0.88	0.88	0.88	0.88	1.00	0.73	0.81	0.84	0.88	0.88	0.88	0.88
	LH	0.75	0.67	0.69	0.71	0.88	0.78	0.81	0.82	0.88	0.70	0.75	0.78	0.88	0.88	0.88	0.88
	RH	0.75	1.00	0.88	0.86	0.88	0.88	0.88	0.88	0.75	0.86	0.81	0.80	1.00	1.00	1.00	1.00
	WR	0.75	0.86	0.81	0.80	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88

Table 11(a): Performance Analysis of Classifiers in Each Region for Paired Sad Emotion (Gamma Band)

Emotion	Region	Ensemble				k-NN				Linear Discriminant				Logistic Regression			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93
	P	0.75	1.00	0.88	0.86	0.75	1.00	0.88	0.86	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
	T	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	0.88	0.86	1.00	0.89	0.94	0.94
	C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	0.75	0.67	0.38	1.00	0.69	0.55
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	1.00	0.94	0.93
	LH	0.88	1.00	0.94	0.93	0.88	1.00	0.94	0.93	0.63	0.83	0.75	0.71	0.75	0.86	0.81	0.80
	RH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.67	0.63	0.57	0.50	0.67	0.63	0.57
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	0.88	0.86	0.63	0.56	0.56	0.59
S	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94
	P	1.00	0.80	0.88	0.89	1.00	0.80	0.88	0.89	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
	T	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.88	0.89	0.88	1.00	0.94	0.93
	C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.75	0.80	1.00	0.62	0.69	0.76
	R	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	1.00	0.89	0.94	0.94
	LH	1.00	0.89	0.94	0.94	1.00	0.89	0.94	0.94	0.88	0.70	0.75	0.78	0.88	0.78	0.81	0.82
	RH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.60	0.63	0.67	0.75	0.60	0.63	0.67
	WR	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.88	0.89	0.50	0.57	0.56	0.53

Table 11(b): Performance Analysis of Classifiers in Each Region for Paired Sad Emotion (Gamma Band)

Emotion	Region	Naïve Bayes				Neural Network				SVM				Tree			
		TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC	TPR	PPV	ACC	FSC
P	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.86	0.81	0.80
	P	0.88	0.88	0.88	0.88	0.88	1.00	0.94	0.93	0.88	0.88	0.88	0.88	0.75	0.86	0.81	0.80
	T	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93
	R	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93	1.00	0.89	0.94	0.94
	RH	1.00	0.80	0.88	0.89	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94
	WR	1.00	0.89	0.94	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94
S	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	0.78	0.81	0.82
	P	0.88	0.88	0.88	0.88	1.00	0.89	0.94	0.94	0.88	0.88	0.88	0.88	0.88	0.78	0.81	0.82
	T	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94
	R	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93
	LH	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	0.94	0.88	1.00	0.94	0.93
	RH	0.75	1.00	0.88	0.86	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93
	WR	0.88	1.00	0.94	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.94	0.93

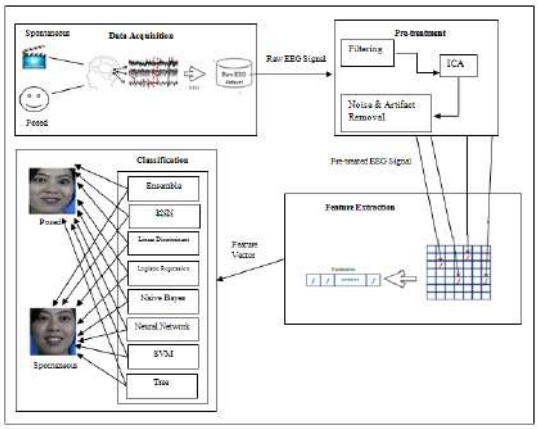

Figure 1: Temporal Characteristics based Classification of Posed and Spontaneous Emotion (*TC²PSE*)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of Virtual Reality Based Training (VRBT) and Proprioceptive Training for Improving Dynamic Balance in Chronic Ankle Instability (CAI) Patients - A Comparative Study

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Received: 20 Feb 2021 Revised: 13 Mar 2022 Accepted: 22 Mar 2022

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ABSTRACT

Chronic ankle instability (CAI) is define as, the tendency toward repeated ankle sprain and improper treatment, recurring symptoms like pain, weakness, giving way feel. CAI classified into mechanical ankle instability and functional ankle instability. Balance is most commonly affected in CAI due to loss of proprioception. To find out the effectiveness of virtual reality based training (VRBT) and proprioception training in CAI for improving dynamic balance. 30 CAI subjects were selected by talar tilt and anterior drawer test and 15 consist in Group A-VRBT and 15 in Group B-proprioception training for 10 weeks. Pre -Post-test measure by star excursion balance test (SEBT) and foot and ankle disability index and Sport modules (FADI-S). Group A and Group B had improvement in dynamic balance and functional activity. Group A shows significant difference from group B in all outcome measures. The independence sample t-test in anterior p-value .000 less than .05, in P-L p-value .017 less than .05, in P-M p-value .038 is less than .05, and in FADI-S p-value .000 less than .05. Proprioception improved in both group as well as feedback, reaction time also increase in VRBT group. It was concluded that virtual reality based training is more effective than proprioception training for improving dynamic balance in chronic ankle instability.

Keywords: CAI, VRBT, Proprioception Training, Dynamic Balance, SEBT, FADI-S

INTRODUCTION

Bipedalism is the main form in human locomotion where a human move by means of legs with hand swing [1]. An ankle-foot complex is one of the most important region of a human body in bipedal locomotion and it is extremely complicated structure including bone, multi-articular muscle, tendons, and other soft tissues such as walking,

running, jumping because it is unique one that physically interacts with the environment [2]. If any injury occurs in our lower extremities it effects our locomotion e.g. sprain, compartment syndrome, fracture, tendinopathy etc. During heel -strike to mid-stance phase of gait cycle more weight passes to ankle joint. So, that ankle injury is very common during this phase of gait cycle [3]. Ankle sprain is the common injury in lower extremities and mostly all person experiences it once in a life time even at activities of daily living, during sports, or deformitiesmay also play a role in the predisposition to the lateral ankle sprain [4,5,]. The usual mechanism of lateral ligament injury is inversion and plantarflexion of foot while the body center of gravity rolls over the ankle.[6] If the ankle ligament does not heal adequately or recurrent ankle sprain occur may develop instability due to loss of mechanoreceptors located in the joint [6,7]. The tendency toward repeated ankle sprain and improper treatment, recurring symptoms like pain, weakness, giving way feel has been termed as chronic ankle instability (CAI). The ankle will feel loose and unstable [8]. CAI classified into mechanical ankle instability(MAI) and functional ankle instability(FAI). MAI is characterized by pathological laxity of lateral ankle ligament. And FAI is characterized by impaired proprioception, neuromuscular control, postural control without ligamentous laxity [9]. When the ankle ligament is damage, there is loss of mechanoreceptor activity provide essential feedback to the brain and decreased muscle strength of invertor and evertor muscle [7]. This reduces the proprioception at the ankle joint and loss of balance [7,10]. Mechanoreceptors located in joint capsule, muscle tendon and skin. Studies have shown decreased proprioception abilities in patients with CAI [7].

The manifestation of functional instability include impairment in sensorimotor control, with has been previously quantified by the mean of static and dynamic balance task [4]. Talar tilit test and anterior drawer test are test for assessing the CAI [11]. CAI mostly seen in the individual with recurrent ankle sprain, and those does not take proper treatment. International figures report that ankle sprains with are basically weight-bearing injuries represent 15-20% of all sport injuries and remaining presentation to daily activities, accident [12]. An age of 10-19 years old is associated with higher rates of ankle sprain in male and female. Between 15-24year-old have higher rate of ankle sprain in male than female, whereas over thirty-year-old have higher rate in female than male [13]. Numerous treatment facilities available in CAI to improve balance. Proprioception is a neuromuscular process concerned with internal kinesthetic awareness of body position and movement [14,15]. Proprioception training challenges the ability of the targeted joint to detect and react to afferent input regarding joint position.

Virtual reality technology is developed on a computer hardware and software environment, to generate visual, audio and haptic feedback and obtain an interactive experience in the visual space, which strives to give users the impression of a real environment. It focuses on three characteristics-autonomy, interaction, and sense of being [16]. Star excursion balance test (SEBT) is a dynamic test to assess physical and functional performance of the lower extremities [17]. Foot and ankle disability index and sports modules (FADI-S) designed to evaluate the functional limitation in sports and activities of daily living related to foot and ankle conditions [18]. So, using SEBT and FADI-S can assess the patient's functional limitation and dynamic balance in CAI patients. If ankle get injured, proprioception affects and balance loss. So, it is necessary to retrain the dynamic balance and retain the normal ankle function. This study gives more emphasis for improving balance in CAI patients. My endeavor is to increase the dynamic balance and improve functional activities.

BACKGROUND FOR THE STUDY

The chronic ankle instability (CAI) occurs due to recurrent ankle sprain and without proper treatment. The CAI are very common in sports and also happen in activities of daily living at young age in both gender. Due to CAI, balance is also affect and its difficult in day to day life. For sports person due to CAI they will be unable to participated in the competitions.

In this study VRBT and proprioception training is use for CAI. Hence the need arises to evaluate which one is more estimate in chronic ankle instability.

METHODOLOGY

In this study materials are used X-box one console with kinetic 360(Microsoft), CD (Kinect adventures, your shape fitness evolved)exercise mat, ball, wobble board, minitrampoline, elastic strap, SEBT measurement tool (inch tape),FADI-S Scale and assessment form. This was an experimental study. The subjects from the city of Calicut, Kerala. Subjects diagnosed with chronic ankle instability by doing talar tilt test and anterior drawer test. 30 subjects were selected by convenient sampling technique. Study duration1 year**,** Treatment duration 10 weeks. Inclusion criteria are age 14-30years, both genders, diagnosed grade I and II ankle sprain, history of ankle sprain past 6 months to 1year. Any injury or surgery done in ankle joint, deformity at lower extremity, other musculoskeletal condition likefracture, osteoporosis, neurological condition like parkinsonism disease, ataxia, stroke, muscular dystrophy, myasthenia gravis, cardiopulmonary conditions- [American Heart Associated Classification Class C and D], according to Manual Muscle Test< 4/5 grade, color blindness, pregnancy, tumors in the area to be treated for the study were excluded. Star excursion balance test (SEBT) and Foot and Ankle disability index and sports modules (FADI-S)were used to measure dynamic balance in CAI patients. 30 subjects were assessed and who fulfilled the inclusion and exclusion criteria were selected from the population. And divided into 2 group, each group contain 15 subjects. Pretest value of SEBT and FADI-S of Group A and Group B were taken and compared each other. Group A were given VRBT and Group B were given proprioception training. Before treatment pre-test value and after 10-week training post-test value were taken.

PROCEDURE

SEBT has eight directions based on protocol three directions anterior, anteromedial and anterolateral directions were measure and also challenge to subject in balance, postural control, strength, range of motion and proprioceptive abilities.Tap is placed into three directions as y shaped. Each line is separated by a 45-degree angle. While maintain balance, person stance on one leg in center and using other leg to reach as far as possible in three directions. FADI-S is a questionnaire scale contain 136 total score given to the individuals for grade the score.
Some individual if complaing pain after treatment ice pack is given for 15 minutes.

Group A- Virtual Reality Based Traninig
Training periods: 2 times per week, 24min per session, 2repetation of 3min, 60s break of each repetitions

Games: Rally Ball, Reflex Ridge, River Rush, Light Race
Week 1-4 in basic fee play mode (9 levels). Week5-7in intermediate fee play mode (9 levels). Week 8-10in advanced fee play mode (9 levels). 2 repeats of 3 min Break 60s between repetitions for all games.The participant's movements were monitored by a motion detecting camera as they played XBX games.Participants had the opportunity to choose the order in which they would play the balance games, without allowing them to change their time engagement.

Group B - Proprioception Training
Training period: 2 times per week, and 24 min per session consisted of six stations, 2 repetitions of 45s, 60s break to next repetitions.
Exercise 1:Maintain the balance on single leg stance on exercise mat with the contralateral leg flexed. lower and raise the body. Distributed load on the foot.
Exercise 2:Jump from one leg to the other on an exercise mat and control landing for 4 seconds. Raise the contralateral leg.
Exercise 3: Maintain balance while walking on inclined surface
Exercise 4: Maintain balance in single-leg stance on wobble board. The contralateral leg is rested on an inclined surface nearly without being loaded.
Exercise 5: In mini trampoline patients have to jump and maintain the balance while landing down.
Exercise 6: Maintain balance in single-leg stance elevating the contralateral leg against resistance of an elastic strap.

The games and exercises varied each week starting with the easiest level and ending with the most difficult level.

TECHNIQUES OF DATA ANALYSIS AND INTERPRETATION

SPSS20.0 software was used. Kolmogorov-Smirnov test was done to find out the normality.Paired t test was used as parametric test to find out the intra group significance.Independent t-test were used to analyze inter-group significance.Mann Whitney test were used to analyze inter-group significance.

RESULT

Group A And Group B

Evaluation of SEBT (ANTRIOR), (POSTERIO LATERAL), (POSTERIO MEDIAL)

By comparing the mean value of pre test and post test values of SEBT 3 set, post value which is greater than the pre test value,(Table 1) which indicates that there is an increase in balance. Also by analyzing the pre test and post test values by paired t-test, the p-value is less than .05,(Table 1) which indicates that there is significant difference between pre test and post test values.

Evaluation of FADI-S

By comparing the mean value of pre test and post test values of FADI-S, post test value which is greater than the pre test value(Table 1)which indicates that there is an increase in function. Also by analysing the pre test and post test values by paired t-test, the p-value is less than .05, (Table 1) which indicates that there is significant difference between pre test and post test values of FADI-S.

When comparing the post test values of SEBT ANTERIOR, SEBT POSTERO LATERAL, SEBT POSTERO MEDIAL and FADI-S of both group A and group B (Figure1) through analysis of inter group significance; using independent sample t-test: SEBT ANTERIOR have the p-value .000 (α = .05)(Table 2), SEBT POSTERO LATERAL have the p-value .017 (α = .05)(Table 2), SEBT POSTERO MEDIAL have the p-value .038 (α = .05)(Table 2)and by using Mann Whitney testFADI-S have the p-value .000 (α = .05) (Table 2). This shows that group A shows significant difference from group B in all outcome measures. Hence, we can reject null hypothesis & accept the alternate hypothesis that, "there is significant difference in effectiveness of Virtual Reality Based Training(VRBT) and Proprioceptive Training For Improving Dynamic Balance In Chronic Ankle Instability (CAI) Patients".

DISSCUSSION

This study is to find out the effectiveness of virtual reality based training (VRBT) and proprioception training for improving balance in chronic ankle instability (CAI) patients. In 2014, Nikalos V has evaluated the effect of X-box Kinect intervention on balance ability for previously injured young competitive male athletes by using overall stability index and limits of stability**.** He concluded that XBK is improve balance ability in injured ankle [16]. Cailbhe Doherty has evaluated a study dynamic balance deficit in individuals with chronic ankle instability compared to ankle sprain copers one year after a first-time lateral ankle sprain injury. Has founded that individual with CAI have problem with balance, that ankle joint motions is reduced capacity of the stance limb [19]. Proprioception training affect the mobility and balance function by altering proprioceptive receptors input information and improves the control of musculoskeletal motor system and balance function [20,21,22]. Proprioception consist of both sensory and motor nerve that send and receive impulses to and from central nervous system from stimuli with in the muscle spindle, Golgi tendon [23]. These impulses transmit vital information in a given muscle and the relative position of a body part during a given movement [24,25].

In virtual reality main principal is neuroplasticity, mirror neuron, reaction time, and feedback. Virtual reality stabilizes and accurately control the movements which improve functional ability and balance [26,27]. Previous

studies also reported that motivation and active participation produces better functional results [28]. Gyeong H C [29] and Fathy A E [30] has done a study on VR training is effective for improving balance and also reduce risk of fall. Evidence suggest that individuals with CAI will have difficulty in balance. Using proprioception training for increasing balance recurrence of ankle sprain can be prevent. In this study, dynamic balance and functional activities is improvements in VRBT when compared to proprioception training. According using of outcome measure of SEBT and FADI-S. Therefore, the study is favoring the alternate hypothesis. Hence the study reveals that VRBT shows greater improvement than proprioception training on dynamic balance in CAI patients. Limitations of the study are sample size was small, Grade I and II CAI population is only taken, follow-up of VRBT and proprioception training was not done, dynamic balance and functional ability were only studies, the results in this study cannot be generalized to the whole ankle instability population, Lack of control group. Based on recommendations for future research the Comparison of VRBT and proprioception training can be done in a larger population, using another outcome measure for assessing dynamic balance, further studies can be done with either male or female only, Long term follows up study to analyses the long-lasting effect.

CONCLUSION

Both interventions showed good improvement in dynamic balance and functional activity over a period of ten weeks treatment. Based on outcome measure and statistical analysis virtual reality training has greater improvement in balance as compared to proprioception training. From this study, it concluded that virtual reality based training is effective for improving dynamic balance in chronic ankle instability as compared to proprioception training.

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Table 1: Intra GroupAnalysis of descriptive data for SEBT and FADI-S

Group	Measures	Test	Mean	Std. Deviation	Paired-t value	Degree of freedom	P value
Group A	SEBT (ANTERIOR)	PRE TEST	82.0867	10.86790	-18.126	14	0.000
		POST TEST	90.7267	10.01388			
	SEBT (POSTERO LATERAL)	PRE TEST	92.5600	10.70272	-10.087	14	0.000
		POST TEST	100.613	9.25881			
	SEBT	PRE TEST	89.9033	5.23486	-6.060	14	0.000

	(POSTERO MEDIAL)	POST TEST	94.2400	6.71191			
	FADI-S	PRE TEST	89.2333	2.99492	-7983	14	0.000
		POST TEST	95.0367	.91153			
Group B	SEBT (ANTERIOR)	PRE TEST	82.6067	10.64555	-15.888	14	0.000
		POST TEST	88.5067	9.99046			
	SEBT (POSTERO LATERAL)	PRE TEST	92.5933	11.02377	-18.126	14	0.000
		POST TEST	98.4400	9.46164			
	SEBT (POSTERO MEDIAL)	PRE TEST	89.3073	5.16861	-3.763	14	0.002
		POST TEST	92.3867	6.42716			
	FADI-S	PRE TEST	87.1267	1.75600	-11.954	14	0.000
		POST TEST	89.5067	1.52525			

Table 2: Inter-Group Siginifance for SEBT and FADI-S

SL. NO	MEASUREMENT	TEST	STASTICAL T/Z VALUE	P VALUE
1	SEBT- ANTERIOR	INDEPENDENT SAMPLE T-TEST	-4.535	.000
2	SEBT(POSTERO LATERAL)	INDEPENDENT SAMPLE T-TEST	-2.541	.017
3	SEBT (POSTERO-MEDIAL)	INDEPENDENT SAMPLE T-TEST	-2.182	.038
4	FADI-S	MANN-WHITNEY TEST	-3.991	.000

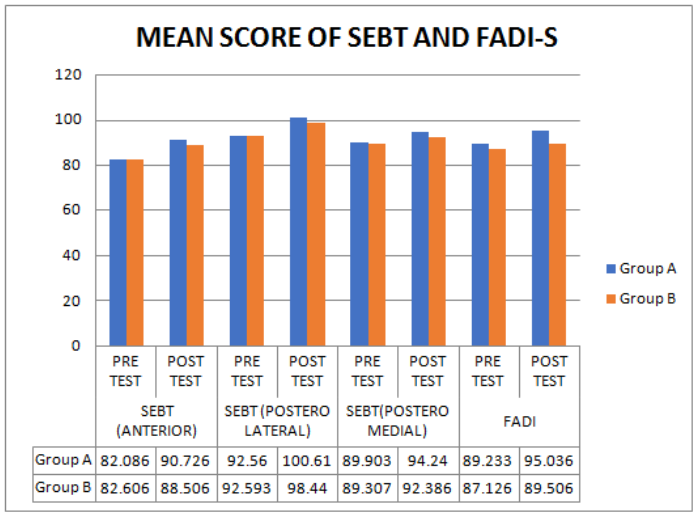

Figure 1: Mean score of SEBT and FADI-S

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Perceptions about Self: A Qualitative Study on Adolescent Girls

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Received: 28 Jan 2022 Revised: 19 Feb 2022 Accepted: 22 Mar 2022

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ABSTRACT

The current research aims to investigate the influence of media and celebrities on young girls of India. With the rapid modernization and influence from the west, there has been a change in how young girls perceive themselves in terms of their bodies and physical features. With the advent of size-zero trend and heavy influence from media in all walks of life – from purchasing commodities to defining beauty, there is a widespread dissatisfaction among young girls in terms of their bodies. The research, with the help of 15 face-to-face semi-structured interviews, reveals the impact of both media and celebrities on these young girls. While some girls understand that the influence is a negative one, more than half fall prey to media sensationalization. The study also explains how media and celebrities together construct the definitions of beauty for young girls who wish to adhere to it in order to be in the current trend and not feel out of place, especially in terms of having thin bodies. The findings also suggest an impact on self-worth and self-esteem of these young girls and how an anti-fat attitude has begun to develop and the perpetrators being media, mainstream Hindi cinema and celebrities. The findings are then discussed where plausible reasons of media influence and stability of identities is talked about.

Keywords: body Image, media influence, celebrity culture, social representations, social identity, thematic analysis, semi-structured interviews.

INTRODUCTION

The pervasiveness of media and the fact that it needs to attract attention all the time can have some disturbing consequences. One such consequence is a disturbed body image. Body image refers to a person's perceptions, thoughts and feelings about his or her own body(Fisher, 1990). Body image is thus a very subjective concept and easily influenced by many social factors, one of them being media. In the times where the media is so determined on focusing on beauty that all it has to offer is thin bodies and fair skins, the beauty myth does seem to have undergone

some serious stereotyping (Mayne, 2000). Global influence on Indian culture, especially from the west, has caused widespread dissatisfaction among young girls of India. A very new concept until a decade ago, the 'zero figure' is the latest trend that has hit India hard. Westernization of media and its aim to capitalize on the concerns of girls regarding their bodies has just reached to another level. Research has shown that this idea of thinness has been taken by Indian girls in an unhealthy manner recently, causing a strange obsessive tendency to be thin even if it calls for extreme steps like starvation (Talukdar, 2012; Becker, 2004). The perpetrator of this beauty myth of thin bodies is undoubtedly the local India media that is under heavy influence from the west. Constant modernization of dressing, emphasis on thin bodies as attractive, losing weight as a necessity for success in career- especially heroines of mainstream Hindi cinema that serve as role models for many young girls and social construction of the concept of femininity as being influenced by slenderness are just some causes of severe bodily dissatisfaction among young girls in India.

The entire scenario is like a vicious cycle. On one hand, media is promoting thin ideals through advertisements, local television programs, movies and other print and electronic media that are reinforced in the young minds. On the other hand, derogatory images of fat people are used in order to attach a stigma of worthlessness to them, reinforcing the thin ideal all the more (Himes and Thompson, 2007; Frouts and Burggraf, 1999). The thinness ideal is embedded so much deep down that issues like inability to reduce the post pregnancy weight for an actress also becomes a worth-to-be-discussed topic and a matter of serious speculation (Smitha, 2013). Girls have lost their sense of originality and self-comfort to the extent that they have begun to seek solace in maintaining a public image that is positive while their body size is constantly touching the negative. This indeed is a matter of serious concern. Dissatisfaction with one's own body creates negative attitudes about oneself creating a perception of self that is not very ideal. This also causes a marked decrease in the levels of self-esteem, confidence and self-worth in an individual (Wilcox and Laird, 2000). In Indian context this becomes all the more highlighted due to the pressure on girls to compete with international beauty idols. Since a female body is under constant scrutiny and is the point of focus in almost all social activities, it becomes very important to have a thin and desirable body structure. This cultural impact is mostly responsible for young girls being unhappy with their bodies (Shroff and Thompson, 2004). This vicious cycle thus operates at two levels- locally and internationally, where the influence remains the media and celebrities who create the thinness norm globally. The current research thus tries to investigate deeper into how the media and celebrities have impacted the self-perceptions of body of the young girls of India. It also tries to extract how media influences the ideas of beauty that surround our everyday lives and impact our most basic actions like buying preferences. This research tries to bring together various psychological concepts of social identity, personal identity, social representations, and identification processes vis-à-vis self-schema theory and social comparison theory in explaining why young girls of India are continuously dissatisfied with their physical appearance and bodies.

The main hypothesis of this investigation is that exposure to thin ideals in media have a negative impact on the way young girls (15-19 years) view themselves with respect to their bodies. The constant objectification of female body in media has led to a rat race of getting thin and even if a girl is thin as per the standards, she might not be satisfied with her body. In this process, what might be an issue of concern are misperceived norms of thinness that impact the personal and social identity of an individual in very wrong ways. Thus, on the whole, the dissertation tries to explore the influence of celebrities and other 'thin' role models on the self-worth and self-esteem of young girls anticipating a negative impact already. Moreover, it also tries to gauge the influence of media in decisions as small as buying preferences to issues as complex and important like construction of socially acceptable standards of beauty thereby seeking an understanding of social construction of definitions of beauty.

METHODOLOGY

The current research is a qualitative research conducted on young Indian girls in order to understand the impact of media and celebrity culture on their self-perception and body satisfaction. Essentially, qualitative research helps to

uncover a different perspective from the point of view of the social actor and new perspectives on the event can be gained by the researcher (Denizen and Lincoln, 1994). Qualitative methods are useful to bring the researcher and the respondent close to each other as each of them help the other understand the event better (Easterby-Smith et. al, 2002). In context of this research, a qualitative study was best suited because every girl had her own satisfactions and dissatisfaction and it was hard to quantify it on the basis of 'how much' or 'how less' (Gephart, 2004). It also required a comfort level between the researcher and the respondent due to the sensitivity of the topic. Lastly, a qualitative study provides a better understanding of the experiences lived by the respondent (Saunders et. al, 2003) as well places more regard on the authenticity of the experience (Silverman, 2006).

USING SEMI-STRUCTURED INTERVIEWS

The method of qualitative research used was semi-structured interviews. The advantage of a semi structured interview is two fold. Firstly, It helps in understanding and mapping the "respondents' life world" (Gaskell, 2000: 39) and secondly it refrains from suggesting a perspective and focuses on the perspective of the respondent (Patton, 1990). Also, a semi-structured interview is based on a flexible topic guide developed beforehand. This helps the researcher in gathering rich information by asking open-ended questions allowing the conversation take a flow (Silverman, 1997) as well maintaining a core structure that is crucial for the research to maintain its focus. Since the current research was completely based on the experiences of young girls, semi-structured interviews were chosen as the best-suited research design to tap the anxieties of these girls due to media pressures and celebrity influence.

INTERVIEW GUIDE

The entire interview with the respondent was based on a topic/interview guide. This guide was prepared under guidance of an academic supervisor in the beginning of the research process after having an ethical approval of the proposed research. The interview questions revolve around the experiences of young girls regarding their bodies when they are exposed to ultra-refined bodies on print and electronic media. The questions also try to understand 'are the notions of beauty and a thin body associated with each other'. The topic guide has been made keeping in mind the influence of mainstream Hindi cinema on girls and questions also seek to gauge impact of actresses on young girls. The interview guide revolves around body image issues mainly but tries to gauge a perspective on media commercialization and its impact on buying preferences and socially constructed ideas of beauty. This adds another dimension of influence of media specifically on young girls. Finally, the interview guide tries to understand how a girl sees herself after having seen the best on paper and on screen all the time.

RESEARCH SAMPLING

After considering the time available and other constraints like time required for transcribing and analyzing the data, the sample size was settled at 15 interviews. The type of sampling done was purposive sampling (Dane, 1990) as the researcher opted for young girls who could converse in English fluently and were aged between 16 to 21 years. Thus, in a way, the sample was also criterion based as the researcher included in her sample, young girls who met the above conditions.

RECRUITMENT OF PARTICIPANTS

For the current research, young Indian girls were selected who were studying in either senior school or undergraduate college. All of them well versed with English and were representative of a larger population of young girls who were experiencing body image issues due to influence from the media. The age profile was a bracket of 15 to 19 years and all these girls contributed immensely in understanding the self-perceptions and body images in the current light of media pressures and celebrity culture.

PROCEDURE

The interview was conducted on 15 Indian girls and the participants were approached on emails and phones. Facebook was also used as a medium to reach out to young girls and asked about their interest in contributing to the study. The participants who agreed to participate were then approached for the interview to be conducted at a pre-decided place and time. The venue was mostly on the interviewee's choice and the interview ranged between 30 to

50 minutes approximately. The interview flagged off with a rapport formation and a debriefing about the study provided by the interviewer. The participants were given the briefs on a sheet that included everything they needed to know about the interview and were asked to sign the consent form to mark their approval for the same. They were informed about the audio recording of the interview and the confidentiality of their identities and data. The participant was made aware that she had the right to skip any question or stop the interview anywhere if she felt uncomfortable. After obtaining the approval from the participant, the interview was conducted and recorded simultaneously. Once the interview was done, the recordings were transcribed and protected. To ensure confidentiality of data, the interview transcripts contained no names at all. They were numbered and only number references were used for analysis. Any names used or identifiable matter was anonymized. However, the basic details like age, gender, and level of education were included.

ANALYSIS OF DATA

The first step of data analysis was conversion of audio data into transcribed/written data. The method of analyzing data that was opted for the current qualitative study was that of thematic analysis. The Attride-Sterling model (2001) was used as the guiding analysis technique. The process included breakdown of the text into codes and creating a framework. This is followed by identification of the themes and redefining them. The next step is to construct thematic networks by arranging the themes into basic, organizing and global themes. This thematic network generation requires repetitive reading of the material (Fereday and Cochrane, 2006). The most important characteristic of thematic analysis is study of links and patterns that emerge in the data and are very relevant to the context of research (Braun and Clarke, 2006). The process of thematic analysis was applied to each interview transcript separately and coding was done. Manual coding was chosen due to two reasons, Firstly, manual coding facilitated critical engagement of the researcher with the data and secondly the data was manually manageable and did not require a software use. Since the interview guide was same for all the participants, the results obtained were relatively similar. The codes were then pooled in for all the data and combined in order to understand the emerging patterns within it. The basic themes (lowest order premises), organizing themes (clubbing of basic themes into more abstract themes) and global themes (all encompassing themes) were identified and explored to explain the entire data.

RESEARCH FINDINGS

The motive of the research was to find the impact of media and celebrity culture on the self-perceptions of young girls of India. After conducting a thematic analysis, four global themes emerged, namely, 1) Body image issues, 2) Impact on self-worth, 3) Media influence and 4) Social construction of beauty. Following are the research findings under the four global themes:

GLOBAL THEME 1- BODY IMAGE ISSUES

This theme tries to understand how young girls of India perceive themselves in terms of their body shape and size and how their perceptions lead to a sense of personal satisfaction or dissatisfaction among them. This theme shall provide insights into the daily struggles many young girls face in terms of their bodies and its representation that happen to be consistent across majority of the participants.

BODILY DISSATISFACTION

Research findings indicate that there is a widespread dissatisfaction in young girls of India regarding their bodies. They seem to be unhappy with the way they look and their body shapes and sizes and are dissatisfied or uncomfortable with it. One of the participant revealed, *"I don't think I'm a cent percent. I'll be honest I think I'm close to 70'ish. There are a certain things that make me ecstatic about how I look and there are some things that I just don't like."* Dissatisfaction was also found in girls who perceived their body as near perfect but were not hundred percent satisfied with it. Another one remarked, *"I perceive my body as fit. Neither skinny… nor fat but with a little stomach that will not be flat irrespective of how much I gym or exercise…..It doesn't go only and annoys me to death."*On the whole mostly all participants had some or the other dissatisfaction with the bodies and longed for *"attractive bodies"*

GAINING/LOSING WEIGHT

Analysis of the data indicates that a lot of girls experienced weight issues, which impacted their clothing preferences as well and weren't satisfied with their current bodies. Losing weight was associated with obtaining attractive bodies more than it was associated with health concerns. One of the participants states, "*A nice body is a prerequisite for wearing clothes you want to. Because of my height and plump abdomen, I avoid wearing short tees and other outfits like low waist jeans.*"Similarly, another one believed that "*My body really cannot pull off everything. I need to be very careful with what I choose to wear.*" A lot of young girls showcased that they received male attention after having lost weight. There were also some girls who reported events that led to weight gain and were not happy with that. The events were like accidents due to which physical mobility reduced, exams and stress that led to excessive eating etc. The weight gain, on a majority, seemed to impact clothes choices and overall satisfaction with bodies of these girls.

FIGHTING THE BODIES

Analysis revealed that the participants have tried to lose their weight by either modifying their eating patterns or by working out and exercising. They did agree a lot of times to have controlled their food intake but the work 'dieting' was always negatively associated and very few agreed to have 'dieted'.

Changing eating patterns

Research findings indicate that a lot of participants opted to change their eating patterns like controlling calories and avoiding junk food in order to maintain their respective weights and keep bodies in shape. A participant expressed her thoughts by saying, "*I make it a point to limit my indulgences in sweets….I avoid junk food like burgers and patties and the oily stuff….We just need to control our calorie-loaded-food intake to keep our weight in check, and make exercising an essential part of our lifestyle."*Participants also revealed their ideas on dieting as method to reduce weight and keep bodies in shape. While some explicitly agreed to have opted dieting "*at various stages of her life like before entering college, birthdays and weddings*", mostly denied indulgence in dieting and considered it as an unhealthy practice. Another participant revealed, "*I am perpetually on a diet I guess. But my mom obviously makes sure I take in the required nutrition."*Thus, the eating patterns were generally changed or mostly compromised to keep the weight in check and body in shape. Favorite items of food like chocolates and sweets took a back seat.

Working out and exercising

Participants showed a high regard to exercising and working out as a tool to stay fit and keep body in shape. Almost all the participants believed that exercising was the only way to maintain health and to lose weight. Gym was seen as a very popular means to work out followed by Yoga/power Yoga.One of the participant expressed, "*It is necessary to exercise to keep a fit mind and body, but there is no doubt that it does take a lot of time and energy, as they say no pain, no gain."*Exercising was thus a universally acceptable way of losing weight and keeping oneself fit.

GLOBAL THEME 2- SELF WORTH

Across all the participants, one common factor that emerged was impact on self-worth due to body shape and size. While thin girls or satisfied girls seemed to be more confident of themselves and higher on self esteem, the dissatisfied or the obese ones had inhibitions, felt out of place and got relatively more upset on comments about body. There was a clear and a direct interrelation between physique and percepts of self-esteem barring a few exceptions.

A SATISFIED BODY IMAGE AND SENSE OF SELF

A more confident sense of self that is secured despite criticisms is witnessed in girls who believe they are thin and beautiful. A participant remarked, "*A sleek figure always gets me compliments (Laughs). I am already fair and a perfect body with it just adds to my beauty altogether. I really like wearing nice clothes and flaunting my figure"* despite being critiqued for being too skinny. However, there are girls, who despite having thin bodies have developed an unhealthy personal identity. Thin and satisfied girls took criticism in their stride and were motivated by the comments and did not upset themselves much. A positive sense of self was witnessed.

A DISSATISFIED BODY IMAGE AND SENSE OF SELF

Findings indicate that girls who are dissatisfied with their high weight and body shape are usually more hesitant and low on confidence. One of the participants shared her story and said, "*It used to pinch me inside to be fat. I felt cursed...There have been days when I thought of killing myself. I didn't speak up in gatherings and I hated to go out and have fun. It was the worst phase of my life."* Due to their body shape, these girls succumbed to getting hurt by criticism. The girls who claimed they were fat and dissatisfied were the ones to use comparisons with friends more, sometimes felt "*out of place*" and "*were hesitant to open up*". Some of the dissatisfied participants used a defense mechanism to maintain a positive self-identity against all odds.

GLOBAL THEME 3- MEDIA AND CELEBRITY INFLUENCE

The current research reveals awareness among all the participants regarding the thin images media promotes and the influence it has in daily decision making processes. While some have reported being healthily influenced by the TV, advertisements and other electronic and print media, some have retained their personal preferences and choices over the current trends. A negative impact on body image was seen in some participants after the size zero trend while some participants completely abhorred the idea of getting thin to levels of malnourishment.

MEDIA AND CELEBRITY INFLUENCE ON DECISION MAKING

Mostly all participants have agreed to be influenced by what media showcases and considers media as a tool for spreading awareness. A participant commented*, "Whatever ads I see on the TV and whatever stuff they promote becomes my buying list ultimately."* Another one revealed*, "I think advertisements just play the role of creating brand awareness. So, of course when I buy anything then that brand comes into my mind as a choice."* However, participants do believe at the same time that celebrity endorsements cannot always be trusted as the celebrities are being paid to advertise the products. Issues like personal preference, personal suitability etc. also come on the surface.Thus, based on the research, social media is a means to provide options and alternatives through advertisement that impacts the choices an individual makes. However, mostly all participants felt that the celebrities are just used as a promotion tool and their appeal cannot be trusted just based on what media promotes it as.

MEDIA AND CELEBRITY INFLUENCE ON BODY IMAGE

Results indicate an impact of media and celebrities on the body image of the participants. While a lot of participants secretly wish that they have an actress-like body, some participants have discarded the idea of acute thinness as just a publicity stunt and unhealthy. Majority of them recognize that in past five years, media has just promoted shorter clothes and thinner bodies which influences them in one way or the other. A participant voiced, *" It has made people think that being thin and having zero size is desirable. I felt that way once…Then my mom treated me with two tight slaps… It emphasizes on the importance of being thin to look good....and the world does not work like that."* However, another one felt, *"And size zero changed the entire industry, don't you think? All actresses got thin and everyone wanted to be thin and good-looking…. I remember it was around four years back when all we girls used to talk was about Kareena Kapoor…."* Thus, the research revealed that media was not only promoting thin body ideals, especially mainstream Hindi Cinema, many were being influenced by this fad and saw their bodies in comparison to these role-models and other girls. This affirmed the concepts of the Self-schema theory and Social-comparison theory discussed earlier.

GLOBAL THEME 4- SOCIAL CONSTRUCTION OF BEAUTY

The research highlights acceptance of one common idea in mostly all participants and that is of a standard of viewing thin as beautiful. Many young interviewees explicitly stated that they regarded thin body as an ideal body because it is good to look at or is attractive. Moreover, majority articulated as fat not being trendy and cool. There was evidence of existence of a pervasive idea where fat bodies are to be ridiculed and thin is socially desirable. Lastly, majority of young girls also agreed that body is a determinant of external beauty but internal beauty is also of equal importance.

DEFINITIONS OF BEAUTY

Most interviewees across the range of 16 to 21 years believed in two definitions of beauty. While body shape and size was a determinant of external beauty, inner beauty was marked by goodness of a person, warmth he/she exuded, personality etc. Research findings show a process of socialization where 'thin is beautiful' is the standard norm while considering the behavioural traits of a person as well. A participant believed, *"body shape and size is an important aspect for a girl's physical appearance, but it can never over-ride her internal gentle heart and character's beauty. I don't know, beauty is a very subjective concept."*On the contrary, another participant defined beauty as "*Beautiful doesn't only describes outer beauty, it also reflects how a person is from within. If a beautiful girl has an ugly heart, she will be seen as an ugly girl, no matter how beautiful she is in reality."* However, the majority lied with body as being a very important determinant of beauty. Beauty was mostly defined in terms of body and physical features while some also took into account the inner beauty or the beauty of the person from within.

DISCUSSION

In the context of the current research, body weight and physical appearance plays a pivotal role. It plays a rather influential role in determining self-worth and self-esteem levels of young girls and has derived its influencing power from the media and celebrities. The research findings form a cycle whereby media is the proprietary source of body image issues in young girls. This in turn impacts the self-worth, or so to say, self-perceptions of many young girls. Thus, the global themes are all connected to each other. The age range chosen in the research is from 16 to 21 years that is of crucial importance. It is an important phase in the process of identity development as it marks the beginning of all future decisions an individual is going to take and the course of action he/she would follow (Phinney, 2000; Erickson, 1968). The criticism in the research interviews against 'size zero' and 'celebrity endorsements' does not mean that there is no influence of them on these young girls. For example, participants realize that media is portraying wrong images of girls and is promoting the thin ideal, yet they nominated an actress who was thin and petite as a person who they can call beautiful. On being asked why, reasons were simple; the actress either had a great body or a great personality that came from a great body.

Media is like an invisible blanket that has enveloped the process of identity development of young girls. These girls, obviously unaware of the media influence on them, go on changing their perceptions about themselves and their bodies with respect to the benchmarks that have been set. This process in these young girls can be explained with the help of following plausible arguments. Firstly, there is a lot of social pressure on a female body. In Indian context, it is even more profound as the body shape and size is not only a determinant of beauty but also fertility. It is an indicator of how much the girl is in demand in terms of marriage (Austin, 2012). However, with the current westernization, girls are trying to balance out their work life as well as their potential married life and body shape and size is a very crucial factor in both the areas of concern. A second possible reason could be, with the process of westernization so rampant, Hindi Cinema, along with television, magazines, newspapers and other media have changed their depiction of girls. The participants in the research realized that over the past five years the clothes have become shorter on screen and girls have become thinner in all walks of life. With media as the most influential weapon, it is impossible for young girls to remain averse to the ideals of thinness and western values of beauty. Thirdly, when same-sex ideals are exposed to young girls again and again, they are bound to model their behavior by either observing them or imitating them. This explanation echoes with Bandura's social learning theory (Bandura, 1977). The theory outlines three factors that are important for learning to take place through modeling. 1) The person being modeled must be of a higher status, 2) The person learning from modeling must lack power, status and skill and 3) The more important certain behavior is, higher are the chances that it will be modeled (Feist and Feist, 2006). Why modeling and observation are potential reasons for body image issues is because girls on media are passing across a message of thin body type as ideal (Harrison and Cantor, 1997) which is fostering the socially constructed notion of 'thin is beautiful' to even greater degrees. This causes young girls to personally identify with the same-sex celebrities and with friends who follow the thinness norm religiously. As a result, girls start viewing themselves from the eyes of the other and try to portray themselves according to the socially acceptable norms. This personal

identification and self-portrayal is a cause of media influence and the effect of which is a disturbed body image and direct impact on self-worth of young girls. There is an ardent need to develop a different and a new aesthetic sense among men and girls whereby fat and thin does not determine the beauty and a girl can enjoy her irrespective of what size she is. Body shape and size must not be a matter of concern unless it has an impact on health. Secondly, the social focus given to a girl's body needs to be redefined. Till the time a girl's body is treated as an object of desire whereby the desire is heightened by a thin and toned body, there will be undue emphasis on body shape and size with severe consequences.

CONCLUSION

The study effectively demonstrates the negative impact of media and celebrities on young girls of India. It shows that in order to maintain a positive identity and meet the pressure of changing representations of a girl's body in mainstream media, a young girl can face several debilitating consequences, negative perceptions of self being one of them. In a country like India where the trend of thinness was started by an actress, a huge impact of media and celebrities gets highlighted which provokes young girls to be thin in order to look beautiful and receive male attention. Thus, the research draws attention towards a very sensitive and all-pervasive issue of body image in Indian context.

RECOMMENDATIONS FOR FURTHER RESEARCH

There is a need for further investigation to understand the role of peers and family and their unconscious impact on self-perceptions of young girls. And calls for a study of both rural and urban populations in order to understand the impact of media and celebrities onthem. It can be very informative if the study is integrated with personality studies that try to find out the traits of girls who were able to cope with media pressures and maintained healthy self-perceptions.

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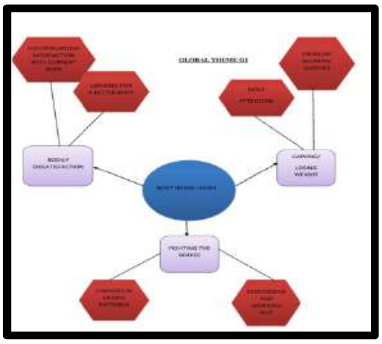

Fig. 1 Global Theme 2- Self Worth

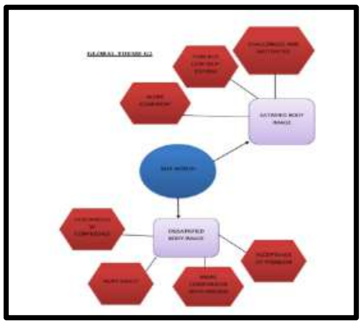

Fig. 2 A Dissatisfied Body Image and Sense of Self

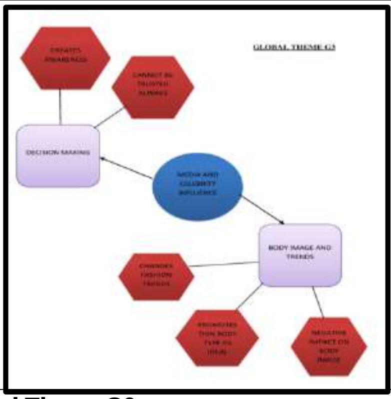

Fig. 3 Global Theme G3

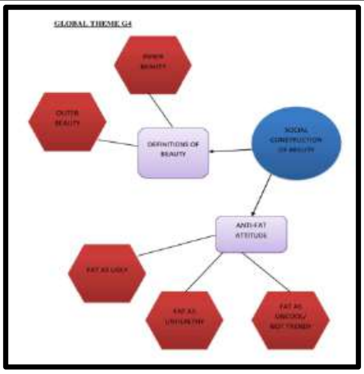

Fig. 4 Definitions of Beauty

Fig. 5 Appendix A- Coding Framework

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Comparative Study to Assess the Health Status among Smokers and Nonsmokers at Selected the Community Area at Karaikal

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Received: 01 Feb 2022 Revised: 26 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

A comparative study to assess the health status among smokers and nonsmokers at selected the community area at karaikal. To assess the health status among the smokers, 2. To assess the health status among the nonsmokers, 3.To compare the health status among smokers and nonsmokers and 4. To associate the health status smokers and nonsmokers with selected demographic variables. The aim of the study of to compare the health status among smokers and nonsmokers above in community area. Anon-experimental descriptive design, simple random sampling techniques was used, data collection was structured questionnaire method in selected areas at keezhakasakudi, karaikal. Revealed that smokers 14 (93.3%), 1 (6.7) had moderate health status. Non smokers that among 15(100) of smokers all had moderate health status. Compare the health status of smoker and non smokers 14 (93.3%) had poor health status and non smoker 15(100) had moderate health status. There is no association between the health status among smokers and non smokers with age, sex, occupation, physical changes and disease condition. The comparison of health status among smokers and non smokers using questionnaire method in selected community area karaikal. This study revelaed that identification of health status among smokers and non smokers.

Keywords: Health, Smokers, Non-smokers,

INTRODUCTION

"Smoking is injurious to health" Smoking, the act of inhaling and exhaling the fumes of burning plant material. A variety of plant materials are smoked, including marijuana and hashish, but the act is most commonly associated

with tobacco as smoked in a cigarette, cigar, or pipe. Tobacco contains nicotine, an alkaloid that is addictive and can have both stimulating and tranquilizing psychoactive effects. Smoking soon spread to other areas and today is widely practiced around the world despite medical, social, and religious arguments against it. Smoking is a practice in which a substance is burned and the resulting smoke is breathed in to be tasted and absorbed into the bloodstream. Most commonly, the substance used is the dried leaves of the tobacco plant, which have been rolled into a small rectangle of rolling paper to create a small, round cylinder called a "cigarette". Smoking is primarily practised as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the bloodstream and reach bodily tissue. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gases and include the pharmacologically active alkaloid nicotine; the vaporization creates heated aerosol and gas into a form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs. In some cultures, smoking is also carried out as a part of various rituals, where participants use it to help induce trance-like states that, they believe, can lead them to spiritual enlightenment.

Smoking is one of the most common forms of recreational drug use. Tobacco smoking is the most popular form, being practised by over one billion people globally, of whom the majority are in the developing countries.[1] Less common drugs for smoking include cannabis and opium. Some of the substances are classified as hard narcotics, like heroin, but the use of these is very limited as they are usually not commercially available. Cigarettes are primarily industrially manufactured but also can be hand-rolled from loose tobacco and rolling paper. Other smoking implements include pipes, cigars, bidis, hookahs, and bongs. Smoking is the inhalation of the smoke of burning tobacco encased pipes and cigars. Casual smoking is the act of smoking only occasionally, usually is a social situation or to relieve stress. A smoking habit is a physical addiction to tobacco products. May health experts now regards habitual smoking as a psychological addiction, too, and one with serious health sequence.

A smoker is a person who at the time of the survey, smokes any tobacco products either daily or occasionally. (i.e) smokers can be either daily occasional smokers. A daily smokers is a person who smokes any tobacco products at lead once a day. An occasionally smoker is a person, who smokes but not every day occasion a smokers can be reduce, continuing occasional smokers or experiments. Non smokers is a person, who at the time of the survey, does not smoke at all. Non smoker can be ex smoker, never smokers or occasional smokers. Ex smoker is a person, who was formerly a daily smoker but currently does not smoke at all. A never smoker is a person wo either has never smoked at all or has never smoked at all or has never been a daily smoker and has smoked less than 100 cigarettes in life time An ex-occasional smoker is person who was formerly an occasional, but never a daily smoker and who has smoked 100 or more cigarettes in her life. Cigarettes certain about 600 ingredients when they burn, hey generate more than 7000 chemical, according to the American lung association. Many of these chemicals are poisonous and at least 69 of them can causes cancer. Many of the same ingredients are found in cigars and tobacco used in pipes and hooakes. Smoking mainly of cigarettes causes cancer of the lung upper respiratory tract, esophagus bladder and pancreas and probably of the stomach, liver and kidney smoking is linked in leukemia and may also causes cancer of the colon and rectum other organs.

Statement of the problem

A comparative study to assess the health status among smokers and nonsmokers at selected the community area at karaikal.

Objectives

- To assess the health status among the smokers.
- To assess the health status among the nonsmokers.
- To compare the health status among smokers and nonsmokers.
- To associate the health status smokers and nonsmokers with selected demographic variables.

MATERIAL AND METHODS

Research approach
Research approach used for their study was quantitative approach.

Research design
Research design is a blue print for conducting the study that maximizes control over factors that could interfere with validity of the finding.

Setting
The study was conducted at keezhakasakudi

Population
Population in all elements (individual objective objects on substances) that meet certain criteria in for in a study. The population is a well defined set that has certain specified properties. The population was this study was adult.

Sample size
A subset of population selected to participate on a study In their study size was 150.

Sample technique
Sampling is the process of selecting representative units of population for the study in a research. In this study convenient sampling technique was used.

Sample criteria
Sample where selected with the following predetermined set criteria during the period of the study.

Inclusive criteria
- Age group between (25-650) years
- Present during data collection
- Who were able to understand Tamil.

Data Collection
This chapter deals with the analysis band interpretation of the smokers and non smokers regarding health status. This study results were tabulated, analysed and presented based on the data collected from the smokers and non smokers. The data were collected regarding their level of health status.

RESULTS

Table 3 shows that among 110 (73%) of smokers had moderate and 40 (27%) of poor health status. Table 4 Shows that among 150 smokers 30 (20%) of the smokers had moderate health status and 120 (80%) smokers had poor health status. Table 5 show that there was a significant association between health status among smokers and non-smokers with selected demographical variables such as quit smoking and history of smoking.

DISCUSSION

The chapter discusses the major finding of the study and reviews them in relation to the findings derived from the result of present study. The aim of the study of to compare the health status among smokers and non smokers above in community area. Overall level of health status was analyzed from the collecting data. The results revealed that

smokers 14 (93.3%), 1 (6.7) had moderate health status. Non smokers that among 15(100) of smokers all had moderate health status. Compare the health status of smoker and non smokers 14 (93.3%) had poor health status and non smoker 15(100) had moderate health status. There is no association between the health status among smokers and non smokers with age, sex, occupation, physical changes and disease condition.

CONCLUSION

The comparision of health status among smokers and non smokers using questionnaire method in selected community area karaikal. This study concluded that more of the person have smokers and non smokers identification of health status into two groups and smokers have major health issues compare to non-smokers.

Recommendations

A study to assess the risk factors of smoking habits. A study to assess the effectiveness of diversional therapy among smokers. Comparative study to assess the pulmonary status among smokers and non smokers. A comparative study to assess the mental status among smokers and non smokers.

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Table 1: Distribution Level of Health Status among Non Smokers

N=150

S.NO	LEVEL OF HEALTH STATUS	FREQUENCY	PERCENTAGE (%)
1.	Moderate 27-53%	110	73.0
2.	Poor 54-79%	40	27.0

Table 2: Distribution Level of Health Status among Smokers

N=150

S.NO	LEVEL OF HEALTH STATUS	FREQUENCY	PERCENTAGE
1.	Moderate 27-53%	30	20.0
2.	Poor 54-79%	120	80.0

Table 3: Distribution of health status Am bong smokers & non smokers with selected demographic variables.

S.NO	DEMOGRAPHIC VARIABLES	X2 VALUE	P. VALUE
1.	Age 25-35 years 35-45 years 45-55 years 55-65 years	4.286	.232
2.	Education Illiterate High school Higher secondary Graduate	1.224	.542
3.	Occupation Coolie Business Government employer employer	1.224	.542
4.	Food pattern Veg Non veg	1.224	.268
5.	History of smoking 2-4 years 5-7 years 7-9 years More than 10 years	1.224	.268
6.	Cigarette smoking 8-10 cigarette 1-3 packets 4-5 packets More than 5 pockets	.536	.464
7	Days a week take of take cigarette One day or less 2-4 days Almost every day	1.224	.268
8.	When began to smoke Below 20 years 21-25 years 26-35 years 36-45 years	1.224	.542
9.	Quit smoking Yes No	-	-

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Web Data Mining is Considered A Sub-Approach to Data Mining, with A Focus on Gathering Information from the Web.

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Received: 09 Jan 2022 Revised: 12 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

To discover marketing- related navigation patterns, you need to develop a data mining algorithm that can discover successional access patterns from weblogs. In recent times, with the development of Internet technology, the growth of the World Wide Web has exceeded all prospects. A lot of information is available in different formats, making getting content a daunting task. One possible approach to problem working is web- grounded mining (WUM). Web mining is the operation of data mining to web data, and web operation mining is an important part of web mining. This system aims to support decision makers' opinions through a clear division of duties. It enables the analysis of web information by rooting, opting, recycling and modelling vast quantities of data. We operation mining promising approach for landing stoner web access geste, detecting intriguing and common stoner access patterns from web logs. Successional website access pattern mining has been the focus of data mining exploration for over a decade in a wide range of operations.

Keywords: Datamining, Web usage mining, Sequential pattern, Data preprocessing, weblog, web data

INTRODUCTION

Generally, access to user pages is sequential. Markov chains were used to model the sequential navigation behavior of users on a website. The main function of Markov chains is that the current state always depends on the previous state. The concept of gray relationship pattern analysis using Markov chains is used to analyze user navigation behavior. They can be estimated statistically easily. The Markov chain model is also generative, so navigation tours

can be derived automatically. Markov chain models can also be customized on the fly with additional user navigation information. When used in combination with a web server, the same model can be used to predict the likelihood of future links being displayed based on the history of the links accessed. Different types of data need to be managed and organized for efficient access by different users. Various data mining methods are used to reveal hidden information on the web. However, web mining is not just about applying data mining technology to data stored on the web. You need to modify the algorithm to suit your web needs. You need to use a new approach that better matches the properties of your web data. In addition to data mining algorithms, artificial intelligence, information retrieval, and natural language processing technologies can be used efficiently. In this way, web mining has evolved into an independent field of study.

Web Mining

The overall process of discovering potentially useful and previously unknown information from web mining of web documents and services can be seen as an extension of standard data mining to web data. Web analytics relies on three common sets of information: previous usage patterns, levels of shared content, and related cache link structures that correspond to three subsets of web mining. That is, (a) web-based mining, (b) web content mining and (c) web structure mining. In web usage mining, pattern recognition consists of several steps, including statistical analysis, clustering, classification, and more. For example, in e-commerce, analyzing web usage data helps organizations understand their customers' web browsing patterns. E-commerce-specific processing, such as, helps you make better strategic marketing decisions. Most of the current research focuses on pattern discovery, but spends little effort on detailed pattern / trend analysis. This depends on the web environment and the intelligent paradigm under consideration. The most commonly used technique for web usage mining is mapping rules. Basically, this technique focuses on the relationships between web pages that are often displayed together in a user session. Pages that are called together are always combined into a single server session. Association rules help you rebuild your website with access logs. The access log usually contains information about requests approaching the web server. The main drawback of this technique is that some rules can be completely irrelevant due to the fact that so many rule sets are created together.

Classification consists primarily of assigning specific datasets to some predefined classes. The main goal of web usage mining here is to develop this type of profile of users / customers assigned to a particular class / category. That's why we need to extract the best features for the class in question. Classification can be implemented by various algorithms. These include support vector machines, K-nearest neighbors, logistic regression, decision trees, and more. Clustering is a technique for grouping many things with similar characteristics / properties. There are two main types of clusters. The first is the used cluster and the second is the page cluster. Page clustering is easy to do based on usage data. Usage-based clustering allows you to automatically group items that you access / purchase together.

A cluster of user groups plays an important role in providing personalized services to your website. To cluster user groups, you need to explain the actions of the browser. Pattern recognition and analysis was performed based on data analysis and graphics workspace tools such as Origin version 8. With the help of pattern recognition and analysis, it is easier to predict relevant useful information and knowledge. We analyzed web user access and server usage patterns. Statistical / text log file data was used in experiments provided by webalizer, one of the most popular analysis tools on web servers. You can use web structure mining to find the link structure of hyperlinks. It is used to identify data that directly links to either a web page or a network. In web structure mining, people consider the web to be a directed graph, and web pages are hyperlinked vertices. The most important application in this context is the search engine Google, which uses the Page Rank algorithm to estimate the ranking of results. If it is frequently linked by other strongly related pages, it will characterize the page as very relevant. Structure and content mining methods are usually combined.

Web usage mining is used to extract useful data, information, and knowledge from weblog records to help you discover patterns of user access to web pages. In mining (use of web resources), an individual considers recording requests from visitors to a website. It is often collected as a web server log. The content and structure of a collection of web pages follows the intent of the page author, but individual queries show how consumers view these pages.

WEB MINING METHODOLOGY

Data mining is the process by which a company discovers patterns in its data and generates insights related to its business needs. This is essential for both business intelligence and data science. There are many data mining techniques that organizations can use to turn raw data into actionable insights. These include everything from state-of-the-art artificial intelligence to the basics of data preparation, both of which are important for maximizing the value of your data investment**.**

Usage Mining allows companies to generate productive information about the future of business functions. Part of this information can be derived from the information gathered about lifetime user value, marketing strategies between products, and the effectiveness of advertising campaigns. The usage data collected allows companies to achieve more effective results for their businesses and increase sales. Usage data can also help you develop marketing skills that outperform your competitors and promote your company's services and products to a higher level.

Pattern Discover Tools

There is a lot of useful and useless information on the Internet. It is very difficult to define useful information for a particular user, which changes from time to time. Useful information at one particular time may not be useful at another time or in another situation. The web itself. About the days of new technology. The Internet uses freestyle media that accepts structured, unstructured, ordered, and unordered forms to provide information on the Web, so you can not only find relevant information, but also depending on your interests. Plan them. It is also an important issue today and is known as web personalization. Pattern detection is a way to leverage the information and web services gained through customer navigation exploration to tailor the content of a website or website to the requirements of every individual user or group of users or organizations.

Web Content Mining

Intelligent analysis of web content refers to the detection of useful information from web content, such as text, images, videos, etc. Two methods used in web content mining are an agent-based approach and a database approach. Intelligent Search agents search for information according to a particular query using domain characteristics and user profiles. Information agents used a variety of techniques to filter data according to their predefined instructions. Personalized web agents learn user preferences and discover documents related to those user profiles. In a database, the data can be organized and manipulated according to a schema and defined domain. It can conduct content mining on unstructured data such as text.

Mining unstructured data yields unknown information. Text mining is the extraction of previously unknown information by extracting information from various text sources. Content mining requires the use of data mining and text mining techniques. Basic content mining is a type of text mining. Techniques used in text mining include information extraction, topic tracking, summarization, classification, clustering, and information visualization. Web content mining is the process of mining necessary and useful data from a web page. This is primarily related to text mining, as most web content consists of text. It also focuses on the content of web pages such as images, text, and other attached media files. Preprocessing of website content is done here. WCM is used in a variety of web applications to identify web objects that have a common pattern or property.

Extracting specific information from unstructured raw data text of unknown structure is known as web content mining. Several information extraction tools are provided to identify and collect items with content such as: B. Text extraction and wrapper guidance. This is the process of extracting information from structured records such as web documents, videos, audio, text, lists and tables. The process of extracting useful information from the content of a web document.

Web Structure Mining

Web structure mining shows the relationship between the user and the web. Recognize the link structure of hyperlinks at the level between documents. It also helps determine the structure of the document used to reveal the structure of the web page, allowing you to compare the schema of the web page. A crawler is a program that visits a website, reads its pages and other information, and creates search engine index entries. All major search engines on the web have such programs, also known as "spiders" or "bots." Search engines often use crawlers to collect information available on public web pages. There are two types of crawlers. You can use web structure mining to detect the link structure of hyperlinks. The purpose of structural mining is to create a structural summary of websites and similar web pages. I'm interested in the structure of hyperlinks on the web. This type of mining is applied at the document level and the hyperlink level. Web structure mining plays a very important role in the mining process. Web size and dynamic unstructured content make it difficult for researchers to extract useful knowledge. This website produces large amounts of data in various formats, including valuable information. For example, the web server log contains information about user access patterns that you can use to customize the information and improve the design of your website. The World Wide Web is certainly the largest data source in the world. The use of global web networks has increased the role and impact of society on everyday life and has led to rapid and unprecedented development in many areas such as finance, banking, commerce, education and society. Due to the huge amount of existing data, there is an urgent need to apply new methods for extracting information and knowledge from the Web.

The use of web mining and structured mining can provide strategic results for marketing websites to promote sales. The more traffic that is directed to a particular site's web pages, the more often the site is revisited and remembered by company-provided information or product-related search engines. This also allows your marketing strategy to produce more productive results by navigating pages that link to the home page of the site itself. Web structure mining is essential to using a website as a business tool. For example, the role of a website link is similar to the role of a citation in academic literature. Popular articles are often quoted. Many hyperlinks that point to pages attract the attention of web users, as well as citations to articles for scholars. In fact, the Web is an example of a social network, a network of entities such as individuals and organizations that connect (or interact) in different ways. The terms popularity, authority and fame are at the heart of social networks. There is an approach called bibliometrics used in library science and informatics to analyze the value of scientific publications. Web mining can be defined as finding and analyzing useful information from the World Wide Web. This is a very active area of research, including the application of data mining techniques to the content, structure, and usage of web resources. If a website is directly linked to another website or the websites are adjacent, I would like to find out the relationship between these websites. Relationships can be categorized into either type because they are related by synonyms or ontology, can have similar content, and both are on the same web server and are created by the same person. Another task of web structure mining is to discover the hyperlink hierarchy or network nature of websites in a particular domain. This helps generalize the flow of information across websites that may represent a particular domain, making query processing easier and more efficient.

Weblog

Server log files are a record of web server activity. In response to those requests, it provides details about the file request to the web server. Weblogs are maintained by the web server and contain information about who visits your site. The World Wide Web is the largest and most well-known source of information that is easily accessible and searchable. The computer that delivers your website is called a web server. The web server stores all the files needed

to display a web page on the user's computer. All individual web pages together form the integrity of the website. Educational institution (university / college) management is struggling to meet the expectations of students. Some enrolled students may be interested in work. Some are enthusiastic about advanced education and business ventures, while others are. To reach the goal, college threads need to understand the attitudes of the students and then help shape them. Weblogs often have the property of being a kind of "protocol of our time" under certain aspects. In general, weblogs are dedicated to one or more topics, or usually the ones you are currently interested in, and can usually be created as individual or collective comments on each topic. Weblogs can consist of people's recorded ideas (a kind of diary) or complex collaborations that are open to everyone. Each log entry contains the client IP, username and password, access time, HTTP request method used, URL, protocol used, status code, number of bytes transferred, and referrer. Not all log entries are meaningful, so weblogs are cleaned up by excluding all image, video, and audio requests, not just failed page requests. • Session detection. The system was designed to meet the growing demand for intensive dialogue with human users. This interaction is based on a powerful mining language that allows experienced users to express background knowledge, guide miners, and gradually improve or readjust the detection process according to the mining results obtained after each query. A mining language like LOGML is used.

CONCLUSION

Data mining is a concept, and - help you find the information your large data warehouse needs using a variety of techniques. It also analyzes historical data and uses to improve future strategies. Web Data Mining is and is considered as asub-approach to data mining focused on gathering information from the Web. The web is a large domain that contains various forms of data. There are three types of web mining methods. Web usage mining aims to explore web page usage patterns in order to learn more about the relationships between users or documents in a web system. Web structure mining uses information transmitted through hyperlinks in each document to investigate only the relationships between web documents. You can use web server logs to track customer behavior on your website. The information available regarding log file analysis is often incomplete or subject to multiple interpretations. Web usage analysis extracts knowledge from web server logs. Analyzing web server logs is one of the key challenges in providing web intelligence services. By detecting sequential patterns in the web access log, you can predict future user access. In clustering, user groups or pages are recognized based on their similarities. Classification classifies new users into one of the predefined groups based on maximum likelihood.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Conventional and Microwave Assisted Synthesis of 4-Arylidene Curcumin Analogues by using Green Catalyst

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Received: 22 Feb 2022 Revised: 12 Mar 2022 Accepted: 25 Mar 2022

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ABSTRACT

We repot here, synthesis and comparative study of some curcumin analogues by conventional and non-conventional method and concluded that Microwave irradiated, acid catalyzed, under green solvent like PEG 400 is best combination for good to excellent yield of products in very less time.

Keywords: Comparative study, Curcumin analogue, MWI, Conventional and Non-conventional methods.

INTRODUCTION

Curcumin consisting central methylene moiety with most reactive proton held by carbon atom. Aromatic benzaldehyde in presence of ethanolic sodium hydroxide offers 4-arylidene substituted curcumin. Pharmaceutically these analogues were synthesized and found more active than curcumin [1]. Replacement of an acidic proton from the central methylene with benzylidene derivatives proved to be as effective antimalarial as curcumin. The 4-hydroxy-3-methoxy-benzaylidene derivative of curcumin was more active than curcumin. This suggested that the presence of electron donating group (-OMe) at *meta* position of 4-hydroxy-3-methoxy-benzylidene derivative of curcumin appears to play an important role for the potency of antimalarial compounds.[2].

MATERIALS AND METHODS

General

The commercial sample of curcumin was purchased from S. D. Fine Chemical Limited, Mumbai, Maharashtra. Solvents were used during experimentation was of Analytical grade purchased from Spectrochem of Loba, India and used further without distillation. Starting materials were check by Thin Layer Chromatography (TLC) for their

purity purpose. Separation or formation of products was initially confirmed by TLC techniques. Mobile phase selected by trial and error method. Silica plate was used TLC Silica gel 60G, F_{254} Plates by Merck. IR was recorded for compounds synthesized in the laboratory and for validation of isolated curcumin from Curcuminoides. Absorption spectra recorded in the range of 400-4000 $cm^{-1}$with KBr pallets, JASCO-8000 FT-IR spectrophotometer. Recorded spectra further substantiated by matching with reported values. Compounds synthesized during laboratory experimental work analyzed by using proton NMR and Carbon NMR. Deuterated solvents mostly used as DMSO-*d6*, unless it is mention. Tetra methyl Silane (TMS) was used as internal standard. Actual scanning was done by Burker Advance DRX 300 FT-NMR.

Experimental

Two routes were used to obtain 4-arylidine curcumin analogues. First, started from curcumin and aromatic aldehydes and second, from three moles of aromatic aldehydes and acetylacetone.

Curcumin-4-arylidine synthesis from Curcumin and aromatic benzaldehyde

Route 'a': Curcumin (2 mmol), 4-methoxybenzaldehyde (2.2 mmol) was added to cooled 20% of ethanolic KOH solution in one portion. Reaction was stirred at low temperature and progress of reaction was monitor by TLC, and reaction stop when no significant change has been observed. Reaction mixture was poured into acidic crush ice-water portion wise with constant stirring and left over ice water bath for another few hours. Obtained yellow crystals were filter off as crude product and purified with column chromatography. (Yield 38%)

Route 'b': Curcumin (2 mmol), 4-methoxybenzaldehyde (2.2 mmol) was added in toluene consisting pyridine (0.1mmol; 8mg) and acetic acid (0.16mmol; 9mg). Reaction was reflux for 140°C for 18 hrs. Progress of reaction was monitor by TLC. After completion of reaction, contain transfer to separating funnel and wash with water. Organic layer dried over anhydrous Na_2SO_4 and concentrated under vacuum. Thus obtained crude yellow colour crystals were purified with column chromatography. (Yield 27%)[3]

Route 'c': Curcumin (2 mmol), 4-methoxybenzaldehyde (2.2 mmol) was added in DMF containing Alum (5 mmol) and ammonium acetate (5 mmol) in one portion with stirring. Reaction was reflux for appropriate time (Table 1, 3)andmonitorby TLC. After completion of reaction, allowed contains to attained room temperature and pour into crush-ice-water mixture with gentle stirring and left in ice-water bath for next 3 hours. Thus obtained crude product filter off and purified with column chromatography. (Yield 89%)

Synthesis of4-arylidene-1, 7-bis(4-methoxyphenyl)hepta-1,6-diene-3,5-dione derivatives from aromatic benzaldehyde and acetylacetone.

Route 'd': 4-methoxybenzaldehyde (3.1 mmol), was added to cooled 20% of ethanolic KOH solution in one portion to this drop wise acetylacetone (1mmol) was added. After completion of addition reaction was stirred at low temperature and progress of reaction was monitor by TLC, and reaction stop when no significant change has been observed. Reaction mixture was poured into acidic crush ice-water portion wise with constant stirring and left over ice water bath for another few hours. Obtained yellow crystals were filter off as crude product wash with light petroleum and purified with column chromatography. (Yield 14%)

Route 'e': 4-methoxybenzaldehyde (3.1 mmol), was added to cool DCM containing acetylacetone (1mmol) to this drop wise piperidine (catalytic) was added with constant stirring. After completion of addition ice-water bath was removed and allowed reaction to stirred and monitor by TLC after regular time interval. When no significant change has been observed, diluted with adding excess of DCM and contains were wash with water with little acetic acid to remove all piperidine from organic layer. After anhydrous Na_2SO_4 treatment, DCM distilled off using Rota evaporator to afford crude product. Purified with column chromatography (Yield 40%)

Route 'f': Mixture of 4-methoxybenzaldehyde (3.1mmol) and acetylacetone (1 mmol) were added to PEG-400 and stirred for few minutes for homogenous mixing. To this acetic acid (catalytic) was added and contains were irradiated with Microwave at 600W for appropriate time. **(Table 2, 3.)** After each successive 10 sec. irradiation 5 sec.

were set as rest period. Reaction was monitor by TLC and after completion pour to cool water and left for 3-4 hours without stirring. Thus obtained yellow colour crude product were filter off and purified by column chromatography.

RESULTS AND DISCUSSIONS

Describe new methods for the synthesis of 4-arylidene curcumin and 4-arylidine curcumin analogues (Reaction Scheme 2.6.1).For synthesis of 4-arylidene curcumin analogues, alum ($KAl(SO_4)_2{\cdot}12H_2O$), ammonium acetate and DMF used as catalyst-solvent and reflux condition. Whereas, curcumin 4-arylidene analogues catalytic Acetic acid in PEG-400 when introduce microwave irradiation at power 600Wfor 2 minutes, offers satisfactory yield. Both described methods are productive. A model reaction was performed to optimized reaction condition. Curcumin and 4-methoxy benzaldehyde were kept as fixed reacting partner. Various reaction conditions were tried including non-conventional techniques like Microwave irradiation and solvent free method. 4-methoxy benzaldehyde was preferred as one fix component to avoid workup difficulties after completion of reaction, which usually occurs with free hydroxyl (–OH) aldehydes. Addition preference does not show any significant effect on yield of product. Alum is naturally occurring substance exhibiting versatile catalytic properties [4-6]. Table 1 shows numbers of solvent catalyst combinations were used to achieve optimum yield of reaction.

Curcumin is unstable in alkaline *p*H [7]. Two phenolic –OH and one enolic –OH susceptible for alkaline *p*H, KOH catalyzed reaction, not surprisingly, gave low productivity. This explanation for low productivity may extend upto workup stage, after product formation, pour to ice-water and neutralized, this water-alcohol solvent mixture not allowed all products to precipitate out. Workup procedure for alcoholic KOH when was modified with evaporation of alcohol from reaction mixture in reduce pressure, offers dark red-brown coloured sticky product. So, overall requirement was aprotic-polar solvent, non-alkaline condition and easy water workup, all this was achieved by using DMF as solvent Alum and ammonium acetate as catalyst. (Table 1; Entry 6) Series of reactions were performed with applying optimized reaction condition (Table 3; Product Nos. 40-45).Describe method is productive with simple handling procedure, use of naturally occurring Alum enhance green impact of present methodology. Dimethyl formamide (DMF) aprotic polar solvent allowed reaction contains to attained 150 °C temperature.

Present methodology consists of one component as curcumin hence resultant diarylheptanoid back-bone was fixed and unaltered throughout the derivatisation. Second methodology describes offers choice of diarylheptanoid backbone selection. Three moles of Aromatic aldehyde and one mole of acetylacetone (Scheme 1). 4-methoxy benzaldehyde and acetylacetone was taken as fix starting materials for model reaction. Various reaction conditions were applied to optimized reaction condition with respect to yield, easy handling procedure and time. Various reaction conditions were applied as shown in Table 2. Base catalyzed reactions (Table 2; Entry 1,2,3,4 and5) exhibits related low yield than acid catalyzed reaction (Table 2; Entry 6), this may be explain with respect to structure of acetylacetone as more numbers of acidic proton (three sets) and couple of carbonyl functionality may increases self-condensation probability strong pH change reaction. Microwave technique was used and found productive (Table 2; Entry 7). These optimized reaction conditions were used for further derivatisation.

CONCLUSION

In brief, in comparison of conventional and non-conventional method, microwave irradiated, acid catalyzed, under green solvent like PEG 400 method is best combination. It is very effective, efficient, and advanced with time reducing over convention method for synthesis of curcumin and its analogues with good to excellent yield.

ACKNOWLEDGMENT

The authors are thankful to Principal and Management of Badrinarayan Barwale Senior College, Jalna-431213 and Vivekanand College Aurangabad-431001 for encouragement for this research work and providing all necessary laboratory facility.

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Table 1 Showing time and yield of product from reaction of curcumin-4-arylidene from Curcumin and 4-methoxy benzaldehyde model reaction.

Sr. No.	Reaction condition	Time	Yield[a] of products
1.	EtOH, KOH, rt.	12 hours	38%
2.	Toluene, pyridine, reflux	18 hours	27%
3.	Toluene, NaH, stirred at rt	12 hours	17 %
4.	DMF, AcOH, pyridine, stirred at rt	24 hours	27%
5.	DMF, AcOH, AcONa, reflux	12 hours	49%
6.	DMF, Alum, $CH_3CO_2NH_4$, reflux	4 hours,	73%
		8 hours	89%
7.	DMF, AcOH, $CH_3CO_2NH_4$, reflux	9 hours	68%
8.	Toluene, pyridine, MWI	2 min.	Sticky mass
9.	DMF, Alum, $CH_3CO_2NH_4$, MWI	2 min	53%

[a] Isolated yield

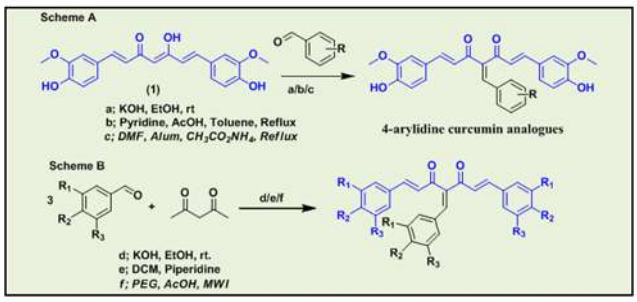
Table 2 Showing time and yield of product,4-arylidene-1,7-bis(4-methoxyphenyl)hepta-1,6-diene-3,5-dione derivatives. From aromatic benzaldehyde and acetylacetone.

Sr. No.	Reaction condition	Time	Yield[a] of products
1.	EtOH, KOH, stirred at 5°-rt.	8 hours	48%
2.	DMC, Piperidine, rt	8 hours	57%
3.	EtOH, NaOEt, 5°-rt.	7 hours	71 %
4.	DMF, KOH, stirred at rt	8 hours	64%
5.	PEG, KOH, stirred at rt	8 hours	67%
6.	AcOH, HCl, reflux	6 hours	71%
7.	PEG, AcOH, MWI	2 min.	91%
8.	PEG, KOH, MWI	2 min.	59%

[a] Isolated yield

Table 3.Showing reaction condition and yield of product.

Products No.	Alum, $CH_3CO_2NH_4$, DMF, Reflux		Products No.	AcOH, PEG-400, MWI-600W	
	Time in hr.	Yield (%)		Time in min.	(Yield (%)
(40)	9	68	**(46)**	2 min.	91
(41)	12	60	**(47)**	2 min.	77
(42)	10	77	**(48)**	2 min.	92
(43)	9	86	**(49)**	2 min.	93
(44)	9	90	**(50)**	2 min.	72
(45)	10	93	**(51)**	2 min.	94



Reaction Scheme 1. Synthesis of 4-arylidine curcumin analogues from Curcumin and substituted aromatic aldehydes and condensation of three moles of aromatic aldehydes with acetylacetone.

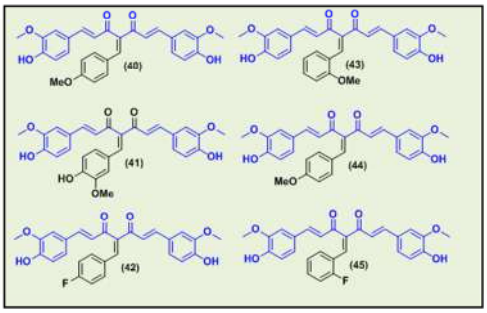


Figure 1 Structure of 4-arylidene curcumin derivatives.

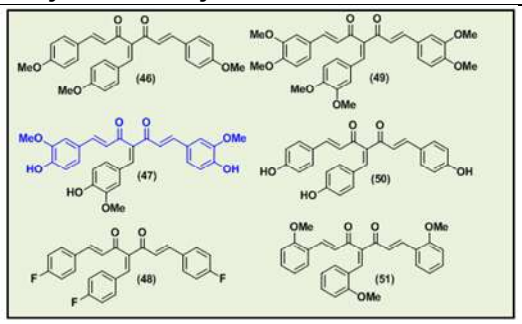


Figure 2. Structure of 4-arylidene-1, 7-bis(4-methoxyphenyl) hepta-1, 6-diene-3, 5-dione derivatives.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Towards Development of a Smart Elephant Detection System to Mitigate Human-Elephant Conflict in South Bengal, India - A Proposal

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Received: 09 Feb 2022 Revised: 03 Mar 2022 Accepted: 22 Mar 2022

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ABSTRACT

In South Bengal a remarkable number of human and elephant lives have been lost due to human elephant conflict in recent years. Therefore to save human lives it is important to reduce human elephant conflict. In this paper we propose a Bluetooth low energy beacon, commonly known as BLE beacon, based system for early detection of elephants near human habitat so that conflicts between them can be reduced. Beacons can function without Internet connection. Thus a major advantage of this system is early detection of an elephant without an Internet connection is possible. Distributive nature of the proposed system enables local people to automatically update the system with information about elephant's presence in their nearby surroundings which makes the elephant detection system robust and effective.

Keywords: Human Elephant Conflict, BLE Beacon, Special Microcontroller board, Smartphone, Raspberry Pi.

INTRODUCTION

In India expansion of human settlement into forest area increases the problem of common place sharing between animal and human [1]. As a result of such sharing human elephant conflict (HEC) has become a serious concern of recent times in India. From 2014 to 2019 a total of 2361 peoples were killed by elephants (fig 1) in India. West Bengal has the highest number of human deaths due to HEC during this period. Southern West Bengal (henceforth SWB) is a part of East-Central elephant habitat of India. The region is moderately populated. The forest area is fragmented and mostly surrounded by villages and agricultural fields. Thus there is almost no continuous passage for the

elephants from one patch of forest to another. Therefore any movement of Elephants from one patch of forest to another either naturally or during driving is bound to cause human elephant conflict and damage to the agricultural crop. In recent years the increased man-elephant conflict in the districts of SWB has become a serious challenge for the forest staff of this region [2]. The four districts Purulia, Bankura, West Midnapore and West Barddhaman of Southern West Bengal are severely affected due to elephant depredation. Therefore a smart elephant detection system is necessary to mitigate this problem.

BLE beacon

Bluetooth low energy beacon is a small coin cell battery operated Bluetooth device that periodically broadcast small data packets containing UUID number of the beacon and some advertising information which a Smartphone can see [3]. A beacon's operation is like a lighthouse. In the sea ships are able to identify a lighthouse by its light. The lighthouse however, neither is able to communicate with the ships nor does it know which ships are seeing its light. Similarly, a beacon broadcasts a radio signal, receiving which BLE-enabled devices like a Smart phone can know about its presence in the area. The beacon is unable to communicate or to identify the devices which are receiving its signal [4]. BLE-enabled devices, such as single-board computers like Raspberry Pi, or a Smartphone, listen to the signal and through applications; they are able to trigger some actions. Beacon operating protocols support three ranges of distance like far, near and immediate [5]. On average in far mode a Bluetooth 5.0 supported beacon can transmit BLE signals up to 500 meters in open space. A plethora of BLE beacons are available in present day market. They are tamper proof, water proof, and are well functioning in extreme weather condition also. Cost of beacons ranges from $15 to $35 in present day market. In the forest regions of SWB tallest trees are not congested. A plenty of bushes with low height shrubs are there. In such environment beacons can perform their normal function moderately. Since the forest areas in SWB are divided into small patches mostly surrounded by villages so an Internet connection is available in almost every region of forest. Information update into the system is thus possible from almost every corner of forest due to availability of an Internet connection. BLE beacons need not require any Internet connection for its normal functioning and thus instant detection of an elephant requires no Internet connection. An Internet connection is needed only for update of information in the system.These features with long lasting battery life (2-5 years) and cheap cost may make a BLE beacon a suitable alternative of radio collar for designing a low cost framework of elephant detection system in SWB.

Different technology enhanced Elephant tracking approaches and their pros and cons:

Radio Collar: GPS enabled radio collars have been tested in many Indian states like Assam, Chattisgarh, Karnataka, Uttarakhand and West Bengal. A radio collar can transmit location information about elephants. It weighs approximately 8 kg and worn around neck of an elephant. Generally an adult from a herd is chosen by forest officials and sedative administered to it to worn the collar before revival of the elephant. Collaring is still in use. Sometimes an accelerometer is added with the collar which provides additional information about the elephant i.e. whether the elephant is running, walking or eating etc. The main problem with radio collaring is its high cost. In India a radio collar costs approximately rupees five lac. It is also reported by Indian forest officers that most of these collars become non functional after few years, which may result a huge loss. Video Cameras: A fully automated method to track wild elephants was proposed by [6]. They had used wildlife video cameras. Elephant's color model with dynamism from a few training images were used in their method. Based on the color model with various backgrounds and lighting conditions, they place elephants in video sequences. To improve effectiveness of their approach, temporal clues were developed by them from the video. They have showed with their method steady detection of elephants are possible. The system is also suitable for other animal species. Hence this method addressed that usage of video cameras is one of the easiest way to monitor elephants. However deployment of such wildlife video camera in every corner of a forest will incur huge cost and thus become impractical.

Seismic Sensors: A review of available literature on elephant tracking using seismic sensors is discussed by [1]. Their analysis covers different paths of elephant tracking with seismic sensors. However proximity detection may not be always accurate with these sensors.

Infrasonic Sound: Members from a herd of elephants emit infrasonic waves to communicate among themselves [7]. Without significant power attenuation these low frequency sound waves can propagate longer distances and therefore detection of these sound waves is possible from several hundred meters away [8]. With Eloc nodes [9] have proposed a method of elephant detection using these infrasonic sounds. However automated update of information about elephant's presence by local people with this method is not possible.

MATERIAL AND METHODS

In our proposed model as a broadcasting device BLE beacon and as receiving device smart phone, Tablet computer, Laptop, specially designed microcontroller board (hence after called SMB) or Raspbery Pi is required. Setting up of such SMB incurs low cost. In present day market a Raspbery Pi costs 5000-6000 INR in India. Therefore deploying such receiving units in forest region is possible. Such deployment is necessary for automatically updating information about elephants. These receiving units will also help those people who have to enter deep forest for their livelihood, providing necessary information about elephant. They need not depend on information from forest department. Beacons can be fixed on elephant body with strong bio friendly adhesive, failing which a collar with beacons may be considered. A remotely controlled unmanned drone should be used to fix a beacon on an elephant body. The standard height for well functioning of a beacon is 9 feet. Therefore best fitted position for the beacon is the finger tip like point on top of elephant head. Alternatively top portion of their ears also can be chosen. Today's modern beacons weigh between 30-60 grams which is neglible load for an elephant. The highest body point on their head is approximately 11 feet. After fixing a beacon on an elephant, the forest department every time should upload UUID numbers of these beacons in a remote server database which we call database-1.

Role of Beacon: Beacons are small devices (approx 3cm x 5cm x 2cm) that after certain interval of time, usually in milliseconds, broadcast their UUIDs to nearby receiving devices like Smartphone, Tablet computer, or SMB. Role of Receiving Unit: Smartphone used by local people can be used as receiver of broadcasted UUID by a beacon. Generally elephant herd enter South West Bengal from neighboring states Odisha and Jharkhand through specific elephant corridor as shown in figure-2. In addition to Smartphone forest department should deploy SMB or Raspberry Pi enabled receiving units in strategic points along these elephant corridors (figure-2) which are shared by both local people and elephants. Identifying probable points of HEC receiving units should be deployed there. After receiving the broadcasted UUIDs roadside receiver SMBs should display a suitable message with the time of reception, so that an individual can look into it and be informed about the time when an elephant has passed the area.

Beacons transmitted UUID is usually a 16 bit number to identify the beacon. Therefore a Smart phone app also should be developed to initiate some necessary action after reception of such UUID in forest department deployed receiving units or in an individual's Smartphone. In the SMB its firmwire should be configured to initiate the same.
Role of App and SMB: The app must download database-1 from the remote server at the time of installation. In case of SMB at the time of deployment forest officials should store the database in it. It should be designed in such a way that whenever a new UUID is recorded in database -1 by forest officials instantly or within few minutes the number should be recorded into the SMB or the app database. After receiving UUID from a beacon the receiving unit or the smart phone app first compare the received UUID with those which are stored in their database i.e., database-1. If a match is found and the receiver is a smart phone then it will trigger an alarm to warn its owner about the presence of an elephant in nearby surroundings. The SMB can display the information into its display board. In addition to that the app and the receiver SMBs also send a data packet in the format given in table 2 to entry into another database (which we call database-2) in the remote server. Database-2 is to be maintained only in the server. Here one problem may arise when an elephant enter into a locality almost similar information may be posted into database-2 by several smart phones from a limited area. There must be a mechanism to remove such entries from database-2 keeping only one record.

Database-2 will play a multi faceted role in mitigating human elephant conflict. Any time forest officials can get latest position of residential individual elephant or a herd by manipulating data from this database and can act accordingly. The app should also have an interface to provide some information like "when elephants were passed nearby places? Or in a particular area whether there is elephant or not?" etc, to local people. Most of the events of conflict reported in leading newspapers highlight that sudden face to face meet with elephants is main reason behind human death. So in such cases automatically triggered warning messages in local people's smart phone or the display units of SMBs will be highly beneficial. Since beacons can transmit signal without Internet connection therefore generation of such warning messages in a distant user's smart phone require no internet connection. Thus instant detection of an elephant is possible.

RESULT AND DISCUSSION

BLE beacons are widely used in various Internet of Things based indoor and outdoor applications. Common practise is a beacon should be fixed in a static position. Anybody with a receiver (Smart phone) passes through nearby places receive advertising packets from the beacon. In our proposed method our approach is just the reverse of this common approach. Here beacons are in dynamic mode when the elephant is moving. Bluetooth signal may not cover enough distance in dense forest region. Therefore to test the longest possible distance between a beacon and a receiver in typical jungle area in SWB we run a dummy protocol in a forest area near human settlement. We have also performed the same experiment in open space. We have used a beacon simulator app (developed by Vincent Hiribarren and downloaded from google play store on 12.12.2021) installed in two Smart phone. One phone is kept in simulator mode and running with a normal speed keeping the phone in an average height of 11 feet. The other phone is fixed in a place in scanning mode. The signal strength (RSSI values) with corresponding distance is measured in feet. Result is summarised in fig-3. From the result we see that covering distance is approximately 20% less than that in open space. Therefore for a 500 meter range we can assume that a beacon signal can be detected from a distance of 400 meters. Safe distance from a wild elephant to be maintained is 100 meter (https://www.wildcard.co.za/essential-tips-for-safe-elephant-viewing/ retrieved on 15.01.2022) which proves good efficacy of our method of elephant detection.

CONCLUSION

A low cost hypothetical model for detection of elephant in South Bengal is proposed in this paper. The most lucrative part of this model is the ability of local people to update the system in real time. They will also able to get information about presence of elephant in their locality in real time. So if any NGO implement this model, peoples of South West Bengal may get benefited from it. However due to lack of different technical support we were unable to implement it. So a rigorous checking is necessary before actual implementation.

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Table 1: Proposed Format of Database-2 Schema to be stored in Server Computer

UUID Number	Latitude	Longitude	Date	Time
Beacon special ID	Receiver position	Receiver position	Current Date	Current Time

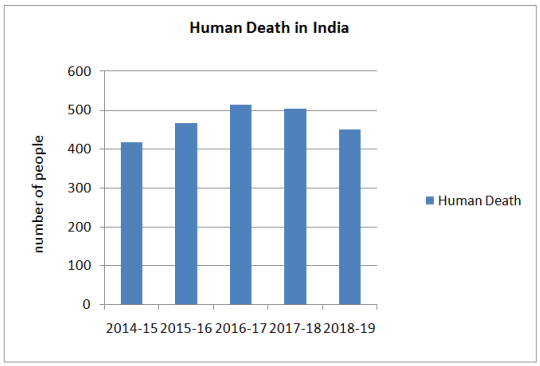

Figure 2. Human Death in India

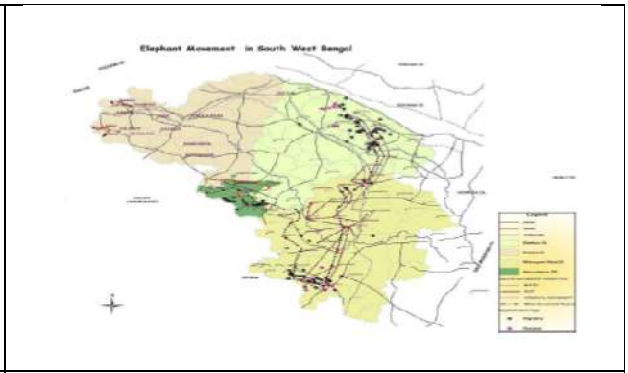

Figure 2: Elephant Corridor in South West Bengal

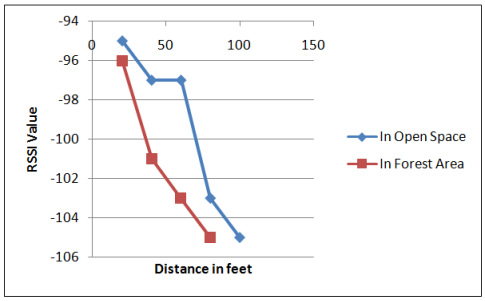

Figure 3. signal strength

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Design and Comparison of Controller Mode Effect for Dynamic Mode of Active Front End Converter

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Received: 03 Feb 2022 Revised: 15 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

The present paper represents the design and comparison of Dynamic mode in terms of %THD by using various controller modes on single-phase front-end converter by varying values of P, I, and D. The single-phase Front-End Converter model is prepared and implemented on MATLAB Simulink for verification of its stability and calculation of %THD. The basic is to identify the various mode of active front-end converters like motoring, regenerative and dynamic. The understating of the basic operation of the Dynamic mode, implementation of the various control algorithm is performed for comparison.

Keywords: Control algorithm, %Total Harmonic Distortion (THD), Proportional (P) action, Integral(I) action, Derivative(D) action, Active Front End Converter (AFEC).

INTRODUCTION

The ongoing research work on AFEC and controller algorithm outcome is compared in the paper presented on %THD, power factor instrument, peak overshoot, steady-state error, and stability in AFE converter. The previous research work of different control algorithms is referred to in this paper and the effects on the performance and dynamic performance of such an AFEC have been observed and compared with the suggested algorithm. The main focus of this paper is to propose a different algorithm that provides an improvement in % THD. The basic diagram of FEC is shown in figure 1. This is because harmonics at two times the carrier frequencies will be 180^{o} phases shifted.

Hence, flux is produced by these cancelling each other at the input transformer secondary side [1]. PID controllers are extensively used for industrial system control.In the proportional term, proportional gain (Kp) is multiplied by the required correction means error. In integral action, the accumulative error would add quickly to give the system a chance to respond, thereby not allowing it to ever stabilize. The derivative term makes an adjustment based on the rate at which the plant output is changing from its set point.

$$Output(V) = K_p e_p + K_p K_I \int e_I + K_p K_d + V_o$$

Such a controller has extra tuning freedom and as a result additionally a much wider place of parameters that stabilize the plant below manage, and gives enhancements on top of things loop robustness [5].

Reviewed Paper

The previous research work is referred to understand the past and present scenario and can be compared with present research work. So that various papers on AFEC and Control algorithms are referred. This paper affords a new goal feature for mixed PI and repetitive manipulate parameters optimization the usage of SGA for a three-segment enhances PFC rectifier. Multiple simulation examples are done primarily based totally on extraordinary emphasis elements γ to test the importance of the proposed goal feature. Moreover, the three-phase boost PFC rectifier overall performance is evaluated in phrases of THD reduction and dynamic overall performance indices (like peak overshoot, steady-state error, rise time, settling time) upgrades the usage of the separate assessment features. The proposed goal feature is as compared with present goal features, particularly ISE, IAE, ITSE and ZLG. Simulation outcomes confirmed that our proposed goal feature outperform present goal features to achieve optimized PI and RC parameter values. The development percentage of the proposed SGA-PIR controller for THD is 59.28% as compared to ZLG at $\gamma = 0.3$. Similarly, the dynamic overall performance parameters inclusive of Mp and tr are stepped forward with the aid of using 13.03% and 2.53%, respectively, on the value of better settling time whilst $\gamma = 0.6$ is set [4].

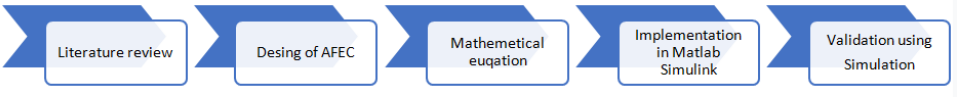
Conventional converters (using diode, thyristor) have numerous hazards like excessive harmonic cutting-edge, low energy factor. In this paper, the unmarried section of the front end converter (FEC) using IGBT as a switching tool is reported. Various components of the unmarried section FEC including, PLL layout, modelling, controller layout for cutting-edge management and DC bus voltage manage are studied. Second-order generalized integrator - section locked loop (SOGI-PLL) is hired to generate the unit vector for reference body transformation. The machine modelling is carried out in a synchronous reference body (SRF). Further, cascaded management scheme such as internal cutting-edge-manage loop and outer voltage-manage loop is applied for cutting-edge, and voltage management in SRF. Pole-0 cancellation and symmetric strategies are used to attain the parameters of the cutting-edge and voltage controllers. Unipolar Sine triangle pulse width modulation scheme is hired for switching the IGBT devices. Model modelling and the layout method of the front give-up converter are offered in detail. The machine is simulated on MATLAB Simulink to validate the layout parameters and the converter indicates the overall performance beneath the temporary and constant nation conditions. A unity energy factor (UPF) at the grid aspect is executed with bidirectional energy float capability [2].

In this work, research is performed in estimating the capability of IPD controller as an AGC controller. It is for integrating RE alongside traditional thermal-primarily based totally strength plants. The managed overall performance of IPD is in comparison with the PID and PI controllers. All controller has tuned the usage of an evolutionary method genetic set of rules with the aid of using incorporating a step load perturbation in each area. The comparison between the two is included in the paper. The truth is that IPD controller calls for equal computational and structural necessities as the opposite traditional controllers even as owning vast blessings afford it a promising approach to the trouble of AGC [3].The major objectives are as under.

- Design and prepare Matlab Simulink Implementation of Frond End Converter using control algorithm.
- Comparison of %THDfor designed mode of PID, P(1 +(I + D)) and IPD.

METHODOLOGY

To derive the mathematical equation is the first step and after that calculate component values of AFEC. The mathematical equation is represented in terms of the block diagram. The Unity modulus method was used to determine the value of AFEC's control algorithm which gave the maximum value of different parameters of the controller algorithm, which was determined by the trial-and-error method depending on the maximum value. To create an AFEC model, its mathematical equation was first created and this mathematical equation was converted to the modelling file in the Simulink model in MATLAB. The License version of Government Engineering College, Gandhinagar, India of MATLAB version R2012A is used for simulation. Flow chart of methodology is shown below.



The PID controller Simulink model is shown in figure 2[1].
The suggested controller Simulink model is shown in figure 3 and 4.
Parameter values are as shown in below table 1.
The block diagram of voltage control loop is shown figure 5.
The Dynamic model of FEC is shown in figure 6.

RESULT AND DISCUSSION

The result of %THD is shown in figure 7 for PID controller.
The result of %THD is shown in figure 8 for IPD controller.
The result of %THD is shown in figure 9 for P(1 + (I+D)) controller.
The previous paper used simulation results to show the result of %THD for PID control algorithm is 1.15% for values of P= 2, I=30 and D =-0.01. The other proposed calculated algorithm simulation result of %THD for the IPD control algorithm is 0.81% for values of P=8.4, I=200, and D=0.0005.The proposed calculated algorithm simulation result of %THD for P(1 + P(I+D)) control algorithm is 0.79% for values of P= 5, I=30, and D=0.01.

CONCLUSION

The simulation was performed by using various values of P, I, and D. The PID and P(1 + (I+D)) algorithm system are stable for a certain range of parameters where the IPD algorithm system is marginally stable. The simulation results show that the IPD algorithm reduced the %THD from 1.91 to 0.79%. The results proved that the P(1 + P(I+D)) algorithm reduced the harmonics.

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Table 1: Parameter values

Parameter	**Values**
Input applied Voltage V_s (rms)	1432 Volts
Supply Frequency	50 Hz
Output Voltage	2800 Volts
Input peak voltage; V_s(peak)	2025V
Switching Frequency, f_{sw}	660Hz
Rated Power	1400 W
DC Link Capacitor	9600 μF
efficiency of converter (η)	98 %
Values of P	Range from 2 to 12
Values of I	Range from 30 to 1800
Values of D	Range from 0.0005 to 0.01
K_v	1/2800
G	2240
ξ	0.7
K_I	1/1410
T	$1.1515\,x\,10^{-3}$
V_{dc}^*	0.9, 1, 1.1
R	0.1 ohm
L	1.78 mH
Modulation index	0.8
ccc	Values
Input applied Voltage V_s (rms)	1432 Volts
Supply Frequency	50 Hz
Output Voltage	2800 Volts
Input peak voltage; V_s(peak)	2025V
Switching Frequency, f_{sw}	660Hz
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T	$1.1515\,x\,10^{-3}$
V_{dc}^*	0.9, 1, 1.1
R	0.1 ohm

L	1.78 mH
Modulation index	0.8

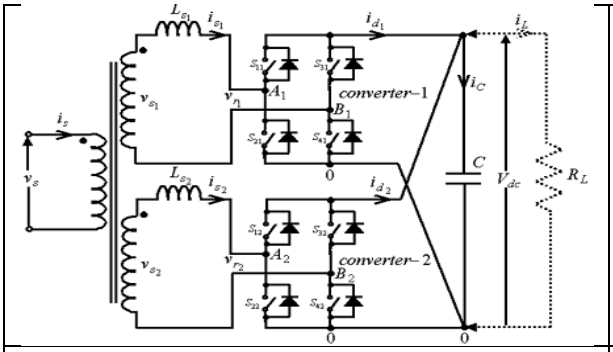

Figure1 Basic FEC Diagram

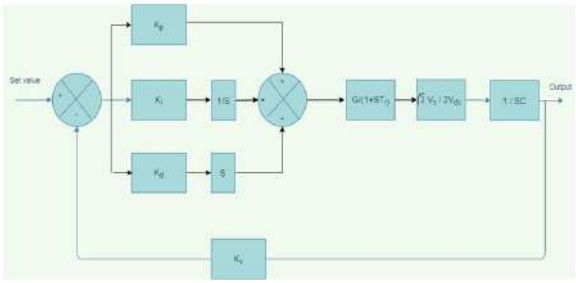

Figure 2. Block diagram of FEC using PID controller

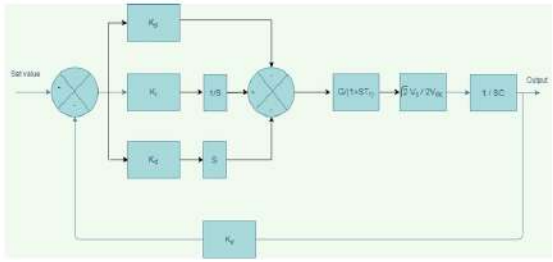

Figure 3. Block diagram of FEC-IPD controller.

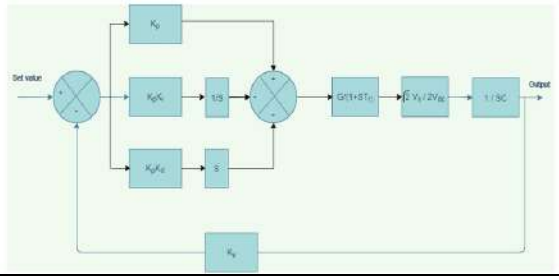

Figure 4. Block diagram of FEC-P(I+D) controller.

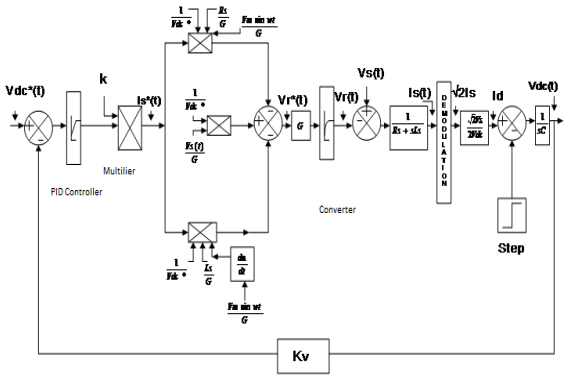

Figure 5. MATLAB-Simulink model of FEC using PID controller

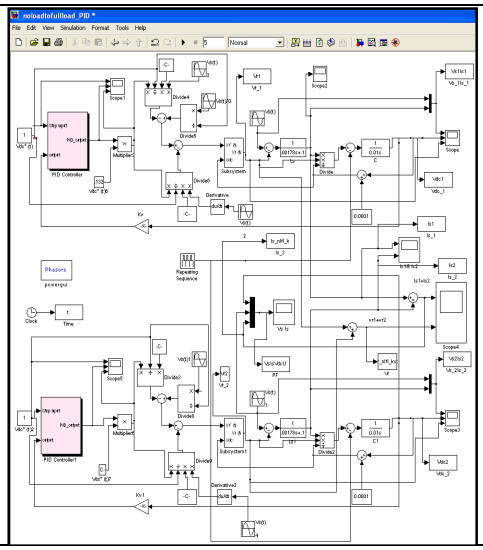

Figure 6. Simulink model of Dynamic Mode

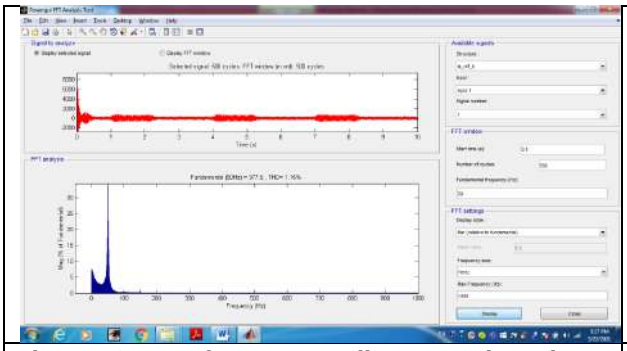

Figure 7. % THD for PID controller -Dynamic mode

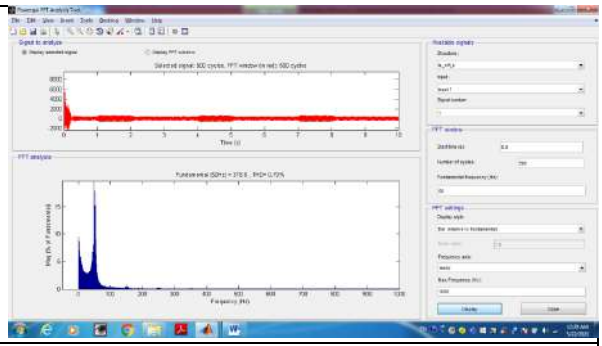

Figure 8. % THD for IPD controller -Dynamic mode

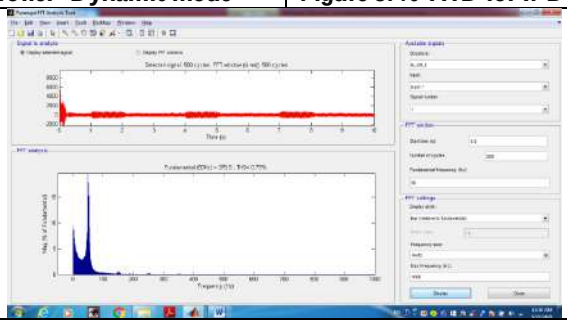

Figure 9. % THD for P(1 + (I+D)) controller -Dynamic mode

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Simultaneous Estimation of Atorvastatin and Aspirin by Dual-Wavelength Spectrophotometric Method from Tablet Dosage Form

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Received: 07 Jan 2022 Revised: 27 Jan 2022 Accepted: 18 Feb 2022

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ABSTRACT

Atorvastatin calcium (ATR) and Aspirin (ASP) are beneficial in combination for elderly people in various health management conditions. Aim of present study is to develop simple, accurate, and precise method for simultaneous quantitative estimation of ATR and Aspirin from combined tablet dosage form. Method involves simultaneous equation, using methanol as common solvent. Calibration curves determination for both drugs has been carried out in 0.1 N HCl, phosphate buffer pH 6.8, and methanol as solvent. Linearity range was observed in the concentration range of 20-120µg/ml for ASP when scanned in 224 to 264 nm ranges (R^2 = 0.999) and 10-60µg/ml for ATR when scanned at ranges of 247 to 284 nm (R^2 = 0.998) respectively. Percent concentration estimated for ASP and ATR were 100.13±1.8218 and 99.98 ± 0.98, respectively. The method was found to be simple, economical, accurate and precise and can be used for quantitative estimation of ATR and ASP.

Keywords: ATR, ASP, accuracy, HPLC, methanol, Linearity.

INTRODUCTION

The number of drugs and drug formulations introduced into the market has been increasing due to increase in rate of diseases. The pharmaceutical formulations with combinations of drugs have shown an increasing trend to counteract other symptoms specific to one drug and formulation, and hence analytical chemist will have to accept the challenge of developing reliable methods for analysis of drugs in such formulation. Drug development involves estimation of drugs from pharmaceutical formulation and biological samples which has got immense role in drug discovery. Pharmaceutical industries are typically depending upon quantitative chemical analysis to ensure that the

raw material used and the final products obtained meet the required specifications. Analytical methods are required at every step of drug development process in which quantification is ofprime importance in the quality control and quality assurance of drugs and drug products. In the analysis of formulations containing two or more drugs, one drug can interfere in the estimation of another drug. To avoid such interferences separation of components of mixture by extraction is usually carried out which makes the procedure time consuming and complicated and often lacks accuracy. Therefore it is worthwhile to develop such methods of analysis, which can estimate both the drugs in combination without prior separation. Atorvastatin (ATR), a statin drug which chemically known as [R-(R∗, R∗)]- 2-(4-fluorophenyl)-dihydroxy-5-(1-methylethyl)-3-phenyl4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, used to reduce blood cholesterol [1]. It inhibits HMG-CoA (3-hydroxy-3- methylglutaryl-coenzyme A) reductase and resulted decreases cholesterol synthesis and ultimately increases expression of low-density lipoprotein receptors [2, 3]. On other side, Aspirin is belongs to salicylates, known as acetylsalicylic acid or 2-Acetoxybenzoic acid. It is used to reduce pain, fever, or inflammation and also widely used for the prevention of cardiovascular disease [4-6].

Atorvastatin calcium, Fenofibrate and nicotinic acid are available in the market as combined tablet dosage form [7-9]. Similarly Atorvastatin calcium, in combination with Aspirin is also available in tablet as well as capsule dosage form. Literature survey reveals that spectrophotometric method and a stability-indicating LC method were reported for determination of ATR in various pharmaceutical preparations in combination with multiple drugs [10-12]. Thereafter, several spectrophotometric methods have been reported for aspirin assay [13-14]. But there are no methods reported forsimultaneous estimation of Atorvastatin calcium and Aspirin in combined tablet dosage form. Hence, in the present work, development of precise, accurate, simple, reliable and less time consuming UV spectrophotometric and chromatographic methods for estimation of Atorvastatin calcium from their combined dosage form has been carried out. The results are within the limits according to ICH guidelines used for validation of analytical methods [8]. The developed methods can be used for analysis of the stated formulations in quality control and quality assurance laboratories.

MATERIALS AND METHOD

To develop the two UV-Spectrophotometric methods for simultaneous estimation of Atorvastatin Calcium (ATR) and Aspirin (ASP) from combined tablet dosage form, methanol was used as solvent (HPLC grade, Merck). Atorvastatin Calcium and Aspirin were collected from Vergo Pharmaceuticals. Pvt. Ltd, Goa and Ethicare Pharmaceuticals Pvt. Ltd, Mumbai as free samples respectively.

Equipments required for the experiment

UV visible double beam Spectrophotometry (Jasco UV-630), Analytical balance (Digital) (Shimadzu AX-200), Hot air oven (Sai Enterprises Works,Mumbai, India), digital pH meter (Lab India) and Bath Sonicator (PCI Analytics Private Limited).

Method

The instrument used for the present study was PC based Jasco V-630 UV-Visible Double Beam Spectrophotometer with 1cm matched pair quartz cell and spectral bandwidth of 2 nm.

Selection of Solvent System

Methanol was selected as a solvent system for study of developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Drug Solution

Standard stock solution containing ASP and ATR was prepared by dissolving 20 mg of ASP and 10 mg of ATR separately in 100 ml of methanol solvent and sonicated for 10 minutesto get stock solution containing 200 µg/ml of ASP and 100 µg/ml of ATR in two different 100ml volumetric flasks.

Procedure for Determining the Sampling Wavelength for Simultaneous Analysis

ASP and ATR (20 µg/ml each) were scanned separately in a wavelength range of 200 nm to 400 nm. In this method two wavelengths were selected for each drug in such a way so that thedifference in absorbance is zero for another drug. Aspirin was determined by plotting the difference in absorbance at 224 and 264 nm (difference is zero for Atorvastatin) similarly for the determination of Atorvastatin the difference in absorbance at 247 and 284 nm (difference is zero for Aspirin). Methanol was taken as solvent. Overlain spectrum of ASP and ATR is shown in figure-1 and 2.

Procedure for Making Mixed Standards

The standard stock solutions of ASP and ATR were used to prepare mixed standards. From standard drug solutions six working standard solutions of ASP with concentrations of 20, 40, 60, 80, 100, 120, µg/ml and ATR with concentration 10, 20, 30, 40, 50, 60, µg/ml. were prepared. The composition of mixed standards is given in Table-1.

Procedure for Plotting Calibration Curve

The above six mixed standard solutions were scanned at the selected analytical wavelengths and the calibration curve for both the drugs was constructed. Calibration curve for ASP was plotted against concentration by taking absorbance difference at 224-264 nm in zero order mode from the spectra of mixed standards, while calibration curve for ATR was plotted against concentration by taking absorbance difference at 247-284 nm in zero order mode. ASP and ATR obeyed Beer's law in the concentration range 20-120µg/ml and 10-60µg/ml respectively. By using quantitative modes of instrument slope, intercept and correlation coefficient values for calibration curve was obtained for both the drugs (Figure-3 and 4).

Analysis of Tablet Formulation

Marketed tablet formulation A-Vin-AS (Bestochem) containing ASP 75 mg and ATR 10 mg were analyzed using this method. From the contents of 20 tablets, an amount equivalent to 75mg of ASP and 10mg of ATR was weighed and dissolved in 60 ml of solvent in 100 ml volumetric flask. The solution was filtered through Whatmann filter paper no. 41 and then final volume of the solution was made up to 100 ml to get stock solution containing 750 µg/ml of ASP and 100µg/ml of ATR. After appropriate dilutions, the absorbance was measured and the concentration of each analyte was determined with the equations generated from calibration curve for respective drugs. Result of tablet analysis is shown in table-2.

Method Validation

Linearity study: Linearity study was carried out by plotting the calibration curve for both the drugs. ASP and ATR was found to be linear, in concentration range 20-120µg/ml and 10-60µg/ml respectively. The results of linearity study are reported in table-3 and 4.

Accuracy: Accuracy was determined by performing recovery studies by spiking different concentrationsof pure drug at three levels i.e. 80%, 100% and 120%. From the tab stock solution 750 µg/ml of ASP and 100µg/ml of ATR final dilution of tab 80µg/ml of ASP and 40 µg/ml of ATR Further addition of pure drug 80, 100 and 120% to final solution Results of recovery studies indicated that the method is rapid, accurate and reproducible. Results are shown in table-5.

Precision: The repeatability of the method was confirmed by performing analysis of drugs was repeated for 6 times with the same concentration as the 80 µg/ml of ASP and 40µg/ml of ATR. Intra-day and Inter-day precision also done at 3 conc. level, for ATR preparing conc. 10µg/ml, 30µg/ml, 60µg/ml, for ASP preparing conc. 20µg/ml, 80µg/ml, and 120µg/ml, were the values of relative standard deviation (R.S.D.) was calculated. The data obtained fromprecision experiments are given in table-6, 7 and 8 for repeatability, intra-and inter- day precision studies.

LOD: Based on the Standard Deviation of the Blank, the measurement of the magnitude of analytical background response was performed by analyzing the six replicates of blank samples and calculating the standard deviation of these responses. LOD = $3.3\ \sigma/S$.

LOQ: Based on the Standard Deviation of the Blank, the measurement of the magnitude of analytical background response was performed by analyzing the six replicates of blank samples and calculating the standard deviation of these responses. LOQ = 10σ/S. The result of LOD and LOQ are shown in table-9.

RESULTS AND DISCUSSION

Quantitative estimation of atorvastatin calcium and aspirin

For quantitative estimation of ATR and ASP, simultaneous equation method and the two wavelengths were chosen from the overlain spectra of ATR and ASP that are shown in below figure-1.

Method Validation

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for analyte. Result of repeatability, LOD and LOQ, intraday precision, inter day precision was calculated. The zero order spectra of pure drugs were found to be overlapping making their simultaneous determination by dual wavelength method possible. Dual wavelength spectrophotometric method was considered to be ideal to facilitate their quantitativedetermination. It was observed during initial study that zero order spectra of both drugs can be used for the estimation of ASP and ATR in their combined dosage form. The method utilizes six mixed standard solutions which were scanned in a wavelength range of 200-400 nm against methanol as blank. In the proposed method, for the estimation of ASP and ATR two wavelengths were selected for each drug in such a way so that the difference in absorbance is zero for another drug. Aspirin was determined by plotting the difference in absorbance at 224 and 264 nm (difference is zero for Atorvastatin) similarly for the determination of Atorvastatin the difference in absorbance at 247 and 284 nm (difference is zero for Aspirin). Methanol was taken as solvent. Linear regression data showed a good linear relationship over a concentration range of 20-120µg/ml for ASP and 10-60µg/ml for ATR. For both the drugs six point calibration curves were generated. Data obtained from repeatability, interday and intraday studies, showed high degree of precision of an analytical method under normal operational conditions. Result of tablet formulation showed percent relative standard deviation valuesin the average range of 1.8218 for ASP and 1.3034 for ATR. The results indicated excellent average recoveries, at 80%, 100%, 120%. For, ASP 100.21%, 100.01%, 99.60% and for ATR 98.45%, 99.80%, 100.06% were resulted. Recoveries obtained for two drugs do not differ significantly from 100%, showed that there was no interference from common excipient used in formulation indicating accuracy and reliability of method. Lower limit of detection (LOD) for ASP and ATR was found to be 0.0858 and 0.04753 µg/ml respectively and limit of quantization (LOQ) for ASP and ATR was found to be 0.144 and 0.2.

CONCLUSION

In present study, from the observation of the validation parameters, it was concluded that the developed method is simple, accurate, reliable, and economical for the simultaneous quantitative estimation of atorvastatin calcium and aspirin from combined dosage form using UV spectrophotometric method.

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Table 1: Concentration of Mixed Standard of ASP and ATR

Standard No.	1	2	3	4	5	6
Concentration of ASP (µg/ml)	20	40	60	80	100	120
Concentration of ATR (µg/ml)	60	50	40	30	20	10

Table 2: Regression Equation Data for ASP, Y=A+B×C

Regression Equation Data for ASP, Y=0.010c+0.641	
Slope(B)	0.010
Intercept(A)	0.641
Correlation Coefficient	0.999

Table 3: Regression Equation Data for ATR, Y=A+B×C

Regression Equation Data for, ATR Y=0.016c-0.036	
Slope(B)	0.016
Intercept(A)	0.036
Correlation Coefficient	0.998

Table 4: Results of Tablet Analysis

Analyte	Label Claim (mg/tab)	%Label Claim Estimated* (Mean ±% R.S.D.)
ASP	75	100.13±1.8218
ATR	10	99.94±1.3034

*Average of nine determinations, R.S.D.: Relative Standard Deviation

Table 5: Results of Recovery Study

Label claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Concentration recovered (Mean ± S.D)	%Recovery Estimated (Mean ± % R.S.D.)
ASP 75 mg	80	60	60.12 ±0.3702	100.21±0.3670
	100	75	75.01 ±0.7823	100.01±0.7778
	120	90	89.62 ±1.25	99.60±1.2550
ATR 10 mg	80	8	7.12 ±0.3821	98.45±0.3881
	100	10	9.82 ±1.8147	99.80±1.8147
	120	12	12.02±1.5888	100.06±1.5879

Table 6: Results of Repeatability

Analyte	Label Claim (mg/tab)	%Label Claim Estimated*(Mean ±% R.S.D.)
ASP	75	99.54±0.4615
ATR	10	99.17±1.2994

*Average of nine determinations, R.S.D.: Relative Standard Deviation

Table 7: Results of Intraday Precision

Time	%Label Claim Estimated* (Mean ± %R.S.D.)	
	ASP	**ATR**
T1	99.94± 0.8799	99.45 ± 1.1723
T2	99.64± 0.5001	99.23± 0.9383
T3	99.65± 0.4455	99.54± 0.6750

*Average of nine determinations, R.S.D.: Relative Standard Deviation

Table 8: Results of Inter day Precision

Day	%Label Claim Estimated* (Mean ± %R.S.D.)	
	ASP	**ATR**
Day 1	99.12±1.0522	99.55±1.2822
Day 2	99.38±0.8463	99.53±1.0596
Day 3	99.34±1.0220	99.61±1.3332

*Average of nine determinations, R.S.D.: Relative Standard Deviation

Table 9: Limit of Detection and Limit of Quantization

LOD (µg/ml)*		LOQ (µg/ml)*	
ASP	**ATR**	**ASP**	**ATR**
0.0858	0.04753	0.144	0.268

*Average of nine determinations

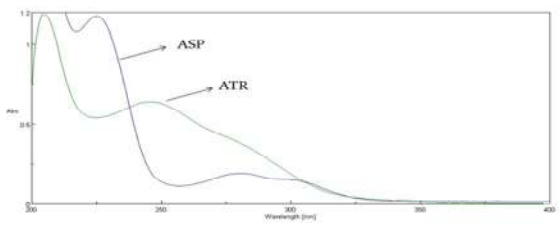

Figure 1: Overlain Spectra of ATR and ASP

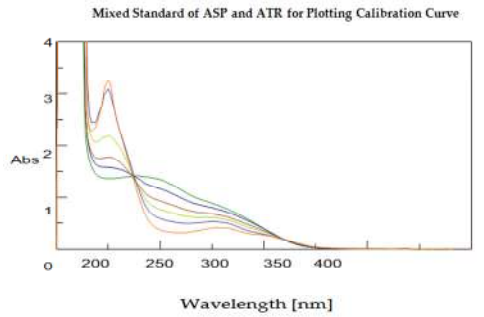

Figure 2: Overlain spectra of mixed standard shows linearity

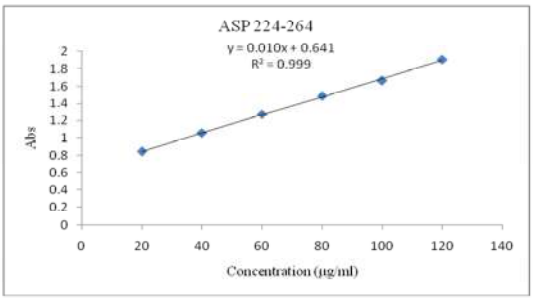

Figure 3: Calibration curve for ASP

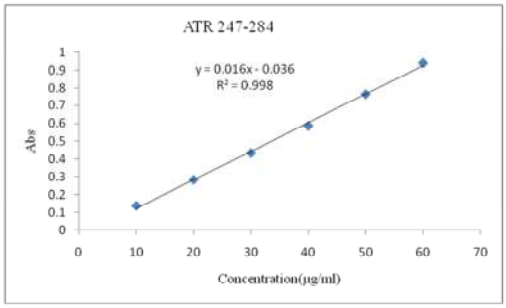

Figure 4: Calibration curve for ATR

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Evaluation of Surface Roughness of Flowable Composite Resin after Brushing Simulation with Fluoridated and Remineralising Toothpaste - An *In Vitro* Study

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Received: 10 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Composite resin is the material of choice for anterior tooth restorations in adult and pediatric patients. Surface roughness is a crucial property in the success of dental restoration.Tooth brushing may influence the wear and surface roughness of different types of composite resins. The aim of this study is to evaluate the surface roughness of flowable composite resin after brushing simulation with fluoridated and remineralising toothpaste. The in vitro study was carried out in White Lab, Saveetha Dental College and Hospital, Chennai, India. Eight disc shaped samples of flowable composite resin restorative material with 2 mm of thickness were prepared using a customised mould. After obtaining the surface roughness value prior to brushing, the flowable composite resin samples were subjected to brushing simulation. (ZM3.8 SD Mechatronik brushing simulator). 4 flowable composite resin samples were brushed with colgate fluoridated toothpaste (group 1) and another 4 flowable composite resin samples were brushed with enafix remineralising toothpaste (group 2). The surface roughness value after brushing simulation was again determined using the stylus profilometer. Mean Ra for Colgate and Enafix group was 0.00050 and 0.00000 respectively, Mean Rq for Colgate and Enafix group was 0.00075 and 0.00050 respectively, Mean Rz for Colgate and Enafix group was 0.00775 and 0.00700 respectively. Independence sample T test was

used p value of less than or equal to 0.05 is considered significant. P value for Rq and Rz parameters was 0.356 and 0.506. Hence, not statistically significant. There was no variation in the surface roughness of flowable composite resin samples after brushing simulation with Colgate and Enafix toothpaste.

Keywords: composite resin, surface roughness, brushing simulator, stylus profilometer, innovative measurement

INTRODUCTION

Dental composites are artificial substances that integrate polymeric matrices with a dispersion of glass, mineral or resin filler via means of coupling agents. Just like dental amalgam, they are used to repair tooth shape and structure affected by trauma and dental caries(1,2). Composites also can be used to cement crowns and veneers. While the amalgam is phasing out in dentistry, composites have grown to be one of the maximum extensively used esthetic restorative substances(3). It is the material of choice for anterior tooth restorations in adult and pediatric patients(4). Composite resin polymerization can change from viscous liquid to viscoelastic solid. These modifications occur when composite resin monomers react to external energy to form polymers. Surface roughness is a crucial property in the success of dental restoration(5). The resin composite structure and the characteristics of the resin particles have a direct impact on the smoothness and the susceptibility of the material to extrinsic staining. The surface roughness of the composite is usually dictated by size, hardness and quantity of load particles, which influence the mechanical properties of composites, the flexibility of organic matrix, hardness and size of the abrasive

Tooth brushing may influence the wear and surface roughness of different types of composite resins. Tooth brushing plays a prime role in maintaining the oral hygiene of the individual. Dentifrices can damage the surface of composite resin restoration, thereby making it rougher and more prone to dental plaque accumulation, staining of restoration, soft tissue inflammation and development of recurrent dental caries. To achieve cleaning, the abrasive systems in dentifrices have to provide a certain degree of abrasiveness with respect to the surface of the teeth. The amount of surface wear due to tooth brushing depends on certain parameters such as brushing habits, usage of hard, medium or soft toothbrush and abrasive types seen in dentifrice(6,7). The surface wear and roughness can have an impact on the life of the dental restoration. The aim of this study is to evaluate the surface roughness of flowable composite resin after brushing simulation with fluoridated and remineralising toothpaste.

MATERIALS AND METHODS

The in vitro study was carried out in White Lab, Saveetha Dental College and Hospital, Chennai, India. Eight disc shaped samples of flowable composite resin restorative material with 2 mm of thickness were prepared using a customised mould. (Figure 1) The surface roughness prior to brushing of the prepared flowable composite resin circular discs were determined using a Stylus profilometer - Mitutoyo SJ 310, 2μm tip/60°angle, device was moved physically on the surface of the flowable composite resin material to obtain the values prior to brushing. The values of Ra (Roughness average), Rq (Root mean square) and Rz (Roughness height) were recorded for each sample. After obtaining the surface roughness value prior to brushing, the flowable composite resin samples were subjected to brushing simulation. Samples were placed in a brushing simulator (ZM3.8 SD Mechatronik). (Figure 2) The samples are subjected to 8-9 hours of brushing which is equal to around three years of brushing and around 30000 cycles. 4 flowable composite resin samples were brushed with colgate fluoridated toothpaste (group 1) and another 4 flowable composite resin samples were brushed with enafix remineralising toothpaste (group 2). The surface roughness value after brushing simulation was again determined using the stylus profilometer. The surface roughness values before and after brushing simulation were obtained and tabulated. The results were then analysed using SPSS software version 22.0 and were graphically represented.

RESULTS

Ra, Rq and Rz values of the flowable composite resin samples before and after brushing simulation was analysed. Independent T test was done for comparison between the groups.(Table 1, figure 3) Mean Ra for Colgate and Enafix group was 0.00050 and 0.00000 respectively, Mean Rq for Colgate and Enafix group was 0.00075 and 0.00050 respectively, Mean Rz for Colgate and Enafix group was 0.00775 and 0.00700 respectively. Independent sample T test was used p value of less than or equal to 0.05 is considered significant. P value for Rq and Rz parameters was 0.356 and 0.506. Hence, not statistically significant.

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications (8–17),(18–21),(22–26),(27). The effect of brushing simulation with various dentifrices on surface roughness of various types of resin composites has been investigated in many studies. However, no studies have compared the surface roughness of composite resin after brushing simulation with fluoridated and remineralising toothpaste. Hence we did this novel study. In the colgate and enafix toothpaste group, surface roughness values before and after brushing simulation did not show any deviation. The results were statistically not significant. In a study, 60 disk-shaped specimens were made of microfill, nanofill, minifill hybrid and nanohybrid composite resins. They were subjected to brushing simulation with Colgate Total, Colgate baking soda & peroxide whitening Colgate tartar control and whitening toothpastes. They found a significant increase in surface roughness of composite resin after brushing with all dentifrices. In our study we did not find any changes in surface roughness values before and after brushing simulation of flowable composite resins.(28).

Thirty six samples of Z350XT and Empress Direct composite resin material subjected to brushing simulation using dentifrices such as Oral-B Pro-Health Whitening, Colgate Sensitive Pro-Relief and Colgate Total Clean Mint. The samples were subjected to 5000, 10000 and 20000 cycles of simulated Tooth brushing in a brushing simulator. The higher the brushing simulation time cycles used, greater was the surface roughness of the composite resin samples. The dentifrice Oral-B Pro-Health Whitening caused an increased surface roughness in the composite resins samples when compared to the other groups. Another study investigated the alteration of surface roughness of the nanofill composite was caused by simulated brushing associated with the use of mouthrinses. It was concluded that the surface roughness of the nanofilled composite resin tested can be influenced by the mouthrinse associated with Tooth brushing(29). The surface roughness (Ra), the morphology and composition of filler particles of different composites submitted to Tooth brushing and water storage was analysed in a study. Finishing and polishing resulted in similar Ra for all the composites, while Tooth brushing and water storage increased the Ra of all the tested composite resin materials(30,31). The current study has limitations such as small sample size, we did not use multiple toothpastes and no comparison was done with soft, hard and medium toothbrushes. In the future, randomized control clinical studies which deal with multiple parameters in different types of composite resin restorative materials are also required to prove the clinical efficacy of the various toothpastes.

CONCLUSION

There was no variation in the surface roughness of flowable composite resin samples after brushing simulation with Colgate and Enafix toothpaste. This indicates that brushing simulation does not have any effect on the surface roughness property of flowable composite resin restorative material.

ACKNOWLEDGEMENT

The first author is thankful to the White lab of the Saveetha Dental College for providing support for the conduction of the study.

Conflict of Interest

The author declares that there was no conflict of interest in the present study.

Source of Funding

The funds were provided by:

- Saveetha Dental College and hospitals, Saveetha University of Medical and Technical Sciences, Saveetha University, Chennai.
- Sri Thirumalaipathy Transport.

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Table 1 : Mean. std. deviation and significance testing between groups

Parameters	Groups	N	Mean	std.deviation	Significance
Mean diff Ra	Colgate	4	0.00050	0.000577	-
	Enafix	4	0.00000	0.000000	-
Mean diff Rq	Colgate	4	0.00075	0.000500	0.356
	Enafix	4	0.00050	0.000577	
Mean diff Rz	Colgate	4	0.00775	0.000957	0.506
	Enafix	4	0.00700	0.000816	

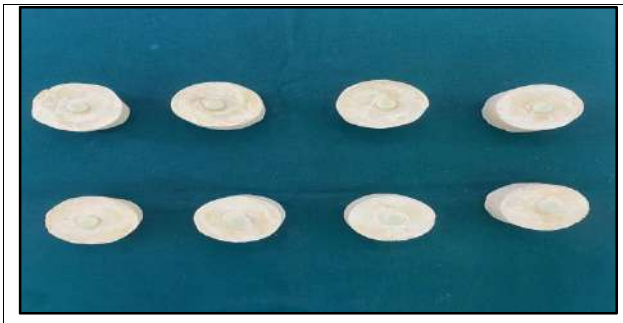

Figure 1: Flowable composite resin samples

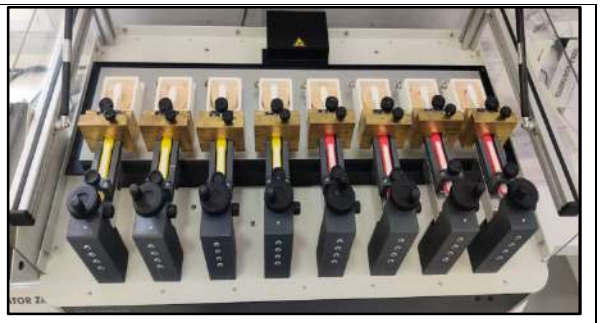

Figure 2: Composite resin samples subjected to brushing simulator

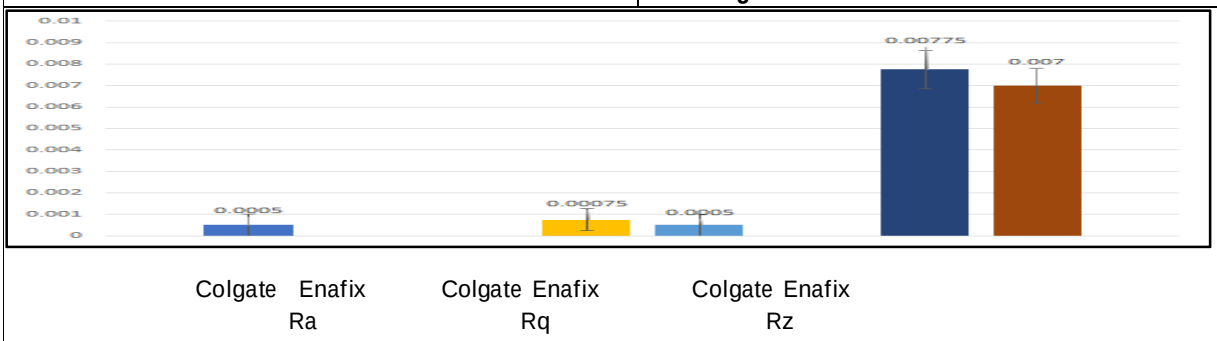

Figure 3: This bar graph depicts the association between the type of paste used and the mean difference in surface roughness. In Ra Blue denotes the mean value of Colgate respectively, in Rz yellow and sky blue denote the mean value of Colgate and Enafix, in Rz dark blue and brown denote the mean value of Colgate and Enafix. Rq and Rz values were decreased in enafix group

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Influence of Zinc Enriched Organic Manures on the Growth, Yield and Nutrient Uptake by Groundnut in Coastal Soil

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Received: 08 Jan 2022 Revised: 12 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

Coastal sandy soils are well known for the deficiency of micronutrients especially zinc. In groundnut production and improving the yield, nutrient uptake and quality of the Zn plays a vital role. A field experiment was conducted to find out the influence of zinc enriched organic manures on the growth, yield and nutrients uptake by groundnut in coastal soil. The experiment was carried out in a farmer's field at Manalmedu coastal village near Chidambaram Taluk, Cuddalore district, Tamil Nadu during December– March 2020. The initial fertility status of experimental soil was pH – 8.37, EC- 1.73 d Sm^{-1}, organic carbon 2.7 g kg^{-1} and represented low status of Zinc (0.71 mg kg^{-1}). The various treatments included were T_1 –Control (100% NPK/ RDF alone), T_2–RDF + FYM @ 12.5 t ha^{-1} , T_3 – RDF + Composted coirpith (CCP) @ 12.5 t ha^{-1}, T_4– RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1}, T_5– RDF + $ZnSO_4$ @ 25 kg ha^{-1} + Composted coirpith (CCP) @ 12.5 t ha^{-1} , T_6– RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% Foliar spray, T_7– RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% Foliar spray, T_8– RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % Foliar spray and T_9– RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % Foliar spray. The experiment was carried out in a Randomized Block Design (RBD) with three replications, using groundnut variety VRI 2 as test crop. The results clearly indicated that, T_9– RDF + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % Foliar spray twice at Pre flowering and flowering stages along with zinc enriched composted coir pith (ZnECCP @ 6.25 t ha^{-1}) application significantly increasing growth, yield and nutrients uptake by groundnut. This treatment recorded the highest pod yield of 2142 kg ha^{-1} and haulm yield of 2958 kg ha^{-1} as compared to control (100 per cent NPK alone).

Keywords: Coastal soil, Zinc enriched organics, Growth, Yield, Nutrient uptake, Groundnut.

INTRODUCTION

Low micronutrient status of the soil leads to poor crop production in coastal soils. This is mainly due to the leaching of nutrients, poor organic matter and less soil management. Zinc is one of the essential elements for both plants and humans as over more than 100 billion peoples are suffering from zinc deficiency in world wide. Above 40 % of yield loss of groundnut is due to zinc deficiency (Ramprosad Nandi *et al.,* 2020). The zinc deficiency in groundnut is most common in coastal sandy soil it cause irregular mottling and yellow – ivory intervinal chlorosis in upper leaf. Zinc is plays a vital role in the cofactor for synthesis of many enzymes, carbohydrate, improves protein metabolism, better nodulation activity and overall it improves the plant health. Further, Zn is recognized as a key element in promoting growth, yield and quality of groundnut (Habbasha *et al*., 2013). Organic matter helps in increasing adsorptive power of soil for cations, anions and micronutrients. These adsorbed ions are released slowly for the benefit of crop during entire growth period. Organic manures are such a valuable by product from various sources of agriculture and allied sectors which are notable changes on physical, chemical and biological properties of the soil in a great manner. Farm yard manure and composted coir pith are easily available and less cost. Use of organic manure is essential for plants and soil because it improves microorganism's activity which is symbiotic relationship with plants and also acts a chelating agent where nutrients are released in slowly and required for plant in various growth stages. By the way when zinc is applied in the form enriched with organics which improve the soil fertility as well as groundnut yield in a significant manner (Maharnor *et al*., 2018). Hence, in the present study was undertaken to find out the influence of zinc enriched organic manures on the growth, yield and nutrient uptake by groundnut in coastal soil.

MATERIALS AND METHODS

A field experiment was carried out in a farmer's field during December – March, 2020 at Manalmedu coastal village, to find out the influence of zinc enriched organic manures on the growth, yield and nutrients uptake by groundnut in coastal soil. The various treatments included were, T_1 –Control (100% NPK/ RDF alone), T_2 –RDF + FYM @ 12.5 t ha^{-1} , T_3– RDF + Composted coirpith (CCP) @ 12.5 t ha^{-1} , T_4– RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1} , T_5– RDF + $ZnSO_4$ @ 25 kg ha^{-1} + Composted coirpith (CCP) @ 12.5 t ha^{-1} , T_6 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% Foliar spray, T_7 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% Foliar spray, T_8 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % Foliar spray and T_9 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0% Foliar spray. The experiment was laid out in a randomized block design (RBD), with three replications, using groundnut variety VRI 2. The experimental soil had sandy texture with pH- 8.37; EC- 1.73 d Sm^{-1}; organic carbon- 2.7 g $kg^{-1}$and Zinc status of 0.71 mg kg^{-1}. The alkaline $KMnO_4$–N; Olsen-P and NH_4OAc-K, were low (159.24 kg ha^{-1}), low (9.73 kg ha^{-1}) and medium (184.23 kg ha^{-1}) status, respectively. Calculated amount of inorganic fertilizer doses of Nitrogen (17 kg N ha^{-1}), Phosphorus (34 kg P_2O_5 ha^{-1}) and Potassium (54 kg K_2O ha^{-1}) were applied through urea, single super phosphate and muriate of potash, respectively. Calculated quantities of organic manures like FYM, composted coirpith (CCP), zinc enriched FYM (ZnEFYM) and zinc enriched composted coirpith (ZnECCP) were applied to the soil as per treatment schedule. Required quantities of Zinc Sulphate as per the treatment schedule were incorporated. Foliar application of $ZnSO_4$ @ 0.5 per cent and Humic acid (HA) @ 1.0 per cent at Pre Flowering Stage (PFS) and at Flowering Stage (FS) was applied as per the treatment schedule. The biofertilizer *Rhizobium* @ 2 kg ha^{-1} was applied to all the experimental plots. The plant samples were collected at different critical stages of crop growth and analyzed for major (N, P and K) and micronutrients (Zn) contents (Jackson, 1973). The total uptake of individual nutrients was computed by multiplying the respective nutrient content with DMP. At the harvest stage, the pod and haulm samples were separately analysed for the above nutrients and uptake were calculated. At harvest stage, pod and haulm yield were also recorded.

RESULTS AND DISCUSSION

Growth Characters

The application of zinc enriched organics + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % foliar spray along with recommended NPK fertilizers was significantly and positively influenced the growth characters *viz.,* plant height and dry matter production at different growth stages of flowering stage (FS), peg formation (PFS) and at harvest stage (HS) of groundnut. The combined application of recommended dose of fertilizer (RDF) + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil application + foliar spray of $ZnSO_4$ @ 0.5% + Humic acid (HA) @ 1.0 per cent twice at pre flowering stage and at flowering stage (T_9), recorded the highest plant height (59.84 cm) and dry matter production (5012 kg ha^{-1}) at the harvest stages of groundnut, respectively. This was followed by the treatments T_8 (RDF + ZnEFYM @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% + HA @ 0.5%), T_7 (RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5%), treatment T_6 (RDF + ZnEYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5%), treatment T_5 (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + CCP @ 12.5 t ha^{-1}) and T_4 (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1}) which recorded a mean growth characters like plant height (57.72, 55.57, 53.36, 51.18, and 48.97 cm), and dry matter production (4854, 4693, 4527, 4354 and 4189 kg ha^{-1}) of groundnut at harvest stage, respectively. This was followed by the application of organics and RDF alone or organics alone or RDF/ Zn alone and without enriched organics and humic acid or Zn foliar spray treatments. The treatment T_3 (application of RDF + CCP @ 12.5 t ha^{-1}) and T_2 (application of RDF + FYM @ 12.5 t ha^{-1}) recorded the lowest growth components as compared to without micronutrients treatments. The control treatment T_1, application of 100% recommended dose of NPK alone (without micronutrient, HA and organics) produced the shortest plants and dry matter production of groundnut over all other Zn enriched organics treatments.

In coastal sandy soil, application of recommended dose of fertilizer (RDF) + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil application + foliar spray of $ZnSO_4$ + Humic acid (HA) @ 0.5 per cent twice at pre flowering and at flowering stage recorded the highest plant height and dry matter production. This might be due to the significant improvement in plant height may be due to significant improvement in nodulation. This observation was in accordance with those of Saha Bholanath *et al.* (2015) and Christopher Aboyeji *et al.* (2019). Indeed, they reported that application of zinc enhances the plant growth enhancement through auxin and better dry matter production. Zinc improved DMP though the nodulation and N fixation by enhanced root growth and by activation of several enzyme systems and auxins. Whereas, Zinc influenced the nitrogen and carbohydrate metabolism of plants which might have contributed for the better growth of groundnut (Sai surya gowthami and Ananda. (2019). The integrated application of NPK + enriched organics in the presence of zinc increased the plant dry matter production. The applied nutrients by their effect on metabolism of cell, promoted the meristematic activity of the crop and its better uptake would result in increased dry matter accumulation. These result obtained in the present investigation was in agreement with the findings of Kheravat *et al.,* (2018) and Poonia *et al*., (2018).

Groundnut Yield

The significant influence of RDF + zinc enriched organic manures along with foliar application of $ZnSO_4$ and humic acid in increasing the pod and haulm yield of groundnut was well evidenced in the present study. The yield realized under the nutrient poverished coastal sandy soil, the highest pod yield (2142 kg ha^{-1}) and haulm yield (2958 kg ha^{-1}) was recorded with combined application of recommended dose of fertilizer + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 per cent twice at pre flowering and flowering stage (T_9). This was followed by the treatments T_8, (RDF + ZnEFYM @ 6.25 t ha^{-1} through soil application and foliar application of $ZnSO_4$@ 0.5% + HA @ 1.0%), T_7 (RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5%) and T_6 (RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5%) which recorded the pod (2074, 2002, and 1927 kg ha^{-1}) and haulm (2859, 2757 and 2659 kg ha^{-1}) yield of groundnut, respectively. This was followed by the treatments arranged in the descending order like T_5>T_4>T_3 and T_2. These treatments were also statistically significant. Among the various Zn enriched treatments, the treatment (T_9), 100% recommended dose of NPK + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil + foliar spray of $ZnSO_4$ and Humic acid @ 0.5% twice recorded a pod and haulm yield of 2142 kg ha^{-1} and 2958 kg ha^{-1} which

was 27.63 and 28.22 per cent increase over control or 100 per cent NPK alone. The control treatment T_1, 100 per cent NPK alone recorded a lower pod (1550 kg ha^{-1}) and haulm (2123 kg ha^{-1}) yield of groundnut, respectively.

The groundnut yield increased with application of 100% recommended dose of NPK + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$@ 0.5% and Humic acid @ 1.0% twice. This might be due to the application of Zn enriched organics helped in the slow and steady rate of nutrient release into soil solution to match the required absorption pattern of groundnut thereby increased the yield. This corroborates the earlier report of Kadam *et al*., (2018). Further the rapid mineralization of N, P and K from inorganic fertilizers and steady supply of these nutrients from Zn enriched coirpith, might have met the nutrient requirement of crop at the critical stages in addition, the beneficial influence of Zn through activation of various enzymes and basic metabolic rate in plants, facilitated the synthesis of nucleic acids and hormones, which in turn enhanced the pod yield due to greater availability of nutrients and photosynthates. These results are in agreement with those of Reddy *et al.,* (2011) and Shubhangi *et al.,* (2014). Foliar application of $ZnSO_4$ and humic acids at pre flowering and flowering stages of crop growth were effectively absorbed in the plant system and translocated into sink which resulted in more number of pods $plant^{-1}$. Further, increased in photosynthesis during growth stages might be contributed for greater assimilates supply to the pods which resulting in better seed setting and also betterment of higher pod yield of groundnut. The results are in conformity with Jeetarwal *et al*. (2014); Kalita *et al*. (2015) and Gulam masthan vali *et al*., 2020

Major nutrients uptake (NPK)

NPK uptake

The NPK uptake of groundnut at all the critical stages of crop growth and in pod and haulm was significantly increased with RDF + zinc enriched organic manures along with foliar application of $ZnSO_4$ and humic acid. Among the treatments evaluated, the combined application of recommended NPK and Zn enriched organics through soil along with foliar spray of $ZnSO_4$ and humic acid (T_9), which registered the highest N (79.90 and 62.37 kg ha^{-1}) P (10.47 and 13.21 kg ha^{-1}) and K(34.21 and 69.26 kg ha^{-1}) by pod and haulm, respectively. This was followed by T_8– RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % foliar spray, T_7 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% and T_6 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% foliar spray which recorded a lowest NPK uptake as compared to above said treatments. This was followed by the treatments arranged in the descending order like $T_5 > T_4 > T_3$ and T_2. These treatments were also statistically significant. The lowest NPK uptake was registered in control (100% NPK alone).

The NPK uptake was increased by combined application of NPK + Zn enriched organics through soil and foliar spray of $ZnSO_4$ @ 0.5% twice along with organics as compared to sole application of Zn either through soil or foliar. This is because of the fact that the micronutrient like zinc is involved in nitrogen fixation and translocation into plant parts, which might have increased the N content of plants. The higher nitrogen absorption may also be due to stimulatory effect of zinc on nitrogen uptake. In line with the present study, Kabir *et al.*, (2013); Kamdi *et al.,* (2014) and Meena *et al.,* (2017) also reported similar results. The addition of NPK fertilizers along with foliar spray of humic acid (HA) enhanced the uptake of NPK by groundnut. This might be due to increased availability of these nutrients released from applied fertilizers. Further, humic substances as humic acids applied through foliage would have been easily absorbed and translocated in the plants without any loss which resulted in enhanced nutrients uptake by the crop. Similar findings were observed by Heba Mohamed Noman *et al*., (2015). The higher P content and uptake may be due to the solubilization of native phosphorus by the organic acids namely, lactic, glycolic, citric and succinic acid present in ZnECCP. In addition, applied NPK resulted in better root growth and increased physiological activity of roots to absorb more phosphorus. These findings are in conformity with Verma *et al.* (2017) and Nkaa *et al.* (2014).

The overall improvement of NPK uptake with application of recommended NPK along with Zn enriched organic manures increased the absorption power of the soil for cations and anions, particularly nitrogen and phosphate. These ions are released gradually during entire growing period of the crop which might have increased concentration as well as improved the plant growth and accumulation of greater biomass which helped to increase

the uptake. In line with the present study Elayaraja and Singaravel (2016) also reported similar results. Further added Zn enriched organics improved the organic carbon content of soil through decomposition which helped in the release of organically bounded both macro and micronutrients in soil. These findings are in agreement with Jahiruddin *et al*. (2014) and Arulrajsekaran *et al*. (2021).

Zinc uptake

The influence of the combined application of RDF along with Zn enriched organic manures and $ZnSO_4$ + HA foliar spray had significant influence on zinc uptake by groundnut at all the critical stages *viz.,* flowering, pod formation and at harvest stage in grain and haulm. Among the various treatments, the highest Zn uptake by pod (322.68 g ha^{-1}) and haulm (224.44 g ha^{-1}) was recorded with the application of RDF + ZnECCP @ 6.25 t ha^{-1} through soil and foliar spray of $ZnSO_4$ @ 0.5% + Humic acid @ 1.0% twice (T_9). This was followed by application of RDF + ZnEFYM @ 6.25 t ha^{-1} through soil and foliar application of $ZnSO_4$ + HA@ 0.5 per cent (T_8), application of RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% (T_7), and application of RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% (T_6) which recorded a Zn uptake of 311.79, 311.79, 300.77, and 289.67 g ha^{-1} by pod and 216.90, 209.32 and 201.77 g ha^{-1} by haulm, respectively. This was followed by the treatments which received organics + $ZnSO_4$ along with recommended NPK (without Zn and HA foliar) applied treatments. The treatment T_5, (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + CCP @ 12.5 t ha^{-1}) and T_4 (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1}) recorded a lowest Zn uptake of groundnut as compared to above said treatments. This was followed by the treatments T_3 and T_2. These two treatments were also statistically significant. The control (100% NPK alone) treatment recorded the lowest Zn uptake at all the critical stages of groundnut.

The application of RDF + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% and humic acid @ 1.0% twice significantly increased Zn uptake by groundnut. The increased these nutrients might be due to addition of micronutrients as impurities along with fertilizers. Further the application of Zn enriched organics might have increased the availability through enhanced mineralization and chelation action which have increased the absorption and utilization of these nutrients. The earlier reports of Nayek *et al.,* (2015) and Prashantha *et al.,* (2019) support the present findings. Further, increased uptake of Zn by groundnut pod and haulm were noticed in the treatment supplied with ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% and humic acid @ 1.0% twice. As uptake being a parameter associated with the DMP, increased growth and yield characters due to humicacid and $ZnSO_4$ resulted in the production of higher dry matter production with this treatment. These findings corroborate the earlier results of Kulhare *et al*., (2014) and Jat *et al.,* (2015).

CONCLUSIONS

The results of the present investigation clearly indicated that for increasing the growth, yield and nutrient uptake by groundnut, the treatment which received RDF + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% and Humic acid @ 1.0% twice at pre flowering and flowering stage would be beneficial.

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Table 1. Effect of Zn enriched organics on the growth and yield of groundnut

Treatments	Plant height (cm)			Dry matter production (kg ha^{-1})			Yield (kg ha^{-1})	
	FS	PFS	HS	FS	PFS	HS	Pod	Haulm
T_1	21.19	29.17	42.49	1464	1882	3698	1550	2123
T_2	22.28	30.78	44.61	1508	1968	3866	1632	2225
T_3	23.39	32.42	46.75	1550	2043	4021	1710	2323
T_4	24.48	34.08	48.97	1588	2138	4189	1782	2438
T_5	25.69	35.69	51.18	1622	2230	4354	1856	2549
T_6	2688	37.35	53.36	1664	2318	4527	1927	2659
T_7	28.02	38.97	55.57	1703	2400	4693	2002	2757
T_8	29.14	40.71	57.72	1741	2478	4854	2074	2859
T_9	30.23	42.36	59.84	1776	2550	5012	2142	2958
SE_D	0.54	0.95	1.12	21	28	93	23	35.25
CD (p=0.05)	1.02	1.57	2.04	32.8	68	152	65	94

Table 2. Effect of Zn enriched organics on the nutrients uptake by groundnut

Treatments	Nitrogen (kg ha^{-1})				Phosphorus (kg ha^{-1})				Potassium (kg ha^{-1})				Zinc (g ha^{-1})			
	FS	PFS	HS		FS	PFS	HS		FS	PFS	HS		FS	PFS	HS	
			Pod	Haulm			Pod	Haulm			Pod	Haulm			Pod	Haulm
T_1	37.59	47.89	57.74	44.92	6.38	5.32	4.49	6.38	30.86	39.97	17.17	37.58	245.54	316.26	234.41	163.96
T_2	39.44	50.21	60.54	47.16	6.71	6.20	5.20	7.20	33.95	44.09	19.42	41.64	256.82	330.84	245.38	171.55
T_3	41.22	52.58	63.37	49.32	7.07	7.04	6.01	8.01	37.13	48.51	21.63	45.66	268.13	345.35	256.51	179.13
T_4	43.04	54.92	66.12	51.44	7.42	7.86	6.79	8.84	40.38	52.90	23.81	49.65	279.49	360.01	267.60	186.68
T_5	44.81	57.21	68.89	53.69	7.86	8.65	7.49	9.77	43.59	57.22	25.97	53.63	290.83	374.65	278.55	194.20
T_6	45.65	59.52	71.71	55.91	8.24	9.41	8.25	10.68	46.75	61.49	28.09	57.58	302.15	389.22	289.67	201.77
T_7	47.47	61.86	74.49	58.09	8.66	10.14	8.96	11.49	49.88	65.71	30.18	61.47	313.44	403.87	300.77	209.32
T_8	49.26	64.17	77.21	60.25	9.05	10.86	9.73	12.36	52.99	69.92	32.22	65.38	324.66	418.48	311.79	216.9
T_9	50.98	66.44	79.90	62.37	9.42	11.51	10.47	13.21	56.07	74.11	34.21	69.26	335.93	433.06	322.68	224.44
E_D	0.82	1.15	0.97	0.77	0.11	0.36	0.34	0.38	1.55	1.98	0.96	1.91	4.19	5.41	4.0	2.80
CD (p=0.05)	1.69	2.21	2.66	2.07	0.31	0.65	0.60	0.79	2.98	4.09	1.89	3.72	11.19	14.43	10.75	7.48

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

γ −Open Sets in Tri Topological Space

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Received: 04 Feb 2022 Revised: 16 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

The cause of this paper is to introduce and examine the properties of tri- γ- open set in tritopological space. In this paper we also learn about tri- γ- locally closed set and relationship between tri-b-open set and tri- γ- open set.

Keywords: tri- b-open set, tri-α- open set, tri- locally closed, tri- γ- open set .

INTRODUCTION

D. Andrijevic and M.Ganster [3] delivered a category of generalized open sets in a topological spaces, the so referred to as γ-open set. The magnificence of γ-open sets includes all semi-open sets, pre-open sets, and b-open sets. In 1963 J.C Kelly [4] introduced the idea of bitopological spaces. Tri topological space is a generalization of bitopological spaces. The examiner of tri-topological space changed into first initiated via Martin Kovar [6]. Palaniammal [7] and Hameed [5] studied separation axioms in tri-topological spaces and gives the definition of tri open set in tri topological spaces. Tapi [9] introduced semi open and pre open set in tri topological space. Priyadharsini [8] introduced tri-b open sets in tri topological spaces. In 2013 Baby Bhattacharya and Arnab Paul[10] introduced "On bitopological γ- open set". The motive of this paper is to introduce the γ- open set in tritopological space and also take a look at the properties of this set and investigate the connection among tri γ-open set, tri locally -closed set , tri γ-locally closed set and tri b-open set .

Preliminaries

In the course of this paper by X we mean tritopological Space (X,τ_1, τ_2, τ_3).

Definition :2.1([7]).
Let X be a nonempty set and τ_1, τ_2 and τ_3 are three topologies on X. The set X together with three topologies is called a tri topological space and is denoted by (X,τ_1, τ_2, τ_3).

Definition :2.2 ([5]).
A subset A of a topological space X is called tri- open set if A $\in \tau_1 \cup \tau_2 \cup \tau_3$ and complement of tri- open set is tri-closed set.

Definition:2.3 ([7])
A subset A of a bitopological Space X is
(i) tri semi-open set if A ⊆ tri-cl(tri-int(A))
(ii) tri pre-open set if A ⊆ tri-int(tri-cl(A))
(iii) tri α-open set if A⊆ tri-int(tri-cl(tri-int(A)))
(iv) Tri regular- open set if A = tri- int(tri-cl(A))

Definition :2.4([8]).
Let (X,τ_1, τ_2, τ_3) be a tri topological space, a subset A of a space X is said to be tri b open set if A ⊂ tri-cl(tri-int(A)) ∪ tri- int(tri-cl(A)).
The Complements of all the above mentioned open sets are called their respective closed sets. The family of all tri open sets [tri semi open, tri α-open,tri-regular-open, tri b-open] of X will be denoted by tri O(X)[resp. tri SO(X) , tri α O(X),tri RO(X), tri b O(X)].

Definition:2.5 ([8])
Let (X,τ_1, τ_2, τ_3) be a tri topological space and let A ⊂ X. The intersection of all tri b closed sets containing A is called the tri -b closure of A and denoted by tri- b-cl(A). tri b-int (A) is the union of all tri -b open sets contained in A, tri b – cl(A) is the intersection of all tri b closed sets containing A.

Lemma:2.5.1([8])
Let (X,τ_1, τ_2, τ_3) be a tri topological space and let A ⊆ X. Then
(a) [tri-bcl(A)]^c = [tri-bcl(A)^c] .
(b) [tri-bint(A)]^c = [(tri- bcl(A)^c] .

Remark:2.5.2 In a tri topological space tri-b open set and tri-locally closed are independent.

Tri- γ- open set:

Definition: 3.1 A subset A of (X,$\boldsymbol{\tau_1, \tau_2, \tau_3}$) is said to be tri $\boldsymbol{\gamma}$-open set if for any non empty tri pre-open set B in X such that A ∩ B ⊆ tri-int(tri-cl(A ∩ B)).

Example: 3.1.1 Let X={a,b,c}, $\boldsymbol{\tau_1}$= {∅, X, {a,b}}, $\boldsymbol{\tau_2}$= {∅, X}, $\boldsymbol{\tau_3}$= {∅, X,{a}}
Tri-$\boldsymbol{\gamma}$-O(X) = {∅, X,{a},{a,b},{a,c}}.

Remark: 3.1.2 In a tritopological (X,$\boldsymbol{\tau_1, \tau_2, \tau_3}$) a tri-open set and a tri $\boldsymbol{\gamma}$-open set are independent as seen in the following examples.

Example:3.1.3 Let X= {a,b,c}, $\boldsymbol{\tau_1}$= {∅, X}, $\boldsymbol{\tau_2}$ ={∅, X,{b},{a,b}}, $\boldsymbol{\tau_3}$= {∅, X,{c},{a,c}}
tri-O(X) ={∅, X,{b},{a,b},{c},{a,c} },
tri-Cl(X) = {∅, X,{c},{b},{a,c},{a,b}}
tri -PO(X)= {∅, X,{a},{b},{a,b}},
tri - $\boldsymbol{\gamma}$-O(X)= {∅, X, {a},{b},{a,b}}
Here,{a,c} is tri-open set but not a tri $\boldsymbol{\gamma}$-open set.

Example: 3.1.4 Let X= {a,b,c}, τ_1= {∅, X,{c},{a,c}}, τ_2 ={∅, X,{a}}, τ_3= {∅, X,{b,c}}
tri-O(X)= {∅, X, {a},{c},{a,c},{b,c},
tri -PO(X)= {∅, X,{a},{b},{c},{a,c},{b,c}},
tri - γ-O(X)= {∅, X,{a},{b},{c},{a,c},{b,c}},
Here, {b} is tri-γ-open set but not a tri-open set.

Definition:3.2 A subset A of (X,τ_1, τ_2, τ_3) is called
(i) Tri locally closed if A= U ∩ V, where U∈ tri-open and V is a tri-closed set.
(ii) Tri locally γ – closed if A= U ∩ V, where U∈ tri-open and V is a tri γ -closed set.

Remark:3.2.1 Tri γ-open set and tri locally closed sets are independent as seen from the following example.
Example: 3.2.2 Let X= {p,q,r,s}, τ_1= {∅, X,{q,r},{p,s}}, τ_2 ={∅, X,{p,q,r}},
τ_3= {∅, X,{p},{p,q}}
tri -O(X)={∅, X,{p},{p,q},{q,r},{p,s},{p,q,r}}
tri-Cl(X)= {∅, X,{q,r,s},{r,s},{p,s},{q,r},{s}}
tri - γ-O(X)= {∅, X, {p},{q},{p,q},{p,r},{p,s},{q,r},{q,s},{p,q,r},{p,q,s},{q,r,s}}
tri -LC(X)= {∅, X,{p},{q},{r},{s},{p,s},{q,r},{p,q},{r,s},{p,q,r},{q,r,s}}
Here, {p,q,s} is tri γ-open set but not tri locally closed.
Similarly, tri- γ-cl(X)= {∅, X,{p},{r},{s},{p,s},{p,r},{q,r},{q,s},{r,s},{p,r,s},{q,r,s}
tri-L- γ-cl(X)= {∅, X,{p},{q,},{r},{s},{p,q},{q,r},{p,s},{p,r},{p,q,r}}
Here, {q,r,s} is not a tri locally γ – closed but {q,r,s} is a tri γ-open set.

Definition: 3.3 A space is called tri- extremely γ - disconnected space if tri γ- closure of each tri γ-open set is tri γ-open.
Example: 3.3.1 Let X= {a,b,c}, τ_1= {∅, X}, τ_2 ={∅, X,{b,c}}, τ_3= {∅, X,{c}}
tri – γ O(X)= {∅, X,{c},{b,c},{a,c}}, tri – γ Cl(X)= {∅, X,{a,b},{a},{b}}
Hence every tri γ-closure of each tri γ-open set is tri γ-open set.

Theorem: 3.3.2
For a subset A of an tri extremely γ - disconnected space X, if A is tri γ-open set and tri- locally closed set, then A is tri-open.
Proof: Let A be tri γ-open set and tri locally closed set. So
(A∩ B) ⊆ *tri-int(tri-Cl(A∩ B)) and A=* $\boldsymbol{U \cap tri - Cl(A)}$.
Then (A∩ B) ⊆ *tri-int(tri-Cl(A)* ∩ $\boldsymbol{tri - Cl}$*(B))*
⊆ *tri-int(tri-Cl* ($\boldsymbol{U \cap tri - Cl(A)}$) ∩ *tri-Cl(B))*
⊆ *tri-int(*$\boldsymbol{U \cap tri - Cl(A)}$ ∩ *tri-Cl(B)) (since X is tri extremely* γ *–disconnected)*
⊆ *tri-int (A∩ tri-Cl(B))*
⊆ *tri-cl(tri-int (A∩ B))*
= *tri-int(A∩ B)*
Hence (A∩ B) ⊆ *tri-int (A∩ B)*
Therefore A is tri-open.

Preposition: 3.3.3 Let H be a subset of X, H is tri locally γ-closed set iff there exists an tri- open set U ⊆ X such that H = U ∩ tri γ cl-(H).
Proof: Since H is tri locally γ-closed ⇒ H = U ∩ $\boldsymbol{F}$, where U is tri-open and F is tri locally γ-closed. So H ⊆ U, H ⊆ F
H⊆ tri γ-cl (H) ⊆ tri γ-cl (F) = F

Hence H ⊆ U ∩ tri **γ**-cl(H) ⊆ U ∩ tri **γ**-cl(F) ⊆ U ∩ F = H.
Hence H =U ∩ tri **γ**-cl(H).
Conversely since tri **γ**-cl(H) is tri **γ**-closed and H = U∩ **tri γ**-cl(H) then H is tri locally **γ**-closed.

Preposition:3.3.4 The union of any family of tri **γ** -open set is a tri **γ** -open set .

Proof: Let *A* and *B* be any two tri γ- open set. So there exist two non empty pre-open set C and D, we have
(*A* ∩*C*) ⊆tri- int(tri-*cl*(*A* ∩*C*)).
(*B* ∩ *D*) ⊆tri- int(tri-*cl*(*B* ∩ *D*))
Now,(*A*∪*B*) ∩ (*C* ∪*D*) = (A ∩ C) ∪ (B ∩ D)
⊆ *tri-int(tri-cl*(A∩ C)) ∪ tri-int(tri-cl (B ∩ D))
⊆ *tri-int(tri-cl*(A∪ B)) ∩ tri-int(tri-cl (C ∪ D))
⊆ *tri-int(tri-cl*((A∪ B) ∩ (C ∪ D))).
Hence the union of any family of tri γ-open set is a tri γ-open set.

Preposition:3.3.5 The intersection of any tri γ-open set is a tri γ-open set .
Proof: Let *A* and *B* be any two tri γ- open set .
So, for any tri pre open set *C*
(A ∩ C) ⊆ tri- int(tri-cl(A ∩ B)) and (B ∩ C) ⊆ tri- int(tri-cl(B ∩ C)
Now, (A ∩ B) = A ∩ (B ∩ C) .
By the definition of tri γ -open set (A ∩ (B ∩ C)) is a tri pre open set. Hence A ∩ B is a tri γ-open set.

Preposition: 3.3.6 Let A be a subset of (X,τ_1,τ_2,τ_3) and if A is tri locally γ-closed then
(i) tri γ cl(A)- A is tri γ- closed.

(ii) [A ∪ (X- γ cl(A)] is tri γ- open set.
(iii) A ⊆ γ int (A ∪ (X-γcl(A))
Proof: (i) If *A* is an tri locally γ-closed, then there exist an tri open set U such that

A=U ∩tri γ*cl*(*A*)
Now, *tri* γ*cl (A) – A= tri* γ*cl(A) – [U* ∩*tri* γ*cl(A)]*
= tri γ*cl (A)* ∩*[(X-U)* ∪ *(X-tri cl(A))].*
= tri γ *cl(A)* ∩ *(X-U), which is tri* γ*- closed.*(by proposition 3.3.3)
(ii) Since *tri cl(A)-A is tri* γ *- closed,* then *[X-(tri* γ *cl(A)-A]* is tri γ -open .
And *{ [X –tri cl(A)-A] = (X- tri cl(A))* ∪ *(X* ∩ *A) = A* ∪ *[X-tri cl(A)]}.*
Hence *[A* ∪ *(X-cl(A))]* is tri γ-open.
(iii) It is clear that A ⊆ *[A* ∪ *(X-tri* γ *cl(A))] = tri* γ *int [(A* ∪ *(X-* γ *cl(A))].* Hence the proof.

Remark:3.15 Tri **α**-open set and tri locally **γ**-closed are independent as seen from the following example.
Example:3.16 Let X= {p,q,r,s}, $\boldsymbol{\tau_1}$= {∅, X,{p,q,s}}, $\boldsymbol{\tau_2}$ ={∅, X,{q},{q,s}},
$\boldsymbol{\tau_3}$= {∅, X,{r}}, tri **α**-O(X)={ ∅, X,{q},{r},{p,q},{q,s}{p,q,s}}
tri L-**γ** Cl(X) = {∅, X,{q},{r},{s},{p,r},{p,s},{q,s},{p,q,s},{p,r,s}}
Here, {p,r,s} is a tri locally **γ** -closed set but not tri **α**-open set. And also {p,q} is a tri **α**-open set but not tri locally- **γ** closed set.

(ii) [A ∪ (X- γ cl(A)] is tri γ- open set.
(iii) A ⊆ γ int (A ∪ (X-γcl(A))
Proof: (i) If *A* is an tri locally γ-closed, then there exist an tri open set U such that

$A = U \cap \text{tri } \gamma cl(A)$

Now, $tri\ \gamma cl\ (A) - A = tri\ \gamma cl(\ A) - [U \cap tri\ \gamma cl(A)]$

$= tri\ \gamma cl\ (\ A) \cap [(X-U) \cup (X-tri\ cl(A))].$

$= tri\ \gamma\ cl(A) \cap (X-U)$, *which is tri* γ*- closed.*(by proposition 3.3.3)

(ii) Since $tri\ cl(A)-A$ *is tri* γ *- closed,* then $[X-(\ tri\ \gamma\ cl(A)-A]$ is tri γ -open .

And $\{[\ X - tri\ cl(A)-A] = (X-\ tri\ cl(A)) \cup (X \cap A) = A \cup [X-tri\ cl(A)]\}$.

Hence $[A \cup (X-cl(A))]$ is tri γ-open.

(iii) It is clear that $A \subseteq [A \cup (X-tri\ \gamma\ cl(A))] = tri\ \gamma\ int\ [(A \cup (X-\ \gamma\ cl(A))]$. Hence the proof.

Remark:3.15 Tri $\boldsymbol{\alpha}$-open set and tri locally $\boldsymbol{\gamma}$-closed are independent as seen from the following example.

Example:3.16 Let X= {p,q,r,s}, $\boldsymbol{\tau_1}$= {∅, X,{p,q,s}}, $\boldsymbol{\tau_2}$ ={∅, X,{q},{q,s}},

$\boldsymbol{\tau_3}$= {∅, X,{r}}, tri $\boldsymbol{\alpha}$-O(X)={ ∅, X,{q},{r},{p,q},{q,s}{p,q,s}}

tri L-$\boldsymbol{\gamma}$ Cl(X) = {∅, X,{q},{r},{s},{p,r},{p,s},{q,s},{p,q,s},{p,r,s}}

Here, {p,r,s} is a tri locally $\boldsymbol{\gamma}$ -closed set but not tri $\boldsymbol{\alpha}$-open set. And also {p,q} is a tri $\boldsymbol{\alpha}$-open set but not tri locally- $\boldsymbol{\gamma}$ closed set.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

On a Bulk Arrival Vacation Queue with Exceptional First Service and Server Breakdowns

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Received: 21 Feb 2022 Revised: 14 Mar 2022 Accepted: 25 Mar 2022

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ABSTRACT

We have considered a multiple vacation queueing model in which customers arrive in batches of random size according to a time homogeneous Poisson process and server is subject to breakdown while serving. Server resumes operation immediately after repair. After each busy period the server goes for a vacation and after returning from a vacation the server activates only if customer are in the queue, otherwise takes another vacation. First service of each busy period is exceptional. Service times, repair times, times to breakdown and length of vacation time follows exponential distribution. This type of queueing model finds its application in many fields like transportation network, computer communication networks, maintenance and in medical services. Using generating function techniques mean queue length and average number of customer in the queues are obtained.

Keywords: Bulk Arrival, Exceptional Service, Vacation, Server Breakdown, Repair, Generating Function Technique.

INTRODUCTION

This paper demonstrates a bulk arrival multiple vacation queueing system where first customer of each busy period receives exceptional service. System is subject to breakdown with constant rate and server goes on vacation at the instant when the queue becomes empty. Because of vast application of bulk arrival queues with variable service rates many researchers have contributed in this field. The concept of first exceptional service M/G/1 queueing system was introduced by Weltch [1964]. For determining stationary probabilities of queue length, a computational scheme was explored by Li Huan et.al. [1996] where service depends on the number of customer arrivals. Yutaka Baba [1999] investigated M/G/1 queue and presented the scheme for moments of queue length and sojourn time distribution,

where first N customer receives exceptional service. Gray et. al. [2000] contributed by studying vacation queue model with service breakdowns. This model was characterized by different arrival rates during vacation, active service and breakdowns. Ghimmere et. al. [2011] studied M/M/1 queue with heterogeneous arrival and departure. Niranja and Indhira [2016] presented an excellent review on classical bulk arrival and batch service queue model. They provided enough information about the work to analyse the model of congestion problems. Bharathidass et. al. [2018] examined a single server Erlangian bulk service queue with certain concepts like vacation, break down and repair. In this paper they derived state probabilities and the expected number of units in the system under different positions. Ayyappan & Karpagam [2019] studied the behavior of a non markovian bulk service queueing model with unreliable server, immediate feedback, N-policy, Bernoulli schedule multiple vacation and stand-by server. Khurana & Sharma [2019] considered a M/M/1 queueing system where first N customers of each busy period receive exceptional service. Using generating function technique mean queue length formula and average number of the customer in the system was obtained and then Khurana & Sharma [2020] proposed the model with coxian-2 type service model. Anyue Chen et. al. [2020] studied a modified Markovian bulk-arrival and bulk-service queue incorporating general state-dependent control. The stopped bulk-arrival and bulk-service queue was first investigated, and the relationship between this stopped queue and the full queueing model is examined and exploited. Agrawal S. K. et. al. [2020] worked upon the tri-cum biserial bulk queue model linked with a common server with fixed batch size. They have assessed various queuing model performances using the probability generating function technique and other statistical tools.

Model Discription

This paper studies a single server multiple vacation queueing model which is subject to server breakdowns, while in operation. Service is resumed after a repair process. Customers arrive in batches of random size according to a time homogeneous Poisson process but with different rates during vacation, service and breakdown state of the system. The first customer of each busy period receives an exceptional service and server goes on vacation at the end of each busy period. Uninterrupted service times, repair times, vacation times and time to breakdown following a repair or vacation have exponential distribution. The generating function approach is used to find mean queue length, mean waiting time in queue and system, average number of customers in the system.

Assumptions & Notations

λ_0: composite arrival rate during vacation

λ: composite arrival rate during active service

λ_1: composite arrival rate during breakdown

ν: vacation rate

μ_1: service rate for first customer of a busy period

μ_2: service rate for second or following customer of a busy period

b: breakdown rate

r: repair rate

c_{1k}: probability of batch size k when composite arrival rate is λ_0

c_{2k}: probability of batch size k when composite arrival rate is λ

c_{3k}: probability of batch size k when composite arrival rate is λ_1

$\overline{c_1}, \overline{c_2}, \overline{c_3}$: mean batch size during vacation, active service and breakdown respectively.

$Q_0 = \frac{\lambda_0}{\lambda_0 + \nu}$

$\lambda_0, \lambda > 0, \quad \lambda_1 \geq 0$

$\mu_1, \mu_2, b, r > 0$

States for the model

(0, i): i customer in the queue and the server is on vacation. $i \geq 0$

It's probability is$P_{o,i}$

(1, i, 1):i customer in the system during active service and customer being served is first of the busy period. $i \geq 1$

Its probability is $P_{1,i,1}$

(1, i, 2):i customer in the system during active service and customer being served is second or following customer of the busy period. $i \geq 1$

It's probability is $P_{1,i,2}$

(2, i, 1):i customer in the system during repair process and the customer which was being served was first of the busy period. $i \geq 1$

It's probability is $P_{2,i,1}$

(2, i, 2): i customer in the system during repair process and the customer which was being served was second or following customer of the busy period. $i \geq 1$

It's probability is $P_{2,i,2}$

Normalization condition is

$$\sum_{i=0}^{\infty} P_{0,i} + \sum_{i=1}^{\infty} P_{1,i,1} + \sum_{i=1}^{\infty} P_{1,i,2} + \sum_{i=1}^{\infty} P_{2,i,1} + \sum_{i=1}^{\infty} P_{2,i,2} = 1$$

Partial generating Function for the model

We define following generating functions for the model

$$F_0(z) = \sum_{i=0}^{\infty} P_{0,i}\, z^i,$$

$$F_{1,1}(z) = \sum_{i=1}^{\infty} P_{1,i,1} z^i, \qquad F_{1,2}(z) = \sum_{i=1}^{\infty} P_{1,i,2} z^i,$$

$$F_{2,1}(z) = \sum_{i=1}^{\infty} P_{2,i,1} z^i, \qquad F_{2,2}(z) = \sum_{i=1}^{\infty} P_{2,i,2} z^i,$$

Finally $F(z) = F_0(z) + F_{1,1}(z) + F_{1,2}(z) + F_{2,1}(z) + F_{2,2}(z)$ is the generating function for the queue length distribution.

$$C_1(z) = \sum_{i=1}^{\infty} c_{1i} z^i, \qquad C_2(z) = \sum_{i=1}^{\infty} c_{2i} z^i, \qquad C_3(z) = \sum_{i=1}^{\infty} c_{3i} z^i$$

Balance Equations for Queue Length Distribution

For subsequent analysis the balance equations of related steady state probabilities are

$$\lambda_0 P_{0,0} = \mu_1 P_{1,1,1} + \mu_2 P_{1,1,2} \quad (1)$$

$$(\lambda_0 + \nu) P_{0,i} = \lambda_0 \sum_{k=1}^{i} P_{0,i-k}\, c_{1k}, \; i \geq 1 \quad (2)$$

$$(\lambda_0 + \mu_1 + b) P_{1,1,1} = \nu P_{0,1} + r P_{2,1,1}, \quad (3)$$

$$(\lambda + \mu_2 + b) P_{1,1,2} = \mu_1 P_{1,2,1} + \mu_2 P_{1,2,2} + r P_{2,1,2}, \quad (4)$$

$$(\lambda + \mu_1 + b) P_{1,i,1} = \lambda \sum_{k=1}^{i} P_{1,i-k,1}\, c_{1k} + \nu P_{0,i} + r P_{2,i,1}, \; i \geq 2 \quad (5)$$

$$(\lambda + \mu_2 + b) P_{1,i,2} = \lambda \sum_{k=1}^{i} P_{1,i-k,2}\, c_{2k} + \mu_1 P_{1,i+1,1} + \mu_2 P_{1,i+1,2} + r P_{2,i,2}, \; i \geq 2 \quad (6)$$

$$(\lambda_1 + r) P_{2,1,1} = b P_{1,1,1} \quad (7)$$

$$(\lambda_1 + r) P_{2,1,2} = b P_{1,1,2} \quad (8)$$

$$(\lambda_1 + r) P_{2,i,1} = \lambda_1 \sum_{k=1}^{i} P_{2,i-k,1} c_{3k} + b P_{1,i,1} \quad i \geq 2 \quad (9)$$

$$(\lambda_1 + r) P_{2,i,2} = \lambda_1 \sum_{k=1}^{i} P_{2,i-k,2} c_{3k} + b P_{1,i,2} \quad i \geq 2 \quad (10)$$

Now using generating function technique, we get from equation (2)

$$F_0(z) = \sum_{i=0}^{\infty} P_{0,i}\, z^i = \frac{P_{0,0}}{1-\rho_0 C_1(z)} \quad , \tag{11}$$

From equation (3) and (5), we get

$$\{\lambda + \mu_1 + b - \lambda C_2(z)\}F_{1,1}(z) = \nu[F_0(z) - P_{0,0}] + rF_{2,1}(z) \tag{12}$$

From equation (4) and (6), we get

$$\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z}\right\}F_{1,2}(z) = \frac{\mu_1}{z}F_{1,1}(z) + rF_{2,2}(z) - \lambda_0 P_{0,0} \tag{13}$$

From equation (7) and (9), we get

$$F_{2,1}(z) = \frac{b}{\lambda_1 + r - \lambda_1 C_3(z)}F_{1,1}(z) \tag{14}$$

From equation (8) and (10), we get

$$F_{2,2}(z) = \frac{b}{\lambda_1 + r - \lambda_1 C_3(z)}F_{1,2}(z) \tag{15}$$

Then, from equation (12) and (14), we have

$$\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}F_{1,1}(z) = \nu[F_0(z) - P_{0,0}]$$

$$F_{1,1}(z) = \frac{\nu\rho_0 P_{0,0} C_1(z)}{\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\{1 - \rho_0 C_1(z)\}} \tag{16}$$

And from equation (13) and (15), we have

$$\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}F_{1,2}(z) = \frac{\mu_1}{z}F_{1,1}(z) - \lambda_0 P_{0,0}$$

$$F_{1,2}(z) = \frac{1}{\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}}\left[\frac{\nu\rho_0 \mu_1 P_{0,0} C_1(z)}{z\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\{1 - \rho_0 C_1(z)\}} - \lambda_0 P_{0,0}\right] \tag{17}$$

Generating function F(z) using equation (14) to (17) is

$$F(z) = \frac{P_{0,0}}{1 - \rho_0 C_1(z)} + \left(1 + \frac{b}{\lambda_1 + r - \lambda_1 C_3(z)}\right)\left(F_{1,1}(z) + F_{1,2}(z)\right)$$

Where $F_{1,1}(z) + F_{1,2}(z) = \dfrac{\nu\rho_0 P_{0,0} C_1(z)\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}z + \nu\rho_0 \mu_1 P_{0,0} C_1(z) - \lambda_0 P_{0,0} z\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\{(1 - \rho_0 C_1(z))\}}{z\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\{1 - \rho_0 C_1(z)\}}$

Since at $z = 1$ numerator of equation (20) vanishes, queue length distribution exist. Now we observe the denominator to find the roots associated. Denominator

$$z\left\{\lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\left\{\lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}\right\}\{1 - \rho_0 C_1(z)\}$$

Let $A = \lambda + \mu_1 + b - \lambda C_2(z) - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}$ and $X = \lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}$

Now $A = \lambda(1 - C_2(z)) + \mu_1 + b\left(1 - \frac{r}{\lambda_1 + r - \lambda_1 C_3(z)}\right)$

$$= \lambda(1 - C_2(z)) + \mu_1 + \frac{b\lambda_1(1 - C_3(z))}{\lambda_1 + r - \lambda_1 C_3(z)}$$

$$= \frac{\{\lambda(1 - C_2(z)) + \mu_1\}\{\lambda_1(1 - C_3(z)) + r\} + b\lambda_1(1 - C_3(z))}{\lambda_1(1 - C_3(z)) + r}$$

Also $X = \lambda + \mu_2 + b - \lambda C_2(z) - \frac{\mu_2}{z} - \frac{rb}{\lambda_1 + r - \lambda_1 C_3(z)}$

$= \lambda(1 - C_2(z)) - \frac{\mu_2}{z}(1 - z) + \frac{b\lambda_1(1 - C_3(z))}{\lambda_1 + r - \lambda_1 C_3(z)}$

$= (1 - z)\left[\lambda\{1 + \sum_{i=1}^{\infty}(1 - c_{21} - c_{22} - c_{23} - \cdots - c_{2i})z^i\} - \frac{\mu_2}{z} + \frac{b\lambda_1}{\lambda_1 + r - \lambda_1 C_3(z)}\{1 + \sum_{i=1}^{\infty}(1 - c_{31} - c_{32} - c_{33} - \cdots - c_{3i})z^i\}\right]$

Let $X = (1 - z)Y$

At $z = 1$,

$$Y = \lambda - \mu_2 + \frac{b\lambda_1}{\lambda_1 + r} + \lambda\sum_{i=1}^{\infty}(1 - c_{21} - c_{22} - c_{23} - \cdots - c_{2i}) + \frac{b\lambda_1}{\lambda_1 + r}\{\sum_{i=1}^{\infty}(1 - c_{31} - c_{32} - c_{33} - \cdots - c_{3i})\}$$

Since series $\sum_{i=1}^{\infty}(1 - c_{21} - c_{22} - c_{23} - \cdots - c_{2i})$ *and* $\sum_{i=1}^{\infty}(1 - c_{31} - c_{32} - c_{33} - \cdots - c_{3i})$ are convergent at z=1, so we can conclude the condition for existence of steady state queue length is $\frac{b\lambda_1\overline{C_3}}{\mu_2 r} + \frac{\lambda\overline{C_2}}{\mu_2} < 1$

Using $F(1) = 1$ (Normalization condition)

i.e. $\left[F_0(z) + F_{1,1}(z) + F_{1,2}(z) + F_{2,1}(z) + F_{2,2}(z)\right]_{z=1}$ (18)

After some tedious algebra, we get

$$P_{0,0} = \frac{\mu_1 r(1-\rho_0)^2}{(1-\rho_0)(r\mu_1 + \nu\rho_0 r + \nu\rho_0 b) + (r+b)\nu\rho_0 E} \quad (19)$$

Where $E = \frac{(1-\rho_0)\left(r\lambda C_2^{(1)}(1) + b\lambda_1 C_3^{(1)}(1) - r\mu_1\right) + r\mu_1 C_1^{(1)}(1)}{\left(-r\lambda C_2^{(1)}(1) - b\lambda_1 C_3^{(1)}(1) + r\mu_2\right)}$

Mean Queue Length

In this section, mean queue length is evaluated by $F'(1)$

$$L = \frac{d}{dz}\Big|_{z=1} = \frac{\varrho_0 P_{0,0}}{(1-\varrho_0)^2} C_1^{(1)}(1) + \left(1 + \frac{b}{r}\right)\frac{d}{dz}\left[F_{1,1}(z) + F_{1,2}(z)\right]_{z=1} + \frac{b\lambda_1}{r^2} C_3^{(1)}(1)\left[F_{1,1}(1) + F_{1,2}(1)\right]$$

Where

$$F_0(1) = \frac{P_{0,0}}{1-\rho_0}$$

$$F_{1,1}(1) = \frac{\nu\rho_0 P_{0,0}}{\mu_1(1-\rho_0)}$$

$$F_{1,2}(1) = \frac{\nu\varrho_0 P_{0,0}}{\mu_1(1-\varrho_0)^2} E$$

$$\frac{d}{dz}F_{1,1}(Z)\Big|_{z=1} = \frac{\nu\rho_0 P_{0,0}}{\mu_1^2(1-\rho_0)^2}\left[\mu_1 C_1^{(1)}(1) + (1-\rho_0)\left\{\lambda C_2^{(1)}(1) + \frac{b\lambda_1}{r} C_3^{(1)}(1)\right\}\right]$$

$$\frac{d^2}{dz^2}F_{1,1}(Z)\Big|_{z=1} = \frac{\nu P_{0,0}}{\mu_1^3}\left[\left\{\frac{-2\varrho_0^2\{C_1^{(1)}(1)\}^2\mu_1}{(1-\varrho_0)^3} + \frac{\varrho_0 C_1^{(2)}(1)\mu_1}{(1-\varrho_0)^2} + \frac{\varrho_0\mu_1}{(1-\varrho_0)}\left\{-\lambda C_2^{(2)}(1) - \frac{b\lambda_1}{r} C_3^{(2)}(1) + \frac{2b\lambda_1^2}{r^2}\left\{C_3^{(1)}(1)\right\}^2\right\}\right\}\right.$$
$$\left. - 2\left\{\frac{\varrho_0 C_1^{(1)}(1)\mu_1}{(1-\varrho_0)^2} - \frac{\varrho_0}{(1-\varrho_0)}\left\{-\lambda C_2^{(1)}(1) - \frac{b\lambda_1}{r} C_3^{(1)}(1)\right\}\right\}\left\{-\lambda C_2^{(1)}(1) - \frac{b\lambda_1}{r} C_3^{(1)}(1)\right\}\right]$$

$$\frac{d}{dz}F_{1,2}(Z)\Big|_{z=1} \frac{\mu_1}{2\left\{-\lambda C_2^{(1)}(1) + \mu_2 - \frac{b\lambda_1}{r} C_3^{(1)}(1)\right\}^2}\left[\left\{-\lambda C_2^{(1)}(1) + \mu_2 - \frac{b\lambda_1}{r} C_3^{(1)}(1)\right\}\left\{-2F_{1,1}'(1) + 2F_{1,1}(1) + F_{1,1}''(1)\right\}\right.$$
$$\left. + \left\{-F_{1,1}(1) + F_{1,1}'(1)\right\}\left\{-\lambda C_2^{(2)}(1) + 2\mu_2 - \frac{b\lambda_1}{r} C_3^{(2)}(1) + \frac{2b\lambda_1^2}{r^2}\left\{C_3^{(1)}(1)\right\}^2\right\}\right]$$

Mean no. of customer in the queue

$$L_q = \sum_{i=0}^{\infty} ip_{0,i} + \sum_{i=1}^{\infty}(i-1)p_{1,i,1} + \sum_{i=1}^{\infty}(i-1)p_{1,i,2} + \sum_{i=1}^{\infty}(i-1)p_{2,i,1} + \sum_{i=1}^{\infty}(i-1)p_{2,i,2}$$

CONCLUSION

In this paper we have studied bulk arrival multiple vacation queueing model where first customer of each busy period receives exceptional service which is subject to breakdown. The condition for existence of steady state queue length is $\frac{b\lambda_1 \overline{C_3}}{\mu_2 r} + \frac{\lambda \overline{C_2}}{\mu_2} < 1$. We have derived mean queue length and average number of customer using generating function techniques. The queueing model under our study is characterized by several variants features, so it may be applied to many real life situations like transportation network, computer communication networks, maintenance as well as in medical services. Also, we have taken batch size to be random, which allows different queueing systems to use this model according to their needs and evaluate system performance measures for better management of the concerned queue.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Naturalist's Guide to Online Portals: A Compilation

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Received: 03 Feb 2022 Revised: 13 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

Online portals serve as a basic tool for identification of flora and fauna. Apart from basic taxonomic applications, they serve as a repository and their database can be utilised for conservation purpose. It is a unique repository of information on India's biodiversity. Large database of world biodiversity. Encourage professional and amateur/upcoming scientists from around the world to publish. eBird is among the world's largest biodiversity-related science website. 'HBW and Bird Life International Illustrated Checklist of the Birds of the World'. ENVIS serves as a single-stop web-enabled repository of comprehensive environmental information with collection.

Keywords: ENVIS, biodiversity, World', Bird Life.

INTRODUCTION

Online portals serve as a basic tool for identification of flora and fauna. Apart from basic taxonomic applications, they serve as a repository and their database can be utilised for conservation purpose. They are open source and can be easily accessed by anyone. Portals like iNaturalist and India Biodiversity Portal are used by naturalists to observe, record and upload their identifications instantly and directly to the online platform. These identifications may also have spatial and temporal attributes (Ex: GPS coordinates, time of observation, date and season). The uploaded identifications are subject to scrutiny and are reviewed by other naturalists and researchers. This web of interconnectivity opens the door for suggestions, asking help for identification, constructive criticisms and corrections which helps in the learning process. Young amateur naturalists of India require proper guidance and direction to further their career and online portals can support them to fill the gaps in their knowledge. This article tries to provide an unbiased list of some online portals that can be easily accessed by clicking on the links provided below. It is also important for young naturalists to keep in mind that:

- Photographs are not the best method for identification.
- Efficacy of species identification from photographs depend on the image resolution, type of camera, time of day and lighting conditions.
- Some errors might be present in the portals due to lack of knowledge/identification skill of the uploader (the errors may remain uncorrected, may persist and can even be passed on).
- Many species (especially insects) require laboratory analysis for identification (Ex: microscopic identification of sex comb in drosophila, comparison of mounted genital plates in coleopterans etc.). Photographic identification will not suffice.
- All credit goes to the editors, regulators and owners of the respective websites.

What follows is a brief description of each online portal along with the open source link.

Biodiversity

India Biodiversity Portal

It is a unique repository of information on India's biodiversity. It consists of a repository of more than 58,000 species and 1.44 million individual observations (As of 20th November 2020). The online Portal aims to:

- Aggregate data through public participation.
- Provide open and free access to biodiversity information.

Database includes

Mammals, birds, Reptiles, Amphibians, Fish, Arthropods, Molluscs, Plants, Fungi and others.

Unique attributes

Detailed taxonomic descriptions of insects (especially coleopterans) along with high quality photographs of the specimens, geographic distribution information and maps are attached.

iNaturalist

It is a large database of world biodiversity which includes more than 54 million individual observations and 306,076 unique species (As of 20th November 2020). The online portal aims to:

- Encourage public participation to record observations.
- Share information with fellow naturalists.
- Provide discussion platform for findings.

Database includes

Protozoans, Fungi including lichens, Plants, Insects, Arachnids, Molluscs, Ray- finned fishes, Mammals, Reptiles, Amphibians, Birds and others.

Unique attributes

Includes database of vast array of arachnids (more than 8,500 species worldwide); Easy to install app for Android and iOS platforms.

Zooniverse

Enables everyone to take part in cutting edge research across many fields across sciences, humanities and more. The datasets are useful to the wider research community. The online portal aims to:

- Provide opportunities to unlock answers and fuel discoveries.
- Encourage public participation through volunteering.
- Accelerate important research by working together.
- Create and enhance the bridge between volunteers and professionals.

Unique attributes
Includes eMammal, which helps to classify organisms through camera trap photos; includes various other departments such as Arts, Humanities and Space.

Biodiversity Atlas – India:
Parent website to Butterflies of India, Moths of India, Odonata of India, Cicadas of India, Reptiles of India, Amphibians of India, Birds of India, and Mammals of India: each of which is rich in database.
The online portal aims to

- Provide Biodiversity documentation.
- Promote Citizen Science.
- Foster Biodiversity Informatics and Research.
- Promote outreach and education.

Database includes
Butterflies, Moths, Odonatans, Cicadas, Reptiles, Amphibians, Birds and Mammals of India

Unique attributes
Extensive and beautiful database of butterflies of India.

Journal of Threatened Taxa
Platform for quick and timely publication of research, findings, reviews and other aspects of science related to conservation and taxonomy. The online portal aims to:

- Encourage professional and amateur/upcoming scientists from around the world to publish.
- Encourage publication of manuscripts: Essays, Monographs, Articles, Reviews, Communications, Short communications, Notes, Conservation applications, Data papers, Viewpoint, Peer commentaries, Response and reply, Corrections, Addenda, Special series and Book reviews.

Unique attributes
Includes subject areas such as Ecology, Behaviour, Physiology, Methodology, Veterinary, Pathology, Management, Models, Data and Conservation biology.

Namami Gange

National Mission for Clean Ganga (NMCG) was registered as a society on 12th August 2011 under the Societies Registration Act 1860. It acted as implementation arm of National Ganga River Basin Authority (NGRBA) which was constituted under the provisions of the Environment (Protection) Act (EPA), 1986, Govt. of India. The online portal aims to:

- Encourage public participation in conservation of the Ganges.
- Accomplish the mandate of National Ganga River Basin Authority (NGRBA).
- Ensure effective abatement of pollution and rejuvenation of the river Ganga by adopting a river basin approach to promote inter-sectoral co-ordination for comprehensive planning and management.
- Maintain minimum ecological flows in the river Ganga with the aim of ensuring water quality and environmentally sustainable development.

Unique attributes
Includes detailed guide (open source pdf. files) for identification of Birds, Amphibians, Crocodilians, Fishes, Mammals and Chelonians (turtles) of the Ganges River.

Young Ecologists Meet & Interact (YETI)

Committee of students and researchers of ecology, evolution, behaviour and conservation in India. The online portal aims to:

- Serve as a friendly and inspiring platform for ecologists to present and discuss their work, exchange ideas and even strike up prosperous collaborations.
- To discuss and explore new projects, provide or obtain information on ecological pursuits.

Unique attributes
Provides open source software which can help with data, collaboration and computational efforts we might require while conducting research or while learning new concepts; registered members get important information regarding workshops, conferences, internships and job vacancies through mail.

Join Mailing List
https://www.freelists.org/list/meetyeti#:~:text=Young%20Ecologists%20Talk%20and%20Interact%20(YETI)%20is%20a%20first-,volunteers%20from%20Bangalore%20and%20Mysore.

National Centre for Biological Sciences, TIFR
Premier Research Institute in Bengaluru. One of the best websites for attending events (both offline and online) pertaining to Life Sciences.

Unique attributes: Enables students to attend lecture series/conferences conducted by cutting edge researchers; website includes open source archives (collecting public centre for the contemporary history of biology in India).

Ornithology
Basic Ornithology
Research and Conservation by IISER Tirupati
Website has a series of educational videos pertaining to ornithological research and conservation. These lectures are a part of a course titled 'Basic Ornithology: Research and Conservation' which was conducted in December 2019 at IISER Tirupati, India. The online portal aims to:

- Educate and encourage students to pursue a career in ornithological research and conservation.
- Cover major topics in avian biology such as anatomy, evolution, behaviour, populations, field and laboratory techniques, as well as research concepts and study design.
- Provide open source videos for educational purpose.

Unique attributes
Video course content covers basics of ornithology essential for an avid birder which will be helpful for identification of bird species, understanding bird behaviour and avian conservation biology.

The Cornell Lab of Ornithology – eBird
eBird is among the world's largest biodiversity-related science website. It has more than 100 million bird sightings contributed annually by eBirders around the world and is managed by The Cornell Lab of Ornithology. Website/app helps in documenting worldwide bird distribution, abundance, habitat use, and trends through checklist data. Birders have to just enter when, where, and how they went birding, and finally fill out a checklist of the birds seen and heard during the excursion. The online portal aims to:

- Encourage public participation to find more birds.
- Keep track of bird lists, photos, and sounds.
- Explore latest sightings from around the world.

- Promote people to join the world's largest birding community.
- Contribute to science and conservation.

Database includes
Identification for almost all avian species worldwide.

Unique attributes
Easy to install app for Android and iOS platforms; website includes Merlin Bird ID (bird packs can be downloaded for different regions); also includes species maps and bird sounds for species identification.

Birdlife International
It is a global partnership of conservation organisations (NGOs) that strives to conserve birds, their habitats and global biodiversity, working with people towards sustainability in the use of natural resources. The online portal aims to:

- Encourage public contribution to raise awareness on conservation issues.
- Respond to specific conservation issues.
- Promote individual regional programmes in addition to global programmes.
- Foster partnerships and collaborate on common priorities.

Unique attributes
It is a one of a kind website for the study of avian taxonomy and species identification. Open source .pdf files can be downloaded which includes taxonomy published in two volumes of the 'HBW and BirdLife International Illustrated Checklist of the Birds of the World'.

Important Bird and Biodiversity Areas in India Priority sites for conservation (Book)
An open source document published by BNHS that is essential for aspiring avian conservationists of India.
Database includes: Important Bird and Biodiversity Areas in India; Priority sites which includes the States/UT's: Andaman and Nicobar Islands, Andhra Pradesh and Telangana, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Lakshadweep, Madhya Pradesh, Maharashtra, Manipur, Mizoram, Nagaland, Odisha, Pondicherry, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand and West Bengal.

Authors of the book
Asad R Rahmani, Zafar-ul-Islam, Raju Kasambe.

Birds of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Herpetology
Reptiles of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Amphibians of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Dakshin Foundation
Dakshin Foundation is a not-for-profit, charitable, non-governmental organisation committed to environmental sustainability and social justice.
Unique attributes: A good repository of chelonian literature.

Lepidopterology
Patanga Suchaka
It is an artificial intelligence-based butterfly and moth identification website; Uses advance algorithms to identify a species as butterfly or moth. This database is collated by the School of Ecology and Conservation (SEC), University of Agricultural Sciences (UAS), Bengaluru. An android app of Patanga Suchaka is available on the Google Playstore for identification and contribution of images of butterflies and moth species.

Database includes
It includes a database of 1011 butterflies and 1111 moths species for identification.

Unique attributes
Easy to install app for Android platform; website includes distribution of butterflies and moth species in India.

Butterflies of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Moths of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Mammalogy
Mammals of India
Daughter website of Biodiversity Atlas-India (https://www.bioatlasindia.org/)

Marine Mammal Research & Conservation Network of India
One of the best websites for taxonomy and identification of cetaceans (whales, dolphins and porpoises), sirenians (manatees and dugongs), pinnipeds (true seals, eared seals and walruses) and otters. The website also contains more than a thousand sightings of marine mammals along with high quality images.

Database includes
Identification for almost all marine mammal species in India.

Unique attributes
contains detailed identification guide for marine mammals along with length, distinctive marks and colouration, teeth, prominent features .etc. Also a unique repository of historical data on marine mammals.

Marine life and Ichthyology
Marine Species Identification Portal

Offers information about identification and taxonomy of thousands of different species in the world's oceans and seas.

Database includes
Brachiopods, corals and sea anemones, fishes, jellyfish and related species, hydrozoans, lancelets, marine mammals, molluscs, marine reptiles, sea spiders, sponges, starfish and other echinoderms, tunicates, plants, worms, protists .etc.

Unique attributes
Beautifully illustrated diagrams and high quality pictures; includes description, classification, synonyms, literature, distribution maps and other important links for each species; a very good website for ichthyology.

Environmental Information System
ENVIS Centres
ENVIS serves as a single-stop web-enabled repository of comprehensive environmental information with collection, collation, storage, retrieval and dissemination of the same through a nationwide network of ENVIS Hubs (hosted by the Environment/ Forest Department of State Governments/ UT Administrations) and ENVIS Resource Partners (RPs) (hosted by environment related governmental and non-governmental organizations/institutes of professional excellence).

Botany
Flora of Peninsular India, IISc
It is an online portal for vascular plants of Peninsular India with up-to-date taxonomic and phytogeographic information.

Database includes: More than 16,000 specimens, from vascular plants to lichens.
Unique Attributes: Website includes vegetation types and habitats of India.

eflora of India
It is a non-commercial site, based on the collection of photographic images of plants from members of the group. Images of plant species requiring identification can be sent through email to this group.

Database includes
More than 13,000 species of Indian Flora with more than 2,50,000 images displayed at species' pages along with displaying these at genera & family pages for comparative purposes for easy identification.

Flowers of India
Flowers of India is aimed at having information about all the flowers found in India, with their common names, especially in Indian languages, pictures and habitat, easily available in one place.

Database includes
Information about Flowering trees, Orchids, Grasses, Medicinal Plants, Flowers in ancient literature etc.

Unique Attributes
Easy to install app for Android platform.

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7. https://www.meetyeti.net/
8. https://www.ncbs.res.in/events
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14. https://www.indianreptiles.org/
15. https://www.indianamphibians.org/
16. https://www.dakshin.org/
17. http://www.pathangasuchaka.in/
18. https://www.ifoundbutterflies.org/
19. https://www.mothsofindia.org/
20. https://www.mammalsofindia.org/
21. http://www.marinemammals.in/
22. http://species-identification.org/
23. http://envis.nic.in/index.aspx
24. http://envis.nic.in/ENVIS_html/ENVISSubject/subject.html
25. http://bsienvis.nic.in/Content/enviscentres_491.aspx
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28. http://www.flowersofindia.net/

Fig.1. India Biodiversity Portal	**Fig.2. iNaturalist**	**Fig.3. Zooniverse**
Fig.4. Biodiversity Atlas – India	**Fig.5. Journal of Threatened Taxa**	**Fig.6. Namami Gange**
Fig.7. Young Ecologists Meet & Interact (YETI)	**Fig.8. National Centre for Biological Sciences, TIFR**	**Fig.9. Research and Conservation by IISER Tirupati**
Fig.10. The Cornell Lab of Ornithology – eBird	**Fig.11. Birdlife International**	**Fig.12. Birds of India**

Fig.13. Reptiles of India

Fig.14. Amphibians of India

Fig.15. Dakshin Foundation

Fig.16. Patanga Suchaka

Fig.17. Butterflies of India

Fig.18. Moths of India

Fig.19. Mammals of India

Fig.20. Marine Mammal Research & Conservation Network of India

Fig. 21. Marine Species Identification Portal

Fig.22. ENVIS Centres

Fig.23. eflora of India

Fig.24. Flowers of India

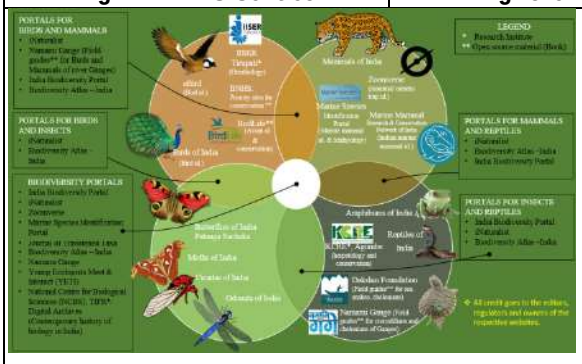

Fig.25. Working towards a sustainable environmental

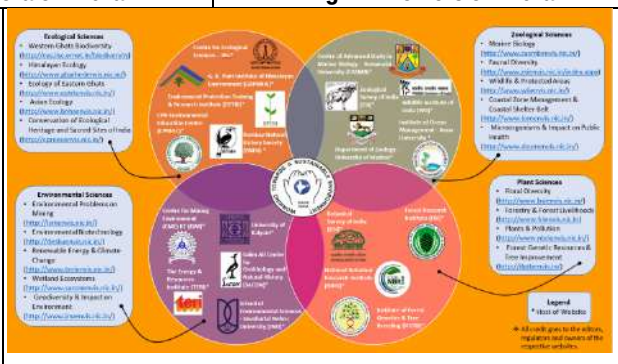

Fig.26.Working towards a sustainable environmental

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Generating Energy Efficient Test Suite using Association Rule Mining for Sustainable Software Testing

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Received: 30 Dec 2021 Revised: 20 Jan 2022 Accepted: 19 Feb 2022

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ABSTRACT

Software Development needs to be optimized in executing the activities and machines usage to provide reliable software product to customers. This may reduce resource consumption from materials to CASE tool usage for making all artefacts relevant to software development life cycle. The Software Testing is a more resource and effort consumption other than all software development phases. The heavy usage of 3ms (Men, Machine and Time) may increase the temperature ratio and thus increase carbon emissions also. To control this, the sustainable software testing to be made with optimal test procedures, test plan, test cases or test suite and optimal testing tools. Hence, the Association Rule mining is proposed in this paper to create optimal test suite for sustainable software testing execution. The objectives and effect of historical test cases are analysed to build an optimal test suite from the test cases repository. The test case reduction may reduce the energy of testing and improving the confidence on results when test suite is built with frequently used test cases on testing certain features of the software product. The idea of sustainable software testing is reducing energy on executing the tasks and improving overall performance of the testing procedures.

Keywords: Sustainable Software Testing, Association Rule Mining, Test Case Reduction, Optimal Test Suite Generation, Energy Efficient Test Suite

INTRODUCTION

Software Development Process is a comprised set of prescribed activities to be done for developing the software system effectively and efficiently. It also has objectives such as 'Customer Satisfaction', 'Software Quality', 'Less Resource Consumption', 'Timely Delivery' and 'Maximum Profit'. To achieve the above objectives and other hidden objectives the software process model will be adjusted within the limited boundary. Generally, the software development process will have the development activities such as 'Communication with Stakeholders', 'Planning the

Project Development', 'Modelling the system', 'Implementing the System Code', 'Performing Software Testing' and finally 'Software Maintenance'. The Project Manager must prepare an effective project plan to feel engineers more comfortable to develop the system with possible deadlines to complete their tasks. Nowadays, CASE (Computer Aided Software Engineering) tools are used to implement the activities in a proper manner. In the market many CASE tools are available with respect to execute the task of development phase activities. But more comfortable and easier one will be selected, and trained people may use the tools to perform their tasks as per the guidelines and principles proposed in Software Engineering [1]. Tools Support is very much required one and, in some situations, it is a mandatory one for producing the expected product within the time boundary. Software Engineers must choose appropriate tools those are perfect and feasible one to implement the work products specified in the project plan. Every software development industries have their own guidelines in maintains the documents to help the activities required in future such as 'Software Maintenance' and 'Software Reengineering'. Hence the selection of procedures, guidelines and tools are playing vital role in software development process.

Data Mining

In all business domains, huge amount of data are collected in different ways from different users and scenarios to place them in a platform to analyse and predict the return on investment. And data may have certain features which may help the data analyst to find the required pattern of data to be taken to simply the weight of data by considering only important features in need of applying in a particular approach to build the solutions. For all these kinds of needs the 'Data Mining' will help the business people to get a required solution in a short time period [2].

Association Rule Mining

Association Rule unsupervised algorithm is about identifying the effect of combining two different features or objects from the data set. The effect may be a 'good decision' or 'good status' or 'good indication' etc. It is a most important algorithm technique in 'Marketing Trends' to reach the very good position by introducing offers like providing an additional item if people purchased another item. For an example, people need mobile phones for their easy communication, and we have different telecom service providers. So, to get more customers the offer may introduce by combining mobile phone and a SIM card from a service provider. Because of this kind of marketing method many people may prefer to buy the mobile phone and they will become the customer of the telecom service provider.

The advantages of using Association Rule Mining are

- The relationships among features or objects will be analyzed
- Suitable composition will be done
- New Strategy or Decision will be made

Hence, the 'Association Rule Mining' technique may be preferred in some problem domains where a perfect rule is to be created for implementing the required activities.

Sustainable Development

Sustainable Development is about the standard method or approach of developing or manufacturing any products and the standard method should be used for long years without any change in the prescribed method. And the method or decision should be accepted by everyone in the field to use it. The 'Sustainable Development' is having different 17 goals among them one of the goals is about introducing a perfect approach to develop a product which consumes required efforts and satisfies the objectives may be 'Money', 'Energy', 'Green environment' and etc [3,4]. Forming the 'Green Environment' is a challenging activity to all countries around the world to make or maintain a pleasant atmosphere which allows all the species would live actively and happily [5, 6]. Hence, the industries which use more machines must find a sustainable process which can help them to meet the objectives of green environment [7].

Literature Survey

Data Mining is used in different application domains for providing support in making all perfect decisions in required time. In Software Engineering domain, different details are collected, created, categorized and organized

for preparing reports and documents required in all software development phase activities. The details may be classified as per the different features identified according to the needs in a particular situation. And to take all business solutions the input details are processed by using data mining algorithms. In that wise, the association rule mining plays a vital role in promoting the methods and techniques wherever they are suitable to use them. Software Testing is about conducting different exercises on the software which is under test to bring all types of defect which disturbs the quality of the product and product deliver to the customer or software market. Hence building test suite is an essential task in conducting the required software testing procedures [8]. The defect classification is an essential activity in software testing to form suitable test cases according to the class of defect will be treated to identify from the software under test. To do this the Association Rule mining is applied with Fuzzy Classifier and Artificial Bee colony algorithm. This method may promote the accuracy on classifying the software artefacts based on all possible defects [9]. This is extended with Artificial Neural Networks techniques to improve the results in terms of accuracy and performance level in making classification based on defects. The test case reduction is a required activity to collect test cases which are highly required to test the major parts of the software. In implementing this, the association rule mining is applied in selecting test cases to build reliable test suite [10].

Associate Rule Mining for Sustainable Software Testing

The goals of Software Testing are 'Finding Maximum Defects', 'Effective Resource Allocation', 'Effective Testing', and 'Satisfying Customer Expectations'. Achieving first goal such as 'Finding Maximum Defects' is in the hand of test engineer by considering all the possible ways to test the software system using 'Manual Testing' and 'Automated Testing' methods [11]. The requirement of this is searching for suitable testing policy, test strategy and testing process to make a proper testing plan. Test Manager who participated from the 'Requirement Analysis' phase in software development process may be able to create the testing plan for 'System Testing' and 'Acceptance Testing' for ensuring that the customer expectations are satisfied. The 'Automated Testing' will be suggested to test engineers to execute a testing type with the help of testing tools and optimized test suite for getting the defects from the application under test. The frequently used test cases are identified from the test repository for a particular system feature to be tested by the testing team. The test cases once designed for functionality to be tested that will be verified before it is applied to the testing procedure. Only approved test cases will be taken finally for tracing requirements to detect defects present in Functionality under test. Test cases could be designed as per the characteristics of requirements to be traced and behaviour to be monitored. The sample test cases designed for 'Sign Up' functionality which comes with 'User Name', 'Password' and 'OTP' are given below.

Test Cases for Sign Up task: To detect maximum defects in 'Sign Up' functionality the above test cases are verified and the test cases will be combined to build a test suite which can be suggested for conducting testing on the developed functional unit [8]. The possible test suites from the above test cases are given below.

Test Suites and Coverage Details: From the above set of test suites the item set can be made as like the below.
The usage frequency of each test case is obtained by verifying their use in different test suites already used on testing 'Sign Up' task. For example, TC1 is used in test suites such as TS1, TS4, TS5, TS7, TS8, TS9, TS10, TS11 and TS12. So TC1's usage frequency is 9. In the same way it is identified for remaining test cases from the test suites' table.

Test Cases and Usage Frequency: Then, combining two test cases and finding their usage frequency from the test suite table.

Two Test Cases Combination and Usage Frequency: In the same way, test cases combination made for 3, 4 and 5 for recording their usage frequency by refer the test suite table. And if frequency is less than two those can be omitted because of less usage in previous products testing.
The test cases having frequency '1' will be rejected so therefore remaining test cases listed in given table -V.

The 5 test cases combination and usage frequency is given in table-VII. Now suppose the tester selecting TC1, TC2 as a mandatory then he has to decide what test case he can take from TC3 to TC6 created for testing the 'Sign Up' process.

Hence, the rules will be generated from the above sample to find the confidence value
TC1, TC2 => TC3 = set (TC1, TC2, TC3) / set (TC1, TC2) = 2/3 *100 = 60%
TC1, TC2 => TC4 = set (TC1, TC2, TC4) / set (TC1, TC2) = 0/ 3 * 100 = 0 %
TC1, TC2 => TC5 = set (TC1, TC2, TC5) / set (TC1, TC2) = 0/3 * 100 = 0%
TC1, TC2 => TC6 = set (TC1, TC2, TC6) / set (TC1, TC2) = 0/3 * 100 = 0%
From the above we may come to a conclusion that the confidence level 60% is obtained when TC3 is considered to include with TC1 and TC2.
The confidence level for including 'TC4' or 'TC5' or 'TC6' with test suite {TC1, TC2, TC3} can be computed to take a decision to increase the test suite size.

RESULTS AND DISCUSSIONS

The energy difference can be obtained by finding the difference between from the energy required in conducting testing with all test cases TC1 to TC6 and the test cases TC1, TC2 and TC3. There is a 50% of energy difference will be possible when the test cases reduction happened from the whole test set TC1 to TC6 for obtaining all types bugs. This energy reduction through making optimal test cases from the whole test case library could be an initiative in saving our environment from the carbon emission [12]. The temperature will be reduced when the cycles of testing is reduced to execute with test minimal but optimal test cases. And the finalized test cases will be an energy efficient and sustainable one in making testing activity more perfect in order to deliver quality and reliable product.

CONCLUSION

In this paper, the association rule mining is proposed to create the test suite which is highly, frequently used with consuming less energy and providing all expected bugs present in the software under test. Association Rule mining is one of the Data Mining approach to create market strategy where more profit could be obtained. This objective is used in making sustainable software test suite which may be a reduced test suite but optimal one in conducting the testing activities with less energy consumption in all perspectives from number of testing iterations to testing tools to be used.

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Table 1: Test Cases for Sign Up task

Test Cases	Input	Output	Condition
TC1	{'validusername', 'valid password', 'valid OTP'}	{valid user, open next service}	Database connection required
TC2	{'invalidusername', 'invalid password', 'invalid OTP'}	{error msg, "Invalid username}	Database connection required
TC3	{'validusername', 'invalid password', 'invalid OTP'}	{error msg, "Invalid user Credentials}	Database connection required
TC4	{'validusername', 'validpassword', 'invalid OTP'}	{error msg, 'invalid OTP'}	Database connection required
TC5	{'validusername', 'invalidpassword', 'valid OTP'}	{error msg, 'invalid credentials'}	Database connection required
TC6	{'invalidusername', 'validpassword', 'invalid OTP'}	{error msg, 'invalid username'}	Database connection required

Table 2: Test Suites and Coverage Details

Test suite ID	Test Cases	Coverage
TS1	TC1	Able to test only valid input
TS2	TC2	Able to work for invalid input
TS3	TC3	Able to work for invalid input
TS4	TC1, TC2	Able to work for both valid and invalid input conditions
TS5	TC1, TC3	Able to work for both valid and invalid input conditions
TS6	TC2, TC3	Able to work for invalid input
TS7	TC1, TC2, TC3	Able to work for both valid and invalid input conditions
TS8	TC1, TC4	Able to work for both valid and invalid input conditions
TS9	TC1, TC5	Able to work for both valid and invalid input conditions
TS10	TC1, TC6	Able to work for both valid and invalid input conditions
TS11	TC1, TC4, TC5, TC6	Able to work for both valid and invalid input conditions
TS12	TC1, TC2, TC3, TC4, TC5, TC6	Able to work for both valid and invalid input conditions
TS13	TC4, TC5	Able to work for invalid input
TS14	TC4, TC6	Able to work for invalid input
TS15	TC5, TC6	Able to work for invalid input
TS16	TC2, TC4	Able to work for invalid input
TS17	TC2, TC5	Able to work for invalid input
TS18	TC2, TC6	Able to work for invalid input
TS19	TC3, TC4	Able to work for invalid input
TS20	TC3, TC5	Able to work for invalid input
TS21	TC3, TC6	Able to work for invalid input

Table 3: Test Cases and Usage Frequency

Items	Usage Frequency
TC1	9
TC2	8
TC3	8
TC4	7
TC5	7
TC6	7

Table 4 a: Two Test Cases Combination and Usage Frequency

Items	Usage Frequency
TC1, TC2	3
TC1, TC3	3
TC1, TC4	3
TC1, TC5	3
TC1, TC6	3
TC2, TC3	3
TC2, TC4	2
TC2, TC5	2
TC2, TC6	2
TC3, TC4	2
TC3, TC5	2
TC3, TC6	2
TC4, TC5	3
TC4, TC6	3
TC5, TC6	3

Table 4 b

Items	Usage Frequency
TC1, TC2, TC3	2
TC1, TC4, T5	2
TC1, TC5, TC6	2
TC2, TC3, TC4	1
TC2, TC4, T5	1
TC2, TC5, TC6	1
TC3, TC4, TC5	1
TC3, TC5, TC6	1
TC4, TC5, TC6	2

Table 5: 3 Test Cases Combination and Usage Frequency

Items	Usage Frequency
TC1, TC2, TC3	2
TC1, TC4, TC5	2
TC1, TC5, TC6	2
TC4, TC5, TC6	2

Table 6: 4 Test Cases Combination and Usage Frequency

Items	Usage Frequency
TC1, TC2, TC3, TC4	1
TC1, TC3, TC4, TC5	1
TC1, TC2, TC4, TC5	1
TC1, TC2, TC4, TC6	1
TC2, TC3, TC4, TC5	1
TC2, TC4, TC5, TC6	1
TC3, TC4, TC5, TC6	1
TC4, TC5, TC6, TC1	2

Table 7: The 5 test cases combination and usage frequency

Items	Usage Frequency
TC4, TC5, TC6, TC1	2

Items	Usage Frequency
TC1,TC2, TC3,TC4,TC5	1

Items	Usage Frequency
TC1, TC2, TC3,TC4,TC5, TC6	1

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Biodegradable Electromagnetic Interference Shielding Enclosure using Banana Fiber

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Received: 27 Dec 2021 Revised: 13 Jan 2022 Accepted: 19 Feb 2022

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ABSTRACT

A biodegradable electromagnetic interference (EMI) shielding enclosure for protecting electronic device is proposed. The fabric is made of banana fibre to absorbs the radiated EMI and a passive filter adjoined to suppresses the conductive EMI. A carbon graphite additives and a conductive material is coated in the fabric, increasing the thermal and electrical conductivity of the fabric. This enclosure also includes a small circular opening in the bottom layer to connect the electronic equipment placed inside the enclosure to equipment placed outside. The radiated EMI absorbed by the fabric is dissipated as heat through dielectric property of banana fiber and conducted EMI of the electronic gadget is prevented by low pass filter attached with fabric. The effectiveness of the shielding enclosure is analyzed using experimental setup carried out in the laboratory and the results are presented.

Keywords: Electromagnetic interference, Shielding, Enclosure, Banana fiber, Bio-degradable.

INTRODUCTION

Electrical and Electronic Equipment have become an integral part of engineering and technological innovations these days. Due to the advent of solid state technologies, several equipment of such kind are packed in a confined space in many projects [1]. This equipment packed together may emit electromagnetic radiations intentionally are accidentally and affect the performance of each other resulting in malfunctioning or data losses [2]. This equipment's

can also create negative effects on human health. The human beings exposed to electromagnetic wave environment may affected by rise in eye temperature, leukemia, brain tumors, Alzheimer's disease, sleeping problems, and depression [3]. Nowadays, every company in the electronics industry is faced with EMI demands. The use of electronic devices is increasing, as the exposure to wide range of frequencies. Radiation and immunity have to be taken into account in early stages of the development of new products [4]. In many cases EMI problems cannot be solved at the PCB level alone and instead, enclosures and cables have to be shielded as well. An Electromagnetic shield can be deployed over each electronic equipment to protect the sensitive equipment from malfunctioning, to prevent data losses and to avoid human beings getting exposed to harmful electromagnetic radiations [5]. The Electromagnetic wave comprises of electric field (E) and magnetic field (H). Whose phase positions are oscillating perpendicular to each other and also perpendicular to the direction of energy propagation as shown in figure 1. These electric and magnetic fields can be arrested by means of reflection or absorption through EMI shielding mechanism. Shielding is typically used in enclosures in order to isolate the electrical devices inside them from external influences. Enclosures come in solid and perforated forms depending on the application. Faraday cage enclosures are bulky and add weight to a device. This makes them impractical for use with small devices like phones tablets and computer components. In this article an electromagnetic shielding enclosure comprises of a biodegradable material is deployed to protect from Electromagnetic interference [6]. This biodegradable EMI shielding enclosure embedded over the electronic gadget. EMI radiated from the gadget is absorbed by the EMI shielding enclosure and conductive EMI is absorbed by passive filter embedded in it.

MATERIALS AND METHODS

Material

In this article a biodegradable electromagnetic shielding enclosure fabric made up of banana fibre, carbon graphite additive and reinforced flexible conductive fibre is developed. Whereas, the banana fiber is a natural fiber and it contains (63-64) % of cellulose and results in fast degradation rate (cellulose with 56.3 %) as compared to bio synthesized fiber (PLA 49.5%) [7]. The tensile strength of the banana fiber is high as compared to bio synthesized fiber. The carbon additive used in this experiment is prepared from the same banana plant. This carbon graphite additive further increases the conductivity of fabric [8].Further increase the conductive nature of fibre it is coated with metals like copper, aluminum or silver. The copper coating will "metallize" the non-metallic surface, which can make it electrically conductive and provide additional benefits such as strengthening and protecting the substrate. At higher frequency, the conductivity of the banana fibre increases, absorbing the high frequency electromagnetic waves [9].Thus, reducing the EMI noise radiated from the device kept in it.

Methodology

The natural fiber extracted from banana plant and it has high dielectric strength in the order of 10 even at 10Hz frequency and conductivity increases at high frequencies [10]. This non-conductive fiber contains 63-64 of cellulose and results in fast degradation rate of 56.3 after its life time it does not pollute the environment unlike artificial and synthetic fibers [11,12]. Further conductive coating is prepared with carbon graphite combination applied on the banana fiber. The carbon graphite additive is prepared with at least 80% to 90% of carbon and 20% to 10% of graphite mixer and coated over the banana fiber to increase the electrical and thermal conductivity at power frequency and high frequency. Again the in order to increase the strength of fabric 5% or less of reinforced flexible conducting fiber is intimately knitted with conductive coated banana fiber. This reinforced flexible conducting fibercoated conducting metal like copper, aluminum or silver. Further fabric is attached with passive filter in order mitigate the conductive EMI and the setup is grounded to earth to avoid electro static effect. The perspective view of proposed EMI shielding enclosure is shown in figure 2. The enclosure includes the isolation area. The enclosure has front layer, bottom layer closed completely and the top layer is left open. The top layer can be closed by using the flip cover. The device may be implemented as any electronic device that requires to be electromagnetically shielded for any purpose. The filter prevents the conductive EMI noise entering from the charger to the device. However, the connector used to connect charger and the corresponding device is body earth to ground the EMI noise.

RESULTS AND DISCUSSION

The fibre provides high dielectric strength, thereby observed EMI is dissipated as heat quickly. Since, the carbon graphite additive composed of activated carbon extracted from banana plant, thereby providing effective EMI shielding and exhibits biodegradable property. The biodegradable EMI shielding enclosure fabric comprises at least 70% to 80% of banana fibre, 25 % to 15 % of carbon graphite conductive additives composite and 5% or less of reinforced flexible conducting fibre is developed.*The conducting material is used with a diameter of* 1 micro meter (950 MHz). The dielectric constant of banana fiber having a permittivity of 5 to 35 and the dielectric loss is comparatively high at high frequency which is an advantage to dissipate the absorbed EMI as heat.The low pass filter comprising of one or more passive element like capacitor and inductor is attached with the fabric which, filter out the conductive EMI. The Nano air hole is present in the fabric, wherein, dissipate the heat generated from the EMI of holding device. This reinforced flexible conducting fibre is at least 1µm diameter, thereby reducing the skin depth at radio frequency results in reduced weight and maximum utilization of conducting path; further the reinforced flexible conducting fibre is made up of at least two ply conducting fibre and cross section is in rectangular shape thereby increases the shielding effectiveness. As shown in figure 3.

Besides, the fabric is flexible due to the usage of banana fibre and reinforced flexible conducting fibre, thereby enclosure fit into holding device. The carbon graphite additive act as outer conductive layer and banana fibre act as an inner core thereby outer layer absorb the EMI and inner core provides high resistance to dissipate the heat results increasing the EMI shielding. The proposed work deals with both radiated and conducted EMI. The radiated EMI is mitigated through the natural banana fiber coated with carbon graphite and conductive EMI is suppressed by using passive filter directed to ground.In order to validate the shielding effectiveness of the biodegradable EMI shielding enclosure fabric an experimental investigation is carried out in the laboratory. The experimental setup to measure the EMI generated and absorbed in the EMI shielding enclosure is shown in figure 4 below.

The fibre has high permittivity i.e., 35 to 5 at the radio frequencies range 300MHz to 3GHz which intern high resistivity results more collision of electrons and more losses thereby EMI to heat dissipation rate is high. It employs 1µm diameter of flexible conductive fiber which reduces the skin depth at radio frequency range of 300 MHz to 3 GHz which results less weight and maximum utilization of conducting path thereby providing increase in shielding effectiveness. In order to prove EMI shielding effectiveness the proposed work, it is tested with different types of antenna at more than 800 MHz and the test results are tabulated below in Table 1. From the above results it can be clear that the shielding enclosure reduces the gain when compared without EMI shielding. The shielding effectiveness of the biodegradable electromagnetic shielding fabric has been tested with different antenna configuration and it is found gain is reduced to 3 db. Thus, the EMI radiated from the gadget is absorbed by the proposed EMI shielding enclosure and conductive EMI is absorbed by passive filter embedded in it. The devices outside the enclosure are not affected by the EMI noise radiated from the device kept in it.

CONCLUSION

Electromagnetic shielding enclosure is fabricated with a biodegradable mesh. The mesh is formed by banana fibre coated with conductive material coating. The conductivity of the banana fibre increases at higher frequency. This property aids in absorbing the high frequency electromagnetic waves effectively. The EMI radiation are absorbed by the enclosure, the equipment around the EMI shielding enclosure are protected. Also, the equipment placed inside the enclosure is protected from the EMI noise radiated from the surrounding equipment. This absorbed EMI is radiated as heat through banana fiber. Shielding is a fast way to comply with legal requirements issued by government to prevent electromagnetic interference. Shielding are used when dealing with sensitive measurements that can be affected by ambient fields as well. Thus, shielding is evidently a wide-spread phenomenon and necessity in the electronics industry to meet today's emission standards.

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Table 1.Comparison between Antenna Measurement and Isotropic Radiation (3db)

Types of antenna	Degree	Carbon graphite coated banana fiber fabric shielding	Direct measurement (without shielding)
		Gain (db)	Gain (db)
Parabolic	0	0	0
	90	2.964	3.074
	180	2.448	3.263
	270	2.695	3.509
	360	2.164	3.843
L/2 Dipole (Vertical)	0	0	0
	90	2.022	3.083
	180	2.114	3.594
	270	2.203	3.690

	360	2.022	3.814
L/2 Dipole (Horizontal)	0	0	0
	90	2.312	3.077
	180	2.545	3.640
	270	2.657	3.699
	360	2.150	3.640
YEGI-UDA (Vertical)	0	0	0
	90	2.569	3.010
	180	2.694	3.375
	270	2.527	3.902
	360	2.775	3.964
YEGI-UDA (Horizontal)	0	0	0
	90	2.218	3.010
	180	2.407	3.735
	270	2.120	3.565
	360	2.313	4.057
L/4 Loop	0	0	0
	90	2.068	3.286
	180	2.159	3.545
	270	2.622	3.760
	360	2.114	3.907

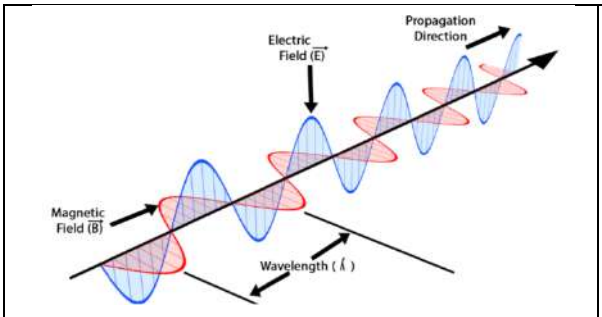

Figure 1. Electromagnetic Wave.

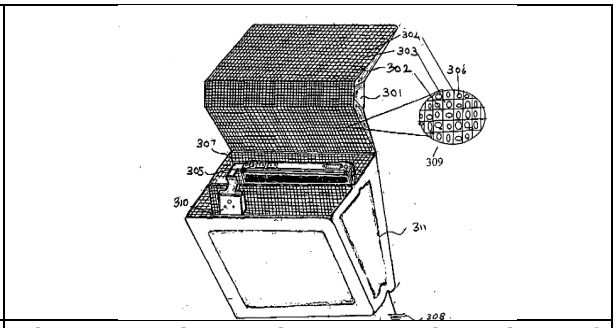

Figure 2. Shows the perspective view of proposed work.

Figure 3. Proposed EMI shielding enclosure designed for Mobile.

Figure 4. Experimental setup to measure the EMI generated.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Opportunistic Observations of Avifauna in Zoological Survey of India, APRC Campus, Senki Valley, Itanagar, Arunachal Pradesh

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ABSTRACT

Eighteen bird species were observed opportunistically during May-October 2017at Arunachal Pradesh Regional Centre of the Zoological Survey of India, Itanagar. The species observed belong to Least Concern (LC) status of the IUCN.F uture studies on the diversity dynamics, seasonal trends and the relationship with microclimate needs to be undertaken.

Keywords: Arunachal Pradesh, Opportunistic, IUCN, microclimate, avifauna.

INTRODUCTION

Birds are excellent markers of an ecosystem's ecological health and are necessary to evaluate the state of the local landscape in order to identify critical factors of bird community structure for avian conservation (Bilgrami 1995, Kattan and Franco, 2004).Birds may be identified opportunistically either by their call or by their appearance. Information on distribution, occurrence and occupancy of bird species can be gathered from opportunistic observations (Ex. Citizen Science) documented via biodiversity databases (Graham *et al*., 2004;Rueteet *al*., 2017).The North eastern state of India, Arunachal Pradesh is the main constituent of Eastern Himalayan biodiversity hotspot region (Daniel *et al*., 2014).The present observations are a pioneer attempt to document and explore the diversity of avifauna from the study site part of the Itanagar Wildlife Sanctuary in Itanagar the capital of Arunachal Pradesh. The Sanctuary listed as one of region's IBA is reported to have 182 bird species (BirdLife International, 2022).

MATERIALS AND METHODS

Arunachal Pradesh Regional Centre of the Zoological Survey of India (APRC ZSI)is adjacent to the Dikrong River in the Senki valley. It has an area of about 7 acres (comprising of the office campus, residential quarters campus and a mini-forest) and is part of the Itanagar Wildlife Sanctuary with Tropical evergreen forest. The opportunistic observation of birds reported in the present study was during May to September2017.The birds were photographed and identified using field guide (Grimmett *et al*. 2011).

RESULTS AND OBSERVATIONS

Nineteen species belonging to fifteen families under five orders are listed (Table 1)photographed during the opportunistic observations in APRC ZSI campus. Two species each in Accipitridae, Estrildidae, Muscicapidae, Pycnonotidae and one each belonging toColumbidae, Cuculidae, Cisticolidae, Hirundinidae, Laniidae, Nectariniidae, Paridae, Passeridae, Phylloscopidae, Sturnidae, Strigidae were observed (Figure 1). The insectivorous Barn swallows were observed swiftly flying over the green patches in the APRC campus and often seen in large social groups sitting on wires in the nearby Ganga Market. Black-eared kite flocks were flying about in circles before settling at the roost. The White-Rumped Munia and the Eurasian Tree Sparrow were found collecting nest material indicating their breeding the area. The Brown Hawk Owl was the only nocturnal species observed. All species are listed under Least Concern (LC) category of the IUCN in their conservation status. The present data provides baseline information on the existence and types of birds in the APRC campus in a valley adjacent to a riverine ecosystem. Future studies on the dynamics of seasonal trends and the relationship with microclimate needs to be undertaken.

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Table 1. Birds observed in APRC ZSI Campus, Itanagar

Sl. No.	Common Name	Scientific Name-Order	Family
		Accipitriformes	
1	Shikra	*Accipiter badius* (Gmelin, 1788)	Accipitridae
2	Black-eared Kite	*Milvus migranslineatus*(J. E. Gray, 1831)	
		Columbiformes	
3	Eastern Spotted Dove	*Spilopeliachinensis*(Scopoli, 1786)	Columbidae

		Cuculiformes	
4	Common Hawk-cuckoo	*Hierococcyxvarius* (Vahl, 1797)	Cuculidae
		Passeriformes	
5	Common Tailorbird	*Orthotomussutorius* (Pennant, 1769)	Cisticolidae
6	White-rumpedMunia	*Lonchurastriata* (Linnaus, 1766)	Estrildidae
7	Scaly Breasted Munia	*Lonchurapunctulata*Linnaeus, 1758	
8	Barn Swallow	*Hirundorustica* Linnaeus, 1758	Hirundinidae
9	Long-tailed Shrike	*Laniusschach* Linnaeus, 1758	Laniidae
10	Oriental Magpie-robin	*Copsychussaularis* (Linnaeus, 1758)	Muscicapidae
11	Common Stonechat	*Saxicolatorquatus*(Linnaeus, 1766)	
12	Purple Sunbird	*Cinnyrisasiaticus*(Latham, 1790)	Nectariniidae
13	Great Tit	*Parus major* (Linnaeus, 1758)	Paridae
14	Eurasian Tree Sparrow	*Passer montanus* (Linnaeus, 1758)	Passeridae
15	White-spectacled Warbler	*Phylloscopus intermedius* (La Touche, 1898)	Phylloscopidae
16	Red-vented Bulbul	*Pycnonotuscafer* (Linnaeus, 1766)	Pycnonotidae
17	Red-whiskered Bulbul	*Pycnonotusjocosus* (Linnaeus, 1758)	
18	Common Myna	*Acridotherestristis* (Linnaeus, 1766)	Sturnidae
		Strigiformes	
19	Brown Hawk Owl	*Ninoxscutulata*(Raffles, 1822)	Strigidae

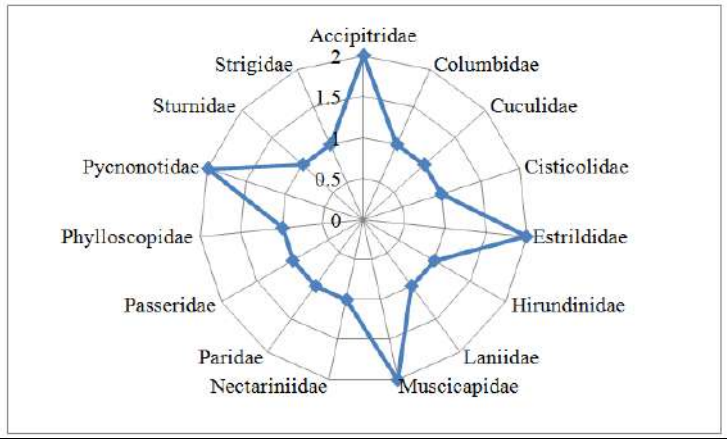

Fig. 1. Number of Species in Each Family

Fig. 2. *Accipiter badius* (Gmelin, 1788)

Fig. 3. *Milvus migranslineatus* (J. E. Gray, 1831)

Fig. 4. *Spilopelia chinensis* (Scopoli, 1786)

Fig. 5. *Hierococcyx varius*

Fig. 6. *Orthotomus sutorius*

Fig. 7. *Lonchura striata*

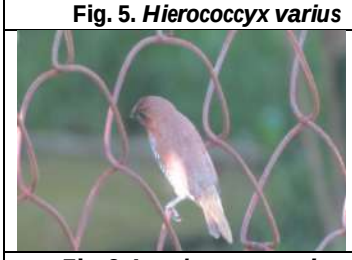

Fig. 8. *Lonchura punctulata*

Fig. 9. *Hirundo rustica*

Fig. 10. *Lanius schach*

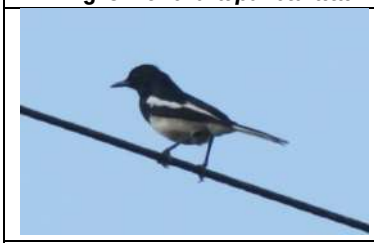

Fig. 11. *Copsychuss aularis*

Fig. 12. *Saxicola torquatus*

Fig. 13. *Cinnyris asiaticus*

Fig. 14. *Parus major*

Fig. 15. *Passer montanus*

Fig. 16. *Phylloscopus intermedius*

Fig. 17. *Pycnonotus cafer*

Fig. 18. *Pycnonotus jocosus*

Fig. 19. *Acridotheres tristis*

Fig. 20. *Ninox scutulata*

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Assessment of Site Suitability for Artificial Groundwater Recharge using Geospatial Technology in Varthur Catchment Area of Bengaluru, South India

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Received: 07 Jan 2022 Revised: 28 Jan 2022 Accepted: 21 Feb 2022

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ABSTRACT

The groundwater scarcity is due to increasing population, urbanization, and expansion of agricultural activities, so need to obtain more attention to groundwater resources. Artificial recharge of groundwater plays a vital role in the sustainable management of groundwater resources. This study aims to assess the suitable sites for the artificial recharge of groundwater were delineated using geospatial techniques in the Varthur catchment. Weighted normalization analysis was carried out in the following thematic layers viz. Land use/land cover, soil, slope, drainage density, lithology, and lineament density. Each thematic layer and individual classes were assigned proper weightage and a score based on their relative contribution to groundwater recharge. Finally, all the thematic layers were integrated by the weighted index overlay (WIO) method. The groundwater recharge map thus obtained was divided into five zones as very high (5.94%), high (22%), moderate (30.82%), least (28.82), and poor (13.02%). The least effective recharge potential is in the eastern parts of the study area due to the low infiltration rate. This is in order to overcome the water shortage and to improve the storage capacity of the groundwater aquifer as well as increase the level of the groundwater table.

Keywords: recharge conditioning factor, geospatial technology, normalized weight, Varthur catchment.

INTRODUCTION

Groundwater is one of the most important natural sources of water for irrigation, drinking, and other uses. In this regard, groundwater plays a vital role in overcoming this shortage. Due to over-exploitation of groundwater without proper recharge mechanism and scanty rainfall, the water table of the open or dug wells in the region are being gone into deeper parts of the surface and many a time even some of them were dried up. In order to increase

the level of the water table of the regions, it was necessary to adopt newer methods of groundwater recharge. Artificial recharge and rainwater harvesting are suitable solutions to address groundwater scarcity apart from demand-side management. The refilling of an aquifer with water from the earth's surface is known as groundwater recharge. Groundwater recharge occurs naturally and artificially. The process of infiltration of water percolates from the surface into the aquifer layer results in natural recharge. Artificial recharge is the use of water to artificially replenish the water supply in an aquifer. In the recharge studies selection of sites for artificial recharge is a very important task (Das, 2003). Several studies have been conducted for the determination of areas most suitable for artificial recharge (Krishnamurthy and Srinivas 1995; Krishnamurthy et al. 1996 and Saraf and Choudhury 1998; Balachandar et al. 2010; Sarup et al. 2011; Amanpreet Singh et al. 2013; Rajasekhar et al. 2018; Mohanavelu Senthilkumar et al. 2019). The majority of the studies involving the identification of suitable sites for artificial recharge have utilized weighted index overlay methods (Saraf and Chowdary 1998; Agarwal et al. 2013). The weighted overlay index method has been implemented in several key groundwater provinces in India (Srivastava and Bhatacharya 2006; Ravishankar and Mohan 2005). For the various themes, a set of weights and their individual features was decided based on expert knowledge considering their relative importance from the artificial recharge viewpoint (Alivia Chowdhury et al. 2010).

Groundwater recharge zones are identified and explored using geospatial technology, which is an important technique for the water resources management system. In recent years, the role of remote sensing and GIS techniques has received much attention with regard to artificial recharge (Shobharam Ahirwar et al. 2020; Suresh et al. 2015). They considered a varying number of thematic maps, such as geology, geomorphology, drainage density, slope, aquifer, permeability, fluctuations of water level or depth to groundwater level and lineament density, etc. Various layers are prepared and weighted overlay analysis is performed to determine the sites suitable for artificial recharge. The groundwater in the major part of the area is being continuously exploited for the purposes of domestic and irrigations. Thus, proper planning for groundwater resources management of the area is required. Usually GIS based studies are employed to find out suitable areas for the augmentation of recharge to groundwater (Ramasamy and Anbazhagan, 1997; Krishnamorthy et al, 1996; Ravi and Mohan 2006; Anbazhagan et al, 2005). For identifying the recharge methods in the study area, a numerical Weighted Parameter Rating (WPR) and the weighted index overlay method was adopted to delineate the suitable sites for artificial recharge.

Study area

The study area Varthur Lake is located in southern Karnataka, between 12°48′24.52″ and 12°53′59.85″ North latitude and 77°24′59.95″ to 77°30′6.72″ East longitude, and covers an area of 241 square kilometres. (Figure 1) with an annual precipitation total of roughly 900 mm, it receives precipitation from both upper east and southwest storms. Parts of the Arkavathi river watershed to the west and the South Pennar River to the east have decreased Bengaluru's water supply. Weathering, fracture pattern, geomorphological setting, and rainfall all influence the adaptability, presence, and aquifer replenishment of groundwater events. The Bangalore urban district has a crystalline storm cellar, which is made up primarily of gneisses and rocks that are influenced by important dykes. Along the city's eastern outskirts, these arrangements have been changed to laterite. The city's household and business needs are heavily reliant on groundwater.

METHODOLOGY

In the present study, geospatial techniques were used for the identification of suitable sites for artificial recharge zones by considering various thematic layers such as available space for recharge, geology, slope, geomorphology, , drainage density, lineament density, and aquifer thickness have been prepared in ArcGIS obtained from various data sources. According to their proportional importance in groundwater occurrence, migration, and infiltration, each thematic map and class were assigned a proper weight and ranking. Then, all the thematic maps were converted into raster format so that they can be easily integrated and processed in a GIS environment. Finally, all the thematic maps were integrated and reclassified into very high, high, moderate, least, and poor groundwater recharge sites by weighted overlay analysis in ArcGIS 10.2.

Normalized Weights

The normalised weight is a groundwater potential indicator based on a multi-parameter analysis. The normalised weight was calculated by dividing the allocated weight of a parameter feature class by the geometric mean. The formula is written as follows:

Normalized weight=(Assigned weight of a parameter)/(Geometric mean) …1

The groundwater recharging zone is indicated by the normalised weighted map. The class with the highest weight is considered a very high suitable zone, while the class with the lowest weight is considered a less suitable or unsuitable zone for groundwater recharge. The map of each theme layer was categorised using normalised weights of distinct attributes of thematic layers. Ranks attributed to certain aspects of the many themes.

Recharge Condition factors

Ten groundwater conditioning factors were classified based on expert knowledge and literature review. Then, the rank of each condition factor class was determined according to expert knowledge and, subsequently, feature normalized ranks were extracted (Rahmati et al. 2014The normalised weights and ranks attributed to distinct aspects of the individual components were supplied individually.

Available Space for Recharge: Available space is one of the factors, which affects the artificial recharge of groundwater. Available space between the ground surface and water table is crucial, because it is the storage space for induced recharge. Water bearing capacity of an aquifer mainly depends upon the available space in the aquifer. The space between the ground surface and water table is called as the available space. Available space is the most important parameter because it serves as potential storage space of induced recharge. Below the water table, the pore spaces will be filled with water and hence no space will be available for the storage of water. The recharged water has to be stored at a depth from the ground surface otherwise it will create swampy or marshy condition. The space between the water table and at 4 m below the ground surface is the available space and it is found out by subtracting a value of 4m from the mean yearly depth to water table. The volume of empty space that is available for recharging per square kilometre is found out from the thickness and the area (Table 1). In the study area, the available space was found from the average annual depth to water table and was divided into fiveclasses and ranked from 1 to 5 (Fig.2).

Geology: It is a well-known fact that the geological setting of a region has a significant impact on the distribution and occurrence of groundwater (Krishnamurthy and Srinivas, 1995). The two types of rock in the study area are classified peninsular gneiss and dolerite dyke. Texture of the rock is the most important factor to hold and transmit capacity of water through the rocks (Table 2). The study area geology is assigned weightage as 5 and ranks are also assigned based on the recharging capacity (Fig.3).

Slope: Slope is one of the important factors controlling the infiltration of groundwater into subsurface. In the gentle slope areas, the runoff is slow which enhance the percolation into subsurface, whereas, high slope areas facilitate high runoff and hence less infiltration (Prasad et al. 2008). So the slope parameter has been assigned 10 ranks are assigned in subclass of the study area (Table 3). The present study area is highly undulating and slope varies from less than 0 to >6. In the study area, slope is divided into five classes and respective ranks are also given (Fig.4).

Geomorphology: The landforms are the result of the various endogenic and exogenic forces operating on the Earth crust, which directly or indirectly affect the hydrological conditions (Reddy and Gajbhiye, 2004). The geomorphologic units of the basin are divided into structural hills, residual hills, shallow pediment, moderately pediment, pediment inselberg complex and valley fills (Table 4). Among these, valley fills and flood plains are very good locations for induced recharge. The landforms usually undergo a series of evolutionary changes and the final shape, geometry, interior and exterior properties and structures, etc., are the end result of such evolutionary changes. Hence, few landforms are able to store groundwater and some others are unstable to do so. Those land forms which are able to store a considerable amount of groundwater can promote artificial recharging of groundwater. So, the

geomorphic land forms are assigned 10 weightage and ranks were assigned against the recharging capacity of the landforms. Various land forms are identified and grouped into four classes and ranks are also assigned (Fig.5).

Drainage Density: The drainage density is an expression of the closeness of spacing of channels and it provides quantitative measure of length of stream within a square grid of the area expressed in terms of length of channels per unit area (km/sq.km). Drainage density characterizes the run off or the quantum of rain water that could infiltrate. Drainage density is significant in the case of artificial recharge because it indirectly indicates the permeability and porosity of the terrain. More the drainage density, the higher would be runoff and vice versa. So, the drainage density is the important parameter for artificial recharge, hence 10 weightage has been assigned (Table 5). In the study area, the drainage density ranges from < 1 to > 6.9 km/km2. It is classified into 3 classes and the ranks assigned to them (Fig.6).

Lineament density: Linear features such as drainages, linear vegetation, weaker plains, secondary porosity, and permeability influence groundwater availability and flow directions. Remote sensing data provides a synoptic view of a broad surface region, which aids in the understanding of lineament's occurrence. Landsat TM data was used to create a linear features spatial map. The higher the lineament density, the better the groundwater prospecting prospects. Lineament density in the research area ranges from 0 to 186 km/sq.km, as indicated in Table 6. It is categorised into five classes using the equal interval approach, with 94.22-186.29 km/sq.km receiving the highest weight and 0-15.64 km/sq.km receiving the lowest. (Fig.7).

Aquifer thickness: Aquifer thickness plays a vital role in the distribution and occurrence of groundwater. The porosity and permeability of aquifer rocks are both affected by the aquifer (Ayazi et al. 2010; Chowdhury et al. 2010). Weathering and fracturing, as well as secondary porosity, transformed the rocks into aquifers (Sener et al. 2005). The thickness of the aquifer was determined using the geophysical resistivity method. (Table 7, Fig.8).

Groundwater recharge integration

The groundwater recharge zones is a dimensionless quantity that applies to perform the groundwater recharge mapping in an area. The weighted linear combination technique was used to determine the groundwater recharge as follows (Adiat et al. 2012; Shekhar and Pandey 2014)

$$GWP = Asr_{Nr} + Geol_{Nr} + Sl_{Nr} + Geom_{Nr} + dd_{Nr} + ld_{Nr} + At_{Nr} \quad (2)$$

The groundwater recharge values were grouped into five classes of poor, least, moderate, high, and very high using the ArcGIS quantile classification method. In this classification method, each class contains the same number of features. Also, quantile method was applied by several researchers due to its efficiency (Nampak et al. 2014; Tehrany et al. 2014).

Numerical Weighted Parameter Rating (WPR)

The various thematic layers were integrated with one after the other through GIS using the numerical weighted parameter rating (WPR) and weighted index overlay method. The following order of sequence has been adopted to derive the final integrated map:

Available space for recharge (I1)	+	Geology (I2)	= O1
O1	+	Slope (I3)	= O2
O2	+	Geomorphology (I4)	= O3
O3	+	Drainage density (I5)	= O4
O4	+	Lineament density (I6)	= O5
O5	+	Aquifer thickness (I7)	= O6

Where, I1, I2… and O1, O2… are the input and output layers respectively

In the first step, Available space for recharge (I1) and geology (I2) layers are integrated by the union option. The integrated output layer (O1) comprises polygons of the drainage density layer and polygons of the slope layer and after union it resulted in new polygons having attributes of both the layers. Adding these two layers derived the weight of each polygon in the integrated layer (O1). In the next step, the O1 layer was intersected with the geomorphology (I3). In this step, the integrated layer O2 was generated by adding drainage density. The O2 layer was integrated with polygons of the geomorphology (I4). Layer I5 involving polygons made around the available space was integrated with layer O3 by the union option. The polygons in the integrated layer (O4) contain the composite detail of all the thematic layers together numerically having maximum weight of 2.66 and minimum weight of 0.85 with standard deviation 2.5.

Composite Suitability Index (CSI)

The delineation of places that are ideal for the development of water harvesting systems has been aided by grouping high-ranking polygons from all thematic layers. Based upon the standard deviation, the polygons were grouped into classes suitable for construction of groundwater recharge structures. A Composite Suitability Index (CSI) has been calculated for each composite unit by multiplying weightage with the rank of each parameter and summing up the values of all the parameters. Categorization of the CSI is achieved by ranging the CSI into 5 classes.

Class 1 Maximum > CSI >= 3 σ
Class 2 3σ > CSI >= 2 σ
Class 3 2σ > CSI >= 1 σ
Class 4 1σ > CSI >= Minimum

Where σ standard deviation

Those polygons, having cumulative weight 0.85 to 2.66 in the final integrated layer were classified as unsuitable for artificial recharge. The polygons classified as least suitable category have the cumulative weight 1.61 to 1.85, whereas moderately suitable category has the weights 275 to 337.5. The polygons classified as highly suitable category have the cumulative weight 1.85 to 2.04.

RESULTS AND DISCUSSION

The index values calculated for each class are used to delineate various groundwater recharge zones, while overlaying the individual thematic layers one over the other. Based on the analysis, the study area has been divided into five classes with respect to the scope for artificial recharge to augment groundwater conditions. The four classes are poorly suitable, least suitable, moderately suitable, highly suitable and very highly suitable for artificial recharge (Fig.9). The highly suitable zones for artificial recharge are observed in the northwestern and central parts, and less suitable zones are found in the northern and southern parts of the study area. For prioritization of recharge zone the high and moderate zones are further classified into Priority-I and less and unsuitable classified into Priority-II (Table 8).The classified artificial recharge zone further divided in the three classes for prioritization of recharge zones. The most suitable zones classified for priority I, the suitable and moderately suitable zones classified for priority II and least suitable and poorly suitable zones are further classified into Priority-I for sustainable development in the study area (Fig.7.4).

CONCLUSIONS

Varthur catchment of was selected for groundwater management studies using remote sensing and GIS technology. In the present study, the delineation of artificial recharge methods, such as analytical weighted parameter rating approach and weighted index overlay method were used to delineate suitable sites for artificial recharging. A value is derived from the rank and weights. This value was used to mark out various priority zones sites for artificial recharging. Overall, it can be concluded that RS, GIS and MCDM techniques are powerful tools for evaluating groundwater potential which can help prepare a suitable and cost-effective groundwater exploration plan for a basin or catchment areas. The groundwater resource map and sustainable watershed management plan is very useful to maintain Varthur catchment for sustainable environment in future.

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Table 1 Rank and weights of the Available Space for Recharge

Class	Assigned rank	Feature Normalized Weight (Nr)
4.70-7.44	1	0.067
7.44-9.08	2	0.133
9.08-10.76	3	0.200
10.76-12.52	4	0.267
12.52-17.29	5	0.333
Total	**15**	

Table 2 Rank and weights of the Geology

Class	Assigned rank	Feature Normalized Weight (Nr)
Peninsular Gneiss	5	0.833
Dolerite dyke	1	0.166
Total	**6**	

Table 3 Rank and weights of the Slope

Class	Assigned rank	Feature Normalized Weight (Nr)
0-0.80	9	0.400
0.08-1.39	6	0.280
1.39-2.00	5	0.200
2.00-2.76	2	0.080
2.76-6.59	1	0.040
Total	**23**	

Table 4 Rank and weights of the Geomorphology

Class	Assigned rank	Feature Normalized Weight (Nr)
Deep pediment	9	0.237
Moderate pediment	8	0.211
Pediment inselberg complex	3	0.079
Residual hill	1	0.026
Shallow pediment	6	0.158
Structural hill	1	0.029
Valley fill	10	0.263
Total	**38**	

Table 5 Rank and weights of the Drainage density

Class	Assigned rank	Feature Normalized Weight (Nr)
0-0.43	9	0.375
0.43-0.99	7	0.292
0.99-1.56	5	0.208
1.56-2.24	2	0.083
2.24-4.12	1	0.042
Total	**24**	

Table 6 Rank and weights of the Lineament density

Class	Assigned rank	Feature Normalized Weight (Nr)
0-15.64	1	0.037
15.64-43.74	3	0.111
43.74-69.53	6	0.222
69.53-94.22	8	0.296
94.22-186.29	9	0.333
Total	**27**	

Table 7 Rank and weights of the Lithology

Class	Assigned rank	Feature Normalized Weight (Nr)
6.80-8.05	3	0.094
8.05-8.87	5	0.156
8.87-9.84	7	0.219
9.84-11.42	8	0.250
11.42-14.58	9	0.281
Total	**32**	

Table 8 Artificial recharge zones and their priorities

Sno	Artificial recharge zones	Area (sq.km)	Area (%)	Priority
1	Poorly suitable (0.87-1.61)	16.66	5.94	Priority-I
2	Least suitable (1.61-1.85)	61.73	22.00	Priority-I
3	Moderately suitable (1.85-2.04)	86.45	30.82	Priority-II
4	Highly suitable (2.04-2.24)	79.16	28.22	Priority-III
5	Very high suitable (2.24-2.66)	36.52	13.02	Priority-III
		280.53	100.00	

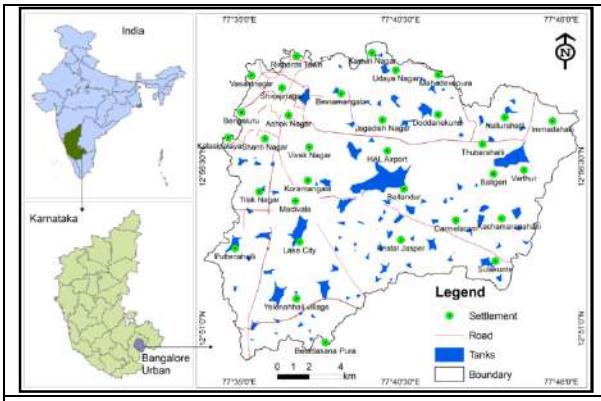

Figure 1: Location of the Varthur Catchment of Dakshina Pinakini River Basin, Karnataka

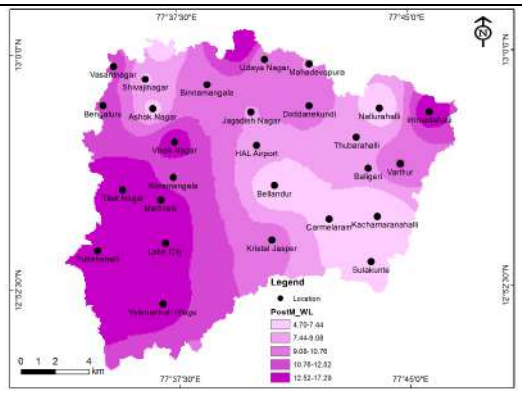

Figure 2: Available Space for Recharge in the study area

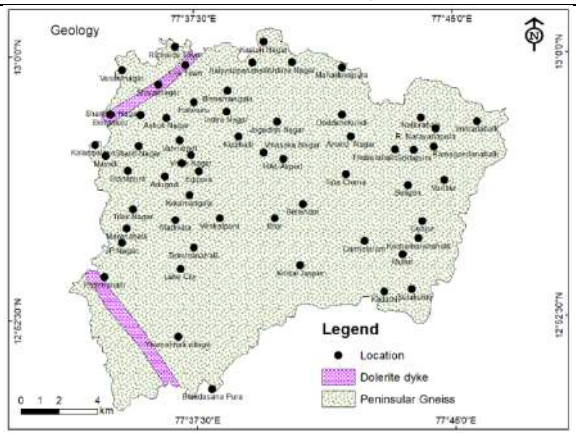

Figure 3: Geology of the study area

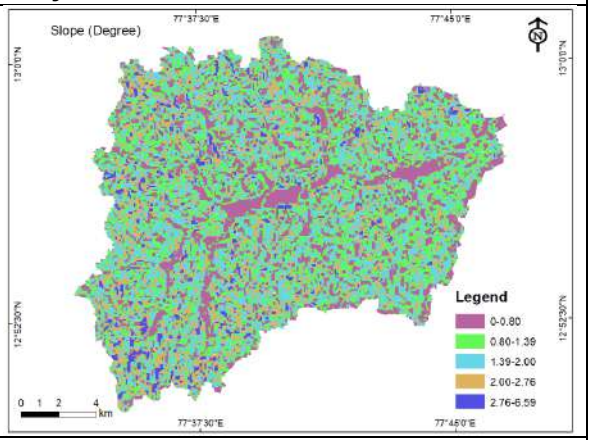

Figure 4: Slope (degree) in the study area

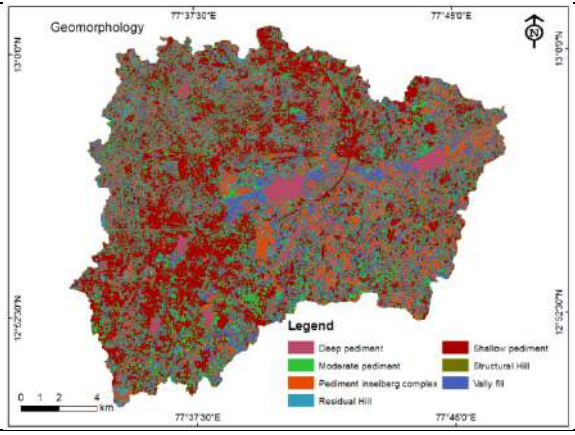

Figure 5: Geomorphology in the study area

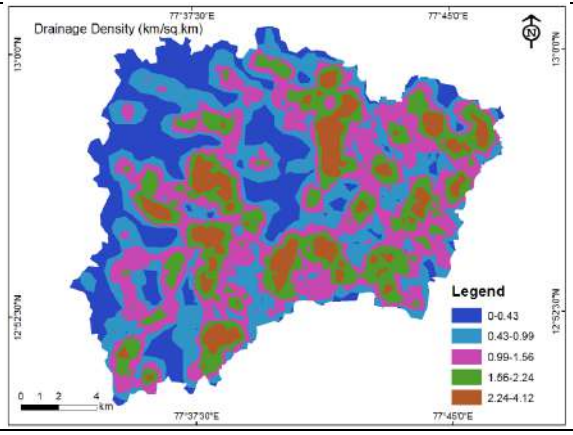

Figure 6: Drainage density in the study area

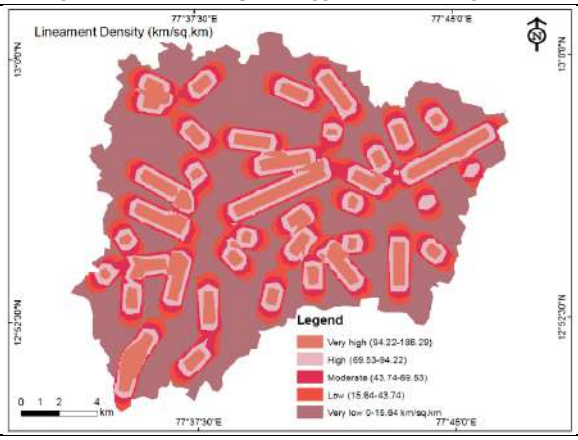

Figure 7: Lineament densities in the study area

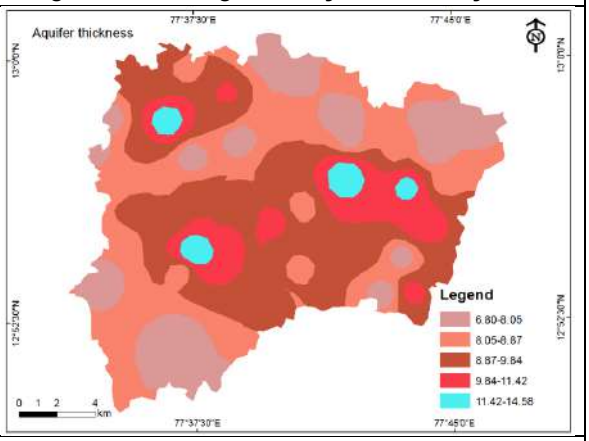

Figure 8: Aquifer thicknesses in the study area

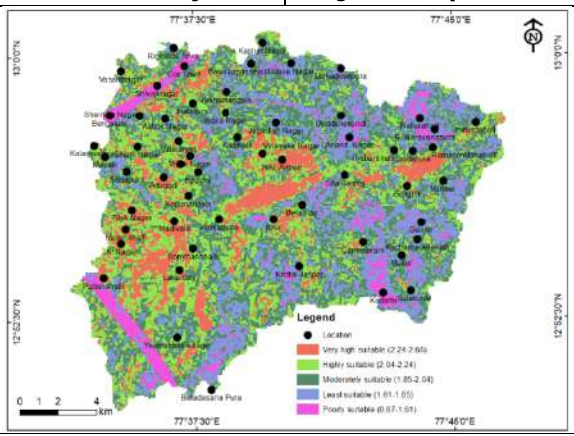

Figure 9: Groundwater artificial recharge zonation mapping in the study area

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Family Functioning Aiding Depressive Symptoms in New Fathers

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Received: 18 Jan 2022 Revised: 05 Feb 2022 Accepted: 21 Feb 2022

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ABSTRACT

Childbirth in India is a family affair which is not merely associated with the concerned parents. The present study is to assess the post partum depressive symptoms in fathers and its relationship with factors of interpersonal relationship with spouse and family environment. The objectives of the study are to explore the relationship of paternal postpartum depression with factors involved in marital relations and family environment. Also to find out the components of marital relations and family environment predicting post partum depressive symptoms. In this descriptive correlation and multiple regression study, a total of 592 fathers from 1 month to 6th month postnatal were contacted through hospitals and chain referral method. The tools used in the study were EPDS, MAQ and FES for analyzing post natal depressive symptoms, marital relations and family environment respectively. Marital relationship components and some of the family environment components were found to be playing part in predicting depressive symptoms in fathers during post partum period. The study helps in throwing light on the ignorant and tabooed issue present in India. Longitudinal study to assess the marital relationship and family environment in pre and post partum period is recommended.

Keywords: Marital Relations; Family Environment; Social Support; Father's Post partum depression; India

INTRODUCTION

Although conditioned to hide their emotions but evidence suggests that men do experience mood disorders post child birth. With the addition of new member in the family, fathers are expected to provide greater material support, thus hiking the finances of the family and eventually the work stress too [1]. The transitional phase of turning to parenthood is stressful which can lead to depression in both men and women in approximately similar ways [2].

Researchers reported in their study about the support received by male partners of depressed women as less, which led them to experience fear, confusion, frustration, helplessness, anger and uncertainty about the future [3]. This altogether can result a disrupted family. Studies reported the prevalence rates of paternal post partum depression to range from 5.3% to 31.7% around the world [4]. It was reported that the prevalence of maternal post partum depression as 22%, which is quite high [5]. Although the prevalence of post partum depression in fathers is not yet known in India, but the studies indicate the strong correlation between maternal post partum depression and the paternal post partum depression. In addition to the mentioned studies, the "contagion effect" suggests that, depression in one partner is transferred to another partner too if they are living together [6]. Parenthood is the major transitional phase in the life of any person which impacts nearly all aspects of psycho- social factors [7].

Marital Relationship and Postpartum Depression

Relationship satisfaction with spouse is suggested as the third strongest factor for predicting post partum depression in fathers [8]. It was noticed that during stressful period or in a stressful situation, men tend to disentangle the interactions with spouse or beloved and the possibility of expressing and eliciting negative emotions while interacting with partners increase [9,10]. Conflict in the marital relationship of the new parents could lead as the predictive factor of post partum depression rather being influenced by depression [11]. The support networks of men are sporadic in comparison to women and thus they turn to their partners for all the support [12]. Through research it was emphasized on the importance of a loving marital relationship stating that with the positive and pleasing bond in marriage, the confidence to deal with new demands increase and hence the fathers tend to face less anxiety [13]. To explore further, here in the present study the couple relationship would be measured by the questionnaire specially designed for the couple's marital adjustment.

Family Environment and Postpartum Depression

A family is a system of social interactions, interdependence and patterns that exist between members of families. Naturally, the family connects together emotionally and the parent is an emotional unit for decision-making and problem-solving in the family [14]. When compared the level of stress experienced by both mother and fathers, it was concluded that both the parents experience approximately similar stress level, although the support received by fathers is way less as compare to mothers [15]. Also in the study on 80 first time parents in Poland, it was analyzed that paternal post partum depression carries a significantly negative correlation(r=-0.68) with experiences and expectations related to family and social life post birth of child [16]. Studies exploring the impact of family environmental components onto paternal post partum depression are really scarce. Post partum depression in fathers is not transient; rather it is a gradual process which increases if not taken care [8]. The present study is to assess the post partum depressive symptoms in fathers and its relationship with factors of interpersonal relationship with spouse and family environment.

Objectives

The objectives of the study are

1. To explore the relationship of paternal postpartum depression with dimensions of family environment.
2. To analyze the relationship of paternal postpartum depression with components of marital relations.
3. To examine the components of marital relationship and family environment in predicting depressive symptoms during post partum period.

METHOD

Sample

A total of 592 new fathers were approached through Max plus Hospital, Panipat and Saket Hospital, Jaipur. Along with this, the chain referral method was also used for contacting the fathers. Inclusion criteria for the study was comprised of following requirements i.e a) fathers of atleast 4 week old and a maximum of 30 weeks old infant, b) fathers living in India, c) couples living together, d) fathers being able to read and write English, e) The exclusion

criteria comprised a) Personal or family history of psychiatric illness of any of the parent, b) birth deformity in child, d) pre term delivery. Participants were briefed about the purpose and process of the study. Proper instructions were given regarding filling up of the designed questionnaire so as to avoid any confusion and asked to put up the signature on the consent before handing over the filled questionnaire. The affected fathers (n= 109) were further analyzed using for their marital relationships (using Marital Adjustment Questionnaire by Dr. Pramod Kumar & Dr. Kanchan Rohtagi) and family environment (using Family Environment Scale by Bhatia & Chadha)

Tools used for the Study

Postpartum Depression measurement in fathers: To assess the depressive symptoms in post partum period, the Edinburgh postnatal Depression Scale [17] is used for both the mothers and fathers. The scale originally designed for mothers is also validated for fathers [18]. This scale is self report evaluation and consists of 10 statements. Each statement is rated through 4 pointlikert scale. The minimum and maximum score that could be obtained on the scale is 0 and 30 respectively. The cut off value recommended for screening the fathers with depressive symptoms is 10. The cut off score with 10 or more can identify the one with current episodes of depression with a sensitivity of 89.5% and specificity of 78.2% [19]. The value for Cronbach's alpha for Edinburgh Postnatal Depression Scale is 0.87.

Marital Adjustment Questionnaire: The marital relationship of the respondent new parents was measured using Marital Adjustment Questionnaire (MAQ) designed and standardized by Dr. Pramod Kumar and Dr. Kanchan Rohtagi [20]. The questionnaire consist total 25 statements with the option of YES/ NO response. The reliability coefficient of the questionnaire was calculated using test retest method and it was noted as 0.84. Whereas, using split half method with spearman formula, it was measured as 0.70.The questionnaire measures three components of interpersonal relationship i.e Sexual component, Social component and Emotional component. The statements in the questionnaire are divided as 4, 9 and 12 for sexual component, social component and emotional component respectively.

Family Environment Scale: The family environment of the respondent new couples was measured using Family Environment Scale (FES) constructed and standardized by Harpreet bhatia and N. K. Chadha [21]. The scale measures three dimensions of family i.e Relationship dimension, personal growth dimension and system dimension. Relationship dimension further measures cohesion, expressiveness, conflict and acceptance & caring in the family. Personal growth dimension measures independence and active- recreational orientation in the family, and System dimension measures organization and control in the family.

Statistical Analysis

Pearson product moment correlation method and multiple regression was used to statistically analyze the obtained data. Along with the descriptive statistics was used to find out the mean, median, average deviation and standard deviation of the scores.

RESULT

Representative characteristics of the sample

In a sample of total 109 fathers facing postpartum depressive symptoms, maximum percentage of the fathers (i.e 75.9%)were of the age above 30 years. Only 29.36% fathers were of the age below 30 years.(Table 1) The average age gap among the husband and wife taken for the study was around three years. Using the Kuppuswamy scale [22], the researchers have targeted only the middle and upper socio economic status population. The reason behind targeting such sample was lack of knowledge of English language in lower socio economic status population. Near about 65.14% fathers had no previous experience of fatherhood. (Table 1). The sample doesn't contain transgender child and only girl and boy child fathers were part of the study with 56.88% as the girl child and rest 43.12% was of boy child. (Table 1)The fathers taken for the study were residing in India. The occurrence rate of paternal post partum depression has been reported to be within 12 months post birth of child by several studies, where 3 months to 6

months are found most crucial for the occurrence of postpartum depressive symptoms in fathers [23]. Here in this study also the percentage of fathers facing depressive symptoms was higher of three to six month old infant father. (Table 1).

Factors contributing to postpartum depressive symptoms

The Edinburgh Postnatal Depression scale helped in screening the fathers who were showing depressive symptoms post child birth. Pearson product moment correlation method aided to identify the relationship of postpartum depressive symptoms with the subtle components of marital relationship with spouse. It was noticed that the postpartum depressive symptoms were found to be significantly correlated ($r= -0.349$, $p< 0.001$) with the emotional component of spousal relations in marriage. Emotional component was found to be inversely correlated with depressive symptoms post child birth i.e. the lesser the emotional bonding between husband and wife, larger would be the symptoms of postpartum depression in fathers. (Table 3)Sexual component is one of the important aspects of marriage. This component was also analyzed associated with depressive symptoms in fathers after the birth of child. Through Pearson product moment correlation method, sexual component was noted as inversely correlated ($r= -0.197$, $p=0.04$) at 95 % level of confidence with the postpartum depressive symptoms. (Table 3) In further analysis, the sexual component and emotional component in the spousal relationship were found significantly directly correlated ($r= 0.269$) with each other at the p value of 0.01. (Table 3)

While scrutinizing for the family environment, the detailed components of family environment were analyzed using the Pearson product moment correlation method. As described above, the three main dimensions of family environment were declared in the Family Environment Scale used in this study. All three dimensions were found related with the depressive symptoms of father in some or other way. Although not every factor in the dimensions were significantly correlated, but four factors among all originated to be significantly correlated with the paternal postpartum depressive symptoms. In the Relationship dimension of family environment, paternal postpartum depressive symptoms were found minimally effected by cohesion and extraversion component in the environment of family. Whereas, the depressive symptoms in father were established to form a significantly inverse relationship ($r= -0.342$, $p< 0.001$) with acceptance and caring factor in the family i,e, more the family accepts the new member or a change in the family, lesser are the chances for the father to be susceptible to depression. (Table 3)While analyzing for the scores of conflict factor in Family Environment Scale, it was specified that higher the score of conflict indicates the less conflict in the family. Here through correlation method, depressive symptoms in fathers were scrutinized as significantly inversely correlated ($r= -0.403$, $p< 0.001$) with conflict component of family environment, which indicates the higher score of conflict would led to lower score of postpartum depression (Table 3).

Considering the interpretation of Family Environment Scale and the relationship of conflict and postpartum depressive symptoms, it could be concluded that conflict has significantly direct relationship with depressive symptoms in new fathers. Conflict was found to have a direct relationship ($r=0.201$, $p= 0.036$) with cohesion in the family at 95 % level of confidence (Table 3). This actually indicates that conflict share an inverse relationship with the cohesion in the family i.e. higher the cohesion, lesser would be the conflict in the family. On the contrary, acceptance and care factor in the family is directly correlated ($r= 0.299$) at 99% level of confidence with the cohesion the family. (Table 3) In the dimension of personal growth in the family, active recreation was found to have minimal relation with depressive symptoms in fathers whereas independence was found to play an important role in indicating the postpartum depressive symptoms in fathers. Pearson product moment correlation states that independence in the family and paternal postpartum depressive symptoms share significantly inverse relationship with each other. ($r= -0.295$, $p= 0.002$). (Table 3) The system dimension of family does play an inseparable part as the control factor of system dimension evolved as significantly directly correlated ($r= 0.442$, $p< 0.001$) with depressive symptoms in fathers after child birth. (Table 3) Both the factors of system dimension share some relationship with the cohesion factor of Relationship dimension i.e. organization factor was significantly directly correlated ($r= 0.365$, $p< 0.001$) with cohesion in the family while control factor was inversely correlated ($r= -0.195$, $p= 0.042$) with cohesion in the family at 95% level of confidence.

DISCUSSION

The present study is unique and first, at the best of our knowledge, to analyze the relativism between postpartum depression in fathers and the detailed components of marital relations and family environment. This could be the epitome for showing the significance of spousal relations and environment of family in the life of a man. In this study, more percentage of fathers screened for depressive symptoms during postnatal period were the father of three to six month old infant (Table 1). Hence, the current study also proves the validity of research carried by Paulson in 2010 [24]. The study conducted by Ramchandani and his fellow beings in 2011 concluded the importance of parental disharmony harmony in the marital relationship is associated with the risk of depression in postpartum period and vice versa [11]. Validating the mentioned studies, the current study also analyzed the relationship of marital components i.e. sexual components and emotional component with the depressive symptoms of father. It was argued by Don and Mickelson that while adjusting for the new demands faced post child birth, both the mother and the father could face the challenges in their interpersonal relationship with each other [13]. The present study was validating the challenges faced after child birth by the father, which could hamper the sexual and social component of marital life of the father.

Family Systems theory [13] explains whenever a major transition for ex- child birth takes place in the family, a new pattern of interactions happen in the members of family which aids the family members to adjust as per the new demand. The theory further states that these new interactive patterns and arrangement for the adjustment to new demand might not be pleasing and can affect the relationship of family members or the marital relationships. Keeping in view this point, this research explored the association of family environment with that of depressive symptoms of fathers after birth of child. In the study conducted by Ramchandani and his fellow mates in 2011, EPDS scores of depressive symptoms in fathers were found to be associated with affection(r= -0.224, p= 0.002), consensus (r= -0.200, p= 0.009), cohesion (r=-0.204, p= 0.006) [11]. Here, it contradicts the results with the given study, as no significant relationship was found between cohesion in the family and the depressive symptoms faced by fathers during postpartum period. The current study clarified the importance of detailed components of marriage and family in the life of father. Considering the association of the studied components with postpartum depressive symptoms, it is not exaggerating to say that these components if controlled can minimize the chance of depression. Hence, Special awareness programs could be organized to spread a word on postpartum depression and coping strategies on post partum depression in India. Families should be counseled in the prenatal period itself to prepare them for the upcoming event. Health professionals could play a key role in this aspect.

Strength & Limitations

Although plenty studies are there on assessing the relationship of postpartum depression with marital relations but a few studies have targeted the detailed aspects of marital relationship. Here in the study the researchers have analyzed the relationship of depressive symptoms with the sexual, social and emotional component of marital health. Also to the best of knowledge, studies relating to the components of family environment in detail are not yet being carried out. Hence this study is a good add on in this respect. The questionnaire chosen for the study is validated on Indian culture, in fact is designed on Indian culture itself. The study conducted was carried out through out in the summers and winters and hence can conclude that the study is free from the biased effect of season on mood. Also since the study is father oriented, the responses are taken from fathers only. The data taken for the study is from the same time i.e cross sectional and hence doesn't signify correctly whether the issues in marital and family relationship aroused due to inclusion of child in the family or were present earlier too.
Further research area is open for this topic.

ACKNOWLEDGEMENT

Appreciation for the study goes to the hospitals for allowing the access to fathers visiting the hospitals. Also the key informants are highly appreciable for providing the contact details of father. The questionnaire was slightly time

taking and contained few personal questions. Authors are grateful to all the new fathers who gave their precious time for the study by filling up the questionnaire.

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Table 1

Sample characteristics	
Variables	%(N= 109)
Primiparous Fathers	65.14%
Age of father less than or 30 years	29.36%
Age of father above 30 years	75.9%
Nuclear family	54.12%
Joint Family	45.9%
Child(Girl)	56.88%
Child (Boy)	43.12%
Infant Age (1-3 months)	34.82%
Infant Age (3-6 months)	65.18%
Family monthly Income less than or 60,000 INR	32.11%
Family monthly income above 60,000 INR	67.9%

Table 2

Descriptive Statistics					
Variables	**Mean**	**Median**	**Minimum**	**Maximum**	**Standard Deviation**
Postpartum Depression	14.55	14	10	26	3.9
Marital (Sexual component)	2.08	2	0	4	1.11
Marital (Social component)	7.36	8	3	9	1.48
Marital (Emotional component)	8.96	9	1	12	2.16
Cohesion	53.29	54	32	65	6.98
Expressiveness	34	35	15	49	6.58
Conflict	45.76	47	24	60	8.07
Acceptance & caring	47.87	48	25	60	7.25
Independence	30.46	31	11	43	7.34
Active- recreation	27.60	28	10	39	5.58
Organization	6.97	7	2	10	1.9
Control	13.11	13	6	19	3.37

Table 3

Pearson product moment correlation

C11= Sexual component, C12= Social component, C13= Emotional component, C21= Cohesion, C22= Extraversion, C23= Conflict, C24= acceptance & caring, C25= Independence, C26= Active recreation, C27= Organization, C28= Control

	PPD	C11	C12	C13	C21	C22	C23	C24	C25	C26	C27
C11	-0.197***										
C12	0.049	-0.046									
C13	-0.349*	0.269**	-0.054								
C21	-0.178	-0.034	-0.052	0.092							
C22	-0.058	0.181	0.048	0.091	0.055						
C23	-0.403*	0.051	-0.079	0.162	0.201***	0.151					
C24	-0.342*	-0.022	-0.222***	0.171	0.299**	0.076	0.542*				
C25	-0.295**	-0.073	-0.037	0.125	0.155	-0.058	0.228***	0.317**			
C26	-0.030	0.122	0.097	0.115	0.004	0.355*	-0.104	-0.211***	0.017		
C27	-0.009	0.257**	-0.092	0.266**	0.365*	0.093	0.044	0.022	-0.012	0.060	
C28	0.442*	0.157	0.079	-0.061	-0.195***	0.117	-0.309**	-0.393*	-0.555*	0.097	0.006 0.948

* Significant at 0.001 p value
**Significant at 0.01 p value
***Significant at 0.05 p value

Table 4

Multiple Regression Analysis

C11= Sexual component, C12= Social component, C13= Emotional component, C21= Cohesion, C22= Extraversion, C23= Conflict, C24= acceptance & caring, C25= Independence, C26= Active recreation, C27= Organization, C28= Control

	Coefficient	Coefficient SE	T	P
PPPD (Predictor Constant)	21.9	4.85	4.52	0.000
C11	-0.757	0.30	-2.50	0014
C12	-0.036	0.21	-0.17	0.866
C13	-0.467	0.15	-3.02	0.003
C21	-0.054	0.05	-1.07	0.287
C22	0.003	0.05	0.05	0.959
C23	-0.104	0.05	-2.25	0.027
C24	-0.016	0.06	-0.28	0.777
C25	0.001	0.05	0.02	0.982
C26	-0.034	0.06	-0.54	0.588
C27	0.327	0.19	1.76	0.082
C28	0.427	0.12	3.67	0.000

S= 3.17 **R-Sq= 40.8%** **R-Sq(adj)= 34.1%**

The regression equation is

PPPD = 21.9 - 0.757 **C11** - 0.036 **C12** - 0.467 **C13** - 0.0538 **C21** + 0.0027 **C22**
- 0.104 **C23** - 0.0161 **C24** + 0.0012 **C25** - 0.0336 **C26** + 0.327 **C27** + 0.427 **C28**

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Investigating Optical Properties of Novel Heterostructure Aluminium Doped Barium Hexaferrite (AlBaM) Cobalt Zinc Ferrite (CZFO) Nanocomposite

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Received: 19 Jan 2022 Revised: 17 Feb 2022 Accepted: 09 Mar 2022

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ABSTRACT

The novel heterostructure $(Al_{0.5}Ba_{0.5}Fe_{12}O_{19})_{1-x}/ (Co_{0.6}Zn_{0.4}Fe_2O_4)_x$ with X= 0.1, 0.2 have been synthesized by using the ball mill technique. Further characterization is done using XRD, SEM, EDAX, FTIR and UV analysis. The XRD Analysis revealed successful hexagonal and spinel formation in the synthesized heterostructure composite. The cell volume and lattice parameter are calculated with the JCPDS card data. The cell volume, lattice a and c decrease with increase in the hard site concentration. Similarly, the average crystalline size calculated by Scherrer's equation also showed decrease in size of the crystal with increase in AlBaM concentration. Comparison between crystalline size and particle size is done using SEM studies. Form UV analysis the band gap energy is found using Tauc's Plot which is 2.563eV for AlBaM-CZFO 80-20 and 2.285eV for AlBaM-CZFO 90-10 respectively.

Keywords: Doped Barium Hexaferrite, Cobalt Zinc Ferrite, Sol-gel Citrate, Sol-gel Auto combustion, Ball milling, Bandgap Energy.

INTRODUCTION

Nano Magnetic composites have various uses in the field of hard disk, supercapacitors, media recording, microwave absorbing, permanent magnets, and refrigeration magnets [1]. Generally, nanocomposites are made by joining two materials with dissimilar geometric arrangements (0-3,2-2,1-3 types) using appropriate synthesis method [2], [3]. Among them the 0-3 type nanocomposite is greatly utilized due to its simple synthesis process and less reaction time [4]. Mainly the M-Type Barium hexaferrite has attained maximum attention due to its high magnetic property [5].

The cobalt ferrite attains ferrimagnetic nature when doped with transition metals [5], [6]. Similarly, many scientists have done numerous studies in electrical, dielectric, optical and magnetic properties of barium hexaferrite nanocomposites [6]–[16]. Yet to the best of the author's knowledge none have synthesized Aluminium doped barium hexaferrite nanocomposite material. Recently our group studied the optical properties of BTO and Barium hexaferrite based nanocomposites. Among them the copper doped barium hexaferrite with cobalt zinc ferrite composite. The study of band gap energy from Tau's plot using UV analysis revealed maximum band gap of 2.8456 eV which increases with the soft site concentration [17], [18]. In the present study, AlBaM-CZFO is prepared using ball milling technique in which individual hard and soft sites were prepared using sol-gel auto combustion and sol-gel citrate method respectively. XRD is used to find the formation of hexagonal and spinel structure in the synthesized heterostructure. The cell volume and lattice parameter are calculated with the help of JCPDS card data. Similarly, the average crystalline size was calculated by Scherrer's equation. Comparison between the size of the crystal and particle is done using particle size distribution obtained from SEM analysis. The optical bandgap of novel heterostructure Aluminium doped barium hexaferrite cobalt zinc ferrite nanocomposite (AlBaM-CZFO) were studied using Tauc's plot from UV analysis.

Synthesis

Preparation of Hard site material: The Aluminium -substituted barium ferrite particles were prepared using the sol-gel auto combustion method (figure 1). Where, stoichiometric amounts of nitrates of barium, ferric and aluminium were dissolved in deionized water followed by the addition of citric acid. The pH value is maintained to 7 using ammonia solution. The solution was heated upto 80 °C in a hot plate until a viscous gel formed. Then the temperature was increased to 100 °C to form brittle powder. Finally, this precursor powder was calcined at 850 °C in muffle furnace for 1 h to get the final aluminium doped barium hexaferrite particles.

Preparation of Soft site material: Cobalt Zinc ferrite nanoparticles were prepared by auto-combustion synthesis (figure2). Where Under fierce stirring, the nitrates of Zinc, Cobalt and Ferric are mixed in stoichiometric amounts. The ratio of citric acid to the metal nitrates is 1:1. The mixture is kept under constant stirring and temperature is gradually increased until a viscous gel form. Later the solution is kept under fierce stirring and the temperature is increased to 75^{0}C until the solution evaporated to dryness. Finally, the dried precursors were ground with mortar and pestle and was annealed at 800 °C for 5 h to get the desired Cobalt Zinc ferrite nanoparticles.

Preparation of nanocomposite material: The hard site AlBaM is combined together with the soft site CZFO at different weight ratios [$(Al_{0.5}Ba_{0.5}Fe_{12}O_{19})_{1-x}/(Co_{0.6}Zn_{0.4}\ Fe_2O_4)_x$] with X=0.1,0.2 with the help of planetary ball milling technique. These are denoted as AlBaM-CZFO90-10 and AlBaM-CZFO 80-20 respectively. The prepared nanocomposites are sintered at 800^{0}C for 3 hours before sending them for further characterization studies (figure 3).

Experimental Techniques

The following characterization is done over the prepared nanocomposites. The Structural analysis were determined using XRD (3rd generation Empyrean, Malvern Panalytical) with Cu Kα (λ=1.540598 Å) radiation. FTIR (Shimadzhu, IR affinity 1A) spectra were analyzed within 4000-400 cm-1 range to confirm Spinel and M-type hexaferrite metal-oxygen bond. Particle size distribution & morphology was studied using SEM Jeol JSM 6390model with EDX for composition analysis. The optical properties of the nanocomposite were analyzed within 200 to 800nm using UV-2400PC Series with slit width 1.0nm and light source of wavelength 360 nm. From the UV analysis the bandgap energy was calculated using tauc's plot.

XRD Analysis: The structural analysis of all the prepared samples are carried out using XRD. All peaks are indexed in accordance with JCPDS card number 71-1376 and 88-2152 for AlBaM and CZFO respectively. The formation of hexagonal structure with space group of P63/mmc for AlBaM and cubic structure with Fd-3m space group for CZFO respectively were confirmed. The cell volume and lattice parameter a and c for all the prepared samples are calculated and it matches with the standard JCPDS card data. The figure 4 represents the XRD peaks for

AlBaM-CZFO 80-20 and 90-10 respectively. The lattice parameter and cell volume are measured and presented in table 1 below, which shows that the values of a, c and cell volume decreases with increase in the concentration of AlBaM. The average crystalline size is measured using Scherrer's equation (as shown in the table 2). Where to calculate the average size for AlBaM-CZFO the peaks (202),(220),(310),(302),(206),(302) is used by substituting K=0.89. here we can clearly see that for AlBaM-CZFO, the crystalline size decreases with increase in hard site concentration.

Morphological Analysis: The SEM image and histogram of particle size are shown in figures 5. The surface of the sample appears to be having dense microstructure with good crystal nature of uniform distribution which is joined together due to agglomeration. The size of the grain calculated from the SEM image shows higher values than the size of the crystal calculated using XRD. This may be due to agglomeration within the particles in the sample. The EDAX analysis was performed on the surface of all the samples (table 3). The EDX spectrum (shown as in figure 6) reveals the presence of Aluminium, Barium, Cobalt, Zinc, Fe and Oxygen elements in AlBaM CZFO 90-10 and 80-20 samples respectively, and it confirmed that no element/s (impurity) is/are present in the sample other than Al, Ba, Co, Zn, Fe and O.

FTIR Analysis: The FTIR spectrum of AlBaM-CZFO is shown in figure 7 along with absorption table (table 4). The band V_1 was found between 522- 601cm^{-1} which is due to tetrahedral Zn^{2+} ion stretching complexes. The band V_2 between 408-460 cm^{-1} is due to octahedral complexes which correspond to the presence of Fe–O stretching vibrations confirming the presence of M–O stretching band in ferrites metal-oxygen bonds and are attributed to the formation of hexaferrite structure. The observation of these bands for the samples confirms the ferrite structure in the prepared nanocomposites. It is obvious that these bands vary to some extent on increasing the ratio of hard/soft content, which could be due to coupling of both the phases in the composites. The difference in the V_1 and V_2 bands could be related to the difference in Fe^{3+}-O^{2-} distances for A and B sites. The peaks 543 cm represents the characteristic peaks of Cobalt Zinc Ferrite and the peak 596 represent Al–O bending vibrations in the tetrahedral sheet. The band V_3 from 650 to 740 corresponds to the characteristic absorption of Fe-O bond which confirms Fe-O stretching modes. The band at 856 cm-1 is assigned to divalent octahedral metal ion–oxygen ion complexes which represent Ba–O stretching. The band at 1433 represents O-H bending which further provides an evidence for the formation of M- type ferrite nanoparticles.

Optical Analysis: The optical properties of the nanocomposite were analyzed within 200 to 800nm using UV-2400PC Series with slit width 1.0nm and light source of wavelength 360 nm. From the UV analysis the bandgap energy was calculated using tauc's plot as shown in figure 8. It is found that the bandgap for AlBaM-CZFO 90-10 (2.2854eV) is lesser than AlBaM-CZFO 80-20 (2.5632eV). Thus, the bandgap energy Increases with the decrease in the hard site concentration.

CONCLUSION

A novel nanocomposite of Al doped barium hexaferrite/ Cobalt zinc ferrite is successfully synthesized using Planetary ball milling technique. Generally, the optical parameters in nanocomposites are greatly affected via synthesis, temperature, and the concentration of AlBaM and CZFO sites. The XRD Analysis revealed successful hexagonal and spinel formation in the synthesized heterostructure composite. The cell volume, and the lattice a and c are calculated with JCPDS card data. The cell volume, lattice a and c decrease with increase in the hard site concentration. Similarly, the average crystalline size calculated by Scherrer's equation also showed decrease in size of the crystal with AlBaM concentration. The SEM and histogram of particle distribution are investigated showing good crystalline nature with dense microstructure. It is interesting to find a uniform distribution in grain size within the sample. From the EDAX spectrum confirms the purity of the prepared sample. The presence of band V_1, V_2 and V_3 in FTIR analysis confirmed the formation of both Metal-Oxide bonds and hexagonal structure in the prepared sample. The study of band gap energy from Tau's plot using UV analysis revealed maximum band 2.2854eV for AlBaM-CZFO 90-10 nanocomposite which decreases with the soft site concentration. These novel results are amazing and unique according to the Author point of view.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENT

We, the authors are grateful to our President, Chancellor, Chief Executive Officer, Vice Chancellor and Registrar of Karpagam Academy of Higher Education, Coimbatore, India for providing facilities and encouragement.

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Table 1. Lattice parameter and cell volume for AlBaM CZFO 80-20 and AlBaM CZFO 90-10.

(JCPDS:71-1376) for AlBaM (JCPDS: 88-2152) for CZFO	a	c	Cell Volume (V)
Standard Value (AlBaM)	10.81	8.707	881.1515
Standard Value (CZFO)	8.396	=	591.8577
Calculated Value AlBaM CZFO 80-20			
AlBaM(002)	-	8.819576	907.5546
AlBaM (310)	10.90052	-	
CZFO (311)	8.37097	=	586.5801
Calculated Value AlBaM CZFO 90-10			
AlBaM(002)	-	8.81524	902.4974
AlBaM(310)	10.87278	-	
CZFO (311)	8.325643	=	577.103

Table 2: Average crystalline size of AlBaM-CZFO nanocomposite calculated using Scherrer's equation.

Sample	Conc. H-S	Peaks Taken	Range of particles (nm) From	Range of particles (nm) To	Average size (nm)	Inference
AlBaM CZFO	**80-20**	(202), (220), (310), (302), (206), (302)	12.23	31.65	22.31	Decreases with increase in hard site
	90-10		12.08	26.63	19.94	

Table 3: Quantitative data extracted from the EDX spectra of AlBaM-CZFO Nanocomposite.

Sample	Element	90-10			80-20		
		Apparent Concentration	Weight %	Atomic %	Apparent Concentration	Weight %	Atomic %
AlBaM-CZFO	Al	1.03	5.84	7.57	1.28	6.65	8.50
	Ba	10.28	33.85	8.62	10.54	31.89	8.02
	Co	0.76	2.57	1.53	0.95	2.93	1.71
	Zn	0.03	0.11	0.06	0.58	1.80	0.95
	Fe	8.35	28.09	17.60	8.79	27.03	16.71
	O	15.41	29.54	64.62	16.67	29.71	64.10
Total		100.00			100.00		

Table 4. FTIR Table for the prepared nanocomposite.

Absorption (cm^{-1})	Functional Group
V2 (408-460)	Fe-O Stretching confirming M-O_{octa}
V1 (522-601)	M-O_{tetra}
543	Characteristic peak of $CoFe_2O_4$
596	Al-O bending
V3 (650-740)	Characteristic peak of Fe-O
856	Ba-O Stretching
1433	O-H deformation, Evidence for M-Ferrite nanoparticles formation

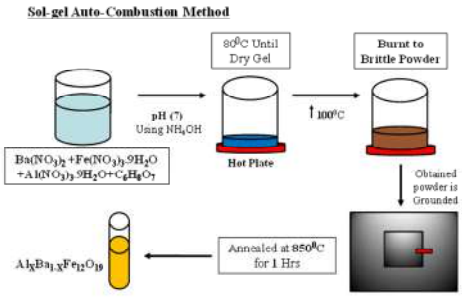

Figure 1. Synthesis of Aluminium doped $BaFe_{12}O_{19}$ (AlBaM).

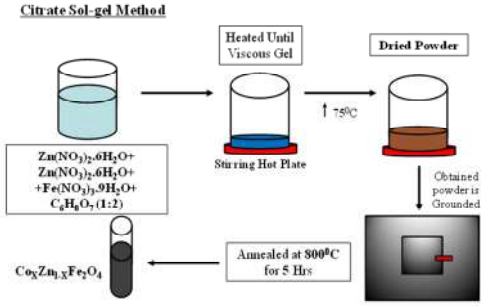

Figure 2. Synthesis of Cobalt doped Zinc Ferrite (CZFO) by Citrate Sol-Gel Method.

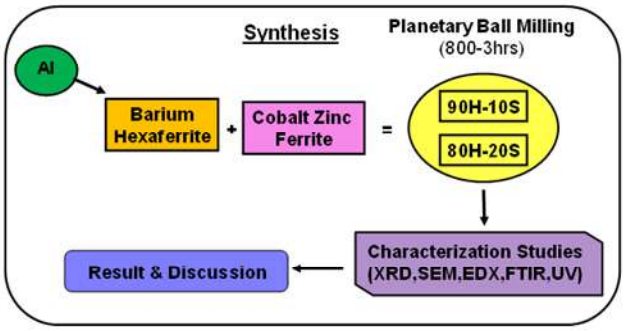

Figure 3. Synthesis of Nanocomposite AlBaM-CZFO.

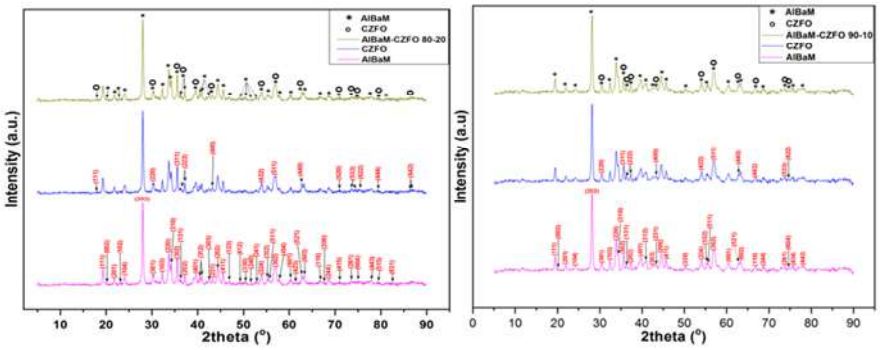

Figure 4. XRD patterns for AlBaM-CZFO 80-20 and 90-10 respectively.

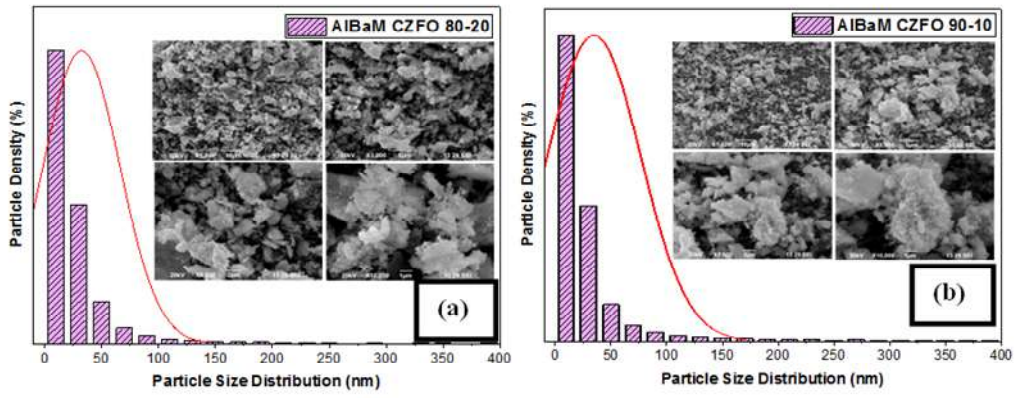

Figure 5. Typical SEM image with histogram of size of the particles of AlBaM-CZFO nanocomposite.

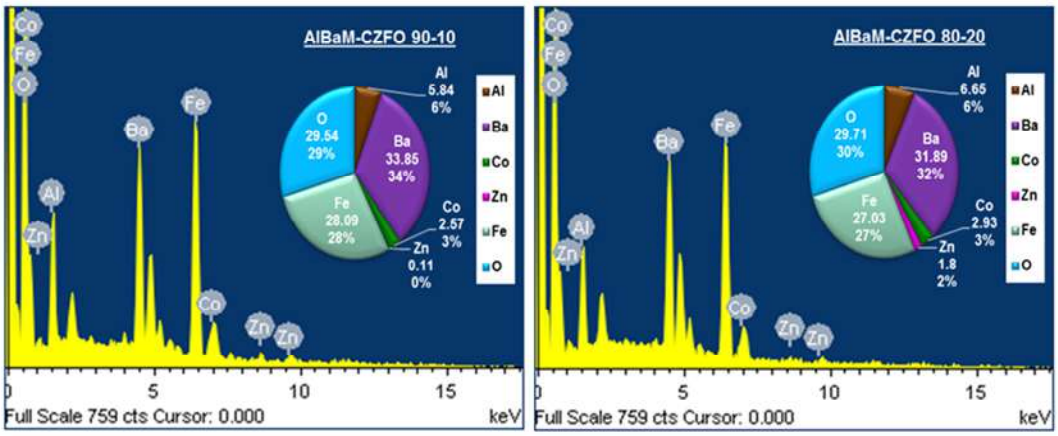

Figure 6. EDAX analysis for AlBaM-CZFO revealing the existence of Al, Ba, Co, Zn, Fe & O elements along with weight %.

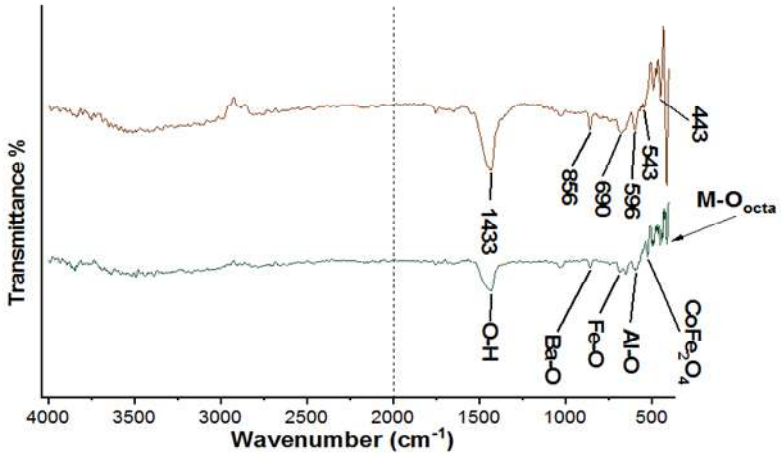

Figure 7. FTIR Graph of AlBaM-CZFO 90-10 and AlBaM-CZFO 80-20

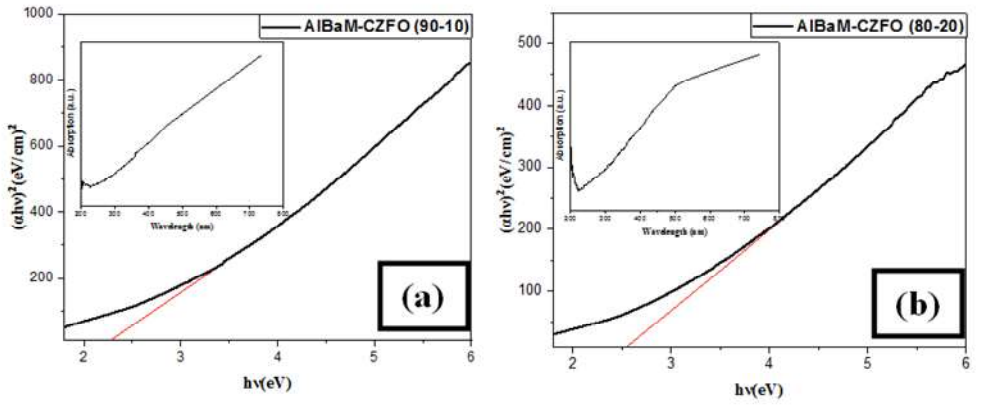

Figure 8. Absorption vs wavelength with Tauc's plot for (a) AlBaM-CZFO 90-10 and (b) AlBaM-CZFO 80-20 respectively.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effectiveness of Foot Massage and Hand Massage on Pain Management among Post Operative Client

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Received: 21 Feb 2022 Revised: 06 Mar 2022 Accepted: 25 Mar 2022

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ABSTRACT

Surgery is a life event of dramatic significance which disrupts an individual's personal, physiological and economic lives as well their physical bodies. pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or is described in terms of such damage. Pain experience involves sensory, emotional and cognitive phenomena. One of the non-pharmacology therapy in the management of pain is relaxation. The relaxation induced through hand foot massage can be helpful for reducing the postoperative pain. The study was aimed to assess the Effectiveness of foot massage and hand massage on pain management among postoperative client. Quantitative research approach and Quasi experimental pretest, posttest with control group research design was adopted for this current study. The population of the study was post-operative client during first postoperative day. 60 clients were selected by convenience sampling technique, with 30 clients in each group: group I received foot massage and group II received hand massage. Pre- and posttest pain levels were assessed by using a visual analog scale. The duration of intervention was 10-15 minutes and given in two times at an interval of 2 hours. The study result proved that foot massage and hand massage were effective on postoperative pain management in both group I and group II. Comparing the effectiveness of hand massage and foot massage it showed statistically significant at $p < 0.05$. The study finding revealed that foot massage was effective in reducing pain among post operative clients. The study concludes that foot massage was effective in reducing postoperative pain hence the nurse as a care giver can explore the massage as a complementary approach to provide cost effective and quality care in pain management.

Keywords: Effectiveness, Foot Massage, Hand Massage, Postoperative Pain, Pain Management and Postoperative client

INTRODUCTION

Surgery can cause many issues such as discomfort, pain, anxiety, nausea, and vomiting. Among these, the big postoperative concern is pain. Post-cesarean section pain is characterized as acute, i.e., it provides a subtle beginning with a predictable end and is closely related to the tissue damage caused by the inflammatory reactions arising from a traumatic event that causes pain [1]. Pain is a dynamic, multifaceted phenomenon in which a specific, unique experience can be difficult to define or explain and sometimes hard to perceive and understand by others. Pain often leads to debilitation, diminished quality of life, and depression [2]. Pain is one of the most common complaints of postoperative patients worldwide. Despite the drugs and anesthetic techniques available, the prevalence of post operative pain is still high. Nearly more than half of the postoperative clients experienced moderate to severe pain on first postoperative day even though sedative drugs had been administered. A large survey reported that of 300 patients, 86% experienced postoperative pain; of whom 75% had moderate/severe pain during the immediate postsurgical period. Although about 88% received pain medications 80% of them experienced adverse effects and 39% reported moderate/severe pain even after receiving it [3,4,5].

Pain is one of the commonly experienced symptoms by patients with acute and chronic conditions. Nurses have a crucial role to control postoperative pain. The two methods most commonly used for pain control are pharmacologic and non-pharmacologic. However, the pharmacological approach may not entirely relieve all aspects of postoperative pain. Therefore, non-pharmacological methods such as massage may potentially reduce the postoperative pain [6]. The use of complementary nonpharmacologic interventions such as massage has been suggested in the clinical practice guidelines of the Society of Critical Care Medicine given their opioid-sparing and analgesia-enhancing potential. Massage has been defined as the manual manipulation of muscles and soft tissues of the body through the application of various systematic and rhythmic hand movements [7]. Complementary therapies are commonly used treatment modalities for pain relief in present days. Massage is a technique that applies pressure to parts of the body by stroking, stretching, pulling and kneading. Its aims to offer relaxation mentally and physically. Massage may concentrate on the muscles, the soft tissues, or on the acupuncture points. Massaging hands and foot stimulates the body to come back in balance. Massage can provide several benefits to the body such as increased blood flow and reduces muscle tension [8].

The purpose of massaging is to assist the treatment procedures by affecting the locomotor system and the nervous system as well as cardiovascular system. Massaging results in a variety of comforts such as general relaxation in the body, deep breath, resting, and drowsiness. The unspecialized nerve cell endings that initiate the sensation of pain are called nociceptors; these are sensory receptors that send signals of pain and are generally located at the surface of internal tissues and beneath the skin, densely in hands and feet. Therefore, applying foot and hand massages is considered to be a significantly appropriate method in pain reduction [9]. Hand and foot massage is one of the cheapest and cost effective methods to reduce pain among post-cesarean mothers. Foot and hand massage stimulates the nerve fibers to produce pain-relieving endorphins. Hand massage is a type of reflexology in which parts of the hands are rubbed with the fingertips, knuckles, and blunt with the purpose of stimulating nerve endings for various organs believed to be present in the hands. Foot massage is a technique which is used for relaxation and to alleviate the sore soles and arches [10]. Therefore, the researched aimed to investigate the effectiveness of hand and foot massage on relieving pain among postoperative clients.

Statement of the Problem

A study to assess the effectiveness of Effectiveness of foot massage and hand massage on pain management among postoperative client.

MATERIALS AND METHODS

It was an quantitative, quasi experimental pretest, posttest with control group design. The study participants were 60 postoperative clients admitted in the post operative unit at VMMC&H, Karaikal. A total of 60 samples and 30 in each group who fulfills the inclusion criteria (postoperative day 1) were selected by using a convenience sampling technique. The content validity of the tool was obtained from the experts from Medical and Nursing field. The tool includes two parts on part I was demographic variables and part II was assessment of pain by visual analog scale. An informed written consent was got from the participants. Pretest was conducted for both group-I and group -II. foot massage was given to hand group I, massage to group II. In group I after the pretest assessment of pain the first time of intervention (foot massage) was performed for 10-15 minutes in each foot and posttest –I level of pain was assessed immediately. The second time of intervention was performed at an interval of 2 hours and posttest-II level of pain was assessed. In group II (hand massage) after the pretest assessment of pain, the first time of intervention was performed for 10 minutes in each hand and posttest-I level of pain was assessed immediately. The second time of intervention was performed at an interval of 2 hours and posttest-II level of pain was assessed. The collected data were analyzed by using SPSS software The both descriptive statistics and inferential statistics were calculated.

RESULT

Table.1 shows that frequency and percentage wise distribution of the post operative clients in both group-I and the group-II. Also depicts the association with the pretest level of pain with selected demographic variable. Among Group-I clients demographic variable like age in years had significant association at $p \leq 0.05$ where as in Group-II clients demographic variable like sex had significant association at $p \leq 0.05$. Table 2 indicates the pre- and posttest I and posttest II mean, SD levels of pain for two groups. In group I, the pre- and posttest 1 and posttest II mean, SD values were 5.687, 4.442, & 2.568 respectively. In group II, the pre- and posttest 1 and posttest II mean, SD values were 5.632, 3.8 and 1.956 respectively. The calculated paired t test value were P1&P2 11.256 and P1&P3 10.563. It shows that statistically significant in group- I where as in group-II calculated paired t test value were P1&P2 12.365 and P1&P3 11.128. It also shows that statistically significant. The calculated un paired t test value was 15.656. It shows that statistically significant.

DISCUSSION

The present study result reveals to evaluate the effectiveness of foot massage and hand massage on postoperative pain. The study result proved that both foot and hand massages were most effective in management of post operative pain in that compare to hand massage the foot massage was slightly shows high significant value in the reduction of postoperative pain. The following studies were supported the present study result. W. Shebi Mol1, et al., (2020) stated that the hand and foot massage ass effective in reducing the level of pain perception among Lower Segment Caesarean Section mothers [11]. A study was conducted by Sozen et al., (2020) found that a significant reduction in the need for analgesics for the patients in the foot massage group and hand massage group compared with the control group ($P<0.05$) [12]. A Similar type of study was conducted by Koraş, K et al., (2019) and revealed that Foot massage decreased postoperative pain and anxiety levels in patients undergoing laparoscopic cholecystectomy surgery [13]. Babu J et al., (2019) concluded that foot massage is effective in reducing pain in the incision site among post-cesarean mothers [14]. Xue, M et al., (2016) also supported the Post-operative massage intervention can reduce anxiety and pain in patients after caesarean delivery [15].

CONCLUSION

The study findings are proved that hand massage and foot massage are more effective in reducing the post operative pain. Further studies may be conducted by using different type of nonpharmacological measures like music therapy, hot massages etc as a management strategy in reducing the postoperative pain.

Ethical consideration
Obtained approval from the Institutional human ethical committee, the concerned authority and got written informed consent from the study participants.

Acknowledgement
The author has grateful for the Dean, Hospital management, Ward Nurses colleagues and study participants for their cooperation.

Financial support and sponsorship
Nil

Conflicts of interest
There are no conflicts of interest

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Table 1. Frequency, Percentage and Association between the pre test level of pain among post operative client.

DEMOGRAPHIC VARIABLES	GROUP-I (n=30)		GROUP-II(n=30)		Chi square
	F	%	F	%	
1.Age in years					
a) 25-30	4	13	8	27	11.523*
b) 36-40	18	60	14	47	
c) 41-50	6	20	5	17	
d) Above 51	2	7	3	9	
2.Sex					
a) Male	20	67	25	83	10.258*
b) Female	10	33	5	17	
3. Education					
a) Primary	15	50	18	60	5.365
b) High School	10	33	5	17	
c) Degree	5	17	7	23	
4. Diet					
a)Vegetarian	14	47	10	33	4.895
b) Non vegetarian	16	53	20	67	
5.Religion					
a) Hindu	16	53	19	63	6.854
b) Muslim	8	27	5	17	
c) Christian	6	20	6	20	

Table 2. Comparison of pre and post tests mean, SD on level pain among postoperative client

GROUPS	TEST	MEAN	SD
GROUP-I Foot Massage	PRETEST (P1)	5.687	0.775
	POST TEST-I(P2)	4.442	1.102
	POST TEST-II(P3)	2.568	1.156
GROUP-II Hand Massage	PRETEST(P1)	5.632	0.725
	POST TEST-I(P2)	3.8	0.945
	POST TEST-II(P3)	1.956	1.086

Table 3. Comparison of post test-II mean, SD on level pain among postoperative client

GROUPS		MEAN	SD	Unpaired t value
GROUP-I Foot Massage	POST TEST-II	2.568	1.156	15.656
GROUP-II Hand Massage	POST TEST-II	1.956	1.098	

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Assess the Factors Influencing Compliance among Patients with Chronic Obstructive Pulmonary Disease

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Received: 02 Jan 2022 Revised: 20 Jan 2022 Accepted: 08 Feb 2022

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ABSTRACT

A descriptive non experimental study was conducted to assess the factors influencing compliance among patients with chronic obstructive pulmonary disease. A total of 70 study participants who fulfils the inclusion criteria were selected by non probability convenient sampling technique at Government Head Quarters Hospital, Cuddalore. The data were collected through demographic & factors influencing compliance questionnaire and analyzed through descriptive & inferential statistics. The result reveals that majority of the study participants 37.1% knows that lung condition require regular follow up, 47.1% unable to follow dietary recommendation, 43.8% says I want to live longer, 55% got sick after performing breathing exercise, 39.6% my condition requires regular exercise, 75.4% health education given very slowly during follow up session. The study concluded that majority of COPD patients had good compliance in terms of adhering treatment.

Keywords: Hospital, COPD, patients, lung, treatment.

INTRODUCTION

Chronic obstructive pulmonary disease is a common, preventable, and treatable NCD characterized by persistent respiratory symptoms and airflow limitation due to abnormalities in the airway and (or) alveoli. According to the Global Burden of Disease study between 1990 and 2017, there was a global reduction in mortality due to maternal and neonatal disorders, enteric infections, and tuberculosis and a rise in deaths due to non communicable diseases (NCDs). As reported in the study, the leading causes of early deaths among NCDs are ischemic heart disease, stroke, and chronic obstructive pulmonary disease (COPD). Worldwide cases of COPD were over 250 million. From 1990 to 2015, the global prevalence of COPD increased by 44.2%.

In a systematic review and meta-analysis, Halbert et al. reported the pooled prevalence of COPD from 37 studies is 7.6%, and pooled prevalence from 26 spirometry estimates is 8.9%. Meta-analysis estimates from 123 globally searched studies reported the COPD prevalence rates among people aged 30 years and older is 11.4%. In 2015, nearly 3.2 million global deaths were reported due to COPD (i.e., 5% of overall deaths) with an increase of 11.6% in deaths due to COPD as compared to 1990. Globally in 2015, 63.9 million disability-adjusted life years (DALYs), which is defined as the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability were reported due to COPD. So it is essential that COPD patients should follow adherence to treatment regiment in order to take care for themselves and prevent complication to lead an independent quality of life especially in pandemic situation of COVID 19.

NEED FOR THE STUDY

COPD is the second most common cause of NCD-related deaths in India, with the age-specific prevalence of COPD increasing rapidly after the age of 30 years. It was noted that the prevalence of COPD among individuals between 5 and 29 years ranged from 0.1%–0.9%, which increased to 1.6%–28.3% among the population aged 30 years or more. From 2007 to 2017, the percentage change in death and premature death due to COPD is over 39% and 37%, respectively. In India, COPD is the second most leading cause of DALYs with the 36% mean percentage change in the number of DALYs from 1990 to 2016. The rate of DALYs per case due to COPD was 1.7 times higher than the global average in 2016. Previous research studies from India suggest the economic impact in terms of direct and indirect cost is on the higher side (direct medical cost: Rs. 29,885 ± 11,995.33 or US$300–500 approximately; direct nonmedical cost: Rs. 7,441.25 ± 2,228.90 or US$90–155 approximately) and is associated with absenteeism at the job for a significant duration of time. A study conducted on COPD patients by Mahmood et al. in Uttar Pradesh reported that 56.5% of patients were nonsmokers, indicating the major role of second-hand smoke and other risk factors. A self-management intervention has shown to be effective in improving compliance in chronic disease populations. These factors have given impetus for the investigator to select and conduct the study related to chronic obstructive pulmonary disease.

STATEMENT OF THE PROBLEM

Assess the factors influencing compliance among patients with chronic obstructive pulmonary disease.

OBJECTIVES

- Assess the factors influencing compliance among patients with chronic obstructive pulmonary disease.
- Find association between the factors influencing compliance among patients chronic obstructive pulmonary disease with selected demographic variables.

ASSUMPTION

- Chronic obstructive pulmonary disease patients may not adherence to treatment session and who are not following therapeutic regimen may get complications.
- Chronic obstructive pulmonary disease patients may have inadequate practice have influence on non compliance.
- Financial status and social support may have influence on compliance.
- Aging and psychological factors can cause non compliance.

LIMITATIONS

- The sample size is limited to 70 COPD patients.
- The period of study is limited to 4 weeks.
- This study is limited to population who are residing in and around Cuddalore.
- This study is limited to population who are willing to participate in the study and able to communicate in Tamil or English.

RESEARCH METHODLOLOGY

- **Research Approach** - Quantitative research approach
- **Research Design** - A descriptive non experimental study design
- **Setting** – Government Head Quarters Hospital, Cuddalore.
- **Population** - Both male and female patients with COPD who were willing to participate in the study.
- **Sample** – Both male and female patients with COPD who are taking treatment in Government Head Quarters Hospital, Cuddalore.
- **Sample size** - 70 male and female general population
- **Sampling Technique** - Non probability convenient sampling technique

INCLUSION CRITERIA

- Peoples who are in the age group of 30 years and above.
- Peoples who are residing in and around Cuddalore.
- Peoples who are available during the data collection time.
- Peoples who are willing to participate in the study and able to communicate in English or Tamil.

EXCLUSION CRITERIA

- Peoples who were not residing in and around of Cuddalore.
- Peoples who are below 30 years of age.
- Peoples with hearing impairment and mentally challenged.

DESCRIPTION OF TOOL

The tool used for data collection was an interview technique. It consists of two parts:

- Part I - Demographic Data (10)
- Part II – Factors influencing compliance questionnaire (6)

DATA COLLECTION PROCEDURE:

- **Formal written permission** for conducting the study was obtained from The Joint director of Medical and Rural Health Services and Family Welfare, Cuddalore District
- **All participants were informed** about the study.
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- The main study was conducted between **01. 06. 2021** at **30.06. 2021** at Government General Hospital, Cuddalore.
- **Seventy study participants who met the inclusion criteria** were selected by using convenient sampling technique.
- The data were obtained from the demographic variables & compliance questionnaire
- The time spent for each study participant is 30 hour.

DATA ANALYSIS & INTERPRETATIONS:

ORGANISATION AND PRESENTATION OF DATA:

The analysis of data was organized and presented under the following sections.

- **SECTION A:** Distribution of the study participants by their demographic variables
- **SECTION B:** Distribution of the study participants factors influencing compliance among patients with COPD.
- **SECTION C:** Association between the factors influencing compliance among COPD patients with selected demographic variables.

SECTION A: Distribution of the study participants by their demographic variables

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SECTION A: Distribution of the study participants by their demographic variables

Figure No.1: Reveals that majority of the patients belongs to the (57.1%) age group of 41 – 50 years and most of them were female 52.9%. Most of the subjects (70%) were Hindu and had (70%) primary education. Figure No.2: With regard to the occupation majority of patients (44.3%) were homemakers and had a monthly income of (55.7%) earns more than Rs. 16000 and most of the subjects 62.90% were not had family history of COPD. Figure No. 3: Shows that majority of the subjects 90% of them receive the health information through health personnel. Regarding diet most of them were non vegetarian 88.6% and had financial support 67.1% by family property.

Figure No. 4: Reveals that majority of COPD patients were never miss the COPD treatment follow up due to the factors such as 37.1% lung condition require regular follow up and 24.3% medical professional told me to come for regular follow up. Figure No. 5: Reveals that majority of the subjects are not adhere to the prescribed diet due to the factors such as 47.1% unable to dietary recommendation and 15.7% prescribed diet is not palatable. Table No. 1: Suggest that the minority of the subjects (45.7%) were never missing the medications due to the factors such as (43.8%) because I want to live longer, (34.3%) taking medications is important to keep my body healthy. Table No.2: Emphasizes that minority of subjects 28.6% were not finds any difficulty with breathing exercise due to the factors such as (55%) got sick after performing breathing exercise and (40%) used alternative method to control breathing.

Table No 3: Reveals that (75.7%) are performing the regular exercise due to the factors such as (39.6%) told my condition requires regular exercise, (18.9%) to improve well being and maintain cardiac endurance. Table No 4: Shows that the COPD patients (87.1%) understanding the instructions due to the factors such as (75.4%) were told health education given very slowly during follow up session and (24.6%) booklets were given to read. Table No. 5: Shows that the non-significant p-value for all the variables infers that the above selected demographic variables have no influence on the factors influencing compliance among COPD patients.

CONCLUSION

Identified the factors influencing compliance among the COPD patients.

- Never miss the COPD treatment follow up due to the factors such as 37.1% lung condition require regular follow up.
- Not adhere to the prescribed diet due to the factors such as 47.1% unable to dietary recommendation.
- Never missing the medications due to the factors such as (43.8%) because I want to live longer.
- Not finds any difficulty with breathing exercise due to the factors such as (55%) got sick after performing breathing exercise
- Regular exercise due to the factors such as (39.6%) told my condition requires regular exercise.
- Understanding the instructions due to the factors such as (75.4%) were told health education given very slowly during follow up session.

IMPLICATIONS

The present study enhance the health personnel to provide guidance and counseling programme which will help the chronic obstructive pulmonary disease patients receiving regular follow up in maintaining good compliance and quality of life and also reducing economic burden in making certain policies.

Nursing Education

The present study emphasizes the importance of client education for future nursing personnel regarding factors influencing compliance among COPD patients. This can be achieved through the integration of the subjects in the curriculum which includes the technological advancement, changing trends and concept related to theory as well as practice of nursing care.

Nursing Service

The nurses are the key persons of health team who plays a vital role in health promotion and maintenance. The newly appointed staff nurse can be given opportunity to learn and insist the factors influencing compliance among COPD patients and quality of life along with the experienced staff nurses who can insist the recent advancement technology and practice in the field.

Nursing Administration

In-service education should become a regular pattern to the nursing personnel to keep them abreast with the prevention of COPD related complications and enhance compliance. The staff nurses from the Pulmonary department are stressed the opportunity to learn the need to promote compliance and quality of life among COPD patients by the nurse administrator. Pamphlet, handouts, information booklet regarding chronic obstructive pulmonary disease should kept at COPD clinic for the use of patients and attenders and significant others.

Nursing Research

The essence of research is to build a body of knowledge in nursing as it is an evolving profession. The finding of the present study serves as a basis for the professionals to conduct further studies. Nurse researchers can motivate nursing students to do more studies on this particular aspect.

RECOMMENDATIONS

Based on the study findings the following recommendations have been made for the further study.

- A comparative study can be conducted between the COPD Vs Lung transplantation patients to identify the factors influencing compliance.
- A comparative study can be conducted between the literate and non literate COPD patients to identify the factors influencing compliance.
- A study can be conducted using structured teaching programme among COPD patients to enhance the compliance.

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Table No.1: Distribution of the COPD patients have ever missed their medications.

n=70

Have you ever missed the prescribed drugs	Frequency	Percentage
Yes	38	54.2
Yes- Cost of medications is too high	12	31.6
Yes- Inconvenience	1	2.6
Yes- Simple forgot	16	42.1
Yes- Were away from house	4	10.5
Yes- Were busy with other things	5	13.2
No	32	45.7
No- Because I want to live longer	14	43.8
No- To relieve the signs and symptoms	1	3.1
No-Medical professional told me to do so	6	18.8
No-Taking medications is important to keep my body healthy	11	34.3

Table No. 2: Distribution of the CODP patient's find difficulty with pulmonary rehabilitation breathing exercise.

n=70

Have you find any difficult with breathing exercise	Frequency	Percentage
Yes	50	71.4
Yes- Not interested	6	12.0
Yes-I take lot of medicines to control breathing	4	8.0
Yes-I was unable to do breathing exercise	40	80.0
No	20	28.6
No- Breathing exercise very important to keep my body healthy	1	5.0
No-I got sick after I have performed breathing exercise	11	55.0
No-Used alternative method to control breathing	8	40.0

Table No 3: Distribution of the COPD patients able to perform regular walking exercise.

n=70

Have you able to perform your regular walking?	Frequency	Percentage
Yes	53	75.7
Reasons		
Yes- Medical professional told me to do so	8	15.1
Yes-Improve my well being	10	18.9
Yes- I fully understand my condition requires regular exercise	21	39.6
Yes-Routine exercise can enhance feelings of self esteem	4	7.5

Yes-To maintain cardiac endurance	10	18.9
No	17	24.3
No- I feel very weak for doing exercise	4	23.5
No- I was physically uncomfortable	1	5.9
No- Fatigue	6	35.3
No-Not interested	6	35.3

Table No 4: Distribution of the COPD patients have understanding of the instructions given by health care professional team.

n=70

Have you understand the instruction given by the health team?	**Frequency**	**Percentage**
Yes	61	87.1
Reasons		
Yes-Health education slowly during follow up session	46	75.4
Yes-Booklets were given to read	15	24.6
No	*9*	*12.9*
No- I was unable to remember	9	100

Table No 5: Association between the factors influencing compliance among COPD patients with the selected demographic variables.

n=70

Variables		**No. of subjects**	**Mean**	**Standard deviation**	**Kruskal Wallis test value**	**p- Value**
Age	41 – 50 Years	40	17.8	1.63	0.059	0.808 **NS**
	Above 50 Years	30	17.7	1.55		
Gender	Male	33	18.1	1.41	1.550	0.213 **NS**
	Female	37	17.5	1.71		
Education	Non Literate	14	18.1	1.29	2.871	0.238 **NS**
	Primary	49	17.5	1.67		
	Secondary	7	18.6	1.27		
Source of Health information	Books	7	17.2	2.05	0.690	0.406 **NS**
	Health personnel	63	17.8	1.53		
Family History	Yes	26	18.1	1.39	0.952	0.329 **NS**
	No	44	17.6	1.68		
Occupation	Dialy wages	4	18.2	1.50	3.294	0.348 **NS**
	Home maker	31	17.5	1.75		
	Self employ	21	18.2	1.27		
	Employ	14	17.6	1.59		

NS – Non Significant

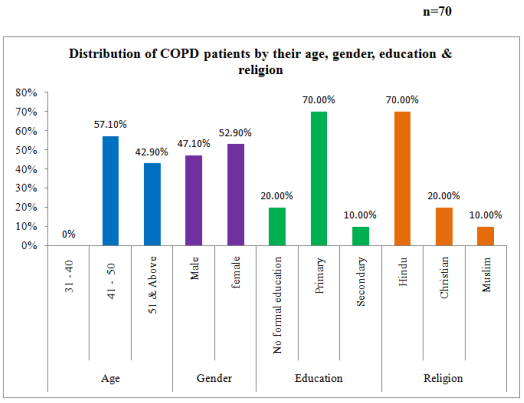

Figure No. 1: Distribution of the study participants by their demographic variables including age, gender, education & religion.

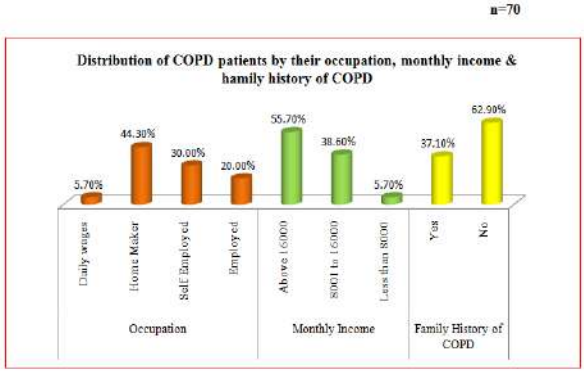

Figure No. 2: Distribution of the study participants by their demographic variables including occupation, income & family history of COPD.

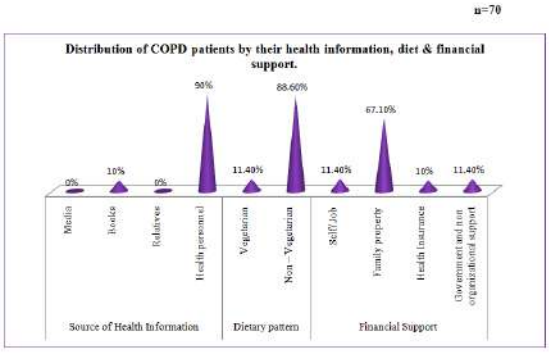

Figure No. 3: Distribution of the study participants by their demographic variables including source of getting health information, diet & financial support.

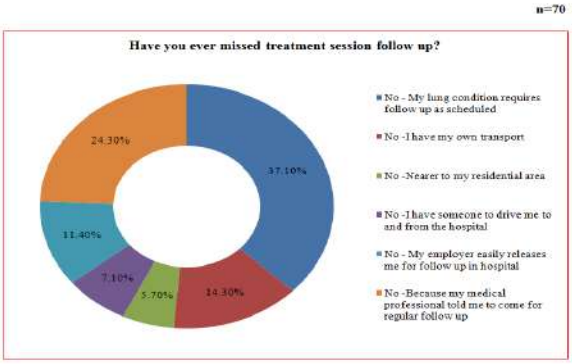

Figure No. 4: Distribution of the COPD patients have ever missed the treatment follow up session to identify the factors influencing compliance.

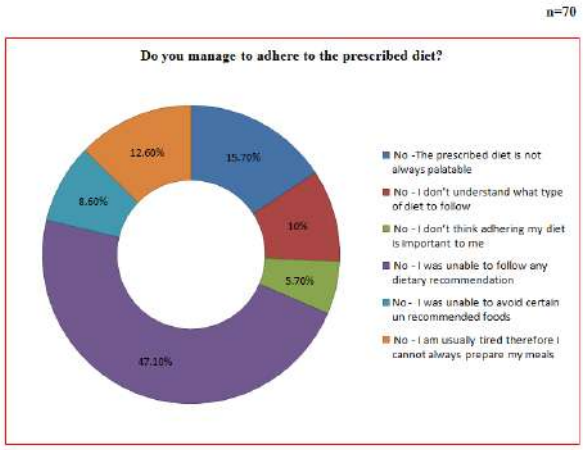

Figure No. 5: Distribution of the COPD patients have adhered to the prescribed diet to identify the factors influencing compliance.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Design, Development and Implementation of an Enrolment Web Based Portal: Agile Methodology Design Observance and Pandemic Counter

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Received: 18 Feb 2022 Revised: 28 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

The administration perceived enrolment as the major transaction, and manual operations were the major problem for the institution. Enrolment for all types of education is mandatory. It is a requirement after the students are accepted into one program/course and before they are admitted to a particular subject. The enrolment portal is of great necessity in today's education in view of the current pandemic where all countries are submerged in. The study generally aimed to design and develop an enrollment web-based portal: an Agile Methodology Design Observance and Pandemic Counter. This design was envisioned to cater to the needs of one of the satellite campuses of a soon-to-be university institution. Specifically, the study sought to design and develop two different modules, such as the student module and the evaluation committee module. The system observed developmental research design, particularly Agile methodology systems development. The Agile method came in during conceptualization and finalization. It was observed that the study addressed the long-standing desire of the administration to design a portal that responded to the problem of manual enrolment. This initiative aligned with other neighboring institutions and conformed to the call from different accrediting agencies for efficient academic transactions. The design itself is a manifestation of countering the pandemic problems since students would no longer physically visit the school for enrolment but rather do enrolment at home. It was observed that there was 100% enrolment of the old students with a higher new student enrollment. Since the present system's development is independent and can stand alone, conduct further studies to evaluate the acceptability level of its usability efficiency and portability among the internal and external customers and enahance its capabilities like adding of grading queues. Extend the developed system not only to other satellite campuses but to other neighboring institutions with problems with manual enrolment.

Keywords: Design, development and implementation, Web Based Portal, Agile Methodology, Pandemic Counter

INTRODUCTION

Each of the world's higher education institutions (HEIs) aimed to provide superior services to both internal and external clientele. They devise whatever means to meet the needs of their customers, particularly students. They're seeking for a variety of connections to help them expand their horizons and keep their status of greater heights. Aside from conforming to standardization for quality education and excellence, they have subjected themselves to any accrediting agencies for the purpose of aligning themselves with, if not surpassing, other established and recognized HEIs. To boast of productivity, they send their staff to any sorts of professional development they can find. At all times, they strive to be a better service provider. As a tertiary education institution, the satellite campus of Surigao State College of Technology aspires to become one of the top institutions in the Caraga region in terms of education quality and excellence. In light of the high enrolment rate and the health and safety concerns brought on by the pandemic, they are seeking for the best way to digitize their manual enrolment process. They are insuring that, despite the epidemic, 100% of the population will continue to enroll because the manual method will be replaced by online transactions. The administration perceived enrolment as the institution's most important transaction, and the institution's most significant issue is the institution's manual operations.

In the Philippines, over 27 million learners were affected by COVID-19, with enrolment set to drop by 20% from the previous year. Other countries have put in place initiatives to increase enrolment during COVID-19. Accordingly, Italy is allowing more students to come back to in-person learning. They launched Project Outdoor Education in the Ivrea "comune," reopening schools by using many outdoor spaces (including parks, playgrounds, and museum gardens) with teachers focusing on socio-emotional well-being as well as academic needs. The Philippines put in place a re-enrollment strategy focused on flexibility and lowering barriers to enrolment. Notably, they made an enrolment form available digitally and physically in kiosks near schools and barangay halls. (UNESCO, 2020). With this, the administration is looking for a tool that can counter the pandemic crises that the institution is part of because they perceive that the crisis would lead to a decrease in enrolment. UNESCO (2020) emphasized that the risk of temporary reluctance to re-enroll will increase and could lead to drop-out if the health (physical and mental) of students and their families is impacted by COVID-19 or if there are concerns that safety protocols are not followed at school. This tool is an enrolment web-based portal. Enrolment for all types of education is mandatory. It is a requirement after the students are accepted into one program/course and before they are admitted to a particular subject. The enrolment portal is of great necessity in today's education to counter the pandemic crisis that all countries are drowning in. As emphasized by Ademola et al. (2013), portal technology is used as a tool to enhance institutional efficiency and productivity. The portal is seen as a system to get ourselves (institutions) organized to better form lifelong impressions.

Perceived and known problems prompted the researchers to design, develop and implement an Enrolment Web Based Portal with Agile methodology design observance and Pandemic Counter. The initiative of the administration brings forth the realization of its desire to be a model of excellence and to provide better services to clients at all times. This design aligned with the government's initiative of lowering barriers to enrolment.

General Objectives

The study generally designed and developed an Enrolment Web Based Portal: Agile Methodology Design Observance and Pandemic Counter.

Specific Objectives

Specifically, the study sought to design and develop two different modules, such as the student module and the evaluation committee module**.** These modules conformed to the institutional processes, observed the Agile methodology design and countered the pandemic crisis.

MATERIALS AND METHODS

Analysis

The study used the modeling tool to determine the system's functional requirements. This tool is a use case diagram modeling tool. This modelling tool is perceived as appropriate during analysis because it portrays and diagrams the major transactions of the administration when it comes to enrolment. In the Figure 1, it can be seen that there are two actors interacting with the system. The first one is the student actor. The process of enrollment begins when students start to enroll. They have to register first for a new student by filling out the necessary information and selecting an item to answer for the old student. Once students intend to enroll, they have to choose from available subjects to enroll in. The system directs their enrolment to the enrolment committee for verification purposes, after which it goes directly to the students for notification of their status. Both students and evaluators can view or print the certificate of registration, indicating that they are already enrolled.

Design

The study observed the developmental design to design and develop the Enrolment Web-Based Portal for two defined modules. The researcher used the concept of System Development Life Cycle (SDLC) during its design, specifically the Agile model, from conceptualization to implementation.J. Valacich, J. George, and J. Hoffer (2012) define the Agile model as short iterative cycles and extensive testing, with active user participation in establishing, prioritizing, and verifying requirements.

Development

During development, the system was developed with the use of the open source PHP language as the front end and the open source MySQL database as the back end. MySQL was used because the researchers believed and perceived that MySQL was easy and efficient to use. This claim was confirmed in the study of Rani and Jos (2016). According to them, MySQL is easy, fast, and efficient and can store a large number of records with little configuration. The study was supplemented with open source JavaScript to improve the design's appeal, simplicity, clarity, and readable.

Implementation

The study was a yearlong and installed at the office of the registrar and selected support offices. The enrolment successfully catered to old and new students on the campus. This has been used for two semester enrolments already. Because of the presence of an Enrolment Portal on campus, the implemented protocol against COVID-19 was followed. Conceptually, implementation was the longer and harder task to take, but because of the full support of the administration, it was made easy and fast. Thus, the tasks were easily completed during implementation. Figure 2 shows the flow of implementation. The system uses a web-based architecture in deploying the system. The students will visit www.ssctmainitfreevar.org to fill in the necessary information and select corresponding subjects to be enrolled in. The request will be sent via the internet and the Internet Service Provider will pass it to the server. The server then sends information to the students with a pending status and that their enrollment is now for evaluation by the enrollment committee. The enrollment committee now will evaluate the information filled in and the subjects selected for enrollment by the students and approve or disapprove the enrollment. The registrar then prints the data for reporting purposes.

RESULTS AND DISCUSSIONS

This part presents different figures to justify the claimed objectives. This presents the two different modules with their descriptions and functions in the system.

First Module, the Student module
Figure 3 is the enrolment/registration page. This is the first page where the students are asked to fill out the given form. It requires their personal information. There is a need to complete the form to proceed to the next step. This form will always be the first to be used. However, in the next usage of the enrolment, they will only have to fill out selected items on the form. Those entries that would need an update, like the semester field, have the majority of the same entries. This scenario differs from the manual process because they are required to fill out the new form for every enrolment. This form is to be filed in different offices, particularly the registrar and the dean's offices, for future use.

Enrollment/Registration Step 2 Page
This is the next page displayed after filling out the personal information of the student. On this page, it displays the subject offerings for the current semester. Students can select among the offered subjects that they would like to enroll in. The system allows students to request a subject if the subject they would like to enroll in is not in the offering, but this request is subject to the approval of the enrolment committee in charge in consideration of the prerequisite, allowable number of students per semester.

Enrollment/Registration Step 3 Page This is the final step page where students are informed of their enrollment status, like "Process completed! Your enrolment is now for evaluation by the enrolment committee "or in queue for pending status, which they can view on the main page. This page provides an announcement at its right on the date of the last enrolment. The figure 6 is the "View Subject Status Page." This is the individual student's page that displays their information and their enrolled subjects with the corresponding status, whether it is approved or disapproved by the committee. It provides an announcement at its right indicating the enrolment schedule per level contribution.

Second Module, the Evaluation Committee Modules
Figure 7 is the login page. This is the page where the user is asked for their user name and password. This is an important feature; the login authentication through their username and password protects the system from people or malicious activities. Figure 8 is the main (Index) committee page. After logging in, they will be directed to this page where they can view all students by status, course, and year level. They can also search for specific students. They are given basic statistics as to the number of enrollees by status, course, and year level. Figure 9 is the Evaluation Page. This page displays all the pending status of the registered students by course. The committee can view subjects in order to either verify the list of enrolled subjects or delete the student's record.

Figure 10 is the Subject Approval Page. This page lets the evaluation committee approve or disapprove the subjects being chosen by the students for enrollment in consideration with the pre-requisite subjects. This page allows the evaluator to add subjects requested by the students if the subjects can be taken by the students. Figure 11 is the Enrollment Report. This page contains a sample enrollment report for the registrar, accounting, student, and parent. Figure 12 is the Registration Report. This page is a sample of report as a copy for registrar and accounting office.

CONCLUSIONS AND RECOMMENDATIONS

It was observed that the study addressed the long-standing desire of the administration to design a portal that responded to the problem of manual enrolment. This initiative aligned with other neighboring institutions and conformed to the call from different accrediting agencies for efficient academic transactions. The design itself is a

manifestation of countering the pandemic problems since students would no longer physically visit the school for enrolment but rather do enrolment at home. It was observed that there was 100% enrolment of the old students with a higher new student enrollment. All of this is because of the emergence of the Enrolment Portal. Since the present system's development is independent and can stand alone, conduct further studies to evaluate the acceptability level of its usability efficiency and portability among the internal and external customers and enahance its capabilities like adding of grading queues. Extend the developed system not only to other satellite campuses but to other neighboring institutions with problems with manual enrolment.

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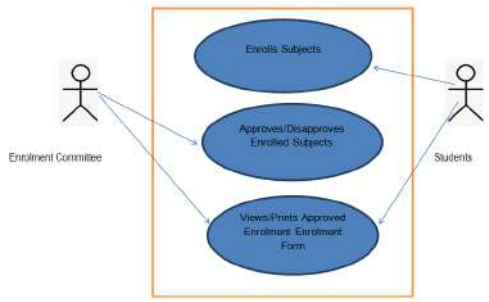


Figure 1. Use Case Diagram of the System

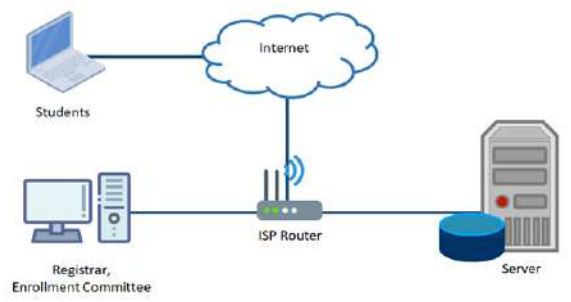


Figure 2. Flow of Implementation

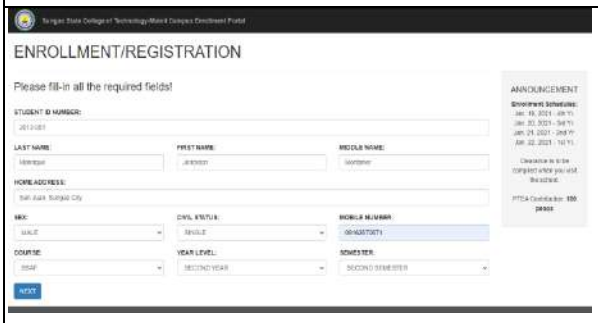


Figure 3. Enrollment/Registration Step1 Page

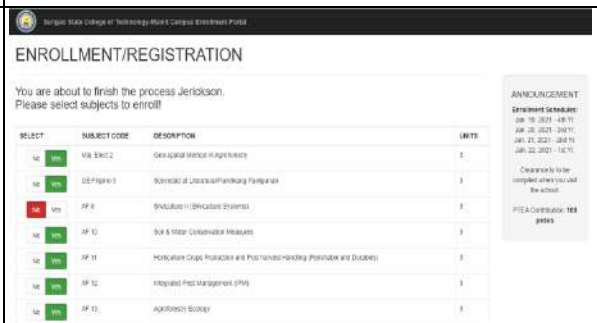


Figure 4. Enrollment/Registration Step 2 Page

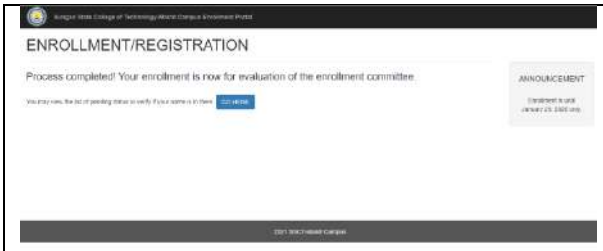


Figure 5. Enrollment/Registration Step 2 Page

Figure 6. View Subject Status Page

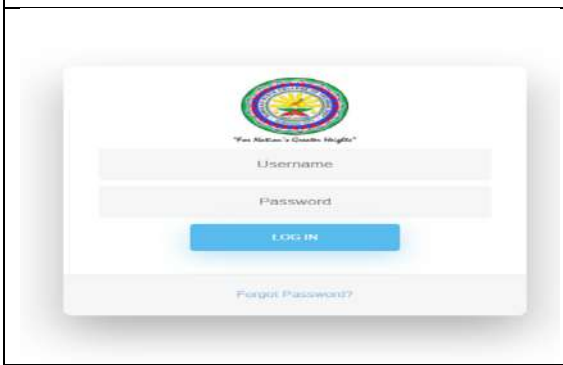


Figure 7. Login Page

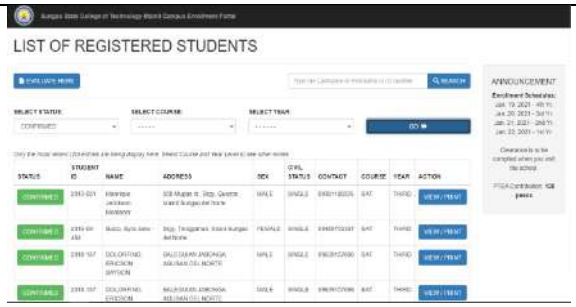


Figure 8. Main (Index) Committee Page

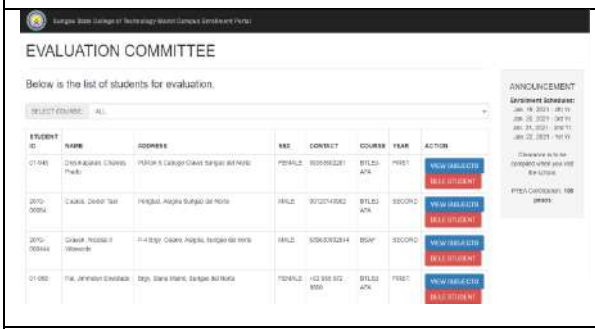


Figure 9. Evaluation Page

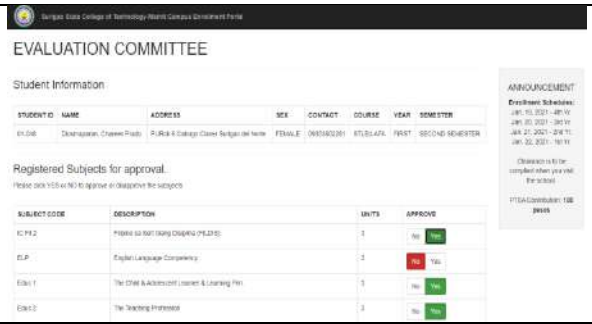
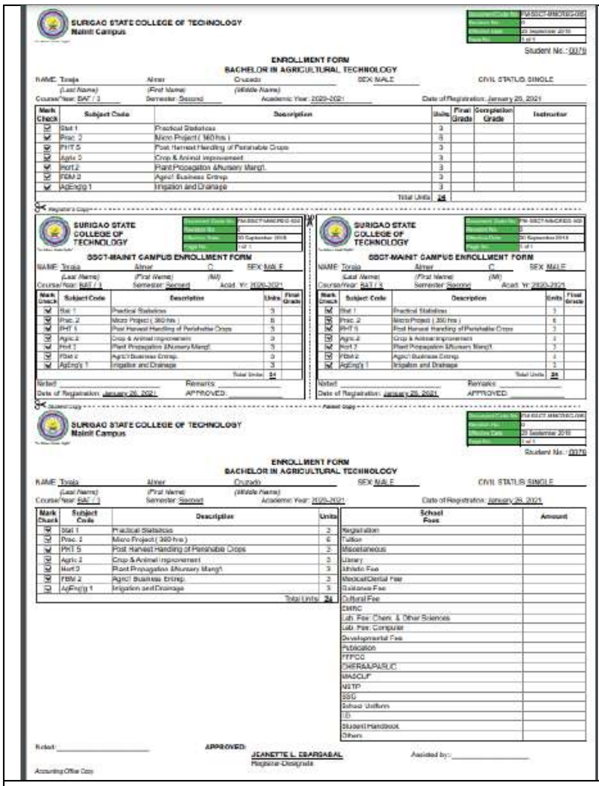


Figure 10. Subject Approval Page

Renz M. Buctuan and Unife O. Cagas



SURIGAO STATE COLLEGE OF TECHNOLOGY
Mainit Campus

Student No.: 0079

ENROLLMENT FORM
BACHELOR IN AGRICULTURAL TECHNOLOGY

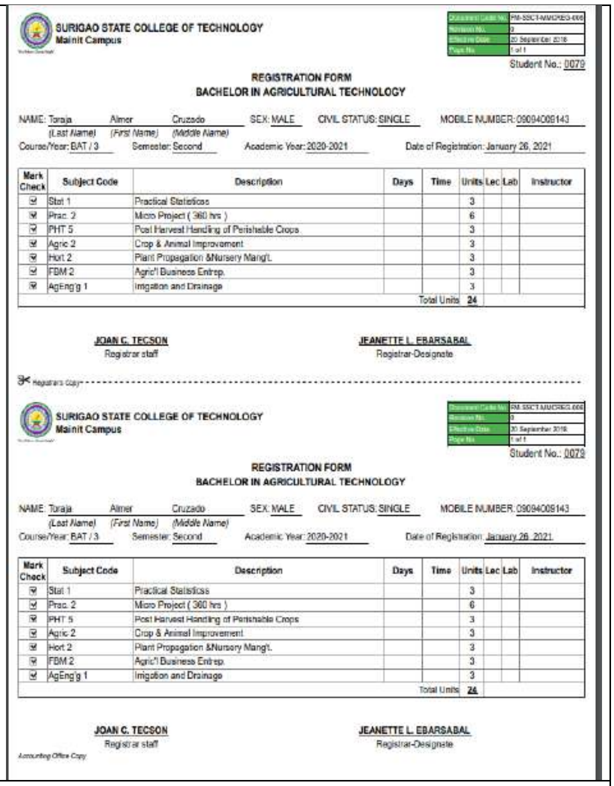
NAME: Toraja (Last Name) Almer (First Name) Cruzado (Middle Name) SEX: MALE CIVIL STATUS: SINGLE
Course/Year: BAT / 3 Semester: Second Academic Year: 2020-2021 Date of Registration: January 26, 2021

Mark Check	Subject Code	Description	Units	Final Grade	Completion Grade	Instructor
☑	Stat 1	Practical Statistics	3			
☑	Prac. 2	Micro Project (360 hrs)	6			
☑	PHT 5	Post Harvest Handling of Perishable Crops	3			
☑	Agric 2	Crop & Animal Improvement	3			
☑	Hort 2	Plant Propagation &Nursery Mang't.	3			
☑	FBM 2	Agric'l Business Entrep.	3			
☑	AgEng'g 1	Irrigation and Drainage	3			
		Total Units	24			

SSCT-MAINIT CAMPUS ENROLLMENT FORM

APPROVED:
JEANETTE L. EBARSABAL
Registrar-Designate

Figure 11. Enrollment Report



SURIGAO STATE COLLEGE OF TECHNOLOGY
Mainit Campus

Student No.: 0079

REGISTRATION FORM
BACHELOR IN AGRICULTURAL TECHNOLOGY

NAME: Toraja (Last Name) Almer (First Name) Cruzado (Middle Name) SEX: MALE CIVIL STATUS: SINGLE MOBILE NUMBER: 09094009143
Course/Year: BAT / 3 Semester: Second Academic Year: 2020-2021 Date of Registration: January 26, 2021

Mark Check	Subject Code	Description	Days	Time	Units	Lec	Lab	Instructor
☑	Stat 1	Practical Statistics			3			
☑	Prac. 2	Micro Project (360 hrs)			6			
☑	PHT 5	Post Harvest Handling of Perishable Crops			3			
☑	Agric 2	Crop & Animal Improvement			3			
☑	Hort 2	Plant Propagation &Nursery Mang't.			3			
☑	FBM 2	Agric'l Business Entrep.			3			
☑	AgEng'g 1	Irrigation and Drainage			3			
				Total Units	24			

JOAN C. TECSON
Registrar staff

JEANETTE L. EBARSABAL
Registrar-Designate

Registrar's Copy

Accounting Office Copy

Figure 12. Registration Report

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of Early Physiotherapy Intervention for Head and Neck Squamous Cell Carcinoma – A Case Report.

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Received: 09 Dec 2021 Revised: 12 Jan 2022 Accepted: 24 Feb 2022

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ABSTRACT

To determine the effects of early physiotherapy intervention for head and neck squamous cell carcinoma. The purpose of this study is to describe early baseline characteristic for a consecutive patient who receives physical therapy with a complaints of temporomandibular dysfunction in head and neck squamous cell carcinoma patients . And also to describe pattern of changes in predicted clinical outcomes across physical therapy episodes of care and regular follow-up. This study helps to setup early physical therapy to improve the function of TMJ and muscles of mastication. The main symptoms that the patients suffer from restricted mouth opening, stiffness, fatigue, and pain of the jaw, severe swallowing and speech problem. positive findings of decreased TMJ joint ROM and cervical ROM. Patient has performed clinical functional evaluation with pre and post-treatment with the follow-up of about 3 months assessment. The therapy includes self trained normal jaw ROM with limited condylar rotation, active vertical mandibular movement, cold therapy, passive motion device, grades of joint distraction ,massage of masseter muscle. For pain intensity Numerical pain rating scales , 11cm plastic ruler marked in millimeters, inclinometer for cervical range of motion. CO- QOL questionnaire scale, Gothenburg trismus questionnaire. We concluded that the patients with oral squamous cell carcinoma were found to be effective in providing early physiotherapy intervention which improves ROM, muscle control and co-ordination to enhance the functional mobility.

Keywords: oral squamous cell carcinoma , restricted ROM, temporomandibular disorder.

INTRODUCTION

The updated cancer prevalence reported an increase in the incidence of oral cancer [1]. Inspite that, it was regarded as the sixth most common cancer worldwide [2]. Oral squamous cell carcinoma is about 90% of the histologic type of oral cancer [3] and may or may not be preceded by oral potentially malignant disorders. Squamous cell carcinoma is one of the most common malignant tumor of oral cavity and it is leading death causing carcinoma. Adenoid squamous cell carcinoma (ASCC) prevalence is about 2%-4% of all squamous cell carcinoma (SCC) cases. Initially it was described by Lever in 1947 as adenoacanthoma of the sweat glands [2]. The sun-exposed areas of the skin, particularly on the head and neck of elderly men, are more commonly affected [1]. It arises from dysplastic oral squamous epithelium. Therefore, it is more frequently seen among the men than women ,when they are exposed to high risk of habit like tobacco chewing or smoking and alcohol consumption. About 95% of all oral carcinoma are malignant in nature. Therefore the annual rate of incidence and mortality rate might varies based on different genders, age group, races. usually oral squamous cell carcinoma of mandibular region, will have lower survival rate than the other carcinoma. It is managed by a combination of surgery, radiotherapy, and chemotherapy, depending on the place, stage and, TNM classification [14,15]. Quality of life is a major component that deals with the management of patients with squamous cell carcinoma and it involves a psychological approach. Physical examination such as stench, pain, and oozing may result in severe psychological symptoms, worsening the wounds [7].

CASE REPORT

A 46year- old male patient were reported to dental OPD department with the chief complaints of painful non healing ulcers over the left mandibular region for past 2 months. Initially it develops gradual in onset and gradually progressed to attain present size and he also gave a history of burning sensation on taking spicy food. The patient is under antibiotic and analgesic medication for about one week after consulting with a nearby hospital. pain was throbbing and continuous in nature and it usually aggravates on opening the mouth ,during mastication and brushing teeth. History of betel quid chewing since 20years with a frequency of 5 times per day and quid it in right buckle secular region. On examination - there is a facial asymmetry noted extra-orally and intra-orally, there is a ulcerated erythematous rough mass extending from tooth 23 to 30region. The positive findings of this ulcertic proliferative lesion on right buccal mucous is diagnosed as carcinoma of buccal sucular region. Later, Patients presented with enlargement and changes in consistency of lesion causing discomfort and dysfunction. Since the patient is followed up with chemotherapy treatment for about 2 months. On progression of the lesion ,the patient underwent a surgical procedure - "partial glossectomy with cervical node dissection''. Now Postoperatively (POD-2), patient is referred for physiotherapy treatment to enhance the range of motion, muscle power ,co-ordination to improve his functional mobility.

CLINICAL DESCRIPTIVE

Data were collected within the clinical routine, after the surgery they are assessed for post operative rehabilitation from POD-2 onwards. The activity of mouth was obviously limited and patient suffered from severe pain for past 2 days (post-op status). He had a difficulty in opening or closing the mouth, difficulty in chewing, aching facial pain. Patient self assessed the level of pain using numerical Pain rating scale. An 11cm plastic ruler marked in millimeters was used for all measurements. The 6 active measurements were opening, overbite, right & left excursion, protrusion and overjet (vertical measurement) and this could measures the intercisal distance between the two edges . neck range of motion during flexion, extension, lateral bending and rotation are measured using inclinometer. Oral cancer - Quality of life questionnaire were deigned and it has high reliable ,statistical validity which usually covers the basic aspect of health related quality of life with 30 questions. Its five function scales are physical functioning , role & emotional functioning, cognitive functioning , social functioning . Gothenburg trismus questionnaire (after and before trismus intervention) it is self administered questionnaire which has good validity and reliability and it is composed of 3 domains containing 13 items : jaw related problems (6items),eating limitations (4), muscular tension(3)..the domains and single items ranges from 0-100,where 100 equates to symptomatology and 0 represent

no symptoms .oral mucosa is evaluated according the Schubert scale. our study obtained a consent from MADHAV UNIVERSITY. The patient also signed a statement of consent to participate in the study and to use photography.

CLINICAL FINDINGS

Initially, from the day 2 of postoperative , patients presented with restricted TMJ ROM , reduced cervical ROM, limitation in swallowing and speech, salivary secretion problems.

THERAPEUTIC INTERVENTION

Rehabilitation protocol is based on three phase - early phase, intermediate phase, late phase.

EARLY PHASE

The main aims of this phase is to preserve mobility within a restricted range and to prevent muscular inhibition, and reduce pain and inflammation without putting too much stress on the joint through the number of repetitions and intensity. It also to avoid the creation of aberrant adhesions and overexerting the capsular tissue and muscles.This mainly adhered by recommending a no chew diet. Exercises repetition should be limited to 3 to 5 per day. Numbing, edema and inflammation are reduced by applying cold pack to the joint on a regular basis which helps to relieve pain. In a recent RCT study , Thienpont stated that there was no clinical advantage to using cryotherapy over conventional cold packs in patients who had a knee arthroplasty. ROM exercises are encouraged with limited condylar rotation to prevent TMJ luxation because this can exacerbate inflammatory response through TMJ stretching. Active vertical mandibular movement with the tongue in contact with the palate is a possible workout since it confines the movements to solely condylar rotation. it is also referred as "goldfish" exercises. To preserve good symmetry, the mandible can be passively dropped and closed with a finger, or slowly, doing actively opened and closed while staring in a mirror. Simple insertion of numerous tongue blades without further activation. Finally, some moderate joint mobilization, such as grade I and II joint distraction. 9 A "no chew diet" is recommended to avoid muscle overexertion, and harmful Para functions should obviously be avoided at all times.

INTERMEDIATE PHASE(1-3WKS)

This phase focuses on increasing ROM which also improves muscle control, coordination, and performance in order to achieve functional mobility. Here exercises can be gradually raised to 5 to 10 per day, where the repetitions can be lowered. In revalidation, this allows for a greater "high-intensity endurance" change. Now pain therapy is still very crucial so Cold therapy can be continued (e.g., after certain workouts) to relieve joint pain, and local moist heat application can also be employed. Heat therapy not only relieves muscle tension and pain, but it also enhances collagen fiber extendibility and reduces tissue viscosity, which can help with stretching exercises. Thereby increasing local blood flow and metabolism, tissue repair can be accelerated. The goal of heat therapy is to produce muscular relaxation; as a result, heat is delivered directly to the muscle rather than the joint and utilized for 20 minutes before the workout programme which allow the muscles to relax as much as possible. Lin41 discovered that combining pretreatment heat and post treatment cold application gave greater results in overall ROM than using only heat.It should be emphasized, however, that this study only looked at knee motion, so TMJ results may vary.

Condylar rotational exercises are continued since they greatly contribute in obtaining a symmetrical mouth opening, as demonstrated by Oh et al42 in TMD patients. Muscle coordination can also be improved by completing coordination exercises in front of a mirror. When completing vertical movement exercises, the patient should try to keep the midline of the lower jaw on this line after drawing a straight vertical line on the mirror. Other exercises, like active opening and closure or protrusive and retrusive activities, can be encouraged with the use of a mirror. 43 In this phase, stretching can begin with both rotational and translative movement. Translative mobility is feasible when the tongue is no longer forced against the palate. Placing dental cotton rolls or a pen between the molars (in case of protrusion and retrusion) or the incisors (in case of protrusion and retrusion) can help with active horizontal mandibular movements (in case of active lateral movement).Active assisted exercises like cross-fingered exercise, in which the mouth is actively opened which can aided by both thumb and index finger, and the "hook-pull," in which the index finger hooks in the floor of the mouth, aiding the downward mandibular movement during opening, can

help with active vertical mandibular movement (Figs. 3,4). The index fingers are placed over the condyles allows the patient to feel the condyles' translative movement, which aids with opening and closing guiding. Passive workouts like manual finger stretching or using a passive motion device like the TheraBite® system can also be considered. The joint distraction and muscle massage are continued. Chewing gum can be used once the inflammation are subsided, because it produces dynamic movement in both the horizontal and vertical planes which strengthens the masticatory muscles.

LATE PHASE -(>4 Weeks)

The phase mainly focused on achieving both smooth and symmetrical lower jaw movement. Further, remaining imbalances and asymmetries, ROM are addressed therefore it can leads to the restoration of normal joint kinematics. The number of repetitions are depends on the kind and concentration of the exercise, which in-turn increase the overall load and even more toward muscle strength. To improve stability, isometric contractions of the jaw in a neutral position are encouraged. It can be accomplished by either attempting to open the mouth from an occlusal position which applies upward manual pressure to the mandible, preventing the lower jaw movement, or by applying upward/lateral manual pressure to the mandible while the lower jaw maintains its position through muscle activity. It emphasizes muscle building and loading through both active and passive activities, as well as it also develops muscle endurance. We can also proceed with forced opening exercises and strengthening activities like active resistance exercises. Joint mobilization can be progress to grade III or even IV intensity. The diet can now progress from a liquid to a solid state.

FOLLOW-UP AND OUTCOME

In the past few year, concept of conservative treatments became the primary end point. Appropriate management has been driven not only by innovations in the delivery of medical care and also by the developments of technology tools. On the other hand, postoperative treatment follow-ups gains the importance in clinical performance. Here the patient is followed up for about 2months with regular days. From the day of post-op status, patients presented with limited trismus, restricted cervical motion. Therefore day 2 of post-operative to week 1 , our role is to reduce pain and joint stiffness to improve functional mobility (PRE NPRS – 8,). We usually start up with ROM exercise with limited condylar rotation , goldfish exercise, mild joint distraction are encouraged to improve the functional mobility as well as to prevent adhesion. Cryo-therapy is initiated to reduce edema, inflammation , and to relieve pain. Here the pain is subsided after a week which is assessed by NPRS (POST VALUE 6). Normally it ranges from 52-55mm. initially from the day of POD TMJ range of motion are 1.opening – 2.2mm, 2.left excursion – 0.8mm, 3. Right excursion – 1.1mm, 4.protrusion- 0.9mm ,5. Overbite -0.2 , 6.overjet – 0.2.Along with this cervical range of motion are noted .limited cervical flexion(30deg), lateral flexion(25), rotation (30deg). From week 1-3, gentle stretching exercise are begin with the above protocol. During this period , we can increase the repetition of exercise per day . the exercise should be done in front of mirror to enhance the movement co-ordination. Hot pack therapy also advisable before the rehabilitation mainly to enhance the muscle relaxation. This protocols are followed up-to 3weeks. Therefore the TMJ joint range should be measured by the end of this week. Here 1.opening – 3mm,2.left exercusion-1.2mm, 3. Right excursion - 1.5mm, 4. Overbite – 0.8mm, 5. Over jet - 0.8mm, 6. Protrusion – 1.2mm. cervical flexion is about 40dgre, lateral rotation is 35deg, rotation is about 40deg. On evaluating, TMJ and cervical range of motion are increased little bit. From 4week , isometric contraction of the jaw , joint distraction of grade 3 or 4 are encouraged to improve the range of motion. TMJ joint : 1.opening – 9mm, 2.left exercusion-3mm, 3. Right excursion - 2.5mm, 4. Overbite – 1.8mm, 5. Over jet - 1.8mm, 6. Protrusion – 3mm. cervical flexion is about 50dgre, lateral rotation is 40deg, rotation is about 50deg. This rehabilitation protocol are followed up for about 8weeks ,at the end of the session again the range of motion for TMJ and cervical motion , pain intensity , quality of life questionnaire and Gothenburg trismus questionnaire were assessed to enroll the improvements that are enhanced in this studies. AT 8 week , Here TMJ ranges are 1.opening – 13mm,2.left excursion- 6mm, 3. Right excursion - 4mm, 4. Overbite – 3mm, 5. Over jet - 3mm, 6. Protrusion – 2.5mm. cervical flexion is about 50dgre, lateral rotation is 40deg, rotation is about 65deg. NPRS – 3 , Gothenburg questionnaire score is 0(no symptoms of trismus). Therefore the above values determines that there is a improvement in the ranges from day 1 to 2months. Figure 1 and 2 shows the patient attempting for mouth opening.

DISCUSSION

In this study , early physiotherapy intervention in head and neck squamous cell carcinoma has showed a positive effect of structured exercise protocol was persistent at 2 months follow up. They improved the mouth opening, less trismus related symptoms and also improved the quality of life . Trismus is one of the major problem in oral carcinoma. It is well known that trismus results in functional interference with oral hygiene, speech and nutritional intake .This subsequently contributes to difficulties in obtaining any dental treatment, potential weight loss and malnutrition,(4,5,6)therefore this factor contributes a negative impact on quality of life and risk of depression further it could limit in mouth opening can interfere with social activities and ability to work. (7,8).

Our data states that there is a improvement in mean mouth opening of 13 mm, seem to be a clinical importance for the patients with the improved functioning . Earlier research would supports that a difference in mouth opening of at least 5 mm can be interpreted as clinical relevant and not the result of inter-rater differences in measurement technique or normal variation [9]. This research was demonstrated that aside from improved mouth opening, the intervention group consistently reported less problems with pain, eating limitation, jaw-related problems, problems with teeth, and a better functional mobility and global quality of life. In the case , there is a risk of mastication muscle contracture and atrophy, and TMJ fibrosis resulting in reduced mandibular range of motion. A rational stated that why the early exercise intervention for trismus have a positive effect when it is performed on a regular basis . where it activates the muscles of mastication and stretching of the TMJ can counteract shortening of muscles, maintain circulation and increase the range of motion of the TMJ.

exercise is important in order to maintain mouth opening capacity and also that regular follow-up and MIO assessment is important to maintain patient motivation for continued exercise, thereby further reducing trismus-related symptoms and improving HRQL. The GTQ was useful as an endpoint for trismus and it is valuable in order to detect both patients in need of, and the perceived effect of rehabilitative measures, such as exercise intervention.
The main strength of the study is to determine the early intervention in head and neck squamous cell carcinoma has improved the functional mobility and as well as quality of life in an individual .

PATIENT PERSPECTIVE

Initially I was not able to open my mouth ,there is a restriction of movement and pain due to surgical excision . From the day 1, I wouldn't able to open my mouth , swallow or chewing activities are difficult in nature . This might triggers the functional activities to be avoided initially. After taking physiotherapy treatment I might feel that there is a improvement in my functional activities like chewing , swallowing , mouth opening , etc. Now I can do all my functional activity without any discomfort and pain .

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Figure 1 - Mouth oppening of patient before and after myofascial release

Figure 2 -Device based intervention for increasing mouth opening.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

DDR: A Deep Learning Approach for Diabetic Retinopathy Detection using Fuzzy C Means Clustering

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Received: 15 Dec 2021 Revised: 14 Jan 2022 Accepted: 26 Feb 2022

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ABSTRACT

Diabetic Retinopathy (DR) is blood vessel leakage in the retina. Visible impairment can ensue from the loss of blood from these vessels. Diabetic studies have concentrated on expanding the number of patients with the disease and improving their ability to test different retinal variables over the past few decades. Retinal biometrics includes examining the structure of the eye and measuring the flow of blood in the retina. DR is a sometimes sight-threatening condition, causing blindness. The earlier the diabetes is diagnosed, the better because it preserves vision. Therefore, this investigation suggests using digital imaging and electronic manipulation of photographs for the pre-diagnostic evaluation of DR type. The key aim is to distinguish non-proliferative Diabetic Retinopathy (NPDR) on the sight film at the instant of printing. This study presents a new method named (DDR-Deep-Learning for Diabetic Retinopathy) for DR utilizing segmentation at the Gaussian level. The training data is provided with equal representation and the CNN algorithm classification. The DDR method proposed results suggest that the Optic Disc Centre and Diameter are true to ground reality. This proposed system has shown the highest overall accuracy by utilizing the same results, with an accuracy of 98.5%.

Keywords: DR, DDR, CNN, FCM Clustering, Retinopathy Detection

INTRODUCTION

According to the World Health Organization (WHO), DR caused an estimated 5 million blind individuals, accounting for about 5% of blindness worldwide [3]. Early detection may help avert blindness. This article describes a methodology for early identification and grading of DR using deep learning [1]. DR is a serious eye condition that affects a large number of people. It is the most prevalent cause of legal blindness in industrialized nations' working-age population [6] [7]. DR develops when diabetes destroys the blood vessels inside the retina, resulting in the

leakage of blood and fluids into the surrounding tissue. Microaneurysms, haemorrhages, hard exudates, and cotton wool patches (a.k.a. soft exudates) are all caused by this fluid leakage [9], [11]. DR is a quiet condition that patients may not identify until alterations in the retina have advanced to the point that therapy becomes difficult, if not impossible [12]. Mediated growth manifests as a visible symptom in 80% of people with diabetes over lengthy periods. According to recent studies, less than 90% of the current events could be avoided if they were investigated and provided with appropriate care and observed despite these unpleasant discoveries [13]. It's even possible that leaky capillaries will harm the retina. An injury of the eyes will result in complete vision loss (the jelly-like center point of the eye). It may also affect the vision by reflecting light from the retina [14]. To help diagnose and prevent this condition among patients living in rural regions where medical screening is difficult, Aravind Eye Hospital has initiated a project to use Artificial Intelligence and Deep Learning to detect DR automatically [15].

Numerous traits, as well as their location, play a significant role in the categorization of DR. Early detection and treatment may significantly lower the incidence of blindness by 95% [16]. Early diagnosis of DR with an automated screening method allows laser treatment to prevent or postpone vision loss, encouraging diabetes management and lowering health care expenditures [17]-[20]. The purpose of this paper is to explain the application of semantic segmentation to the fundus of an artery-venous retinal vascular. The technology can tell the difference between an artery and a vein in retinal pictures. The last component of the system can calculate the accuracy and amount of time used. The ground truth is divided into five segments: context, artery, vein, both, and unsure. Blue signifies an artery; red suggests a vein, white indicates an unknown, and green shows that both arteries and veins traverse the pixel. Black serves as a backdrop. Segmentation on a semantic level is confined to two categories: artery and vein.

The severity of DR is categorized in this article using CNN. Our objective is to transfer knowledge across several neural networks, compare the architectures, and select the ideal network to aid clinicians in illness diagnosis and treatment. This innovation has the potential to ease the diagnostic strain on physicians significantly. The accurate identification of DR is critical for the disease's early detection. There are some challenges and issues in previous work that has been done so far. Few features are not considered yet. Our study focuses on such retinal image features that earlier researchers do not consider. In previous work, there is no such algorithm that focuses on each class's overall accuracy. The following is the structure of the paper. Section 2 presents a detailed discussion of DR detection and segmentation approaches. Section 3 details the recommended technique. Section 4 presents the assessment findings and analysis using other methodologies. The article closes with Section 5.

BACKGROUND STUDY

Benita Jeglin & Hariharan [2] innovative algorithms for microaneurys in medical image analysis have been demonstrated. Ensembles include internal pre-processing and pre-extraction. The method improves the original picture. Blood vessels are identified using the edge detection system. By combining many different edge detection methods, several levels of DR can be discerned. Charu Sharma & Geeta Kaushik [3] suggested using neural networks and fuzzy clustering to identify segments. A test proved that retinal scans could identify and show DR with no delay. However, this approach fails in noisy areas, resulting in inaccurate segmentation. E. V. Carrera et al. [4] Retinal image processing has the potential to play a significant role in the diagnosis of DR. The findings were positive, and a future clinical study will incorporate the algorithms given in this paper into a tool for diagnosing DR. D. K. Elswah et al. [5] outline a three-stage process for automated DR grading. Pre-processing, feature extraction, and classification were all included in the framework. Experiments demonstrate that system performance may be greatly improved by using data augmentation and data balance.

Falguni & Rajvi [6] conducted an exhaustive report on DR. Many scientists have pointed out that DR retinal detachment triggers retinal diseases. DR results in almost 25,000 cases of permanent visual loss per year. The first symptom of DR indication is leakage of retinal veins. Referred to as an individual who has diabetes for people with diabetes, the spectacle affects all eyes. You may reduce the risk of loss of blindness by using proper screening techniques at the beginning of the production process. Ganesh & Basha [7] Kirsch's edge

detection algorithm has detected a possible DR tale. The Kirsch template is utilized in extracting the blood vessels from the picture. After processing the resulting photographs, it discovers new blood vessels in the retinal imagery by doing initial vessel isolation, feature detection, and classification of the vessels in the pictures Jahiruzzaman & Aowlad Hossain [10] overall, the vast majority of the fundus photos provided an appropriate way to calculate exudates and haemorrhages. Reducing the chroma with the K-compression approach is effective in fundus videos. There is no set definition of "creative" for assessment purposes. Sensitivity and consistency are taken into account when gauging the results. Using the fuzzy logic classifier, the figure comes to 96.67%. V. Saravanan et al. [12] will build a computer-aided detecting method. Due to our implementation's optimization technique, the same technology may be expanded to identify more types of lesions. To avoid misclassification of drusen as exudates, post-processing must be performed. Singh A. Gautam et al. [13] showed that biological image processing might be a beneficial tool for humanity in detecting DR early and noninvasively. Complex and time-consuming human work is decreased by using basic and straightforward image processing algorithms. Based on their white pixel counts, fundus eye photographs may be classed as DR or NDR. The patient is not required to see a doctor or clinician diagnose DR, saving both time and money.

V. Suryawanshi & S. Setpal [14] DR develops and is the leading cause of blindness. Early detection and treatment may prevent a person from becoming blind. This article proposes a new method for training and testing using the MESSIDOR and DRIVE datasets. The proposed method utilizes precise GLCM features and transforms the points into higher dimensions using a Gaussian function before feeding the points into a two-layer feed-forward network for training. Wu, Y., and Hu, Z. [18] the approach of migrating learning, fine-tuning the new dataset using Keras' built-in pre-training model to accomplish classification based on the degree of DR. As can be observed from the above table, the accuracy rate increases as the complexity of the network structure increases, indicating that the inception V3 network performs the best. Then, it can be observed that the accuracy rate rises as the number of training rounds grow, indicating that the number of training rounds should be increased accordingly. Additionally, the authors can observe that the correctness of the training and the pace of learning have a link.

S. Yu et al. [19] suggested a deep learning-based technique for detecting exudates at the pixel level. The candidate points for exudates are first retrieved using morphological ultimate opening approaches and then given to trained CNN deep networks for classification. For both the training and test sets, the approach obtained a high level of pixel-level accuracy. Zhang et al. [20] using a computer-aided diagnostic. Since diabetes mellitus is a chronic illness, it can ultimately get worse. It induces retinal disease and is very harmful to the eyes. When the authors start in DR, the microaneurisms are first identified, and fundus imaging is performed. Two different approaches are used to detect the microaneurys in the fundus and retinal pictures, which use multi-scale similarity and dynamic thresholding. This method uses micro and algorithm and microanalyses to distinguish between legitimate and fraudulent microannuem candidates. Two well-known public databases, including Retinopathy Study and Regular PDR, are also used to research DR. This procedure has been empirically determined to be more reliable and successful.

DEEP LEARNING METHOD FOR DIABETIC RETINOPATHY

IMAGE ACQUISITION

The inside surface of the eye is the retinal fundus photos. The opposite portion of the lens is specified: retina, visual record, macula, fovea, and back pin. These photographs can be examined by ophthalmoscopy and checked. Datasets are collected from kaggle.com

PRE-PROCESSING

The pre-processing step involves the color transfer and filtering. The retinal photographs can be taken as RGB images. These RGB images are transformed into grey images. RGB photos are transformed into grey images since the reference images are removed from their hue and saturation. Pre-processing method is the preliminary diagnostic stage. The photographs of the input fundus are pre-processed before being added to the segmentation process. The

blurred images are rectified as an initial pre-processing phase. The transfer of color space, the reproduction of images, and the improvement are included in pre-processing. This study takes initial measures, such as backdrop removal, contrast improvement, noise reduction, and optical disc elimination. These measures are important as the access database is sometimes noisy and lit because of odd noise and camera configurations. The sensitivity and time use of fundus images and retinal images can be increased by cropping backdrop and noisy areas. The color picture is transformed to a color space transfer grey scale model, and the gray scale fundus image is pre-processed. The sensitivity modification is done using the 2D median filter in fundus pictures. Moreover, the noise in the transformed fundus picture is eliminated during the image acquisition, and the results are enhanced in the pre-processing phase. The 2D medium filter is nonlinear, and the sound reduces without blurring the rough edges when used.

Template Matching

To locate the optical disc centroid, the size of the input picture of the optical disc differs from dataset to dataset, and the proposed procedure recalls the value for the original retinal images to maintain the Fr detail amplitude within the HSI color space. Then, the morphological closure procedure is performed on Fi to eliminate blood vessels, accompanied by the correlation of Ft and Fc with the closed picture Fc. In this study, the complete Pearson-R tip, distance regulation, and form priority. The proposed approach used two primary optic disc center detection methods for the diabetic retina problem: (1) the matching of the prototype and (2) the relocation of the optical disc centre.

Locating the Optic Disc Centre

The estimated center of the optical disc is located by detection of the Fci peak as the prototype matching process. The center of the optical disc picture (Fodc) and the suggested process scenario aimed to detect retinopathy. It's over. Since the center of the optic disc is generally around the blood artery. The level set method is vulnerable to initialization, and the knowledge on the blood vessel is collected to reset the centroid. First, the closing procedure is performed to clear the blood vessel from the grey level picture of Fr. The disparity between the closed picture and the grey level image is calculated. The contrast correction feature improves the picture due to the poor contrast. Then a global modified picture threshold is measured, and the blood vessel threshold method is implemented. The blood vessel was divided. This thesis is aimed at segmenting the optical disc (Fvr). Let al be the property of the image and let alone the signed distance function (SDF). Each term of the functional energy model has various aspects of the issue. The first word ES is a previous term used to adjust for the strength of the homogeneity of the optic disc after the displacement of the blood vessel. EE's second word includes the edge details obtained from the picture taken from the vessel since the optical disc contains important edge information. The last word ER is a time limit that allows the optical disc border to remain smooth.

Optic disc and optic cup segmentation

The detection of glaucoma is usually performed by measuring the ratio between the optic disc (OD) and the optic cup (OC). The normal ratio between optic disc and optic cup is 0.3. If it is increased then there is an indication of glaucoma.

$$g(y,x) = \alpha f(y,x) \text{-------(1)}$$

$$g(y,x) = \left\{\frac{1, if\ f(x,y) > T}{0, if\ f(x,y) \leq T}\right\} \text{ ---------(2)}$$

$$m = \sum_{i=0}^{L-1} i.p(i) \text{ ---------(3)}$$

$$\sigma = \sqrt{\sum_{i=1}^{L-1} (i-m)^2\ p(i)} \text{ ------(4)}$$

In this case, 2 called as the variant or the normalized two-order moment as p(i) states the probability of the appearance i and as the function of opportunity. L states the highest border values and m refers to the mean value.

$$Tdisc = mean + 2 * std \text{----------(5)}$$

$Tcup = mean + 4 * std$ ----------(6)

Figure 4 depicts the person's input picture with the layers of retinal information and optic disc cover information, each of which has a distinct shape and perspective image that has been processed. The pre-processed picture is shown in Figure 5.

SEGMENTATION IMAGE

The morphological operations must be done for the segmentation phase. During morphological operations, the closed and dilated images are shaped and added to the binary image. It is used to extend the field boundary in the first pixels. The image's form and features may segment the picture. The tiny elements are initially separated, and the closed picture should be. The optical disc is observed and measured by an optical disc count of the number of pixels. The red channel may be seen to show that the blood vessels are less conspicuous. The gradient vector flow can be allocated for a more robust image. Figure 6 illustrates the semantic segmentation output used to identify the layers, optics, and other components individually. Semantic segmentation does certainly aid in identifying the specific portion and edge part separately. Additionally, the LBF area is geographically distinct. Figure 7 illustrates the final result for locating the optimum local area and distorted field using the DDR approach and the optic cup feature and determining if a person is impacted or the process is just starting.

Shape-prior Term

The pressure within the optical disc is uniform because of the vessel elimination residues. The shape-prior concept is used to adjust the homogeneity intensity inside an optical disc. The outline of the optical disc is usually spherical. After that, Fbvs are added to prune tiny brunches to maintain the key arcade and (cx, cy) as an estimated center of the optical disc. The tests show that cy is already situated at the centre of the optical disc. This means that the suggested approach keeps unchanged the y value of the estimated centre (cy') and searches for a new cx value by Fo(cx'). Reset picture of the optical disc (Fodcr)

Edge-Based Term

The suggested approach includes edge-based knowledge in the energy formulation to precisely identify the optical disc border. Where the coefficients are unchanged for clauses 0 and paragraph. The first word L(g), calculated the integral line of the function g and the null-level limits of os bis. As the fixed degree oscillates at the limits of the optical discs, the term is reduced to the minimum when a feature (g) oscillates with the weighted field of the system oscillating(x,y). This method would therefore accelerate the motion of the zero-level contours in the process of evolution.

Distance-Regularization Term

The optical disc border is located; the proposed solution must be precise when the border is removed. Therefore an energy functional distance regularization term is applied, which shows the high-intensity data.

Convolutional shape local binary: CNN

This approach converts an image to grayscale and a crisp picture, giving the image a reshaping similar to the image's pixel length, intensity, and magnitude. In this scenario, the image is in the same outlined view of data as the formulated image data for a clustered area is pre-processed again, to begin with the local binary bit pattern and texture classification, such that each portion of the image clusters in a matrix format in which all matrix structure information is clustered locally and globally, and thus computed as a matrix. Generally, several local heads and clusters are formed around displaced regions. The DDR approach accentuates the centre force zone depending on the separated matrix. Each average head is determined and highlighted in a single array based on its strong functional performance. The associated matrix intensity centre values were extracted independently to allow for segmentation of the area into afflicted and common sections.

Algorithm: CNN

Step 1: get input image to estimate the length and width and reshape it into a standard shape

Step 2: sum of feature (f) is the input for the fatal of the image

Step 3: convolution neural network

A. Input layers → dimension of image structure is given as one of input
B. No of dimension → dimension of the image in the different category are categorized
C. Separate layers are trained in unlike fields from convolution layer, pooling layer, dense layer, output layer
D. each layer of information feature with array matrix
E. Each array matrix has neurons and linked layers and which has equality label
F. The knowledge base and information graph of the structure is defined, then the collective field as (s) is noted
G. Feature data s = i+o layer,

Step 4: END

RESULTS AND DISCUSSION

The suggested framework was implemented using the MATLAB tool. Retinopathy with diabetes is a condition that affects diabetic eyes. Blindness can occur if not handled promptly. Lubis says that the Retinopathy of diabetes is systemic microangiopathy marked by capillary damage and blockage. Given the retina of the eye demands that the blood supply works properly in its operation, it is clear why this condition attacks patients with diabetes. The Retinopathy of diabetes itself is graded as Non-Proliferative DR and Proliferative DR. The presence of DP is usually detected by a qualified ophthalmologist through a physical test. This approach is very successful but needs several experts involved. As the number of diabetic patients continues to increase, the number of DR patients continues to increase. Therefore doctors need sufficient time to detect particular patients?

SAMPLE IMAGE

The photos are taken from the standard calibration level 0 DR database and DIARETDB1 - the standard DR database calibration level 1. The reference images are from DIARETDB0. Ninety-nine fundus photographs are taken from this description database below. Pre-processing a picture is an important method for each classification since it prepares the image for further steps. At first, the RGB image was transformed into a greyscale image to simplify the operation. Any noise may be introduced in any observation of a picture. You will process this by fluttering the picture with the medium filter and removing the noise in the image. The median filter algorithm is used to eliminate image n, set the threshold for extracting blood vessels, and measure the diameter of the fundus pictures for blood vessel classification. The visual lighting method clarifies and sees the eye independently. The picture's contrast can be extended for visible screening after completing the optical illumination phase. Then for the sample picture and contrast, the image's histogram has been created. The threshold value for the extended picture may be selected. Two threshold values are selected to distinguish the optical disc and the context of the film. The negative Laplacian operator is taken to detect the edges. The operator uses a regular mask, which contains positive elements in the middle of the picture and negative elements in the corner and makes the mask -1.

SEGMENTATION IMAGE

Morphological operations shall be conducted for the segmentation phase. During morphological work, the closed image and the dilated image are shaped and added to the binary image. It is used to extend the field boundary in the first pixels. The image's form and features may segment the picture. The tiny elements are initially separated, and the closed picture should be. The optical disc is observed and measured by an optical disc count of the number of pixels. The red channel may be used to make the blood vessels less prominent. The gradient vector flow can be allocated for a more robust picture

PERFORMANCE EVALUATION

The retinal images are defined by the optical discs, CNN, and Gaussian stages in this segment. The CNN classifies the DR picture, whether mild or moderate DR, extreme DRNP, or PDR retina. Output is measured based on sensitivity, specificity, and precision. Finally, many classifier outputs are contrasted, and the visual output bag is the highest. The Gaussian level clustering is shown in Figure 16, where the X-axis represents the latent information and the Y-axis indicates the index computation The accuracy comparison table in Figure 20 compares the current authors to the planned DDR system. Thus, the results of our suggested technique indicated that the Optic Disc is quite near to the centers identified by experts using CNN.

CONCLUSIONS

This study presented a new methodology named as (DDR-Deep Learning method for Diabetic Retinopathy). for detecting DR by using CNN and Optic disc localization and optic cup segmentation. People with diabetes require screening to avoid lack of vision. Methods of medical image processing and advanced detection algorithms are used to detect retina abnormalities. Blindness is the world's most common cause, according to DR. For early detection and recovery patients, the screening method is critical. The present analysis is used to automatically diagnose the DR. Recognition algorithms, including an optical disc, blood vessels, exudates, microaneurysms, and hemorrhages in colored retinal or fundus pictures, are used in the proposed method for automated identification of DR with 98.5% accuracy. The different phases of DR are listed in the scheme suggested. The procedure is generally carried out in three phases. Feature extraction is used to identify normal/abnormal and hard/soft exudates, including field, average, perimeter, entropy, variance, autocorrelation, and cross-correlation. Upon segmentation, characteristics are removed, and diabetic identification is carried out using neural recognition networks. The preliminary phase in designing an automatic cluster method for the FCM is the quick and accurate segmentation of DR in different imagery conditions. The accurate and exact segmentation of the location of the optical disc increases the correct diagnosis. To increase the accuracy of diabetic prediction, further studies.

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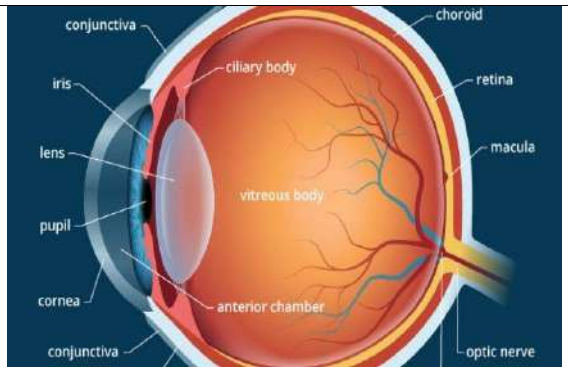

Figure 1 Structure of the human eye

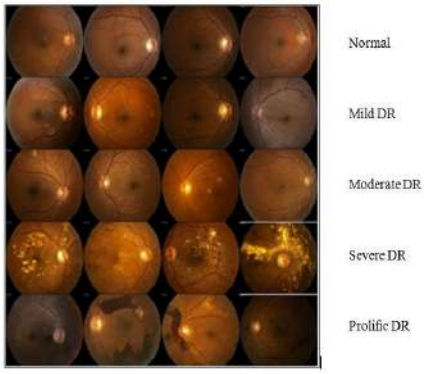

Figure 2 Stages of DR

Figure 3 Sample Retina image

Figure 4: Image used as an input

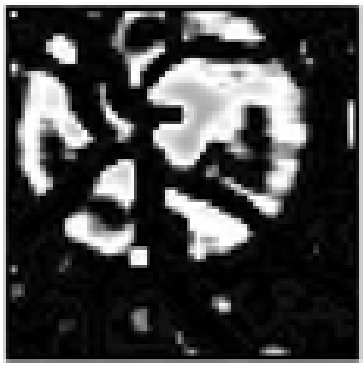

Figure 5: Image that has been pre-processed

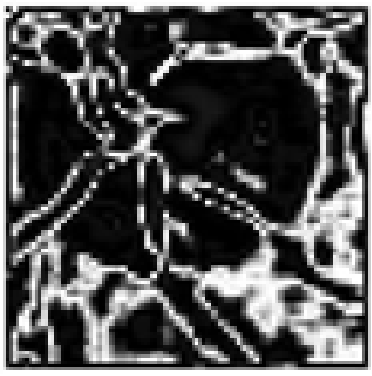

Figure 6: Semantic Segmentation

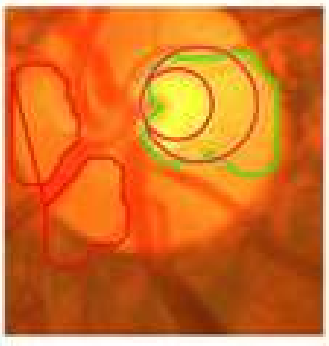

Figure 7: Affected region of the problem

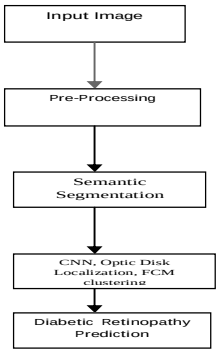

Figure 8: Architecture Diagram

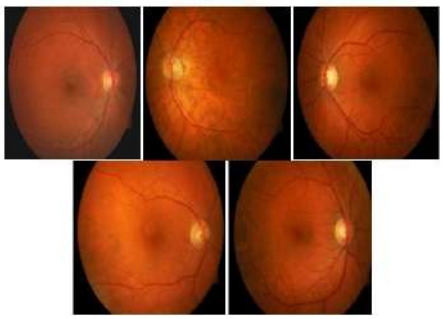

Figure 9 Mild DR Sample images

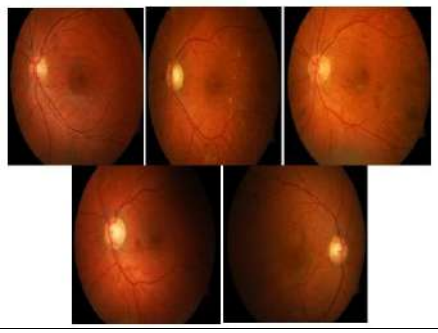

Figure 10 Moderate DR Sample images

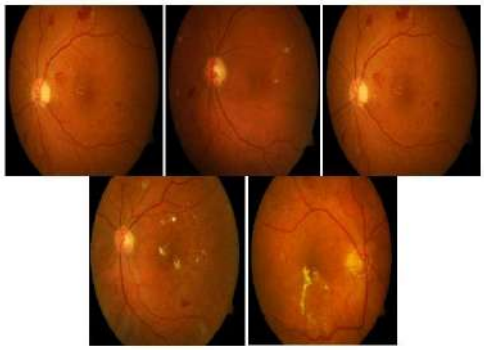

Figure 11 Severe DR Sample images

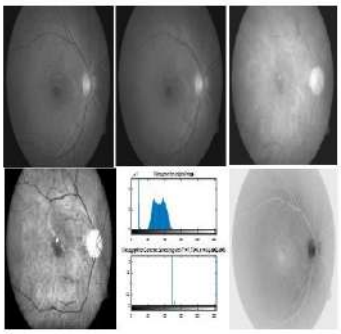

Figure 12 Pre-processed image of mild DR sample1 Row 1: Gray Scale Image, Median Filtered Image, Illuminated Image. Row 2: Contrast Stretched Image, Histogram, Negative Operator

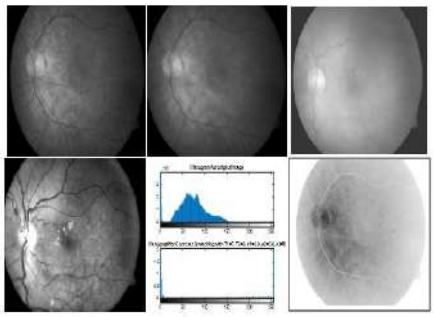

Figure 13 Pre-processed image of mild DR sample 2 Row 1: Gray Scale Image, Median Filtered Image, Illuminated Image. Row 2: Contrast Stretched Image, Histogram, Negative Operator

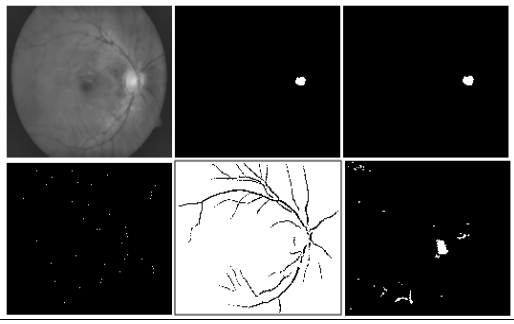

Figure 14 Segmented Image of Mild DR Sample1 Row 1: Morphological Closed Image, Optic Disc Detected Image, Dilated image. Row 2: Microaneurysms Detected Image, Blood Vessel Detection, Hemorrhages Segmented Image

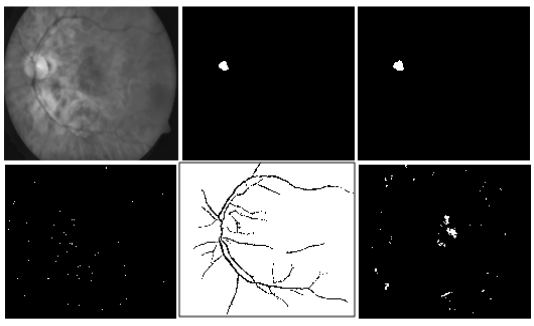

Figure 15 Segmented Image of Mild DR Sample2 Row 1: Morphological Closed Image, Optic Disc Detected Image, Dilated image

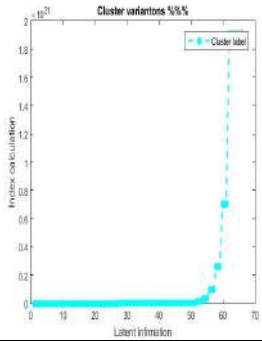

Figure 16: Percentage of cluster variations

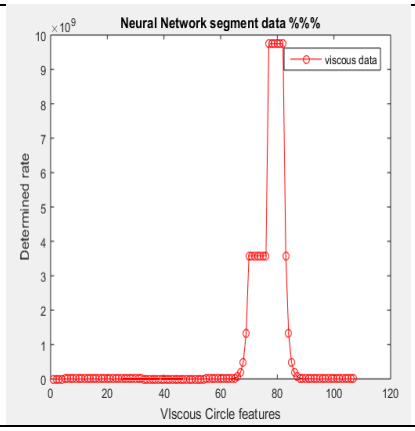

Figure 17: Segmentation of the optic disc at the level of neural networks The optic disc localization using viscous data using CNN is shown in Figure 17, where the X-axis represents the Viscous Circle features and the Y-axis represents the Determined rate.

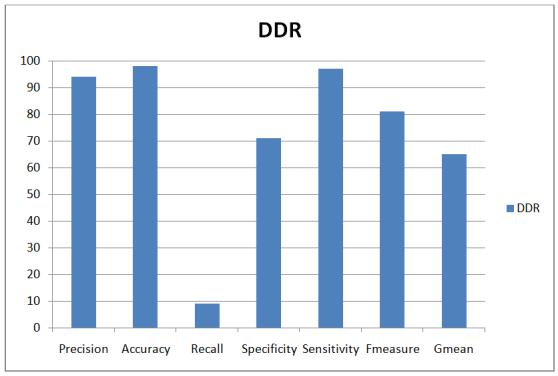

Figure 18: Performance Metrics

Figure 19: Confusion Matrix

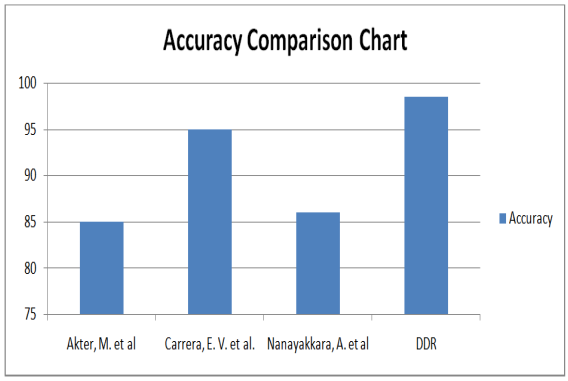

Figure 20: Comparison of prediction accuracy

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Multilevel Inverter System with Switched Capacitor Unit

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Received: 12 Feb 2022 Revised: 26 Feb 2022 Accepted: 26 Mar 2022

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ABSTRACT

The inverter is a power converter device which is used to convert DC power into AC power based on the required output voltage and frequency. The conventional inverters are converting a dc source into ac having two voltage levels only. The multilevel inverter is one of evolution in the history of power converters as it will produce output voltage of different levels. These multilevel inverters are gaining importance because of it can handle high power and lower harmonic contents in the output voltage and current. The conventional cascaded multilevel inverter will need a higher number of switches and the complexity in designing control system will be increased. This paper proposes a new 5 level inverter with switched capacitor unit for a single phase load with neutral point clamping. A multi carrier modulation technique is used to generate the pulses for the switches in the inverter. The voltage gain of 1.5 is achieved using the proposed 5 level inverter model.

Keywords: Power converter, Multilevel inverter, Switched capacitor, Neutral point clamped, multi carrier modulation

INTRODUCTION

The power conversion system with standard structure, large capacity and high reliability are needed in the fields of industrial drives, electric vehicles and renewable energy systems nowadays. The concept of multilevel inverter was introduced to handle the large capacity power system with low harmonic distortion. The multilevel inverters differ from conventional inverters in the means of output waveform and topology. The introduction of multilevel inverters made the interfacing of power electronic switches directly to the high and medium voltage grids. It reduces the

switching losses, as the voltage across the power electronic switches is reduced. With all the merits, multilevel inverter is predominant power conversion system in all the fields. The conventional multilevel inverters are having different topologies such as Cascaded H-Bridge, Flying Capacitor and Diode Clamped ML inverter. Each of them is having different advantages of their own.Diode clamped multilevel inverters utilizes the diodes to provide voltage boosting. This reduces the switching stress on power electronic switches. The drawback in this topology is that its output voltage is half of the input dc voltage [1]. To get maximum boosting the number of switches and capacitors need to be increased. The major application of this topology is when dc and ac transmission lines are interfaced [2-4]. Cascaded H-Bridge inverter consists of many h-bridge inverters connected to give the multilevel output. The number of output levels of the inverter is based on the number of dc sources present in the topology. If there are S dc sources present, then the number of levels in the output waveform is 2S+1. The number of switches required for the corresponding levels is 4S. The major issue in this topology is it needs separate dc sources for each h-bridge. It does not need any diodes or capacitors for clamping [5-8]. The flying capacitor type multilevel inverter is similar to the diode clamped ML topology with a difference of using capacitors instead diodes to limit the voltage levels. The input dc voltage is divided by the capacitors used in this configuration. To produce k level output, the flying capacitor inverter uses 2k-2 switches and k-1 capacitors in its configuration. The general application of this type of topology is in static var generation [9-11]. The above discussed three configurations are general structure in Multilevel Inverter topology. The general outcome is to synthesize a sinusoidal voltage from several levels of voltage from dc source. The usage of these inverters not only achieves high power ratings but also enable them to use it in renewable energy sources such as photovoltaic, windmill, etc. The conventional multilevel inverters are in usage since 1975[12]. Mostly all the topologies are changed and new topologies are emerged as per the requirement. The new topologies reduce the number of switches used and increase the efficiency. In this paper, the new five level inverter with switched capacitor configuration is designed and simulated in the MATLAB environment.

Proposed Configuration

The proposed five level inverter configuration is depicted in fig.1. The proposed topology is having a boosting ability and the output voltage is 1.5 times the input voltage. The circuit consists of seven switches and three capacitors to produce the five level output. By variably switching the power electronic switches in the circuit, the required boosting can be achieved. The charging and discharging of capacitors is also achieved based on the switching sequence of the power electronic switches. In the circuit IGBTs are used as switches to increase the power handling capacity. The table 1 shows the switching modes and the output voltage for different switching modes. The switching is done using the pulses given to the IGBT switches. The pulse generation is through level shift pulse generation technique. There are several other pulse generation methods available such as Sinusoidal PWM, Space Vector PWM, Harmonic injection modulation, Phase shifted PWM, Level shifted PWM, etc. Among the mentioned types of pulse generation technique, the phase shifted and level shifted PWM are multicarrier PWM techniques. The Level Shifted PWM (LSPWM) technique is used for pulse generation for the switches in the proposed topology. There are three methods of LSPWM [13]

- Phase Disposition (PD)
- Phase Opposition Disposition (POD)
- Alternate Phase Opposition Disposition (AP-POD)

Phase Disposition

The method used in this work is Phase Disposition where the phases of all the carrier waves used are in phase with each other as shown in fig.2. The number of carrier waves required to generate pulse is m-1, where m is the number of levels in the output. The five level inverter requires four carrier waveforms to generate pulses. The frequency of the carrier waveform is selected as 5 kHz. The pulses are generated by comparing the carrier waveform with the reference waveform using comparator block in the MATLAB SIMULINK. The pulses for positive carrier waveforms are shown in fig.3 and the pulses for negative carrier waveforms are shown in fig.4. Based on the switching modes, the pulses are connected to the switches and the switches will be operated to get the desired output from the proposed multilevel inverter.

Modes of Operation

As there are five level of output, there are five modes of operation of the prosed circuit. In each mode, the corresponding switches will be turned on/off using the generated pulses. The three capacitors are used to charge and discharge according to the mode of conduction of switches. The dc input voltage is 100V and the capacitors used with the value of 4700µF.

Mode A of Operation: In mode A, the switches S2,S3,S4,S7 are going to conduct. The output voltage across the RL load is $-1.5V_{dc}$. In this mode, the output voltage is boosted to 1.5 times in the negative magnitude.The current flow path during this mode of operation is shown in fig.5. The boosted output voltage is supplied from the two capacitors which is having the charge of V_{dc} and $0.5V_{dc}$.

Mode B of Operation: In this mode, the switches S4, S5 and S7 are conducting. The output voltage is $-0.5V_{dc}$. The current flow path is shown in fig.6.

Mode C of Operation: In this mode of operation, the output voltage is 0. The load connected to the circuit is not supplied with any source. The states of charging and discharging of capacitors makes the voltage across the load as zero. The switching condition for this mode of operation is shown in fig.7.

Mode D of Operation: The output voltage in this condition is 0.5 times the V_{dc}. The switches S1, S2 and S6 are conducting in this condition. The current flow through the load in this mode of operation is reversed compared to mode A and mode B operation. Hence the output voltage level is shifted to positive magnitude from zero level in this mode of operation. The capacitor voltage supplies the load and the voltage across the load is $+0.5V_{dc}$.

Mode E of Operation: In this mode of operation, the switches S1, S3, S5 and S6 are conducting as per the pulse sequence. The output voltage across the load is 1.5 times the V_{dc} and in positive magnitude. The boosting of voltage is due to the discharging of capacitors connected during this mode of operation through the load. The switching state of this mode of operation is shown in fig.9.

RESULTS AND DISCUSSION

Proposed topology in MATLAB/SIMULINK

Fig.10 shows the simulation of the proposed topology of five level multilevel inverter with switched capacitors. The IGBT switches are used and connected in a manner that the anti-parallel diodes across the switches are allowed to flow the reverse current due to inductive load. The anti-parallel diodes are also used to improve the commutation condition of the switches. The resistive-inductive branch is connected across the circuit as a load. The voltage drop across the load is measured and displayed. The switching pulses are generated using level shifted method using pulse generators and comparators as shown in fig.11. The five level output voltage generated from the proposed topology is shown in fig.12. The input 100V is boosted to 150V which is 1.5 times the input voltage. The proposed model inverts and boosts the DC voltage. The output voltage is having a THD of 24.83%. The output voltage can be further smoothened to sinusoidal waveform by improving the number of levels in the output. The obtained output is done using seven switches which are switched according to the required level of output.

CONCLUSION

The multilevel inverters have been used in industrial drives, renewable energy systems, UPS, FACTS and electric vehicle applications. A five level multilevel inverter is proposed in this paper. The simulation of five level multilevel inverter is successfully done in MATLAB environment. The proposed topology reduces the switching count for five level output, the manufacturing cost and the capacitor voltage balancing.

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Table 1. Switching Modes

Mode	Switches							Output Voltage
	S1	S2	S3	S4	S5	S6	S7	
A		ON	ON	ON			ON	-1.5Vdc
B				ON	ON		ON	-0.5Vdc
C	ON	ON					ON	0
D	ON	ON		ON	ON	ON		0.5Vdc
E	ON		ON		ON	ON		1.5Vdc

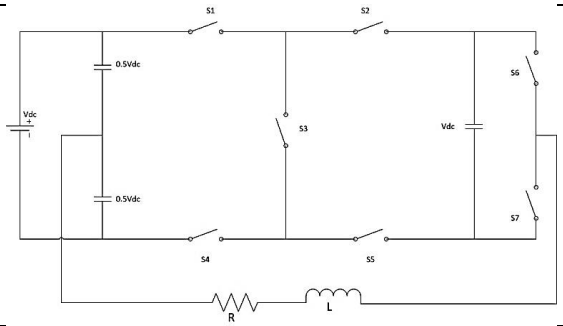

Fig 1: Proposed five level inverter topology

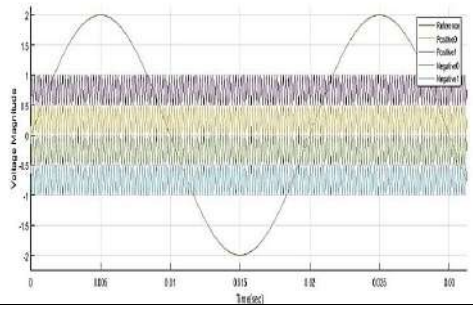

Fig 2: Level Shifted PWM Waveform

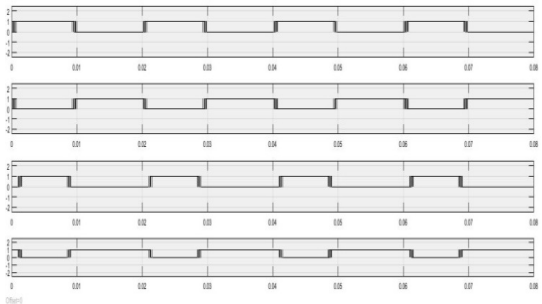

Fig 3: Pulses from positive carrier waveforms

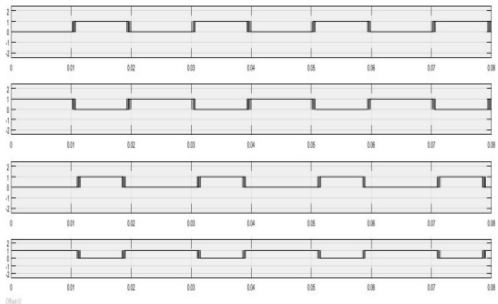

Fig 4: Pulses from negative carrier waveforms

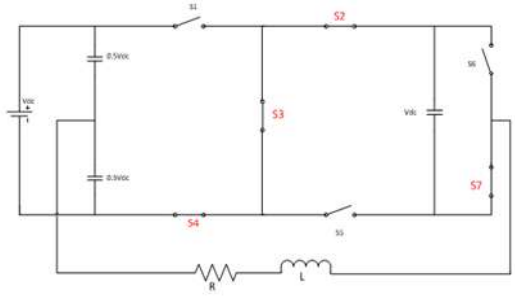

Fig 5: Switching condition in Mode A operation

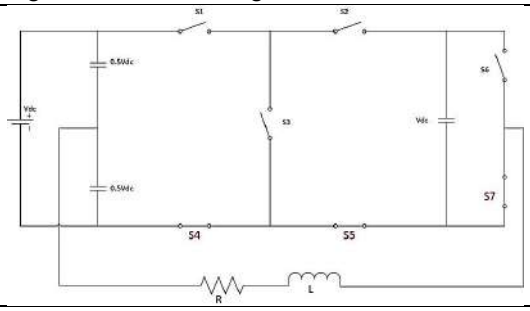

Fig 6: Switching condition in Mode B operation

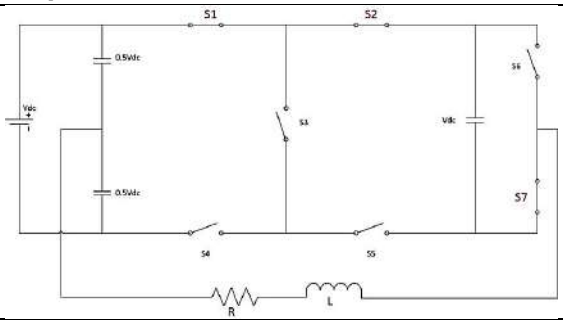

Fig7: Switching condition in Mode C operation

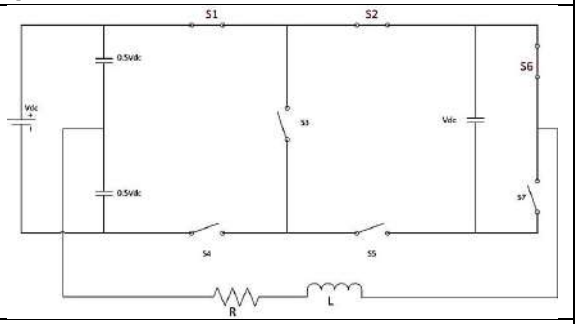

Fig 8: Switching condition in Mode D operation

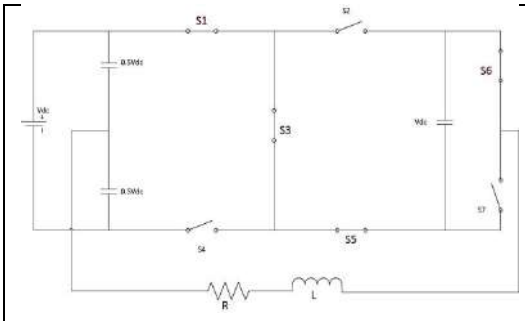

Fig 9: Switching condition in Mode E operation

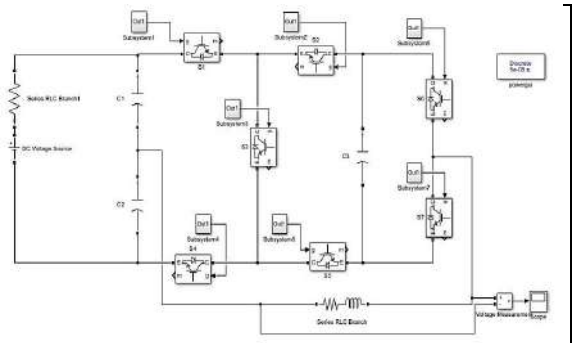

Fig 10: Five level multilevel inverter topology

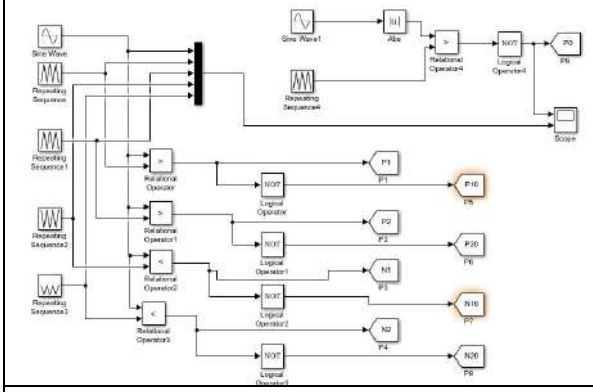

Fig 11: Pulse generation block

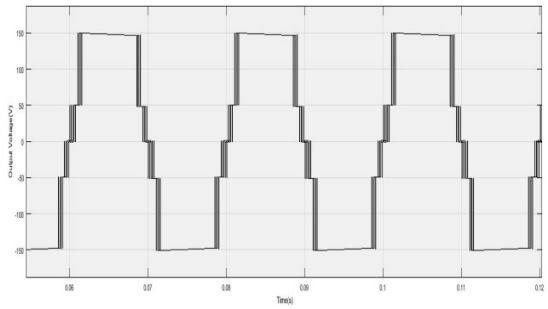

Fig 12: Output voltage of proposed model

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Characterization of Uniform and Hybrid Fuzzy Cellular Automata with Null Boundary

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Received: 08 Jan 2022 Revised: 03 Feb 2022 Accepted: 23 Feb 2022

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ABSTRACT

We study the characterization Fuzzy Cellular Automata with Restricted Vertical neighborhood and Von Neumann neighborhood of null boundary conditions over the field Z_2 in Uniform Fuzzy Cellular Automata and Hybrid Fuzzy Cellular Automata. Transition fuzzy matrix for uniform and hybrid fuzzy cellular automata with null boundary condition is obtained.

Keywords: Fuzzy matrix, Two-Dimensional Cellular automata, Von Neumann Neighborhood, Restricted Vertical neighborhood, Ternary field, Transition rule matrix, Matrix algebra, Null boundary condition, Reversibility.
Subject Classification: 37B15, 20M35, 15A04

INTRODUCTION

Cellular automata are discrete dynamical systems that exhibit a variety of dynamical behaviors, although they are formed by simple basic components. Cellular Automata or shortly (CA) were first used for modeling various physical, Biological processes and on computer science. The concept of cellular automata was initiating the early1950's by John Von Neumann and Stanislaw Ulam. The father of CA is John Von Neumann [13] working on self replication and attempting to provide a reductionist theory of biological development, Von Neumann was trying to conceive a system capable of producing exact copies of itself. Now biology prima facie appears to be the realm of

fluidity and continuous dynamics. In the early 1960's Moore [53] and Myhill [55] proved the Garden of Eden theorems stating conditions for the existence of so-called Gardens of Eden, that is, patterns that cannot appear on the lattice of a CA except as initial conditions. Gustav Hedlund [31] investigated CA within the framework of symbolic dynamics. In 1970 the mathematician John Conway introduced his aforementioned Life game [11], arguably the most popular automaton ever, and one of the simplest computational models ever proved to be a universal computer. In 1977, Tommaso Toffoli used CA to directly model physical laws, laying the foundations for the study of reversible CA. Cellular Automata are also called Cellular Space, Tessellation Automata, Homogeneous structures, Cellular structure, Tessellation structures and Iterative arrays [14].

A cellular automata is a model of a system of "cell" objects with the following characteristics.
∗The cell lives on a grid.
∗Each cell has a state. The number of state possibilities is typically finite. The simplest example has the two possibilities of 1 and 0 (otherwise referred to as "ON" and "OFF" (or) "alive" and "dead").
∗Each cell has a neighborhood. This can be defined in any number of ways, but it is typically a list of adjacent cells.
The 2D finite cellular automata consists of (m × n) cells arranged, where each cell takes one of the values of the fieldZ_2. The relative positions of the cells are called neighbor of the center cell. The state of these neighbors is used to compute the new state of the center cell.

The paper is organized as follows. In the 2nd section, the concept used in the paper is formally defined. In 3rd section, the algebraic structure of restricted vertical neighborhood and Von Neumann neighborhood of 2D fuzzy cellular automata is obtained. In 4th section, the rule of the uniform fuzzy cellular automata with restricted vertical neighborhood is studied. In 5th section, using both Von Neumann and Restricted Vertical Neighborhood the fuzzy matrix of the hybrid fuzzy cellular automata is obtained.

Preliminaries

Definition 2.1. [9] A Null Boundary CA is the one in which the extreme cells are connected to logic zero state.
Definition 2.2. [10] If the same rule is applied to all the cells in a CA, then the CA is said to be uniform or regular CA.
Definition 2.3. [3] If in a CA the diffe rent rules are applied to different cells, then the CA is said to be hybrid CA.
Definition 2.4. [11] Restricted Vertical Neighborhood *(RVN)*: The Restricted Vertical Neighborhood (RVN) cellular automata rule is the class of CA's where a cell can have either top (or) bottom dependency but not both, this class of CA is referred to as Restricted Vertical Neighborhood CA.
Definition 2.5. Fuzzy cellular automata
In, [1] A fuzzy cellular automata (FCA) is defined by the tuple (A, Q, u, F, M) where,
∗ m dimensional array A of cell.
∗ Q is a finite set of cell states.
∗The neighborhood u is a K-tuple of state of cells.
∗ $F = \{f | f : Q^k \to Q\}$ is a set of local transition rule.
∗ $M = \{\mu | \mu : Q^k \times F \to [0,1]\}$ is a set of grades of the local transition

Definition 2.6. [4] **Cellular Automata:** Cellular automata are formally defined
as quadruplets $A = \{d, S, N, f\}$
∗ $d \in Z_+$ is the dimension of the cellular space, then each point $n \in Z^d$ is called a cell.
∗ $S = \{0,1,2, p-1\}$ represents the finite state set and the state of any cell at any time must be taken from S.
∗ $N = (\vec{n_1}, \vec{n_2}, \vec{n_m})$ is the neighbor vector, where $\vec{n_i} \in Z^d$ and $\vec{n_i} \neq \vec{n_j}$ when $i \neq j (i, j = 1,2, m)$. Thus, the neighbors of the cell $\vec{n_i}$ are the m cells, i = 1,
2, ..., m.
∗ $f: S^m \to S$ is the local rule, which maps the current states of all neighbors of a cell to the next state of this cell.

A configuration is a mapping $C: Z^d \to S$ which assigns each cell a state. Make C^t denote the configuration at time t, then the state of cell n at time is $C^t(\vec{n})$ and its state at time (t + l) goes like this.

$$C^{t+1}(\vec{n}) = f(C^t(n_1), C^t(n_2) \to \to, C^t(n_{\overline{m}}))$$

now we consider the case in which the local rule f is a linear function $C^{t+1}(\vec{n}) = f(C^t(n_1) \to + C^t(n_2) \to + \ + C^t(n_{\overline{m}}))$

$= [\lambda_1 C^t(n_1^{\to}) + \lambda_2 C^t(n_2^{\to}) + \ + \lambda_m C^t(n)]$ (mod S)

Where $\lambda_i \in S$ is the rule co-efficient for neighbor $n_i^{\to}$, i = l, 2, … , m.

In [7] they consider the information matrix $C^{(t)} = \begin{pmatrix} X_{11}^{(t)} & \cdots & X_{1n}^{(t)} \\ \vdots & \ddots & \vdots \\ X_{m1}^{(t)} & \cdots & X_{mn}^{(t)} \end{pmatrix}$ The matrix $C^{(t)}$ is called the configuration of the 2D finite CA at time t.

Neighborhood Structure

In this section, we explain the neighborhood structure and there local rule transition.

3.1. Restricted Vertical Neighborhood *(RVN)*

In 2D CA theory, the *RVN* comprises the 3 cells surrounding the central cell on two-dimensional.

In figure 3.1, we show the *RVN* which comprises 3 cells which surround the center cell $X_{(p,q)}$. The state $X_{(p,q)}^{(t+1)}$ of the cell $(p,q)^{th}$ at time (t + 1) is define by the local rule function.

$f: Z_2^3 \to Z_2$ as follows,

$$X_{(p,q)}^{(t+1)} = f(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)})$$
$$= \left(X_{(p,q+1)} + X_{(p,q-1)} + X_{(p-1,q)}\right) \quad \ldots (1)$$

The grates of the local transition: $Q^3 \times F \to [0,1]$

$$\mu[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}), f[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}), (y_{(p,q)})]] = \frac{y_{(p,q)}}{100} = t_{(p,q)}$$

3.2. Von Neumann Neighborhood

The Von Neumann neighborhood comprises the 4 cells surrounding the central cell on a 2D square lattice.

In figure 3.2, we show the John Von Neumann with neighborhood which comprises 4 square cells surrounding the center cell $X_{(i,j)}$. The state $X_{(i,j)}^{(t+1)}$ function: $Z_2^4 \to Z_2$ as follows.

$$X_{(p,q)}^{(t+1)} = f(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}, X_{(p+1,q)})$$
$$= \left(X_{(p,q+1)} + X_{(p,q-1)} + X_{(p-1,q)} + X_{(p+1,q)}\right) \quad \ldots \quad (2)$$

The grates of the local transition: $Q^4 \times F \to [0,1]$

$\mu[(X_{(p,q+1)} + X_{(p,q-1)} + X_{(p-1,q)} + X_{(p+1,q)}), f[(X_{(p,q+1)} + X_{(p,q-1)} + X_{(p-1,q)} + X_{(p+1,q)}), (y_{(p,q)})]] = \frac{y_{(p,q)}}{100} = t_{(p,q)}$

Uniform Cellular Automata

In this section, we explain the fuzzy transition of the local rule

4.1 Transition fuzzy matrix of the 2D Uniform fuzzy cellular automata

Here we obtain the fuzzy matrix of 2D finite fuzzy Cellular Automata with *RVN* rule over the field Z_2 under the null boundary condition. The rule matrix which takes the $t^{(th)}$ finite configuration matrix $C^{(t)}$ of order m × n to the $(t + 1)^{th}$ state $C^{(t+1)}$.

Theorem 4.1. Let M = (A, Q, u, F, M) be the uniform fuzzy cellular automata and let m ≥ 3 and n ≥ 3. Then the rule matrix from $Z_2^{mn} \to Z_2^{mn}$ corresponding to the 2D uniform fuzzy cellular automata in restricted vertical neighborhood that takes from configuration the state $C^{(t)}$ of order (m × n) to the $(t + 1)^{th}$ state $C^{(t+1)}$ is given, we will prove that corresponding fuzzy matrix of grades of the local transition is given,

$$FM = \begin{pmatrix} t_{11} & t_{12} & \cdots & t_{1,n-1} & t_{1n} \\ t_{21} & t_{21} & \cdots & t_{2,n-1} & t_{2n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ t_{m1} & t_{m2} & \cdots & t_{m,n-1} & t_{mn} \end{pmatrix}_{(m \times n)}$$

Proof.

Let M = (A, Q, u, F, M) be a fuzzy cellular automata

Where,

A- 2 Dimensional array of cells.

$Q = \{X_{i,j}\}$, i = 1,2,3, m and j = 1,2,3, ..., n is a finite set of cell state u-is a neighborhood of state of cells

$f : Q^3 \times Q$ be a local transition rule

$\mu -$ is the set of grades of the local transition $\mu : Q^3 \times F \to [0,1]$ is given by, $\mu[(X_{(1,2)}), f[X_{(1,2)}, (y_{(1,1)})]] = \frac{y_{(1,1)}}{100} = t_{(1,1)}$

$$\mu[(X_{(1,3)}, X_{(1,1)}), f[X_{(1,3)}, X_{(1,1)}, (y_{(1,2)})]] = \frac{y_{(1,2)}}{100} = t_{(1,2)}$$

$$\mu\left[(X_{(1,q+1)}, X_{(1,q-1)}), f[X_{(1,q+1)}, X_{(1,q-1)}, (y_{(1,q)})]\right] = \frac{Y_{(1,q)}}{100} = t_{(1,q)}, \qquad (2 \le q \le n-1)$$

$$\mu[(X_{(1,n-1)}), f[X_{(1,n-1)}, (y_{(1,n)})]] = \frac{Y_{(1,n)}}{100} = t_{(1,n)}$$

$\mu\left[(X_{(p,2)}), f[X_{(p,2)}, (Y_{(p,1)})]\right] = \frac{Y_{(p,1)}}{100} = t_{(p,1)}$, for $(2 \le p \le m-1)$

$\mu[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}), f[X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}, (Y_{(p,q)})]] = \frac{Y_{(p,q)}}{100} = t_{(p,q)}$

$$\mu\left[(X_{(p,n-1)}, X_{(p-1,n)}), f[X_{(p,n-1)}, X_{(p-1,n)}, (Y_{(p,n)})]\right] = \frac{Y_{(p,n)}}{100} = t_{(p,n)}, \qquad (2 \le q \le n-1)$$

Finally, we have

$$\mu[(X_{(m,2)}), f[X_{(m,2)}, (Y_{(m,1)})]] = \frac{Y_{(m,1)}}{100} = t_{(m,1)}$$

$\mu[(X_{(m,q+1)}, X_{(m,q-1)}, X_{(m-1,q)}), f[X_{(m,q+1)}, X_{(m,q-1)}, X_{(m-1,q)}, (Y_{(m,q)})]] = \frac{Y_{(m,q)}}{100} = t_{(m,q)}$ $2 \le q \le n-1$

$$\mu[(X_{(m,n-1)}, X_{(m-1,n)}), f[X_{(m,n-1)}, X_{(m-1,n)}, (Y_{(m,n)})]] = \frac{Y_{(m,n)}}{100} = t_{(m,n)}$$

Finally we get the fuzzy matrix FM.

Example 4.1 If we take m = 3 and n = 3, then we get fuzzy matrix FM of 2D finite fuzzy CA with RVN rule over the field Z_2 be as follows, $FM = \begin{pmatrix} t_{11} & t_{12} & t_{13} \\ t_{21} & t_{22} & t_{23} \\ t_{31} & t_{32} & t_{33} \end{pmatrix}$

Solution. $\mu[(X_{(1,2)}), f[X_{(1,2)}, (y_{(1,1)})]] = \frac{y_{(1,1)}}{100} = t_{(1,1)}$

$$\mu[(1,0,0), f[(1,O,O)(1)]] = \frac{1}{100} = 0.01$$

$$\mu[(X_{(1,3)}, X_{(1,1)}), f[X_{(1,3)}, X_{(1,1)}, (y_{(1,2)})]] = \frac{y_{(1,2)}}{100} = t_{(1,2)}$$

$$\mu\left[(1,1,0), f[(1,1,O)(2)]\right] = \frac{2}{100} = 0.02$$

$$\mu[(X_{(1,2)}), f[X_{(1,2)}, (y_{(1,3)})]] = \frac{y_{(1,3)}}{100} = t_{(1,3)}$$

$$\mu[(0,1,0), f[(O,1,O)(1)]] = \frac{1}{100} = 0.01$$

$$\mu[(X_{(2,2)}, X_{(1,1)}), f[(X_{(2,2)}, X_{(1,1)}), (y_{(2,1)})]] = \frac{y_{(2,1)}}{100} = t_{(2,1)}$$

$$\mu[(1,0,1), f[(1,O,1)(2)]] = \frac{2}{100} = 0.02$$

$\mu[(X_{(2,3)}, X_{(2,1)}, X_{(1,2)}), f[(X_{(2,3)}, X_{(2,1)}, X_{(1,2)}), (y_{(2,2)})]] = \frac{y_{(2,2)}}{100} = t_{(2,2)}$ $\mu[(1,0,1), f[(1,O,1)(2)]] = \frac{2}{100} = 0.02$

$$\mu[(X_{(2,2)}, X_{(1,3)}), f[(X_{(2,2)}, X_{(1,3)}), (y_{(2,3)})]] = \frac{y_{(2,3)}}{100} = t_{(2,3)}$$

$$\mu[(0,1,1), f[(O,1,1)(2)]] = \frac{2}{100} = 0.02$$

$\mu[(X_{(3,2)}, X_{(2,1)}), f[(X_{(3,2)}, X_{(2,1)}), (y_{(3,1)})]] = \frac{y_{(3,1)}}{100} = t_{(3,1)}$

$$\mu[(1,0,0), f[(1,0,0)(1)]] = \frac{1}{100} = 0.01$$

$\mu\left[(X_{(3,3)}, X_{(3,1)}, X_{(2,2)}), f\left[(X_{(3,3)}, X_{(3,1)}, X_{(2,2)}), (y_{(3,2)})\right]\right] = \frac{y_{(3,2)}}{100} = t_{(3,2)}$ $\mu[(0,1,1), f[(O,1,1)(2)]] = \frac{2}{100} = 0.02$

$$\mu[(X_{(3,2)}, X_{(2,3)}), f[(X_{(3,2)}, X_{(2,3)}), (y_{(3,3)})]] = \frac{y_{(3,3)}}{100} = t_{(3,3)}$$

$$\mu[(0,1,1), f[(O,1,1)(2)]] = \frac{2}{100} = 0.02$$

$$FM = \begin{pmatrix} 001 & 002 & 001 \\ 002 & 002 & 002 \\ 0.01 & 0.02 & 0.02 \end{pmatrix}$$

Theorem 4.2. Let M = (A, Q, u, F, M) be the uniform fuzzy cellular automata and let m ≥ 3 and n ≥ 3. Then the rule matrix from $Z_2^{mn} \to Z_2^{mn}$ corresponding to the 2D uniform fuzzy cellular automata in Von Neumann neighborhood that takes from configuration the state $c^{(t)}$ of order (m × n) to the $(t + 1)^{th}$ state $c^{(t+1)}$ is given, we will prove that corresponding fuzzy matrix of grades of the local transition is given,

$$FM = \begin{pmatrix} t_{11} & t_{12} & \cdots & t_{1,n-1} & t_{1n} \\ t_{21} & t_{21} & \cdots & t_{2,n-1} & t_{2n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ t_{m1} & t_{m2} & \cdots & t_{m,n-1} & t_{mn} \end{pmatrix}_{(m \times n)}$$

Proof. The proof of the Theorem 4.2 can be obtained by following similar steps as in the proof of Theorem 4.1.

Hybrid Cellular Automata

In the present study, we work with special 2D CA defined by hybrid rule over the field Z_2 under null boundary condition. We will determine the fuzzy transition matrix rule $C^{(t)}$ of matrix of order $m \times n$.
Case (i). m is even, the fuzzy matrix FM is given in the following theorem

Theorem 5.1. Let $M = (A, Q, u, F, M)$ be the hybrid fuzzy cellular automata and let $m \geq 3$ and$n \geq 3$. Then the rule matrix from $Z_2^{mn} \to Z_2^{mn}$ corresponding to the 2D hybrid fuzzy cellular automata in Von Neumann and restricted vertical neighborhoods that takes from configuration the state $C^{(t)}$ of order $(m \times n)$ to the $(t + 1)^{th}$ state $c^{(t+1)}$ is given, we will prove that corresponding fuzzy matrix of grades of the local transition is given,

$$FM^e_{HYD} = \begin{pmatrix} p^e_{11} & p^e_{12} & p^e_{1,n-1} & p^e_{1n} \\ p^e_{21} & p^e_{22} & p^e_{2,n-1} & p^e_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ p^e_{m1} & p^e_{m2} & p^e_{m,n-1} & p^e_{mn} \end{pmatrix}$$

Proof.
Let $M = (A, Q, u, F, M)$ be a fuzzy cellular automata
Where,
A-2 Dimensional array of cells.
$Q = \{X_{i,j}\}$, i = 1,2,3, m and j = 1,2,3, ..., n is a finite set of cell state
u- is a neighborhood of state of cells $f : Q^4 \times Q$ be a local transition rule for odd rows and $f : Q^3 \times Q$ be a local transition rule for even rows
$\mu -$ is the set of grades of the local transition $\mu : Q^4 \times F \to [0,1]$ and $\mu : Q^3 \times F \to [0,1]$ is given by,

$$\mu[(X_{(1,2)}, X_{(2,1)}), f[(X_{(1,2)}, X_{(2,1)}), (y_{(1,1)})]] = \frac{y_{(1,1)}}{100} = p_{(1,1)}$$

$\mu[(X_{(1,q+1)}, X_{(1,q-1)}, X_{(2,q)}), f[(X_{(1,q+1)}, X_{(1,q-1)}, X_{(2,q)}), (y_{(1,q)})]] = \frac{y_{(1,q)}}{100} = p_{(1,q)}, 2 \leq q \leq n-1$

$$\mu[(X_{(1,n-1)}, X_{(2,n)}), f[(X_{(1,n-1)}, X_{(2,n)}), (y_{(1,q)})]] = \frac{y_{(1,n)}}{100} = p_{(1,n)}$$

When m is odd

$\mu[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}, X_{(p+1,q)}), f[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}, X_{(p+1,q)}), (y_{(p,q)})]] = \frac{y_{(p,q)}}{100} = p_{(p,q)}, 2 \leq p \leq m-1$

$$\mu[(X_{(2,1)}, X_{(1,1)}), f[(X_{(2,1)}, X_{(1,1)}), (y_{(2,1)})]] = \frac{y_{(2,1)}}{100} = p_{(2,1)}$$

$\mu[(X_{(2,q+1)}, X_{(2,q-1)}, X_{(1,q)}), f[(X_{(2,q+1)}, X_{(2,q-1)}, X_{(1,q)}), (y_{(2,q)})]] = \frac{y_{(2,q)}}{100} = p_{(2,q)}\ 2 \le q \le n-1$
$\mu[(X_{(2,n-1)}, X_{(1,n)}), f[(X_{(2,n-1)}, X_{(1,n)})), (y_{(2,n)})]] = \frac{y_{(2,n)}}{100} = p_{(2,n)}\ 2 \le q \le n-1$
when m is even
$\mu[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}), f[(X_{(p,q+1)}, X_{(p,q-1)}, X_{(p-1,q)}), (y_{(2,n)})]] = \frac{y_{(p,q)}}{100} = p_{(p,q)}\ 2 \le p \le m-1$
Finally we get the fuzzy rule matrix FM^{e}_{HYD}.

Case (ii). m is odd, the fuzzy matrix FM is given in the following theorem

Theorem 5.2. Let $M = (A, Q, u, F, M)$ be the hybrid fuzzy cellular automata and let $m \geq 3$ and $n \geq 3$. Then the rule matrix from $Z_2^{mn} \to Z_2^{mn}$ corresponding to the $2D$ hybrid fuzzy cellular automata in Von Neumann and restricted vertical neighborhoods that takes from configuration the state $c^{(t)}$ of order $(m \times n)$ to the
14
$(t+1)^{th}$ state $c^{(t+1)}$ is given, we will prove that corresponding fuzzy matrix of grades of the local transition is given,

$$FT^{o}_{RYD} = \begin{pmatrix} p^{o}_{11} & p^{o}_{12} & p^{o}_{1,n-1} & p^{o}_{1n} \\ p^{o}_{21} & p^{o}_{22} & p^{o}_{2,n-1} & p^{o}_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ p^{o}_{m1} & p^{o}_{m2} & p^{o}_{m,n-1} & p^{o}_{mn} \end{pmatrix}$$

Proof. The proof of the Theorem 5.2 can be obtained by following similar steps as in the proof of Theorem 5.1.

CONCLUSION

In this paper, 2D fuzzy cellular automata of Restricted Vertical Neighborhood and Von Neumann Neighborhood are studied over the field Z_2. The fuzzy matrix of 2D fuzzy cellular automata is computed.

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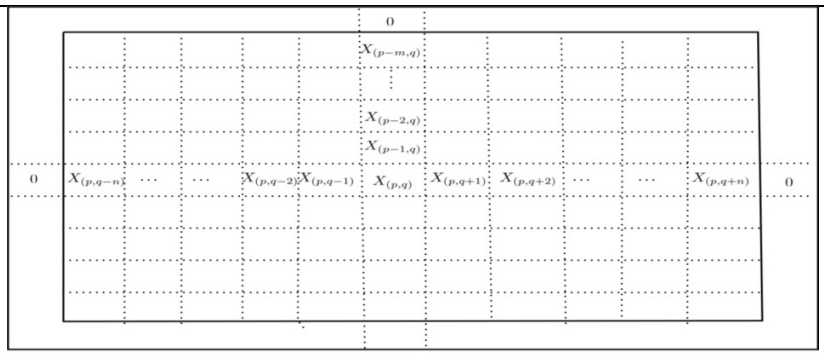

Figure 3.1. Elements of null boundary for Restricted Vertical neighborhood surround the center $X_{(p,q)}$ of $CA_{(m\times n)}$

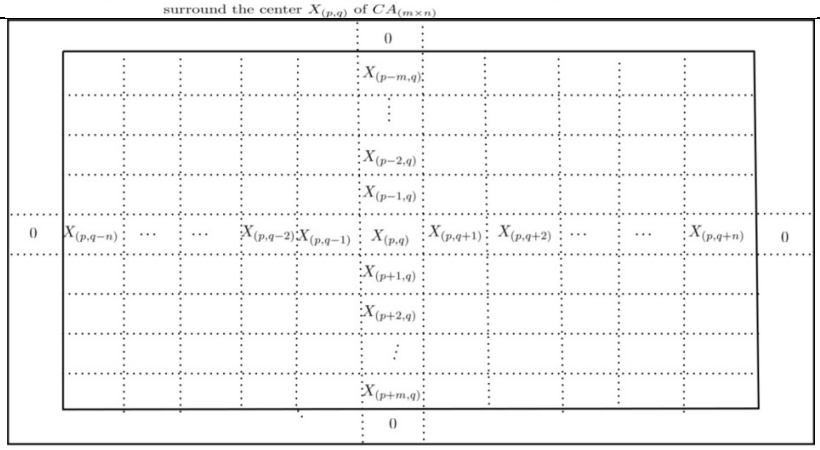

Figure 3.2 Elements of null boundary for Von Neumann neighborhood surround the center $X_{(p,q)}$ of $CA_{(m\times n)}$

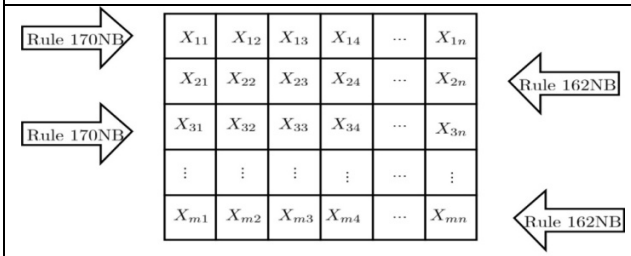

Figure 5.1. Hybrid rule means that it is applied 170NB and 162NB respectively for each rows when m is even on $(m \times n)$ CA.

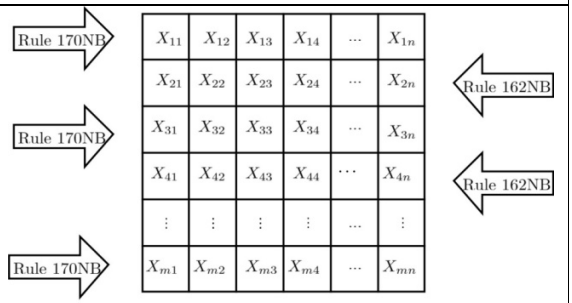

Figure 5.2 Hybrid rule means that it is applied 170NB and 162NB respectively for each rows when m is odd on $(m \times n)$ CA.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Colour Stability of Bulk Fill Composite Resin after Brushing Simulation with Two Different Toothpastes - An *In Vitro* Study

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Received: 08 Jan 2022 Revised: 10 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Composite resin has been used for nearly 50 years as a restorative material in dentistry. Colour stability is a prime property that assesses the success or failure of dental restorative treatment. The aim of the study is to evaluate the colour stability of bulk fill composite resin after brushing simulation with two different toothpastes. For this study, A2 shade of bulk composite resin restorative material was light cured in a disk mould of dimension 10 mm, 4 mm thickness and height of 4 mm. The samples were removed from the mould and the composite disk was then finished and polished. Totally 8 samples were made and divided into 2 groups. The prepared bulk composite resin samples were mounted using die stone and were subjected to brushing simulation with two different toothpastes. Group A was tested for the color stability after brushing simulation with dabur toothpaste and group B was tested for the color stability after brushing simulation with pepsodent toothpaste. Eight disk shaped samples were placed in a brushing simulator (ZM3.8 SD Mechatronik). Colour stability was checked using an instrument named Vita EasyShade Spectrophotometer. The colour stability was checked on 2 different sessions, pre-brushing and post-brushing simulation (24 hours). For the comparison of color stability of bulk composite resin sample pre brushing and post brushing simulation, the Delta E values were calculated. P value was calculated using SPSS version 2.3 and T-test was done. The mean delta E value for bulk

composite resin restorative material after brushing simulation using Dabur toothpaste was 20.77 . The mean delta E value for bulk composite resin restorative material after brushing simulation using Pepsodent toothpaste was 20.74. P value is 0.880(> 0.05), hence statistically not significant. Within the limitations of the study, it can be concluded that superior color stability was found in bulk composite resin samples subjected to brushing simulation with pepsodent toothpaste when compared to dabur toothpaste.

Keywords: Brushing simulation, Colour stability, Toothpaste, Composite resin, Spectrophotometer, Innovative measurement

INTRODUCTION

The demand for esthetic dentistry is consistently increasing. It might be due to the patient's desire to look for esthetically attractive healthy smiles. The will for whiter and brighter smiles has led to the development of dental products, especially whitening agents and composite resin. Composite resin has been used for nearly 50 years as a restorative material in dentistry. Use of this material has recently increased as a result of consumer demands for esthetic restorations, coupled with the public's concern with mercury-containing dental amalgam. Composite is now used in over 95% of all anterior teeth direct restorations and in 50% of all posterior teeth direct restorations.(1) Composite resins are mercury-free and esthetically appealing restorative materials that are commonly used by clinicians. Composite resin varies depending on the form and content of the filler, which determines its handling qualities and physical properties. In medicine, fiber-reinforced composites have been used in orthopedics as implants, osseous screws and bearing surfaces. (1). Tooth color is a crucial parameter that plays a role in facial beauty. Some recent studies have shown that 17% to 53% of individuals from different populations are not satisfied with their tooth color.(2,3) Brushing simulator is a mechanical device which can simulate various brushing movements and various toothpastes can be subjected to simulation. Change in colour of the composite of teeth in the anterior arch which is supposed to improve the aesthetic profile of the patient ends up being a disaster and this might be the reason why many patients come for the treatment of their previous dental work. Other habits like smoking, drinking and sometimes food can affect the colour of the composite, these are factors which are greatly influenced by the lifestyle of the patient(4). Colour stability is a prime property that assesses the success or failure of dental restorative treatment. Substances like alcohol, acidic food, tooth paste and mouth rinses can cause etching in the composite, which could lead to changes in the colour of the composite resin.(5),(6) The aim of the study is to evaluate the colour stability of bulk fill composite resin after brushing simulation with two different toothpastes.

MATERIALS AND METHODS

For this study, A2 shade of bulk composite resin restorative material was light cured in a disk mould of dimension 10 mm, 4 mm thickness and height of 4 mm. The samples were removed from the mould and the composite disk was then finished and polished. Totally 8 samples were made and divided into 2 groups. The prepared bulk composite resin samples were mounted using die stone and were subjected to brushing simulation with two different toothpastes. Group A was tested for the color stability after brushing simulation with dabur toothpaste and group B was tested for the color stability after brushing simulation with pepsodent toothpaste. Eight disk shaped samples were placed in a brushing simulator (ZM3.8 SD Mechatronik). The samples were subjected to 8-9 hours of brushing which is equal to around three years of brushing and to around 30000 cycles in total among which 10000 cycles were performed in the linear X axis, 10000 cycles in the linear Y axis and the last 10000 were further subdivided and 5000 cycles were performed in the clockwise direction and the remaining 5000 cycles in the anti-clockwise direction. The change in the colour stability of the bulk composite resin was analysed. Colour stability was checked using an instrument named Vita EasyShade Spectrophotometer. The colour stability was checked on 2 different sessions, pre-brushing and post-brushing simulation (24 hours). The discs were washed in distilled water before checking the

colour stability. We had only collected the L, a, b values from the colour analyser (Vita EasyShade Spectrophotometer) for the colour stability of the composite samples. For the comparison of color stability of bulk composite resin sample pre brushing and post brushing simulation, the Delta E values were calculated. The total colour difference Delta E for each bulk composite resin material sample was calculated using the following equation $\Delta E(L^* a^* b^*) = [(\Delta L^*)^2 + (\Delta a)^2 + (\Delta b)^2]$. P value was calculated using SPSS version 2.3 and T-test was done.

RESULTS

In this study we have calculated the colour stability of bulk composite resin restorative material after brushing simulation using two different toothpastes (Dabur and Pepsodent). The mean delta E value for bulk composite resin restorative material after brushing simulation using Dabur toothpaste was 20.77 . The mean delta E value for bulk composite resin restorative material after brushing simulation using Pepsodent toothpaste was 20.74. P value is 0.880(> 0.05), hence statistically not significant. (Table 1, Figure 1)

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications(7–16),(17–20),(21–25),(26). Facial aesthetics and physical appearance have great potential to affect one's social life. Therefore, even small imperfections in dental aesthetics might lead to a fear of negative public reactions and cause appearance-based insecurity. Enhancing your smile includes oral care routines and restorations designed to deal with any harm which could have damaged the tooth surfaces. Brushing can also affect the color and optical properties of composite resin. Influence of red wine, soft drink, sugar cane spirit or artificial saliva on the color stability of composite resin was evaluated in a study. Red wine and soft drinks caused alterations in the color stability of composite resins after brushing simulation.In our study, the Delta E values of pre and post brushing simulation were collected and was calculated, which made it evident that both toothpaste influenced the change in the colour of the composite due to brushing, in which toothpaste A (Dabur) affected the colour stability of the bulk composite resin slightly greater than that of the toothpaste B (pepsodent). (27) A study evaluated surface gloss and color changes of six bulk composites after brushing simulation. Gradia Direct LoFlo brand showed excellent color stability after toothbrush simulation. Color changes of bulk composites caused by brushing abrasion was acceptable on the premise that 3.3ΔE units were considered as acceptable threshold values. (28) The effects of brushing simulation and artificial accelerated ageing on the color stability of aesthetic restorative materials was analysed in a study. They used nanohybrid composites and dentifrices. They concluded that abrasiveness of the toothpaste does not change the color of the composite resin material.(29)

Toothpaste abrasiveness and brushing time period may increase color changes of resin composites.(30) Composite specimens were subjected to mechanical brushing for 58,400 cycles in a study. They found that the longer the brushing time and toothpaste abrasiveness, the greater will be the color changes of nanocomposite resins.(31) The limitations of the study was less sample size and we did not compare the color stability of different types of composites. Further study is required to correlate the other variables such as time, temperature, ageing, PH of the staining solutions, characterisation and understanding the mechanism of discolouration.

CONCLUSION

Within the limitations of the study, it can be concluded that superior color stability was found in bulk composite resin samples subjected to brushing simulation with pepsodent toothpaste when compared to dabur toothpaste.

ACKNOWLEDGEMENT

We would like to thank our college and management for their constant support in completing this research work.

Conflict Of Interest
The author declares that there were no conflicts of interest.

Source of Funding
The funds were provided by:

- Saveetha Dental College and hospitals, Saveetha University of Medical and Technical Sciences, Saveetha University, Chennai.
- Sarkav health services Pvt Limited, Chennai.

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Table 1 : Mean. std. deviation and significance testing between groups

Groups	Mean	Std. Deviation	Significant value
Dabur	20.7702	1.23556	0.880
Pepsodent	20.7425	1.30434	

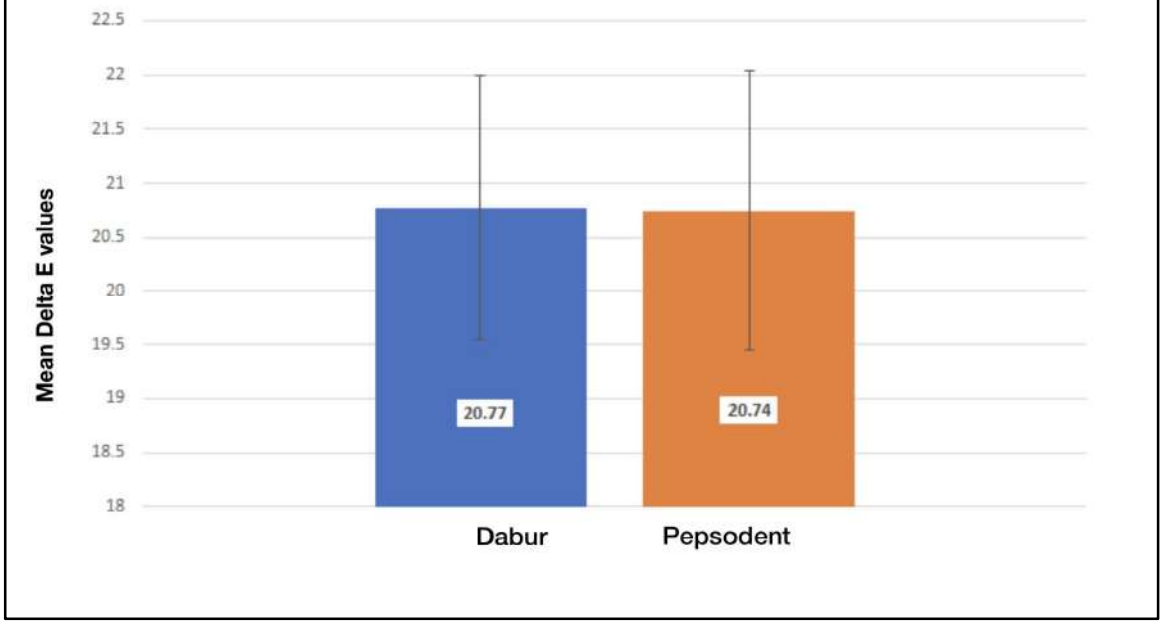

Figure 1: Bar graph shows the mean value of colour stability of bulk composite resin restorative material when brushed with two different toothpastes (Dabur and Pepsodent). X axis represents the toothpaste material, and the Y axis represents the Mean Delta E value. Blue denotes dabur and orange denotes pepsodent group. Mean delta E value was less for the pepsodent group. Hence, bulk composite resin brushed with pepsodent had better color stability. P value is 0.880(< 0.05), hence statistically not significant

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Role of Indigenous Traditional Knowledge in the Conservation of Biodiversity: A Review

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Received: 30 Dec 2021 Revised: 19 Jan 2022 Accepted: 17 Feb 2022

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ABSTRACT

Plants are preserved by the native individuals that fill in as a wellspring of wild consumable food as roots, tubers, rhizomes, seeds, products of the soil rural and plant parts. Medicinal plants play a central role, not only as traditional medicines used in many cultures, but also trade commodities which meet the demand of often distant markets. Demand for a wide variety of wild species is increasing with growth in human needs, numbers and commercial trade. With the increased realization that some wild species are being over-exploited, a number of agencies are recommending that wild species be brought into cultivation systems. Cultivation can also have conservation impacts, however, and these need to be better understood. Medicinal plant production through cultivation, for example, can reduce the extent to which wild populations are harvested, but it also may lead to environmental degradation and loss of genetic diversity as well as loss of incentives to conserve wild populations

Keywords: Biodiversity, ethnic individuals, medicinal plants, genetic diversity

INTRODUCTION

Humans mostly depend on the nature for basic needs like cloth, shelter and food in addition to also flavored items, fragrance products fertilizers. Especially before the introduction of chemical medicines or synthetic medicines, man relied on the healing properties of medicinal plants. To the globally increased population, medicinal plants play an important role in health care system of people especially in developing countries. Medicinal plants are the "backbone" of traditional medicine, on an average more than 3.3 billion people in the developing countries utilizes

medicinal plants on a regular basis. In India and China the herbal medicine has a continuous history with long usage. The development and recognition of medicinal plants, financial support for the cultivation and drug identification from these plants are need of hour in both industrialized and developing nations (WHO, 1998). Medicinal plants became the future hope for the production of safe medicine (Hamburger and Hostettmann, 1991). We have number of modern drugs, but still it is genuinely urgent to discover and develop new therapeutic agents to overcome the newly evolved diseases. It is identified that acceptable therapy is available only for one third of the known human ailments so, new drug discovery is necessary. Traditional plant medicines still enjoy significant position in the modern-day drug industries due to minor side effects as well as the synergistic action of the combination of compounds. The WHO endorses and promotes the use of herbal drugs in national health care programs because they are easily accessible within the reach of a common man and are time tested and thus considered to be much safer than the modern synthetic drugs. The phytochemical-pharmacological research work has recently yielded effective solutions to certain diseases which synthetic drug industry has failed to afford. The most important research work on *Artimisia annua, Cathranthus roseus, Taxus spp., Lantana camara* and *Baccopa spp. etc.*,. Such plants were earlier considered as poisonous or useless, but now have been found to contain molecules of high drug values and are considered as medicinal herbs of great significance.

Distribution of medicinal plants

India, with its geographic area of about 329 million hectares and meeting ground of three major bio-geographic realms, the Indo-Malayan, Eurasian and Afro tropical, is fortunately endowed with a wide spectrum of biodiversity. India has 2.4% of world's area with 8% of global bio-diversity. It is one of 12 mega diversity hot spot regions of the world. Other countries included are Brazil, Columbia, Chcina, South Africa, Mexico, Venezuela, Indonesia, Ecuador, Peru, USA and Bolivia (Scippmann, 2002). Medicinal plants were distributed in diverse habitats, in Indian subcontinent, nearly 70% of these found in tropical forests of both Eastern and Western Ghats, Chota Nagpur plateau, Aravalis, Vindhyas and the Himalayas. Among the Himalayas, Kashmir Himalayan region, settled within the Northwestern folds is recently designated as global biodiversity hotspot of the Himalayas (Mittermeier *et al.* 2005). Indian forests comprise 90% of medicinal plant diversity whereas, 10% of known medicinal plants restricted to non-forest habitats. Some of the most important medicinal plants of Kashmir, Himalayas include *Dioscorea deltoidea, Rheum Emodi, Arnebia benthamii, Inula racemosa, Datura stramonium Aconitum heterophyllum,, Artemisia spp., Podophylum hexandrum, Juniperus macropoda, Hypercum perforatum, Hyoscyamus niger, Sassurea spp*., and *Picrorhiza kurroa* etc., The important aromatic plant species include Caraway *(Carum cervi),* Saffron *(Crocus sativus),* Siya zira *(Bunium persicum),* Garlic *(Allium sativa),* Coriander *(Coriandrum sativum),* Mint *(Mentha spp.)*, Fennel *(Foeniculum vulgare)* and Hare's foot *(Trigonella foenum-graecum).* Hamilton (2003) reported that 44% flora present in India is used for medicinal purpose.
Globally 72000 plant species are used for medicinal purpose (Table l). According to International Union for Conservation (IUCN), 1500 species are medicinal plant species, 3000 species are globally traded and 900 species are cultivated (Scippmann *et al.,* 2006). 70-90% of the species are collected from the wild. 50-70% of the medicinal plant biomass is sourced from the wild collections. But 72% of the and also but making them threatened (Rao and Rajput, 2015) (Table 2).

Socio cultural aspects

There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences (Liu *et al.,* 2005). It is considered essential that technologies for the domestication are developed that adjusted to the specific social values and livelihood assets of intended groups (Weirsum, 1997). Main scope for cultivation of medicinal plants is its contribution towards poverty alleviation. The stimulation of domestication of medicinal plants should not be regarded as a process of transfer of professionally developed cultivation techniques, but rather as a process of change in institutionally embedded local resource use and management practices. In Kenya Maasai tribe heavily depend on indigenous medicine as primary healthcare (Kiringe, 2005) 90% of people rely on herbal medicine and have vast knowledge on ethano-medicinal practices to treat and mange diverse illness in both human and livestock. The existence of traditional medicine depends on plant species diversity and the related

knowledge about their use as medicine in local people. So, both plant species and traditional knowledge are important to herbal medicine trade and pharmaceutical industry.

The Ayurvedic system was practiced in Indian continent, where three humors- phlegm, bile and flatulence and hot and cold is also important. In chinese system of medicine also it is believed that the balance between the hot and cold of the body is the base of health for example when a man fainted with sun stroke, food items will be given which are cold to body like plant soups, and for instance when a mother gives birth to child, she have to eat heat given products like meat. In some cultures, there is a religious belief that, supernatural powers and spirits will affect human body and those gods or spirits were the major cause of illness (Ember and Peregrine, 2002). Plants which are medicinally important are included in Indian cultural and religious rituals eg. *Ficus religiosa*, *Azadiracta indica*, *Cyanodon dactylon*, *Artemesia sp.,Calotropis gigantea*etc.In some festivals related to Hindu religion, plants which are medicinally valuable represent god and sacredness eg. *Aegle marmelosa* (bael), *Ocimum sanctum* (tulasi) (Prakash, 2013). The special significance of socio cultural aspects is that medicinal plants conservation improves rural livelihoods, culture and economy (Hamilton, 2004).

Conservation through sacred groves

Traditional beliefs along with religious and cultural practices have played a vital role in preserving bio-diversity of several forests and have conserved several flora and fauna that come under the sacred groves. Odisha is blessed with the abundance of natural resources and it holds the strongest tradition that makes support conservation of biodiversity. All kinds of human activities are prohibited in the sacred groves. The areas of forest are dedicated to deities and their temples that are run by the tribal communities. These socio-cultural beliefs have helped to traditionally preserve these patches of unadulterated forest. According to the definition given by IUCN, Sacred forests structure is a piece of venerating nature and is considered as "Holy Natural Sites". Despite the fact that, individuals related with such hallowed forests are by and large uneducated, they have fastidiously drawn out their customary traditions, customs, functions and method of timberland life. Consecrated forests are likewise supplies of numerous conventional prescriptions. With this acknowledgement, there has been a fast advancement in the investigation of assets having a place with hallowed forests just as their preservation. These practices are for biological significance as well as for socio-social and strict meaning of the state. Keeping this in see, an endeavor has been made to visit some chosen sacrosanct forests of the state and specify the normal and endemic bio-wealth having a place with those forests.

Conservation of plants for fermented foods and beverages

Fermented foods and beverages have been used for centuries. Among food plants, beverage, alcoholic and non-alcoholic beverages occupies an important social life of tribal communities. These beverages are devoured during significant services are frequently offered to Gods and dieties. Cooling and invigorating beverages arranged from different plant species, for example, *Aegle marmelos*, *Curcuma augustifolia, Mangifera indica*, are utilized during summer as non-cocktails.

Plants conserved in abandoned sites of shifting agriculture

Shifting agriculture is one of the customary frameworks of agriculture that can be followed back from the Neolithic time frame. It is otherwise called the 'slash and burn agriculture'. Various clans possessing various spaces of the state are working on moving development. These clans are Bondo, Didayi, Koya, Gadaba, Paroja, Soura, Juang, and so on. Majority of these ancestral networks are known as Primitive Tribal Groups (PTGs). Following a time of development, the land is deserted for quite a while for recovery of soil fertility. During this period ranchers ordinarily move to other land. The tribales don't perform total felling of woods however they hold a few valuable types of green and rural significance, for example, *Mangifera indica, Citrus spps, Musa spps, Phyllanthus embilica, Zea mays, Saccharum spps*. A few valuable plants like *Ardisia polycephala, Ardisia cripsa, Casearia glomerata, Meliosma ipnata, Rhus spps, Phoenisx spps,* and so on are colonized at deserted locales (Rajiv, 2000).

Plants conserved for their therapeutic value

Medicinal plants have assumed an essential part in numerous old conventional frameworks of prescription around the world. Customarily, individuals accepted on plant based medication for their essential medical care. Utilization of plants or plant parts for different sicknesses by the rustic and ancestral individuals of Odisha is a typical practice. These locally wild plants are gathered and saved for their therapeutic worth. The delicate leaves of *Alagium salvifolium* are seared with oil and applied remotely to alleviate agony and growing of joints. Juice extricated from the leaves of *Coccinia indica* and *Leuca saspera* is applied remotely to treat skin illnesses like Scabies and skin ejections. During prescription either the entire plant or a solitary part are recommended for a specific sickness. Further, it was seen that now and again meds are endorsed in mix with other plant parts. Furthermore, numerous different substances, for example, matured rice water, milk; rock salt, etc are blended in with the plant items while planning of the medication.

Plants rationed by tribals as wild natural products, seeds, bulb, roots and tubers for consumable reason

The clans of this area are reliant up generally on wild assets for their food and other day by day needs. The area is wealthy in wild consumable plant assets. During the hour of shortage, they rely upon unusual eatable plant parts like leaves, shoots, youthful twigs, roots, rhizomes, tubers, blossoms, natural products, seeds, and so forth.

CONCLUSION

Medicinal plants occupy a vital sector of health care system in India and represent a major national resource. The dedicated medicinal plants are used by various tribal's and local people to cure different ailments ranging from simple injuries, wounds, cuts, fever, diarrhoea, ulcers, swelling, bone fractures, potency, antidote, skin care, night blindness, toothache, asthma, cough & cold. Hence, there is an immense need for conservation of diversity of medicinal plant wealth with high use value for the present and fore coming generations by adapting proper conservation measures, which are essentially required to design better conservation strategies to preserve and conserve the existing biodiversity

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Table 1. Status of useful medicinal plant species in the World

Country	Medicinal plant species	Percentage over total number of plant species
China	4,941	18.9
India	3,000	20.0
Indonesia	100	4.4
Malaysia	1,200	7.7
Nepal	700	10.0
Pakisthan	300	6.1
Phillippines	850	9.5
Sri Lanka	550	16.6
Thailand	1,800	15.5
Vietnam	1,800	17.1
USA	2,564	11.8

(Hamilton, 2003)

Table 2. Number of medicinal plant species recorded from different countries

Country or Region	No. of species of medicinal plants
China	11,146
India	7,555
Mexico	2,237
North America	2,572
Global	52,896

(Hamilton, 2003)

Table 3: List of plants conserved by tribals as home to god and goddess

S.No	LOCAL NAME	VERNACULAR NAME	SCIENTIFIC NAME	FAMILY	NAME OF GOD AND GODESS RESIDING IN PLANTS
1	Aam	Amra	*Mangifera indica*	Anacardiaceae	Lord Vidhyadhara
2	Arjun	Arjun	*Terminalia arjuna*	Combretaceae	Lord Brahma
3	Bijapura	Nibu nedica	*Citrus medica*	Rutaceae	Lord Brihaspati
4	Bilva	Belo	*Aegle marmelos*	Rutaceae	Lord Shiva
5	Basil	Tulsi	*Ocimum sanctum*	Lumiaceae	Goddess Lakshmi
6	Baka	Agasti	*Sesbania grandiflora*	Fabaceae	Lord Narayan
7	Nimba	Neem	*Azadirachta indica*	Meliaceae	Serpent King
8	Nilapadma	Kamal	*Nelumbi nucifera*	Nymphaceae	Godess Ambika
9	Karavira	Kerabi	*Nerium indicum*	Apocynaceae	Lord Ganesh
10	Sweta-padma	Madar	*Calotropis gigantean*	Asclepiadeceae	Lord Shiva

Table 4: Local names of the fermented food items

SL. NO	LOCAL NAME	DESCRIPTION
01	Pakhala	Cooked rice water. A type of fermented food.
02	Sambar	A type of legume pest based local mouth appetizer normally eaten with morning snacks.
03	Handi	Earthern pot with large mouthed pitcher.
04	Bakhar	A mixture of rice flour, yeasts, and medicinal plant parts.
05	Handia	Fermented cooked rice.
06	Karadi	Fermented bamboo shoot.
07	Mahuli	Country liquor.
08	Jantani	Semi solid mixture prepared from rice flour and jiggery.

Source: Ramesh, Manas. Indigenous Fermented Foods and Beverages of Odisha, India

Table 5: Table showing list of tribes practicing shifting cultivation in different areas of the state

S. No	Tribe	District	Area
01	Bondo	Koraput	Khairput area of Malkangiri sub-division
02	Parenga	Koraput	Puttasingi area of Koraput Sub-division
03	Kondh	Phulbani	Kandhamal Sub-division
04	Juang	Keonjhar	Telkoi, Harichandrapur area of Keonjhar Sub- division
05	Paudi Bhuyan	Sundergarh	Bonsai Sub- division

Source: Balaram Dash. Shifting Cultivation among the Tribes of Orissa

Table 6: Table showing list of plants used by the tribals for their medicinal value

S. No	LOCAL NAME	SCIENTIFIC NAME	FAMILY NAME	AILMENTS
1	Ankota	*Alangium salvifolium*	Cornaceae	Pain And swelling of joints
2	Belo	*Aegle marmelos*	Rutaceae	Diarrhea
3	Bansulee	*Thalictum minus*	Ranunculaceae	Conjuctivities
4	Apamarga	*Achyranthes aspera*	Amaranthaceae	Pneumonia
5	Poi	*Basella rubra*	Basellaceae	Constipation
6	Toria	*Brassica campestris*	Brassicaceae	Bronchitis
7	Khora	*Nepeta longibracteata*	Lamiaceae	Hyperacidity
8	Chare Jam	*Syzygium jambos*	Myrtaceae	Jaundice
9	Jada	*Ricinus communis*	Euphorbiaceae	Headache
10	Patala garuda	*Rauwolfia serpentina*	Apocynaceae	Blood pressure

Table 7: List of plants conserved by tribals for edible purpose

S.No	LOCAL NAME	SCIENTIFIC NAME	FAMILY NAME	USES
1	Bathuasaga	*Chenopodium album*	Amaranthaceae	Leaves as vegetables
2	Ban Aalu	*Dioscorea alta*	Dioscoreaceae	Tubers as vegetables
3	Kantakeera	*Amaranthus sipinosus*	Amaranthaceae	Leaves as vegetables
4	Kalama saga	*Ipomoea acquatica*	Convulvulaceae	Leaves as vegetables
5	Baelo	*Aegle marmelos*	Rutaceae	Fruits are roasted and consumed
6	Pita Alu	*Dioscorrea bulbifera*	Dioscoreaceae	Tubers are vegetables
7	Bhalia	*Semecarpus anacardium*	Anacardiaceae	Fruits as vegetables
8	Tangeni	*Xylia xylocrpa*	Mimosaceae	Seeds as vegetables
9	Mahua	*Madhuca indica*	Sapotaceae	Flowers are vegetables
10	Tetul	*Tamarindus indica*	Fabaceae	Flowers are vegetables

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Almost Block Diagonal Algorithm for Two-Point Boundary Value Problem

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

In this paper, orthogonal spline collocation method (OSCM) has been used for two-point boundary value problem. Hermite cubic basis functions are used to approximate the solution for linear and boundary value problem. We have explained the efficiency of ABD solver for two-point boundary value problem. Using ABD solver we can solve the linear system with O(N) operations.

Key words: Orthogonal cubic spline collocation methods (OSCM), Two-point boundary value problem, Hermite Cubic basis functions, and Almost block diagonal (ABD) matrix.

INTRODUCTION

Orthogonal Spline Collocation method is a Gelarkin Method that uses a block diagonal linear system to obtain the coefficients of a polynomial equation by using a block diagonal linear matrix to find the value of unknowns which could be calculated using the matrix inverse determination and by collecting the values of the coefficients from the first highest order term as the solution to the problem and then move on to the lower order term to determine the derivative coefficients and hence in turn we obtain the solutions by collecting their values.

Our equation under study is as follows:

$$y'' + k^2 q(x) y = f(x), \quad x \in [a,b] \qquad (1.1)$$
$$\alpha_a y(a) + \beta_a y'(a) = g_0, \quad \alpha_b y(b) + \beta_b y'(b) = g_1 \qquad (1.2)$$

where α_a, β_b ,α_b , β_b, g_0, g_1 are known constants and k^2 is a wave number. The coefficient $q(x)$ is assumed to be piece-wise constant or piece-wise continuous and has finite jump across the interface = x_i , where $x_i \in (a,b)$.

The orthogonal spline collocation approximation for problem (1.1) - (1.2) is defined as follows: Find an approximation $y_h \in M_1^3(I)$ such that

$\alpha_a y(a) + \beta_a y'(a) = g_a,$

$Ly_h(\xi_i) = f(\xi_i), \quad i = 1,2,\dots,2N \qquad (2.2)$

$\alpha_b y(b) + \beta_b y'(b) = g_b.$

The collocation points are $\{\xi_j\}_{j=1}^{2N}$, where

$$\xi_{2i-1} = x_{i-1} + \frac{1}{2}\left(1 - \frac{1}{\sqrt{3}}\right)h_i, \quad and \quad \xi_{2i} = x_{i-1} + \frac{1}{2}\left(1 + \frac{1}{\sqrt{3}}\right)h_i, \quad i = 1,2,\dots,N.$$

We define the functions $v_j(x), s_j(x) \in M_1^3(\pi), i,j = 0,1,\dots,N,$ by

$v_j(x_i) = \delta_{ij}, \; v_j'(x_i) = 0$ and $s_j(x_i) = 0, \; s_j'(x_i) = \delta_{ij}, \; i,j = 0,\dots,N,$

where $\delta_{ij} = 1$, if $i = j$ and $\delta_{ij} = 0$, if $i \neq j$. Then the set $\{v_i\}_{i=0}^N \cup \{s_i\}_{i=0}^N$ forms a basis for $M_1^3(\pi)$, which we order in the form $\{v_0, s_0, v_1, s_1, \dots, v_N, s_N\}$.

Since only four basis functions $v_{i-1}, s_{i-1}, v_i, s_i$ are nonzero on $[x_{i-1}, x_i]$, the coefficient matrix of the collocation equations structures out as

$$\begin{bmatrix} D_0 & & & & & \\ S_1 & T_1 & & & & \\ & S_2 & T_2 & & & \\ & & \ddots & \ddots & & \\ & & & S_N & T_N \\ & & & & D_1 \end{bmatrix}$$

where $D_0 = [\alpha_a, \beta_a]$, $D_1 = [\alpha_b, \beta_b]$, and, for $j = 1,2,\dots\dots,N,$

$$S_i = \begin{bmatrix} Lv_{i-1}(\xi_{2i-1}) & Ls_{i-1}(\xi_{2i-1}) \\ Lv_{i-1}(\xi_{2i}) & Ls_{i-1}(\xi_{2i}) \end{bmatrix}, \quad T_i = \begin{bmatrix} Lv_i(\xi_{2i-1}) & Ls_i(\xi_{2i-1}) \\ Lv_i(\xi_{2i}) & Ls_i(\xi_{2i}) \end{bmatrix}.$$

Almost Block Diagonal Algorithm

In case of a cubic base we see that the convergence is in the order of 4 but for a quartic we find the convergence order to be 5 with also simultaneous exhibition of super convergence property at the nodal points of the mesh. We can observe from the numerical experiments that the convergence rate also is determined by the step size we take in to consideration and it also becomes evident that in some cases we find too much of an overlap in the solution we have obtained numerically vs the solution that we have obtained from exact solution and hence there might be a small deviation from what we see in the trend of the same method applied to various differential equations with boundary values and interface conditions. We also see that even if we have slightly deviating values of convergence we gradually see the rate converging to the one that has been theoretically predicted. A common conclusion that can be drawn is that the variation in step size is a determining factor in the improvement of convergence of a given algorithm to a particular problem.

But however we cannot assure if the convergence rate of one problem of a particular step size could correspond to a convergence rate of another differential equation with same step size having theoretically same order because the determining factor in rate of convergence is the matrix that we build and the collocation points that come with it along with the boundary and interface conditions. However the methods of OSCM that we employ to the differential equations are not only accurate but also fast since everything involved can be carried out by simple computations in less time. However there are restrictions that come in the form of software that we use and the matrix size and the related computations associated with it. But none the less the accuracy we obtain even with the standard limitations is noteworthy for its accuracy and rapid output.

Also the numerical results we obtain are class 1 functions and this stands in support of the existence and uniqueness theorem. The OSCM that we have employed here could also be used to solve differential equations with higher order rates and higher super-convergence rates if we shift from cubic/quartic to a higher order base polynomial.And the solution and accuracy will still be valid regardless of the system of coordinates we use. And thus we can say the OSCM methods are widely preferred over the B Splines. Also one can safely observe that the time taken for the computations take longer time if we try to increase the number of sub intervals in the chosen subspace.
The OSCM is of extreme importance due to it's simplicity and wide applicability in diverse fields.

Numerical Example
Consider the problem
$u_{xx} + 6u_x + u = cosx, x \in (0,1)$

With boundary conditions
$u(0) = 1,\ u(1) = \cos(1)$

CONCLUSION

In this paper, we have applied an orthogonal cubic spline collocation methods (OCSCM) to one-dimensional fourth-order linear boundary value problems. One numerical experiment is performed and obtain the fourth order convergence atthe grid points.

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Table 1. we have shown the error in tabular form.

N	$L^{\infty}(u)$	order	$L^{\infty}(u')$	order
20	3.4197e-06		3.9003e-06	
40	4.6034e-07	6.0598e+00	4.9001e-07	6.1165e+00
60	1.2008e-08	6.0834e+00	6.0052e-08	6.0356e+00
80	5.5006e-09	6.0075e+00	1.8001e-09	6.0009e+00
160	7.8901e-10	5.9900e+00	7.0095e-10	6.0015e+00
320	9.1117e-12	5.9115e+00	2.8641e-11	5.9990e+00

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Novelty of Three Parameter Weibull Distribution

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Received: 26 Nov 2021 Revised: 03 Jan 2022 Accepted: 25 Feb 2022

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ABSTRACT

From an economic standpoint, a proper estimate of the potential wind energy at a certain location is critical since it determines the project's pricing effectiveness and aids in projecting future incomes and revenues. The two-parameter Weibull distribution is widely accepted as an adequate model and is thus widely used in the wind industry. The Weibull conformity for low wind speed is important in some circumstances when the possibility of null wind is significant. The three-parameter Weibull distribution, which takes into account the frequency of null winds, tends to better depict wind ranges with high percentages of null wind speeds and so produce better results. To evaluate the Efficiency, the parameters of two and three-parameter Weibull distributions are estimated in this article.

Keywords: Wind speed, Weibull distribution, Wind Energy, WPD algorithm, Statistical Tests, Efficiency factor, Wind turbine.

INTRODUCTION

When analysing lifetime data, the exponential, Rayleigh, linear failure rate, or Weibull distribution are frequently used. These distributions have a number of desirable features and physical interpretations that make them useful in a variety of situations. In this study, we introduce the modified Weibull distribution with three parameters, a new three-parameter distribution function. We present a statistical characterization of the three-parameter Weibull distribution as well as a mathematical technique for computing the Efficiency factor. Frechet [3] was the first to identify the Weibull distribution, and Rosin and Rammler [6] were the first to apply it, which provides a close

approximation to the probability laws for natural phenomena. Waloddi Weibull [14] later paid more attention to this method for wind energy applications, not only because of its higher flexibility but also because of its simplicity. Celik [2] did a study to predict wind energy using Weibull representative wind data rather than observed data in time-series format, and found that the estimated wind energy is highly accurate. For a case study in North Dakota country areas, Zhou et al. [17] successfully evaluated wind speed distribution models. Abbas et al. [1] used statistical analysis to establish the best-fitting distribution of wind speed in Pakistan. Two-parameter Weibull, Gamma, Lognormal, and Rayleigh distributions, as well as three-parameter Burr and Frechet distributions, were fitted to wind speed data, with parameters for each distribution determined using the maximum likelihood technique. Stathopoulos et al. [9] used the two-parameter Weibull distribution to forecast wind power both numerically and statistically. In the scientific literature, a great number of studies have been published that employ the two-parameter Weibull approach to characterise wind speed frequency distributions. The Weibull distribution is commonly used nowadays for wind energy estimation and wind region research.

The basic two-parameter Weibull function is not a universal model in theory. Furthermore, because wind patterns change due to terrain variation, no single model can be relied on to provide the best results at all locations. As a result, Takle and Brown [10] recommended several changes to improve the two-parameter Weibull function that was presented to improve modelling at low wind speeds. In several research, Tuller and Brett [11] developed a three-parameter Weibull function, which exhibited higher fitness and flexibility than the standard Weibull function. The Type I extreme value distribution and three parameter Weibull functions were advocated by Xiao et al. [15] as alternate probability density functions to the two parameter Weibull. Although all three probability density functions are acceptable for characterising the probability distribution of extreme wind speed data, statistical analyses reveal that the Type I and three-parameter Weibull functions are more relevant than the two-parameter Weibull. This is supported by evidence from Hong Kong's extraordinary wind speeds.

Arnab Sarkar et al. [8] devised a method for determining the range of wind speeds for which the Weibull model is appropriate, as well as for fitting the upper wind speed data into a suitable statistical distribution such as the Type I extreme value distribution (Gumbel) and the three parameter Weibull distribution. The technique was justified using Ahmadabad's hourly mean wind speed data. Wais [13] examined two and three parameter Weibull distributions for wind energy evaluation. The least square approach is used to approximate the parameters. In wind energy estimation, he advocated that the three parameter Weibull distribution be used instead of the two parameter Weibull distribution. He also devised some more analysis to back up his findings.

The survey of literature as presented in the above sections clearly depicts that

- A large number of studies has been published presenting the use of a variety of probability density functions to describe wind speed frequency distributions. The Weibull distribution function is comprehensively used for delineating the wind power potential at a destined site. Two parameter Weibull function is the most, widely used and accepted function in the specialized literature on wind energy.
- Initially, the two-parameter Weibull function was used due to its appeal of a wide array of applicability, flexibility and usefulness for describing the frequency of occurrence of high wind speeds. But some authors have indicated that the Weibull function should not be used in a generalized way, as it is unable to represent some wind regimes.
- A three-parameter Weibull function has also been utilized in some studies and was found to dispense improved fitness and flexibility than the classical two parameter Weibull function. So there is an opportunity for bettering the three parameter Weibull distribution in the field of wind resource assessment.
- Of all the researchers who have worked in the field of wind energy assessment, only few have used the three parameter Weibull distribution considering the value of $\varepsilon \neq 0$ while the ubiquitous rest have used the two parameter Weibull distribution to determine the wind resource assessment considering the value of $\varepsilon = 0$.
- Though three parameter Weibull distribution was proposed by Tuller and Brett in as early as the year 1984, researchers have concentrated only in the two parameter Weibull. A copiousnumber of papers has been

published on classical Weibull. Only few researchers have concentrated on the three parameter Weibull. So was my profound interest in it, for I have turned to it, to develop a mathematical model which aids in finding the capacity factor, which sequentially gives a better result in the assessment of wind resource.

- No researcher has come up with a mathematical model to estimate the Efficiency factor for wind energy assessment using three parameters until now.
- In the sections to follow, a mathematical model achieving the above requirements is proposed using three parameter Weibull distribution.
-

PROBABILITY DENSITY FUNCTION

The Weibull distribution which is a three parameter function can be expressed mathematically as

$$f(v) = \frac{k}{c-\varepsilon}\left(\frac{v-\varepsilon}{c-\varepsilon}\right)^{k-1} \exp\left[-\left(\frac{v-\varepsilon}{c-\varepsilon}\right)^{k}\right]$$

where

- v is the wind speed.
- k is the non-dimensional shape parameter.
- c is the scale parameter.
- ε is the location parameter.

The Weibull shape parameter k indicates the breadth of a distribution of wind speed. A small value of k indicates very variable winds, while constant winds are characterized by a larger k. Typical Weibull k value for most wind conditions ranges from 1 to 3. On the other hand the Weibull scale parameter c shows how 'windy' a location is, or in other words how high the annual mean speed is. These two Weibull parameters determine the wind speed for optimum performance of a Wind Energy Conversion System as well as the speed range over which it is expected to operate. The location parameter, as the name implies, locates the distribution along the wind speed axis. The location parameter ε is the minimum wind speed $v \geq \varepsilon$. The dimensions of c and ε are the same as v (m/s)[8].

ESTIMATION OF WEIBULL PARAMETERS -WPD ALGORITHM

The WPD (Wind power density) algorithm has defined a requirement to fit the Weibull distribution with measured wind speed data [5]. The WPD algorithm does not attempt to directly fit the measured frequency histogram, but rather demands that:

1. The mean power density of the fitted Weibull distribution be equal to that of the observed distribution.
2. The proportion of values above the mean observed wind speed be the same for the fitted Weibull distribution as for the observed distribution.

Let's consider the first requirement. The following equation gives the mean power density of the Weibull distribution, assuming constant air density:

$$WPD = \frac{1}{2}\rho(c-\varepsilon)^3\Gamma\left(\frac{3}{k}+1\right)$$

We can also write an equation for the mean power density of the observed wind speeds, again assuming constant air density:

$$WPD = \frac{1}{2N}\rho\sum_{i=1}^{N}(v_i-\varepsilon)^3$$

Requirement number 1 says that these must equate, so we can write:

$$(c-\varepsilon)^3\Gamma\left(\frac{3}{k}+1\right) = \frac{1}{N}\sum_{i=1}^{N}(v_i-\varepsilon)^3$$

Solving this for c gives:

$$c = \sqrt[3]{\frac{\sum_{i=1}^{N}(v_i - \varepsilon)^3}{N\,\Gamma\left(\frac{3}{k}+1\right)}} + \varepsilon$$

Now for requirement number 2, we begin by defining a symbol *X* to represent the proportion of the observed wind speed that exceeds the mean observed wind speed. The cumulative distribution function F(*v*) gives the proportion of values that are less than *U*, so 1-F(*v*) is the proportion of values that exceed *V*. Requirement number 2 states that:

$$1 - F\left(\bar{v}\right) = X$$

The following equation gives the cumulative distribution function of the Weibull distribution

$$F(v) = 1 - \exp\left[-\left(\frac{v-\varepsilon}{c-\varepsilon}\right)^k\right]$$

since the equation holds for any wind speed v, it holds for the mean wind speed. So we can substitute and rearrange to get :

$$1 - F\left(\bar{v}\right) = \exp\left[-\left(\frac{\bar{v}-\varepsilon}{c-\varepsilon}\right)^k\right]$$

so we can write:

$$\exp\left[-\left(\frac{\bar{v}-\varepsilon}{c-\varepsilon}\right)^k\right] = X$$

Taking logarithm of both sides gives:

$$\left(\frac{\bar{v}-\varepsilon}{c-\varepsilon}\right)^k = -\ln X$$

now we can substitute the value of c to get an equation whose only unknown is k:

$$\left[\frac{\bar{v}-\varepsilon}{\sqrt[3]{\frac{\sum_{i=1}^{N}(v_i-\varepsilon)^3}{N\,\Gamma\left(\frac{3}{k}+1\right)}}}\right]^k = -\ln X$$

when performing this algorithm to fit the Weibull distribution, first calculate X. Then solve the above equation iteratively to find the k parameter. Then calculate the parameter c using k.

STATISTICAL TESTS

The performance of estimation methods is to be investigated with the aid of statistical tests since a proper statistical analysis of wind data is a very important step in performing a wind resource assessment [5]

EXTRAPOLATION

Before the installation of a wind turbine, it is essential to estimate energy output at various heights in order to assess the economic growth of the project. The Weibull parameters are estimated to the wind turbine height by the following expression [15].

$$k_h = \frac{k_{10}}{1 - 0.0881 \ln\left(\frac{z_h}{z_{ref}}\right)} \quad and \quad c_h = c_{10}\left(\frac{z_h}{z_{ref}}\right)^{\alpha}$$

where

- k_{10} and c_{10} are the shape factor and scale factor at a height of 10m.
- k_h and c_h are shape and scale factors at desired heights.
- The wind shear exponent α is given by $\alpha = 0.37 - 0.0881 \ \ln(c_{10})$

POWER DISTRIBUTION

Wind energy is currently making a significant contribution to India's electric power generation. India is becoming one of the world's leading countries in terms of wind energy development and deployment. India will achieve tremendous potential in the future, according to the rise of wind energy. Wind is a natural resource that can be harnessed by mechanically transforming wind power into electricity with the use of wind turbines. Wind energy is an indirect type of solar energy. The Weibull Distribution is discovered to be more suited than the other distribution in the literature review. The power density can be calculated using this best-fit probability distribution [4]. According to Chang [12], the parameters of the Weibull probability distribution function characterise wind speed for a proposed wind project site. Three characteristics, namely the cut-in, rated, and cut-off speeds, can be used to define the turbine's power-performance curve. The cut-in speed is the speed at which the turbine initially begins to revolve and generate power. The rated output wind speed is the wind speed at which it hits the limit. The cut-off speed is the wind speed at which the wind turbine shuts down. Assume $V \sim Weibull(k, c, \varepsilon)$.. The wind speed of a wind turbine producing power P can be calculated as follows:

$$V_{turbine} = \begin{cases} 0 & V_{turbine} < v_{cut-in} \\ V & v_{cut-in} \le V \le v_{rated} \\ v_{rated} & v_{rated} < V < v_{cut-off} \\ 0 & V \ge v_{cut-off} \end{cases}$$

where v_{cut-in} , v_{rated} & $v_{cut-off}$ are specified by the manufacturer.

We define $P_{turbine}$ as

$$P_{turbine}=\begin{cases}0 & V_{turbine}<v_{cut-in}\\ \frac{1}{2}\rho AC_P\eta V^3 & v_{cut-in}\le V\le v_{rated}\\ \frac{1}{2}\rho AC_P\eta v_{rated}^3 & v_{rated}<V<v_{cut-off}\\ 0 & V\ge v_{cut-off}\end{cases}$$

It is evident that $V_{turbine}$ and $P_{turbine}$ are discontinuous random variables, but we can still compute their distribution functions and moments.

Also $P_{turbine}$ has Cumulative distribution function

$$F_{P_{turbine}}(v)=\begin{cases}0 & -\infty<v<0\\ 1+\exp\left(-\left(\frac{v_{cut-off}-\varepsilon}{c-\varepsilon}\right)^k\right)-\exp\left(-\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right) & 0\le v\le\frac{1}{2}\rho AC_P\eta v_{cut-in}^3\\ 1-\exp\left(-\frac{1}{(c-\varepsilon)^k}\left(\frac{2v}{\rho AC_P\eta}\right)^{\frac{k}{3}}\right)+\exp\left(-\left(\frac{v_{cut-off}-\varepsilon}{c-\varepsilon}\right)^k\right) & \frac{1}{2}\rho AC_P\eta v_{cut-in}^3\le v\le\frac{1}{2}\rho AC_P\eta v_{rated}^3\\ 1 & \frac{1}{2}\rho AC_P\eta v_{rated}^3\le v<\infty\end{cases}$$

Also $P_{turbine}$ has mean,

$$\begin{aligned}E[P_{turbine}]=\left(\frac{1}{2}\rho AC_P\eta\right)\Bigg[&(c-\varepsilon)^3\left(\Gamma\left(1+\frac{3}{k},\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)-\Gamma\left(1+\frac{3}{k},\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\\&+3\varepsilon(c-\varepsilon)^2\left(\Gamma\left(1+\frac{2}{k},\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)-\Gamma\left(1+\frac{2}{k},\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\\&+3\varepsilon^2(c-\varepsilon)\left(\Gamma\left(1+\frac{1}{k},\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)-\Gamma\left(1+\frac{1}{k},\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\\&+\varepsilon^3\left(\exp\left(-\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)-\exp\left(-\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\\&+v_{rated}^3\left(\exp\left(-\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)-\exp\left(-\left(\frac{v_{cut-off}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\Bigg]\end{aligned}$$

The expected power output of a wind turbine can be found from the above equation if we know the rotor swept area of the turbine. But we are in need to find the mathematical model, which is independent of turbine parameters. This is the prerequisite for the installation of a turbine, where Efficiency factor comes into play. This factor estimation does not require the involvement of any of the technical components of the wind turbine. Efficiency factor is defined as “The ratio of the expected output power to the rated power of a wind turbine, over a period of time” [7].

$$Efficiency\quad Factor=\frac{E[P_{turbine}]}{\frac{1}{2}\rho AC_P\eta v_{rated}^3}$$

$$Efficiency\quad Facor = \left(\frac{1}{v_{rated}}\right)^3 \left\{ (c-\varepsilon)^3 \left(\Gamma\left(1+\frac{3}{k}, \left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right) - \Gamma\left(1+\frac{3}{k}, \left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\right.$$
$$+ 3\varepsilon(c-\varepsilon)^2 \left(\Gamma\left(1+\frac{2}{k}, \left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right) - \Gamma\left(1+\frac{2}{k}, \left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)$$
$$+ 3\varepsilon^2(c-\varepsilon) \left(\Gamma\left(1+\frac{1}{k}, \left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right) - \Gamma\left(1+\frac{1}{k}, \left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)$$
$$\left. + \varepsilon^3 \left(\exp\left(-\left(\frac{v_{cut-in}-\varepsilon}{c-\varepsilon}\right)^k\right) - \exp\left(-\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right)\right)\right\}$$
$$+ \left[\exp\left(-\left(\frac{v_{rated}-\varepsilon}{c-\varepsilon}\right)^k\right) - \exp\left(-\left(\frac{v_{cut-off}-\varepsilon}{c-\varepsilon}\right)^k\right)\right]$$

The proposed model is based on a probability density function of the output power generated by the wind turbine. In order to validate the model, the value obtained by the proposed model was compared with the results of the two parameter Weibull. This model has the tactical advantage that, it can easily be implemented in a variety of computer programs and that it requires a computing time, which is considerably less, than in the case of simulation or numerical methods.

Data source

Selection of wind site depends on many factors like wind resource, wind regime, geographical location, topography, forest or private land, accessibility, nearness to grid, etc. The first step in every wind energy project is the identification of suitable sites and prediction of the economic viability of the wind project.With data provided by the Ministry of New and Renewable Energy, the government of Tamil Nadu started a wind power plant at Viralimalai on 20/12/2000 which functioned up to 25/03/2002. The value of the average wind speed, they measured was nearly 3.27 m/s with mean wind power as 45 W/m^2 at 20m and as 65 W/m^2 at 50m heights. It was declared that the power gained would not be sufficient and hence, the calculation of wind power was dropped at Viralimalai station. In this study, mean wind speed data for Viralimalai station, Pudukottai district with mast height of 10m are collected from the meteorological society for the year 2017 with mean average wind speed 5.7130 m/s.

RESULTS AND DISCUSSION

The recorded wind speed data at Viralimalai station are fitted to the two and three parameter Weibull distributions. The parameters involved in the distributions are estimated by WPD algorithm, and the validity of the methods were verified using statistical Tests. The estimated values of Weibull parameters statistical tests are listed from Table 3.

The k parameter of the Weibull shape denotes the breadth of a wind speed distribution. Wind speeds tend to vary over a wide range of speeds with lower k values, whereas wind speeds tend to stay within a limited range with larger k values. The k values in our investigation ranged from 2.1071 to 2.6342. For most wind situations, typical Weibull k values range from 1 to 3. The Weibull scale parameter c, on the other hand, indicates how 'windy' a location is, or how high the yearly mean wind speed is. The mean wind speed of Viralimalai station has c values ranging from 4.9261 to 6.0231, according to our study. The wind speed for optimum performance of a wind energy conversion system, as well as the speed range across which it is intended to function, are determined by these two Weibull parameters. Low RMSE values suggest successful forecasts, while higher values indicate deviations. The output value of R^2 for the most accurate parameter estimation is the highest. Because the RMSE should be as small as

feasible, the three-parameter Weibull distribution provides the best fit for the Viralimalai station. The three parameter Weibull probability density curve is closer to the histogram of the wind speed data, as seen in Fig.1. However, in Fig. 2, the probability density curve of the Two parameter Weibull distribution is somewhat deviated to the left of the histogram, confirming that the Three parameter Weibull distribution performs better than the Two parameter Weibull distribution. This is due to differences in the k and c values. The results demonstrate that the three approaches provided are effective in evaluating the parameters of the Weibull distribution for the data supplied. For the two-parameter Weibull distribution model, however, the maximum likelihood method is the most accurate. Because of the characteristics, wind speed and wind power density rise as altitude increases in both models. In addition, the three-parameter Weibull distribution produces a greater wind power density value and better performance.These calculations are of utmost importance at the time of installation of turbines [9]. For a given wind regime, with known k and c parameters, we are free to select the values of v_{cut-in} , $v_{rated}$and $v_{cut-off}$ that maximizes the expected output power, and thereby can maximize the efficiency factor. Considering the typical Weibull PDF, the efficiency factor of Viralimalai station for the year 2017 is estimated by the parameters which are reported in Tables 7. The resultant capacity factor is substantially larger when the three parameter Weibull distribution is used instead of the standard two parameter Weibull distribution, as seen in Table 6. In the two parameter Weibull distribution function, the fact that the likelihood of occurrence of wind speed is overestimated is taken into account. As a result, the government underestimated the Viralimalai station's capacity factor and ceased wind monitoring there from 2000 to 2002. This attained 27 percent is the wind turbine's efficiency, regardless of altitude. As a result, extrapolation for this factor is pointless [16]. The wind speed frequency distribution in that location was studied using a mathematical formulation based on the three-parameter Weibull probability distribution function. The model established here can be used in other areas where the wind speed distribution is represented by the Weibull probability density function. Because the two-parameter Weibull wind speed distribution is not very accurate for particular wind regimes, it should not be generalized. According to the examination of wind data, the capacity factor for wind power plants to be erected in Viralimalai must be computed using the three parameter Weibull rather than the normal two parameter Weibull. Otherwise, the risk of the efficiency factor being underestimated is high.

CONCLUSION

In Tamil Nadu, a number of project developers and public institutions are conducting research in order to promote and negotiate wind generating projects. Manufacturers of wind turbines employ a simple calculation to measure the wind power density, with anemometers mounted at a height of 10 metres determining the average wind speed. I would like to depart from this process because I am aware that a mathematical model is required for correct wind power density calculation. The data from the Viralimalai wind monitoring station in India was used to validate a model devised in this study, which has luckily come to fruition. The results show that, in contrast to the two-parameter Weibull distribution, the three-parameter Weibull distribution produces superior results for the maximum likelihood of the null wind, and that it can be offered as an alternate wind energy estimating technique. Additional research backs up this claim. This method enables the designer to select the best location for a wind turbine, taking into account factors such as maximum power generation and minimum wake effect. The location is appropriate for the installation of modest wind turbines. The statistical approach to determine the efficiency factor for wind power plants to be installed in Viralimalai must be computed using the three parameter Weibull probability density function instead of the two parameter Weibull probability density function, according to the study of wind data. Otherwise, the efficiency factor will be underestimated, resulting in an overestimation of the manufacturing cost. Depending on the average and maximum wind speeds at a given site, turbine makers fix the factors and build turbines with a specific efficiency factor. Overestimating or underestimating the efficiency factor can result in significant financial losses for both manufacturers and landowners. The goal of this model was to show that the Viralimalai station, which had been declared unfit for wind energy harvesting, had been greatly underestimated in terms of its ability to generate wind energy, and that if and when a Wind Energy Conversion System is used in that

region, it will almost certainly come to fruition. The same is true for all locations and Wind Energy Conversion Systems all around the world. Despite the fact that the Viralimalai station served as the nodal point for this model's development, the model may be used to any and all wind energy conversion systems everywhere in the world.

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Table 1 Statistical Tests

Root mean square error	$RMSE=\left[\frac{1}{N}\sum_{i=1}^{N}(F_i-F_w)\right]^{1/2}$
R^2 Test	$R^2=\frac{\sum_{i=1}^{N}(F_i-\bar{v})^2-\sum_{i=1}^{N}(F_i-F_w)^2}{\sum_{i=1}^{N}(F_i-\bar{v})^2}$
Here • F_i is the observed cumulative probabilities. • F_w is the Weibull cumulative probabilities. • N is the number of observations.	

Table 2 Monthly mean wind speed data

Year	2017				
Month	$\bar{v}$(m/s)	σ	Month	$\bar{v}$(m/s)	σ
January	2.2857	1.6660	July	7.7097	2.1556
February	1.5417	1.2954	August	8.4839	2.332
March	5.1613	2.3046	September	5.4138	1.8999
April	5.8667	2.8405	October	4.1333	3.7810
May	9.3226	2.2338	November	3.8095	2.2642
June	10.5667	2.8333	December	4.2608	2.6665
Mean wind	5.7130				

Table-3 Estimated values of Weibull parameters and Statistical Tests

Distributions	Estimation method-WPD algorithm			Statistical Tests	
	k	c	$\mathcal{E}$	RMSE	R^2
Two parameter Weibull distribution	2.1071	4.9261	-	0.9989	0.0012
Three parameter Weibull distribution	2.6342	6.0231	0.2777	0.9162	0.0371

Table 4 Extrapolated k,c and Wind power density(WPD)

Height (m)	Two parameter Weibull distribution				Three parameter Weibull distribution				
	k	c (m/s)	Mean wind speed (m/s)	WPD (W/m^2)	k	c (m/s)	$\mathcal{E}$	Mean wind speed (m/s)	WPD (W/m^2)
10	2.1071	4.9261	5.7130	103	2.6342	6.0231	0.2777	5.7130	167
20	2.3214	4.2314	5.8519	174	2.6895	7.1977	0.2777	6.2871	198
30	2.4753	5.7416	6.1774	211	2.7165	7.6805	0.2777	6.8260	259
40	2.6002	6.2106	6.9161	245	2.7805	8.0660	0.2777	7.4256	311
50	2.6991	6.9984	7.0257	284	2.8327	8.6554	0.2777	7.8602	387

Table 5 The cut-in, rated and cut-off speed

Power curve values	Wind speed(m/s)
v_{cut-in}	2
v_{rated}	5
$v_{cut-off}$	20

Table 6. Efficiency factor value for two and three parameter Weibull distributions

PDF	Capacity factor
Two parameter Weibull	19%
Three parameter Weibull	27%

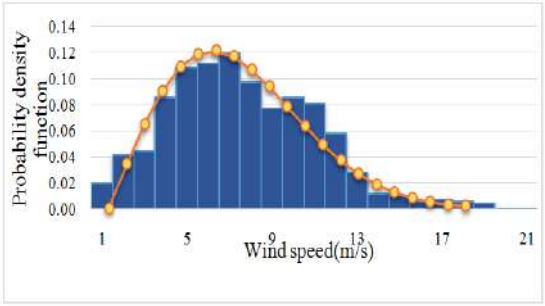

Fig.1 Two parameter Weibull probability density function curve by moment method and histogram of observed wind speed

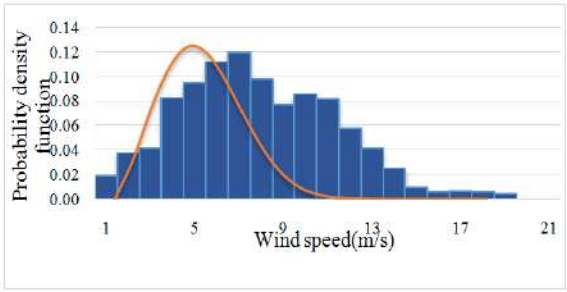

Fig.2. Three parameter Weibull probability density function curve by moment method and histogram of observed wind speed

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Evaluation of Surface Roughness of Composite Resin Material Polished With Two Commercially Available Polishing Kits

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Received: 10 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Introduction: In the present world of esthetic dentistry, good finishing and polishing plays an important role in determining the quality of composite restorations. Smooth surface of the composite restoration ensures the clinical durability, good esthetic appearance with natural enamel tissue and at same time it also prevents the discoloration and staining of the restoration. The aim of the study is to evaluate surface roughness of composite resin material polished with two commercially available polishing kits. Composite discs were divided in two different sampling groups in which each group contained 5 samples. These samples were polished using two different polishing kits which were a shofu super snap mini kit and a shofu composite finishing kit. After polishing the samples with 2 different types of polishing kit the surface roughness is checked by using the device named stylus profilometer (SURFTEST SJ-310). The values of Ra, Rq and Rz were obtained for both polished composite resin samples. All the data were tabulated and comparison of surface roughness was done using the SPSS software 23. Independent T test done, in which the mean value of Ra of both polishing kits is 0.005, Rq value of both polishing kits is 0.007 and then mean of Rz of the shofu super snap mini kit is 0.0664 and mean for the shofu composite finishing kit is 0.0672. The significant value for the Rz was 0.117. Hence statistically not

significant. Composite resin material polished with shofu super snap mini kit and shofu composite finishing kit did not show much deviation in surface roughness values.

Keywords: composite restoration, composite polishing kit, surface roughness, stylus profilometer, innovative measurement

INTRODUCTION

In the present world of esthetic dentistry, good finishing and polishing plays an important role in determining the quality of composite restorations. The main benefits of the finishing and polishing of the restoration includes minimal irritation in hard and soft tissue natural tooth surface aesthetics, helps to decrease the potential for corrosion and it has the least chance of trapping food debris (1). Goal of finishing and polishing restoration is to obtain desired anatomy of the tooth, proper occlusion of the tooth, it decreases the roughness and scratches and at same time the surface should be tolerated by the oral tissue with the resistance for the bacterial adhesion and excessive plaque accumulation (2). During composite restoration, the finishing is followed by the polishing procedure (3). Finishing is done during the composite restoration to remove the excess and for contouring the tooth surface (4). If once the process of finishing the gets over the procedure gets continued with the polishing, polishing is very much helpful to give the glossy appearance to the tooth surface (5). The irregularities will be found if the finishing is not done correctly, this can lead to the clinical related problems such as secondary caries, gingival irritation and accumulation of plaque in the teeth (6). The esthetics and longevity of the composite restoration depends on the high quality of finishing and polishing of the restoration (7). The appearance of the composite restoration depends on its finishing methods and polishing techniques (8). Surface roughness is micromorphology created by various physical processes used for surface modification. Surface roughness of the restoration depends on its mechanical properties of the composite and the flexibility of the packing materials (9). Most of the studies were based on the different types of polishing systems with multiple number steps which gave the smoothest surface finishing (10).

Profilometers provide two-dimensional information, also the surface roughness can be measured by using nanoscale by qualitative methods, such as scanning electron microscopy (11). In the recent atomic force microscopy has been largely used in dentistry to study surface characteristics of different resin composite materials (12). There is no previous research carried out on comparative evaluation of the quality of polished surfaces of composite restoration with two commercially available polishing kits. The aim of the study is to evaluate surface roughness of composite resin material polished with two commercially available polishing kits.

MATERIALS AND METHODS

This in-vitro study was done at White lab, material research centre at Saveetha dental college, Chennai. Sample preparation was done by using silicon mold with the standardized diameter of 10mm and thickness of 2mm width. A total number of 10 normal composite discs were made using a silicone mold. Composite discs were divided in two different sampling groups in which each group contained 5 samples. These samples were polished using two different polishing kits which were a shofu super snap mini kit and a shofu composite finishing kit. After polishing the samples with 2 different types of polishing kit the surface roughness was checked by using the device named stylus profilometer (SURFTEST SJ-310). Stylus profilometer consists of 2□ and 60 degree angulated tip. (Figure 1) The values of Ra, Rq and Rz were obtained for both polished composite resin samples. All the data were tabulated and comparison of surface roughness was done using the SPSS software 23.

RESULTS

Surface roughness values of 5 samples of composite discs polished with shofu super snap mini kit is given in Table 1. Surface roughness values of 5 samples of Composite discs polished with shofu composite finishing kit is given in Table 2. Mean value of surface roughness for both the polishing kits is shown in Table 3, independent T test done, in which the mean value of Ra of both polishing kits is 0.005, Rq value of both polishing kits is 0.007 and then mean of Rz of the shofu super snap mini kit is 0.0664 and mean for the shofu composite finishing kit is 0.0672. The significant value for the Rz value was 0.117. Hence statistically not significant.

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications(13–22),(23–26),(27–31),(32). In the field of dentistry, finishing and polishing procedures plays an important role in the clinical steps for composite restoration which helps to restore the anatomical and morphological forms of the tooth after restoration. Smooth surface of the composite restoration ensures the clinical durability, good esthetic appearance with natural enamel tissue and at same time it also prevents the discoloration and staining of the restoration(33). Polyester strip promotes greater smooth surface for the composite restoration, but when it comes under clinical practice the restorations require final contouring, which is more helpful to removal of excess material for the final finishing and polishing (34). In the present world of dentistry there are many dental finishing and polishing kits for obtaining good surface quality for composite restoration. In this study two polishing systems were used with multiple steps: Super Snap mini kit (Shofu, Inc, Japan)and composite finishing kit (Shofu, Inc, Japan) were used for standardizing the uniform method of material used for polishing and finishing composite restorative surfaces. In another study, surface roughness of different tooth-colored restorative materials such as conventional glass–ionomer cement, compomer, microhybrid and nanofill composite was evaluated. In total, 112 specimens were made and after the light curing and setting cycle, seven specimens from each group which received no polishing treatment were used as controls. Specimens were randomly polished with Sof-Lex disks, Poli-pro disks, and the HilusterPlus systems for 30 seconds. The smoothest surfaces of all materials were obtained with the Mylar strip. They stated that effectiveness of a polishing system on the surface roughness depends on both the polishing system and restorative material (34). Surface roughness of 126 specimens of nano-composites (Filtek Supreme XT, Ceram-X, and Grandio) was analysed in a study. The specimens were randomly polished using PoGo and Sof-Lex systems for 30 seconds after being wet-ground with 1200-grit silicon carbide paper (35). According to them, the current one-step polishing system appears to be as effective as multi-step systems and may be preferable for polishing resin composite restorations.

In another vitro study, the effects of five different two-step diamond impregnated polishing systems (Sof-Lex Spiral, Venus Supra, Komet Spiral, CompoMaster and Shapeguard) on the surface roughness and morphology of a submicron hybrid composite resin material was analysed. It was found that Diatech Shapeguard and Komet Spiral polishing systems produced the lowest surface roughness values. They concluded that similarly designed polishing systems do not produce comparable surface roughness levels (36). The limitations of the current study is small sample size and only 2 brands of polishing kits were used. In future, testing of surface roughness for different type composites and multiple polishing systems can be done.

CONCLUSION

Within the limits of the study, composite resin material polished with shofu super snap mini kit and shofu composite finishing kit did not show much deviation in surface roughness values.

ACKNOWLEDGEMENT

We thank Saveetha Dental College and Hospitals for providing us the support to conduct the study.

Conflict Of Interest

The author declares that there was no conflict of interest in the present study.

Source of Funding

The present study was supported by the following:

- Saveetha Dental College and hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai
- Prompt paper products private LTD

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Table 1 : Surface Roughness Values Of Composite Resin Polished With Shofu Super Snap Mini Kit

	Shofu Super snap kit		
Sample size	Ra	Rq	Rz
1	0.005	0.007	0.057
2	0.005	0.007	0.067
3	0.005	0.007	0.064
4	0.005	0.007	0.071
5	0.005	0.007	0.073

Table 2: Surface Roughness Values of Composite Resin Polished With Shofu Composite Finishing Kit

	Shofu composite finishing kit		
Sample size	Ra	Rq	Rz
1	0.005	0.007	0.066
2	0.005	0.007	0.065
3	0.005	0.007	0.071
4	0.005	0.007	0.068
5	0.005	0.007	0.066

Table 3: mean, std. Deviation and significance testing between groups

Parameter	Group	Mean	Std. Deviation	Significant value
Ra	Shofu super snap	0.0050	.00000	
	Shofu composite polishing kit	0.0050	.00000	-
Rq	Shofu super snap	0.0070	.00000	
	Shofu composite polishing kit	0.0070	.00000	-
Rz	Shofu super snap	0.0664	0.0063	
	Shofu composite polishing kit	0.0672	0.0023	0.117

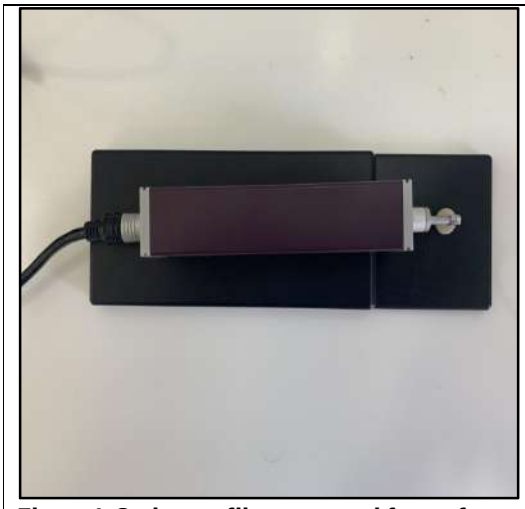

Figure 1: Stylus profilometer used for surface roughness analysis

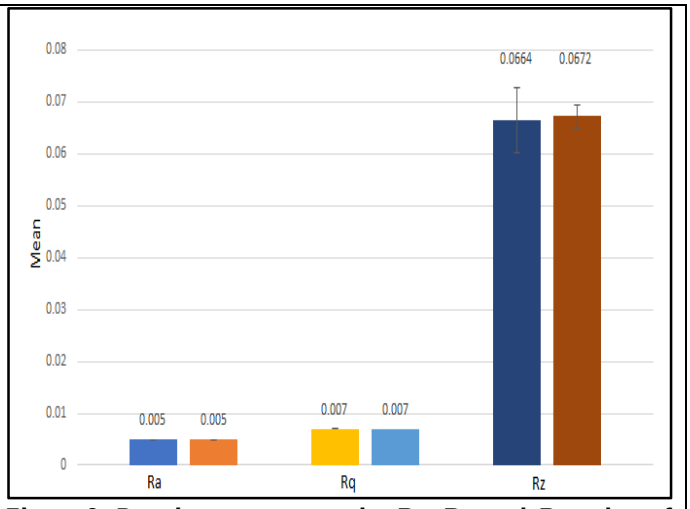

Figure 2: Bar chart represents the Ra, Rq and Rz value of composite resin samples polished with two different polishing kits (First polishing kit - shofu super snap mini kit, Second polishing kit - shofu composite finishing kit). X axis depicts composite samples and the Y axis depicts the mean values. Blue and orange denote the Ra mean difference value of shofu super snap mini kit and shofu composite finishing kit respectively, yellow and sky blue denote the Rq mean difference value, dark blue and brown denote the mean difference value of Rz. Only Rz value was slightly increased in shofu composite finishing kit.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Gesture Recognition Based Interactive Air Canvas System using Open CV

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Received: 12 Jan 2022 Revised: 14 Feb 2022 Accepted: 15 Mar 2022

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ABSTRACT

One of the oldest fascinations of humankind has always been the desire to write in air and in the recent years, this has turned into a competitive research field. Existence of such a system can ameliorate the user-system interface in multiple applications. It can contribute immensely to the advancement of automation process. The current research work mainly focuses on the methodologies to improve the recognition accuracy and cut- down the overall processing time. One of the crucial tasks present in the area of Computer Vision is Object tracking. Due to the evolving innovations, presence of advanced computers as well as accessibility to economical yet better quality video cameras, object tracking techniques has become one of the most sought-aftertasks. This paper represents a virtual air canvas which lets the userto write, draw as well as doodle in the air within the viable range of web camera. The goal of this paper is attained through two major functionalities: The first one deals with detection of the writing tool by the web camera. In this case, the writing tool is a fingertip wrapped with a colored paper. The second functionality is to trace the motion of this writing tool for every frame. It is accomplished with the help of computer vision libraries such as OpenCV. The extended version of the project can include OCR and web based interface that can allow multiple users to access this application.

Keywords: Gesture control, object detection, computer vision, motion tracking

INTRODUCTION

In this continuously evolving world, digitalization is an inevitable process. All the conventional, old fashioned methods are now being replaced by digitalized versions. Eventhings as simple as a normal book are being digitalized as e- books. Initially we were writing books with a paper and a quillthen we evolved by printing books, now we have evolved to digital pdfs. Writing by just using our fingers in plain area without any board is a beguiling challenge that is now feasiblewith the new evolving technologies in the field of ComputerScience. Computer vision is a branch of Artificial intelligence which allows the computers to acquire information from forms of digital visual inputs like photos and videos [1]. It isa branch of CS, which tries to train computer to visualize objects in fastest way possible with the help of optimized algorithms. The libraries and programming language that can help us achieve writing in air are open CV, numpy and python [2]. Open CV is a library that contains functions that helps in computer visualization related tasks. It comprises of various algorithms that help in detecting the object and recognizing the objects. Other tasks include face detection, objection tracking and landmark detection [3]. Numpy is a library that used for efficient mathematical computations [4]. Python is the most trending, beginner friendly, interpreted programminglanguage. The remainder of this paper is divided into the following categories: The following section is a review of other research articlesto which we referred prior to starting our work. After that, theworkflow of our method is described in the next section. Ourproject's outcomes are described in the Results and Discussions section. The section under "Challenges Faced" discusses the technical glitches we encountered while attempting to execute the project. Our research paper is summarized in the conclusion section.

RELATED WORK

The authors in [5] introduce the notion of the augmented reality canvas for information visualization. They are using embedded visualization to achieve human like thinking by computers. The paper title AR-CANVAS is an abbreviation of a bunch of words like Context-data, Artifact, Navigator, Visualization, Activity, and Scene. Accordingly they implemented their methods to achieve above five terms and writing in air in turn. The paper summarizes several possibilities to design air canvas. In [6], the authors proposed a system that can be used as a virtual white board to draw or write. They use fingers as objects. They track the movement of each finger after detecting their presence using python libraries. They then usemasks and do other morphological CV tasks to track the finger movement accurately. Finally a User Interface is madeto make the application more user friendly. This could end upas most effective and fun way to write or draw virtually.

The authors in [7] develop an air canvas by following three main tasks: first of all, they try to detect the writing tool. Then, they track its motion in front of the camera and finallyexamine overall actions of this writing tool. They have discussed about the challenges they faced during the processsuch as: fingertip detection, motion if drawing mode and selection mode and controlling the real-time system. They have also mentioned some major societal problems that can be solved using this virtual canvas such as: People hearing impairment, Overuse of Smartphones and Paper wastage. The concept of a virtual air canvas where a user can draw, write or doodle in air in the viable range of a phone camera isproposed in [8]. They accomplished this goal by dividing theentire process into two major steps: firstly, they try to trace the writing tool in front of the mobile camera. Then, for the system to understand the scribbled characters, they haveadded the functionalities of OCR on the formed pictures. Theapplication is developed using Open Cv with JAVA language. The resultant system is such that the user won't require any external help of materials such as keypad, gloves and markerin order to give input. Hence, the interface is very userfriendly. No sensor or hardware is needed at all, just the camera and the colored tape is enough. The suggested systemis straight-forward, brisk and easy to implement. The limitation of this system is that the existence of a same colordisturbance in the background can lead to false results.

In [9] the authors have explained that air-writing is a formof input that helps a person to write or sketch in free space using their fingertips. Air-writing, like motion gestures, is recorded as a regular stream of information including a seriesof hand and finger motions. A few of the main concepts usedhere are Slope orientation, Open CV and Leap

remote control. The concept of slope orientation is used here where a leap motion is used to capture the trajectory of the motion and plot a continuous set of points. From these points, lines are drawn and the major slopes are identified. Open CV is used to detect and trace the movement of the marker on the camera. The limitations of this project are that each character may have up to four different slope orientation sequences, due to different writing styles of the users. Since, this sensor is highly sensitive to movement of the marker on the screen, straight lines are often difficult to draw. Also, certain characters tend to be identified as the same due to similar slope orientation sequence. For ex: U and V, D and P, and also the letter O and number 0.

The authors in [10] propose a project named as Air Script, whose accuracy when compared to other similar projects was very high with a 96.7% accuracy. This project is considered to be different from other similar ones due to its high interactivity with the user, and also since it can detect all characters drawn on the screen with high precision. It is also said to provide high freedom of movement to the user while drawing on the screen. The creators of Air Script have made it convenient to be used in highly sophisticated environments such as smart factories, classrooms and even laboratories. The user can write anything in the air and it can successfully be converted into documents with desired handwriting which makes it unique.

METHODOLOGY

This paper achieves the basic functionalities of an air canvas through the following states: Drawing State - Here, the system will detect the presence of any color paper wrapped fingertip. In case if it is present, the system will track its path and will store its coordinates. A bullish crossover occurs when the shorter Colour Selection State – By using this functionality, the user can change the colour of the text among the various available colours. This functionality is possible for the colours mentioned with their HSV (hue-saturation values) in the back-end
Clear All Option – This functionality is used, when the user wants to clear the characters drawn and start afresh.

IMPLEMENTATION

The implementation involves three steps namely

1. Detection: In this system, a coloured paper attached to the fingertip (as shown) is used for the purpose of detection. This method is preferred over the fingertip alone to make it more recognizable and dominant.
2. Tracing: The concept of computer vision is used to access the web-camera of the device and to trace the movement of the coloured paper tip on the vicinity of the screen, to draw characters as well as choose different functionalities available. This can be viewed on the tracking window clearly.
3. Perception: The final characters drawn by the user will be displayed on the paint window. The Steps Involved are:

- The individual frames are read, and is converted at every instant to HSV colour space, which enables easy colour detection.
- A few colours HSV values such as red, green, blue and yellow are mentioned in the backend to make the process of colour detection easier.
- The desired colour for the purpose of drawing can be achieved by using coloured paper, or selecting from the options given on the screen.
- All the frames captured continuously via the web camera are stored in an array
- Various morphological operations such as edge detection and enhancement, smoothening filters, colour detection and normalization are applied to get clear output

The frames captured are displayed to the user through the tracking window and paint window simultaneously

RESULTS

The output is obtained for the working code is shown in Fig 2. There are various options on the window to choose thecolours as required as well as to clear the writing and start afresh. Also, due to the various edge enhancement and otheroperations used, the writing is visible clearly on the screen without any disturbances.

For the output, there are 2 windows displayed:

- The first one being the tracking window, which shows the entire camera view along with the characters being drawn
- The second one, is the paint window, which displays only the required final character, assuming there are no disturbances at all.

CHALLENGES FACED

The following challenges were encountered during theimplementation of the work.

1. Since the entire process is based on accurate colourdetection from HSV values, it is highly advised to sit with a background which is plain (preferably white), so that there are no disturbances to the colour detection.
2. Since, this project does not have depth sensing, a few undesired motions of the coloured paper will also be detected and traced on the screen, which can causedisturbances to the characters perceived.
3. If there happens to be more than one point on the screenbeing detected as the same colour required (due to same HSV value), then this also tends to cause disturbances inthe characters on the screen, as the output might have both of them or more, which can be undesired.

Since the motion of the coloured paper attached to the fingertip always starts from any edge of the screen, the same is displayed on the window as well. This leads to undesired additional lines. It can be avoided by covering the coloured paper attached to the fingertip until reachingthe desired start location on the screen.

CONCLUSION

The proposed system has the capability to eliminateconventional ways of writing. With the use of this system,one wouldn't need notebooks or cell phones in order to write down important notes. This system also has thepotential to serve people with hearing impairment or people with difficulty using keyboards. Extended version of this project can be used as an OCR. The generated text can be sent as an email, text message or a simple word document. Some improvements can be done in this project in order to eradicate its limitations. Some of them are: First of all, writing of the words can be made rapid by replacingthe character recognizer by handwriting recognizer. Second, the background colors should not disturb the system from recognizing the writing tool. The systemshould take care of the writing tool only and nothing in thebackground. Third, in order to control the system, numberof fingertips should be replaced with paused hand- gestures.

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Fig 1: Detection using colour paper attached to fingertip.

Fig 2: Writing in the air and front end of the system

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

An Overview on Traditional Herbs - Remedies for Various Diseases

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Received: 30 Dec 2021 Revised: 20 Jan 2022 Accepted: 19 Feb 2022

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ABSTRACT

Plants on the earth are important as human beings are influenced in various ways by plants and their products. Existence of mankind is impossible without plant kingdom as they provide balanced ecosystems, major source of nutrition, preventive aspects of medicine, primitive aspects of health and curative aspect of disease. Medicinal plants or medicinal herbs are used for human kind to cure different ailments since long date ago. Plants synthesizing secondary metabolites known as phytochemicals having active constituents with immense biological activity which are identified to use in different therapeutic actions.

Keywords: medicinal plants, phytochemicals, therapeutic actions

INTRODUCTION

Human beings mostly depend on the nature for basic needs like cloth, shelter and food in addition to also flavored items, fragrance products fertilizers. Especially before the introduction of chemical medicines or synthetic medicines, man relied on the healing properties of medicinal plants. To the globally increased population, medicinal plants play an important role in health care system of people especially in developing countries. Medicinal plants are the “backbone” of traditional medicine, on an average more than 3.3 billion people in the developing countries utilize medicinal plants on a regular basis. In India and China the herbal medicine has a continuous history with long usage. The development and recognition of medicinal plants, financial support for the cultivation and drug identification from these plants are need of hour in both industrialized and developing nations (WHO, 1998). In commercial point of view, contribution of plants to diversified industries is remarkable such as fine chemicals,

cosmetics, pharmaceuticals and industrial raw materials etc. The beneficial properties of plants are recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. The earliest reports carved on clay tablets in cuneiform date from about 2600 BC were from Mesopotamia; identified materials were oils of *Commiphora* species (Guggul), *Cedrus* species (Cedar), *Glycyrrhiza glabra* (Liqorice), *Papaver somniferum* (Poppy capsule) and *Cupressus sempervirens* (Cypress). These are still used today for the cure of diseases extending from cold and cough to inflammation and parasitic infections (Fakim, 2006). The traditional medicine practice is widespread in South East Asia countries like China, India, Japan, Pakistan, Sri Lanka and Thailand. About 40% of the total medicinal consumption is attributed to traditional tribal medicines alone in China. The herbal medicinal preparations are more in demand than mainstream pharmaceutical products in Japan.

Medicinal plants became the future hope for the production of safe medicine (Hamburger and Hostettmann, 1991). We have number of modern drugs, but still it is genuinely urgent to discover and develop new therapeutic agents to overcome the newly evolved diseases. It is identified that acceptable therapy is available only for one third of the known human ailments so, new drug discovery is necessary. Traditional plant medicines still enjoy significant position in the modern-day drug industries due to minor side effects as well as the synergistic action of the combination of compounds. The WHO endorses and promotes the use of herbal drugs in national health care programs because they are easily accessible within the reach of a common man and are time tested and thus considered to be much safer than the modern synthetic drugs. The phytochemical-pharmacological research work has recently yielded effective solutions to certain diseases which synthetic drug industry has failed to afford. The most important research work on *Artimisia annua, Cathranthus roseus, Taxus spp., Lantana camara* and *Baccopa spp. etc.*,. Such plants were earlier considered as poisonous or useless, but now have been found to contain molecules of high drug values and are considered as medicinal herbs of great significance.

Global Scenario

Recently, WHO (World Health Organization) estimated that, 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential as medicinal plants. As per data available, over three-quarters of the world population relies mainly on plants and plant extracts for their health. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the medicinal plants play a major role in the economy of their countries where compared to rest of the world. (https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl).

History

Early medical history is consistent with the history of herbal medicine. The first books written about medicine were the first books written about plants, including the texts of the *Ebers Papyrus*, written in 1500 BC, in which the names of many medicinal plants had been appeared (Ackerknecht, 1973). Herbs and spices are used mainly in culinary preparations, to store food since prehistoric time. Weeds can be used as herbal medicines, such as nettle, dandelion and chickweed (Stepp, 2004). Plant samples from prehistoric burial sites are among the lines of evidence that Paleolithic people had knowledge of herbal medicine. For instance, 60000 year old Neanderthal burial site, "Shanidar IV", in northern Iraq had yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies. A mushroom also found frozen in a body of 5000 years old. This mushroom was probably used against whipworm (Capasso, 1998). The works of Hippocrates (459–370 BC) contain 300 medicinal plants classified by physiological action. Wormwood (*Artemisia*) and common centaury (*Centaurium umbellatum Gilib*) were used against fever, garlic against intestine parasites, opium, henbane, deadly nightshade, and mandrake were used as narcotics, sea onion, celery, parsley, asparagus, and garlic as diuretics, oak and pomegranate as astringents (Gorunovic, 2001).

The Greek physician Dioscorides, who worked in the Roman army, documented more than 1000 recipes for medicines using over 600 medicinal plants in *De materia medica*, 60AD; this formed the basis of pharmacopoeias for 1500 years. Drug research makes use of ethno botany to search for pharmacologically active substances in nature,

and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes (https://en.wikipedia.org/wiki/Medicinal_plants). In herbal medicine different medicinal plants will be used studying their medicinal uses. The term herb referring to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma, and root or may be gums, as well as non-woody plants also. These are used in different ways like food, flavonoid, medicine or perfume and in various spiritual and religious activities (https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl). The isolation of serpentine in 1953 from the Indian plant *Rauwolfia serpentine* root was a remarkable innovation in the history of hypertension treatment and lowering of blood pressure. During 1971-1990 fresh medicines *i.e.,* artmisinin, Zguggulsterone, ginkgolides, lectinam, E-guggulsterone, teniposide, ectoposide, plaunotol and nabilone appeared all around the world. The medicines which were presented during 1991-1995 include irinotecan, toptecan, paclitaxel and gomishin etc. The Vinblastine was used for the treatment of leukemia in children, Hodgkins choriocarinoma, non-Hodgkins lymphomas was isolated from the *Catharanthus rosesus* (Jones, 1998). The manufacture of morphine on industrial scale by E. Merck, Germany in 1826 marks the beginning of commercialization of plant-derived drugs (Galbley and Thiericke, 1999).

In Bible and also in Jewish book, the Talmud there is note of aromatic plants like myrtle. The history of medicine in India can be traced back to the remote past in the Vedic period. The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc (Tucakov, 1964). "The Rig-veda perhaps the oldest repository of human knowledge, having been written in 4500-1600 B.C. claims about 99 medicinal plants, the Yajur-veda having description of 82 plants and the Sam-veda too have the description of medicinal plants. Atharva-veda deals with 288 plants almost all having medicinal value and were used for deadly diseases" (Kapoor, 2003). In addition to these ancient sources, other ancient works on medicinal value of plants are as: Charaka Samhita, Susruta Samhita, Vagbhata I, Vagbhata II, Vagbhata III, Madhavakara, Cakrapanidatta, Sarangadhara and Bhava Misra.

Folklore and Traditional Medicine

The importance of traditional medicine as a source of primary health care was first officially recognized by world health organization (WHO) in 1978 in UN at its Alma Ata Conference (Dey *et al.,* 2007). To understand Traditional medicine (TM) in easy way as curing of different diseases or for healing of wounds using traditional knowledge of different tribes or communities followed in different regions using locally available flora or medicinal herbs passed down from generation to generation is known as traditional medicine. TM is the oldest form of health care in the world and was used in prevention, and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases. TM is also variously known as complementary and alternative, or ethnic medicine, and it still plays a key role in many countries (WHO, 2000). But appropriate processing and dose regulation through clinical trials is required to acquire drug efficacy to minimize side effects and to increase its precision over particular disease.

Traditional medicine usage is prevalent in most of the rural areas. They have immense knowledge in folklore and ethano-medicine but usage is rapidly diminishing partly due to change in life style and exposure to western culture especially usage of allopathic system usage. India has one of the richest plant medical traditions in the world. Individual tribes and ethnic communities in different parts of the world preserved different versions of indigenous or traditional knowledge (Bruchac, 2014). There are estimated to be around 25,000 effective plants based formulations, are used in folklore and are known by rural communities of India. There are over 1.5 million people are practicing traditional medicinal system using different medicinal plant species for preventive and curative purpose. It is reported that, an average of 7800 medicinal drug manufacturing units in India are using around 2000 tonnes of herbs as raw material annually and the largest users are India and China (Ramakrishnappa, 2002 and Rawat, 2002). Indigenous Knowledge about the identity and the use of medicinal plants has been circulating chiefly among practitioners of traditional medicine or the benefactors of such practices. Localized knowledge and

experiences gained through generations, such as the types, distribution, ecology, methods of management and methods of extracting the useful medicinal plants properties are disappearing annually due to lack of written documents, the death of seniors, migration of people due to drought and social problems, urbanization, the influence of modern medicines and the influx of different cultures (Regassa, 2013).

The Ayurvedic system was practiced in Indian continent, where three humors- phlegm, bile and flatulence and hot and cold is also important. In chinese system of medicine also it is believed that the balance between the hot and cold of the body is the base of health for example when a man fainted with sun stroke, food items will be given which are cold to body like plant soups, and for instance when a mother gives birth to child, she have to eat heat given products like meat. In some cultures, there is a religious belief that, supernatural powers and spirits will affect human body and those gods or spirits were the major cause of illness (Ember and Peregrine, 2002). Plants which are medicinally important are included in Indian cultural and religious rituals eg. *Ficus religiosa*, *Azadiracta indica*, *Cyanodon dactylon*, *Artemesia sp.,Calotropis gigantean* etc. In some festivals related to Hindu religion, plants which are medicinally valuable represent god and sacredness eg. *Aegle marmelosa* (bael), *Ocimum sanctum* (tulasi) (Prakash, 2013).

Uses

1. Plants like tulsi, aloe, turmeric and ginger are present in our daily life as home remedies and also used to prepare anticancer and ant obesity drugs. Some of that are recognised for their therapeutic values. Some of these plants include green tea, walnuts, pepper and mint etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste etc.
2. Herbs like basil, fennel, chives, cilantro (coriander), mint, thyme, oregano, fenugreek, dill, Rosemary, Variegated Sage are medicinally important antipyretic, antifungal, and antibacterial properties along with nutritional value, can be planted in kitchen. These are very easy to grow and smell amazingly having insect repellent properties.
3. Many herbs are used as blood purifiers to alter or change a long-standing disease by eliminating the metabolic toxins and also to remove the free radicals by having radical scavenging activity eg. turmeric, aloe. These are also known as 'blood cleansers'. eg. green tea. Certain herbs improve the immunity of the person, can cure fever due to its antipyretic properties eg.kalmegh
4. Wood and bark plants like sandalwood and cinnamon having astringent properties.
5. Spices like cardamom, coriander and ginger are possessing appetizing qualities and also they also remove the gas from stomach.
6. Plants like kalmegh, sarpagandha and sage act against poisons and snake bites.
7. Herbs like rosemary, thyme, coriander, kasturi methi, basil, mint, oregano etc. give pleasant aroma to food, rich in nutritive values and possess antioxidant, antibacterial and also antibiotic properties that inhibiting growth of harmful bacteria.
8. Ginger, tulasi, cloves etc. are used in cough syrups because they are known for their expectorant property, because they eject mucus from the bronchial system.
9. Due to a narcotic property in opium latex, used in cough syrups as sedative drug (sleep inducing). But opium seed which is free of narcotic properties rich in unsaturated fatty acids like linoleic acid.
10. Fenugreek seed mucilage (galactomannan), aloe mucilage (glucomannan), are identified for their anti obesity and diabetic properties. Mostly aloe known as sunburn plant, to treat burns and it is famous for cosmetic preparations eg. aloe gel, soaps, moistrisers etc.
11. Aloe juice extract in combination with other medicinal fruits like amla and jamun used as blood purifier.

Continued growing economic importance of medicinal plants and plant based pharmaceuticals in developing countries and for most of medicinal plants are indigenous to these countries have to identify the importance of medicinal plants and motivate the policy makers to sustain the funding of research on medicinal plants will boost the foreign exchange of developing countries and also increase the national health system by curing of endemic diseases. And further improves the healthcare delivery system by making available essential pharmaceuticals at affordable prices to the majority of population (Bukar *et al.,* 2016). Human societies have been in close contact with their

environments since the beginning of human origin and used the ingredients of the environment or nature to obtain food and medicine (Lorigooini *et al.,* 2018). As human life style is running away from nature and forgetting that without nature we cannot survive. As medicinal plants are natural products and eco-friendly and locally available and are giving less side effects than compared to synthetic drugs. Although medicinal plants and spices had been used for their medicine, flavouring and aromatic qualities for centuries, need a physician help in some cases especially in usage of narcotic crops like opium, datura and belladona. There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies (Singh, 2015). Growing of medicinal plants in home gardens enriches the biodiversity and also increases human empowerment.

CONCLUSIONS

Keeping in view of the growing importance of ethno botanical studies, it is necessary to collect the information's about the knowledge of traditional medicines which is preserved in tribal and rural communities that can be used for curing various diseases. Phytochemical and biochemical research with a continuous endeavor for the revival and spreading of Indian medical inheritance for the welfare of the society at large is needed.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Experimental Investigation on High Strength Self-Compacting Concrete

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Received: 11 Dec 2021 Revised: 19 Jan 2022 Accepted: 24 Feb 2022

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ABSTRACT

An attempt is made to understand the behaviour of High Strength Self-Compacting Concrete in its fresh and hardened state incorporating mineral admixtures with different percentages of silica fume and fly ash. Experiments were carried out using 12 mixes with different w/c ratios. Four replacements of OPC with fly ash and silica fume of 5%, 10%, 15% and 20% were cast cured for 28 days. The experimental program is carried out in three stages. First, for each mix, fresh properties (slump flow, T-500, V-funnel test and L-box test) are checked. In the second stage, cubes and cylinders were cast and kept in fresh water for curing. Then in the third stage, these samples were used for testing mechanical properties (compressive strength, split tensile strength and modulus of elasticity). The results obtained indicate a direct impact of percentage content of fly-ash and silica fume on the properties of fresh and hardened SCC.

Keywords: Self-Compacting Concrete· Fly ash· Silica fume· Slump flow· T-500· V-funnel test· L-box test

INTRODUCTION

Rapidly increasing use of chemical and mineral admixtures, developments in the area of construction technology and the better understanding of the behaviour of fresh and hardened concrete have contributed to our ability and confidence in using concrete in more and more challenging conditions. It has led to the development of special concretes, special construction methods and improvement in concrete properties. Given the dense reinforcement around which concrete is required to move, or the complicated geometry of the formwork, and the distances over which concrete needs to be pumped, makes a high demand on the workability of the concrete, and practically

"flowing" concrete is required. Needless to say, in order that the concrete does not segregate, this workability and flow ability strains the ability of the fluid phase to carry with it the denser aggregate fraction to the limit. Self-Compacting Concrete (SCC) provides a solution by overcoming this problem as it can flow, compact by itself without any need of vibration or another mean of compaction and fills completely on to the formwork with no segregation [1-4]. SCC can reduce the considerable number of the skilled work force and need of good quality control; thereby it reduces the time of construction. It was also reported that Self-Compacting concrete is more economical than economical than conventional concrete [5]. However, because of its requirement of "highly flowing nature", proper care should be taken so as to achieve filling and passing ability without any segregation [6]. High Strength SCC (HSSCC) requires a lower water to binder ratio with higher cement content and limiting the size of coarse aggregates [7-8]. The production of HSSCC also requires suitable chemical admixture to reduce water content by decreasing interparticle friction but maintaining required workability and also supplementary cementitious materials so as to fill the voids to make the concrete denser increasing its compressive strength [1, 9-11]. In the present work, the procedure followed to produce a cost-effective and high strength SCC is briefly discussed. The objectives of the present work in this experimental investigation is to produce controlled HSSCC and compare its relative performance with HSSCC incorporating fly ash and silica fume with different percentages i.e. 5%, 10%, 15% and 20% at the different water to binder ratios.

MATERIALS

The production of HSSCC requires materials of proper quality. Selection of materials for HSSCC is of paramount importance. In addition to cement water and aggregate, constituents like mineral and chemical admixtures are a must for the production. Chemical admixtures are usually used to control the properties like slump whereas mineral admixtures are used for enhancing the concrete strength. Selection of cement is of utmost importance in HSSCC production as the chemical and physical characteristics of cement affects the compressive strength of concrete more than any other single material. In the present experimental work, Ordinary Portland Cement (Ultra-Tech cement) conforming to IS: 12269-1987 has been used. Fly ash and silica fume were incorporated as supplementary cementitious material. They together act as a binder in the concrete. Silica fume was collected from Shri Sai Durga Enterprises, Bangalore having a specific gravity of 2.15 and the fly ash was obtained from Thermal Power Station, Raichur. The chemical and physical properties of OPC, fly ash and silica fume are given in Table 1. The fineness of mineral admixtures was checked by wet sieving over a 45-μm sieve at every 2 hours as per ASTM C430-08 (2009a). After 20 hours, it was observed that passing was more than 90%, better than the amount of OPC passing 45-μm sieve.

Graded crushed aggregates with a maximum particle size of 12 mm with fineness modulus of 6.78. and natural river sand with fineness modulus of 3.43 was used to produce HSSCC. The specific gravity of sand and Coarse Aggregates (CA) was 2.62 and 2.70 respectively. Major tests were carried out on the sand and CA to check specific gravity, water absorption and fineness modulus and are presented in Table 2. Master Glenium Sky 8233, a high range water reducing super plasticizer was used to increase the workability and reduce water content in the concrete. In this experimental work, super plasticizer was obtained from BASF chemicals, Bangalore. Physical and chemical properties of Master Glenium Sky 8233 are tabulated in table 3. The viscosity modifying agent Master matrix 2, which is used to make the concrete more viscous and prevent segregation was provided by the BASF Company, Bangalore. The dosages of viscosity modifying agent for various applications typically range from 0.1 to 1.5% by weight of cement. If the dosage of VMA is higher than the requirement, it will affect the setting time and stability of entrained air.

Mix Proportions and Casting

A series of trial mixes (Table 4) were prepared in order to achieve a target strength of 70 MPa by varying super plasticizer at 2%, 2.5 % and 3% to obtain optimum dosage and water to binder ratio in the range of 0.34 to 0.4. For all the mixes, fresh properties were checked as per EFNARC guidelines to satisfy the conditions of SCC. After obtaining

the results, a final mix proportion (TT3 in table 4) was finalized and variations were done by replacing cement with fly ash and silica fume at 5%, 10%, 15% and 20%. These contents were finalized based on the available literature so as to achieve a compressive strength of 70 MPa. A total of 12 HSSCCs were produced to study the performance of SCC and compare their properties as given in table 5.

Mix Proportions and Casting

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Testing and Discussion

Testing of all the mixes was done in both fresh states and hardened states. For Fresh state properties, EFNARC (2002) guidelines were followed. Flow-Ability, Filling-ability and Passing- Ability are the major characteristics of SCC in the fresh state. After mixing, all the mixes were tested for these 3 key parameters. A number of cubes and cylinders were also cast for testing compressive, split tensile strength and elastic properties.

Flow-Ability: The flowing ability of all the mixes was examined with respect to slump flow test and T 500 test as per EFNARC specifications. For slump flow test, the procedure is same as that of normal concrete except there is no need for compaction. After lifting the cone, the concrete spreads out and the diameter is measured (Figure 1). Table 6 shows the slump flow values of conventional SCC concrete and SCC with different mineral admixtures. According to European guidelines the slump flow values are in between 600mm to 800mm to be classified as SCC. From table 6 and figure 2, it can be seen that the presence of silica fume in SCC makes the concrete mix more homogenous and stiffer as compared to the SCC with fly ash because of its reactive nature. The slump flow value decreases in a small amount for SCC with fly ash (IF1, IF2, IF3, IF4) and values of slump flow vary from 702 mm to 685mm i.e. 2.42% decrement in slump values. The slump flow values for SCC with silica fume (IS1, IS2, IS3, IS4) decreases significantly as the amount of silica fume increases from 5% to 20%. The reason behind the reduction of slump values is the highly reactive nature of silica fume. The value of slump flow varies from 684 mm to 650 mm, i.e., 4.98% reduction in slump flow values. The reduction of slump values for SCC with silica fume is doubled as compared to SCC with fly ash. For every 5% of addition of silica fume as a cement replacing material, the slump flow values are reduced by 2.11%. The SCC with a combination of both fly ash and silica fume (IC1, IC2, IC3, IC4), the slump flow values are reduced from 692mm to 670mm, i.e. 3.17% reduction in slump flow values. Hence, from above observation, it is clear that in presence of silica fume the slump flow values are rapidly reduced as compared to the fly ash.

The T50 slump flow time is a time required for concrete for the 50-cm slump flow. The lower value of time indicates the greater flow ability. According to European guidelines the T500 time has minimum 2 seconds and a maximum of 5 seconds. The T500 time test results are tabulated for various mix proportion in table 7. From table 7 and figure 3, it can be seen that for SCC with fly ash (IF1, IF2, IF3, and IF4), T500 time did not vary significantly because the fly ash is chemically inactive at an early age of concrete. SCC with silica fume (IS1, IS2, IS3, IS4) gives higher value (4.06 sec to 5.00 sec) as compared to the SCC with fly ash (3.52 sec to 4.20 sec). The test results for SCC with a combination of silica fume and fly ash (IC1, IC2, IC3, and IC4) are nearly equal to the SCC with fly ash (IF1, IF2, IF3, IF4). The results

showed that the concrete spreads uniformly and set quickly because of the higher dosages of super plasticizer and lower w/c ratio. All the mixes satisfied the requirement as specified by EFNARC.

Filling ability: To check the filling ability of all the mixes of SCC, V-Funnel test was carried out as per the guidelines. The lower the flow time, better is the ability to fill. EFNARC guidelines give the range of 6 seconds to 12 seconds for SCC criteria. All the mixes tested were in this range satisfying the guidelines and the results were similar to T500 tests. The results are tabulated in table 8. From table 8 and figure 4 it can be seen that for SCC with fly ash (IF1, IF2, IF3, and IF4), the V-funnel test time did not differ much, values in between 9.50 sec to 10.40 sec have been found. SCC with silica fume (IS1, IS2, IS3, IS4) gives higher value (10sec,10.5sec, 11.32sec, 12sec) as compared to the SCC with fly ash (9.50sec,9.56sec,10.12sec, 10.40sec). The V-funnel test time results for the SCC with silica fume (IS1, IS2, IS3, IS4) are higher because the silica fume increases the viscosity and homogeneity of concrete. IS4 with silica fume 20% gives 12 seconds time indicating viscous concrete but near to the boundary limit of satisfying SCC criteria as per EFNARC guidelines.

Passing Ability: The passing ability of all the mixes was conducted with respect to L-Box test which gives the measurement of the passing ability of HSSCC with regard to blocking ratio H 2 / H 1 . The higher value indicates the greater passing ability of SCC. The higher dosage of super plasticizer helped in achieving the results for passing ability. Table 9 gives results for passing ability tests. From table 9 and figure 5 it can be seen that SCC with fly ash (IF1, IF2, IF3, and IF4), SCC with silica fume (IS1, IS2, IS3, IS4) and SCC with combination (IC1, IC2, IC3, IC4) gives good results for L-box test mainly due to super plasticizer and VMA. The values vary between 0.8 to 1. The L-box test results for the SCC with silica fume (IS1, IS2, IS3, and IS4) gives very less value as the quantity or dosage of silica fume increases, the value varies in between 0.81 to 0.90. The L-box test results for the SCC with fly ash and SCC with the combination of both gives a nearly same value of (H2/H1). The L-box test results for the SCC with silica fume (IS1, IS2, IS3, IS4) are lower than other SCC mixes because the silica fume increases the viscosity and homogeneity of concrete similar to that of filling ability tests.

Effect of Water to Binder ratio

Water to binder ratio is considered as one of the important factor influencing overall properties of all type of concretes. In SCC, here too, it plays a vital role in achieving strength as well as workability. It was seen from the experimental investigation that as the water to binder ratio decreases and binder quantity increases, the workability of HSSCC increases significantly because of super plasticizer and VMA content. This is mainly due to increased paste content and lesser aggregate contents which results in increased binder surface area due to the presence of porous, fine particles of fly ash and silica fume. Thus, the increased paste volume and higher wettable surface area of the binder induces better resistance to SCC flow.

Compressive Strength

The average 7 days and 28-days compressive strength of cube specimens for all mixes are shown in figure 6 to 8. It was found that strength development in concrete specimens containing only silica fume was faster as compared to other specimens. This rate of initial strength gain can be due to using micro silica as the mineral admixture. As the water to binder ratio decreased, increase in compressive strength was observed as the binder content increased which resulted in the higher amount of calcium silicate hydrate (C-S-H) gel improving the physical packing of aggregates. This resulted in higher compressive strengths. It was observed that SCC with 15% silica fume (IS3) gives nearly 4% more compressive strength as compared to conventional SCC, and 5% to 10% more compressive strength as compared to SCC with fly ash. The increase in strength is due to the addition of mineral admixtures having the micro filling ability and pozzolana activity. With the presence of silica fume and fly ash, the concrete becomes dense and voids are reduced by decreasing the pores. Moreover, pozzolana reaction of mineral admixtures influenced the compressive strength of concrete by refining the microstructure of increased binder-paste and improving interfacial bond between binder-paste and aggregates [12]. At 15% replacement by silica fume, a good amount of CaOH 2 liberated from hydration of cement increasing C-S-H gel by pozzolanic reaction with a high percentage of SiO 2 content in mineral admixture.

It was also observed that CaOH 2 content in fly ash systems reached a maximum value after 7-14 days and was decreasing later as a result of its consumption in pozzolanic reaction. This decrease of CaOH 2 content followed the augmentation of strength due to use of fly ash. Figure 9 shows a cube specimen after failure.

qSplit Tensile Test (SPT)

SPT of M70 grade SCC with mineral admixture (silica fume, fly ash), and normal self- compacted concrete were done on cylinders. The split tensile strength of concrete of standard cylinder size 150 mm diameter and 300mm long cylinder specimen is calculated for different curing periods of 7 & 28 days. SCC with fly ash gives higher split tensile strength results and increase in quantity of fly ash in concrete mix results in increased split tensile strength. For SCC with silica fume gives 17% more split tensile strength as compared to SCC with fly ash. The SCC with a combination of both silica fume and fly ash mixes (IC1, IC2, IC3, and IC4) gives nearly 15% more split tensile strength as compared to the SCC with fly ash. From above experimental work, SCC with 15% silica fume (IS3) gives 19.95% more split tensile strength as compared to SCC with fly ash and SCC with a combination of both silica fume and fly ash. Table 10 gives the split tensile results for all the mixes and figure 10 shows a specimen of the cylinder failure.

Modulus of Elasticity (MOE)

Self-Compacting Concrete and conventional concrete shows a similar modulus of elasticity. If the grade of concrete is higher than the modulus of elasticity of that concrete is also higher. For this test, a standard cylinder of 300mm length and 150mm diameter are used and load applied in uniaxial compression. The MOE of SCC was determined from the stress and strain values of SCC concrete. To obtain the modulus of elasticity, the 80% of ultimate strength load was considered. Figure 11 shows the test set up of cylinder specimen and figure 12 shows modulus of elasticity in N/mm 2 for different specimens along with the average curve. In figure 12, horizontal axes can be read as follows I0-1, IF1-2, IF2-3, IF3-4, IF4-5, IS1-6, IS2-7, IS3-8, IS4-9, IC1-10, IC2-11, IC3-12 and IC4-13. It was observed that HSSCC shows higher deformability as compared to conventional concrete. This high deformability observed was mainly due to the higher amount of paste content present in HSSCC as compared to conventional concretes. The reason behind this is due to the fact that aggregates are less deformable than hardened paste and high deformability can be observed in higher paste content in a hardened composition.

CONCLUSIONS

Water to binder ratio, SF and FA content and super plasticizer dosage significantly affected the fresh and hardened properties of HSSCC. Following conclusions can be drawn based on the results of this research:

The results of slump flow test, V-funnel test and L-box test for SCC with silica fume and
SCC with fly ash are less than SCC with a combination of both silica fume and fly ash.

- Presence of silica fume and fly ash require more dosages of super plasticizer because of its extremely high surface area for constant w/c ratio and the same was also observed in the previous literature of low strength SCC.
- The results of this study suggest that the SCC with a combination of both mineral admixtures improve the workability of concrete.
- Maximum compressive strength is obtained from SCC with 15% silica fume (IS3). The increases in strength can be attributed to the improved aggregate –matrix bond. For maximum compressive strength, optimum silica fume replacement with cement in percentage is in between 5% to 15%. The compressive strength reduces when silica fume replacement was above 15% of cement and vice versa.
- Maximum split tensile strength was also obtained from SCC with 15% silica fume (IS3). The durability of SCC is better than other normal concrete because SCC is less porous than normal concrete due to the addition of mineral admixtures.

- The value Modulus of elasticity of SCC with mineral admixture is nearly the same as that of conventional concrete.

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Table 1. Major Physical Properties of Constituent Materials Used in Concrete

Chemical composition	OPC	Fly ash	Silica Fume
SiO_2 (%)	19.3	62.63	91.9
Al_2O_3 (%)	5.2	23.34	0.7
Fe_2O_3 (%)	2.4	3.93	0.3
CaO (%)	61.2	2.04	-
MgO (%)	1.25	1.3	0.1
SO_3 (%)	3.2	0.6	0.1
Na_2O (%)	0.069	0.63	0.06
K_2O (%)	0.62	2.09	0.65
Density (kg/m^3)	3089	2270	2260
Specific surface area BET (10^3 /kg)	0.55	2.14	26.43
Fineness % Retain on 90μ sieve	3%	-	-
Initial setting time (min)	62	-	-
Final setting time (min)	370	-	-
Specific gravity	2.96	2.2	2.15
Compressive Strength (MPA)			
7 Days	45	-	-
28 days	65		

Table 2. Physical properties of fine aggregates and coarse aggregate.

Properties	Fine Aggregate	Coarse aggregate
Specific gravity	2.62	2.70
Water absorption	0.8 %	0.4%
Fineness modulus	3.43	6.78

Table 3. Physical and chemical properties of Master Glenium Sky 8233.

Parameter	Result
Colour	Light brown
Boiling point	>100˚ c
Viscosity (25˚ c)	=50-150 cps
Specific gravity (25˚ c)	=1.2
Soluble in water	Soluble
pH	>=6
Chloride ion content	< 0.2%

Table 4. Trial mix design

Trial	Designation	Cement (kg/m³)	Sand (kg/m³)	C.A (kg/m³)	Water (lit)	W/C	Slump flow (mm)	V-funnel test (sec)	L-box test (H2/H1)
	FT1	575	700	833	196	0.34	-	-	-
1st trial	FT2	575	700	833	196	0.36	-	-	-
SP- 2%	FT3	575	700	833	196	0.38	-	-	-
	FT4	575	700	833	196	0.40	520	-	-
	ST1	575	700	833	196	0.34	-	-	-
2nd trial	ST2	575	700	833	196	0.36	538	46	-
SP- 2.5%	ST3	575	700	833	196	0.38	554	40	-
	ST4	575	700	833	196	0.40	574	35	-
	TT1	575	700	833	196	0.30	590	28	0.98
3rd trial	TT2	575	700	833	196	0.32	640	19	0.92
SP- 3%	**TT3**	**575**	**700**	**833**	**196**	**0.34**	**690**	**10**	**0.86**
	TT4	575	700	833	196	0.36	750	8	0.80

Table 5. Final mix proportion.

Mix proportion[a]	Cement (kg/m³)	Silica fume (kg/m³)	Fly ash (kg/m³)	RCP (kg/m³)	Coarse agg (kg/m³)	Water (kg/m³)	Super plasticizer 3% (kg/m³)	w/c ratio
I0	575	-	-	700	833	196	-	0.34
IF1	546.25	-	28.75	700	833	196	17.25	0.34
IF2	517.5	-	57.5	700	833	196	17.25	0.34
IF3	488.75	-	86.25	700	833	196	17.25	0.34
IF4	460	-	115	700	833	196	17.25	0.34
IS1	546.25	28.75	-	700	833	196	17.25	0.34
IS2	57.5	57.5	-	700	833	196	17.25	0.34
IS3	488.75	86.25	-	700	833	196	17.25	0.34
IS4	460	115	-	700	833	196	17.25	0.34
IC1	546.25	14.38	14.38	700	833	196	17.25	0.34
IC2	517.5	28.75	28.75	700	833	196	17.25	0.34
IC3	488.75	43.13	43.13	700	833	196	17.25	0.34
IC4	460	57.5	57.5	700	833	196	17.25	0.34

[a] I0-Conventional concrete, IF1- 5% Fly Ash, IF2-10% Fly Ash, IF3-15% Fly Ash, IF4-20% Fly Ash, IS1-5% S.F, IS2-10% S.F, IS3-15% S.F, IS4-20% S.F, IC1- 2.5%S.F+2.5% Fly ash, IC2-5% S.F+5% Fly ash, IC3-5%S.F+7.5% Fly ash, IC4-10% SF+10% Fly ash.

Table 6. Slump flow test results.

Sl No	1	2	3	4	5	6	7	8	9	10	11	12	13
Concrete designation	I0	IF1	IF2	IF3	IF4	IS1	IS2	IS3	IS4	IC1	IC2	IC3	IC4
Slump flow test (Diameter in mm)	710	702	695	690	685	690	675	660	650	692	685	680	670

Table 7. T-500 test results

Sl No	1	2	3	4	5	6	7	8	9	10	11	12	13
Concrete designation	I0	IF1	IF2	IF3	IF4	IS1	IS2	IS3	IS4	IC1	IC2	IC3	IC4
T500 time (sec)	3.50	3.52	4.06	4.15	4.20	4.06	4.26	4.45	5.00	3.55	4.10	4.22	4.35

Table 8. V-Funnel test results.

Sl No	1	2	3	4	5	6	7	8	9	10	11	12	13
Concrete designation	I0	IF1	IF2	IF3	IF4	IS1	IS2	IS3	IS4	IC1	IC2	IC3	IC4
V-funnel test value in (sec)	9.34	9.5	9.56	10.12	10.4	10	10.5	11.32	12	9.75	10.15	10.5	11.15

Table 9. Test Results for L-Box test

Sl No	1	2	3	4	5	6	7	8	9	10	11	12	13
Concrete designation	I0	IF1	IF2	IF3	IF4	IS1	IS2	IS3	IS4	IC1	IC2	IC3	IC4
H1 in cm	10.3	10.2	10.1	10	9.8	10	9.7	9.4	9.0	10.1	9.9	9.7	9.2
H2 in cm	9.8	9.4	9.2	8.9	8.5	9.0	8.4	7.9	7.3	9.2	8.8	8.5	7.8
L-box test value in (H2/H1)	0.95	0.92	0.91	0.89	0.87	0.90	0.87	0.84	0.81	0.91	0.89	0.87	0.85

Table 10. Split tensile results

Sl No	Concrete designation	Split tensile strength (N/mm²)	
		7 days	28days
1	I0	2.80	4.25
2	IF1	2.50	3.67
3	IF2	2.60	3.80
4	IF3	2.90	3.96
5	IF4	3.10	4.11
6	IS1	2.70	4.39
7	IS2	2.95	4.67
8	IS3	3.39	4.95
9	IS4	2.90	4.52
10	IC1	2.60	4.11
11	IC2	2.90	4.39
12	IC3	3.00	4.55
13	IC4	3.10	4.70

Fig 1. Measuring diameter for slump flow test

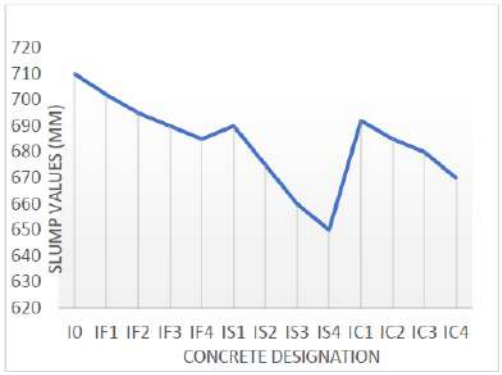

Fig 2. Test results for slump flow tests

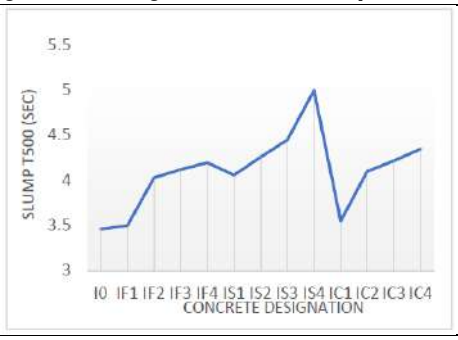

Fig 3. Test results for T-500 test

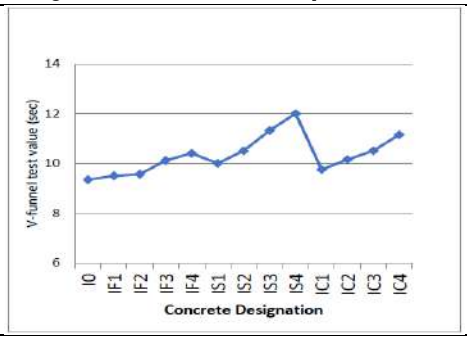

Fig 4. Test results for V-Funnel test

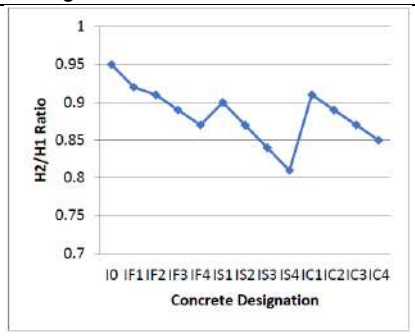

Fig 5. Test Results for L-Box test

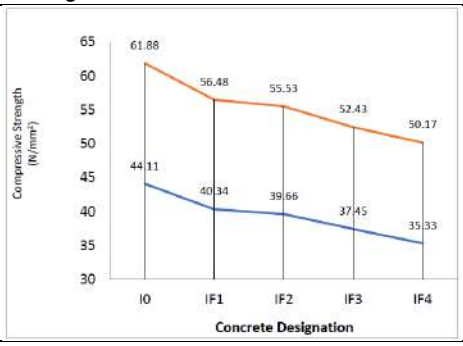

Fig 6. Compressive test results of SCC with fly ash

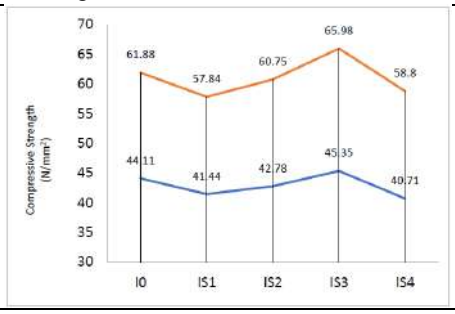

Fig 7. Compressive test results with SF

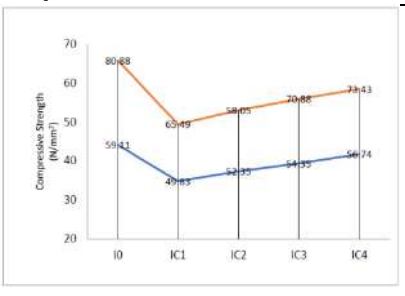

Fig 8. Compressive test results of SCC with SA and FA

Fig 9. Cube specimen after failure

Fig 10. Cylinder Specimen after Failure

Fig 11. Test Setup for MOE

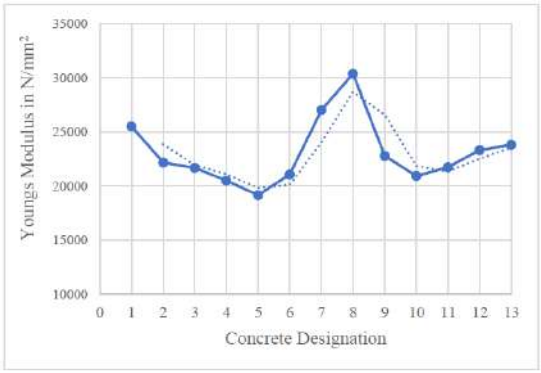

Fig 12. MOE of HSSCC

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Evaluation of Surface Roughness of Bulk Fill Composite Resin after Brushing Simulation with Herbal and Fluoridated Toothpaste - An *In-Vitro* Study

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Received: 11 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Survival of bacteria in the oral cavity depends upon the adhesion of bacteria to any hard surfaces, such as those of teeth, filling materials, dental implants, or prostheses. The simulated tooth brushing abrasion is considered as an established model in the literature, because it is an important in-vitro wear factor, which will be able to simulate a clinical condition. The aim of the study is to evaluate the surface roughness of bulk fill composite resin after brushing simulation with herbal and fluoridated toothpaste. Silicon mould of standard diameter has been made from which composite discs were obtained, then A2 shade of composite restorative material of brand Te-Econom plus was light cured in a disc mould of dimension 10 mm, 4 mm thickness and height of 4 mm. The composite disc was then finished, polished and subjected to brushing simulation. Pre and post brushing surface roughness values were measured using a stylus profilometer. Ra, Rq and Rz values were calculated. Independent sample t test was used p value of less than or equal to 0.05 is considered significant. The mean Ra of herbal toothpaste is 0.00200 and fluoridated toothpaste is 0.00500, which showed a significance of 0.024. Hence, it is statistically significant. The mean Rq of herbal toothpaste is 0.00200 and fluoridated toothpaste is 0.00200. The mean Rz of herbal toothpaste is 0.01725 and fluoridated toothpaste is 0.02450, which showed a significance of 0.893. Hence, it is statistically not significant. It can be concluded that the surface roughness of bulk fill

composite resin material is reduced upon brushing simulation for around 30000 cycles. Samples brushed with fluoridated toothpaste showed more surface roughness after brushing simulation than the herbal toothpaste.

Keywords: Surface roughness, Brushing simulator, Composite resin, Toothpaste, Profilometer, Innovative technique.

INTRODUCTION

Survival of bacteria in the oral cavity depends upon the adhesion of bacteria to any hard surfaces, such as those of teeth, filling materials, dental implants, or prostheses (1). It is widely accepted that the surface roughness of intraoral hard surfaces has a major impact on the initial adhesion and the retention of oral microorganisms. In detail, the rougher surfaces (crowns, implant abutments and denture bases) retain more plaque than smoother ones (2). The roughness of restorative material also have a major impact on the aesthetic appearance, discoloration of restorations, secondary caries occurrence, gingival irritation and wear of opposing and adjacent teeth (3). In patients with less than adequate oral hygiene, variations in surface roughness of provisional restorations may be associated with the onset of subclinical or even clinical inflammation. On the other hand, a smoother surface of restoration ensures patient comfort and facilitates oral hygiene (4). Composite resin restorations are non-toxic and tooth-colored. They are used to restore teeth that have been broken or decayed, and they offer resilience and strength to these weaker regions. Composite fillings can be used on the anterior or posterior teeth, in which it's 'natural appearance has made them a popular choice. Bis-GMA and other dimethacrylate monomers (TEGMA, UDMA, HDDMA), filler material such as silica, coupling agents and photoinitiator are the components of composite resins. The lifespan of resin-based composite restorations has been compared to the longevity of silver-mercury amalgam restorations in several studies. Composite restorations can last as long as amalgam restorations, depending on the dentist's competence, the patient's characteristics, and the type and location of restoration (4).

The simulated tooth brushing abrasion is considered as an established model in the literature, because it is an important in-vitro wear factor, which will be able to simulate a clinical condition (5). The aim of the study is to evaluate the surface roughness of bulk fill composite resin after brushing simulation with herbal and fluoridated toothpaste.

MATERIALS AND METHODS

Sample Preparation

Eight disc shaped samples of restorative material of A2 shade of composite restorative material of brand Te-Econom plus with 2 mm of thickness were prepared using a customised mould. The samples were subdivided into two groups with 4 samples in each group. Group A was tested for the surface roughness with herbal toothpaste and group B was tested for the surface roughness with fluoridated toothpaste.

Brushing Simulator

Eight disc shaped samples were placed in a brushing simulator (ZM3.8 SD Mechatronik). The samples were subjected to 8-9 hours of brushing which is equal to around three years of brushing and to around 30000 cycles in total among which 10000 cycles were performed in the linear X axis, 10000 cycles in the linear Y axis and the last 10000 were further subdivided and 5000 cycles were performed in the clockwise direction and the remaining 5000 cycles in the anti-clockwise direction.

Surface Roughness Assessment

The surface roughness assessment of the prepared A2 shade of composite restorative material of brand Te-Econom plus circular discs samples were done prior to brushing simulation and post brushing simulation using a Stylus profilometer - Mitutoyo SJ 310. It has 2μm tip/60°angle, the device was moved physically on the surface of the composite resin sample material to obtain the surface roughness values prior to brushing. After obtaining the surface roughness value, composite restorative material of brand Te - Econom circular disc samples were placed in the brushing simulator. The surface roughness value after brushing simulation was again determined using the stylus profilometer and Ra, Rq and Rz values were calculated.

RESULTS

In the present study, the surface roughness measurements of the sample pre brushing and post brushing with herbal and fluoride toothpaste were recorded. Independent sample t test was used p value of less than or equal to 0.05 is considered significant. The mean Ra of herbal toothpaste is 0.00200 and fluoridated toothpaste is 0.00500, which showed a significance of 0.024. Hence, it is statistically significant. The mean Rq of herbal toothpaste is 0.00200 and fluoridated toothpaste is 0.00200. The mean Rz of herbal toothpaste is 0.01725 and fluoridated toothpaste is 0.02450, which showed a significance of 0.893. Hence, it is statistically not significant. (Table and figure 1) blue and brown denote the mean value of Rz. It showed Rz values were increased in the fluoridated toothpaste.

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications(6–15),(16–19),(20–24),(25). In clinical restorative dentistry, resin-based composites are often utilised as cosmetic restorative materials. Smooth surface, clinical durability, aesthetics, superior optical qualities, compatibility with natural enamel tissue, surface gloss and preventing discoloration of the restoration are all affected by the filler size and percentage(26). For improved aesthetics and longevity of restored teeth, finishing and polishing tooth-colored restorations is an essential clinical step(27). Because of its aesthetic, physical, and mechanical qualities, resin composites are commonly employed for direct restoration of both anterior and posterior teeth. Because they mediate the interaction of restorative materials with the oral environment, such as bacterial buildup, the surface qualities of materials used in restorations are crucial for their success. Surface roughness is a crucial surface characteristic(28). The surface roughness of a resin composite is determined by the material's composition and porosity, as well as the polishing devices and processes utilised.

Enamel surface abrasion caused by three different dentifrices was analysed using an automated brushing simulator and profilometer in a study. Colgate Swarnavedsakthi, Dabur Herbal and Ayush toothpaste samples were subjected to brushing simulation. A 3D laser profilometer was used to detect the wear in the enamel surface. Pre and Post profilometric readings were compared and they found that Ayush toothpaste group had higher surface abrasion while comparing with other groups such as Colgate Swarna Sakthi and Dabur herbal toothpaste. The higher the brushing time, the higher the surface roughness of composite resins(29). In our study, there was a decrease in the surface roughness of bulk fill composite resin samples after brushing simulation with herbal and fluoridated toothpaste. Fluoridated toothpaste samples showed an increase in surface roughness than composite resin samples brushed with herbal toothpaste. The current study has limitations such as small sample size, comparison was not done with multiple toothpastes and soft, hard and medium toothbrushes. In the future, clinical studies which deal with multiple parameters in different types of composite resin restorative materials are also required to prove the clinical efficacy of the various toothpastes.

CONCLUSION

It can be concluded that the surface roughness of bulk fill composite resin material is reduced upon brushing simulation for around 30000 cycles. Samples brushed with fluoridated toothpaste showed more surface roughness after brushing simulation than the herbal toothpaste.

Conflict Of Interest
None declared

ACKNOWLEDGEMENTS

We thank Saveetha Dental college and Hospitals for providing us the support to conduct the study.

Source of Funding
The funds were provided by:

- Saveetha Dental College and hospitals, Saveetha University of Medical and Technical Sciences, Saveetha University, Chennai.
- Royal hospital, thanjavur.

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Table 1 : Mean. std. deviation and significance testing between groups

Parameter	Groups	Mean	Std.deviation	Significance
Ra	Herbal	0.00200	0.000000	0.024
	Fluoridated	0.00175	0.000500	
Rq	Herbal	0.00200	.000000	-
	Fluoridated	0.00200	.000000	
Rz	Herbal	0.01725	0.002500	0.893
	Fluoridated	0.02450	0.002380	

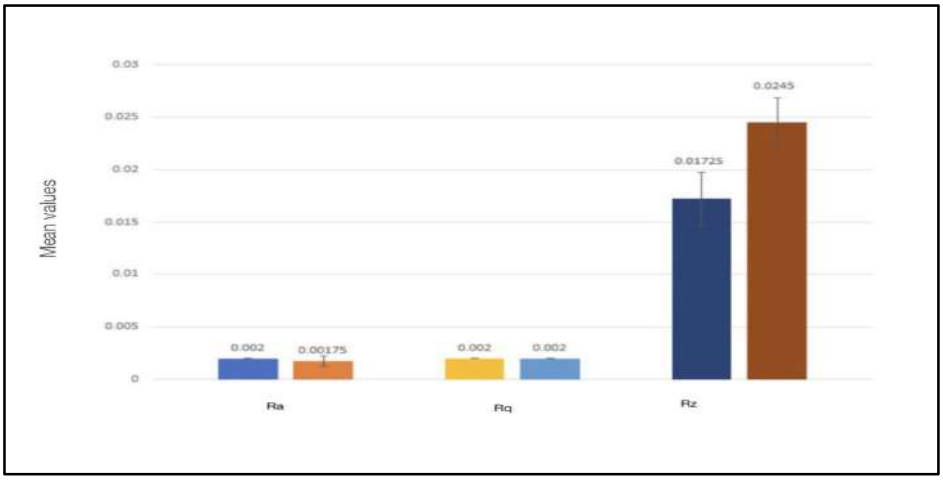

Figure 1: This bar graph depicts the association between the type of composite used and the mean difference in surface roughness. Blue and orange denote the Ra mean value of herbal and fluoride toothpaste, yellow and sky blue denote the Rq mean value, dark

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Almost Block Diagonal Algorithm for Robin Boundary Value Problem

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Received: 04 Jan 2022 Revised: 15 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

In this paper, orthogonal spline collocation method (OSCM) has been used for two-point boundary value problem. Hermite cubic basis functions are used to approximate the solution for linear and boundary value problem. We have explained the efficiency of ABD solver for two-point boundary value problem. Using ABD solver we can solve the linear system with O(N) operations.

Key words: Orthogonal cubic spline collocation methods (OSCM), Two-point boundary value problem, Hermite Cubic basis functions, and Almost block diagonal (ABD) matrix.

INTRODUCTION

Orthogonal Spline Collocation method is a Gelarkin Method that uses a block diagonal linear system to obtain the coefficients of a polynomial equation by using a block diagonal linear matrix to find the value of unknowns which could be calculated using the matrix inverse determination and by collecting the values of the coefficients from the first highest order term as the solution to the problem and then move on to the lower order term to determine the derivative coefficients and hence in turn we obtain the solutions by collecting their values.

Our equation under study is as follows:

$$y'' + k^2 q(x) y = f(x), \quad x \in [a,b] \qquad (1.1)$$
$$\alpha_a y(a) + \beta_a y'(a) = g_0, \quad \alpha_b y(b) + \beta_b y'(b) = g_1 \qquad (1.2)$$

where α_a, β_b ,α_b , β_b, g_0, g_1 are known constants and k^2 is a wave number. The coefficient $q(x)$ is assumed to be piece-wise constant or piece-wise continuous and has finite jump across the interface = x_i , where $x_i \in (a,b)$. The orthogonal spline collocation approximation for problem (1.1) - (1.2) is defined as follows: Find an approximation $y_h \in M_1^3(I)$ such that

$\alpha_a y(a) + \beta_a y'(a) = g_a,$
$Ly_h(\xi_i) = f(\xi_i), \quad i = 1,2,\dots,2N$ (2.2)
$\alpha_b y(b) + \beta_b y'(b) = g_b.$

The collocation points are $\{\xi_j\}_{j=1}^{2N}$, where

$$\xi_{2i-1} = x_{i-1} + \frac{1}{2}\left(1 - \frac{1}{\sqrt{3}}\right)h_i, \quad and \quad \xi_{2i} = x_{i-1} + \frac{1}{2}\left(1 + \frac{1}{\sqrt{3}}\right)h_i, \quad i = 1,2,\dots,N.$$

We define the functions $v_j(x), s_j(x) \in M_1^3(\pi)$, $i,j = 0,1,\dots,N$, by

$v_j(x_i) = \delta_{ij}$, $v_j'(x_i) = 0$ and $s_j(x_i) = 0$, $s_j'(x_i) = \delta_{ij}$, $i,j = 0,\dots,N$,

where $\delta_{ij} = 1$, if $i = j$ and $\delta_{ij} = 0$, if $i \neq j$. Then the set $\{v_i\}_{i=0}^N \cup \{s_i\}_{i=0}^N$ forms a basis for $M_1^3(\pi)$, which we order in the form $\{v_0, s_0, v_1, s_1, \dots, v_N, s_N\}$.

Since only four basis functions $v_{i-1}, s_{i-1}, v_i, s_i$ are nonzero on $[x_{i-1}, x_i]$, the coefficient matrix of the collocation equations structures out as

$$\begin{bmatrix} D_0 & & & & \\ S_1 & T_1 & & & \\ & S_2 & T_2 & & \\ & & \ddots & \ddots & \\ & & & S_N & T_N \\ & & & & D_1 \end{bmatrix}$$

where $D_0 = [\alpha_a, \beta_a]$, $D_1 = [\alpha_b, \beta_b]$, and, for $j = 1,2,\dots\dots,N$,

$$S_i = \begin{bmatrix} Lv_{i-1}(\xi_{2i-1}) & Ls_{i-1}(\xi_{2i-1}) \\ Lv_{i-1}(\xi_{2i}) & Ls_{i-1}(\xi_{2i}) \end{bmatrix}, \quad T_i = \begin{bmatrix} Lv_i(\xi_{2i-1}) & Ls_i(\xi_{2i-1}) \\ Lv_i(\xi_{2i}) & Ls_i(\xi_{2i}) \end{bmatrix}.$$

Almost Block Diagonal Algorithm

In case of a cubic base we see that the convergence is in the order of 4 but for a quartic we find the convergence order to be 5 with also simultaneous exhibition of super convergence property at the nodal points of the mesh. We can observe from the numerical experiments that the convergence rate also is determined by the step size we take in to consideration and it also becomes evident that in some cases we find too much of an overlap in the solution we have obtained numerically vs the solution that we have obtained from exact solution and hence there might be a small deviation from what we see in the trend of the same method applied to various differential equations with boundary values and interface conditions.

We also see that even if we have slightly deviating values of convergence we gradually see the rate converging to the one that has been theoretically predicted. A common conclusion that can be drawn is that the variation in step size is a determining factor in the improvement of convergence of a given algorithm to a particular problem. But however we cannot assure if the convergence rate of one problem of a particular step size could correspond to a convergence rate of another differential equation with same step size having theoretically same order because the determining factor in rate of convergence is the matrix that we build and the collocation points that come with it along with the boundary and interface conditions.

However the methods of OSCM that we employ to the differential equations are not only accurate but also fast since everything involved can be carried out by simple computations in less time. However there are restrictions that come

in the form of software that we use and the matrix size and the related computations associated with it. But none the less the accuracy we obtain even with the standard limitations is noteworthy for its accuracy and rapid output. Also the numerical results we obtain are class 1 functions and this stands in support of the existence and uniqueness theorem. The OSCM that we have employed here could also be used to solve differential equations with higher order rates and higher super-convergence rates if we shift from cubic/quartic to a higher order base polynomial.And the solution and accuracy will still be valid regardless of the system of coordinates we use. And thus we can say the OSCM methods are widely preferred over the B Splines.

Also one can safely observe that the time taken for the computations take longer time if we try to increase the number of sub intervals in the chosen subspace. The OSCM is of extreme importance due to it's simplicity and wide applicability in diverse fields.

Numerical Example

Consider the problem

$$u_{xx} + 6u_x + u = cosx, \qquad x \in (0,1)$$

With boundary conditions

$$u(0) + u'(0) = 1, \quad u(1) + u'(1) = \cos(1) + \sin(1)$$

CONCLUSION

In this paper, we have applied an ABD solverwith OSCM to one-dimensional linear two point boundary value problems. One numericalexperiment is performed and obtain the fourth order convergence atthe grid points.

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Table 1. we have shown the error in tabular form.

N	$L^{\infty}(u)$	order	$L^{\infty}(u^{'})$	order
20	1.8204e-06		8.3950e-07	
40	1.1319e-07	4.0075e+00	5.2196e-08	4.0075e+00
60	7.0650e-09	4.0019e+00	3.2568e-09	4.0024e+00
80	4.4143e-10	4.0005e+00	2.0347e-10	4.0006e+00
160	2.7579e-11	4.0006e+00	1.2712e-11	4.0006e+00
320	1.7743e-12	3.9583e+00	8.3011e-13	3.9367e+00

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Osmolytes: A Journey from a Simpler Molecule to many Significant Roles

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Received: 31 Dec 2021 Revised: 20 Jan 2022 Accepted: 18 Feb 2022

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ABSTRACT

Osmolytes have significant functions in all kinds of life forms at various positions. Few of the literature has been described osmolyte producing microorganisms. *Saccharomyces cerevisiae* has been reported for trehalose production. Whereas some osmolytes are applied to use in diseases like Alzheimer's as osmolytes play a key role in protein folding. Trehalose is generally synthesized by five different pathways but all the *OtsA-OtsB* pathway is generally found in all trehalose synthesizing organisms, even in plants. Molecules like Ectoine and its derivative hydroxyectoine are often synthesized by halophilic bacteria. Considerably few pieces of kinds of literature have been focused on the synthesis of these osmolytes due to their less availability. Ectoine acts as in zwitterion ion form rather than a simpler biological molecule. The development of large scale production of trehalose leads to the various usages of this disaccharide in the food industry. As it is resistant towards heat and acid, and also can stabilize carbohydrates, proteins, flavours, and aroma it can be used to prevent deterioration of food quality thus very important in food industries. This review discussed four different types of osmolytes Trehalose, Betaine-, Glycine betaine, Ectoine- hydroxyectoine, present in various microbes and their biosynthetic pathways. Osmolytes synthesized by PGPR strains have many applications in enhancing sustainable agriculture practices.

Keywords: Plant growth-promoting rhizobacteria (PGPR), Osmolytes, Biosynthesis, Extremophile, Protein, Halotolerant.

INTRODUCTION

Osmolytes are simple low molecular weight compounds found in plants, insects, microorganisms such as bacteria, yeast, some fungi, and in some invertebrates as well. These osmolytes fall into various chemical groups that include carbohydrates, amino acids, methylamines, polyols, sugar, etc. All these organisms produce osmolytes in different kinds of stress conditions i.e. high temperature, extreme salt concentration, drought, etc. for their survival. By producing such osmolytes, bacterial cells allow themselves to adjust according to the external environment(Sleator and Hill, 2002). They have a significant job in all organisms to conserve the function of cell metabolism as well as preserving the environment of the cell (Chaudhuri et al., 2017). Osmolytes can protect of define properties for organisms (i) by increasing protein stability (ii) facilitating protein fabrication (iii) their presence does not influence enzymes and other cellular activities of the organisms(Hasan et al., 2019). Osmolytes have very important functions in all kinds of life forms at various positions. They are also applied in the medical field to treat many diseases that occur due to protein misfolding and protein accumulation like Amyloidosis, Alzheimer's disease, Parkinson's disease, etc.(Chaudhuri et al., 2017). Osmolytes having antioxidant activity show the defensive function in metabolism e.g. taurine. Glucose plays a significant role in the maintenance of redox balancing. Whereas some osmolytes, generally called counteracting osmolytes found in mammalian kidneys and some other organisms, counteract the impact of high intracellular unreal concentration (Khan et al., 2010).

Another major function of osmolyte is to enhance the shelf life of plants and maintain osmotic balance in abiotic stress tolerance. This is regulated by several osmoprotectants such as proline, glycine betaine, and soluble sugars. The safety and survival of the plant depend on the collaboration of vital osmoprotectants. These are also defined as compatible solutes present in cells, without hindering the biological fluidity and metabolism of the cell (Eiberweiser et al., 2015). Osmolytes can also act as zwitterion ions, non-charged molecules, and provide osmotic balance under stress conditions. In halophiles, osmoadaptation takes place by the mechanism of uptake or synthesis of Osmolytes however the uptake is more supportive. Microorganisms related to archaea, bacteria, yeast, filamentous fungi and algae, depend completely on osmolytes for osmoadaptation except for *Halobacteriaceae* and *Halanaerobiales* **Table 1.** To study the structure of living osmolytes, a technique like NMR spectroscopy can be applied from selected samples. The osmolytes also help to preserve the cell and its components from freezing, desiccation, high temperature, and oxygen radicals (Kondepudi and Chandra, 2011). The ability of osmolytes to maintain the native arrangement of proteins is attributed to their water-binding nature. From the surface of the protein, the osmolytes are excluded and however highly hydrated themselves (Eiberweiser et al., 2015). The accumulation of osmolytes will increase in external salinity or osmolarity and these are such types of stress which are attacked by essentially all free-living microorganisms. When cells are exposed to a high osmolarity environment, there is an increase in the osmotic potential of cell cytoplasm due to a wide range of stress(Czech et al., 2018). This review focuses on three main types of osmolytes with their subtypes. Osmolytes are the most valuable molecules in the upcoming time with many applications like from a protein stabilizers to medical importance. Together we have summarized all the presented data on osmolytes, their mechanism, and applications.

TREHALOSE

Trehalose was discovered for the first time in 1832 by H.A. Wigges from ergot of rye. In the macrocosm, it is broadly distributed and it is also isolated from a broad range of lower organisms containing bacteria, cyanobacteria, fungi, nematodes, crustaceans, insects, etc.(Elbein, 1974). In fungi and yeast, it can be found in vegetative cells, spores, and fruiting bodies (Trevelyan and Harrison, 1956). Trehalose has a very significant function in lower organisms as a source of carbon and in higher animals and plants it plays a major role as an osmoprotectant or as a stabilizing molecule. In various abiotic stressful conditions like extreme temperature, high concentration of salt, drought, and

trehalose plays a defensive role to protect the organism (Kosar et al., 2019). Trehalose can also be used as a cryoprotectant for freezing-drying and as a stabilizing agent in dehydration (Chen et al., 2000). Trehalose is also found in plants, invertebrates, and mammals. Not all but some insects have trehalose as their major blood sugar (Wyatt and Kalf, 1957). The structure of trehalose is comprised of two molecular units of glucose which is bonded by alpha, alpha-1, 1 glycosidic linkage disaccharide. It is a non-reducing sugar and is named alpha-D-glucopyranosyl alpha-D-glucopyranoside. It is also known as α,α-trehalose and can be isolated naturally from living organisms. The other two isomers of trehalose are α,β-trehalose and β,β-trehalose which do not occur naturally but can be isolated from koji and hydrolysates of starch (Elbein et al., 2003). In comparison with other disaccharides, trehalose has some exclusive characteristics because of the involvement of both the reducing subunits in the formation of glycosidic linkage (Jain and Roy, 2009).

MECHANISM

In various stress conditions, trehalose has very major role to play. Cassells and Curry in 2001, defined stress as the abiotic or biotic change in the environment that can cause damage, disease, or change in physiology. The mechanism of trehalose is studied in higher and lower organisms that are exposed to various kinds of stress. Trehalose resists the hostile impact of high salt concentration, drought, and the low and extreme temperature on plants(Tapia and Koshland, 2014). During the lack of water, trehalose works as an osmolyte according to solute protecting membrane and proteins and furnishing dehydration tolerance on cells(Cortina and Culiáñez-Macià, 2005). The resistance towards drought conditions can be achieved by increasing the amount of intracellular trehalose in some plants and animal cells Fig 3. For example by inserting *otsA* and *otsB* genes of *E. coli* into tobacco plants an adequate amount of trehalose is produced which leads to performance in growth in drought conditions (Pilon-Smits et al., 1998). Because of 1,1 glycosidic bonding, trehalose remains resistant to acid hydrolysis, therefore acts as a very strong and protecting agent against abiotic stress conditions. During extreme temperature and dried conditions, trehalose forms a glass surrounding the biological molecule and remains stable. By the formation of trehalose glass, a specific environment is created that can implicate biomolecules. In these high-temperature conditions, trehalose can also be present as various forms of crystalline that can implicate water. The most important characteristic which separates trehalose from other disaccharide sugar is its potency to maintain its structural properties while transitioning from one state to another (Vanaporn and Titball, 2020).

GENES AND ENZYMES INVOLVED IN BIOSYNTHESIS OF TREHALOSE

There are many pathways reported for the synthesis of trehalose. Generally, five pathways of biosynthesis are known to occur naturally for trehalose, which are the *otsA-otsB* pathway, *TreP* pathway, *TreS* pathway, *TreY-TreZ* pathway, and *TreT* pathway. Among all of these pathways, the *OtsA-OtsB* pathway is a very famous to be seen in all the prokaryotic and eukaryotic organisms for the synthesis of trehalose. This is the only pathway for trehalose biosynthesis to be found in plants (Paul et al., 2008). In microorganisms, the synthesis of trehalose is made up of two steps. In the first step, the synthesis of trehalose-6-phosphate is catalyzed by trehalose-6-phosphate synthase (TPS) from glucose-6-phosphate and uridine diphosphate glucose (UDPG) Fig.1, UDP is also released in this step. In the next step, dephosphorylation takes place and trehalose-6-phosphate is dephosphorylated by trehalose-6-phosphate phosphatase (TPP) to form trehalose (Iordachescu and Imai, 2008). In *E. coli* and *S. cerevisiae,* this biosynthesis pathway is studied briefly. In *S. cerevisiae* a complex of TPS homolog (TPS1) is formed with TPP homolog (TPS2) and other two controller subunits that is TPS3 and TLS (trehalose synthase long-chain) (Bell et al., 1998). Whereas in *E. coli* TPP and TPS are individual substances. In *E. coli* the reactions of the biosynthesis pathway are catalysed by TPS which is a yield of the *otsA* gene and TPP that is from the *otsB* gene (Kaasen et al., 1992). During the stationary phase and osmotic stress, the transcription of genes *otsA* and *otsB* is induced in *E. coli*. The expression of genes *otsAB*is connected to RpoS which is chief regulator of the stress response. When an organism exposed to various stress, the trehalose biosynthesis pathway can occur in a RpoS-independent manner (McIntyre et al., 2007). In a eukaryotic organism, the pathway for trehalose synthesis is catalyzed by trehalose phosphorylase. This pathway is reversible production of trehalose from glucose-1-phosphate and glucose. This pathway is regulated by gene *treP* and can be seen in fungi *Agaricus bisporus*(Wannet et al., 2000) and protist *Euglena gracilis*(Belocopitow and Maréchal, 1970).

The third alternative pathway was discovered in *Pimelobacter* (Tsusaki et al., 1996) and in *Thermus aquaticus*(Tsusaki et al., 1997). In this pathway α (1-4)linkage in maltose is transformed to α (1-1) linkage to produce trehalose. The gene *treS* is responsible for the catalysis of this reaction (De Smet et al., 2000). Pathways for trehalose production from glycogen are also reported in *Arthrobacter*(Maruta et al., 1996a), *Rhizobium* (Maruta et al., 1996b), and in *Sulfolobus acidocaldarius* (Maruta et al., 1996c). The conversion of α (1-1) linkage from the terminal α (1-4) linked glucose polymer residue takes place by maltooligosyltrehalose synthase which is encoded by *treY*. The hydrolase enzyme MOT trehalohydrolase (encoded by *treZ*) cleaves this terminal disaccharide, and the free trehalose molecule is released (De Smet et al., 2000). The TreT pathway is observed in extremophilic organisms. This pathway is reported in archaea *Thermococcus litoralis* (Qu et al., 2004), *Pyrococcus*(Ryu et al., 2005), and bacteria *Thermotoga maritime* (Worning et al., 2000). This pathway takes place by catalyzation of ADP-glucose and glucose by trehalose glycosyl transferring synthase.

Biosynthesis of trehalose in plants in catalyzed *OtsA-OtsB* pathway. In this pathway, UDP-glc and glc-6-P are catalyzed by TPS, and the formation of trehalose-6-phosphate takes place (Blazquez et al., 1998), this trehalose-6-phosphate is then converted into trehalose by enzyme TPP. In *Mycobacterium,* there are three different pathways reported for the biosynthesis of trehalose. These pathways are *OtsA-OtsB, TreS*, and *TreY-TreZ*(De Smet et al., 2000). Among these, the *TreS* pathway is the major studied pathway for the synthesis of trehalose in *Mycobacterium*, because this pathway is interconnected with *in vivo* production of glycogen and trehalose (Chandra et al., 2011). Similarly in another bacteria *Corynebacterium glutamicum,* it is also found to have these three pathways- *OtsAB, TreS*, and *TreYZ* for the biosynthesis of trehalose (Wolf et al., 2003).

APPLICATIONS

One of the most important aims of trehalose biosynthesis in organisms is to protect themselves and to stabilize the life for their survival from freezing or drought stress conditions(Elbein, 1974). Trehalose plays a major part to defend bacteria from various stress conditions. For example, the increased amount of trehalose in the spores of *Streptomyces griseus* helps to protect against heat and drought stress (McBRIDE and Ensign, 1987). During dry conditions, trehalose plays a very significant role, for example, the cell membrane of *E. coli* is protected by trehalose and it encourages the formation of a glassy state during dehydration (Welsh and Herbert, 1999). At lower temperatures, the production of trehalose in *E. coli* may be induced to protect and survive the cold conditions (Kandror et al., 2002). In some organisms, trehalose is reported as a source of energy during the initial stage of their life, for example, the spore germination stage(Elbein, 1974). For an example incorporation with glycolipids, trehalose in *mycobacteria* can work as a structural constituent (Elbein and Mitchell, 1973). Trehalose subordinates play the role of intermediate molecules in metabolisms and also as constructive molecules in some of the microorganisms (Elbein, 1974). In yeast *Saccharomyces cerevisiae,* trehalose provides resistance towards cold and water stress (Mackenzie et al., 1988) and against oxygen radicals (Benaroudj and Goldberg, 2001).In some insects, trehalose can work as a source of energy for flying (Wyatt and Kalf, 1957).

Unsaturated fatty acids and proteins are protected by trehalose from oxidative impairment (Furuki et al., 2009). Trehalose has a very important function in the neutralization of reactive oxygen species (ROS) and maintaining the anabolism machinery of protein (Chang et al., 2014). Labile proteins are also preserved in drought conditions by trehalose (Elbein et al., 2003). During drought conditions, stabilization of protein and phospholipids may take place in the lipid bilayer. The protein folding and enzyme activity are also affected by the increased amount of trehalose in organisms. Trehalose has many application in food industry. The development of large scale production of trehalose leads to the various usages of this disaccharide in the food industry. As it is resistant towards heat and acid, and also can stabilize carbohydrates, proteins, flavours, and aroma it can be used to prevent deterioration of food quality thus very important in food industries (O'Donnell and Kearsley, 2012). Trehalose is widely used for the maintenance of polyphenol content, antioxidant activity, aromatic unstable compounds (Kopjar et al., 2013), and colour (Umene et al., 2015) in food industries. It can also be found in bread, honey, mushroom, wine, and beer so it can be considered as a food aliment for humans (Burek et al., 2015).

Plant growth promoting bacteria which are producing trehalose has a very major role in protecting bacteria against different stresses, it can help bacteria to survive inside and outside of the host. For example, because of the ability to produce trehalose, some bacteria include *Rhizobium leguminosarum*(McIntyre et al., 2007), *Sinorhizobium meliloti*(Jensen et al., 2005), and *Bradyrhizobium japonicum*(Sugawara et al., 2010) form plant root nodules. Under the stress of hydrogen peroxide, the resistance impact of trehalose has been visualized towards hydroxyl radical in vivo and in vitro by growing *TPS1* tobacco plants (Romero et al., 2002). In one study it is observed that while drought stress condition trehalose is associated with seed oil maintenance (Delorge et al., 2014). Transgenic plants become more resistant to abiotic stress because of the over expression of the trehalose gene. The overexpression of *TPS* and *TPP* genes from *E. coli* can cause resistance towards abiotic stress but still, some plants show stunted growth and pleiotropic changes because of the unfavourable effect of T6P while plant growth and development (Lyu et al., 2013). Trehalose also has some important applications in the cosmetic industry. It can be used in creams and lotions to maintain moisture condition and to preserve the odour of its active components (Ohtake and Wang, 2011). It is also used in deodorants (Higashiyama, 2002).Trehalose can be used as a cryopreservative agent for stem cells and also used to preserve organs and tissues for transplantation. Trehalose can work as an additive agent that increases the shelf-life of vaccines and antibodies. It can also be helpful for the storage of thermolabile enzymes such as DNA polymerase or restriction enzymes at the appropriate temperature (Iturriaga et al., 2009). Consumption of food having trehalose would be good for bone metabolism because it could help to prevent osteoporosis (Higashiyama, 2002).

Trehalose-lipids has so many different applications. It can be used in biomedical or healthcare as antifungal, antiviral, antitumor agents, adhesive agents, immunomodulatory molecules, and vaccines. It has various applications in cosmetics and food also, where it can be used as an emulsifier, solubilizer, suspension, wetting, and foaming agents as explained in Fig 2. The environmental application of trehalose-lipid includes bioremediation of soil, sands and shales, biodegradation of PAHs, and several chlorinated pesticides (Christova and Stoineva, 2014).

ECTOINE AND HYDROXYECTOINE

Osmolytes like ectoine and hydroxyectoine are low molecular weight organic molecule, which has strong water-binding properties and acts as a zwitterion compound. They are combined by various aerobic chemoheterotrophic and halophilic/halotolerant bacteria. Their production is enhanced by the accumulation of the surrounding environmental conditions by improving the stability of biomolecules. Ectoine [(4S)2-methyl-3,4,5,6-tetrahydro pyrimidine-4-carboxylic acid] was first investigated by Galtlinski in *Ectothiorhodospira halochloris* bacteria. It is an extremely halophilic phototrophic eubacterium. Ectoine was discovered by the scientists Inbar and Lapidot in 1988 in an accident when they were working on gram-positive bacterium *Streptomyces parvulus*. Later on, another derivative molecule was derived from the ectoine which was name ashydroxyectoine [(4S,5S)-2-methyl-5-hydroxy-1,4,5,6-tetrahydro pyrimidine-4-carboxylic acid] (Czech et al., 2018). The ectoine and hydroxyectoine are also chemically classified as heterocyclic amino acids and both are naturally occupied as compatible solutes. After it has been discovered some other substance was also found in various moderately halophilic bacteria. In NaCl concentrations, it can be grown at up to 5M. It has been shown that ectoine with some other compatible solute decreases water activity or it can generate high osmotic pressure in a cell with a small concentration (Eiberweiser et al., 2015). The first ectoine was newly found in small amounts and due to this most of the research was hampered for this novel compound, at that time researchers do not have any hope to use it on any immediate commercial scale when the researcher started for the testing of this chemically synthesized ectoine, it was found that ectoine can give interesting results with cosmetic application. Immediately after this invention researchers started screening ectoine-producing microbes for large-scale production(Melmer and Schwarz, 2009).

The search began with a method called “bacterial milking" for the initial production of ectoine from a popular halophilic bacteria *Halomonas elongate.* Large-scale production was obtained by this method optimized by various researchers. However, this method has some drawbacks like corrosion to the equipment because of high salt concentration. As a consequence, a very low amount of growth was obtained hindering downstream processing. To overcome this disadvantage, the researcher has applied transgenic non-halophilic bacteria to enhance the production

of ectoine. The ectoine biosynthetic gene cluster from various halophilic bacteria has been expressed in *Escherichia coli (E. coli)* for boosting the production of ectoine in the scale-up process(Parwata et al., 2019). It has a central intermediate in microbial amino acid metabolism and cell wall synthesis and has a precursor of L-aspartate-β-semialdehyde (Widderich et al., 2016).

MECHANISM

Ectoine and hydroxyectoine are assumed to be dependent on their hydration properties, it has recently been studied by using computer simulations, confocal Raman spectroscopy, and neutron diffraction experiments. It was reported that dry hydroxyectoine behaves more glass-like than ectoine due to the stronger intermolecular OH-bonds and depends on infrared-spectroscopy and electron spin resonance. In recent studies, it was shown that the ectoine is in dielectric spectroscopy measurements. It was strongly hydrated even in the presence of high salt concentrations, where the ions are expected to alter the hydrogen bonding. For ectoine, high sodium chloride (NaCl) concentrations have also been reported using optical Raman spectroscopy and due to the interaction among hydroxyectoine remains unexplored as of yet (Sahle et al., 2018). Ectoine can stabilize different native folded states compared to the unfolded denatured state due to this free energy variation has been occurred for denaturation ((ΔG_{denat}) is lifted in a consequence of an added unfavourable interaction about the solute-containing solvent and also the unfolded state. Expected into their special separation near to the protein surface, the experience of protein stabilization can be described as the decision of the tendency to minimize the surface area(Kunte et al., 2014). The salt in and salt out strategies and can be counteracted in high osmolarity-instigated water efflux. Salt in strategies have high salinity stress to accumulate favourable microorganisms, due to this it can only limit the number of microorganisms. In salt-out strategies, it has an uptake of a high-osmolarity environment or has a restricted set of organic osmolytes, the compatible solutes.

GENES AND ENZYME INVOLVED IN BIOSYNTHESIS OF ECTOINE AND HYDROXYECTOINE

Biosynthesis of ectoine has been carried out by the precursor or a three-step enzymatic reaction which converts the precursor of L-aspartate- β -semialdehyde, this is mediated by microbial amino acid metabolism and the cell wall synthesis(Widderich et al., 2014). The ectoine have ABC enzymes, which includes diaminobutyric acid (DABA), it has enzymatic steps that are catalyzed by L-2,4-diaminobutyrate transaminase (*EctB*), 2,4-diaminobutyrate acetyltransferase (*EctA*), and ectoine synthase (*EctC*) (Bownik and Stępniewska, 2016) to yield the cyclic ectoine molecule [(4S)-2-methyl-3,4,5,6-tetrahydropyrimidine-4-carboxylic acid]. These are the genes that are encoded by the enzymes and are organized as (*EctABC*) from *Halomonas elongata*. It has structural genes which are fundamentally organized in an operon (*EctABC*) and in stress conditions, this operon model is up-regulated in the transcription process and *Ect* genes are triggered in some microorganisms depicted in Fig.1. The 5-hydroxyectoine is served as a compatible solute *in vivo* and *in vitro*, it exhibits protein-stabilizing properties and it has a sub-group of ectoine producers which synthesize a hydroxylated Ectoine. 5-hydroxyectoine, in a biosynthetic reaction and the reaction, is catalyzed by the ectoine hydroxylase *(EctD)*(Bursy et al., 2008). The analysis of enzyme activity was present in the cell-free extract, found in *E. halochloris* and *H. elongate* and it was originally defined in the biosynthesis route of ectoine. Also, form a major contribution to follow the biochemistry of ectoine in the biosynthesis of enzymes. *Salibacillus salexigen* was originally identified by the biochemical properties of ectoine hydroxylase. The biosynthesis gene of ectoine and hydroxyectoine is used to provide the primers for molecular analysis of the *Streptomyces chrysomallu* for *ectABCD* and the *M. halophilus* for *ectABC* genes (Czech et al., 2018).

It is the first step of the enzyme synthesis pathway which began with a reaction catalyzed by the L-2,4-diaminobutyrate-2-oxoglutarate transaminase *EctB*, with the help of precursor L-aspartate- β -semialdehyde. *EctB* made some aminotransferases like dependent enzymes of a pyridoxal-5-phosphate and needed K^+ for its activity and stability. The reversible transfer of an amino group from L-glutamate to the aldehyde group of the substrate, and then it will form the L-2,4-diaminobutyrate (DABA) and 2-oxoglutarate. Both orthologous enzymes *H. elongata* and *Methylomicrobium alcaliphilum* have been reported in *EctB* by the biochemical characterization. The *H. elongate* has optimal catalytical activities and has been recorded at a temperature of 25 °C, high alkalinity pH of 8.6 and KCl concentration of 0.5 M. (Czech et al., 2018). It has a native molecular mass of 21.2 kDa (Schwibbert et al., 2011).

In the second step of the enzyme synthesis pathway of L-2,4-Diaminobutyrate Acetyltransferase EctA is formed by N-γ-acetyl-2,4- diaminobutyrate (N-γ-ADABA) and it will change the DABA and the co-substrate acetyl-coenzyme A. Originally is to report on the enzymatic properties of an EctA ortholog which isolated from *H. elongata*. It has an enzyme that associates with a big family of GCN5-related-N-acetyltransferases (GNAT) which bring the transfer of acetyl group from acetyl-coenzyme A as a donor to a primary amine as an acceptor molecule. It brings the enzyme which showed its highest purified activities at different levels like at temperature of about 20 °C, at pH 8.2 and due to the presence of 0.4 M NaCl. It has three biochemical characters of orthologous forms of methanotrophic or methylotrophic bacteria (*M. alcaliphilum, Methylophaga thalassica*, and *Methylophaga alcalica*) (Czech et al., 2018). It has a native molecular mass of about 45 kDa ((Schwibbert et al., 2011).

In the third step of the enzyme synthesis pathway, it shows a ring closure to form the end product of ectoine. Due to this ectoine synthase, EctC forms a reaction catalyzed due to intramolecular condensation. EctC catalyzes the ring enclosure of ectoine and forms the generation of an intramolecular imino bond and ejecting water molecules from a carbonyl bond in the substrate N-γ-ADABA. The ectoine synthase of *H. elongate* shows the highest enzymatic activity at a pH of 8.5–9.0, a temperature of 15 °C, and in the presence of 0.5 M NaCl. In the purified enzyme forms which it stabilizes in presence of high salt concentration Although a hike in salt concentration and the temperature were correlated. It was observed that with an increase in temperature from 15 °C to 30 °C with respect to salt concentration from 0.77 M to 3 M. the kinetic energy of the enzyme was affected.

Ectoine synthase reaction, measured in crude cell extracts of *Halorhodospira* (formerly *Ectothiorhodospira) halochloris,* and was first described by Galinski and co-workers, who described the ectoine biosynthetic pathway and they have demonstrated its reversibility (Czech et al., 2018). It has a native molecular mass of 15.5 kDa (Schwibbert et al., 2011).The enzyme synthesis pathway of Ectoine Hydroxylase *EctD*. It has a considerable number of ectoine makers which additionally synthesize 5-hydroxyectoine by the position and stereospecific hydroxylation with the help of ectoine. It explains the biochemical basis for the development of 5-hydroxyectoine through studies with the help of purified ectoine hydroxylases (*EctD*) from the moderate halophile *S. salexigens*, it was also reclassified as *Virgibacillus salexigens* and the soil bacterium *S. coelicolor*. It was biochemically analyzed and revealed that *EctD* is a member of the superfamily of non-heme Fe(ii)-containing and 2-oxoglutarate-dependent dioxygenases. The *EctD* has an O_2-dependent hydroxylation in which the substrate ectoine followed by the oxidative decarboxylation of 2-oxoglutarate and then it will form succinate and CO_2, it has an iron cofactor that acts as a catalyst for the activation of molecular oxygen and the enzyme EctD is highly dependent on the presence of molecular oxygen. In *EctD* the stereochemical configuration has a 5-hydroxyectoine which produced in vivo by *S. parvulus* and other is in vitro which purified by *V. salexigens* of *EctD* enzyme and analyzed by H-NMR spectroscopy (Czech et al., 2018). It has a molecular mass of 37.4 kDa (Schwibbert et al., 2011).

APPLICATION OF ECTOINE AND HYDROXYECTOINE

Ectoine and hydroxyectoine are involved in enhancing the shelf life of nucleic acids and many enzymes and protect them in high-stress conditions, or during uneven temperatures in bacteria. A large number of osmolytes are required for cultivable products. *Halomonas elongota* can be grown on media with 10% NaCl and due to this, it accumulates up to 2.25 M ectoine per litre of osmotic volume. It doesn't require any specific growth factors and can grow in a wide range of pH, salinity, temperature, and have a high osmotic shock (Detkova and Boltyanskaya, 2007). Ectoine is the first commercially used as a skincare ingredient and it has a different application that plays a major role, mostly in sun protection and anti-ageing products.. Although ectoine acts as a stabilizer and impart cell membrane function during desiccation conditions. When the cell cycle gets hit due to continuous freezing and thawing, ectoine act as desiccation resistant to the cytoplasm membrane of the cell (Czech et al., 2018).

In recent decades the use of ectoine has been increasing in health care products and other important products. It has been shown that ectoine can suppress the initial UV-A radiation-induced ceramide signal response in human keratinocytes, and it has been examined at different conditions of human skin and because of this skin, it has different properties like- skin hydration, skin elasticity, and skin surface structure. The reduced effects of ectoine are

not only detected in the skin but also in inflammatory conditions of other epithelia (Kunte et al., 2014). There are different ranges of medical-based products which are used to treat allergies and other inflammatory conditions like atopic dermatitis, the treatment of dry eye and dry nose has been evolved in clinical trials and commercialized in the market after successful testing. The treatment of epithelial was derived from the inflammatory diseases, especially nanoparticle-induced, lung inflammation colitis, and tissue protection in ischemia and potentially these all are future applications (Kunte et al., 2014). They can serve as water-attracting and water-structure-forming compounds which are used to equalize macromolecules and the entire cells between their chaperon and glass-forming effects, to protect DNA from ionizing radiation(Czech et al., 2018).

Both osmolytes also have a significant role in *in-vitro* biological applications of osmoregulation and are used in commercial activity (Graf et al., 2008). Compatible solutes have been used or have various ranges in wastewater treatment systems or biological waste to prevent osmotic and other related environmental stress. It also works as the addition of glycine betaine and trehalose, because of this, it got the reaction of ectoine which was evaluated in this regard. The study of ectoine about denitrification has been countered by an increase in salinity and a denitrifying microbial consortium is used. In the denitrification process, ectoine is used to accelerate the process and it was assumed that the nearly ended removal of nitrates and nitrites relative to the control samples in a small-time because of this will increase the activity of degradative enzymes. Increased salinity conditions were used in the Anammox process (by about 40%) and all this happened because of the addition of ectoine.

These all demonstrate the use of compatible solutes in general and about the particular osmolytes like ectoine, for these type of applications, we can also conclude that ectoine can be used in large-scale wastewater treatment systems and biological waste but it all can be acquired when the production cost of ectoine would drop headlong and come equal or opposite with the bulk-chemical glycine betaine(Czech et al., 2018). *Halobacterium* isolated from the rhizosphere of *Sesbania grandiflora* named Bacillus spp PU1^{T} has been reported for Plant growth promoting activity like phosphate solubilization, auxin production, siderophore production. The PGPR activity of bacteria was screen out on four different plants at high temperature by (Tipre et al., 2015)

BETAINE AND GLYCINE-BETAINE

The first discovery of betaine is reported in the 19th century from the juice of beets and then later it was also found in other different organisms (Craig, 2004). Betaine is also named as trimethylglycine because its structure looks as glycine having three extra groups of methyl (Yu et al., 2004). Protein and several other biomolecules that are present in a cell can get affected by various stress. To protect the structure of the protein, plants use several mechanisms that can increase protein stability, prevent aggregation of protein and degrade irreversibly damaged protein. Over expression of chaperones and some stabilizing protein can help to prevent aggregation and stabilize proteins (Wang et al., 2004), it can also be done by the accumulation of osmolytes. Glycine-betaine is a very common osmolyte found in plants, bacteria, and animal cells(Yancey et al., 1982). It also plays a major role in several biochemical as well as cellular processes such as antioxidant activity, macromolecule stabilization process, metabolism of homocysteine, apoptosis, etc (Figueroa-Soto and Valenzuela-Soto, 2018a). Betaine ($C_5H_{11}NO_2$) is an amino acid, a methylated nitrogen compound, having glycine with three methyl groups. It is also known as trimethylglycine (Frank, 2014). it is an amphoteric compound.

Glycine-betaine is a quaternary amine present in zwitterionic compounds, which is discovered by Scheibler in 1869. It is present in many foods such as wheat, beetroot, quinoa, clams, spinach, and mussels and accumulates in fungi, bacteria, plants, and animals. Glycine betaine has many biochemical and cellular processes such as antioxidant activity, macromolecule stabilization process, metabolism of homocysteine, apoptosis, etc(Figueroa-Soto and Valenzuela-Soto, 2018b)**.**

MECHANISM

Betaine is a coherent osmolyte that supplements the water retention of cells, alters inorganic salts, and protects intracellular enzymes from osmotic stress(Yancey et al., 1982). The main function of betaine in plants and

microorganisms is to defend cells against osmotic inactivation. In various stress conditions, betaine stabilizes cellular metabolic functions in some organisms and animal tissues(Chambers, 1995). To protect cells from higher electrolytes concentration and urea, the accumulation of betaine takes place in the kidney via external origin(Moeckel et al., 1994). Glycine-betaine is a major studied osmolyte in plants and bacteria. It has major roles in the kidneys of animal cells whereas in humans it plays a very important role in the liver in homocysteine metabolism as a methyl donor. Because of its protein stabilization nature, Glycine-betaine is also known as osmoprotector. It is involved in cell metabolism therefore also known as cytoprotector. Glycine-betaine plays important role in protein synthesis, which is studied by changes in expressions of the gene, and those expressions are measured by RT-PCR, qRT-PCR, or western blot (Figueroa-Soto and Valenzuela-Soto, 2018a). The accumulation of glycine-betaine in plants occurs in higher concentrations in response to various abiotic stress conditions in order to protect plants. The plant tolerance against various abiotic stress is improved by glycine-betaine via protein and membrane stabilization, transcription and translation processes and by the enhancement of antioxidative enzyme activity (Park et al., 2006).

GENES AND ENZYMES INVOLVED IN BIOSYNTHESIS OF BETAINE AND GLYCINE BETAINE

The biosynthesis of the glycine-betaine takes place by two-step oxidation of choline with the toxic intermediate betaine aldehyde (Hasanuzzaman et al., 2019). The enzymes involved in this process are choline monooxygenase (CMO), choline dehydrogenase, and betaine aldehyde dehydrogenase 1 (BADH1). CMO is a ferredoxin-dependent soluble Rieske type protein whereas BADH1 is NAD+ dependent soluble protein (Roychoudhury and Banerjee, 2016). The formation of choline takes place by the L-serine-ethanolamine pathway that further reduces to betaine aldehyde by CMO in plants and by choline dehydrogenase (Grossman and Hebert, 1989) in animals and some bacteria. In the next step, BADH1 oxidizes betaine aldehyde into glycine-betaine. Cánovas et al., (2000)isolated *betI* gene involved in oxidation pathways of choline to glycine betaine from Halomonas elongate DSM 3043. Other three open reading frame *betIBA* was found arranged in operon for conversion of choline to glycine betaine.

Some microorganisms include bacteria *Arthrobacter globiformis* and *Arthrobacte pascens* and fungi *Aspergillus fumigatus* oxidizes choline to glycine-betaine by using only one enzyme that is choline oxidase (Figueroa-Soto and Valenzuela-Soto, 2018a). In extreme halophilic bacteria such as *Actinopolispora halophilia* and *Ectothiorhodospira halocloris* synthesis of glycine-betaine takes place from glycine by three steps of methylation, which is catalyzed by glycine sarcosine methyl transferase (GSMT) and sarcosine dimethylglycine transferase (SDMT). In this process, S-adenosylmethionine (SAM) is used as a methyl donor (Kimura et al., 2010). The synthesis of glycine-betaine in plants takes place in chloroplasts, the cytoplasm, and/or peroxisome(Nakamura et al., 1997); whereas in animals it is synthesized in cytoplasm and mitochondria(Chern and Pietruszko, 1995).

APPLICATIONS

Betaine is present in almost all kinds of living organisms including plants, animals, and microorganisms. The major function of betaine is as an osmolyte and methyl donor. Betaine provides protection to the cells, proteins, and enzymes from various environmental stress conditions such as drought, high salinity, or high temperature. Glycine-betaine is a determinant of osmolyte. To maintain the cellular osmotic pressure and producing tolerance against various abiotic stress, it is very important to transport it throughout the cell (Roychoudhury and Banerjee, 2016). To resist various environmental stress conditions, many plant species synthesizes glycine-betaine. In protein refolding, glycine-betaine plays a major role as a molecular chaperone (Bourot et al., 2000).Betaine is very useful for *in vitro* shoot multiplication in many plants such as tomato, sweat paper, watermelon, olive trees, and tea (Saini et al., 2012). Glycine betaine acts as a polyamine component that accumulates in many plants under drought stress. Glycine betaine has wide application in medicinal crops under field environmental conditions. Less concentration of GB show less wilting sign in comparison to other plants which is increasing by photosynthesis method (Armin and Miri, 2014).Some studies show that glycine-betaine improves plant tolerance against oxidative stress by increasing antioxidant enzyme activity (Hu et al., 2012).

It is used as feed supplements in poultry for animal feed. It is used as a dietary feed supplement in animal nutrition for more than fifty years (Craig, 2004), a highly valuable feed supplement in the diets of many farm animals, and is

also known as a multi-nutritional agent. Betaine helps birds to resists poor environments and heat stress (Ahmed et al., 2018). In farmed fishes, betaine feed is used as osmolyte which protects fishes from salinity stress (Craig, 2004).Glycine-betaine has a major role in the metabolism of ethanol, carbohydrate, lipid, and homocysteine/methionine (Figueroa-Soto and Valenzuela-Soto, 2018b). It also has several industrial applications such as in the food and cosmetic industries it is used for its zwitterionic, hygroscopic, and osmoprotective characteristics(Mäkelä, 2004). Betaine is also used in the enhanced oil recovery process, which is the central focus process of the energy industry to improve recovery factors for the production of hydrocarbons (Yarveicy and Javaheri, 2019). Betaine takes participation in the methionine cycle as a methyl donor in the kidney and liver of humans (Craig, 2004)

CONCLUSION

There are many biological molecules that are not in sight due to the unavailability of literature. Some of them are osmolytes products which can be in the structure of sugar amino acids and proteins sometimes. Detailed research is required to create a prominent interest in this type of molecule. Those biological molecules not only act as osmolytes but can also be applied in the various biotechnological field as discussed earlier in the application portion of each osmolyte. Sugars like trehalose have tremendous demand in the industry because of their multitasking role. However, ectoine and hydroxyectoine are the least studied osmolytes as they are produced by rare halotolerant bacteria. Perhaps focusing on such osmolyte producing bacteria can encourage their production in the industry with low cost rather than synthesizing chemically. Many researchers are alos focusing on plant growth-promoting rhizobacteria for sustainable and biotic stress tolerant yield of crops.

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Table 1: Description of osmolytes producing microbes with their specific role

Microorganisms name	Osmolytes	What is used for.	Reference paper
Bacillus subtilis	Trehalose	As carbon source	(Whatmore et al., 1990)
Chromohalobacter salexigens	Ectoine & hydroxyectoine	Thermoprotectant	(García-Estepa et al., 2006)
Saccharomyces cerevisiae	Glycerol	Chief osmoprotectant	(Hohmann, 2002)
Listeria monocytogenes	Glycine betaine	Cryoprotectant	(Chattopadhyay, 2002)
Archaeoglobus fulgidus	Glycine betaine	Thermoprotectant	
Saccharomyces cerevisiae	Trehalose	Osmoprotectant	(Kiewietdejonge et al., 2006)
Propionibacterium shermanii	Proline	Protects plants from various stress	(Hayat et al., 2012)
Anaerococcus hydrogenalis	TMAO	Protect against the adverse effects of temperature, salinity, high urea, and hydrostatic pressure	(Romano et al., 2015)
Candida boidinii	Sorbitol	Serves as a storage and transport sugar in most plant families	(Li et al., 2012)
Lactobacillus plantarum	Arabinose	Provide innate immunity to plants	(Bolouri Moghaddam and Van den Ende, 2012)
Lactobacillus leichmanii	Mannitol	Stabilization of the structures of membrane lipids and proteins at low water activity	(Leslie et al., 1995)

Table 2: List of gene transfer for specific osmolytes from selective bacteria aiming to construct transgenic plants to enhance resistance against abiotic stress.

Organisms from which gene is taken	Recipient organism	Osmolyte	Application	Stress	Reference
Escherichia coli	Tobacco and *Arabidopsis*	Mannitol	Resistance against salinity stress	Salinity stress	(Tarczynski et al., 1993)
Methanohalophilus portucalensis	*Arabidopsis*	Glycine-betaine	Promotes tolerance to drought and salt stress	Drought and salinity stress	(Lai et al., 2014)

Grifola frondosa	Tobacco	Trehalose	Enhances photosynthetic activity with higher soluble carbohydrate levels	Dessication and salt stress	(ZHANG et al., 2005)
Malus domestica	Tobacco	Sorbitol	Synthesis of a sugar alcohol	Decreasing water stress	(Tao et al., 1995)
Halomonas elongata	Tobacco cells	Ectoine	Low level of resistance towards osmotic stress under certain level of mannitol	Hyperosmotic stress	(Nakayama et al., 2000)
Arabidopsis	*Petunia hybrida*	Proline	The growth of petunia plants was influenced	Drought stress	(Yamada et al., 2005)
Winter flounder	Tobacco	Proline	Plant growth promotion and resistance against stress	Drought stress	(Kishor et al., 1995)
Arthrobacter globiformis	Tobacco lines	Glycine-betaine	Reduction of oxidative damage under freezing stress	Freezing stress	(Parvanova et al., 2004)

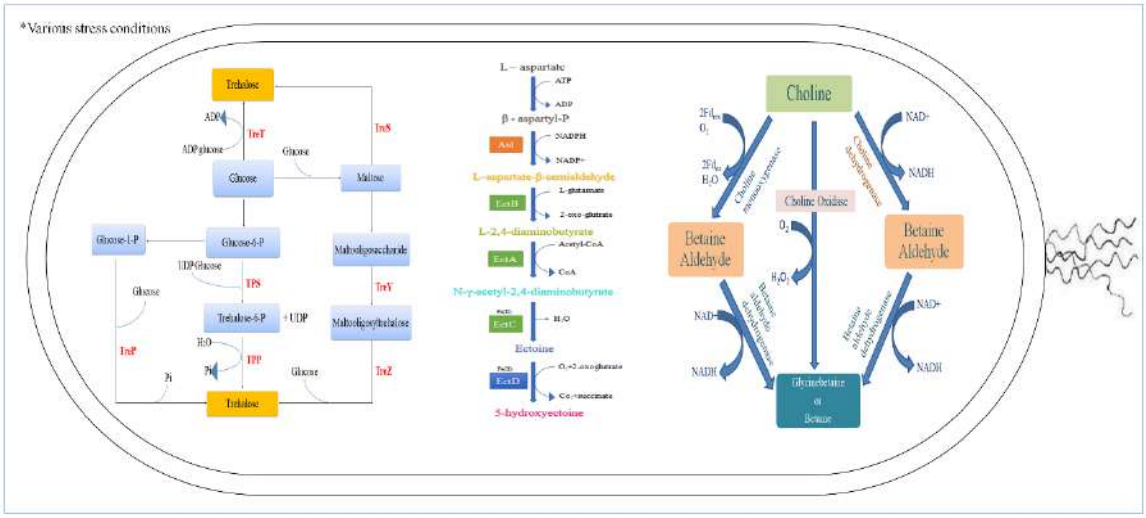

Figure 1: Biosynthesis pathway of osmolytes in bacteria. Due to the accumulation of stress conditions cells release osmolytes like trehalose which is synthesized by three different pathways, whereas ectoine and hydroxyectoine are synthesized subsequently. Betaine and glycine betaine synthesis is initiated byprecursor choline which parts in two different steps involving enzymes necessary for the synthesis of glycine betaine and betaine.

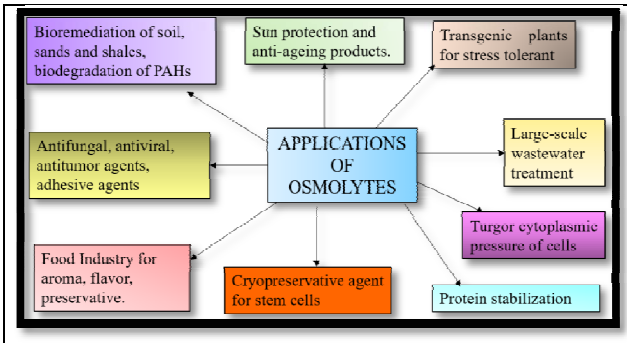

Figure 2: In general applications of osmolytes in different fields of biological sciences

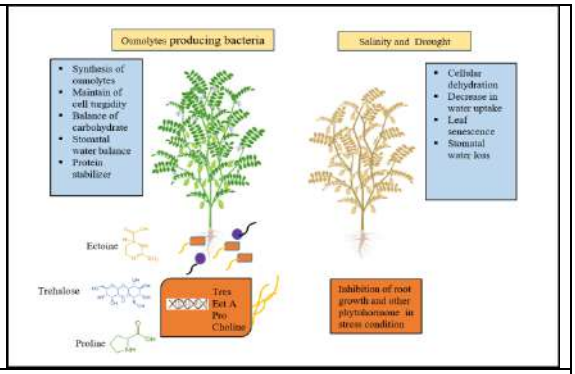

Figure 3: When the plants are treated with osmolytes producing bacteria there is a resistance to stress compared to untreated plants. In drought or salinity stress when osmolytes producing PGPR is added as inoculant they protect the plant by releasing various osmolytes in the rhizosphere and maintain the plant regular growth cycle.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Comparative Analysis of Contrast Enhancement of Retinal Fundus Images using Histogram-Based Methods

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Received: 08 Feb 2022 Revised: 25 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

Retinal fundus images captured by the fundus camera are commonly used for detecting retinal disorders. The captured images highly suffer from various noises and contrast issues that result in image quality deterioration. The distorted images demarcate the visual analysis of the ophthalmologists, and it may lead to inaccurate results. The preprocessing of retinal fundus images can reduce the noise and enhance the image quality by normalizing and adjusting the contrast and luminosity levels. In this paper, histogram-based image contrast enhancement methods such as Histogram Equalization (HE), Adaptive Histogram Equalization (AHE), and Contrast Limited AHE (CLAHE) are used to compare and evaluate the performance on two different Diabetic Retinopathy (DR) datasets, Eye PACS and Messidor-2. The histogram-based techniques are easy to implement and computationally efficient than other enhancement techniques. The Peak Signal-to-Noise Ratio (PSNR), Signal-to-Noise Ratio (SNR), and Structural Similarity Index Measure (SSIM) are the parameters used to assess the performance. It has been found that CLAHE provides better results than HE and AHE by enhancing image contrast and quality.

Keywords: Retinal fundus images, Retinal disorders, Noise, Contrast enhancement, Quality deterioration, Luminosity, Histogram Equalization (HE), Adaptive Histogram Equalization (AHE), Contrast Limited AHE (CLAHE), EyePACS, Messidor-2, PSNR, SNR, SSIM.

INTRODUCTION

In the past few decades, Retinal imaging has created a significant impact with its rapid development in biomedical and patient management. Fundus photography facilitates population-based, mass detection of retinal diseases such as Diabetic Retinopathy (DR), Glaucoma, and Macular degeneration and, so on. Retinal images acquired from the fundus camera can badly be influenced by distinct noises and distortions due to several reasons. Some of them are the inappropriate focus, decentered lens, scattering, and adverse atmospheric conditions, which can pave the way for inadequate quality and image degradations. The qualityless images mislead ophthalmologists in making inaccurate and faulty findings [1]. So, the image quality can be enhanced through various image enhancement techniques. Image enhancement is an arduous task done prior to complex processing. It is one of the low-level image processing techniques which manipulates and performs various operations on the image pixels to make the image well-suited than the real one. The prime objective of image enhancement is to improve the image quality and highlight the specific features required for computation. Some of the image enhancement operations are denoising, deblurring, edge detection, smoothening, contrast/brightness enhancement, sharpening, and so on [2]. This paper focuses on histogram-based image contrast enhancement methods such as HE, AHE, and CLAHE. These methods are evaluated on two different DR datasets, EyePACS and Messidor-2. In this paper, section 2 reviews some of the related works. Section 3 gives a brief description on datasets considered in this work. Section 4 and its sub-sections discuss the contrast enhancement methods such as HE, AHE, and CLAHE. Section 5 analyzes the performance results, and section 6 concludes our work.

Related Works

Khan et al. [3] proposed a HE-based method that reduces the intensity saturation and improves the image quality. In this method, the equalization is performed on a fixed segment rather than a complete one. The fixed image segments are scaled to a full range. However, the proposed method is not time efficient when compared to other techniques taken for consideration. Kim et al. [4] proposed a bi-histogram-based equalization technique that preserves the mean brightness by considering the mean value and performing HE over the decomposed images. However, the proposed method needs a more complex hardware setup than conventional methods. Khan et al. [5] proposed two multi HE-based methods that reduce the intensity saturation and improve the image quality. In this method, the equalization is performed based on mean or median values. The narrow segments are scaled to a full range. However, the proposed method is not computationally efficient when compared to other techniques taken for consideration. Sim et al. [6] proposed a recursive sub-image-based HE, which is more robust, energy preserving, and better image contrast. Due to the strong dependencies of image pixels, it carries vital object structure information in the visual arena. However, the proposed method does not possess much scalability, and the technique is not tested on real-time images. Feng et al. [7] proposed a method based on contourlet where the contourlet coefficients are modified, which can often well detect the edges and contours. For contourlet transform, the shift-variance is concentrated, and optimal parameters are found. However, the shift variance effect needs to be examined well to achieve the best possible results.

Dataset Description

The Kaggle EyePACS and Messidor-2 are the benchmark datasets considered in our work. The EyePACS dataset available at Kaggle [8] contains 88,702 images which was labeled and rated by trained clinicians on a scale of 0-4 (0-No DR, 1-mild DR, 2-moderate DR, 3-Severe DR, and 4-proliferative DR). Messidor-2 [9-11] dataset contains 1748 images which were graded according to their severity levels.

Contrast Enhancement Techniques

In general, the image contrast enhancement techniques are grouped into several classes. Some of them are a filter-based enhancement, transformation-based enhancement, mask-based enhancement, and histogram-based enhancement. In this paper, histogram-based image enhancement techniques such as HE, AHE, and CLAHE are considered and evaluated in our work since it is computationally efficient.

Histogram Equalization (HE)

Histogram Equalization is a simple basic method used for enhancing the image contrast with less computational efforts. It is accomplished by uniformly distributing the image pixel intensities to all the gray levels [12]. HE cannot be used over separate RGB (Red Green Blue) components as it may lead to abrupt changes that produces chromatic imbalance. This method mainly accounts for increasing the global contrast of images. On applying HE over the retinal fundus images, it is found that the results are not satisfactory as images contain high-intensity noises. However, it provides better results for simple images [13, 14].

Adaptive Histogram Equalization (AHE)

Adaptive Histogram Equalization (AHE) is a HE-based adaptive method where various histograms are computed for distinct image segments, which can be used to redistribute the luminance value of the images [15]. This method performs better when compared to HE [12]. Unlike HE, AHE mainly accounts for improving the local contrast and edge details.

Contrast Limited Adaptive Histogram Equalization (CLAHE)

To enhance the low contrast images, Contrast Limited Adaptive Histogram Equalization (CLAHE) was applied [16, 15]. When compared to AHE, CLAHE stands apart with its contrast limiting property by using the clip limit parameter to control the noise amplification. The CLAHE controls the noise amplification by performing histogram clipping at a predetermined region prior to the Cumulative Distribution Function (CDF) computation. Initially, the original image gets divided into smaller non-overlapping regions called blocks or tiles. The two vital parameters used in CLAHE are block size (BS) and clip limit (CL), which controls and produces a significant impact on image quality enhancement. The increase in BS and CL value increases the contrast on low-contrast images, which substantially enhances the image quality. The histogram equalization is applied to each tile, and the actual histogram is clipped as shown in Fig.1. The image pixels (clipped) are redistributed to individual gray levels. Since the image intensities are limited to a fixed maximum, the redistributed and the actual histogram are different. However, the improved and the actual image possess the same minimal and maximal gray level values [15, 17]. The CLAHE image enhancement method consists of the following steps:

Step 1: The original input image is divided into smaller non-overlapping regions called tiles. Let M×N is equal to the number of tiles in total.

Step 2: As per the gray level values, the histogram of each tile is calculated.

Step 3: The contrast limited histogram of each region is calculated by the clip limit value, and it can be expressed as,

$$Np_{avg} = \frac{(NpX \times NpY)}{N_g}$$

Where Np_{avg} is the no. of pixels on average, N_g is the no. of gray levels, NpX and NpY are the no. of pixels in X and Y dimensions, respectively.

The Clip Limit (CL) can be given as,

$$N_{cl} = N_{ncl} \times Np_{avg}$$

Where N_{cl} is the clip limit, N_{ncl} is the clip limit which is normalized [0, 1]. When the no. of pixels is larger than N_{cl}, the image pixels are clipped. Then the histogram clipping rule is applied.

Step 4: The remaining pixels are redistributed; Rayleigh transform is used to enhance the intensity values. The Rayleigh distribution works better for fundus images at higher alpha values [18].

Step 5: The linear contrast stretch can be used for rescaling. The tile regions are combined, and the boundary artifacts are removed using bilinear interpolation.

EXPERIMENTAL RESULTS AND ANALYSIS

In this section, we discuss the experimental results of the image enhancement techniques for two different DR datasets such as Messidor-2 and EyePACS. The histogram-based enhancement techniques such as HE, AHE, and CLAHE are analyzed and evaluated. Fig. 2 and Fig. 3 show the original Messidor-2 dataset image and the CLAHE

enhanced image, respectively. Fig. 4 and Fig. 5 show the original Eye PACS dataset image and the CLAHE enhanced image, respectively. In this work, we have used three different parameters to assess the performance of three histogram-based image enhancement methods such as HE, AHE, and CLAHE. The Peak Signal-to-Noise Ratio (PSNR), Signal-to-Noise Ratio (SNR), and Structural Similarity Index (SSIM) are the metrics considered. The following formulae compares the original image r(x,y) with a test image t(x,y) of same size $[n_x,n_y]$.

$$\mathrm{PSNR} = 10 \cdot \log_{10} \left[\frac{\max(r(x,y))^2}{\frac{1}{n_x \cdot n_y} \cdot \sum_{0}^{n_x-1} \sum_{0}^{n_y-1} [r(x,y) - t(x,y)]^2} \right]$$

$$\mathrm{SNR} = 10 \cdot \log_{10} \left[\frac{\sum_{0}^{n_x-1} \sum_{0}^{n_y-1} [r(x,y)]^2}{\sum_{0}^{n_x-1} \sum_{0}^{n_y-1} [r(x,y) - t(x,y)]^2} \right]$$

$$SSIM(I,I') = \frac{(2\mu_I\mu_{I'} + c_1)(2\sigma_{II'} + c_2)}{(\mu_I^2 + \mu_{I'}^2 + c_1)(\sigma_I^2 + \sigma_{I'}^2 + c_2)}$$

Where, μ_I and σ_I are the average gray values and variance for I. $\sigma_{II'}$ are the covariance between the images I and I', C_1 and C_2 are the two constants. In common, the greater PSNR, SNR, and SSIM values lead to more accurate image enhancement performance. The statistical comparison of PSNR, SNR and, SSIM for three image enhancement methods on two different datasets is discussed further in this section. The PSNR values are compared on Messidor-2 and EyePACS datasets in Fig. 6, which shows that the CLAHE method outperforms the other methods on both datasets. The PSNR values achieved on Messidor-2 and EyePACS datasets are 44dB and 45dB, respectively. The SNR values are compared on Messidor-2 and EyePACS datasets in Fig. 7, which clearly shows that the CLAHE method provides better performance than other methods on both datasets. The SNR values achieved on Messidor-2 and EyePACS datasets are 72dB and 71dB, respectively. The SSIM values are compared on Messidor-2 and EyePACS datasets in Fig. 8, which shows that the CLAHE method outperforms the other methods on both datasets. The SSIM values achieved on Messidor-2 and EyePACS datasets are 0.91 and 0.90, respectively.

CONCLUSION

In this work, some of the histogram-based image contrast enhancement methods such as HE, AHE, and CLAHE are evaluated on two different DR datasets. HE, when applied on retinal fundus images, did not provide satisfactory results because of the high-intensity retinal image noises. AHE provides good results when compared to HE, but both the image quality and background noises are enhanced simultaneously. In CLAHE, contrast enhancement is limited which provides an enhanced image, thereby reducing the image noises. The performance parameters such as PSNR, SNR, and SSIM produce the best results for CLAHE in both datasets. The CLAHE results are greatly influenced by two parameters, BS and CL which turns out to be the reason that CLAHE outperforms the other histogram-based methods.

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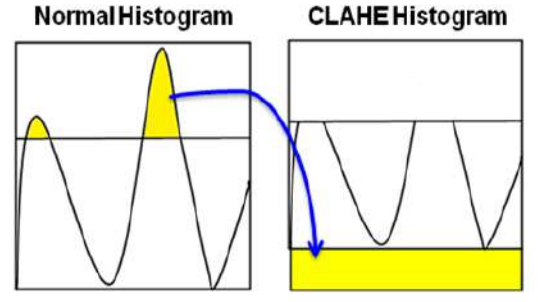

Fig. 1. CLAHE Pixel redistribution

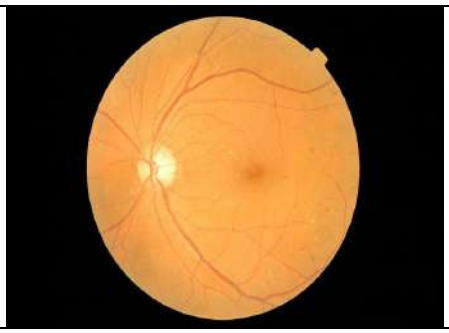

Fig. 2. Original image – Messidor-2 dataset

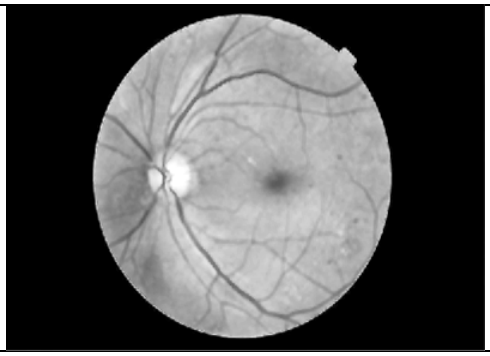

Fig. 3. Enhanced image by CLAHE

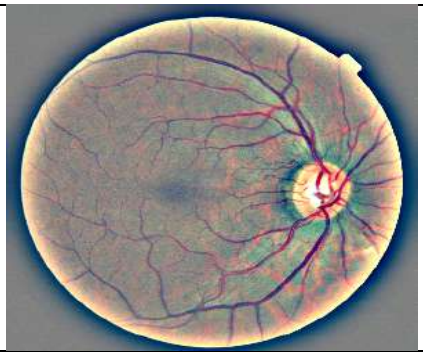

Fig. 4. Original image – EyePACS dataset

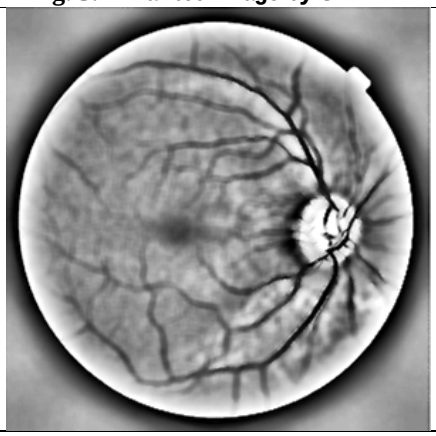

Fig. 5. Enhanced image by CLAHE

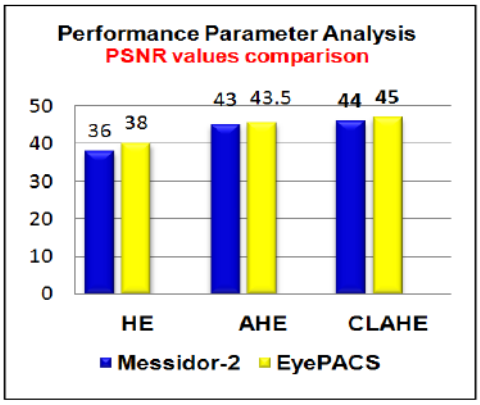

Fig. 6. The PSNR values comparison of contrast enhancement methods on Messidor-2 and EyePACS dataset

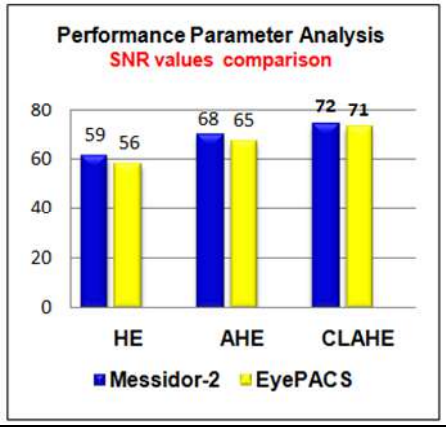

Fig. 7. The SNR values comparison of contrast enhancement methods on Messidor-2 and EyePACS dataset

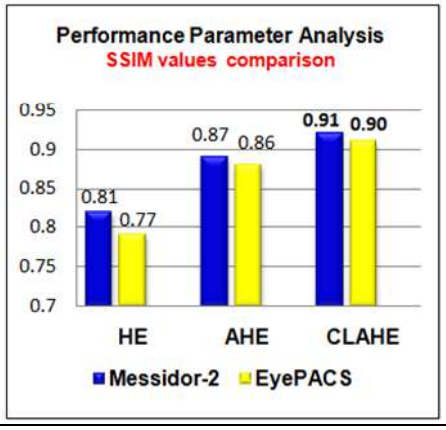

Fig. 8. The SSIM values comparison of contrast enhancement methods on Messidor-2 and EyePACS dataset

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Survey on Trust and Reputation-Based Security Systems in Wireless Sensor Networks

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Received: 14 Feb 2022 Revised: 02 Mar 2022 Accepted: 26 Mar 2022

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ABSTRACT

Wireless Sensor Networks (WSNs) are the major development of Information and Communications Technology (ICT). Nowadays, a remarkable growth is recognized in the utilization of WSN applications like surveillance systems, battlefield applications, healthcare monitoring, atmospheric conditions forecasting, etc. Because of the Sensor Nodes (SNs) constrictions according to their energy, storage, lack of infrastructure and computational abilities, several security challenges have been arisen in those applications. Additionally, the standard security systems with high overhead of computation and communication are infeasible in WSNs. Thus, security in WSNs is still a challenging process. To tackle this, Trust and Reputation-Based Security (TRBS) systems have been proposed for various types of attacks and vulnerabilities in the networks to achieve security requirements. This article presents a detailed survey on TRBS systems for WSNs. At first, security systems based on the estimation of trust and reputations of each node are studied in detail. Then, a comparative analysis is conducted using the strengths and weakness of those security systems to suggest few future directions to the security improvements for WSNs.

Keywords: WSN, Network security, Vulnerabilities, Trust, Reputation

INTRODUCTION

Wireless Sensor Networks (WSNs) are the major category of networks that consist of distributed, autonomous, low-power, low-cost, small-size devices using many SNs for cooperatively gathering and processing the sensed information through an infra structure less ad-hoc wireless network. Typically, WSNs are constructed on a huge amount of independent SNs and a base station which also acts as a gateway to another network. Different types of SNs can create a WSN such as low sampling rate magnetic, thermal, visual, infrared and acoustic. The sensor on each node can able to detect the light, pressure, temperature, etc. Every sensor is equipped with a small battery as a power supply which refers that the network efficiency is highly dependent on the rate of Energy Consumption (EC). These networks are applied in various applications like environmental forecasting, industrial operation monitoring, medical diagnosis, transportation, robotics, etc. In many applications, security plays a fundamental role to prevent the attacks in the networks. Since sensor networks pose unique challenges, security systems used in the traditional networks cannot be directly applied to WSNs because of its unique nature [1]. Normally, SNs are very sensitive to the cost of manufacturing since WSNs have a huge amount of SNs. As a result, most SNs are resource constrained in terms of energy, storage, computation and communication abilities for reducing the SN's cost. On the contrary, SNs deployed in public hostile localities may susceptible to physical attacks by invaders.

In common, invaders are considered to be able to undetectably take SN control and extract all confidential information in the node. Besides, if the size of sensor networks is noticeably large, then the network topology is dynamically modified since few nodes may die out of inadequate energy or malfunction and new nodes may fix the network for maintaining required functionality [2]. Therefore, sensor networks utilize insecure wireless transmission channel. Therefore, conventional security systems are not desirable to prevent the networks from the invaders. The security systems should provide confidentiality defense and authentication features for preventing malicious attacks. Also, it should create a relatively secure design for sensor networks which is a primary problem of whether the WSNs are practical. Thus, the challenges experienced by WSN security are becoming the major research field. For this purpose, the foremost essential traits of nodes such as trust and reputation are estimated to determine the security of the network [3-4]. Over the past few years, numerous researches suggested on TRBS systems in WSNs. This paper studies different TRBS systems in WSNs with their merits and limitations to further enhance the performance of security in WSNs during data transfer.

Survey on Different TRBS Systems In WSN

Anita *et al.* [5] presented a novel two-way Acknowledgement-based Trust (2-ACKT) protocol with Individual (2-ACKT-I) and Group (2-ACKT-G) ACK for reducing the communication overhead and memory requirements for trust analysis in WSNs. In this protocol, the direct trust was computed by using the link layer ACK and a two-hop ACK from a downstream neighbor. Also, the sensors were allowed to be in inactive mode to minimize the EC and avoid the promiscuous mode of functions of sensors. Luo *et al.* [6] proposed a Trust Management Scheme (TMS) for clustered WSNs to forecast the SN's behaviors via analyzing their trust ranges. In TMS, a hash function was employed to create the uniqueness labels for SNs that identifies external invaders. Besides, every node's trust range was adjusted via beta density function to recognize the compromised nodes. Feng *et al.* [7] proposed a credible Bayesian-based TMS (BTMS). In this scheme, the overall trust value was aggregated by both direct and indirect trust information. Also, the former was computed by the modified Bayesian equation and updated by a sliding window. By recommendations from the third party, the latter was computed. Moreover, the indirect trust computation was conditionally invoked based on the uncertainty of direct trust computed through entropy theory and malicious feedbacks were excluded. Simultaneously, different recommendations were properly weighted in light of the trust levels of recommenders.

Fang *et al.* [8] proposed the Beta-based TR Evaluation System (BTRES) for WSN's according to the monitor node's behaviors and allocation of node's credibility. The nodes trust ranges were applied for directly electing the relay nodes and defending the internal assaults threats. Moreover, the collusion attack of compromised nodes was

defended by setting weight and threshold value. Jadidoleslamy *et al.* [9] proposed a fuzzy fully Distributed TMS (DTMS) in WSNs by considering both direct and indirect trust values. In DTMS, the trust establishment functionality was uniformly distributed throughout the network. At first, the neighbor node's behavior was monitored by each node for computing their trustworthiness. Then, the untrustworthy neighbors were detected and eliminated from the network's functionalities. Ye *et al.* [10] proposed an efficient Dynamic Trust Evaluation Model (DTEM) for WSNs that executes a dynamic trust evaluation by dynamically adjusting the weights, direct and indirect trust including the update factors. In DTEM, the direct trust was estimated via the multi-trust such as transfer trust, information trust, power trust, penalty and regulating factors. The indirect trust was evaluated conditionally by the trusted recommendation from a third party. Moreover, the integrated trust was measured by allocating dynamic weights for direct and indirect trust as well as combining them. At last, an update method was proposed by a sliding window based on the induced ordered weighted averaging operator for enhancing the flexibility. The parameters and interactive history windows number were dynamically adapted according to the actual network requirements for realizing the dynamic update of direct trust value.

Zhang *et al.* [11] proposed a novel TMS according to the Dempster-Shafer (D-S) proof concept to identify the malevolent nodes. First, the trust level was measured via the spatiotemporal relationship of the information acquired by SNs in the nearest area. After, the trust framework was built using D-S concept to calculate the number of distributed features of belief, disbelief or ambiguity, direct and indirect trust ranges. Also, a flexible synthesis scheme was used to determine the total trust and recognize the malevolent nodes. Jin *et al.* [12] proposed a multi-agent trust-based attack identification in which node's trust was defined and Mahalanobis distance model was applied to analyze whether the trust was standard or not. The nodes trust was determined and modified according to the combined Beta allocation and a tolerance function. After, the attack nodes were identified to improve the network's scalability and fault tolerance.Li *et al.* [13] suggested a new topology path management and defending invaders in real-time WSNs. First, a non-invasive scheme of positioning virtual SNs was applied and a common topology control scheme was introduced to resist the tolerant attack. Then, a prediction framework was constructed via copula factors for estimating the maximum resources and determining whether it begins the dynamic mitigation or not. Also, the minority game with imperfect approach was employed for overwhelming the assault flows and enhancing the penetrability of the standard flows. Tang *et al.* [14] recommended a Trust-Based Secure Routing (TBSR) mechanism via the traceback scheme to enhance the routing confidentiality and the utilization of accessible power in energy-harvesting WSNs. In TBSR, the data and warning were broadcasted between the origin and sink through disjoint paths in an independent manner to guarantee the confidentiality. Besides, an active selection of marking and logging strategy was implemented in the routing.

Al-Farraj *et al.* [15] proposed an Activation Function-based Trusted Neighbor Selection (AF-TNS) method to improve the network security in resource-constrained WSNs. This AF-TNS has two steps such as trust evaluation with energy constraint and additive metric-based node evaluation for retaining the neighbor's trustworthiness. Also, the random transigmoid function was used to simplify the complex decision-making process of the AF by differentiating the trusted and un-trusted node. Qi *et al.* [16] presented a new trust framework according to the SN's recovery strategy. First, the node breakages were categorized into recoverable and unrecoverable breakages via taken into account the susceptibility, untrustworthiness and active traits of SNs. After, the standard communication outcomes was extended to the trinomial dissemination. The global trust level was grouped via direct and indirect communication outcomes according to the Bayesian cognitive framework. Further, a new trust framework was applied to lessen the breakage chance for task performance. Anwar *et al.* [17] introduced an efficient Belief-based Trust Evaluation Method (BTEM) to defend the malevolent attacks. First, a Bayesian measure was applied to gather the SN's direct and indirect trust ranges and the relationship of the information acquired over a period. Then, indefinite skill was determined in decision-making to achieve the sheltered data transfer and defend the malevolent nodes. Mythili *et al.* [18] suggested a Spatial and Energy-Aware Trusted Dynamic Distance Source Routing (SEAT-DSR) method for enhancing the WSN's lifespan. In SEAT-DSR, a normal clustering was achieved to aggregate the SNs according to the trust, spatial information, power and distance between SNs. Also, a new hierarchical trust scheme was adopted to taken into consider the multiple traits of SNs like data transfer rate, data size, power use and recommendation.

Nie [19] recommended a new dynamic optimization trust framework according to the entropy called Trust-Doe for exploiting the SN's global trust and clustering the SNs into many groups. For every group, the entropy and weights were determined according to the entropy weight scheme and often the SN's local trust was adjusted. Also, the standard variation of the local reputation for various groups was estimated via the dynamic optimization competition scheme to enhance the trust framework's accurateness. Zhao *et al.* [20] developed an Exponential-based TR Evaluation System (ETRES) to analyze the SN's behaviors and describe the exponential distribution of SN's trust. The SN's trust was considered to discover the trustworthy nodes for data transfer and defend the malevolent nodes. Besides, the entropy concept was applied to estimate the ambiguity of direct trust whereas the data transfer was improved via an indirect trust. Further, the belief term was restated to adjust the SNs trust and lessen the risky impacts of the compromised nodes.

CONCLUSION

In this article, a survey on TRBS systems in WSNs is presented in detail. Also, merits and demerits of these systems are discussed to suggest the future directions towards the security improvements in WSNs. From this survey, it is concluded that the ETRES can be easily reduced the risk of internal attacks, particularly on-off attacks. But, there are still few limitations in the ETRES. Though ETRES can efficiently resist the internal attacks, the performance was not effective to tradeoff between energy and security. Therefore, it would require further research to solve these limitations by improving the ETRES for detecting compromised nodes effectively and improving the network security.

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Table 1 summarizes the merits and limitations of surveyed TRBS systems in WSNs.

Ref No.	Mechanism	Merits	Limitations	Performance Metrics
[5]	2-ACKT, 2-ACKT-I and 2-ACKT-G	It is apt for heterogeneous WSNs due to easily identify the malicious attacks by individual SNs.	Memory requirements and time complexity were high.	@No. of communicating nodes=144: Communication overhead=1000; Memory requirements=17000bytes
[6]	TMS using a hash function	Malicious nodes were identified rapidly.	External and internal attacks were not mitigated.	-Nil-
[7]	Credible BTMS	Better defense against on-off attacks, bad mouthing and ballot stuffing attacks.	Energy usage was high.	-Nil-
[8]	BTRES	It can effectively resist the internal attack of compromised nodes.	It does not consider the node's energy, computation and memory constraints.	-Nil-
[9]	Fuzzy fully DTMS	Improved network lifetime and reduced traffic	High computational overhead,	EC=1.62×10^{15}; Residual energy=0.62;

		overhead.	complexity and execution time.	Scalability and fault tolerance=8.34; Execution speed=9.27
[10]	Efficient DTEM	High Detection Rate (DR) for identifying different types of attacks.	High computational burden.	@The fraction of malicious nodes=50%: DR=86%
[11]	Novel TMS based on D-S theory	It can reduce the computation and communication overhead.	Network lifetime was not increased.	@Node distribution density=5: Lifespan of the initial power depleted node=35sec; Fraction of died nodes to alive nodes=2.5%
[12]	Multi-agent trust-based intrusion identification	Better DR and False Positive Rate (FPR).	High computational difficulty.	@No. of attacking nodes=5: DR=97.5%; FPR=5.04%
[13]	Novel topology link control method	Low cost and ease of deployment.	Time delay was high due to high computational complexity.	@Simulation time=29sec: Throughput=1500packet
[14]	TBSR scheme using traceback approach	Less energy use and high routing success rate.	Computational difficulty was high.	@Hops from the sink=5: The success rate of reaching the sink=0.89
[15]	AF-TNS method	Minimum delay and EC.	It does not taken into account the indirect trust estimation.	@Malicious nodes=10: Average delay=8.5msec; EC=18.4Joule; Throughput=149kbs; Network lifetime=390sec; False detective rate=1.54%
[16]	New trust framework using node recovery	Privacy demands were effectively satisfied.	EC and computational complexity were high.	@Interaction rounds=400: Total EC=330J
[17]	Efficient BTEM	Reduced EC.	Less throughput and accuracy.	@Malicious SNs=10%:

				DR=98%; Detection accuracy=80%; Residual energy=91%; Throughput=124kbps
[18]	SEAT-DSR algorithm	Less average end-to-end delay and high DR.	Average throughput was very less.	@Simulation time=90sec: DR=92% @No. of malicious nodes=25: Average throughput=0.25; Average end-to-end latency=23msec; Network lifetime=490sec
[19]	Trust-Doe model	Effective DR and FPR.	Power usage and overhead were high.	@Time intervals=40sec: DR=96.5%; FPR=5.6
[20]	ETRES	It can effectively resist the internal attacks by choosing the reliable nodes.	Performance was not increased efficiently and EC was high.	-Nil-

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Evaluation of Surface Roughness of Tooth after Etching with Two Different Commercially Available Etchants

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Received: 10 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Acid etching in dentistry is an effective way to bond different types of restorations to enamel or dentin. These acidic materials act at the tooth's outer layer, exposing a rough, porous layer that gives the bonding process more surface area. In the present study, the main aim is to compare the surface roughness of teeth after etching with two different commercially available etchants- d tech and Echo-tech with two different timings (15seconds and 20 seconds). In the present study, eight extracted permanent anterior natural teeth samples were used. All the extracted teeth were inspected carefully and any carious, worn or damaged teeth due to any other reason were excluded from the study. Two brands of etchants- d tech and Echo-tech were used. Out of the eight samples, four were etched with d-tech and the remaining samples were etched with Echo-tech. Among each 4 samples, 2 teeth etched for 15 seconds and the other 2 teeth etched for 20 seconds and rinsed in water immediately. The pre etching and post etching surface roughness were measured using Mitutoyo SJ-310 Stylus Profilometer. Ra, Rq and Rz surface roughness values were assessed and compared. The mean difference of Ra is found to be 0.0125 and 0.0075 for D tech and Echo tech, the mean difference of Rq is 0.0175 for both D tech and Echo tech and the mean difference for Rz is 0.1150 and 0.1850 for D tech and Echo tech. Independent 'T' test was done and the p value for Ra is 1.000 (>0.05) which is statistically insignificant, for Rq the p value is 0.549 (>0.05) which is statistically insignificant and for Rz the p value is 0.983 (>0.05) which is statistically

insignificant. From the present study, we conclude that the etchant brand d tech and Echo tech doesn't show much deviation in the surface roughness.

Keywords: Surface roughness, Tooth, Etching, Profilometer, Innovative measurement

INTRODUCTION

Acid etching in dentistry is an effective way to bond different types of restorations to enamel or dentin. These acidic materials act at the tooth's outer layer, exposing a rough, porous layer that gives the bonding process more surface area. It is important to use dental etchants properly so that the acid doesn't stay on the enamel for too long. Acid etchant (more commonly known as acid etch) is available in a phosphoric acid solution or gel and is commonly available in 15%, 34% or 37% concentrations (37% being the most common). Acid etch creates microscopic spaces in enamel (increasing surface roughness) into which the bonding agent/adhesive can flow, aiding the bonding process (micromechanical retention). When enamel etching was introduced in 1955, the recommended time for 85% phosphoric acid (P.A.) etching was 30 seconds(1). Surface roughness is a widely used test method for evaluating the effect of acid etching after preventive procedures on dental hard tissues, and it is widely acknowledged as a comparison feature, quantifying surface texture by randomised amplitude readings. Roughness, like hardness it is a critical feature of teeth because it affects the mechanical attachment of foreign materials to their surfaces. The average surface roughness (Ra) is the most generally reported parameter among those used to quantify surface roughness in dental investigations(2).

Recently, most manufacturers of dental acid-etchants have recommended 15 to 30 seconds when 32% to 40% P.A. is used(3) In the total etch technique, dentin is etched with P.A. and then the etchants must be rinsed off immediately after 15–30 seconds of application time(4). P.A. (37%) can lead to necrosis in the oral mucosa and ulcerative lesions of the periodontal tissue(5). These lesions can spread from the superficial to the deeper layers and can be accompanied by difficulty of swallowing and pronunciation and burning depending on the location. Some studies have been conducted on the hazards of dental acid-etchants (37% P.A.) to the skin and oral mucosa(6). It is obtained by treating calcium phosphate rocks with sulphuric acid, then filtering the resultant liquid to remove the calcium sulfate. The acid is useful in the laboratory because of its resistance to oxidation, reduction and evaporation as glue for dentures. It forms the basis of innovative procedures such as: Resin bonded metal retainers, porcelain laminate veneers, holders of prosthodontics. It also solves the problem arising from the resin such as filtering and marginal pigmentation. The etching composition may include other components such as methylene blue or antimicrobial agents. Similarly an additional 30 seconds etching is suggested for enamel if uniform white frosted appearance is not obtained using the recommended etching time. In some clinical situations, such as fluorosis and amelogenesis imperfecta, a prolonged etching can be needed to achieve a sufficiently etched surface(7). The role of acid etching with phosphoric acid (35–38 percent) in improving the adhesion of resin restorative materials to the tooth structure has been well established and supported by a number of studies(8). Our team has extensive knowledge and research experience that has translated into high quality publications(9–18),(19–22),(23–27),(28) In the present study, the main aim is to compare the surface roughness of teeth after etching with two different commercially available etchants- d tech and Echo-tech with two different timings (15seconds and 20 seconds).

MATERIALS AND METHODS

The in vitro study was performed at White Lab at Saveetha Dental College, Chennai, India. In the present study, eight extracted permanent anterior natural teeth samples were used. All the extracted teeth were inspected carefully and any carious, worn or damaged teeth due to any other reason were excluded from the study. Two brands of etchants- d tech and Echo-tech were used. Out of the eight samples, four were etched with d-tech and the remaining samples were etched with Echo-tech. Among each 4 samples, 2 teeth etched for 15 seconds and the other 2 teeth

etched for 20 seconds and rinsed in water immediately. The pre etching and post etching surface roughness were measured using Mitutoyo SJ-310 Stylus Profilometer using 2µm/60 degree angle tip (Figure 1). Ra, Rq and Rz surface roughness values were assessed and compared.

RESULTS

In the present study, the surface roughness measurements of the sample before and after etching with dtech and Echo-tech were recorded. The mean difference of Ra is found to be 0.0125 and 0.0075 for D tech and Echo tech, the mean difference of Rq is 0.0175 for both D tech and Echo tech and the mean difference for Rz is 0.1150 and 0.1850 for D tech and Echo tech. Independent 'T' test was done and the p value for Ra is 1.000 (>0.05) which is statistically insignificant, for Rq the p value is 0.549 (>0.05) which is statistically insignificant and for Rz the p value is 0.983 (>0.05) which is statistically insignificant. (Table 1, Figure 2)

DISCUSSION

The phosphoric acid etching technique is widely used in dentistry for a variety of purposes(29). In the present study, even the etching difference time of 5 seconds has been found to deviate the surface roughness of teeth. Here, the surface roughness of the tooth shows a deviation of 5% between the 15 seconds and 20 seconds. But the brand differentiation does not show a preferable deviation. In another study, the etching time has been found to affect the surface features of etched tooth tissues. For example, the recommended etching time for dentinal tissue is 15s and observed to compromise the micron tensile bonding strength while using a reduced etching time of 5s. The variation in etching time affects the dental hard tissues at a structural level (such as surface properties) that is also reflected in clinical applications. It can be shown that etching with hydrogen peroxide weakens dentine structure, and that certain offices are damaged by water jet washing and drying under pressure. In the case of etching with phosphoric acid gel , demineralization is insufficient since the acid does not penetrate through dentine channels and tubules due to the high viscosity, resulting in a superficial demineralization that does not provide a suitable substrate for the usage of dental adhesive(30). Etching resulted in the creation of 4 mm holes that remained intact after being washed with water and dried with an air jet, allowing the adhesive to penetrate demineralized dentine channels and tubules(31). In another study, surface roughness and depth profile were determined by AFM at different etching times for different dental fluorosis grades. These evaluations provide different findings compared with those from studies in which only SEM observations were made(32). The basic principles of acid etching are to convert a low surface energy enamel surface into a high-surface-energy enamel surface, as well as to dissolve and demineralize the inorganic [Ca10(PO4)6(OH)2 matrix, resulting in micropores and microgrooves that improve mechanical retention(33). Deproteinization after acid etching greatly improved the shear bond strength in immature permanent teeth(34). Acid etching patterns and bond strength values of primary and immature permanent teeth vary from those of mature permanent teeth, according to previous research(35). The present study possesses limitations such as the study deals only with limited parameters and it also analyses the surface roughness exhibited with the use of etchants only. Further studies with large samples and focussing on many parameters like surface roughness, colour stability is to be done to formulate this useful finding commercially in the market.

CONCLUSION

From the present study, we conclude that the etchant brand d tech and Echo tech doesn't show much deviation in the surface roughness.

ACKNOWLEDGEMENT

The team extends our sincere gratitude to the Saveetha Dental College and hospitals for their constant support and successful completion of this work.

Conflict Of Interest

Nil

Source of Funding

The present study was supported by the following:

- Saveetha Dental College and hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai
- Arcus Pvt Ltd, Erode

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Table 1: It Represents The Mean, Standard Deviation And Significance Between The Groups.

Surface roughness parameter	Groups	Mean	Standard Deviation	Significance
Mean difference Ra	D tech	0.0125	0.00957	1.000
	Echo tech	0.0075	0.00957	
Mean difference Rq	D tech	0.0175	0.01258	0.549
	Echo tech	0.0175	0.01708	
Mean difference Rz	D tech	0.1150	0.28243	0.983
	Echo tech	0.1850	0.27258	

Figure 1: Stylus Profilometer

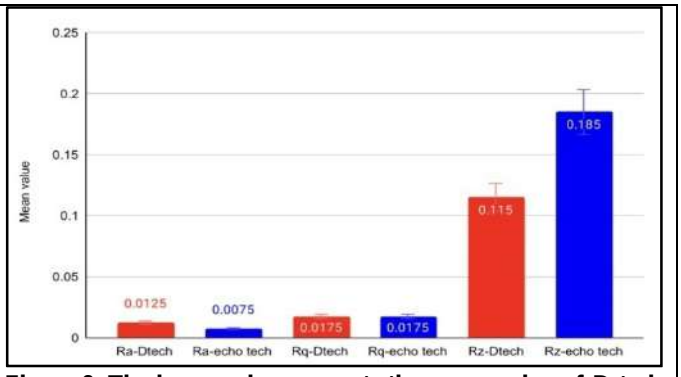

Figure 2: The bar graph represents the mean value of D tech and Echo tech etchants, identified via 't' test. X axis represents the type of etchant, Y axis represents the mean value. Red colour indicates the D tech etchant, Blue colour indicates the Echo tech etchant. Rz values were slightly increased in the echo tech group.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Covid-19 Infected under Alertness using Stochastic Modelling

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Received: 03 Jan 2022 Revised: 27 Feb 2022 Accepted: 26 Mar 2022

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ABSTRACT

This paper deals with the recent COVID19 disease which is threatening the whole world in the last two years. This causes many deaths and a greater fall in the whole economy. This paper identifies the amount of people infected with Covid-19 which has been increasing in recent months at this time of global pandemic spreaded over to various countries. The threshold level of the covid-19 virus is been calculated through a statistical model of the infected person. In this paper the expected time is estimated for covid-19 infection using stochastic model which helps to develop a prevention strategy. This prevention strategy creates awareness to individuals who have been in contact with an unknown person. The limiting level of antigenic diversity is sufficient for the reception prevention procedure in extending the concentrated normal time by presenting the stochastic model under suspicion of random variability followed by Exponentiated Modified Weibull distribution [EMWD]. Parametric values are assessed and statistical data are provided to predict the distribution of COVID-19 and to confirm the results of studies.

Key words: Covid-19, Parameter, Stochastic Model, Cumulative damage.

INTRODUCTION

COVID19 infection is a hazardous infection that broadens common virus to real diseases such as Middle Eastern Respiratory Syndrome (MERS- CoV) and Severe Acute Respiratory Syndrome (SARS - CoV). On December 8, 2019, the Wuhan Government of China announced that health officials (Bakar & Rosbi, 2020) would treat hundreds of new viral cases known as corona virus disease (COVID-19). COVID-19, a new SARS strain (SARS-CoV-2), later became a global pandemic and distributed to various countries. This infection communicates individual person to individual person through tainted individual hacks or sniffles directly affects the respiratory beads. Contaminated beads land in the noses and mouths of persons surrounded very closely are breathed into the lungs to lead potential infection. It is

spread by touching the surface or object containing the virus and then touching your own mouth, nose, or your eyes. It affects the respiratory system and the affected person experience fever, cough, shortness of breath and difficulty breathing. The onset of infection and symptoms last from one to fourteen days. An infected person shows symptoms within five to six days. To prevent the spread of the disease, it is essential to wash your hands and mouth and nose when catching hacking and sniffling and avoid contact with infected people.

Transmission from infected individuals to more and more non-covid19 infected people; Antigenic variations increase. When antigenic diversity threshold traverse a specific level, seroconversion happens immediately after the human immune system collapses. The individuals should generally avoid contacting the other person through direct touch to escape from possible contraction from an infected person and this is accepted universally by everyone who has awareness about the spread of corona virus. This raises alertness among human who may be part of risk. In deriving this model it is assumed that a person with alertness uses the prevention strategy and if the person is not be alert then there is a risk of transmission. An assumption on antigenic diversity threshold is used to estimate the expected time using the stochastic model in deriving the alertness and preventive strategy. The random variable considered here is the threshold level of antigenic diversity which follows an Exponentiated Modified Weibull Distribution.

Contacting a person consciously at touch does not have to be a definite event. Therefore, it is assumed that the probability of infection during contact or direct infection occurs with probability $\beta < 1$. The random variable considered here is the infected covid-19 individual. The estimation of expected time of infected Covid-19 by a simple random sample based on the following assumptions.

Assumption of the model

(i) Covid-19 transmits only through a person touching.

(ii) Anyone touching in which a person who is not alert considered as sure event of transmitting covid-19.

(iii) A person is alert while touching is with probability p, and not in alert with probability q, so that $p+q=1$.

(iv) The damage caused to the immune system is linear and additive.

Description of the model

Here the susceptible population who is in the touching activity or contact considered as the major mode of transmission. One technique for measuring the strength of humor and cell-interference reactions is to determine how quickly the infection can escape from these reactions. The fundamental thought, which we examine as far as cell-intervened reactions, is that cells contaminated by wild-type infection ought to be vulnerable to both viral cytopathic impacts and invulnerable interceded killing, say by CTL reactions, while a 'CTL get away from variation' would just be defenseless to viral cytopathic impacts. The frequency of mutations as a function of time since the onset of infection can be solved to reflect the cases. As one would intuit, these recurrence increments at a rate dependent upon how quickly the getaway freak develops comparative with the wild kind. This rate, called the escape rate, increases in proportion to the rate of CTL killing of wild animals, *k*, and decreases with the survival costs, *c*. Thus, the fastest escapes would occur when the CTL pressure, *k*, is high, and the cost of escape, *c*, is low; whereas when there is weak CTL pressure a significant expense of departure, the break rate ought to be low

Measurable disseminations are valuable to depict and foresee genuine marvels. Measurements writing contain many constant univariate circulations and illustrations of their fruitful applications. Recent developments have focused on creating a new distribution phase by adding one (or more) additional shape (s) to the basic (standard) distribution that makes distribution much easier, especially in studying tail performance. With regards to lifetime disseminations, in the event that G(x) is the total conveyance work (cdf) of the dispersion, the three notable strategies for speculations include: Modified Weibull (MW) circulation is perhaps the main conveyances in lifetime demonstrating, and some notable appropriations are uncommon instances of it. This distribution was introduced by Lai et al. (2003) to which

we allude the per user for a point by point conversation just as uses of the MW dissemination (specifically, the utilization of a genuine informational collection addressing disappointment times to outline the demonstrating and assessment technique)

The reliability function and hazard (failure) rate function of the EMWD distribution are given by

$$R(y) = 1 - F(y) \text{ and } h(y) = \frac{f(y)}{1 - F(y)}$$

The probability density function of EMWD is

$$h(y) = \alpha(\theta + \gamma\beta y^{\beta-1}) e^{-(\theta y + \gamma y^{\beta})} [1 - e^{-(\theta y + \gamma y^{\beta})}]^{\alpha-1}, y > 0 \text{ and } \theta, \beta, \alpha, \gamma > 0.$$

and its distribution function is

$$H(y) = \left[1 - e^{-(\theta y + \gamma y^{\beta})}\right]^{\alpha}, y > 0.$$

Then corresponding survival function is

$$\overline{H}(y) = 1 - \left[1 - e^{(\theta y + \gamma y^{\beta})}\right]^{\alpha}$$

Since Y is taken to EMWD (θ, γ)

It can be shown that

$$P\left[\sum_{i=1}^{k} X_i < Y\right] = \int_0^{\infty} g_k(x)\, \overline{H}(x)\, dx \qquad \text{--------- (1)}$$

Where $\overline{H}(x) = 1 - H(x)$

Put α = 2 and β = 1 in $\overline{H}(x)$, then it becomes

$$\overline{H}(y) = \left[2e^{-y(\theta+\gamma)} - e^{-2y(\theta+\gamma)}\right] \qquad \text{-------- (2)}$$

Substituting equation (2) in (1), we get

$$P\left[\sum_{i=1}^{k} X_i < Y\right] = \int_0^{\infty} g_k(x)\left[2e^{-x(\theta+\gamma)} - e^{-2x(\theta+\gamma)}\right] dx$$

$$= \left[2g*(\theta+\gamma)\right]^k - \left[g*2(\theta+\gamma)\right]^k \qquad \text{On simplification}$$

S(t) = P[no infection in (0,t]] = P[T > t]

$$= \sum_{k=0}^{\infty} \Pr\{\text{k person touching in } (0,t]\}$$

* Pr{the cumulative total < Y}

$$= \sum_{k=0}^{\infty} \left[F_k(t) - F_{k+1}(t)\right] \left[\left[2g*(\theta+\gamma)\right]^k - \left[g*2(\theta+\gamma)\right]^k\right]$$

$L(t) = 1 - S(t)$ is called the prevalence function as mentioned in Jewell and Shiboski (1990).

$$= 2\left[1 - g^*(\theta+\gamma)\right]\sum_{k=1}^{\infty} F_k(t)\left[g^*(\theta+\gamma)\right]^{k-1} - \left[1 - g^*2(\theta+\gamma)\right]\sum_{k=1}^{\infty} F_k(t)\left[g^*2(\theta+\gamma)\right]^{k-1}$$

On simplification

The p.d.f of T is

$$l(t) = 2\left[1 - g^*(\theta+\gamma)\right]\sum_{k=1}^{\infty} f_k(t)\left[g^*(\theta+\gamma)\right]^{k-1} - \left[1 - g^*2(\theta+\gamma)\right]\sum_{k=1}^{\infty} f_k(t)\left[g^*2(\theta+\gamma)\right]^{k-1}$$

Taking Laplace Stieltje's transform of *l*(t) we get,

$$l^*(s) = \frac{2\left[1 - g^*(\theta+\gamma)\right] f^*(s)}{\left[1 - g^*(\theta+\gamma)\, f^*(s)\right]} - \frac{\left[1 - g^*2(\theta+\gamma)\right] f^*(s)}{\left[1 - g^*2(\theta+\gamma)\, f^*(s)\right]}$$ On simplification ... (3)

But the c.d.f of Z is given by

$$F(Z) = \beta q \sum_{n=1}^{\infty} [p + q(1-\beta)]^n \; G_{n+1}(Z)$$

Taking Laplace Stieltjes transform of F(z) is

$$F^*(s) = \int_0^{\infty} e^{-st}\, dF(Z)$$

Hence,

$$F^*(s) = \frac{q\beta G^*(s)}{\left[1 - \left[p + q(1-\beta)\right] G^*(s)\right]}$$

The p.d.f of $F^*(s)$ is,

$$F^*(s) = \frac{q\beta g^*(s)}{\left[1 - \left[p + q(1-\beta)\right] g^*(s)\right]}$$ ----- (4)

Assuming that g ~ exp(c), then

$$g^*(s) = \frac{c}{c+s},\quad g^{*\prime}(0) = -\frac{1}{c} \;\&\; g^{*\prime\prime}(0) = \frac{2}{c^2}.$$ ------ (5)

From equation (4)

$$\frac{df^*(s)}{ds} = \frac{\left\{\beta q\, g^{*\prime}(s)\left[1 - \left[p + q(1-\beta)\right] g^*(s)\right] - \left[-\left[p + q(1-\beta)\right] g^{*\prime}(s)\right]\beta q\; g^*(s)\right\}}{\left[1 - \left[p + q(1-\beta)\right] g^*(s)\right]^2}$$

$$f^{*\prime}(0) = \frac{-1}{c\beta q}$$ ------ (6)

Therefore

$$-\left.\frac{df^{*}(s)}{ds}\right|_{s=0} = -\left\{\frac{2\left[1-g^{*}(\theta+\gamma)\right]f^{*\prime}(0)}{\left[1-g^{*}(\theta+\gamma)f^{*}(0)\right]^{2}} - \frac{\left[1-g^{*}2(\theta+\gamma)\right]f^{*\prime}(0)}{\left[1-g^{*}2(\theta+\gamma)f^{*}(0)\right]^{2}}\right\} \quad \text{------ (7)}$$

Let $g^{*}(\lambda)=\frac{\mu}{\mu+\theta+\gamma}, g^{*}(2\lambda)=\frac{\mu}{\mu+2\theta+2\gamma}$ ------ (8)

Substituting equation (6) and (8) in (7) we get

$$E(T)=\frac{2\left[1-\frac{\mu}{\mu+\theta+\gamma}\right]\left[\frac{-1}{c\beta q}\right]}{\left[1-\frac{\mu}{\mu+\theta+\gamma}\right]^{2}} - \frac{\left[1-\frac{\mu}{\mu+2\theta+2\gamma}\right]\left[\frac{-1}{c\beta q}\right]}{\left[1-\frac{\mu}{\mu+2\theta+2\gamma}\right]^{2}}$$

$$\therefore \quad E(T)=\frac{3\mu+2(\theta+\gamma)}{c\beta q 2(\theta+\gamma)} \quad \text{------ (9)}$$

CONCLUSION

In expected time corresponding in c the parameter of the distribution of interarrival time when q, γ, θ, μ and β are kept fixed. As c increases, the value of $\frac{1}{c}$ decreases that means the interarrival time between contacts become smaller and so there is a corresponding decrease in expected time.

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Table 1. Empirical means

n	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\theta}$	$\hat{\mu}$
100	1.402 (0.244)	3.163 (2.053)	1.151 (1.214)	1.374 (0.351)
200	1.423 (0.167)	2.660 (1.532)	0.915 (0.927)	1.368 (0.272)
300	1.429 (0.144)	2.528 (1.111)	0.859 (0.722)	1.369 (0.243)
500	1.464 (0.097)	2.362 (0.888)	0.724 (0.504)	1.408 (0.192)
1000	1.478 (0.054)	2.049 (0.565)	0.554 (0.329)	1.467 (0.116)

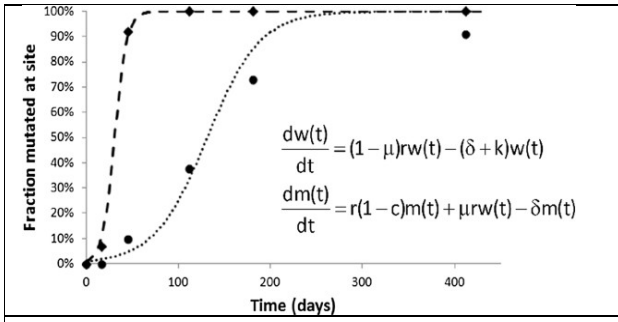

Fig.1.Description of the model

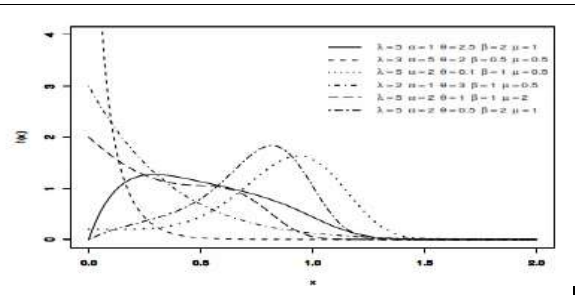

Fig.2. Probability density function

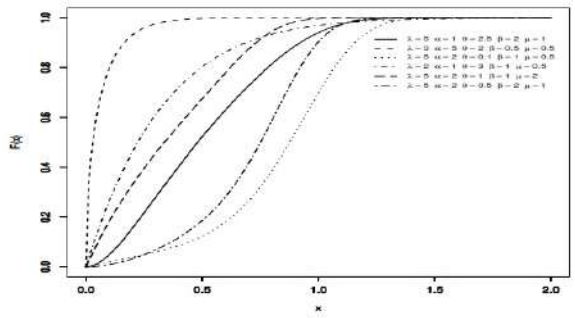

Fig.3. Cumulative distribution function

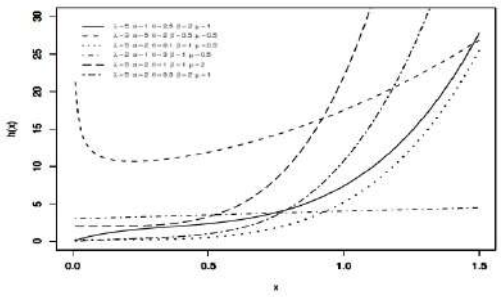

Fig.4. Hazard rate function

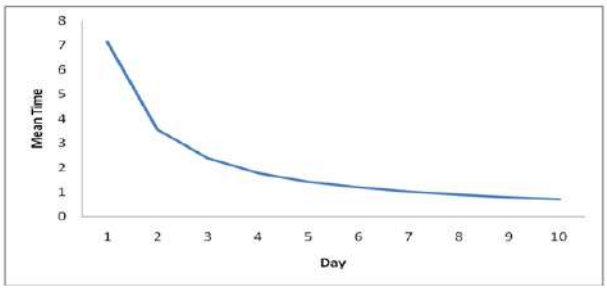

Fig.5. Empirical means

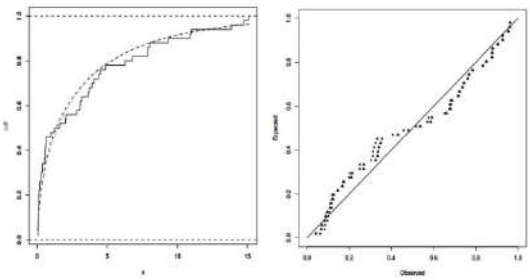

Fig.6. Fitted cumulative distribution function and P-P plot

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Challenges Faced by Teachers towards Mode of Online Teaching and Offline Teaching

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Received: 05 Jan 2022 Revised: 12 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

In the offline mode of teaching, teachers demonstrate the concepts to the students with the help of blackboard, whiteboard, chalks, markers, charts, lecture, textbook, question answer, group discussions etc. Nowadays, the innovative teaching methods are taken into consideration utilizing electronic devices such as smartphones, laptops, computers and various other types of modern devices. The present study is formulated in order to find the difference between teaching attitude of teachers towards offline and online mode. The study sample consists 114 respondents (teachers) teaching in private, public and government schools. The qualitative and quantitative data were collected via online questionnaire prepared circulated among different sectors of teachers. The objectives of the study was to analyze the various teaching methods adopted by different sectors of school teachers of all sectors; public, government and private. The teachers tries to incorporate e-learning apps and e-learning modules in their teaching to students. The study also highlighted the challenges and problems faced by teachers towards offline and online teaching

Keywords: offline teaching, online teaching, e-learning, teachers, challenges

INTRODUCTION

Today's modern era allows utilization of modern devices like smartphone, laptops, tablets, computers which are used to text messages, photos, audio and video files to transmit from one device to another in seconds, latest mobiles and i-phones allow us to connect to the internet through wireless network; e-mails, instant messaging, chat rooms, and blogs have revolutionized the way information is shared [1]. As the letter "e" in e-learning stands for the word

"electronic", e-learning would incorporate all educational activities that are carried out by individuals or groups working online or offline, and synchronously or asynchronously via networked or standalone computers and other electronic devices [2]. There are various types or modalities of e-learning activity. An e-Learning environment which works as an interface between the students and their learning objectives and provides different means to achieve the learning goal [3]. Usually the e-Learning environment can be accessed using a Web browser over the Internet or Intranet and supports several learning strategies and different ways of interaction, communication and collaboration [4]. Additionally e-Learning environments often include administration and management utilities and interfaces to other systems to support the organizational part of learning as well. Thus e-learning has become a very crucial part of this ever changing educational environment [5].

REVIEW OF LITERATURE

Prabhakaran *et al.,* (2021) concluded that the Tamil Medium Students in Tamil Nadu Government Schools are taught using an e-content module of teaching and learning. Many schools lack the necessary infrastructure to deliver electronic curriculum. The facilities to learn the courses are slowly being developed in general government schools. The quality of education provided by State Government schools will be monitored as educational implications of the Federal Government. The study findings revealed that using an e-content learning module improves the learning of a certain language [6]. Alsoud *et al.,* (2021) concluded that E-learning is a way of teaching and learning that allows teachers to give educational materials to their students via internet media, internets, or other computer network media. E-learning is the conversion of a traditional educational system and content to a digital medium. The research revealed that students from remote areas encountered aslew of issues, including technological barriers, poor internet connectivity, and difficult study conditions. [7].

Ann Marie Casanova (2018) concluded that a learning app promotes deep conceptual understanding which improves educational outcome in India. Moreover, it also elucidates that Byjus app revolutionize millions of the students and improves their thinking and learning [8]. Bakr (2011) in the study on Egyptian public schools highlighted that teachers have positive attitude towards computers. No significant differences in terms of gender and teaching experience were reported. Murithi & Indoshi (2011) in their findings reported that both students and teachers had positive attitude towards the use of computer in relation to the Computer Studies curriculum [9].

MATERIALS AND METHODOLOGY

The study used a survey approach in order to examine the challenges faced by teachers towards online and offline teaching methods. The structured questionnaire is prepared based on offline teaching and online teaching. A structured questionnaire is prepared on online mode and circulated among teachers of secondary level.

Data Analysis

The data of 114 respondents was computed and analyzed. For each question, there were few options were given to the respondent in order to choose the best one accordingly. The data was analyzed and results were illustrated in the form of pie chart.

RESULTS AND DISCUSSION

The majority of respondents lies in the age group of 18-25 years which is followed by the age group of 25 to 45 years. The qualification background of 76.3% respondents were bachelors and rest 21.1% have completed their master's degree. As per results illustrated in pie chart, it has been observed that few respondents have completed their doctoral degree. The major portion of teachers towards teaching sector belongs to private schools (66.7%), followed by government schools (20.2%) and the minimum percentage of respondents reported in public schools (13.2%). The

major portion of teachers were 82.5% who teaches secondary level students whereas 17.5% of teachers teaches senior secondary students. 74.6% of teachers show interest in IT sector whereas 25.4% of teachers lack interest in IT sector. Most of teachers prefer offline mode of teaching (75.4%) whereas rest of teachers prefer online mode of teaching (24.6%). As per the demonstration of data, it has been observed that 69.3% of teachers had experience of online teaching as per data results many teachers show huge active participation in IT sector whereas 30.7% teachers do not have online teaching experience. The positive feedback has been given by teachers (36.8%) towards interactive presentations followed by animation videos prepared by 21.1% teachers. Few teachers preferred traditional method of teaching (24.6%) followed by group discussions (14.9%). Majority of teachers (84.2%) prefer teaching from e-learning apps and content as it is easily available on internet and such educational apps contain informative content which is beneficial for teachers as well as for students whereas 15.8% of respondents prefer books. In the scenario of online learning, many teachers faced hurdles in teaching. Many teachers (55.3%) had a problem of less interactive sessions with students. Another major problem faced by teachers was working hours (12.3%). As for longer duration of working hours, they need to be on laptop or in front of camera. The problems faced by teachers towards offline teaching are also taken into account. In offline teaching, visual presentations or animation videos were less (20.2%), there were less utilization of e-learning apps or e-learning content in classrooms (18.4%). 12.3% of teachers had a complaint about less interactive sessions with students in IT.

CONCLUSION

The results of the present study show that teachers are in favor of blending teaching method which involves offline and online mode of teaching with the help of e-learning apps and e-learning content. Moreover, this study reveals that teachers preference more towards offline learning as compared to online learning as they can interact more with students in physical mode. Moreover, they can utilize online educational apps for both online and offline learning. In particular, teacher's attitude is significantly changing from traditional mode of teaching to modern mode of teaching. They emphasize majorly on animated video lectures, utilization of e-learning apps in teaching students and interactive presentations to make students more interested and attentive in classrooms.

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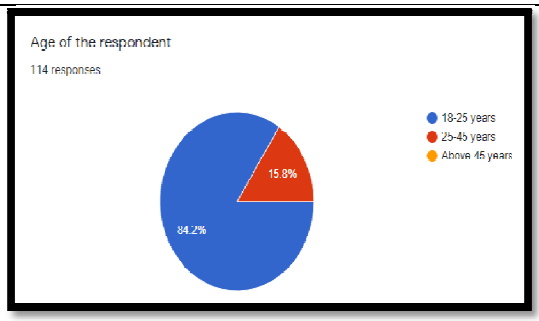

Figure 1: Age of Respondents

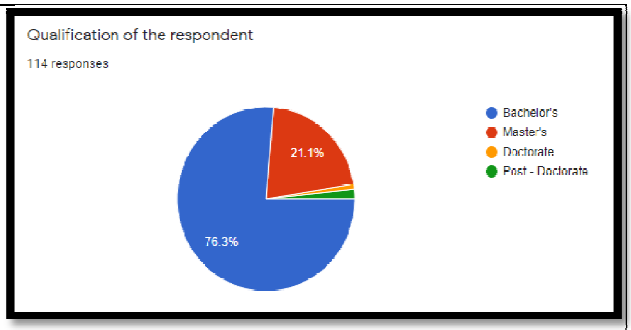

Figure 2: Qualification Of Respondents

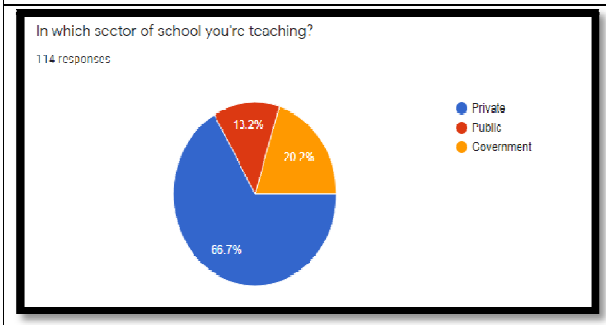

Figure 3: Sector Of Teaching

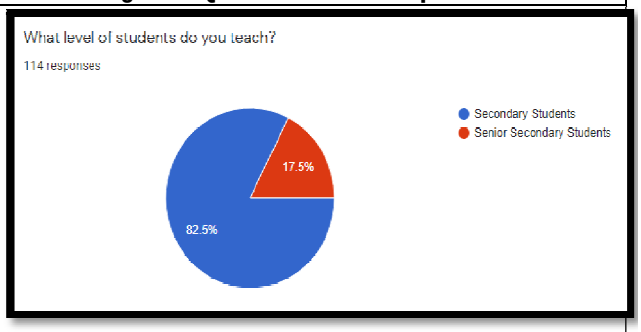

Figure 4: Level Of Teaching

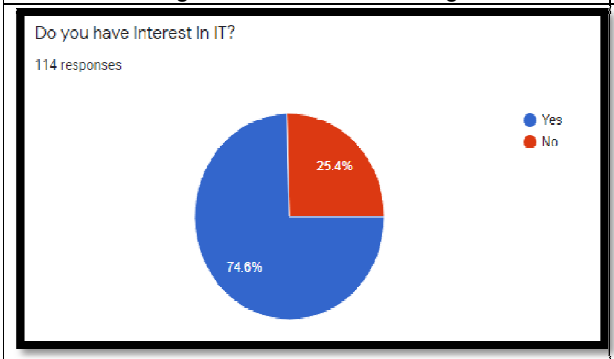

Figure 5: Interest In It Sector

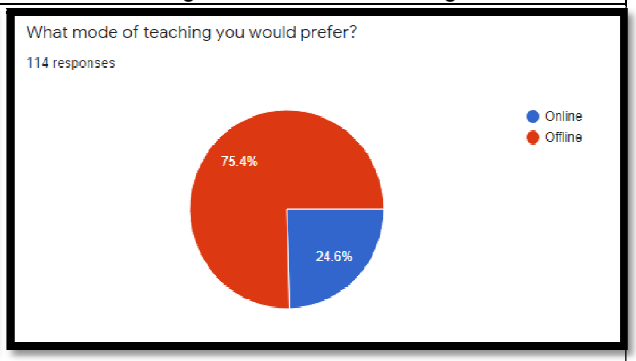

Figure 6: Mode Of Teaching

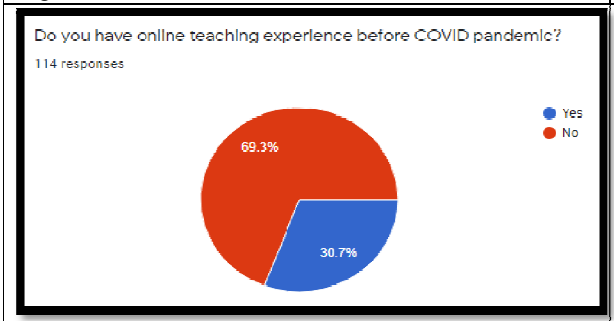

Figure 7: Online Teaching Experience

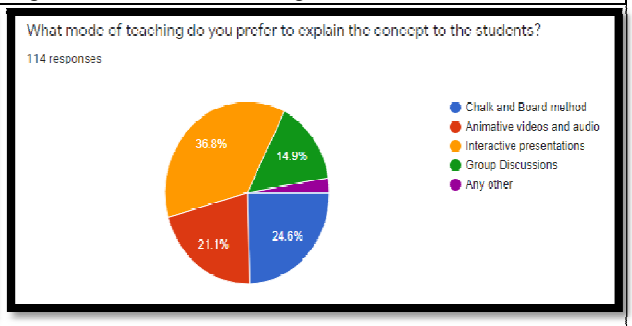

Figure 8: Mode Of Teaching To Students

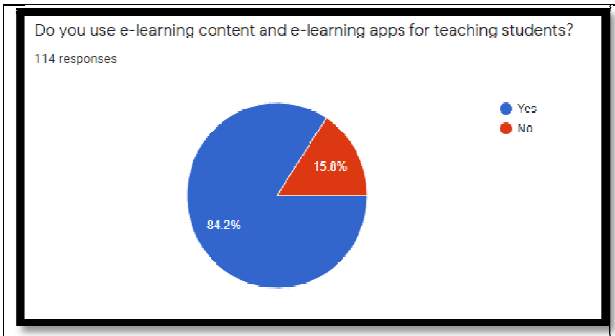

Figure 9: E-Learning Content And E-Learning Apps

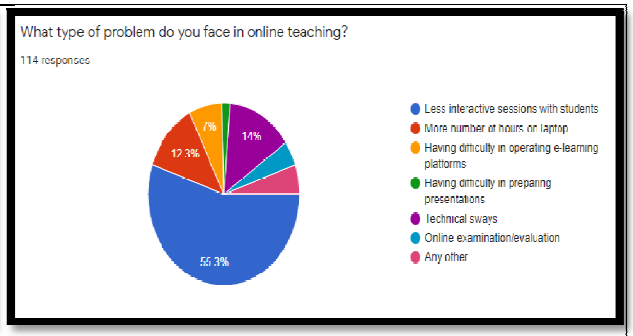

Figure 10: Problems Faced Towards Online Teaching

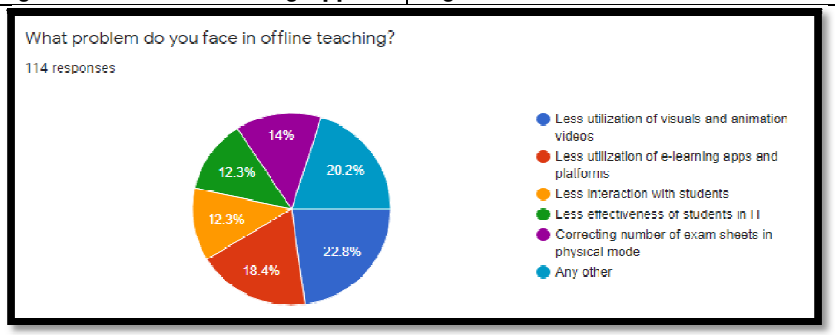

Figure 11: Problems Faced Towards Offline Teaching

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Break-up Mechanism by a Different Approach for Loosely Bond Nuclei

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Received: 10 Jan 2022 Revised: 22 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

The analysis of the breakup experimental data has been done in an innovative way using the concept of dalitz plot which normally people use for the high energy physics and found that it is very much useful to understand the direct and sequential breakup. A montecarlo based simulation has been performed for the above situation keeping the experimental geometry in mind and the experimental data has been reconstructed for comparison. It has been observed that in addition with direct breakup a lot of events are there from the sequential break which need further investigation to explore.

Keyword: CN,BU,SSB,BT

INTRODUCTION

Due to low breakup threshold (BT) the nuclear reaction involving loosely bound projectiles is interesting including its widerange of surprising behavior in experimental measurements [1]. Due to the availability of RIB (Radioactive Ion Beams) Over last decade and with the understanding of breakup new experimental results are coming to picture which are become amajor research focus [2]. Breakup (BU) of Projectile in the coulomb field of target nucleus has renewed the interest due to low threshold energy, also due its application to the radiative capture in the astrophysical scenario. breakup of Projectile changed the accepted picture for two body fusion process involving strongly bound nuclei. People observed that [3] the breakup of projectile can happen either near the vicinity of target which we called as Direct breakup or the breakup will happen at very far from the target called as elastic, inelastic/sequential breakup. In both the cases as explained above the breakup of projectile can happen and it can break in to one or more then one fragments. The heavy Residue which some time we call as the recoil nuclei can be left in ground or in excited state. For a proper understanding and to get a clear idea about the process ideally all the exit channel has to be measured experimentally which is not possible always. Since it is very difficult to measure all

the exit channel in all the experiments , the more contributing channel are measured and always focus has given to measured these channels first. In experimental measurements involving weakly bound nuclei, the cluster (α+x) structure has been found significantly more cross sections[4] then the production cross section of the fragment (x). i.e the measured cross section involving alpha channels are found more compared to others. This indicates that there are other mechanisms which are responsible for the formation of α+x cluster which need to be understood [5]. Presently there is not a single theoretical model exist which explain the breakup process and normal fusion including particle evaporation simultaneously. In the present article we reported a simple but effective approach to understand the breakup mechanism involving weakly bound projectiles. This approach is not common in normal experimental nuclear physics but in high energy physics people use this method. It is a three body process as explained bellow. This involves Monte Carlo technique for the calculation and a continuation of our earlier work reported in [6].

Experimental Details with Simulation

The experiment was performed at LNL(Laboratori Nazionali di Legnaro) Tandem Vande Graaff accelerator. The beam was ^{7}Li having energy varies from 35 and 39 MeV. The currents was varied between 5 and 10 nA. A target of ^{208}Pb having thickness 200 μg/cm^{2}has been used. The particles which were emitted during the reaction were detected by8PLP set up [7]. The set up has two main part called as "WALL" & the "BALL" both covers forward and backward angles. In total 126 Telescope (ΔE and CsI(TI)) has been used in BALL and the WALL has a 11×11 matrix telescope. For the present data analysis the WALL part has been used. The mass and charge has been identified very nicely . For the light particle(α, t, d & p). The mass identification has done in a very nice way. ^{7}Li which came out due to the elastic scattering has completely stopped by the one part (ΔE) of the telescope. The elastic particle has been clearly identified by the ΔEvs Time matrix. For the particle identification (alpha, p,d,t) a ΔE vs Eres matrices has been plotted for each detector. All the different types of particles (alpha, t, d, p & elastic ^{7}Li) has been separated very nicely from each another. It needs to understand the origin of each particles since they are in many varieties. The origin must be inside the different reaction mechanism. A correlation matrix has been generated to identify the breakup event only. A co-relation matrix between α &t for the coincident events has been reconstructed for the further calculation and comparisio. The Dalitz method has been adopted presently to understand the origin of the breakup process of the incident projectiles. A simulation has been carried out using a Monte Carlo techniques keeping two situations in mind: direct breakup and sequential breakup. In case of first situation the projectile directly breakup in target field whereas in second situation it is under go scattering first and then breakup will happen from the ground or any excited stated of the ejectile. This mechanism will happen at very far from target

The recoil nucleus will be either in ground or inexcited state. The keyidea of the Dalitzplot is the conservation of the total angular momentum and energy. The simulation involving Monte Carlo techniques for a Direct breakup event and sequential breakup event has been shown in Fig.1(a) for the incident beam energy of 35MeV. The projectile breakup has been simulated by considering the projectile in it's ground state. The filtration of breakup event has been done using the geometry of 8PLP. To study the three body reaction mechanism for breakup Dalitz plot is an excellent tool for this. The conservation of momentum and energies for the decay particles has been considered for each event in such a way that the Total energy in the rest frame of the decaying particle is equal to Etot= E1+E2+E3. The three energy are associated with the three particles, which will shown as the three sides of an equilateral triangle , where sided are being the possible energies of each one. The triangle are made in such a way that the perpendicular distances to any sides are adjust in a way that the total energy has to be conserve and each combination of (X,Y) shows a possible decay path of the projectile through the intermediate state[8].For direct breakup, nocon strains of energy has imposed as all possible values can happen so a uniform random distribution inside the triangle has been shown in Fig1(a). For this report the full geometry of the experimental setup has been simulated and reconstruction of the coincidence data has been done. The transformation of this breakup events in coincidence mode for direct and sequential breakup situation has been shown in Fig.1 (a). The experimental data has been shown in Fig. 1 (b) as insert The simulated breakup events from the excited state of the projectile for ^{7}Li has been shown in the Fig.1 (b) and it has found that the behavior is simlar to the experimental data (shown insert) . The

Data has shown for beam energy of 35 MeV and by using Dalitz triangle. one can see from Fig.1(a),(b) that the sequential breakup are restricted within a section of a triangle whereas a random continuous distribution has been shown for direct breakup events.

CONCLUSION

The analysis of the breakup using experimental data has been done in an innovative way using the concept of dalitz plot which normally people use for the high energy physics and found that it is very much useful to understand the direct and sequential breakup. A montecarlo based simulation has also performed by keeping the experimental geometry in mind and the experimental data has been used for comparison. It has been observed that in addition with direct breakup a lot of events are there from the sequential break which need further investigation.

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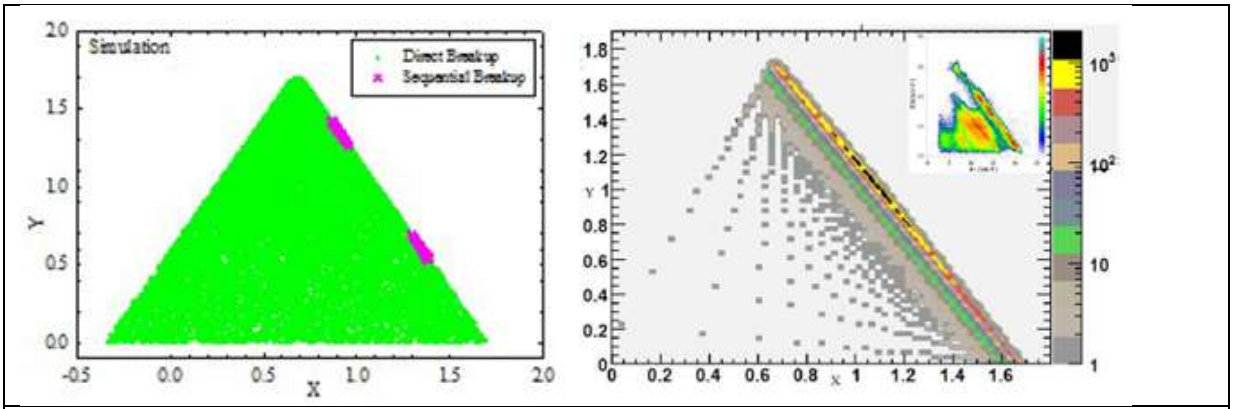

Fig.1 (a) The Dalitz triangle for the continuous direct breakup event where the energy constraints are not there. The side patches are due to the breakup from an excited state, a restriction due to energy conservation. (b) The experimental coincidence data shown in insert and the simulated data for the sequential breakup has shown in the triangle.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Influential Users Detection Techniques on Multiple Social Network Applications: Review

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 20 Mar 2022

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ABSTRACT

Online Social Networks (OSNs) have evolved to be an astounding development with great social and economic significance over the last few decades. Some of popular OSNs like twitter, instagram and face book have over one billion monthly active users in worldwide. Thus, OSNs grabs a massive attention from frequent media users and community-based research works. When integrated with the massive amount of knowledge stored by OSNs, it is estimated to be worth billions of dollars. Hence, a hybrid of technical and social events has evolved around the world, gaining significant socioeconomic influence. OSNs play a significant role in internet marketing techniques by improving the web search functioning, instructions for different filtering systems, and help to fast distribute technology (products) in the industry. It is vital to detect user who have the power to influence the actions of their neighbours; they are referred to as In Fluential users (IFU).Recognizing IFU in OSNs is an important step in accelerating the propagation of information, such as marketing applications, or avoiding the transmission of harmful topics. On several OSNs applications, this survey provides a complete survey of IFU detection strategies. In addition, their benefits and drawbacks are discussed in a tabular form. Finally, an entire survey is summarized and future directions are suggested to increase the IFU detection in various OSNs.

Keywords: Online Social Networks, Marketing Applications, Influential User, Multiple OSNs, Web Search Functioning.

INTRODUCTION

OSNs are fast evolving and playing an increasingly essential role in today's society. OSN enables users to contact with their friends and mutual interest groups, discuss knowledge, and pass it on. Social networking sites are now widely utilised as a tool for connecting with people who share common interests and aspirations. OSNs are rapidly expanding, with some reaching million hundreds of users in recent years. Some OSN sites are popular in specific nations, while others have a global audience. Social networks perform a significant part in the dissemination of data content have investigated [1]. the role of social networks in online data dispersion, as well as the effect of strong and weak relationships in information dissemination.

OSNs have enormous business possibilities in the current era. For evaluating and measuring user activity in OSNs, several data mining and other approaches are used. Various types of corporate companies are attempting to contact their target audience using social networking sites. OSNs provide excellent marketing options for enterprises of all sizes and sorts [2].. OSNs can be used to promote brands, educate customers about products and services, entice new customers, build relationships with existing customers, collect customer feedback, and so on. It has been discovered that approximately 80% of firms currently use social networking or blogging websites to sell their products [3]. The identification of IFU is extremely beneficial and has recently received a lot of attention [4].. Targeting IFU is critical for developing approaches for either speeding up the spread of information in marketing applications or preventing the spread of undesired content such as viruses, online negative behaviours, and rumours [5]..Furthermore, analysing influence patterns is an important aspect in determining the rapid adoption of specific trends. Advertisers can use influence patterns to create highly effective advertisements.

Numerous research papers have been published for the discovery of IFU in OSNs. The search for the best set of important users is a major issue that is dependent on user topological interactions [6].. The ranking of IFU in relation to topological measures is commonly used to identify them. As a result, an efficient topological measuring algorithm is important to detect the significant users in OSNs. In this way, this article presents the detailed survey on various detection models of IFU on multiple OSNs applications. Also, a comparative study is presented to address benefits and drawbacks of those models for suggesting further improvement on detecting the IFU in social media websites.
The following sections of this paper are followed as: Section II studies different techniques for influential user's detection on various OSNs. Section III discusses their benefits and drawbacks. Section IV concludes the survey and suggests the future enhancements.

Survey on Influential User Detection On Various Social Online Networks (SNA)

A data mining approach was developed [7]. based on the Association Rule principle to identify attributes of great influencers in factual data of user profiles and activity logs obtained from a particular online application. The user data was selected from the specific web application as an affiliating gateway for social networking. Then, these datas were pre-processed and converted then to nominal values. By using the Association Rule principle, the threshold values were determined to ensure the statistical significance of the system. However the performance was limited.
A heuristic method called Degree Punishment was developed [8]. to select spreaders sequentially by maximising their propagating influence in undirected systems. This method incorporates simple degree centrality with a punishing technique to eliminate distance between selected spreaders who were too close to optimise the spreading phase. The Susceptible-Infected-Recovered (SIR) model was used to assess the spreading influence of the selected spreader. However, the performance of this method was not effective than other meta-heuristic methods. A multi topic-aware influence maximisation approach [9]. was developed to identify a certain number of important users and assign video clips on diverse topics to them. Various topics were used to represent the different video clips of user's interest. Then, the developed Influence Maximization (IM) was used to solve the video distribution problem. Greedy algorithm was used to solve sub modularity problem of the influence spread function. An upper-bound estimation algorithm was applied to reduce the computational pace of this developed greedy algorithm. However, the computational complexity of this model was high.

The user influence rank (UIRank) technique was presented [10]. to find influential persons in microblogs by interactive information flow and interaction links between users. This method recognises the value of a user's tweet and the characteristics of information distribution in microblog networks. The user influence score findings were assessed from this algorithm using a user follower relationship graph. This method was incapable of training huge important users in micro-blog networks. Millions attributes of social network users and their messages content was investigated [11]. in order to predict influencers in social media platforms. First, Individual influence was evaluated over time using a new approach, and it identifies the individual effect of the majority of users changing over time. Second, the phrase merging method was created to provide a high-quality 'bag-of-words' as input to LDA, ensuring that tokens in the same phrase were classified to the same topic. Third, the each user influence was predicted with a higher performance result. However, the number of topics was fixed and must assign in advance.

Twitter networks were constructed [12]. to identify influencers by analyzing their tweeting behavior. The data of inactive followers was evaluated using various forms of Twitter networks were built with responses and mentions (R/M). A connection between two entities was recognized in this network when one replied to or mentioned another. The author either has something to add to the other account (replies) or believe that mentioning the other account will benefit their audience (mentions). Furthermore, this network model requires more computational time and cost.
A novel Page Rank algorithm [13]. was designed to discover an influential user for evaluating the sentiment polarity of a social network-based topic-based micro blogging community. First, the user's influence in social communities was determined. Then, a Page Rank algorithm was utilised to calculate an impact degree for each community member on social networking sites. The computed value was then used to weight the postings, and the community sentiment polarity value was determined using the weighted postings. However, an accuracy of this method was low.

The development of influential individual Twitter users was explored [14]., and the cause for gaining a large amount of supporters during the severe disasters: Hurricane Harvey, was investigated. Initially, a relationship between a user's gain in followers and the substance of their tweets was analyzed. Then, emerging influencers were separated from ordinary Twitter users in order to investigate the significance of various characteristics that users might utilise to boost their following. Finally, clustering analysis was utilised to identify and profile the people who helped with catastrophe information transmission. However, it does not allow for a casual relationship examination between each budding influencer's tweeting activity and their events. A novel method was introduced [15]. for detecting an influential user to enhance information dissemination in the internet marketing. First, the user trust network geared to online marketing was determined, as well as the user's integrated interest degree in the network comprising isolated users. Then, a time factor model was built to replicate the information spread in online marketing. Finally, a dynamic modelled technique was designed to identify influential people from the processed information diffusion. Furthermore, it is impossible to obtain all data from the network due to insufficient layout or user security access.

A novel overlapping influence (OI) method was developed [16]. for identifying various IFU in multiplex social networks. At first, the concept of spreading rate was defined using the effective spreading shortest path (ESSP) to denote the relative position of users. Next, the collective influence was estimated by taking the topological factor and user location dispersion into consideration. The identified users were central and relatively dispersed with a low overlapping influence. The SIR model was used to assess propagation efficiency in terms of maximum spread influence. This method's effectiveness was occasionally delayed due to weak overlapping affects. A novel approach was created [17]. to recognise IFU and opinion propagation towards the networked social group was extracted. The optimism and pessimism scores of users' personalities were used to identify the network's IFU. An opinion aggregation approach was presented that takes the IFU effect and opinion propagation into account to provide the consensus opinion in signed and unsigned networks. Finally, client feedback was used to generate a rating score for an online purchasing product. If significant people's opinions are incorrect, this approach will be unable to evaluate the aggregation model precisely.

Threshold estimation structure was developed [18]. to identify the IFU using influence-weight and degree dispersion of online social network. Four estimating models were utilised in this method to derive threshold values for the Linear Threshold (LT) and LT-based IM methods for mining IFU on online platforms. Threshold vales were categorized under unique and random individual categories. Under an LT paradigm, this technique might be used to any weight and degree-based IM. When a network was very large, the running time would be higher. Influencer tweets for three childhood vaccines (measles, mumps, rubella (MMR), tetanus, diphtheria, pertussis (Tdap), and human papillomavirus (HPV)) were examined [19]. on Twitter. This method collects negative tweets from both pro- and anti-vaccine communities, demonstrating the popularity of negative sentiment analysis on social media. Then, geo-location clusters of IFU were detected. The detection of important users and their geolocations would offer data to aid in the reduction of online vaccination disinformation and the detection of possible disease epidemic areas. However, this model has a large computational load.

An improved Hybrid Rank algorithm [20]. was developed to score and select relevant spreader nodes in an OSN with the goal of IM. This algorithm combines two elements, such as the Extended Neighborhood Coreness and the H-Index centralities. This method ensures that selected spreaders have a good neighbourhood in order to increase information spread throughout the network. This method was stimulated by utilising the SIR model on various un-directed and directed real-world networks to demonstrate node ranking and the selection of influential spreaders. However, this model had a considerable computational complexity. A new incremental method was proposed [21]. to identify influential nodes in dynamic social networks. Initially, the influential nodes were identified in the original network. Then, the changes in the influential nodes were gathered and analyzed. Finally, an incremental algorithm was developed to update the influential nodes in dynamic social networks. On the other hand, the selected influential node does not show any significant variations in large-sized networks.

A SNet model for expressing a OSNs based on individuals, tags, and relationships between them was presented [22].. A graph-based technique was created to estimate an IFM and the speed of information propagation. The passion point was created to identify the community of users who are interested in the user product and share similar traits. Two primary influential measures were built using theSNet model as an influence extent on other individuals by user relationships and concern for user's tags, and tag propagation through social pulse on the OSNs. However, this model has a tremendous computational expense. A large-scale analysis of influencer behaviour on Instagram was given [23]. to prove the influencer's honesty and ensure compliance with advertisement regulation. A broad characterisation of Instagram influencers were collected and formally categorized according to their behavioural attributes. Then, an automated tool was constructed to detect a sponsored post which fails to explicitly tag them. Finally, a Contextual LSTM Neural Network classifier was created to determine if a post was sponsored or not based on text content and other metadata. However, this model was difficult to train the behavioural attributes.
The origins and implications of opinion leadership were investigated [24]. On Instagram. This research provides an unexpected finding that shows the characteristics of material published on an Instagram account should have in order for the author to be perceived as an authority figure by other users. Multiple patterns were identified to stress the role of opinion leadership on consumer behavioral intentions, and two modifiers were included to demonstrate these links. However, an unanticipated effect such as inclination for online interaction does not increase the influence of opinion leadership.

Anew method was determined to detect IFU on Instagram [25]. by examining User Generated Contents (UGC). This method was classified into three different sections 1)the details of Instagram users were validated asIFU, and then they were divided into influential and non-influential categories. 2) On multiple visual representations and classifications of the image, feature selection and Influential Post Identification were created to separate into influential and non-influential sets. 3) Classifier Development and Influential User Identification would combine many classification approaches to create a final classifier that could be used to locate IFU and posts. However, this model has a large computational cost. A population-based grey wolf optimization method was presented [26]. to detect a near-optimal set of the IFU in online platforms. Entropy metric was used to measure an influentiality of users. Then, a grey wolf optimization method was used to solve the IM problem with cost functions like nodes

influentiality and their distance between them. However, the performance of this method was not effective than other existing methods.

Using network topology derived from user communication relationships, a link analysis technique, and user profile data, IFU were identified [27].. The goal of this methodology was to find popular subject influencers. In a trending subject, communication relationships such as retweet, mention, and reply relationships between Twitter users were retrieved. The user's personalization weight was calculated using profile information and a trending topic community graph with relationships. Finally, IFU were identified using a link analysis approach. The model's efficacy in terms of tweet influences for locating IFU was sometimes restricted. A Weighted Correlated Influence (WCI) method was developed [28]. to calculate the influence ratings of each user in the microblog Twitter network. The content for a specific topic or keyword was created by topical influencers from global social networks that were separated into a pertinent network. Individual profile parameters were integrated in the full chronology, as well as fundamental structural elements discovered by examining the user-user relationship graph. The combined effect of profile behavior and distributed system structure was utilized to assign an influence score to online users. However, the computational time was high.

A Discrete Moth-Flame Optimization (DMFO) technique was presented [29]. to tackle IM in social media. DMFO was used to find IM node sets by layering a local crossover and mutation evolution method on top of traditional moth position updates. A search area selection technique adaptable was used to seed set size to improve the algorithm's convergence speed. An influence estimation model based on total valuation and variance in value of neighbor nodes was devised to quantify the influencing potential of seed sets while taking into account the likelihood of transmission unreliability in social relationships. However, this model was computationally expensive. A multidimensional social influence (MSI) measurement method was developed [30]. to detect influencers in OSNs. Initially, OSNs attributes were examined using three dimensions like structure, information and action and also explores the important factors of users influence. Then, a topic-level MSI (TMSI) and a global-level MSI (GMSI) measurement structure were created by selecting a selecting relevant metrics, weights calculations, and acquiring topic distribution. But, MSI was able to discover influential nodes in a consistent manner with an acceptable computational complexity,

An effective social media influential user was identified [31]. using SNA for a real-world online marketing technique. The multiple centrality indices were compared in order to determine the network's key nodes. The spreading behaviour of individual users in a social network was then computed using a linear threshold model.A network-wide comparison of various centrality indicators would back up this marketing strategy. However, the distribution of information among influencers' followers was not well adjusted.

Comparative Analysis

In this section, a comparative study is presented in Table 1 according to the benefits and drawbacks of different techniques used to detect the IFU which employed in multiple OSNs applications which are studied in above section.

CONCLUSION

OSNs have become a valuable source of large data for researchers interested in analysing and interpreting social interactions among their users. In this paper, the benefits and limitations of recent influential user identification methods in OSNs are explained in this article. From this survey, it is analyzed that many algorithms have been used to detect the IFU on multiple OSNs application for viral marketing applications or administrating the propagation of producing information. From the above systems, the user, structure and content based methods provides the better influential user detection with various OSNs applications. By using user post content, it is beneficial to investigate an influential user for various marketing strategy on social media application. Analyzing the role of IFU is helpful in identifying OSN and user privacy related problems for future developments.

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Table 1 Comparison Of Different Influential User's Detection Techniques On Various Social Network Sites

Techniques Used	Merits	Demerits	Performance Accuracy
Association Rule principle[7].	High level confidence findings of IFU were resulted.	limited performance	Confidence level = 80%
Degree Punishment heuristic method and SIR model[8].	Even it was effective and suitable for large-scale networks.	Performance was not effective than other meta-heuristic methods.	Confidence level = 20%
Multitopic-aware IM model and upper-bound estimation method [9].	Comparatively, this method was much faster than other existing algorithms.	High computational burden	For example, if K = 100, the execution time on high degree (Wiki-Vote) is twice that on small degree (Wiki-Vote) (NetPHY)
User influence rank algorithm[10].	Efficient running time	Less performance in training the large influential user networks	@top 2000; N=4 Precision = 0. 97 Recall = 0.99 F1-measure = 0.68
Phrase Merging Algorithm and LDA[11].	Each user's individual influence might be anticipated with reasonable precision.	Fixed topic values will reduce the accuracy results.	RMSE (gradient boosting) = 1.59
Twitter network and R/M networks[12].	Efficient in identifying all account types that exhibit the closest relationships	High computational expense and time.	Pearson correlation coefficient = 81%

PageRank algorithm and sentimental analysis[13].	It maintains the accuracy of correlations between stock market behaviours and the financial community's sentiment polarity.	Slow performance	correlation coefficient = 51%
Pre-processing and Clustering analysis [14].	This method was effective in sharing and warning the public information for large social networks.	Relationship exploration was not permitted among the tweeting activity of an influencer and their events.	When k=10; interest of the covered nodes (SC) for DBLP = 0.04%
User trust network time factor model and dynamic algorithm[15].	Less computational time	Insufficient system architecture	No. of interested user on twitter influence = 26.36%
Overlapping Influence (OI) method and SIR model[16].	Even for the large size of spreaders, this method provides a significant results with less overlapping influence	Slow performances due to weak overlapping influences.	the ratio of maximum influence (MI) rates between two layers = 1.0 to 3.5 (t).
consensus opinion aggregation method[17].	Consistent performance was resulted throughout the experiment.	The precise results were reduced due to incorrect opinions of influential people.	For two IFU Fuzzy Majority Opinion (FMO) = 93% Ordered Weighted Average (OWA) = 89%
Threshold estimation models and LT-based IM models[18].	The narrower range of smaller threshold values reduced the insufficient influence effect.	Increase in network model, increases the running time.	When compared to other methods, the seeding computational cost was reduced by 30-40%.
Sentiment analysis[19].	Simultaneous identification of IFU was possible.	High computational complexity	Sentiment analysis results by community Negative = 52.27% Positive = 12.37% Neutral = 33.33%
Hybrid Rank algorithm and SIR model[20].	it was an effective tool to IM in online social networks.	High computational complexity	Kendall-Tau's correlation coefficient on Hybrid rank = 26.8
Incremental algorithm[21].	Less computational time.	Influential node does not provide any significant variations in large-sized networks.	For timestamp = 100, influence degree for facebook = 0.12 Email= 0.10
SNet model, Graph-based approach and passion point[22].	This strategy could approach the emerging costumers correctly.	High computational cost.	Number of interactions for post rate = 36%; comments = 47%; Shares = 46%
Contextual LSTM Neural Network classifier[23].	Even with the limited classification task, this model was effective in identifying influencer posts.	Difficultly in training the behavioural attributes.	Accuracy Random Forest = 84% Contextual LSTM = 89%

Partial Least Square (PLS) method[24].	Increased customer intent to interact on Instagram was encouraged, which benefited the opinion leader.	Online interaction propensity does not improve the influence of opinion leadership. The inclination for online interaction has no effect on the influence of opinion leaders.	Dependent variable (R^2) opinion leadership = 0.437 Intention to follow the advice received= 0.317; Intention to recommend an account online= 0.239) and Intention to interact =0.314.
User Interaction structure and content based methods [25].	In order to identify influence users in a social network, this method was more precise and robust than earlier approaches.	High computational cost.	Accuracy on feature stage= 86.50%; Rank stage = 85.23%; Decision stage= 84.39%
Population-based grey wolf optimization method and Entropy metric[26].	Less computational time	Performance was not effective than other existing methods	For K= 10, 202, 30; Grey Wolf based IM(GWIM) = 1.000
Link analysis approach[27].	The chance of significant IFU for a trending topic was retrieved with 100% of effectiveness.	Limited performances was available	Recall = 76%; F1-measure = 79%
Weighted Correlated Influence (WCI) approach[28].	Parameters were effective in generating a range of properties from which the influence score could be computed.	High computational time	Influence score for Rank wise user ID in eigen vectors = 1611
DMFO algorithm, search area selection scheme and influence estimation model[29].	The quality of IM solutions was more efficient to detect an IFU.	Computationally expensive	when K= 15; The developed DMFO for Pages-public figure = 1747.07
a topic-level and global-level MSI measurement method[30].	It was a beneficial method for improving OSN users' reputation and influence.	High computational complexity	MSI model for FBI = 66.17%; LeaderRank= 67.61%.
SNA[31].	Less computational cost.	Information spread among the influencer's followers was not properly optimized.	When node n = 20; Node ranked according to the spreading capability (score) will be 7.5

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Advances of Nanotechnology in Cosmetics

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Received: 17 Feb 2022 Revised: 03 Mar 2022 Accepted: 26 Mar 2022

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ABSTRACT

Cosmetic products are being used as a way to enhance beauty. As of now the use of various products like lipstick, deodorants, shampoo, hair dyes are used to improve the personal well - being and to boost self-esteem. The use of nanotechnology in cosmetic formulation has become an emerging technology. It is being used as an effective way to counteract the limitation of traditional products. The use of nanoparticles in cosmetics come up with enhanced skin penetration, UV protection, prolonged effect. The nanocarriers used in cosmetic include liposome, noisome, nanoemulsions, carbon nanotubes, gold and silver nanoparticles, nanospheres and nanocrystals.

Keywords: Cosmetics, nanotechnology, formulation, nanocarriers, liposome

INTRODUCTION

Cosmetics are preparations that are designed to be placed in contact with various external areas of the human body, or teeth or oral mucous membranes, for the purposes of cleaning, perfuming, changing their appearance, and preserving them in good condition [1]. The significance of cosmetics in modern culture is rapidly evolving, and now considered as a fundamental component of personal life [2]. The cosmetic business is seeing a breakthrough in the form of newer and more advanced cosmetics as technology advances. Nanotechnology has shown to be the most useful for this advancement [3]. Nanotechnology is a branch of science that began in Japan and which manipulates atoms and molecules at the nanoscale (1–100 nanometers) [4]. Nanotechnology is increasingly being used in the field of cosmeceuticals to eliminate some of the limitations associated with traditional products [5]. Nanotechnology has enabled the invention of unique target-related cosmetics for certain areas and actions, resulting in higher-quality goods. Nanotechnology has proven to be effective in improving product efficacy, shelf life, and performance [1]. Cosmetic formulations that use nanotechnology as a delivery mechanism to improve the effectiveness of bioactive compounds are known as nanocosmeceuticals. This method enables for the development of microscopic cosmetic nanoparticles that may be quickly absorbed into the skin, repair damage, and improve product efficacies [6].

Liposomes, niosomes, nanocapsules, nanoemulsions, dendrimers, gold and silver nanoparticles carbon nanotubes are some examples of carriers used in nanotechnology [7].

Why nanocosmetics?

According to current research, nano-sized cosmetic pose no risk to human health, and their use in sunscreens provides significant benefits such as skin cancer protection [28, 8].

The advantage of using nanomaterials in cosmetics are

- Improves stability and penetration of vitamins, or antioxidants
- Enhance tolerance of skin to UV filters
- Make the product more aesthetically pleasing [9,10].

The use of nanoparticles have been found in toothpaste, deodorants and sunscreens. Because of their small size, they have special features that can alter how they interact with the skin [11]. Nanosized particles act as sunscreen reflectors, as depot site in creams, or they can protect actives ingredient or colours from degradation. Silver NPs are utilised in deodorants and hand creams for their antibacterial and preservation properties. Furthermore, NPs can be utilised to add stability to formulations containing chemicals that degrade due to oxidation or other factors [12].

Types of Nanosystem in Cosmetics

Nanocarries and used in cosmetic preparation for efficient delivery of active constituents through skin .These nano-systems can help cosmeceutical active moieties absorb into epidermal layers, allowing for sustained active release [13]. The types of nanocarriers are depicted in figure 1. The general representation of nano carriers and given in figure 2.

Liposomes: Liposomes are commonly used in cosmetic preparations. These are vesicular with an aqueous core and hydrophobic lipid bilayer [5]. Phospholipids are regarded as safe ingredients thus lowering the risk of side effects [14]. Phosphatidylcholine is a phospholipid with softening and conditioning qualities that are used in skin and hair care products such as moisturising lotions and shampoos [4]. Dior's anti-aging cream 'Capture,' launched in 1986, was the first liposomal cosmetic product to hit the market [14]. As the flexible and soft bilayers of liposome may mix easily with the skin lipids, these are useful in cosmetics, where they can ease the creation of various topical formulations and perhaps improve the percutaneous absorption of medicines. This may weaken the lipid in the stratum corneum, as well as their cell membranes, and improve medication absorption into the skin at the same time [15]. Antioxidants such as carotenoids, CoQ10, and lycopene, as well as active components such as vitamins A, E, and K, are combined into liposomes, which improves their stability [4]. The encapsulated medications, release the active ingredient in a regulated manner to protect them from metabolic breakdown and is being used in sunscreen, antiaging cream and in hair loss treatment [16].

Nanospheres: Nanospheres are colloids of size ranging from 10 to 200 nanometer that are arranged in a matrix. These are meshed structures with a central core of colloidal material which makes it a complex systems [3]. These are amorphous or crystalline nanocarriers that can be divided into two categories: biodegradable and nonbiodegradable nanospheres [3,13]. Nanospheres are utilised to transport active chemicals to deeper layers of the skin more efficiently and precisely. Cosmetics such as anti-acne treatments, moisturisers, and anti-wrinkle creams are increasingly including nanospheres [7].

Nanoemulsion: Nanoemulsions or Miniemulsions or submicron emulsions are spherical dispersed particles which are used as carriers. They appear to have a bright future in cosmetics, diagnosis, drug therapies, and biotechnologies [17]. The most essential role of nanoemulsion-based cosmeceuticals is the lower micellar nanoparticle size, which make cosmetic formulations resistant to flocculation, sedimentation, and the Ostwald ripening phenomenon [6]. Oil in water nanoemulsion is proving to be a viable strategy in cosmetics such as make-up removal, face chemical, anti-aging moisturiser, sun-screens etc [4]. The smaller size assures increased availability of active substance reaching the desired site of action [18]. An O/W nano-emulsion containing *Opuntia ficus-indica* (L.), a mill hydroglycolic extract, outperformed conventional formulation in terms of moisturising performance [19].

Gold Nanoparticles: Nanogold are inert, very stable, biocompatible and noncytotoxic with a diameter ranging from 1 nm to 8µm [20]. These come in a variety of configurations including nanospheres, nanoshells, nanoclusters, nanorods, nanostars, nanocubes, branching triangles, and nanotriangles [5]. Since ancient times gold has been used for beautifying and gold nanoparticles has been used in many anti-aging cosmetic products [2]. Nanogold can penetrate into the target tissues because of small size and large surface area [3]. Furthermore these have good drug loading capacity[3,16]. These particles have antibacterial, antifungal, anti-inflammatory and antioxidant properties, thus being used in moisturising creams, facial creams, toothpaste, deodrants, and antiging creams [3,21,22]. The added advantage of nanaogold is that it is nontoxic and do not exhibit photo bleaching [21, 3].

Dendrimers: Dendrimers are micellar branched symmetrical nanostructures with a high density of functional end groups at their periphery, making them suitable for use in pharmaceutics as well as cosmetics [9]. Dendrimers have the ability to penetrate skin barriers and is being used in cosmetic preparations like shampoos, sunscreen antiacne creams and hair gels [23,13]. Cosmetic industry like L'Oreal have patent for production of mascara and nail polish using dendrimers [24]. In an in vitro study performed, it was found that the dendrimer based pre-treatment enhanced the volume an penetration of chlorhexidine gluconate which can be used for treating bacterial infections [25]. It was found that the dendrimer conjugated molecule had UV absorbing properties and developing clear sunscreen formulations [26].

Carbon Nanotubes: Carbon nanotubes are a cylindrical arrangement of carbon atoms used in drug delivery systems that are made by rolling one or more graphite layers. It is of two types: Single walled carbon nanotubes and Multiwalled carbon nanotubes [3]. Carbon nanotubes are a new technology that has been exploited in the cosmeceuticals industry, in skin care which can operate as antioxidants on their own [7,27]. Heavy metals are used in skin whitening products, which are absorbed by the skin and can have negative consequences. In a study carbon nanotubes were used for the quantitative recovery of heavy metals [28]. These have been used as a viable hair dying method for darkening light coloured hair to dark colours like brown or black [29,30]. Various chemically functionalized carbon nanotubes for colouring brows, eyelashes, and so on have been developed [31]. Peptide-based carbon nanotubes, which are made by incorporating a hair-binding peptide on the surface of the nanotube, have a higher affinity for hair due to covalent bonding [32]. On the other side it was found that keratinocytes treated with carbon nanotube materials, exhibited significant phagocytic capacity, oxidative stress, antioxidant depletion, and cytotoxicity [8].

Niosomes: Niosomes are non-ionic surfactant vesicles that are formed by hydrating synthetic non-ionic surfactants with or without cholesterol with particle size from 10 -100nm [33, 34]. The two main components of niosomes are cholesterol and non-ionic surfactants. Cholesterol maintains appropriate shape and rigidity, while non-ionic surfactants aid in niosome production [35,36,37]. Properties such as good chemical and physical stability, low cost, and ease of multiplication are attracting interest in the pharmaceutical and cosmeceutical industries [38,39]. According to one study, quercetin (an antioxidant) loaded niosomes have better water solubility and skin penetration than free quercetin. As a result, niosomes are a promising cosmetic candidate [40]. L'Oreal was the first company to use niosomes in cosmetics and product was named as Lancome [33,41]. Niosomes have lower toxicity and allowed controlled distribution of the loaded active chemicals, which have beneficial effects for skin moisturising and tanning and are commonly utilised in anti-wrinkle, skin-whitening, sunscreen, and moisturising products [41]. In a study with cream containing niosomes loaded with 1 percent w/v methanolic purple glutinous rice extract had superior in vivo anti-aging efficacy on human skin compared to the cream base and before application. Thus it can be developed further as a novel anti-aging cosmeceutical product [42].

Nanocrystals: Nanocrystals are crystal aggregates with sizes ranging from 10 to 400 nm that are made up of hundreds to thousands of atoms that combine to form a cluster and are used to deliver poorly soluble actives [43]. Nanocrystals are now being investigated in the cosmetic market [44]. The nanocrystals can be incorporated into any cosmetic topical formulation, such as creams, lotions, and liposomal dispersions [45]. The application of nanocrystal technology to the delivery of poorly soluble, non-pharmaceutical actives have highlighted its use in cosmetics or

nutraceuticals [46]. It was evident from a study, when compared to commercially available powder, rutin nanocrystals in gel had the highest antioxidant activity [47]. Nano particle based cosmetic products are being formulated and a list of marketed formulation is given in table 1.

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Table 1: Marketed Nanocosmeceuticlas and its uses.

Novel Nano Carriers	Product Name	Marketed By	Uses
Nanospheres	Nanosphere Plus	Dermaswiss	Antiaging
	Lip Tender	Kara Vita	Lip Moisturizer[48]
Nano emulsion	Coco Mademoiselle	Chanel	Prolongs Fragrance Effect [49]
	Nano cream	Sinerga	Wet Wipes[50]
Liposomes	Decorte Moisture Liposome Face Cream	Decorte	Moisturizer
	Decorte Moisture Liposome Eye Cream	Decorte	Moisturizer, Brightens The Delicate Skin Around The Eye[51]
Gold Nanoparticles	Chantecaille Nano Gold Energizing Cream	Chantecaille	Revitalizes ,Promote Regeneration of Cell and Collagen Production, Repairs Sun Damage and Makes Skin Firm
	Chantecaille Nano Gold Energizing Eye Cream	Chantecaille	Antiaging, Promotes Collagen, Reduce Inflammation[52]
	Orogold 24k Nano Ultra Silk Serum	Orogold	Restore Moisture Loss, Improves Wrinkles And Fine Lines[53]
Niosomes	Niosome + Perfected Age Treatment	Lancome	Remove Wrinkles
	Mayu Niosome Base Cream	Laon Cosmetics	Whitening And Moisturizing[5]

Figure 1: Types of nanocarriers used in cosmetics

Figure 2: General representation of nanocarriers

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Surface Roughness of Bulk Fill Composite Resin Before and after Immersion in Different Fizzy Drinks - An *In Vitro* Study

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Received: 08 Jan 2022 Revised: 11 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Consumption of certain beverages may affect the esthetic and physical properties of the resin composite, thereby undermining the quality of restorations. A smooth restorative surface can also help to prevent plaque retention, reducing gingival irritation, surface staining, patient pain and secondary caries. The aim of this study was to evaluate the surface roughness of bulk fill composite resin before and after immersion in different fizzy drinks. We took 12 bulk fill composite resin samples, in which 6 were of Swiss TEC brand and the other 6 of Te Econom Plus brand. A disc was made using a circular mold of standard diameter of 10mm and thickness of 2mm, which is made up of additional silicone material. The composite material was placed and UV light cure was done for 30 seconds. Then the specimen was removed from the mold and smoothen up by using conical bur. All the samples were inspected carefully. Among 12 samples, 6 were immersed in coke and the other 6 were immersed in Appy Fizz for 24 hours. The pre immersion and post immersion surface roughness were measured using a Mitutoyo SJ-310 stylus profilometer. The pre Ra and post Ra for bulk composite resin was 0.003. The pre Rq and post Rq for bulk composite resin was 0.00400 and 0.00350. The pre Rz and post Rz for bulk composite resin was 0.02525 and 0.02058 respectively. Independent t test was done.The p value for Rq was 0.007 (<0.05), so it is

statistically significant, and for Rz was 0.945 (>0.05), so it is statistically not significant. There was a negligible difference in the surface roughness values of bulk fill composite resin samples before and after immersion in appy fizz and coke drinks. This might be due to less time period immersion protocols of bulk fill composite resin.

Keywords: Surface Roughness, Composite resin , Fizzy drinks, Stylus profilometer, Innovative measurement

INTRODUCTION

Surface roughness is one of the components of surface texture (1). In restoration procedures, a surface character, such as roughness, can determine the quality and the clinical behavior of the restoration material. Smooth surfaces can influence the aesthetic aspect of restorative materials. Roughness plays an important role in determining how a real object will interact with its environment. In tribology, rough surfaces usually wear more quickly and have higher friction coefficients than smooth surfaces (1). Roughness is often a good predictor of the performance of a mechanical component, since irregularities on the surface may form nucleation sites for cracks or corrosion (2). Bulk-fill resin composites are light-cured resin composites that can be placed in increments or layers of 4 to 5 mm in depth. These materials have been available to the dental profession for several years now. At first glance, the most important advantage that these materials provide is time reduction during material placement and polymerization, along with reduced technique sensitivity. However, other advantages such as the elimination of voids between increments are also important. Consumption of certain beverages may affect the esthetic and physical properties of the resin composite, thereby undermining the quality of restorations (3). Beverage erosion activity affects composite restorations, leaving a rough surface that impairs the material's optical qualities and increases the accumulation of bacterial plaque and degradation of the restorations surface (4). Erosion reduces the hardness and wear resistance of a material. Furthermore, surface roughness can irritate the gums and raise the risk of secondary infections (5). Dental plaque buildup on the surfaces of restorative materials can operate as a bacterial source, resulting in secondary caries and periodontal disease (6). A smooth restorative surface can also help to prevent plaque retention, reducing gingival irritation, surface staining, patient pain and secondary caries (7),(8). The aim of this study was to evaluate the surface roughness of bulk fill composite resin before and after immersion in different fizzy drinks.

MATERIALS AND METHODS

This in vitro study was carried out in White Lab at Saveetha Dental College, Chennai, India. We took 12 bulk fill composite resin samples, in which 6 were of Swiss TEC brand and the other 6 of Te Econom Plus brand (Figure A and B). A disc was made using a circular mold of standard diameter of 10mm and thickness of 2mm, which is made up of additional silicone material. The composite material was placed and UV light cure was done for 30 seconds. Then the specimen was removed from the mold and smoothen up by using conical bur. All the samples were inspected carefully. Among 12 samples, 6 were immersed in coke and the other 6 were immersed in Appy Fizz for 24 hours. The pre immersion and post immersion surface roughness were measured using a Mitutoyo SJ-310 stylus profilometer with 2µm tip/60 degree angle (Figure C and D). Ra, Rq and Rz values of bulk fill composite resin samples was assessed and compared.

RESULTS

The pre Ra and post Ra for bulk composite resin was 0.003. The pre Rq and post Rq for bulk composite resin was 0.00400 and 0.00350. The pre Rz and post Rz for bulk composite resin was 0.02525 and 0.02058 respectively. (Table 1).

Independent t test was done. The p value for Rq was 0.007 (<0.05), so it is statistically significant, and for Rz was 0.945(>0.05), so it is statistically not significant. (Graph 1)

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications. (9–18),(19–22),(23–27),(28). Food and liquids in the oral cavity expose restorative materials to temperature variations and acidic-base environments (29). The clinical quality and efficacy of restorative materials are determined by surface characteristics like roughness (30). Surface roughness and imperfections make restorations more prone to dental plaque deposition, causing gingival irritation and lowering the aesthetics and lifespan of restorative materials (31). As the pH of the environment lowers, the roughness value increases. As resin-based restorative materials are so widely used, they must be able to withstand the harsh conditions of the oral environment (32). Nano-filled composites have been shown to be more resistant to deterioration than micro hybrid resins due to their smaller particle size and lower surface roughness (33).

In the present study, the 12 composite samples were treated with coke and appy fizz drinks for 1 day. There was reduction in surface roughness of the bulk fill composite resin immersed in both appy fizz and coke drinks. In a study, Nano-filled composites were exposed to four acidic drinks: Gatorade sports drink, Tang fruit flavoured juice, Bindhu Jeera Fizz, and 10% Sucrose solution. They found that surface roughness increased progressively with time with maximum average roughness value (Ra) value seen on the 60th day of acidic drink immersion (33). In another study, bulk fill composite resin samples were immersed in 5 groups of acidic beverages. They found that acidic food simulating liquids and beverages significantly increased the surface roughness of bulk fill composite resin. This is contradictory to our study, where we found a reduction in Rz surface roughness values of bulk fill composite resin (33). The current study has limitations, such as low sample size and limited time frame in immersion protocols. In future, more studies with big sample size are needed, focusing on several characteristics such as color stability and microhardness of bulk fill composites.

CONCLUSION

There was a negligible difference in the surface roughness values of bulk fill composite resin samples before and after immersion in appy fizz and coke drinks. This might be due to less time period immersion protocols of bulk fill composite resin.

Conflict Of Interest

None declared

ACKNOWLEDGEMENTS

We thank Saveetha Dental college and Hospitals for providing us the support to conduct the study.

Source of Funding

The present study was supported by the following:

- Saveetha Dental College and hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai
- Sarkav healthcare services.

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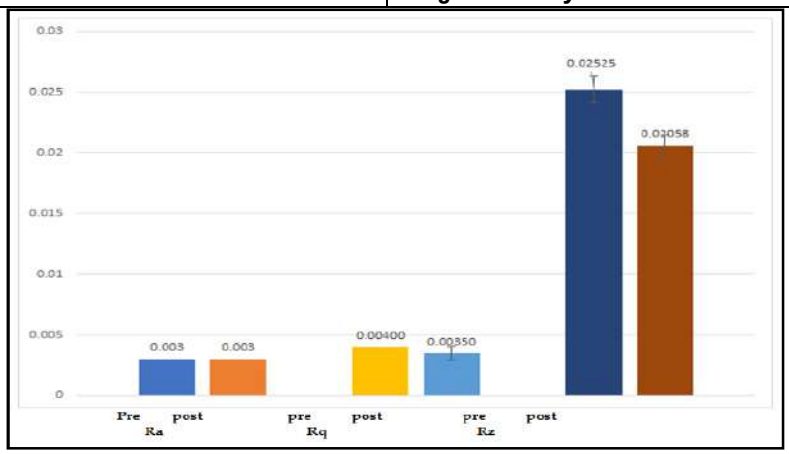
Table 1: Mean. std. deviation and significance testing between groups

S.No	Parameter	Mean	Std. deviation	Significance
1	Pre Ra	0.00300	-	-
2	Post Ra	0.00300	-	-
3	Pre Rq	0.00400	-	0.007
4	post Rq	0.00350	0.000522	
5	Pre Rz	0.02525	0.001138	0.945
6	post Rz	0.02058	0.000900	

Figure A and B: Bulk fill composite resin used for the study.

Figure C and D: Stylus Profilometer for surface roughness analysis.



Graph 1: This bar graph depicts the association between the composite used and the mean difference in surface roughness. Blue and orange denote the pre and post Ra, yellow and sky blue denote the pre and post Rq, dark blue and brown denote the pre and post Rz. It showed that there was a decrease in the post Rq and Rz value.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study on Community Participation in School Education in Andhra Pradesh

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Received: 05 Feb 2022 Revised: 02 Mar 2022 Accepted: 19 Mar 2022

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ABSTRACT

Community Participation is a tool of the improving the school education system, it helps to holistic development of school education. Community Participation was in schools are different shapes; like School Monitoring Committees (SMC's), School Building Committees (SBC's), Parent's committees, Parent Teacher Association (PTA), Mother Teacher Association (MTA), Village Education Committee (VEC) and so on. The Andhra Pradesh state has recently bifurcated state, census 2011 shows that, the Andhra Pradesh total population is 4.95 crores, and the population growth rate is 9.21 percent. The total literacy rate of the state is 67.35 percent, out of this male literacy rate is 74.77 percent and female literacy is 59.96 percent. The school committees have been constituted in the state, by the State Act no. 13 of 1998. At present in Andhra Pradesh, has a 10.93 percent of schools are having School Monitoring and Development Committee's (SMDC's), and totally 28.37 percent of schools having School Building Committee's (SBC's) only, it seems poor community participation in Andhra Pradesh school education system. The methodology of the study is secondary data analysis; the main objective of the study is, to discuss the community participation in school education system, and its impacts. Second objective of the study is, to assess the school education system in Andhra Pradesh. Another objective of the study is, to examine the status of community participation in Andhra Pradesh school education.

Keywords: Andhra Pradesh; Education; Community Participation; United Nations Organisation (UNO); Quality of Education

INTRODUCTION

United Nations International Children's Emergency Fund (UNICEF-1992) said that, "the Education is not an activity to be carried out in isolation. It is an activity to be done in collaboration. Without community participation, education cannot achieve its goals. Community participation can contribute to promoting education. Community participation not only increases ownership but also empowers the communities to take important decisions concerning the future of their children. Education policies give a significant place to 'community participation', which is also considered to be a tool to improve the functioning of school. There is also a need to take initiatives to improve the infrastructure, educational services and educational quality**"** [1]. Shaeffer (1994) points out "seven possible definitions of the term participation. Such as Involvement through the mere use of a service, (such as enrolling children in the school or through primary health care of them children). Involvement through 'attendance' for e.g., parents' meetings at school, another point is Involvement through consultation on a particular issue related to the children, finally Participation as implementers of delegated powers" [2]. Uemura (1999) noted that 'Participation 'in real decision making at every stage', including identification of problems, the study of feasibility, planning, implementation, and evaluation" [**3**].

According to the United Nations Organisation (UNO), "the Sustainable Development Goal (SDG's) number four explains the "Quality Education" to every person in the world. But this goal can be achievable only, when the other socio-economic goals, like SDG's number 1-No Poverty, 2- Zero Hunger, 3- Good Health & Well being, 5- Gender Equity, and 17- Partnership for the goals, are too be achieved. Because the above goals are inter related, and it can be achievable through community participation" [4]. In before that "Universal elementary education (UEE) is a part of Millennium Development Goal (MDG) set by the United Nations, but also a fundamental requirement for the well-being of individuals. However, education can facilitate the achievement of different personal and social goals only when it is of a desired quality" [5]. As per United Nations Education and Socio Cultural Organisation (UNESCO; 2004), "education comprises of a set of processes and outcomes that are defined qualitatively. At the international level, the emphasis on quality of education can be traced back to 1990 when the 'World Declaration on Education for All' recommended that education be made both universally available and improved qualitatively" [5]. Moreover; "the UNESCO were developed the 'Dakar Framework for Action' in the year of 2000, according to that; put forward quality of education as a fundamental determinant of enrolment, retention and achievement. The UNECSO was noted in the year of 2014, the concept of 'quality education' has evolved over the years and it is now a multidimensional concept encompassing factors like learners' characteristics and their social environment, availability of enabling inputs (competent teachers, learning materials and school facilities) and learning outcomes of students"[5]. Planning Commission of India, in his annual report-2011, said that "India has too observed that, the net enrolment ratio (NER) has shown considerable improvement at the primary level and it was at par with the developed countries in the year 2007" [6].

Kothari commission (1964-66) focused on "need of decentralization of school education and involvement of parents and local bodies in the processes of improvement of school" [7]. National Policy on Education (NPE 1986) was emphasised "primarily on decentralized planning and management of Primary education. Furthermore it is emphasis the removal of disparities in educational system, and equalise the opportunities in the school education. Village Committees were further strengthened with the support of Programme of Action (POA) in 1992. In 1992, the 73rd and 74th constitutional amendments provided a basic framework of decentralization of powers and authorities to the Panchayati Raj at different levels" [7]. The Veerappa Moily committee report on CABE Committee on Decentralised Management of Education-1992 was said that, "India was decentralization of educational planning; it is ensuring the greater community participation in education. Community participation is a way to take responsibility and accountability, to identifying the problem, to design and implement programme for the development of the society, its members include from different groups, caste, religion, and sex etc" [8]. Sarva Siksha Abhiyan (SSA, 2001) is a "Government of India's flagship programme for achievement of Universalization of Elementary Education. It also ensures that involvement of community participation for the success of the programme. Many kinds of community-based institutions have been involved such as Parent Teacher Association (PTA), Mother Teacher Association (MTA), Village Education Committee (VEC) and Gram Panchayat" [9].

Present Condition in Andhra Pradesh & India

As per Planning Commission report on elementary and adult education-2001, for 12th five year plan describes that "In India the educational facilities are measured by a set of indicators concerning access. As per norms, a habitation (cluster of households) is entitled to have a primary school, if it has a total population of 300 and more, and has no school within a distance of one kilometre. For upper primary schools, the corresponding norm is total population of 500 and more, and a distance of three kilometres, these norms are often relaxed in case of hilly and tribal areas, difficult terrains and border districts" [10]. According to the Planning commission, "During the period 1950-51 to 1999-2000, the number of primary schools has increased by more than 3 times from 2.10 lakhs in 1950-51 to 6.42 lakhs in 1999-2000 whereas the number of upper primary schools increased 15 times from 1, 36,000 in 1950-51 to 1, 98,000 in 1999-2000. In 1950-51, the ratio of upper primary school to primary schools was 1:15, which has come down to 1:3.2 in 1999-2000. The total number of teachers increased from 6.24 lakh in 1950-51 to 32.17 lakh in 1999- 2000, i.e., registering an increase of more than five times, while the number of female teachers increased from 0.95 lakh in 1950-51 to 11.52 lakh in 1999-2000, an increase of twelve times. The percentage of female teachers to total teachers, which was 15.2% at primary level and 15.1% at upper primary level, has increased to 35.6 and 36.1% respectively" [10]. However, the planning commission noted that "the teacher-pupil ratio (TPR) has worsened over the years. During 1950-51, the TPR in primary schools was 1:24, and in middle schools it was 1:20. In 1999-2000, this ratio has increased to1:43 in primary schools and 1:38 in upper primary schools. Despite increase in the number of habitations and population, both the percentage of habitations and rural population served by primary and upper primary schools / sections within a distance of 1 and 3 Kms. has increased significantly. Of the 10,61,000 rural habitations in the country, 52,800 (about 50 per cent) had a primary school / section each within the habitation itself in 1993-94. About 83.4 per cent habitations, were had a primary school/section within, or at a walking distance of 1 kilometre" [10].

The planning commission of India was describes that "Presently, about 1, 00,000 habitations continue to remain unserved as per prescribed norms. The alternative and innovative programme envisages opening of non-formal education centres in habitations where opening of a full-fledged school is not economically feasible or academically viable. Over a period of time, infrastructure and other facilities in schools have also improved significantly although a large number of primary and upper primary schools continue to suffer from deficiencies making it difficult for schools to function smoothly and preventing them from providing optimal conditions for teaching and learning" [10]. According to the National University of Educational Planning and Administration (NUEPA)'s secondary education report-(2016-17); "national average ratio of upper primary to secondary schools is 0.77 percent, and the ratios from the secondary schools to higher secondary schools are 2.15 percent" [11]. NUEPA report said that "in India total enrolment in 2016-17 is 6.13 crores, in that rural enrolments are 3.98 crores, and urban enrolments are 2.15 crores. As per report, total teachers in India as on April/2017 is 36 lakhs, out of this 22.13 lakhs teachers belongs to rural areas and 13.86 lakhs teachers from urban area. Out of the total teachers, 48.86 percent of teachers are female teachers; the average national girl's enrolment ratio is 47.50 percent" [11]. The above report also noted that "average pupil-teacher ratio is 17, and student-classroom ratio is 43, the average teachers, per school in the country are 14" [11]. As per NUEPA secondary education report-(2016-17), "in Andhra Pradesh the ratios of upper primary to secondary schools are 1 percent. Whereas, the secondary schools to higher secondary schools ratio in the Andhra Pradesh state are 33.70 percent. The above report also said that, the girls to boy's enrolment ratio at national level in 2016-17 are 0.90 percent in secondary level and same 0.90 percent in higher secondary level"[11]. The above report also said that, "the girls to boys enrolment ratio at state of Andhra Pradesh in 2016-17 is 0.93 percent in secondary level, and same 0.0 percent in higher secondary level. Moreover; the transition rates of the, elementary school to secondary school are, 97.81 percent within the state" [11].

Theoretical Framework

"Social Exchange Theory", was propounded by American sociologist "George Homans", he is discussed the social exchange theory, published the paper "Social Behaviour as Exchange". According to Homans, any two-person Relationship can be viewed in terms of cost-benefit analysis, "what I am giving, and what I am getting in return" [12]. The social exchange theory defines that "as a concern of social interactions in exchanges where all participants

seek to maximize their benefits. Within the social work, the professionals may utilize their theory to better understand. Moreover; the interactions with their clients and others, around them diving into the intrinsic rewards they may receive" [12]. According to the Williams (1994); "that until the middle of the last century, responsibility for educating children rested with the community. Although; in some places, the communities still organize and operate schools, for their children today. The Community participation in the education sector is not completely recognized, at the same time, it is not extended systematically in the society" [13]. Paul (1987) noted that, "Community Participation is an active process by which beneficiary or client groups influence the direction and execution of a development project which a view to enhancing their well being in terms of income, personal growth, self reliance, and other values they cherish" [14].

According to the Mistrianingsih and others (2015); "Quality Education is that education in which the social, emotional, cognitive, physical, and mental development of students sensitively focused regardless of race, socio-economic status, geographic location, and race. The key aim of quality education is to produce creativity as well as developed manpower in a form of well-educated personalities like competent teachers, professors, doctors, engineers, scientists, philosophers, critics, poets, educationists, and writers, etc" [15]. The RTE Act 2009 "is the most substantive declaration of the government's commitment and responsibility towards education" [16]. The Act clearly makes "state responsible for providing free and compulsory education to all children in the age group of 6-14 for eight years" [16]. The Act also sets "basic norms in terms of infrastructure, learning facilities and academic calendar to be satisfied for all types of schools. Besides prescribing a pupil – teacher ratio of 30:1 in each school, rather than as an average over a block or district, the Act clearly lays down the academic qualifications, duties and responsibilities of the teachers" [16]. The 2009 Right of Children to free and compulsory education (RTE) Act emphasis the involvement of communities in school governance through SMCs to ensure school quality with equity.

METHODOLOGY

The methodology of the study is mainly based on the secondary data; the researcher did the systematic review of the available data and analysed, presented in a systemic manner. The main objective of the study is, to discuss the community participation in school education system, and its impacts. Second objective of the study is, to assess the school education system in Andhra Pradesh. Another objective of the study is, to examine the status of community participation in Andhra Pradesh school education.

DISCUSSION

According to census 2011, "India has more than 121 crores of population, and the decadal growth rate is 17.70 percent. Whereas, the population density ratio is 382 persons per square kilometre, total geographical area is 32.8 lakh square kilometres. The report also told that; India's sex ratio is 943 females to1000 males, the Indian literacy rate is 73 percent" [17]. As per census 2011, "the Andhra Pradesh total population is 4.95 crores, the population growth rate is 9.21 percent. Whereas, the sex ratio of Andhra Pradesh is 997females to 1000 males, the population density of the state is 304 persons per square kilometre. The total geographical area of the Andhra Pradesh is 1.62 lakh kilometres, the total literacy rate of the state is 67.35 percent, out of this male literacy rate is 74.77 percent and female literacy is 59.96 percent" [17]. When compared with above national and state, literacy rates, the literacy rate has huge differences and education is still one of the problem at grass root well, that too rural women and child. That's why; community participation in the education is one of the solutions to increasing of literacy rate at grass root level.

According to the UDISE report (2018-19), "in Andhra Pradesh 24.71 percent of primary (along with upper primary and secondary and higher secondary) schools are having School Monitoring and Development Committee's (SMDC's), and 37.93 percent of schools having School Building Committee's (SBC's). 19.89 percent of upper primary schools (along with Secondary, Higher secondary) having School Monitoring and Development Committee's (SMDC's), and 35.91 percent of upper primary schools having School Building Committee's (SBC's)" [18]. The report

also said that "3.55 percent of primary (along with Upper Primary and Secondary) schools having School Monitoring and Development Committee's (SMDC's), 13.91 percent of primary (along with Upper Primary and Secondary) schools having School Building Committee's (SBC's). 11.82 percent of upper primary (along with Secondary) schools having School Monitoring and Development Committee's (SMDC's), 30.63 percent of primary (along with Upper Primary and Secondary) schools having School Building Committee's (SBC's). 9.09 percent secondary schools having School Building Committee's (SBC's), and 33.33 percent of secondary (along with Higher Secondary) schools are having a School Monitoring and Development Committee's (SMDC's)" [18]. Overall, "10.93 percent of schools are having School Monitoring and Development Committee's (SMDC's), and totally 28.37 percent of schools having School Building Committee's (SBC's)" [18]. There is the need to improving the enrolment, retention and quality of School education through community participation. Problems are in community participation in school education, which encompasses the problems related to school management committee meetings awareness and developing the school development plans are considered in this study. Necessary suggestions for proper community participation regarding school education are discussed.

CONCLUSION

The above studies said that, community participation in school education is very important. It is helps to holistic development of school education system, such as, quality of education, social audit of school funds and mid day meals programme, etc. In Andhra Pradesh; there is a necessary to increase the number of school monitoring committees, because it is very less in the state. The encourage of community participation in school education, will helps to increase the quality of education, as well as increasing the literacy rate of the state.

Funding: None

Conflict of interest: None

Ethical Consideration: Not Required

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Neem Oil Versus Neem Extracts: An Approach in Comparison of Medicinal Applications

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Received: 11 Jan 2022 Revised: 12 Feb 2022 Accepted: 09 Mar 2022

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ABSTRACT

Neem oil is a fixed oil pressed from fruits and seeds of neem and has been used in pest control due to its insecticidal and medicinal properties. Neem has antiallergenic, antidermatic, antifeedent, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic properties as well as cardiac, diuretic, insecticidal, larvicidal, nematicidal, and spermic properties. Triglycerides make up neem oil, which also contains a lot of triterpenoid chemicals. Stigmasterol, terpinene-4-ol, sugiol, 4-cymene, nimbiol, -terpinene, and vitamin E are all found in neem leaf extract. Various experimental research on the antifungal properties of neem oil have been undertaken. Due to its diverse medicinal activities neem oil has been found beneficial in ayurvedic and allopathic medicine. Despite of the applications, neem oil and extracts are available in limited dosage forms. Many approaches had been used to formulate neem oil and extracts as advanced delivery systems for topical drug delivery.

Keywords: Neem oil, Neem extract, Azadiractin, Antifungal activity, Zone of inhibition

INTRODUCTION

Neem tree (*Azadirachta indica*) a fast growing tree belongs to family 'Meliaceae'is a tropical evergreen tree related to mahagony. Neem oil is a fixed oil pressed from fruits and seeds of neem and has been used in pest control due to its insecticidal and medicinal properties. Neem oil is normally yellowish- brown, golden -yellow, reddish brown, dark brown, greenish- brown or bright red liquid in colour (Figure 1). It has an unpleasantly offensive and strong odour. The oil has a smell of partial combination of peanut and garlic. For proper emulsification oil needs appropriate surfactants due to its hydrophobic nature. It is a non drying oil and its obnoxious odour is due to the presence of

sulfur- containing volatile components [1]. Neem can be used as a solution to many problems such as antiallergenic, antidermatic, antifeedent, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal and spermic. Because of its therapeutic characteristics, it has been utilised in Ayurvedic medicine for over 4000 years [2].

Constituents

Neem oil is composed of *triglycerides* and contains many *triterpenoid* compounds that are responsible for its bitter taste.The major crude bitter principle extracted from neem oil is Nimbidin, which exhibit several of biological activities. From this crude principle isolation of some tetranortriterpenes including *nimbinin, nimbin, nimbolide, nimbidinin and nimbidic acid* has been possible. Nimbidin possess numerous biological activities such as Antifungal,Anti- inflammatory, Antiarthritic, Antipyretic, Hypoglycaemic , antiulcer, spermicidal ,Antibacterial and Diuretic. Anti- inflammatory property is also shown by sodium nimbidate . Azadirachtin is obtained from seed oil which possess antimalarial property [2]. Leaves of neem contain ingredients such as *nimbanene, nimbin,nimbolide, nibandiol, 6-desacetylnimbinene, ascorbic acid,n-hexacosanol , amino acid, 17- hydroxyazadiradione , 7- desacetyl-7-benzoylazadiradone, 7-desacetyl-7 benzoylgedunin and nimbiol*. Antifungal and anti-bacterial activities are shown by the fresh leaves of neem oil which is purified from *quercetin* and *polyphenolic flavanoids* [3].

Antifungal activity

For determination of antifungal activity of neem oil the fungal strains mainly used are *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma viride*. This can be done by using Agar well diffusion method. The movement of the molecules through the matrix that is formed by the gelling of agar is shown by this method [4]. Antifungal activity is determined by measuring *zone of inhibition*. If the drug stops the fungus from growing or kills them, there will be an area around the disk or well where the bacteria have not grown enough to visible is termed as Zone of inhibition. If zone of inhibition is larger then the organism is susceptible. If it is small or no zone of inhibition that indicate resistance. Disc diffusion method is also used for determination of antifungal activity. This approach is appropriate for organisms that grow rapidly at 35-37°C over night [5]. Temperature, hydrolysis, lipase enzyme , oxidation and another compounds in the oil are the main factors affecting quality of neem oil. As temperature increases, quality of oil decreases [6].

Uses:

Neem oil can be used in medicines due to its anti-inflammatory, hypoglycaemic, anti-pyretic, anti-fungal, antibacterial, sperimicidal, diuretic and anti-ulcer properties. It can also be used for the treatment and control of syphilis, eczema, chronic ulcer, leprosy and intestinal helminthiasis in ayurvedic medicines.`Onam cutar tailam` is a preparation containing neem seed oil used for epilepsy in siddha system of medicine. Neem seed oil can be used as a natural insecticide and pesticide. It can also be used in toiletries, insect repellent spray, and lotion, candles wax , cosmetics such as skin care , hair care products like shampoo and hair oils [7]. It also has Antioxidant activity. Neem can be well known for rejuvenating skin. It also has cooling impact on body. Neem oil mitigates and grease up skin. Neem can also be used to impart skin moisture and softness and treatment of psoriasis, malaria, sexually transmitted diseases, vitiligo, viral diseases, heart diseases, AIDS, dendistry, wound healing. It also has antifungal activities, anticancer , hepatoprotective, anti-inflammatory, anti-bacterial [8]. Fungus-caused dandruff can be treated by neem oil. The agar cup method was used to assess the ability to suppress the fungal growth that causes dandruff [9]. Extracts of neem have been discovered to have insecticidal qualities, and it is utilised as a foliar spray [10].

Extraction method

Neem oil is obtained by cold expression of neem seeds while the neem extract is from the mature leaves of neem (*Azadirachta indica*). Neem seed is a component of the Neem tree that contains a lot of oil .The soxhelt extraction technique was used to extract essential neem oil from neem seed utilising various organic solvents and parameters [11].

When neem seeds are extracted with alcohol, the bitter components recovered are primarily composed of nimbidin. It is abundant in the kernel, accounting for around 2% of the total. Bioactive components can be found in many sections of the neem plant although they are concentrated and more easily accessible in seed kernals [12]. In neem oil extraction, the proportions of ethanol-hexane combinations acted as efficient solvent alternatives to hexane alone. These mixtures are more eco friendly, have a lower risk of flammability, less poisonous, and generate more oil than hexane or ethanol [13].

NEEM OIL VERSUS NEEM EXTRACT

The oil is likely the most economically important of all of these goods. It is predominantly made up of triglycerides of oleic, stearic, linoleic, and palmitic acids similar to other vegetable oils. This cold-pressed oil is mostly utilised in the manufacture of lamps, soaps, and other non-edible items. It's black, bitter, and stinky in general. It includes sulphur compounds, which have a strong odour similar to garlic unlike other vegetable oils. The neem leaf extract contains stigmasterol, terpinene-4-ol, sugiol, 4-cymene, nimbiol, -terpinene, and vitamin E, according to the results of phytochemical analysis using HPTLC. Triterpenoids 22, 23-dihydronimocinol and desfurano-6 hydroxyazadiradione, azadiradione, tetraterpenoids (14.15-epoxynimonol), and flavonoids are also found in neem leaves [14].

Various studies are conducted to determine the antifungal activities of neem products. Mossini SA compared the effects of neem oil and neem extracts on mycelial proliferation, sporulation, morphology, and ochratoxin A production using *P. verrucosum* and *P. brevicompactum*. In Yeast Extract Sucrose medium, the impact of neem oil and neem extract was examined at varying concentration. The fungi's growth and sporulation were significantly reduced by oil extracts. The synthesis of ochratoxin A was not inhibited. Neem oil has been demonstrated (Figure2) to be fungitoxic by inhibiting growth and sporulation [15].The higher the neem aqueous concentrations, the greater the suppression of *Candida albicans* growth. In aqueous neem extracts, *Aspergillus niger* was suppressed more effectively than *Candida albicans*. *Candida albicans*, on the other hand, was suppressed more effectively in alcohol neem extract. Differences in antifungal activity are attributable to the existence of active principles extracted by different solvents, which can be modified by plant age, extraction process and extracting solvent type [16]. Methanolic and ethanolic extract of neem leaf show significant antifungal properties. The zone of inhibition is more for methanol extract [6].

Neem oil versus other anti-microbial agents

Neem oil at 15 % concentration shows higher anti- fungal activity than tea-tree oil and coconut oil. The zone of inhibition produced by the oil against *Candida albicans* species is more for neem oil [17]. Neem seed oil's antifungal activity was also compared to that of $CaCl_2$. A 2% aqueous emulsion of clarified neem seed oil was moderately fungicidal against *B. cinerea* and *G. cingulata*, but had minimal effect against P. expansum. Neem seed oil was equally potent as CaCl2 as an antifungal agent [18]. In case of antibacterial activity, when tested against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhi*, even at extremely low concentrations, neem oil demonstrated the highest rate of antibacterial activity than mustard oil. The suppression of growth by Neem oil was greater than 99 percent in all three infections. However, Mustard oil and Black seed oil showed extremely poor sensitivity to *Pseudomonas aeruginosa*. Only dilutions of Neem oil had shown antibacterial efficacy against *Pseudomonas aeruginosa* [19].

EXPERIMENTS OF NEEM OIL CONDUCTED AND ITS ANTIFUNGAL ACTIVITIES

Abiola Grace Femi-Adepoju [20] conducted a study to investigate the effects of neem seed oil on four fungi namely *Fusarium* sp., *Rhizopus* sp., *Curvularia* sp.,and *Aspergillus* species. Petroleum ether extract of neem seed oil was taken for the experimental study. Experimental result showed that the neem seed oil have completely inhibited the growth of *Curvularia* sp. and substantially retard the growth of *Aspergillus* and *Fusarium* sp. There is no notable effect on *Rhizopus* sp. Effectiveness of the oil varied across the organisms which is indicated by their percentage of zone of inhibition. Lowest percentage inhibition was shown by both *Rhizopus* (1.79%) and *Fusarium*(7.56%) sp. Whereas percentage inhibition in *Aspergillus* and *Curvularia* sp. were substantial. Therefore highest inhibitory activity exhibited by *Curvularia* sp. In the treatment and control experiments, the mean radial growths of organisms were compared and shown in Table1.

D.A. Mahmoud [21] performed in Different neem leaf extracts including the nimonol have antifungal efficacy against various significant human diseases. The purpose of this study was to see how aqueous, ethanolic, and ethyl acetate extracts from neem leaves affected the growth of a variety of human pathogens in vitro (Asp*ergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans,* and *Microsporum gypseum*).The existence of a major component (nimonol) was purified and chemically validated by Nuclear Magnetic Resonance (NMR) spectroscopic analysis of ethyl acetate extract using High Performance Liquid Chromatography (HPLC). After pooling out the nimonol, the 20 percent ethyl acetate extract lost some of its antifungal action, and this loss of activity was variable on test pathogens.

Antifungal activity of nimonol

The antifungal activity of pure nimonol when tested independently against the six test human pathogens. When pure nimonol was tested at a concentration of 20%, the results revealed that it has no inhibitory effect on the development of the test fungus. Four *Aspergillus* species are known to cause aspergilloses as well as *Microsporum gypseum* (a dermatophyte) and Candida albicans the cause of dermatophytosis and candidiases. Daniel Iván OSPINA SALAZAR [22] investigated the Antifungal activity of Neem (*Azadirachta indica*: MELIACEAE) extracts against dermatophytes. Several bioassays were carried out using the M38-A2 broth microdilution method on 14 isolates of the dermatophytes *Trichophyton Mentagrophytes*, *Trichophyton rubrum, Microsporum canis* and *Epidermophyton floccosum* in order to assess the antifungal activity of methanolic extracts from the neem tree (*Azadirachta indica* A. Juss.).

Determination of Antifungal activity

The 14 dermatophytes isolates examined with neem extracts showed varying amounts of growth inhibition in antifungal activity testing. There were more than 25 peaks in the chromatographic profile of neem seed oil and leaf extracts, which were more pronounced between 14 and 18 minutes.This experimental study reveals that the development of *T. mentagrophytes, T. rubrum, E. floccosum,* and *M. canis* was inhibited by both neem leaf and seed oil extracts. The antifungal activity of neem leaf extract was developed and evaluated by D.K.Shrivastava [6]. Three fungal species were used in the experiment: *Aspergillus flavus, Alternaria solani,* and *Cladosporium.* The disc diffusion method was used to prepare ethanolic and methanolic extracts in various concentrations (25 %, 50 %, 75 %, and 100 %) and test them against test organisms. This approach is appropriate for organisms that grow rapidly at 35-37°C during the night.The fungicide concentration decreases in a logarithmic manner. The susceptibility is assessed by measuring the zone of fungal growth inhibition surrounding each disc.To examine the toxicity of Neem leaf extract and its antifungal activity, ketoconazole was used. In conclusion, the methanol and ethanolic extracts of *Azadirachta Indica* (Figure 3) were found to inhibit the growth of these 3 fungal species, and the zone of inhibition was observed. The methanolic extract of *Azadirachta Indica* was found to be the most efficient extract against *Aspergillus flavus* among all the extracts tested.

Patihul Husni [23] investigated the anti-scabies efficacy of neem oil in vivo. The efficiency of the cream as an anti-scabies treatment was tested on scabies-infected New Zealand white rabbits. Permethrin cream was utilised as a positive control, and permethrin-free cream was used as a negative control. The effectiveness of neem oil 30% cream is higher than that of neem oil 10 % and 20 % cream.Once a day, the cream was administered and kept on for 8 hours.The rabbit dorsal dermal acute irritation tests showed that 5 percent neem oil cream was useful as an anti-scabies cream with a 20-21 day recovery time. The recovery period was shown to be greater than Permethrin cream (7-8 days), but less than the negative control, which took 30 days to recover. A 30 percent neem oil cream is the best formula and most powerful anti-scabies treatment (Table 2). In conclusion, A. indica cream has a faster recovery period than Permethrin cream and is successful in the treatment of scabies. Simhadri V. S [24] conducted research to determine the extract with the highest antifungal activity, as well as the least dose required to stop the organism from growing. The antifungal activity of *Azadirachta indica* leaf was tested against *Trichophyton rubrum, Microsporum gypseum, Epidermophyton floccosum* and *Candida albicans* using several sequential extracts such as hexane and benzene , ethylacetate, methanol, and aqueous. A disc-diffusion approach was used to assess activity, and a resazurin microtitre indicator method was used to determine the minimum inhibitory concentration (MIC).

Determination of Antifungal activity

The microbiological study was repeated six times. The disc diffusion method is the gold standard for determining the zone of inhibition, while the resazurinmicrotitre method is a viability assay based on fungal organism viability. This research demonstrates potential antifungal action against dermatophytes as well as Candida. As a result, we may deduce that the sensitivity of extracts was concentration dependent, and that as the concentration of extract increased, so did the zone of inhibition. Puneet Sudan [25] explored the Antifungal Potential of *Azadirachta Indica* against *Microsporum Gypseum*. The antifungal potential of various extracts of dried powder of *Azadirachta indica* leaves was examined using the paper disc diffusion method in 25 ml, 50 ml, and 100 ml concentrations with petroleum ether, ethyl acetate, ethanol, and aqueous solvents against *M. gypseum*. As a benchmark, clotrimazole was used. *Azadirachta indica* is a powerful antifungal agent against *Microsporum gypseum*, according to the conclusions of the study. The maximum zone of inhibition was 20.520+ 0.84 mm (Table 3) and 47.720 percent mycelial inhibition (Table 4) against a tested pathogen while using an ethanol extract of fenugreek at 100 ml doses. The best treatment for the tested strain was found to be an ethanol extract of *Azadirachta indica* .

CONCLUSION

According to the studies, neem oil is an excellent antifungal agent. Neem oil contains phytoconstituents that have fungicidal and fungistatic properties. Neem oil extracted from the kernal of the neem seed is more effective than neem extract. The zone of inhibition can be used to evaluate antifungal activity. For better economic and therapeutic application of neem and its products, more research should be conducted.

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Table 1: Mean radial growth of organism

Organism	Mean radial growth (mm)			
	Treatment	Control	Differences	% Inhibition
Aspergillus	2.84	3.56	0.72	20.22
Curvularia	1.50	2.41	0.91	37.76
Fusarium	3.18	3.44	0.26	7.56
Rhizopus	11.00	11.20	0.20	1.79

Table 2: Recovered Time Comparisons in Five Dose Groups

Dose Groups	Average	p-value	Note
Cream 10%	14.00	0.000	There is a difference
Cream 20%	11.50	0.000	There is a difference
Cream 30%	9.50	0.000	There is a difference
Negative control	7.50	0.000	There is a difference
Positive control	31.00	0.000	There is a difference

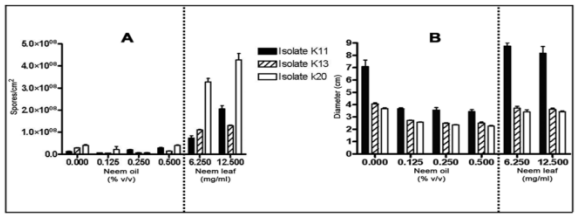
Table 3: *Azadirachta indica's* Mean Zone of Inhibition in Different Solvents (mm)

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
Azadirachta indica	25 µml	7.699 + 0.85	9.891 + 0.86	13.211+0.65	4.565+ 1.20	43.00+0.20
Azadirachta indica	50 µml	12.210 + 0.65	15.130 + 0.86	17.100+0.75	10.560+ 0.50	43.00+0.20
Azadirachta indica	100 µml	14.510 + 0.75	18.360 + 0.72	20.520+0.84	12.210+0.47	43.00+0.20

Table 4: Percentage inhibition (percent) of several *Azadirachta indica* extracts

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
Azadirachta indica	25µml	17.883	23.000	30.720	10.604	100
Azadirachta indica	50µml	27.723	34.577	39.201	23.849	100
Azadirachta indica	100µml	33.744	42.697	47.720	28.395	100

Figure 1: Neem leaf and its oil



At 0.125% NO inhibited diameter growth of K11, K13 and K20 by 48.30%, 33.50% and 30.43%, respectively. At 0.25%, NO extract inhibited diameter growth of K11, K13 and K20 by 49.71%, 39% and 36.14%, respectively. Finally, at 0.5%, NO extract inhibited growth of K11, K13 and K20 by 51.55%, 38.4% and 38.85%, respectively.

Figure 2: Effect of neem oil and neem leaf extracts on sporulation (A) and on colony diameter (B) of Penicillium (isolates K11, K13 and K20) on YES medium

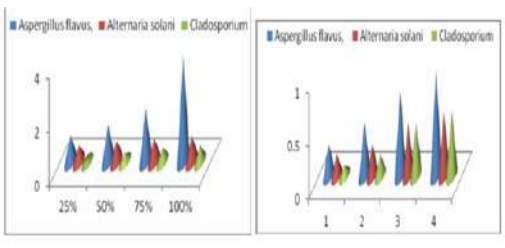


Figure 3: A comparative analysis of the inhibitory zones produced by several(a) methanolic extracts (b) ethanolic extracts on various fungal species

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study of Inclusive / Exclusive Cross Section Involving Loosely Bound Nuclei

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Received: 18 Jan 2022 Revised: 20 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

The inclusive and exclusive cross section has been measured for $^{7}Li+^{208}Pb$ system and it has found larger cross-section for the inclusive alpha compared to the exclusive one. The origin of this inclusive cross section is not fully understood but the cluster structure including the pickup and transfer channel are responsible for this. Here the different particle has been shown with the intensity and showed that in addition with different particles like alpha proton deuteron have emitted from the CN they possess different energy also.

Key words: CN,BU

INTRODUCTION

Using loosely bound nuclei the excitation towards the continuum which is an unbound states for the exit channels is a common phenomenon which has observed in many experimental results involving weakly bound nuclei [1].Around threshold energies , the number of channels which contribute significantly becomes very small and is possible to examine the detail influences towards the reaction mechanisms arises due to structure differences in the nucleus. Because of the above fact it makes different nuclear processes , study of which will lead to fruitful results indicates an emerging area of investigation around the Coulomb barrier regimes. Even if large number of experiment has been performed to study the breakup effect involving^{6}Liand^{7}Li projectiles which indeed loosely bound it has been found that there are limited data are present for both projectiles with same target for a comparison. Specially it has found the alpha production cross-section and more focus has given to understand the alpha channel after an interaction with a heavy target [2]. There are report which are less in number indicates that the direct breakup

contribution is less compared to the transfer breakup like pickup or striping type. [3]. Since the breakup threshold for ^{6}Li is small compared to ^{7}Li one can expect that the breakup will be more for the case of ^{6}Li and specially the alpha production via breakup. The present report explains the experimental approach to measure the alpha particle cross section and also explains the various other types of particle observed during the experiments for ^{7}Li+^{208}Pb reaction.

Experimental detail

A beam of ^{7}Li was delivered by the Laboratori Nazionalidi Legnaro Tandem Van de Graaff accelerator to perform the experiment with incident beam energy varies between 31 & 35MeV.The Beam flux was varied between 5 and 10 nA. The target used was ^{208}Pb having thickness 200μg/cm^{2}and self-supported for the experiment. The emitted Light particles due to nuclear reaction has been detected by a complex setup called 8PLP. It has different parts, the parts on the forward side which covers 2.5^{0} to 34^{0} is called as WALL and "BALL" part covers the angles up to 163^{0} . There are 126 telescopes number of detector (each two called as a telescope) are present in BALL. Each telescope was consists of front side thin SSB detector and a back side thick CsI(Tl) scintillator detector .The total angle between 34^{0} to 163^{0} has been covered by BALL which has divided in to 7rings A, B, C, D, E, F & G. The WALL is consists of a 11×11 matrix telescope . The BALL and the WALL combinedly provided a very nice particle charge and mass separation. Different matrix has been plotted to separate them specially ΔEvs Time(T) has plotted to see all the varieties of particles whereas the ΔE Vs E matrix has been used for charge separation, the elastically scattered ^{7}Li has not found since it has stopped in the ΔE part of the detector..

RESULTS AND DISCUSSIONS

The evaporated Particles which were detected by the detectors placed in BALL and WALL were identified by the following process. :i) Identification by ⊚E (energy loss) method and ii) Particle identification by the Energy and Time method. The two mentioned method has been adopted for each telescope. For the first one it is basically the plot of ΔE,a small amount of energy loss in thin SSB detector and the remaining energy is in thethick E detector which has considered as the x-axis in Eres (residue energy) plots. This provided a very nice particle identification as Shown in Fig.1 . The elastically scattered ^{7}Li has stopped in the ΔE so no any ^{7}Li particle has been found in this plot where as in the E vs Time plot where time is a mix of TOF (time of flight) and PSD (pulse shape discrimination) rise time. Using the above two method a very good separation of particle has been identified including the elastically scattered ^{7}Li. One can see a very nice and clean separation between proton, deuteronandalpha as shown in Fig.1 ,where there is a distribution of energy in each of the case and significant amount of events are concentrated around some energy which indicated that there are reaction mechanics needs to be understood and there are other mechanics besides the breakup which lead to the other particles like proton , deuteron and alpha. The emitted particles have different origins: such as the^{7}Li breakup fromit's resonance state (4.63MeV)into α αnd triton (t); proton pickup of converts the ^{7}Li to ^{8}Be then breaks to two alpha or strip of a neutron andbecomes^{6}Li and breakup to α and deuteron(d). One can see that there are strong patches in the triton and alpha band which indicates clearly the different origin. Using the alpha counts the inclusive cross section has been extracted and same for proton and triton also. The cross section has been extracted for some of the identified channels as $((\alpha+\alpha)+(\alpha+\delta)+(\alpha+\tau))$. Inaddition to cross section a Q values reconstruction has been done to understand the reaction mechanism. The provided mentioned picture Fig.1 is the particle spectra for 35 MeV beam energy and for a special forward angles $(\theta = 20.6^{0}\ \alpha\nu\delta\phi = 346.53^{0}$.

Deuterons and tritons of different energies are clearly separable and are expected to belongs to different types of breakup. Different patches in the triton and deuteron band indicated the breakup through other process including elastic/inelastic breakup. It has observed that the inclusive cross-section is much larger than the exclusive cross section. This implies in addition with the direct breakup channel other process are contribution which needs to be understood very clearly. Similar conclusion has also provided in reports [5].Further more study will require to separate out the contribution from other reaction channels a

SUMMARY

The alpha particle spectra for the inclusive (exclusive) events has been determined including the differential cross-section for $^{7}Li+^{208}Pb$ reaction around 33 and 35 MeV . The particle spectra for one energy has been shown . The exclusive ◎◎ events has also extracted and found that the contribution not able to cover the inclusive one. This is a clear indication that there are other mechanism which are responsible for the larger inclusive cross section including the striping and pickup which need to be understood experimentally- More experimental data will be required for further investigations.

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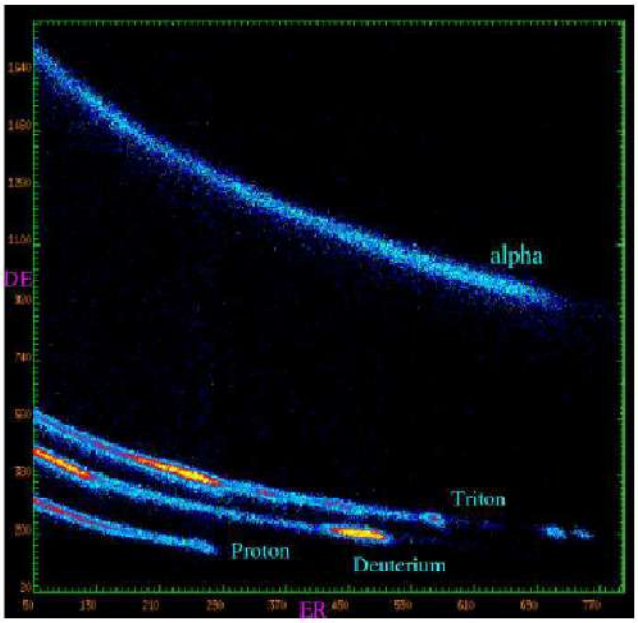

Fig.1 a 2D particle spectra obtained by the E-ΔE Telescope. All the different particles has been shown very clearly with different energies .

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Molecular Interaction Study of Sanitizer at Different Temperatures

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Received: 10 Feb 2022 Revised: 12 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

In this work, density, viscosity, and speed of sound were measured as a function of concentration for the ternary mixtures of Glycerol, Isopropyl alcohol and hydrogen peroxide at T=298 to 328 K and at frequency 2 MHz From the experimental density, viscosity and speed of sound data, the acoustical parameters such as adiabatic compressibility (β), intermolecular free length (L_f), internal pressure (π_i), Rao's constant (R), relaxation time (τ), acoustical impedance (Z), free volume (V_f), etc, have been computed. The results are interpreted in terms of molecular interaction between the components of the mixture. The non-linear variations of acoustical parameters with concentration and temperature indicated the existence of strong molecular interaction in the systems studied.

Keywords: Ternary mixture, ultrasonic velocity, adiabatic compressibility, internal pressure.

INTRODUCTION

The study of intermolecular interaction plays an important role in the development of molecular science. A large number of studies have been made on the molecular interaction by various scientists [1,2]. Ultrasonic velocity affects the physical properties of the medium and hence one can furnish information about the liquid and liquid mixtures. Ultrasonic and viscometric studies of organic, inorganic and bioactive compounds are very useful for understanding the ionic, hydrophilic and hydrophobic interactions in the liquid mixtures. They provide information about solute–solute and solute-solvent interactions in the liquid mixtures. Ultrasonic investigation of liquid mixtures consisting of polar and non-polar components, are considerable importance in understanding the intermolecular interaction between the component molecules and finding applications in several industrial and technological processes [3-9]. The variation in ultrasonic velocity and related parameters throw light upon the structural changes associated with the liquid mixtures having strongly as well as weakly interacting components. This has been studied for various

binary and ternary mixtures [10,11] with respect to variation in concentration and temperature. Hand sanitizers were first introduced in 1966 in medical settings such as hospitals and healthcare facilities. The product was popularized in the early 1990s. Alcohol-based hand sanitizer is more convenient. Hand sanitizer that contains at least 60% alcohol or contains a "persistent antiseptic" should be used. Alcohol rubs kill many different kinds of bacteria, including antibiotic-resistant bacteria and TB bacteria. They also kill many kinds of viruses, including the flu virus, the common cold virus, coronaviruses, and HIV. 90% alcohol rubs are more effective against viruses than most other forms of hand washing. Isopropyl alcohol will kill 99.99% or more of all non-spore-forming bacteria in less than 30 seconds, both in the laboratory and on human skin.

Consumer alcohol-based hand sanitizers, Products with 60% to 95% alcohol by volume are effective antiseptics. In addition to alcohol (isopropanol), hand sanitizers also contain the following

- additional antiseptics such as chlorhexidine and quaternary ammonium derivatives,
- hydrogen peroxides that eliminate bacterial spores that may be present in ingredients,
- emollients and gelling agents to reduce skin dryness and irritation,
- a small amount of sterile or distilled water.

In the present paper we, prepare a Sanitizer by taking Glycerol, Isopropyl alcohol, and Hydrogen Peroxide in different proportions as per the following. Various acoustic and derived thermodynamic parameters are studied for the prepared Sanitizer at different temperatures.

Sample

S-1 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.11%)
S-2 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.12%)
S-3 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.13%)
S-4 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.14%)
S-5 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.15%)
S-6 → Glycerol (1.45%) + Isopropyl alcohol (75%) + Hydrogen Peroxide (0.16%)

MATERIALS AND METHODS

The mixtures of various concentrations in mole fraction were prepared by taking analytical reagent grade and spectroscopic reagent grade chemicals with minimum assay of 99.9% and obtained from E-Merck Ltd (India). All the component liquids were purified by the standard methods [12]. The density, viscosity, and ultrasonic velocity were measured as a function of concentration of the ternary liquid mixture at different temperatures. Ultrasonic velocity measurements were made using an ultrasonic interferometer (Model M-84, supplied by M/S Mittal Enterprises, New Delhi) with the accuracy of ±0.1 $m \cdot s^{-1}$. The measuring cell of interferometer is a specially designed double-walled vessel with provision for temperature constancy. An electronically operated digital constant temperature bath (Model SSI-03 Spl, supplied by M/S Mittal Enterprises, New Delhi), operating in the temperature range of −10°C to 85°C with an accuracy of ±0.1°C has been used to circulate water through the outer jacket of the double-walled measuring cell containing the experimental liquid. The density and viscosity of liquid mixtures were measurement with Rolling-ball micro-viscometer.

THEORY

To understand molecular interactions and structural changes, sound velocity and density data can be used to describe various parameters. These parameters have been computed using the following equations [13-16]

Adiabatic Compressibility (β)

The adiabatic compressibility is the fractional decrease of volume per unit increase of pressure, when no heat flows in or out. It is calculated from the speed of sound (U) and the density (ϱ) of the medium by using the equation of Newton Laplace as,

$\beta = 1/U^2.\varrho$ ---------- (1)

Intermolecular free length (L_f)

The intermolecular free length is the distance between the surfaces of the neighbouring molecules. It is calculated by using the relation,

$L_f = K_T\beta^{1/2}$ ---------- (2)

Where, K_T is the temperature dependent constant and 'β' is the adiabatic compressibility.

Free Volume (V_f)

Free volume in terms of ultrasonic velocity (U) and the viscosity (η) of liquid **[20]** is as follows

$V_f = (M_{eff}.U/K.\eta)^{3/2}$ --------- (3)

Where 'M_{eff}' is the effective mass of the mixture, 'K' is a dimensionless constant independent of temperature and liquid. Its value is 4.281×10^9.

Internal Pressure (π_i)

The measurement of internal pressure is important in the study of the thermodynamic properties of liquids. The internal pressure is the cohesive force, which is a resultant of force of attraction and force of repulsion between the molecules. It is calculated by using the relation,

$\pi_i = bRT (k\eta/U)^{1/2} (\varrho^{2/3}/M^{7/6})$ ------- (4)

Where, 'b' stands for cubic packing, which is assumed to be '2' for all liquids,

'k' is a dimensionless constant independent of temperature and nature of liquids.
Its value is 4.281x109.
'T' is the absolute temperature in Kelvin's is the effective molecular weight,
'R' is the Universal gas constant, 'η' is the viscosity of solution in $N.S.m^{-2}$,
'U' is the ultrasonic velocity in $m.s^{-1}$ and 'ϱ' is the density in $Kg.m^{-3}$ of solution.

Relaxation time (τ)

Relaxation time is the time taken for the excitation energy to appear as translational energy and it depends on temperature and impurities. The relaxation time can be calculated from the relation,

$\tau = 4/3. (\beta.\eta)$ ------- (5)

Where, 'β' is the adiabatic compressibility and 'η' is the viscosity of the mixture.

Acoustic impedance (Z)

The specific acoustic impedance is given by,

$Z = U.\varrho$ ------- (6)

Where, 'U' and 'ϱ' are velocity and density of the mixture.

Gibb's free energy

The Gibb's free energy is calculated by using the relation

$\Delta G = kT.Ln (kT\tau/h)$ ------- (7)

Where, 'τ' is the viscous relaxation time, '*T*' is the absolute temperature,
'k' is the Boltzmann's constant and '*h*' is the Planck's constant.

Molar volume can be calculated by using the relation

$V_m = M_{eff}/\varrho$ ---------- (8)
Where, M_{eff} is the effective molecular weight and ϱ is the density of the solution.

Available Volume
Available volume is the direct measure of compactness and strength of binding between the molecules of liquid or liquid mixture. Schaffs et al. shown that the available volume can be obtained by the relation.

$V_a = V_m(1-U/Um)$ ---------- (9)

Rao's Constant
Rao's constant is also known as molar sound velocity andit is an additive property. It has been found to be invariant with temperature and pressure for un-associated organic and inorganic liquid. R can be evaluated by an equation given by Bagchi et al

$R = V_m.U^{1/3}$---------- (10)

Wada's Constant
Molar compressibility is also known as Wada's constant, which is dependent on adiabatic compressibility and density, is given by the relation

$W = V_m.\beta^{-1/7}$ ---------- (11)

Surface Tension
Surface tension can be calculated by using the relation

$S = 6.3 \times 10^{-4}.\varrho.U^{3/2}$---------- (12)

RESULT

The experimental data relating to density, viscosity and velocity at 298 K, 308 K, 318 K and 328 K for frequencies 2MHz for the mixtures are given in table-I. The calculated values of adiabatic compressibility (β), free length (L_f) and relaxation time (τ)are reported in table-2. Acoustic impedance (Z), Gibb's free energy (ΔG) and Internal pressure (π_i)are reported in table-3. Surface tenson (S), Rao's constant (R) and Wada's constant (W) are presented in table-4. Free volume (V_f), molar volume and available volume for the mixture are presented in table-5.The variation of different parameters with temperature are shown infig.1-4. Variation in ultrasonic velocity in any solution generally indicates molecular association in it. This is due to the interaction between solution–solvent molecules. The value of adiabatic compressibility (β) the ease with which the medium can be compressed is higher in lower concentration. This shows the more available space, due to the density of the medium. Intermolecular free length(L_f) depends upon adiabatic compressibility and shows a similar to that of compressibility's. The L_f is found to be a predominant factor to determining the nature of ultrasonic velocity in liquid mixtures. The intermolecular free length (L_f) is the mean distance between the surfaces of neighboring molecule, also reflects the same trend as that of β. This indicates significant interaction between solute and solvent molecules. With increase in temperature, since adiabatic compressibility increases, it clearly indicates temperature dependence of β. The value of intermolecular free length (L_f) shows the decreasing trend in an increase of temperature. This is in agreement with the observations made by Syal et al.

With increase in temperature, the magnitude of L_f increases [17]. From the same Table 2 it is noticed that as concentration of (H_2O_2) increases, the free volume decreases whereas internal pressure increases. The internal pressure (π_i) is the resultant of force of attraction and force of repulsion per unit area between the components of the

mixtures. Internal pressure. The measured values are in same trend with that of the concentration of solutes, the average available volume between the molecules of mixture is referred as free volume (V_f) reflects the reverse trend as that of internal pressure. However, with rise in temperature, increase in free volume and decrease in internal pressure are noticed in all systems. The increase in free volume shows that the strength of interaction increases gradually with the increase in solute concentration. It represents that there is strong interaction between the solute and solvent molecules. The values of viscous relaxation time (τ) and Gibb's free energy (_*G*) increase with increasing solute concentration, which shows that the molecular interactions occurring between solute and solvent molecules become strong. Gibb's free energy (*G*) values suggest that denser systems of molecules form due to the H-bonding between molecules in the solutions. Rao's constant (*R*) and Wada's constant (W) also show increasing trends with increasing solute concentration. This may be due to the presence of more molecules/ions in the same region, which leads to compact packing of the medium, thus increasing the interactions [18].The values suggest the availability of a greater number of components in a given region thus leads to a tight packing of the medium and thereby increasing the solute–solute interactions [17, 18]. From the same Table 3 the acoustical impedance Z is the product of ultrasonic velocity and the density of the medium, in general decrease with increasing concentration of H_2O_2 as well as temperature. Acoustic impedance (Z) is the impedance offered to sound wave by the components of the mixture. The decreasing trend in the parameters suggests the strengthening of interaction among the component.

CONCLUSIONS

A sequential study of ultrasonic velocity, density and viscosity for systems containing Glycerol, Isopropyl alcohol and hydrogen peroxide has been performed over a large concentration range. The acoustical data provides important information about solute-solvent interactions in solutions. From the experimental findings, different parameters have been evaluated, and the results indicate the existence of solute-solvent (hydrophilic-ionic group) interactions in these mixtures, which increase with increasing hydrogen peroxide concentration.

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Table-1: Experimental values of density, viscosity and ultrasonic velocity at different temperature

	Density (Kg/m^3)				Viscosity(η) NSm^{-2} (x 10^{-3})				Velocity (ms^{-1})			
	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K
S-1	0.897	0.894	0.894	0.886	3.892	2.729	2.163	1.637	1398.9	1374.5	1358.8	1330.2
S-2	0.887	0.874	0.880	0.870	3.452	2.355	1.815	1.435	1360.5	1338.9	1323.2	1301.5
S-3	0.872	0.854	0.865	0.856	3.183	2.240	1.645	1.255	1332.2	1308.5	1289.7	1269.5
S-4	0.859	0.852	0.844	0.833	2.846	2.132	1.552	1.180	1291.8	1278.8	1254.1	1228.2
S-5	0.848	0.840	0.831	0.821	2.837	2.032	1.515	1.163	1247.8	1235.6	1217.6	1188.5
S-6	0.815	0.832	0.821	0.810	2.811	1.925	1.352	1.111	1209.2	1192.3	1173.8	1145.6

Table-2: Calculated values of adiabatic impedance relaxation time and free length at different temperatures.

	Adia. Compressibility x 10^{-10}				Relaxation time (x 10^{-12})				Free length(x 10^{-10})			
	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K
S-1	5.697	5.921	6.058	6.379	2.956	2.154	1.747	1.392	0.468	0.482	0.497	0.513
S-2	6.091	6.383	6.490	6.786	2.803	2.004	1.571	1.298	0.484	0.500	0.515	0.529
S-3	6.462	6.839	6.950	7.249	2.742	2.043	1.524	1.213	0.498	0.518	0.533	0.547
S-4	6.976	7.177	7.533	7.958	2.647	2.040	1.559	1.252	0.518	0.531	0.555	0.573
S-5	7.574	7.798	8.117	8.623	2.865	2.113	1.640	1.337	0.539	0.553	0.576	0.596
S-6	8.392	8.455	8.840	9.407	3.145	2.170	1.594	1.393	0.568	0.576	0.601	0.623

Table-3: Calculated values of acoustic impedance, Gibb's free energy and internal pressure at different temperatures.

	Acoustic Impedance (x 10^6)				Gibb's free energy (x 10^{-20})				Internal Pressure x 10^6			
	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K
S-1	1.255	1.229	1.215	1.179	1.257	1.287	1.321	1.353	4.771	4.156	3.842	3.464
S-2	1.207	1.170	1.164	1.132	1.254	1.284	1.316	1.350	4.522	3.853	3.529	3.239
S-3	1.162	1.117	1.116	1.087	1.253	1.284	1.315	1.347	4.338	3.743	3.364	3.034
S-4	1.110	1.090	1.058	1.023	1.252	1.284	1.316	1.348	4.125	3.688	3.260	2.937
S-5	1.058	1.038	1.012	0.976	1.255	1.286	1.318	1.351	4.154	3.629	3.235	2.936
S-6	0.985	0.992	0.964	0.928	1.259	1.287	1.317	1.353	4.091	3.572	3.088	2.896

Table-4: Calculated values of free volume, available volume and molar volume at different temperature.

	Free volume x 10^{-7}				Available volume				Molar Volume			
	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K
S-1	0.427	2.076	3.038	4.689	20.37	22.92	24.52	27.67	162.1	162.6	162.6	164.1
S-2	0.490	2.490	3.799	5.530	24.54	27.15	28.58	31.18	163.9	166.4	165.2	167.1
S-3	0.536	2.593	4.236	6.513	27.91	31.02	32.60	35.09	166.7	170.3	168.1	169.9
S-4	0.605	2.698	4.433	6.798	32.60	34.26	37.24	40.56	169.3	170.7	172.3	174.5
S-5	0.577	2.754	4.397	6.614	37.74	39.42	41.82	45.55	171.5	173.1	175.0	177.1
S-6	0.558	2.831	4.937	6.703	43.58	44.53	47.18	50.98	178.4	174.8	177.1	179.5

Table-5: Calculated values of Rao's Constant, Wada Constant, and Surface tension at different temperatures.

	Rao's Constant				Wada Constant				Surface tension			
	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K	298 K	308 K	318 K	328 K
S-1	181.3	180.8	180.1	180.5	126.4	126.2	125.7	125.9	29.57	28.70	28.21	27.08
S-2	181.6	183.4	181.4	182.5	126.6	127.7	126.5	127.1	28.04	26.98	26.68	25.74
S-3	183.5	186.2	183.0	183.9	127.7	129.4	127.4	128.0	26.71	25.47	25.24	24.39
S-4	184.3	185.2	185.8	186.9	128.2	128.8	129.1	129.8	25.13	24.55	23.61	22.59
S-5	184.6	185.7	186.8	187.6	128.4	129.1	129.7	130.2	23.55	22.98	22.24	21.19
S-6	190.1	185.3	186.8	187.8	131.7	128.8	129.7	130.3	21.59	21.58	20.80	19.79

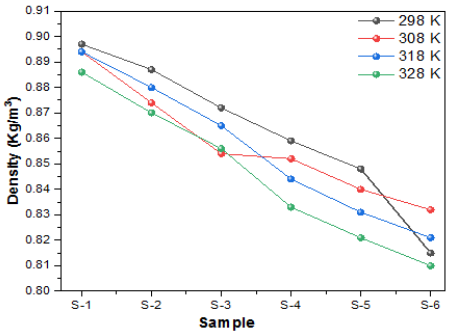

Fig 1: Variation of density with mole fraction of mixtures

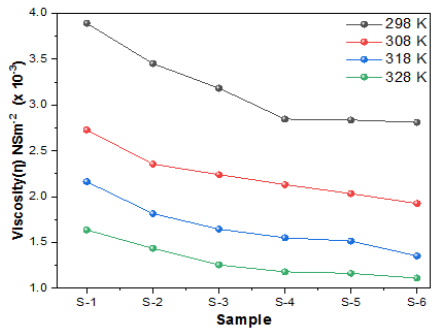

Fig 2: Variation of viscosity with mole fraction of mixtures

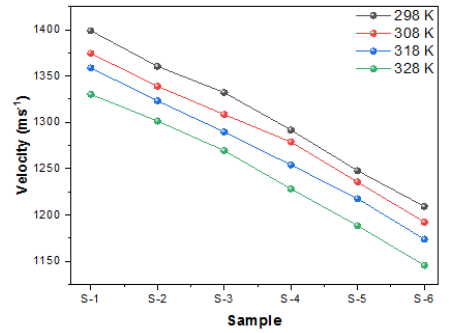

Fig 3: Variation of velocity with mole fraction of mixtures

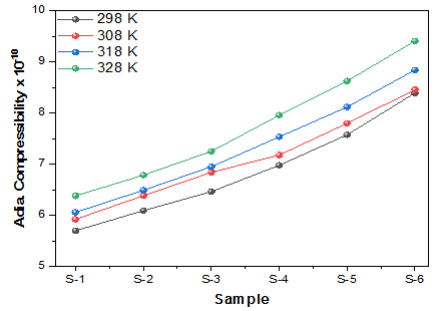

Fig 4: Variation of adiabatic compressibility with mole fraction of mixtures

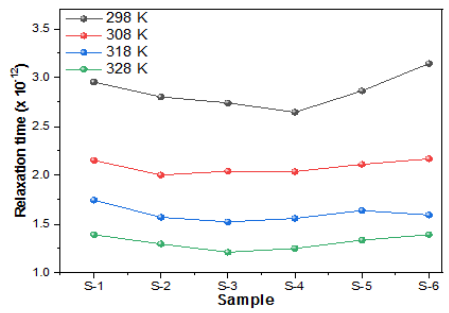

Fig 5: Variation of relaxation time with mole fraction of mixtures

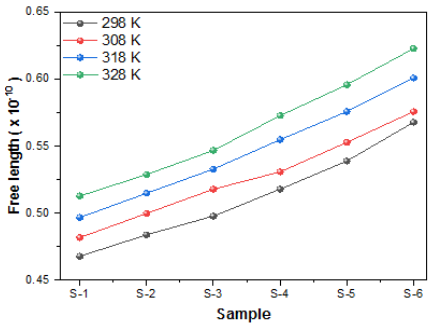

Fig 6: Variation of free length with mole fraction of mixtures

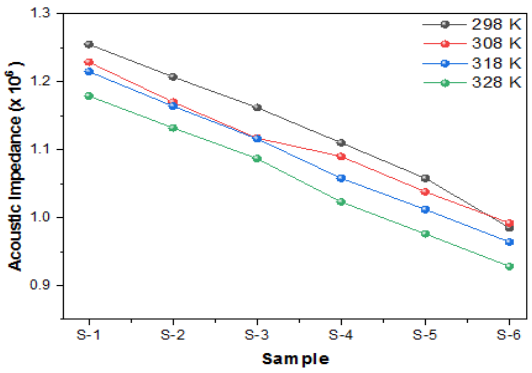

Fig 7: Variation of acoustic impedance with mole fraction of mixtures

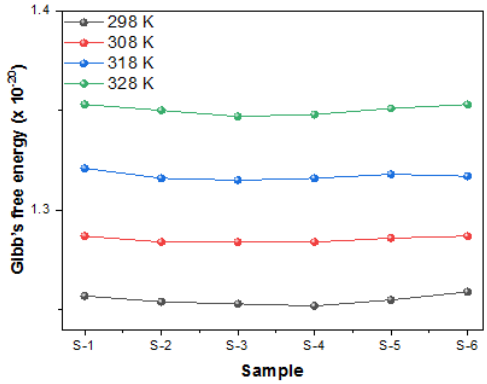

Fig 8: Variation of Gibb's free energy with mole fraction of mixtures

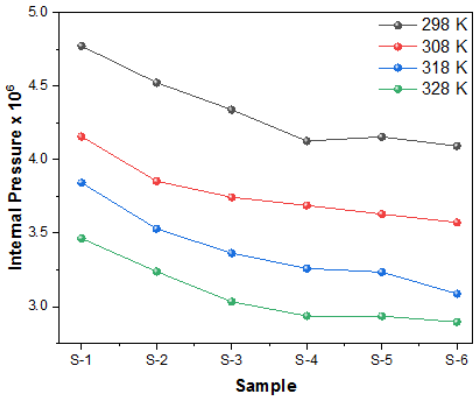

Fig 9: Variation of internal pressure with mole fraction of mixtures

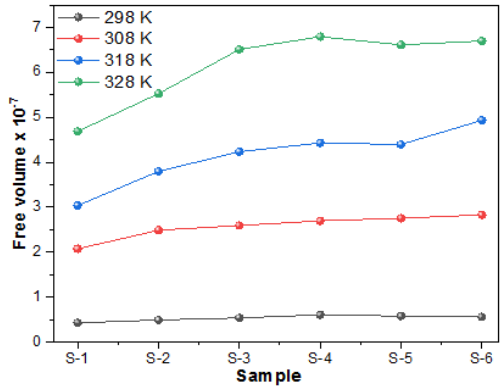

Fig 10: Variation of free volume with mole fraction of mixtures

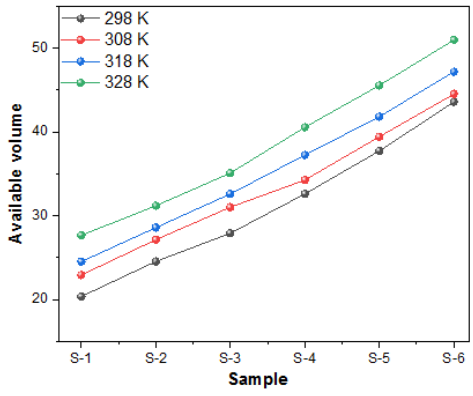

Fig 11: Variation of available volume with mole fraction of mixtures

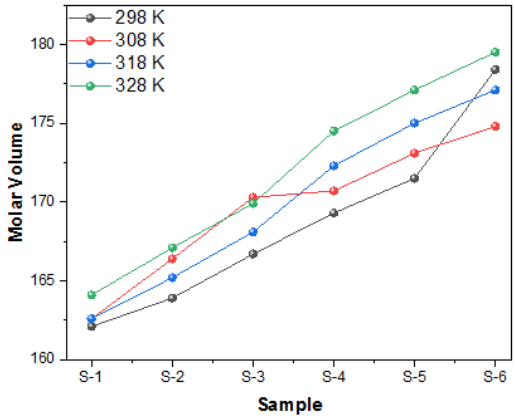

Fig 12: Variation of molar volume with mole fraction of mixtures

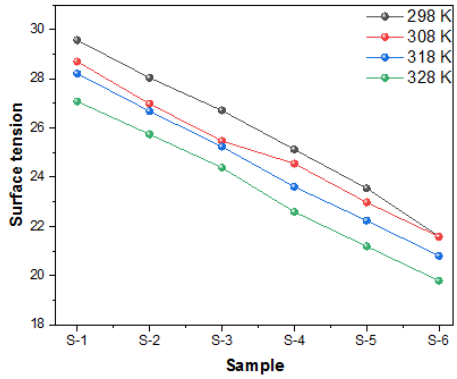

Fig 13: Variation of surface tension with mole fraction of mixtures

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Hot Beverages on Colour Stability of Bulk and Flowable Composite Resin - *An Vitro* Study

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Received: 09 Jan 2022 Revised: 10 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

The color stability of the composite resin is an important property that influences its clinical longevity, which remains an inherent challenge to the material. The aim of the study is to evaluate the effect of hot beverages on the color stability of bulk and flowable composite resin._Two types of commercially available Bulk and flowable composite namely Pyrax and DTech were taken for the study. A total of 10 disc shaped samples, 5 from each type, with dimensions 10 mm and 2 mm in thickness were made. Only the specimens which were found to have smooth surface finish were taken further for performing the experiment. The specimens were immersed in glass beakers containing the tea solution, coffee solution and distilled water separately, 5 samples in each solution. They were checked for colour stability using a spectrophotometer. The mean value of bulk composite is 6.2132 and for flowable is 4.2225 and the standard deviation of bulk is 6.15879 and for flowable 1.22717. Flowable composite resin samples had low delta e values when compared to bulk fill composite. P value = 0.070(> 0.05), so it is not statistically significant. Within the limitations of the study, it can be concluded that coffee and tea have the capacity to stain both, bulk and flowable composites. In comparison, flowable composite resin samples had superior color stability when compared to bulk fill composite after exposure to hot beverages.

Keywords: Colour stability, Coffee, Innovative measurement, Tea, Composite resin, Spectrophotometer

INTRODUCTION

The color stability of the composite resin is an important property that influences its clinical longevity, which remains an inherent challenge to the material. Thus, the purpose of this study was to evaluate the color stability of bulk-fill resins when exposed to hot beverages. Well contoured, aligned, white teeth are considered to give beauty and many dentists have turned their attention towards it in order to achieve it. Even the changes in size, shape, colour and structure of dentition have led to various problems like decreased masticatory efficiency, speech disturbances and psychological problems. Thus, restoring a damaged or decayed tooth is important. Recent technological advancements have been made in the field of dental aesthetics for augmenting and repairing damaged teeth thereby producing a flawless appearance. Composite resins have their indication for posterior teeth restoration established in clinical studies that found excellent performance (1). However, one of the main limitations of composite resins is related to the volumetric shrinkage resulting from polymerization, a property inherent in polymeric materials. This property results in stress forces at the tooth-restoration interface, a consequence generally reduced using a specific cavity insertion protocol known as the incremental insertion technique (2). These polymerization shrink forces are greater than adhesion forces, cracks may be generated and the risk of caries recurrence and failure increases. The incremental insertion technique requires a longer working time, especially in deep cavities, since the maximum incremental thickness has been 2mm. In addition, it is a relatively sensitive technique, with an increased risk of contamination by mouth fluids and air bubble formation between increments (3). Furthermore, bond failure between composite layers and difficulty in insertion into small cavities may also occur. Modifications in the formulation of resin composites have been made to overcome these deficiencies, facilitate the procedure and increase the longevity of dental restorations(4). Among these formulations, the so-called bulk-fill composites or low shrinkage resins or single increment resins with the proposal to fill 4-5mm cavities at once, without influencing the polymerization contraction, the degree of conversion or cavity adaptation. In general, the main property that characterizes this material is the low degree of shrinkage after polymerization as well as the ability to compensate for the highC-factor of some cavities in posterior teeth, resulting in a significant reduction of clinical working time(5). While manufacturers recommend a single 4mm increment fill, many clinicians suspect that the cure depth and mechanical properties may not be satisfactory. Color of resin composites can be negatively affected from hot beverages like tea and coffee consumed. Our team has extensive knowledge and research experience that has translated into high quality publications(6–15),(16–19),(20–24)(25). The aim of the study is to evaluate the effect of hot beverages on the color stability of bulk and flowable composite resin.

MATERIALS AND METHODS

Preparation of Specimens

Two types of beverages were taken for this study. One was coffee and the other one was tea. Two types of commercially available Bulk and flowable composite namely Pyrax and DTech were taken for the study. A total of 10 disc shaped samples, 5 from each type, with dimensions 10 mm and 2 mm in thickness were made. Only the specimens which were found to have smooth surface finish were taken further for performing the experiment.

Immersing Method

The specimens were immersed in glass beakers containing the tea solution, coffee solution and distilled water separately, 5 samples in each solution. The specimen immersed in distilled water was taken as the control for 24 hrs. Then they were rinsed with distilled water and were checked for colour stability using a spectrophotometer.

Calculation of Colour Stability

The L, a, b values were obtained from the Vita EasyShade Spectrophotometer for the colour stability. The ΔE values were calculated in order to determine the degree of alteration in colour at different stages. Then pre and post immersion values were compared and analysed using SPSS software version 23.0.

RESULTS

The table 1 represents the mean delta E value of bulk and flowable composite resin. The mean value of bulk composite is 6.2132 and for flowable is 4.2225 and the standard deviation of bulk is 6.15879 and for flowable 1.22717 and significant value is 0.070. Independent sample t test was used p value of <= 0.05 is considered significant. Flowable composite resin samples had better color stability when compared to bulk fill composite. P value = 0.070(> 0.05), so it is not statistically significant. (Figure 1)

DISCUSSION

Composite resin is a tooth coloured restorative material. The descending order in which the bulk fill group was stained in immersing solutions are as follows: coffee >tea>control. A similar trend was found with flowable composite group also in the following order: coffee >tea >control. Hence it can be seen that coffee stains both the bulk and flowable intensely when compared to tea. Flowable group showed better colour stability when immersed in tea and coffee but they were not considered significant as the p value = 0.07. In another study, 90 disks of nanocomposites resin were used. Tea, Coffee, Cola, Turmeric and Control (artificial saliva) were used as immersion mediums. Post immersion profilometric value was recorded to evaluate roughness brought about by the solutions and spectrophotometric value was recorded to evaluate the color change in samples. Results were statistically analyzed and showed that turmeric has the highest staining potential in all groups (26). In another study where they used 3 different composite and three different beverages in which black tea and coco cola had more mean value than other groups. In another previous study they have taken one bulk composite and flowable composite which was immersed in coffee and in this study it shows that coffee has more staining potential in bulk-flowable composite. The resin composite in another study was immersed in coffee and black tea where the coffee has more mean staining value than tea. In the study where they have used 150 resin composites they have used black tea and milk coffee, in which black tea has more mean value and it stained more than milk coffee (27,28). The limitation of the study was small sample size and the immersing solutions used to compare were also less types. So further studies based on large sample size is to be done for knowing the effects of various beverages on composite resins color stability.

CONCLUSION

Within the limitations of the study, it can be concluded that coffee and tea have the capacity to stain both, bulk and flowable composites. On comparison, flowable composite resin samples had superior color stability when compared to bulk fill composite after exposure to hot beverages.

ACKNOWLEDGEMENT

We would like to thank our college and management for their constant support in completing this research work.

Conflict of Interest

None

Source of Funding

The funds were provided by:

- Saveetha Dental College and hospitals, Saveetha University of Medical and Technical Sciences, Saveetha University, Chennai.
- Palanivel group of companies (dindigul)

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Table 1 : mean. Std. Deviation and significance testing between groups

Groups	Mean	Standard Deviation	Significance Value
Bulk	6.2132	6.15879	0.070
Flowable	4.2225	1.22717	

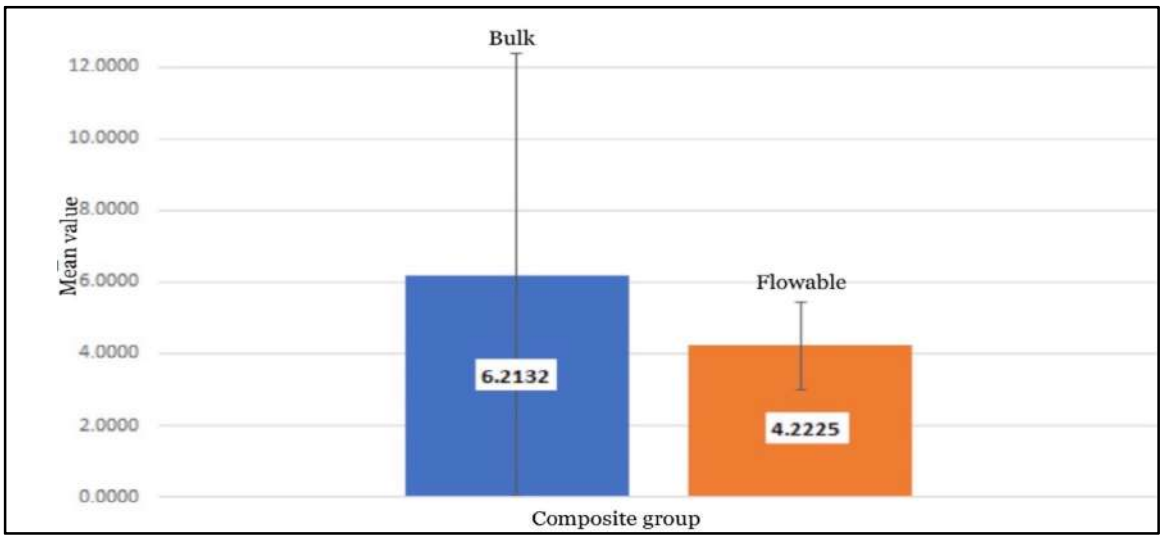

Figure 1: This bar graph shows the association between the composite resin groups and mean delta e value. The X axis represents the composite material and the Y axis represents Mean Delta E value. Blue colour indicates the bulk fill and orange colour indicates flowable composite. Flowable composite resin samples had better color stability when compared to bulk fill composite. P value = 0.070(> 0.05), so it is not statistically significant.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antioxidant and Cytotoxic Effect of Ethanolic Extract of *Nigella sativa* Seeds on HEP2 Cancer Cell Lines

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Received: 28 Jan 2022 Revised: 26 Feb 2022 Accepted: 22 Mar 2022

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ABSTRACT

To analyze the antioxidant and Cytotoxic potential of *Nigella sativa* was evaluated. Free radical scavenging potential of the seed was studied by various *in vitro* assays such as DPPH, ß Carotene and Frap assay. The Cytotoxic activity of *Nigella sativa* was analysed by MTT assay. The ethanol extract is more powerful in scavenging free radicals. The preliminary phytochemical screening of *Nigella sativa* revealed the presence of phenolics, carbohydrates alkaloids, flavonoids and tannins in high amount. The Cytotoxic potential of the ethanol extract was studied on Hep2 cell lines by MTT assay. The extract had an IC50 value of100µg/mL which showed cell viability of 24.67%. From the result, it is clear that ethanol extract of *Nigella sativa* has Cytotoxic effect on Hep2 cell lines. Thus, the study revealed that *Nigella sativa* could be considered as a significant source of antioxidant and Cytotoxic agent

Keywords: Antioxidant, Cytotoxcity , *Nigella sativa*, DPPH assay, Hep2 cell line.

INTRODUCTION

The human system creates reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical, and hydrogen peroxide by many enzymatic systems through oxygen consumption [1]. In small amounts, these ROS can be beneficial as signal transducers and growth regulators [2]. However, during oxidative stress, large amounts of these ROS may favor some human disease conditions such as cancer, hepatic diseases, cardiovascular diseases,

ageing, and neurodegenerative diseases [3]. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS. Antioxidants are compounds that inhibit or delay oxidation of other molecules by terminating initiation or propagation of oxidizing chain reactions [4].A great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties [5]. These properties are attributed to a variety of active photochemical including vitamins, carotenoids, terpenoids ,alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc [6].

Cancer is a complex multifactorial cell disease characterized by abnormal cellular proliferation. Cancer development and progression are dependent on the cellular accumulation of various genetic and epigenetic events [7,8] and is an aberrant net accumulation of typical cells arising from excess proliferation, insufficient apoptosis, or a combination thereof [9]. Cancer development is normally caused by oncogene, tumor suppressor gene, and micro RNA gene alterations [10]. It imposes a serious burden on the public health system, and its treatment and cure are scientifically challenging. Cancer is expected to claim nine million lives worldwide by 2015 [11]. Medicinal plants have a long history in both traditional and modern cancer treatments [12]. Hence, it is possible that traditional medicinal plants can serve as potential sources for developing new drugs and more effective anti-cancer agents for future therapy. Seeds of *Nigella sativa L*., an annual spice, also known as black cumin or black caraway, are a member of Ranonculaceae family and native to some parts of the Mediterranean region. The seeds have traditionally been used in middle eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years [13].The Seeds are used for edible and medicinal purposes and are sold in the markets to be used as a spice , condiment, additive on bread, for cheese and as a native medicine . It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases [14]. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma [15] and popularly used to enhance memory and learning [16]. In view of the reputed efficacies of this seeds, this present study investigates its phytochemical constituents in an attempt to establish its most active form and antioxidant and Cytotoxic potential of *Nigella sativa* was evaluated.

MATERIALS AND METHODS

Collection of Seeds material

The healthy seeds of *Nigella sativa* was purchased from near by herbal Shop in kovilambakkam, Chennai, TamilNadu.

Preparation of Seeds Extract

The plant material seeds of *Nigella sativa* was washed under running Tap water and followed by sterile distilled water. Air dried at room temperaturefor2-3days and powdered (using a Kitchen jar). And about 50g of sample was subjected to added with 250ml of Ethanol and Extracted by using Soxhlet apparatus. The extract was dried and stored in amber coloured Glass jar in a freezer and it was used for further experiments.

Phytochemical evaluation

Photochemical screening is to identify the presence of phyto constituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids .

Estimation of total free phenolics

Total phenolic constituents of seeds extracts were estimated by Folin-Ciocalteau's method using Folin-Ciocalteu reagent. The estimation was done spectrometrically at 760 nm and the results were expressed as gallic acid equivalents (GAE).

Estimation of total flavonoids

Aluminum chloride method was employed to quantify the total flavonoid content in the seeds extracts. The results were expressed as quercetin equivalents (QE).

Estimation of total alkaloids

Total alkaloid content of the seeds extracts was determined. Five gram of the sample was filtered and concentrated to one quarter of the original volume on a water bath after treatment with 200 mL of 10% acetic acid in ethanol. Concentrated NH_4OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH, filtered and weighed.

Estimation of total saponins

Powdered sample (20 g) was treated with 100 mL of 20% aqueous ethanol, heated over a hot water bath for 4 h at about 55°C with continuous stirring. The mixture was filtered and the residue re extracted. The combined extracts were reduced to 40 mL over water bath at about 90°C and the concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added to the combined extracts and washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was heated in a water bath, dried in an oven to a constant weight and the saponin content was calculated as percentage.

Thin layer chromatography

Preliminary identification of photochemical was made by thin layer chromatography (TLC) using silica gel plates (5gm of silica gel dissolved with 90ml of water). The extracts were eluted with chloroform: methanol: water (30:20:4) and the chromatogram was developed by spraying with methanol: sulphuric acid (1:1) and heating to 110ºC. Then Rf value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent.

Free radical scavenging activity on DPPH

Antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to (Eloff *et al*.,1998). Sample extracts at various concentrations was taken and the volume was adjusted to 100 µL with ethanol. Ethanolic solution (5 mL) of DPPH (0.1 mM) was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27ºC. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH activity = (Control OD–Sample OD/Control OD) × 100.

Determination of antioxidant activity - β-carotene assay

The antioxidant capacity was estimated by thermally induced β-carotene bleaching assay was evaluated . A solution of β - carotene was prepared by dissolving 2 mg of β - carotene in 10 ml of chloroform. 2 ml of this solution was pipetted into 100mlconical flask after chloroform was removed under vacuum. 40 mg of purified linoleic acid, 400 mg of tween-40 emulsifier and 100 ml of aerated distilled water were added and shaken vigorously. 4.8 ml of this emulsion were added to test tubes containing 200µl of ethanolic seed extract of BHT was used a control for comparison. As soon as the emulsion was added to each tube, zero time absorbance was measured on UV-VIS spectrophotometer at 470nm. The tubes were then placed in water bath at 50ºC and the measurement of absorbance was continued until the colour of β- carotene disappeared in the control tube which was devoid of the sample. A blank of β- carotene was also prepared for background correction. 1- (Initial absorbance of the sample - Final absorbance of the sample)X100

Ferric reducing antioxidant power (FRAP Assay)

The total antioxidant potential of the sample determined by means of ferric reducing ability (FRAP) assay. FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37˚C for 5 min. Then this solution mixed with certain concentration of the seed extract (0.5mL) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (0.4, 0.8, 1.2, 1.6, 2.0 mM) were used and the absorbance values were measured as for sample solutions. Qualitative and quantitative phytochemical screening: The extracts were subjected to preliminary

Anticancer activity

In oncology research and clinical practice many Cytotoxic assays are used in the assessment of cancer types of individual patients. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay has been described as rapid, simple and reproducible method, widely used in the screening anticancer drugs and to measure the tumor cell proliferation.

RESULT AND DISCUSSION

Qualitative phytochemical analysis

The preliminary phytochemical screening of *Nigella sativa* revealed the presence of phenolics, carbohydrates, alkaloids, flavonoids, sopanins, tannins and steroids, in high amounts followed by glycosides, amino acids and proteins. (Table 1)

Quantitative phytochemical analysis

The major phytochemicals present in the selected seeds extracts were phenols, flavonoids, alkaloids and tannins were quantified. According to the results, suggested with the antioxidant activity of each extract tested . In general, the ethanolic extracts showed higher content of phenols and flavonoids, and likewise higher antioxidant activity. The results of total phenol content, alkaloids, saponins and flavonoids are given in (Table 2).

Thin layer chromatography

The chromatogram developed with 10% methanol in chloroform revealed the presence of five major compounds at Rf value of 0.33; 0.48; 0.56; 0.76; 0.86 as visualized under iodine vapour and UV illumination.

Radical scavenging activity of seed extracts

The DPPH radical is widely used to evaluate the free-radical scavenging capacity of antioxidants. From the dose dependent response curve, DPPH radical scavenging activity of differentent seeds extracts of *Nigella sativa* was observed. The scavenging activity of ethanol extract reached 98%. The ethanol extract of *Nigella sativa* showed good antioxidant and free radical scavenging activity (Figure. 1). Being a stable free radical, the DPPH assay is a simple and rapid method frequently used to evaluate the ability of antioxidants to scavenge free radicals. It gives reliable information concerning the antioxidant ability of the tested compounds to act as free radical scavengers or hydrogen donors. The old electron in DPPH free radicals gives a strong absorption maximum at 517nm. When DPPH becomes paired with hydrogen from a free radical scavenging antioxidant, its purple color fades rapidly to yellow to form reduced DPPH-H [17]. The resulting decolorization is stoichiometric with respect to number of electrons captured.

The comparable beta-carotene bleaching rates of control, BHT (Standard) and ethanolic extracts of seeds. The highest antioxidant activity of seed was found to be 49.87%. The beta carotene bleaching method is one of the most frequently applied methods for determining the total antioxidant property of the extract. In the beta carotene bleaching assay linoleic acid produces hydroperoxides as free radical during incubation at 50ºC and attacks the beta carotene molecules that cause reduction in absorbance at 470nm. Beta carotene in the systems undergoes rapid discoloration in the absence of antioxidant and vice versa in it presence [18]. The presence of different antioxidants can delay the extent of beta carotene bleaching by neutralizing the linoleate free radical and other free radical system. Thus, degradation rate of beta carotene-linoleate depends on the antioxidant activity of the extracts. The result showed the control had substantial and rapid oxidation of beta carotene [19].

Accordingly, the absorbance decreased rapidly in the sample without antioxidant, while the sample extract with the presence of antioxidant retained their color and also absorbance for a longer time. In the present study, free radical scavenging activity was determined by the FRAP (Ferric reducing ability of plasma) method, which depends upon the reduction of ferric tripyridyltriazine (FE(III)-TPTZ) complex to the ferrous tripyridyltriazine. Fe(II)-(TPTZ) by a reductant at low PH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593nm. FRAP values are obtained by comparing the absorbance change at 593nm in test reaction mixtures with those containing range with

antioxidant mixtures .The free radical scavenging power of seed extract of *Nigella sativa* increased with increased amount of extract. There is a clear difference between the control and the sample containing the extract . Among all the extracts the ethanol extract was showing maximum antioxidant power (12.7mmol of feso4/100g dry plant equivalents in *Nigella sativa.*

Anticancer activity

Since, IC50 value for Hep2 (liver) cell line (100 µg/ml) ethanol seeds extract was found to be effective, the reduction percentage of MTT at 48Hrs also estimated for Hep2 (liver) cells. When incubated with the extract, it induced Cytotoxicity in a significant manner which implicit the damage to the member integrity of the cell when contributed with control.

The cytotoxicity was minimized in the extract treated cells and near normal level was attained at various concentrations (25, 50, 100 and 150µg/ ml) and maximum effect was found when treated at 100 µg/ml. From the above results, it was confirmed that *Nigella sativa* ethanol seeds extract at 100µg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies. Tryphan blue is one of the several stains recommended for use in dye exclusion procedure for viable cell counting. This assay is based on the principle that live cells do not take up blue, where as dead cells do and appear as blue under microscope - depicts the viability of cells by Tryphan Blue assay. The viability is measured in terms of percentage was found to decreased 98% in drug treated hepatic cell line. The cell treated with *Nigella sativa* ethanol extract at various concentrations (25, 50, 100 and 150µg/ml) showed protective nature of the extract act against the deleterious effects and the maximum effect was observed at 100 µg/ml. (figure 3). The extract had an IC50 value of100µg/mL which showed cell viability of 22.67%. (Table 3)From the result, it is clear that ethanol extract of *Nigella sativa* has Cytotoxic effect on Hep2 cell lines. Cell cycle arrest is a common feature of cells that are undergoing terminal differentiation and defective proliferation.

CONCLUSION

The present investigation suggests that *Nigella sativa* possesses significant antioxidant and Cytotoxic potential. The antioxidant phytochemicals protect the cells from free radical attack and oxidative damage. Phenolic compounds, including flavonoids, tannin are especially promising candidates for oxidative damage. Thus consuming the diet rich in antioxidant plant foods will provide health protective effects. Lead us to propose *N.sativa* as promosing natural sources of antioxidants suitable for application in Nutritional and pharmaceutical fields, in the prevention of free radical mediated disease. Hence, we can conclude that it has a potential of preventing human diseases in which free radicals are involved . Further mechanistic studies, the seed of *Nigella sativa* can be considered as an efficient source of antioxidant and cytotoxic agents.

ACKNOWLEDGMENTS

The authors wish to thank the Management of Mohamed Sathak college, Sholinganallur.

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Table 1: Phytochemical estimation.

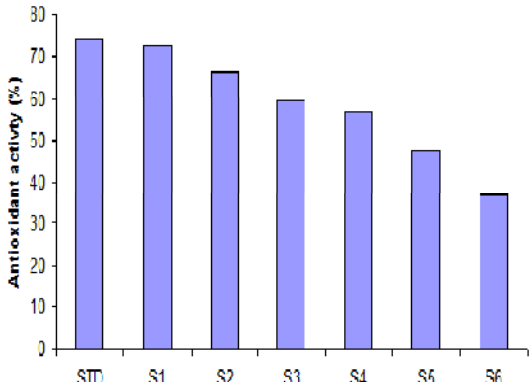
Compound	Result
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Steroids	+
Flavonoids	+
Tannins	+

Table2: Quantitative phytochemical estimation.

Bioactive compound	Result
Total phenols	256.83 μg GAE/g sample
Alkaloids	1.55 mg/g sample
Saponins	0.125 mg/g sample
Tanins	1.32mg/g dry weight
Flavonoids	64.2μg Quercetin equivalent/g

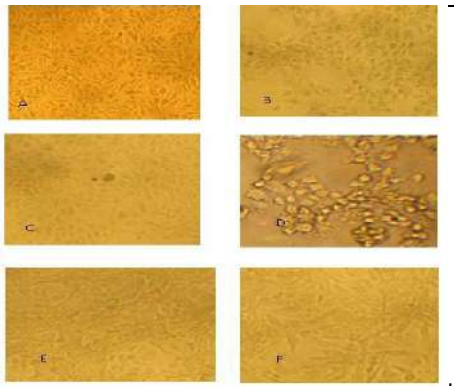
Table: 3% MTT reduction on Hep2 cell line

S. No	Concentration(μg)	MTT reduction (%)
1	Control	99.17
2	25	85.12
3	50	70.47
4	100	45.3
5	150	22.67
6	PC	10.13



Note: S1-Methanol, S2-Ethanol, S3-Ethylacetate, S4-Aqueous, S5- Hexane, S6-Dichloromethane

Fig.1: DPPH Radical Scavenging Assay of *Nigella sativa* Ethanol extract.



Note: A: Control cells (Untreated), B: Ethanol extract 25 µg/ml, C: Ethanol extract 50 µg/ml, D: Ethanol extract 100 µg/ml, E: Ethanol extract 150 µg/ml, F: Cyclophasphamide (Positive control) 180 µg/ml.

Fig.2: Anticancer activity

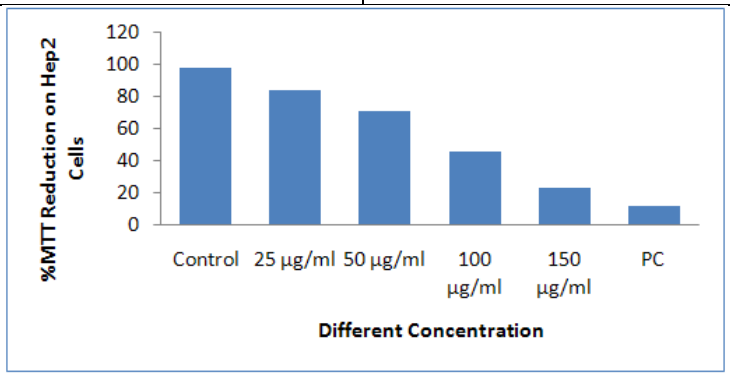


Fig. 3: MTT reduction on Hep2 cell line

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Break-up of Phenomena around the Coulomb Barrier for (^{7}Li, ^{208}Pb)

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Received: 14 Jan 2022 Revised: 20 Feb 2022 Accepted: 26 Mar 2022

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ABSTRACT

A loosely bound particle involving experiment has been performed using the target ^{208}Pb and verities of light particle have been detected using the different types of the detectors. It has found that due to the low breakup threshold the projectile breakup has happened and provided two bands to the energy matrix depending on the origin of the breakup process. A Q-values spectrum has been constructed to know the mechanism in more details and explained the different origin of the process.

Keywords: CN, BU, CS

INTRODUCTION

Many anomalous results have been reported for the reaction of loosely bound nuclei,like^{6}Li, ^{7}Li &^{9}Be, which has been attributed to the lower energy for break-up into constituents. Due to the low breakup threshold the projectile will breaks into it's cluster parts. [1].From the astrophysical interest point of view the breakup of Projectile in the coulomb field of target has renewed interest due to its application for the radiative capture cross section determination. The radiative capture cross sections for light particles say alpha, d and protons are of particular importance which helps to understand the nucleo synthesis process at very low bombarding energy of incident particle. In addition with above, the abundance of the elements are also important to study where the cross section measurement is very important which has a prime role in the stellar burning processes [2]. Due to the availability of the radioactive beam of low intensity it is difficult to perform the experiment whereas the weakly bound nuclei beam can be found in significant amount and their properties are similar to RIB upto certain extent. So the application/study of the loosely bound nuclei Over the last decade has gain interest and it is a major research focus

area[3]. As the Understanding of loosely bound nuclei including their reaction mechanism and also the coupling of this channel to other channel need to be understood , is very important for theoretical point of view also. The breakup of Projectile changed the conventional accepted picture of two body fusion for weakly bound nuclei. It has been reported that the cluster structure has significantly larger cross sections during Measurements involving loosely bound projectile (^{6,7}Li, ^{6}He). Out of many cluster structure the alpha+x cluster structure is very important as it has found that this channel has higher cross section , for the inclusive alpha particle [4] compared to other channel cross section. This cluster structure indicated that there are some other process is happening which need to be understood. This is a continuation of our earlier work [6].

Experimental Detail

The experiment was performed at LNL(Laboratori Nazionali di Legnaro) Tandem Vande Graaff accelerator. The beam was ^{7}Li having energy varies from 35 and 39 MeV. The currents was varied between 5 and 10 nA. A target of ^{208}Pb having thickness 200 μg/cm^{2} has been used. The particles which were emitted during the reaction were detected by8PLP set up [7]. The set up has two main part called as "WALL" & the "BALL" both covers forward and backward angles. In total 126 Telescope (ΔE and CsI(TI)) has been used in BALL and the WALL has a 11×11 matrix telescope. For the present data analysis the WALL part has been used. The mass and charge has been identified very nicely . For the light particle(α, t, d & p) the mass identification has done in a very nice way. ^{7}Li which came out due to the elastic scattering has completely stopped by the one part (ΔE) of the telescope. The elastic particle has been clearly identified by the ΔEvs Time matrix. For the particle identification (alpha, p,d,t) a ΔE vsEres matrices has been plotted for each detector. All the different types of particles (alpha, t, d, p & elastic ^{7}Li) has been separated very nicely from each other. It needs to understand the origin of each particles since they are in many varieties. The origin must be inside the different reaction mechanism. A correlation matrix has been generated to identify the breakup event only. A co-relation matrix between α &t for the coincident events has shown in Fig. 1. A Dalitz plot method has been adopted presently to understand the origin of the breakup process of the incident projectiles.

RESULTS AND DISCUSSIONS

The detected light particles has been identified by : the energy loss ΔE vsEres matrix and ΔE Vs Time (T) matrix. A typical raw spectrum has shown in Fig.1 . From the Fig.1 one can see that each particle has been separated very nicely and clearly. These light particles can come from the evaporation of the compound nucleus and are called as evaporated particles. This particle will try to stay on it minimum potential state. The energy of the two particles which has been detected coincidencely has been plotted in Fig.1 and it has found that there are other state from which the projectile breakup has happened and in fact the contribution from the other states are significant. The kinetic energy of fragments1 and 2 have been used to plot the coincidence events. These are the kinetic energy of the breakup fragements. The plots between the kinetic energiesE1(◎) and E2(t). The(t) coincidence data have shown, one can see that different breakup process has been marked as different (B1 & B2).Strongly population of the bands indicate that the breakup has happened from the ground state whereas inelastic break-up of the target ^{208}Pb is present in strongly populated area in B1.A Q values projection has been extracted from the coincident data to understand the different channel contribution and shown inFig.1(b). The peak is maximum at highest corresponds to ^{208}Pb$^{g.s}$, while the different excited states correspond to the Q spectrum [8].

CONCLUSION

A loosely bound involving experiment has been done using the target ^{208}Pb and varieties of light particle has been detected using the different types of the detector an. It has found that due to the low breakup threshold the projectile has been breaked up and provided two bands to the energy matrix depending on the origin of the breakup process. A Q values spectrum has been constructed to know the things in more details which needs further investigation.

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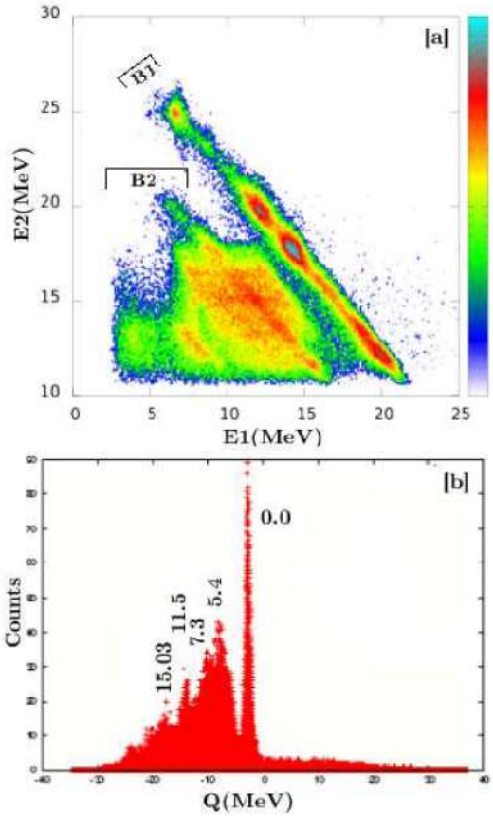

Fig.1 (a) The coincidence data between the alpha and triton. Each band corresponding to the breakup from different process. B1 is from the ground state of Target and B2 is from the excited state (b) A Q value projection of (a) to identify the different channel involvement.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Industrial Accidents and Safety Management in Pharmaceutical Industry A Review

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Received: 20 Jan 2022 Revised: 21 Feb 2022 Accepted: 23 Mar 2022

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ABSTRACT

Hazards is a term associated with an object that can cause injury in a given context or circumstances. Industrial pitfall are a Vital health and safety at work trouble in medicament manufacturing. Chemical protection and sound management of chemicals have made great strides worldwide in recent years. on the same time, the speedy improvement of the manufacturing and distribution of each herbal and fabricated chemicals has caused issues with regard to their effect at the unaffected surroundings and human life. Thus, the pharmaceutical industry holds a unique position. There are practically no other business entities like different types of toxic exposures or the rapid transformation of new chemicals. In the pharmaceutical trade, this energetic state of the issues were created by the use of organic chemical synthesis as a means of integrating a medical product. It's terribly instructive of the work of the healer. Industrial protection is required to envision everything attainable probabilities of misfortunes to stop loss of life and permanent damage to any industrial activity and any mechanical and material damage to a complete company.

Keywords: Hazards, pharmaceutical industry, safety, management, toxic effect, accidents.

INTRODUCTION

Any circumstance established by the industry that could result in employee injury or death, as well as product or property loss, is referred to as an industrial hazard[1]. A succession of significant incidents, including a fire in the Bunce field in the United Kingdom in 2005 due to the release of a fatal toxic gas in Bhopal, India in 1984, and a Deepwater Horizon oil disaster in the Gulf of Mexico in 2010, have resulted in over 2,000 deaths over the previous decades. In Brazil, the Pento Rodriguez Dam accident in 2015 resulted in several deaths and injuries, as well as major

environmental degradation and massive economic losses[2-3]. The industry's serious chemical risks have drew international notice. Industrial security consult to the prevention of harm, loss, and danger in the workplace[4]. Abbreviated the size and ferocity of work related wound requires constructive guidance of work safety and health care[5].

Routes of Industrial Hazards Entry Into the Body

When chemical processing, there is the risk of being exposed to a health hazard. These health concerns are determined by the chemical's effects[6]. Severe health impacts appear very immediately after exposure, whereas persistent toxic effects appear over time. Inhalation, absorption through the skin or eyes, ingestion, and injection are all options[7-8].

Inhalant (Breathing- in)

Hazardous chemicals can be inhaled and Enters the body through the trachea[9]. They enter the body as vapours, gases, mist, or particles (interface depicted in Fig. 1)[6]. Chemicals can create serious health problems when they come into touch with tissues in the upper respiratory tract or lungs. Tissue decomposition. The substances that are taken into the blood circulate and are transferred to the organs.

Osmosis

Almost all synthetic chemics that pass through the skin are fluid. Solid chemicals, gases, and smoke do not normally pass through the skin unless they are first dissolved in the skin's surplus moisture. The second most prevalent route for job chemicals to enter the body is through the skin. Chemicals pass through the epidermis and into the bloodstream[10]. They may cause systemic harm to the organs if absorbed. Small amounts of chemicals can enter the eyes through the soluble, which can enter the eyes if synthetics are sprayed[8]. Many compounds can penetrate the exterior tissues and extend to the arteries, and the eyes are well supplied with blood vessels. Depending on how strong the damaging material is to enter the external tissues the eye may be harmed or damaged[10].

Swallowed

Chemicals can input the belly either via way of means of gulping (ingested) debased physical fluid which has been removed from the lungs or via way of means of eating and ingesting sullied food. Food and drink are most frequently contaminated by contact with unwashed hands, gloves, or clothing, or by allowing them to come into touch with contaminants in the workplace. Chemicals enter the mouth, travel through the oesophagus, and finally into the stomach. Alcohols may pass past the belly partition and into the circulatory system, although the majority of substances pass through the small intestine. The little digestive device has a variety of villi, thin walls, and is loaded with tiny blood veins (vein). Synthetics to pass through the dividers of the villi and into the tiny digestive tract[10].

Injection

Chemical substances can enter the body if the skin is infected or infected with stains. but more unusual in a few workplaces, it will happen if a sharp needle (needle) penetrates the skin and injects a substance (or infection) into the circulatory system, or if an object is bathed in the body during severe stress[6]. The effects will be there as the substance is absorbed into the bloodstream and absorbed into the target organs[8].

Toxic Impact Of Industrial Hazard

Toxicity refers to a substance's or chemical's ability to cause detrimental effects in living beings. Synthetic waste has the potential to lessen toxicity. In many WHO countries, chemical co-operation is crucial. Many member states work hard to meet the strategic approach's goals and demonstrate their health sectors involvement in the effective use of chemical protection to assist mitigate the detrimental impacts of chemicals on health, both directly and indirectly[11].

Impact on the Environment

The pharmaceutical sector, like the chemical industries, has a slew of environmental issues. Air pollution in the workplace or the workplace might smell, and environmental safety is directly or indirectly related to human health. Air enters the lungs after being inhaled through the mouth and nose. Every minute, the average man or woman breaths in and out 12 times[12]. Twelve breaths each bring in around 500 mL of air, or 6 litres of air each minute, as well as any pollutants in the air. More than 2,500 gallons [2,800 L] of air can breathe and leave the lungs in an 8-hour working day. During severe exercise, around 10,000 gallons [10,000 L] of water can be refilled. By removing commercial garbage, they enter the ecosystem. They will not go away until they are treated and decreased completely [8].

Health Repercussions

Harmful gases (CO, CN-) prevent your tissues from receiving sufficient oxygen. Carbon monoxide is 200 times simpler to attach to haemoglobin than oxygen[13]. By preventing the enzymes required for oxygen transfer from the blood to the tissues, cyanide restricts oxygen transmission from the blood to the tissues. Lung cancer is linked to carcinogen substances. Long-term exposure to substances such as silica dust, engine exhaust, or smoke exhaust has been linked to an increased risk of heart disease, stroke, and hypertension[9].

Hazards Classification

Accident time is related to something that may be the cause of employee injuries or that may also cause a shortage of goods, products, etc. , and that is because industries employ a wide range of specialized methods for a wide variety of raw, medium, detritus products, and storage products. The dangers involved are fire, eruption, toxicity, and abode destruction[15].

Fire

A fire is a chemical interaction between air and fuel that releases heat at a specific temperature. The frequency of accidents is very common but the effects are usually minor. People affected by burns get hold of the form of pores and skin rash and conventionally depend on the duration of the ad and the size of the heat[14]. Body structures can be broken both by heat or heat. The three most important factors in heating a fire are fossil fuels (any combustible material), Oxygen (at a concentration of more than 23% in air, the circumstance becomes more dangerous due to grow fire risk.) and temperature[15].

Eruption

Explosions occur on the whole due to the speedy burning of a burning fabric and may lead to chemical response when they liberate excess energy (heat). Examples of these chemical reactions are Polymerizations, instability and thermal interconnection many kinds[16]. There are quite a few types of eruption including gasoline explosions and pollution explosions. A gas explosion is detected when a combustible fuel mixes with air and is revealed to a ignition source. Dust explosion occurs when solid flammable materials, first and foremost metals, in the structure of first-grade powders are heavily mixed with air and ignited[14]. The things that affect the eruption are[17]

1. Particle measurement
2. Chemical possessions
3. humidity
4. Dispersal of clouds

Chemical Hazard

A few flexible and flammable liquids are used in the chemical industry. These liquids evaporate when exposed to room temperature or in excess to create air pollution. The sudden release of toxic fumes has the potential to cause loss of life and serious accidents many miles from the point of release. They are fetch by water and air. The vapour detects hazards and explosives in a fireplace. In addition, they occur unexpectedly in the environment and bring a lack of presence and possessions[16-17].

Combustible Gases

1.Eruption hazard

2. Must keep below lower eruption limit.

Poisonous Feasts

1. Dangerous to mortal health
2. Hand exposure must be limited

Oxygen Displacing Feasts

1. Circular mortal health hazards
2. Deficiency of breathing oxygen

Electrical Hazards

When a human comes into contact with a conductor carrying a current, an electrical mishap can occur. At 1mA, the current level's possible influence on the human body is a minor itching. 5 mill amperes of shock does not appear to be harmful, but it is unsettling. 6-30mA severe shock, loss of muscular control Severe pain (50-150mA), breathing pause, and heavy muscles

Shortness of breath and the possibility of death Muscle contraction and nerve injury occur at 1000-4300mA, and death is a possibility. 10,000mA heart arrest, severe burns, and death are all possibilities. Source The dangers of electricity are numerous[17].

- Short circuits
- Electrical hazards
- Arc hazards and sparks
- Extreme explosions
- Improper cables

Management of Security and Its Responsibilities

The goal of industrial safety is to comprehend the negative consequences of industrial hazards and to explain the risk-danger link. Protecting workers from major industrial hazards is what industrial safety entails. In 2006, the International Conference on Chemical Management was held to improve the process of ensuring that all chemical activities are carried out in a way that protects human health and the environment. Many WHO countries rely heavily on the chemical industry[18]. Many Member States are actively pursuing the Strategic Approach's objectives, including deepening the engagement of their health sectors in efficiently managing chemical safety in order to prevent both direct and indirect detrimental chemical effects on health[11].

Accident Reduction

Risks and accidents in the workplace have become more common as new technology emerge every day and few individuals understand how to use them. Someone with limited expertise is likely to notice this harmful propensity. An accident is a haphazard event involving harmful materials that cause or are likely to cause injury, harm to one's health, or harm to the environment[2]. There are no long-term occurrences included in this (such as chronic pollution). There are a few things to think about[14].

Pre-design Reviews And Refinements: These include the right design, resources and key choices. Less chemicals should be stored, lower values will automatically mean less damage in the event of an accident.

Chemical Hazards: Chemicals are tested based on whether they are compliant, flammable, toxic, explosive hazards and storage.

Process Security Management[8]: Before implementing big policy changes, consider the following. In industrial organisations, managers should aim to build a safety culture, check the reliability of the items, which includes a safe travel and communication, a scratch system, and etc.

Security Inspections: Regular inspections of security procedures and procedures, operation of security systems and gadgets and follow-up measures should be carried out.

Staff Training: Proper staff training and security services should be provided.

Proper Storage: All chemicals and hazardous substances should be kept in a secure location away from children and animals, at a comfortable temperature. If you're storing functional items, make sure they're in a waterproof container[14].

Obligations Of The Employer

The employer bears full responsibility for the prohibited work. It is a widely held belief (for example - an employee has done what an employer told us to do). This creates a culture of dread and suspicion, as well as an unwillingness for employees to question and deviate from control methods. When it comes to enforcing compliance concerns and discussing potential compliance issues linked to security management, employees should be encouraged to take advantage of the chance to open an open door for senior management of the company. There are some responsibilities that employers must fulfil[19].

1. Determine which chemicals are hazardous and compile a list of hazardous chemicals in their workplace.
2. If not provided by a manufacturer, importer, or distributor, obtain Specific Security Spreadsheets (MDDSs) for each hazardous chemical.
3. Implementing risky programs and labels, MSDS and staff training.
4. Labels, MSDS, and official training programmes should be used to communicate danger information to personnel.

Management Of Risks

Controlling fires: Fire safety is an important aspect of industrial safety. We can control fire threats by implementing safety measures[11-12].

1. Smoking is prohibited in the production area.
2. Oxygen in the air can be reduced by combining it with other gases like nitrogen and carbon dioxide.
3. In areas where flammable chemicals are stored, handled, or transferred, identify and control combustible resources.
4. The removal of flammable sources.
5. Build the plant structure with care.
6. Non-combustible brick walls or reinforced concrete walls should be used.
7. Appropriate exit facilities will be provided by the machine building.
8. Proper ventilation
9. Apply a steady stream of water.
10. Adequate fire alarms should be installed.

Hazardous Gas Management

Pressurized gases are poured into cylinders and dispersed to find a suitable location. The critical measures to take are outlined below[17-18].

1. Cylinders should never be unloaded or collided with each other.
2. Valves should be serviced with standard tools, and safety devices should be of high quality. The manufacturer is usually the one that provides these.
3. Protect cylinders from extreme weather, particularly high temperatures.
4. The cylinders must have a standard label indicating the type of gas. Color or pictogram labels indicate gas burning, rust, or heat.

Explosive Control And Fire Control

Fire comes from the industry more often than explosions and toxic release, although the effects of loss of life are usually small. Thus, fire may not be so dangerous. Fire and explosion control should be followed by safety precautions[17].

1. Fire and explosion risks can be reduced by careful planning and careful selection of building materials.
2. Explosions can be limited by non-combustible brick walls.
3. The roof should be constructed in such a way that it can easily lift under explosive force.
4. Fire hazards are reduced by eliminating unnecessary sources such as flames, sparks, smoking, welding, and etc.
5. Installing appropriate temperature alarms, fire alarms, and fire extinguishers, among other things.
6. Inside the pipe, fire extinguishers are installed. These are intended to extinguish the initial fire. There are three stages to the first flames.

Category A fires: Common combustible items cause these types of fires. They're kept in check by employing water, which has cooling and quenching properties.

Category B fires: Fats, oils, flammable liquids, and other combustible materials cause these types of fires. Category B fires should leave a hazy or slick surface.

Category C fires: Electrical equipment cause these types of fires. A firefighting agent makes the environment non-flammable.

The pharmaceutical business faces more health risks than the general public, including major health implications such as cancer, endocrine dysfunction, and liver disease. The possibility of significant amounts of sex steroid exposure is supported by industrial hygiene regulations. In exposed workers, endocrine function is stressed, and reproductive function is disrupted. Asthma at work and contact dermatitis have been the most commonly reported allergies. Asthma in the workplace is a possibility, especially when antibiotics and enzymes are manufactured.

Workplace exposure to birth control tablets has had a negative impact on workers' health, resulting in alterations in liver function and sex hormone levels. Oestrogen exposure may raise the risk of Phytoestrogens in both men and women[20].

Hazardous Waste Awareness

It is our responsibility to educate everyone about safe chemical handling and chemical exposure. Factory workers, transport workers, and importer/distributor employees must be able to recognise the pictogram (as indicated in Table 1) and be informed of safety and security precautions[14]. The MSDS (Material Safety Data Sheet) should be easily accessible to the general public. It's worth noting that it's particularly prevalent in potentially harmful household and automotive products. All of the chemicals we use have the potential to harm us, and we need to know how to prevent being exposed to them. Rust, poisoning, injury, and irritation are the four basic stages of health risk[21]. People should be aware of risk identification in order to protect themselves from their hazardous effects. To avoid accidents and allow as little time as possible on the road, the public should also cooperate with the police and any tanks or big vehicles[14].

Identification and Label

Almost every workplace has industrial dangers. To protect people from the effects of risks, they must be identified and risk management procedures must be implemented[22]. This is a critical first step in any large risk management system. Hazardous photos may be used to alert employees and visitors to the presence of hazardous chemicals[23]. Pictograms assist us in recognising whether the chemicals we use are potentially damaging to humans or the environment. In a few nations, there are standards for identifying and labelling chemical substances, however the details of identification and labelling components differ from country to country[12]. We can keep alert to safety if we can easily read the warning indications of danger. For your industry or workplace, you'll find a decent assortment of hazardous industrial signs and labels here. Hazardous chemical containers at the factory should be labelled, labelled, or labelled with the required information[24].

Hormonaised System On A Global Scale (GHS)

Identification

The GHS was endorsed by the United Nations (UN) in 2003. GHS stands for Globally Harmonized System of Chemical Classification and Labelling. Various nations have different criteria for recognising hazards and including information in data protection labels and sheets. For example, a product may be flammable or poisonous in one country but not in another when it is exported. These distinctions have an impact on both defence and trade[26]. Users with specific needs may receive different label warnings or details of the same chemical data in a secure environment. Manufacturers and distributors in the commercial sector must comply to risk categories, and labelling is costly and time consuming[25]. Small and medium-sized businesses can successfully follow international chemical trade restrictions while avoiding the regulatory burden of compliance. GHS establishes a global risk communication system. It's a standardised and consistent way of identifying and categorising things.

Harmful chemicals stored improperly in a warehouse can cause fires or explosions, or produce injuries by sniffing, looking at the skin or eyes, or by sucking. In situations where flammable chemicals are kept, handled, or moved, ignition equipment must be identified and controlled[27]. As a result, in order to avoid mishaps, particular care must be taken when storing and handling these dangerous gases[17].

CONCLUSION

The global manufacturing and consumption of pharmaceuticals is likely to continue to rise, posing chemical risks. The complete review essay emphasises the industrial and environmental worker's safety and efficacy. Because industrial employees often have a deeper understanding of mortality than the general population, they have suffered major health implications. In the pharmaceutical and chemical industries, safety components should be addressed not only for the sake of workers and property, but also for the sake of neighbours. From the preceding, it should be evident that any pharmaceutical industry, large or little, requires professional counsel on concerns impacting worker health. To determine the link between occupational exposure and health effects, more study is needed. Occupational exposure to the pharmaceutical business currently has few agreed or regulatory requirements.

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Table 1: Globally harmonized system (GHS) pictograms used for chemical hazards

S. No.	Category	Symbol	Pictograms
1	Explosive	Exploding bomb	
2	Flammable	Flame	
3	Oxidising	Flame over circle	
4	Corrosive	Corrosion	
5	Acute toxicity	Skull and crossbones	
6	Hazardous to the environment	Dead tree and fish	
7	Health hazard/Hazardous to the ozone layer	Exclamation Mark	
8	Serious health hazard	Health hazard	

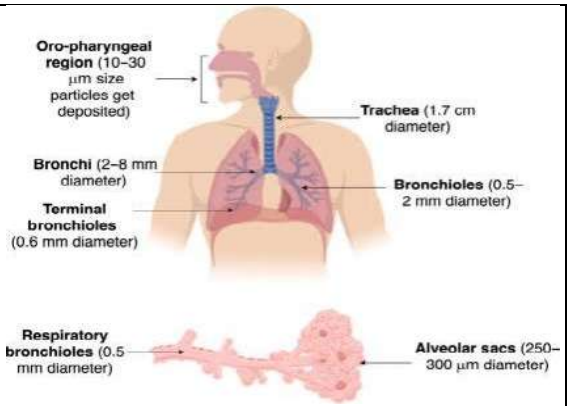

Fig 1: The amount of dust absorbed by the body and their effect on the body

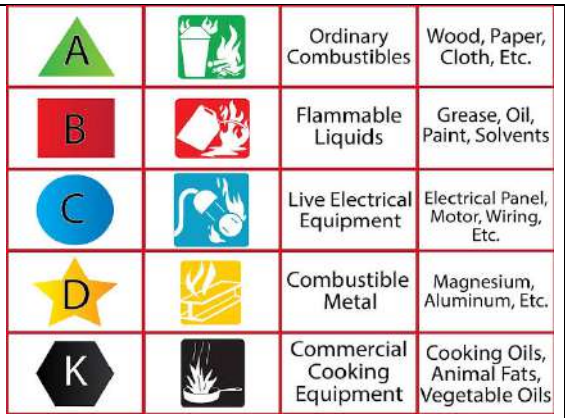

Fig.2 : Explosive control and fire control

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Relation of Antinuclear Antibodies and Anti-thyroid Peroxidase with Standard Cardiometabolic Risk Factors in Women with and without PCOS

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Received: 31 Jan 2022 Revised: 19 Feb 2022 Accepted: 08 Mar 2022

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine condition affecting women of reproductive age. PCOS has been linked to a variety of metabolic and cardiovascular problems, as well as autoimmune disorders, most notably autoimmune thyroid disease. In women with PCOS, serum levels of common autoimmune markers were determined. Antinuclear antibodies and anti-TPO levels are elevated in patients with PCOS, implying a link between the disease and autoimmune responses. 4. It is believed that autoimmune diseases contribute to and influence PCOS risk factors such as obesity, dyslipidemia, hypertension, impaired glucose tolerance, and subclinical vascular disease. If dyslipidemia or other risk factors persist, lifestyle modifications may include diet and exercise, as well as the addition of insulin sensitizers and other medications.

Keywords: Polycystic ovarian disease, metabolic syndrome, antinuclear antibodies Diabetes mellitus, cardiovascular disease, Risk markers, Hyperinsulinemia

INTRODUCTION

PCOS prevalence varies between 10 and 20 percent globally, depending on demographic features and diagnostic criteria [1]. The complicated multifactorial condition is diagnosed when two out of three of the following are present: clinical and/or biochemical androgen excess, anovulation, and polycystic ovaries on pelvic ultrasound. There are almost always antinuclear antibodies (ANAs) in almost all autoimmune reactions [2]. Inflammation, hyper-stimulation of the immune system, and any procedure that causes tissue damage could make ANA production more likely [3]. In this study, we looked at the level of ANAs and Anti-thyroid peroxidase **(**anti-TPO) in the blood of people with PCOS. We wanted to find out how the ANAs and anti-TPO affect the progress of the disease.

MATERIALS AND METHODS

Women with PCOS were included in the study. A total of 180 women were recruited and analyzed. After verbal and written explanations of the study's techniques and dangers, participants gave informed permission to participate. The Institutional Ethical Committee of the Government Medical College, Kozhikode, authorized this research. Each patient who enrolled in the trial signed an informed consent form. Blood glucose was estimated by the enzymatic method using glucose oxidase. The lipid profile was determined using a semi-automatic analyzer (ERBAChem5) based on the enzymatic principle and kits obtained from ERBA diagnostics. The indirect immunofluorescence antibody technique, which is very sensitive, was utilized to detect ANA, with the results reported in terms of fluorescence pattern, the substrate used, and titer of a positive test. The chemiluminescence method was used for the estimation of anti – TPO.

RESULTS AND DISCUSSION

For the comparison of LDL between women with and without PCOS, the Mann-Whitney U statistic is 2436.500, the Z value is -4.619, and the p-value is 0.000. Because the p-value is less than 0.05, we may infer that there is a statistically significant difference in LDL levels between women with and without PCOS. The Mann-Whitney U statistic for comparing Anti TPO levels across women with and without PCOS is 9.000, the Z value is -11.578, and the p-value is 0.000. Because the p-value is less than 0.05, we may infer that there is a significant difference between women with and without PCOS who use Anti TPO.

ANA and FBS have chi-square statistics of 2.356 and 0.125. Because the p-value is greater than 0.05, there is no significant association between ANA and FBS in PCOS women. In both cases, the chi-square statistic is 6.776, with p=0.009. Because the p-value is less than 0.05, we can conclude that ANA and TG are associated with PCOS women. Ana and CL have chi-square statistics of 5.83 and 0.016. Because the p-value is less than 0.05, we can conclude that ANA and CL are associated with PCOS women. Ana and HDL have chi-square statistics of 4.585 and 0.032. Because the p-value is less than 0.05, we can conclude that ANA and HDL are associated with PCOS women. Dietary factors affect ANA and LDL levels. Because the p-value is greater than 0.05, there is no significant association between ANA and LDL in PCOS women.

CONCLUSION

This study shows that people with PCOS have a higher rate of autoimmune thyroid disease than people who are healthy. Because of this, doctors should check for thyroid function and thyroid-specific autoantibodies when they diagnose PCOS, even if there are no symptoms of thyroid problems. The study demonstrates a significant increase in cardiometabolic risk markers in women with PCOS, indicating an increased risk of developing lifestyle diseases such as metabolic syndrome and heart disease.

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Table 01

	Mann-Whitney U	Z	p-value
FBS (mg/dl)	3849.000	-.577	.564
TG (mg/dl)	3242.000	-2.313	.021
CL (mg/dl)	3846.500	-.583	.560
HDL (mg/dl)	2182.500	-5.351	.000
LDL (mg/dl)	2436.500	-4.619	.000
Anti TPO (IU/ml)	9.000	-11.578	.000

Table 02: Chi square tests to check association between ANA and the traditional cardio metabolic risk factors for women with PCOS

	Chi-Square	Df	p-value
FBS (mg/dl)	2.356	1	.125
TG (mg/dl)	6.776	1	.009
CL (mg/dl)	5.83	1	.016
HDL (mg/dl)	4.585	1	.032
LDL (mg/dl)	3.244	1	.072

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prevalence of Abnormal Uterine Bleeding According to New International Federation of Gynecology and Obstetrics Classification in A Teritiary Hospital, Salem-A Cross Sectional Study

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Received: 06 Jan 2022 Revised: 08 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

The purpose of the study was to describe prevalence of abnormal uterine bleeding. The process is one of sexual reproduction in mammals, including humans, in which the male and female organs are physically and physiologically distinct, and the new individual emerges from the fusing of two different sex cells. Up to 30% of women in their reproductive phase experience heavy menstrual bleeding. Menstrual bleeding of unusual frequency, length, or quantity is referred to as abnormal uterine bleeding (AUB). In the absence of recognised pelvic pathology, general medical condition, or pregnancy, abnormal uterine bleeding (AUB) is described as irregular uterine bleeding. It occurs when the usual cyclic pattern of ovulatory hormone stimulation to the endometrial lining is disrupted. PALM-COEIN (pronounced "palm-coien") is a basic/core approach for classifying irregular uterine bleeding. It includes polyp:adenomyosis: leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction endometrial; iatrogenic; and not yet categorised. Menstrual disturbances are a typical gynaecological disease that brings women of reproductive age to the doctor. The cases were distributed based on obesity among 150 cases collected during the study period. Only121 (80.67%) of the women are fat, whereas 29 (19.33%) are non-obese. According to the results of the study, antifibrinolytics are the most commonly used medication class for abnormal uterine bleeding in our tertiary care hospital, and tranexamic acid is the most commonly used therapeutic agent for abnormal uterine bleeding.

Keywords: uterine bleeding, malignancy, hormone, pathology

INTRODUCTION

Abnormal uterine bleeding (AUB) is the most common symptom of gynecological conditions, which is defined as any type of bleeding in which the duration, frequency, or amount is excessive for an individual patient. Outside of pregnancy, abnormal uterine bleeding (AUB) refers to anomalies in the menstrual cycle regarding frequency, regularity, duration, and volume of flow [1] . Abnormal uterine bleeding affects up to one-third of women at some point in their lives, with anomalies most typically occurring during menarche and perimenopause [2]. A normal menstrual cycle occurs every 24 to 38 days, lasts 7 to 9 days, and results in a blood loss of 5 to 80 millilitres [3]. Abnormal uterine bleeding is defined as a change in any of these four characteristics [4]. Older words like oligomenorrhea, menorrhagia, and dysfunctional uterine bleeding should be replaced with more straightforward terms that describe the nature of aberrant uterine bleeding [5]. The International Federation of Obstetrics and Gynecology (FIGO) published revisions to the nomenclature in 2007, followed by changes in 2011 and 2018 [6]. The FIGO systems define abnormal uterine bleeding first, then provide an acronym for the most prevalent etiologies [7]. Menstrual disorders, previously portrayed as dysfunctional uterine bleeding (DUB) and menorrhagia, are now better described as abnormal uterine bleeding (AUB) [8]. The terms DUB and menorrhagia should be discarded [9]. In clinical practice, HMB is defined as 'excessive menstrual blood loss leading to interference with the physical, emotional, social and material quality of life of a woman' [10]. Hence, treatment success ultimately depends on the improvement of the woman's symptoms and quality of life [11]

Atypical vaginal bleeding, also known as abnormal uterine bleeding (AUB), is vaginal bleeding from the uterus that is abnormally frequent, lasts an abnormally long time, is heavier than normal, or is irregular [12]. When there was no underlying explanation for the uterine bleeding, it was referred to as dysfunctional uterine bleeding [13]. Vaginal bleeding is not allowed during pregnancy [14]. It is possible to develop iron deficiency anaemia, which can have a detrimental impact on one's quality of life [15]. Ovulation issues, fibroids, the lining of the uterus expanding into the uterine wall, uterine polyps, underlying bleeding problems, birth control side effects, or cancer are some of the underlying causes [16]. In some cases, more than one group of reasons may apply. The initial step in the investigation is to rule out the presence of a tumour or pregnancy [17]. The diagnosis may be aided by medical imaging or hysteroscopy [18]. The treatment will be determined by the underlying reason. Hormonal birth control, gonadotropin-releasing hormone (GnRH) agonists, tranexamic acid, NSAIDs, and surgery such as endometrial ablation or hysterectomy are all possible options [19]. The volume of menstrual flow is influenced in part by uterine contractions, vascular tone and haemostatic function. Normal menstruation can range from a frequency of between 24 and 38 days, a duration between 4.5 and 8 days, and a volume of blood loss between 5 and 80 ml per cycle. The experience of menstruation is different for every woman. Therefore, defining what constitutes 'abnormal' menstrual bleeding is a subjective assessment for patients and their clinicians. The definition of HMB in the research setting relates to blood loss of more than 80 ml per cycle. This level of blood loss increases the risk of iron deficiency anaemia.

METHODOLOGY

STUDY SITE

The study was conducted in Vinayaka Mission's Kirupanandha Variyar Medical College, Salem.

SOURCE OF DATA

Abnormal Uterine Bleeding patients with classification of AUB[PLAM-COEIN] in a tertiary care hospital.

METHOD

A Cross Sectional study cases of patients 150 with the complaints of AUB. Patients affected with Abnormal Uterine Bleeding with the Patients of aged 17-56 (reproductive age) of gynecology department. The Patients with other comorbid diagnosed as abnormal uterine bleeding.

Patient who attained menopause before 56age were who does not attained menarche at the age 17 and the Patients those who done hysterectomy.

RESULTS AND DISCUSSION

This study focused on the prevalence of abnormal uterine bleeding even though anaemia is one of the significant consequences that women have to face during their ovulatory and an ovulatory cycles. A total of 150 abnormal uterine bleeding patients were hospitalised including in in VMKVMC&H's gynaecology departments from March 2021 to December 2021, and the data from those case sheets were noted, updated on such a periodic manner, and analysed according to the study's needs to yield a result/outcome. This present research seeks to spread awareness among women about the abnormal uterine bleeding and to assist in making changes in lifestyle. The study population were distributed based on their marital status. The majority of the patients were married women (88.67%), with only 11.33 % being unmarried. The study population was divided into two groups depending on their employment status: those who were employed (64%) and those who were not (36%). The study participants were divided into groups based on their surgical data and medical treatment. Only 4 (2.7%) of the 150 patients had surgical treatment, while 146 (97.3%) had medicinal treatment. Heavy monthly bleeding can be handled medically, but if it isn't, surgical interventions are deemed to be managed medically, which is consistent with our findings. The cases were distributed based on obesity among 150 cases collected during the study period. Only121 (80.67%) of the women are fat, whereas 29 (19.33%) are non-obese. The final condition is diagnosed based on the endometrial histopathological of various lesions in 150 cases. The majority of the patients (74.6%) were diagnosed with ovulatory dysfunction (AUB-O), although only 1.3% were diagnosed with coagulopathy (AUB-C). Table No.: 1 show the number and percentage.

According to this cases of past medical history which are most commonly 17.3% of stomach pain,10% of anemic,6% of sinus and 2.6% of dysmonorrehea. Therefore 64% of patient are not had the past medical history. Table No.2 show the number and percentage. In our group of patients, irregular menusturation, stomach pain, and leukorrhea are the most common symptoms of abnormal uterine bleeding. The table no.:3 showed the number and percentage. Calcium and vitamin supplements were the most frequently prescribed drug among 150 prescriptions, and tranexamic acid was the most commonly recommended treatment for irregular uterine bleeding. Calcium >Antifibrinolytics> H2 Blockers > Ferrous sulphate > Analgesic and antipyretics > Antianxiety agents, Anxiolytics, Benzodiazepines > Proton pump inhibitors >prostaglandins> Antiemetic> vaccine.

CONCLUSION

According to the results of the study, antifibrinolytics are the most commonly used medication class for abnormal uterine bleeding in our tertiary care hospital, and tranexamic acid is the most commonly used therapeutic agent for abnormal uterine bleeding. AUB is a common symptom of gynaecological diseases that has a negative impact on women's quality of life. There is currently no report on the Salem tertiary hospital's examination of the cause of a new classification of gynaecological disorders. According to this study, AUB-O is the most common cause of AUB in women aged 17 to 56. A variable menstrual cycle is the most common bleeding pattern, which is commonly characterized by an increase in the volume of flow or longer periods. AUB-O (74.60 percent), AUB-L (11.30 percent), AUB-A (8%), AUB-P (5%), and AUB-C (1.1 percent) are all found in it. Their primary bleeding patterns are affected by thequantity of excessive menstrual bleeding and the length of their periods, and they are linked to age, with the maximum occurrence occurring between the ages of 17 and 56.

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Table no.: 1 Study of population segregation depending on diagnosis of abnormal uterine bleeding.

Sl No.	Types of AUB	No. of Cases	% of Total
1	AUB-A	12	8%
2	AUB-L	17	11.30%
3	AUB-O	112	74.60%
4	AUB-P	7	5%
5	AUB-C	2	1.3%
6	AUB-E	0	0
7	AUB-M	0	0
8	AUB-I	0	0
9	NOTYET CLASSIFIED	0	0
10	TOTAL	150	100%

Table No.: 2 Study of population segregation depending on past medical history

Sl. No.	past medical history	No. of cases	% of total
1	ANEMIC	15	10%
2	DYSMONORRHEA	4	2.60%
3	SINUS	9	6%
4	STOMACH PAIN	26	17.30%
5	NIL	96	64%
6	TOTAL	150	100%

Table no.: 3 Study of population segregation depending on symptoms of abnormal uterine bleeding.

SINO.	Symptoms	No. of Patients	% of total
1	Abdominal Pain	132	88%
2	Heavy Bleeding	80	53.3%
3	Irregular Mensuration	140	93.3%
4	Fatigue	50	33.3%
5	Nausea and Vomiting	25	16.6%
6	Blood Clots	43	28.6%
7	Mood Swings	118	78.6%
8	Sleep Disturbance	101	67.3%
9	Vaginal Dryness	18	12%
10	Fever	68	45.3%
11	Leukorrhea	132	88%

Table no.: 4 Study of population segregation depending on drug usage.

SINo.	Drug	Category	No. of time prescribed (n=150)	(%) of total
1	T.Trapic	Anti fibrinolytics	146	97.3%
2	T.Rantac	H2 blockers	146	97.3%
3	T. Calcium	Calcium	150	100%
4	T. VitC	Vitamin supplement	150	100%
5	T. FST	Ferrous sulphate	4	2.6%
6	T.Para	Analgesic and antipyretics	4	2.6%
7	T.Alprax	Antianxiety agents, anxiolytic, benzodiazepines	4	2.6%

8	T. Pan	Proton pump inhibitors	4	2.6%
9	T.Mesoprostal	Prostaglandins	4	2.6%
10	Inj.TetanusToxoid	Vaccine	4	2.6%
11	Inj. Emeset	Antiemetic	4	2.6%

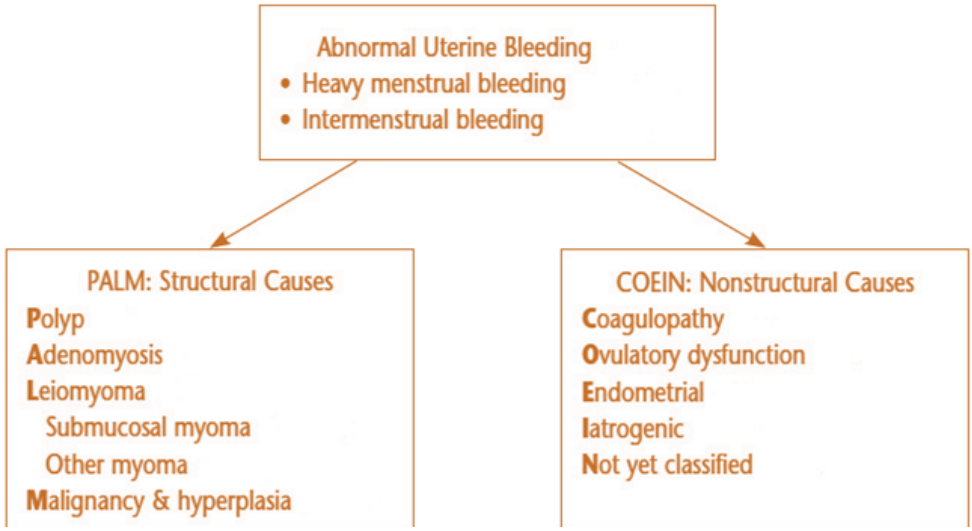

Fig. 1: Types of AUB

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In-silico Predictions and Cytotoxicity Evaluation of Some 2-azetidinone Tethered Flavones as Antibreast Cancer Agents against MCF-7 Cell Lines

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Received: 22 Jan 2022 Revised: 14 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

Flavone scaffold and the 2-azetidinone derivatives are known to induce apoptosis and show anti-proliferation in several human tumour cell lines. The present study emphasizes on the anti-proliferating properties of some substituted flavones clubbed with 2-azetidione derivatives N-(3-Chloro-2-oxo-4-arylazetidin-1-yl)-2-[(4-oxo-2-aryl-4H-chromen-7-yl) oxy] acetamide Derivatives [SLP VI 1(a-c)-2(a,b,d)] in MCF-7 cell lines of breast cancer. Cell growth assays were carried out by MTT reduction test and studies revealed that among all, the compound N- (3-chloro-2-oxo-4-(4-hydroxy, 3- methoxy phenyl)azetidin-1-yl) -2- [(4-oxo- 2- (4-dimethyl amino phenyl) - 4H-chromen-7-yl) oxy] acetamide [SLP VI2b] remarkably inhibited the cell growth in MCF-7 cell lines in a concentration dependent manner with 5.84 µg/mL IC_{50} value. Further, we evaluated Drug likeliness properties by swiss ADME tool and molecular docking of the reported compounds with HER-α and EGFR receptors. The *in silico* and *in vitro* studies revealed that all test compounds are druggable and can be further explored for detailed antibreast cancer investigations. Compound SLP VI2b could be a promising lead for the development of an effective anticancer agent.

Keywords: flavones, 2-azetidinone, MCF-7, molecular docking, HER-α, EGFR.

INTRODUCTION

Flavonoids received great attention due to diverse biological activities and health benefits. They can be classified based on substitution pattern variations, connection position of ring B, annularity of ring C and degree of oxidation, providing an extremely diverse range of derivatives [1]. Flavonoids play protective role against many bacterial, viral infections and degenerative diseases like cardiovascular diseases and cancers [2]. They are also well documented for their hepatoprotective Activity [3], Antimicrobial [4], Anti-inflammatory [5], Antiviral[6], Anticancer[7]. Flavonoids act as antibreast cancer agents through multiple mechanisms [8,9]. The natural, semi-synthetic and synthetic flavones are the largest group of flavonoids with wide range of biological and medicinal uses. Synthesis of flavones has attracted considerable attention because of their potential as lead compounds [10].The unique ability of flavones to modulate various enzyme systems may offer diverse biological activities and reduced risk of developing cardiovascular diseases and cancer [11]**.** The 2-azetidinone structural motifs attracted a great deal of interest due to broad biological activity profile including antimycobacterial[12], anti-inflammatory, analgesic, anticonvulsant, antiviral, antihypercholesterolemic and anticancer activities[13]. The β-lactam derivatives were reported to induce apoptosis and inhibit proliferation in several human tumorous cell lines of colorectal, breast carcinoma, prostate cancer and lung cancer. β-lactams used as tumer targeted pro-drugs and also exhibits anti-proliferation by inducing apoptosis[14].

The recent projected incidence of development of cancer in India is 1 in 9. The global burden and frequency of breast cancer is still rising and constitutes about 10% of all cancers [15].The limitations and challenges of currently available therapies of breast cancer necessitate the identification of novel anti-tumour agents. Impressive number of research papers revealed that MCF-7 is a well characterized, commonly used and suitable model cell line for breast cancer investigations both *in vitro* and *in vivo* than any other breast cancer cell line. MCF-7 cells are important candidates in research for estrogen receptor (ER)-positive tumour cell experiments in breast cancer due to their dependence on estrogen for the proliferation. In the light of facts mentioned above and in continuation of our previous research effort, the present article reports exploration of antibreast cancer effect of N-(3-Chloro-2-oxo-4- arylazetidin-1-yl)-2-[(4-oxo-2-aryl-4H-chromen-7-yl)oxy]acetamide Derivatives [SLP VI 1(a-c)-2(a,b,d)] against MCF 7 cell lines and insilico studies like ADME prediction and Docking studies done on various target proteins involved in breast cancer progression.

MATERIALS AND METHODS

The test compounds of flavones tethered with azetidinone derivatives [SLP VI 1(a-c)-2(a, b, d)] were obtained by the synthetic route [16].The MCF7-Human Breast Adenocarcinoma cell line was obtained From National Center for Cell Science, Department of Biotechnology, Pune, India. DMEM- High Glucose - (#AL111, Himedia) was used as cell culture medium.

Cell Viability Assay

Cell growth assays were carried out with the help of MTT reduction test. 200μL of cell suspension was seeded in a 96-well plate and allowed to grow for about 24 hours. The plate was Incubated for 48 hrs at 37°C in a 5% CO_2 atmosphere after the addition of test agent and MTT reagent was added to a final concentration of 0.5mg/mL of total volume. The conversion of MTT to formazan by metabolically viable cells was measured at 570 nm. The test compounds at a range of concentrations from 12.5, 25, 50, 100, 200 uG/mL were evaluated for the inhibitory effects on cell proliferation in MCF 7 cells by MTT colorimetric assay in vitro using medium control, negative control and positive control (medium with cells and 15uM of Camptothecin).

ADME Prediction

Chem Draw ultra12.0 and chem3Dpro 12.0 softwares were used to draw the structures and 3D structurers of designed molecules respectively. ADME tool, swiss ADME online database software used to predict Molecular properties like log P, polar surface area, number of hydrogen bond acceptors and donors, solubility, Lipinski violations, drug likeliness and synthetic accessibility etc.

Molecular Docking

For molecular docking, the 3D structures of selected compounds were obtained from chem3Dpro 12.0 and converted to PDB format. Crystal structures of target protein molecules which play an important role in cancer progression have been selected from protein data bank. Automated molecular docking was performed to find out molecular interaction and optimized geometry by using docking software Auto Dock Tools 1.5.6 version. The interactions between selected compounds and target receptor were visualized by using Biovia Discovery Studio visualiser.

RESULTS AND DISCUSSION

Cell Viability

The anti-proliferative effect of N- (3-chloro-2- oxo-4-aryl azetidin-1-yl) -2- [(4-oxo-2-aryl -4h- chromen- 7-yl) oxy] acetamide derivatives on human breast cancer cell line (MCF-7) has been evaluated by MTT assay (Table 1). The cells were treated with compounds [SLP VI 1(a-c)-2(a, b and d)] along with positive control camptothecin in the concentration range of 12.5–200 μg/ml for 24 h. Among all, compounds SLPVI 2b showed the highest inhibition towards MCF-7 cells with IC_{50} value 5.84 μg/ml. Compounds SLPVI 2a, 1c and 2d showed moderate activity while other compounds of the series SLPVI 1a and 1b were found to be relatively less active (>100 μg/ ml). The Observations in Statistical data of Cell Cytotoxicity Study suggested that against MCF7 cell line, given Test Compounds [SLP VI 1(a-c)-2(a,b,d)] showed Good cytotoxic potential properties compared to the Standard Drug, Camptothecin. The growth of MCF-7 cells was significantly inhibited in a dose dependent manner in the presence of SLP VI 2b (12.5, 25, 50, 100, 200uG/mL) at 48 hr (Table 2).

Evaluation of Drug Likeness Properties

Some of the ADMET properties/parameters of the compounds [SLP II (1-2) and VI 1(a-d)-2(a-d)] exhibited optimum drug likeness properties and obeyed Lipinski's rule (Table 3).

Molecular Docking Studies

The summary of the docking studies result of some test compounds is given in Table 4 and table 5. The docking score was obtained using Autodock 1.5.6 software and interactions were visualized using BioviaDiscovery Studio Software. Compare to Flavones SLP II 1-2, their corresponding azetidinone derivatives have good binding energies with the target protein. Among all the derivatives SLP VI 2d has highest binding energy of -10.71kcal/mol(figure 2). The interaction energy of Flavones (SLP II 1-2) and azetidinone derivatives SLP VI 1 (a-d)-2(a-d) with Epidermal growth factor receptor (EGFR, PDB id: 2J6M) were good. Among all the derivatives SLP VI 2b has highest binding energy -8.77 kcal/mol (fig 3).

CONCLUSION

The anti-proliferative properties of substituted flavones clubbed with 2-azetidione derivatives [SLP VI 1(a-c)-2(a,b,d)] were effective against MCF-7 cancer cell lines. Cell viability studies revealed that among all, the compound N-(3-chloro-2-oxo-4-(4-hydroxy, 3- methoxy phenyl)azetidin-1-yl) -2- [(4-oxo- 2- (4-dimethyl amino phenyl) -4H-chromen-7-yl) oxy]acetamide [SLP VI2b] remarkably inhibited the growth of MCF-7 cells in a concentration dependent manner with IC_{50} value of 5.84 μg/ml. The results obtained in ADME prediction and docking studies has been supported the invitro evaluation of antibreast cancer activity. All the test compounds showed good binding

affinity towards the target proteins Human Estrogen receptor α and epidermal Growth factor receptor.the present study strongly suggest that compound SLP VI 2b may have possible therapeutic potential against human breast cancer cells and provide the basis for further in vitro and in vivo studies for evaluation of mechanism of action.

ACKNOWLEDGMENT

Authors are thankful to Stellixier Biotech private Ltd, karnataka for MTT assay support.

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Table 1: The IC $_{50}$ (µg/mL) Concentrations of the Test Compounds against MCF7 Cells

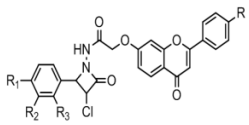
Id	Compound		R	R^1	R^2	R^3	IC_{50}(µg/mL)
1	SLP VI 1a		$-OCH_3$	$-OCH_3$	-H	-H	92.07
2	SLP VI 2a		$-N(CH_3)_2$	$-OCH_3$	-H	-H	61.32
3	SLP VI 1b		$-OCH_3$	-OH	$-OCH_3$	-H	189.02
4	SLP VI 2b		$-N(CH_3)_2$	-OH	$-OCH_3$	-H	5.84
5	SLP VI 1c		$-OCH_3$	$-N(CH_3)_2$	-H	-H	54.59
6	SLP VI 2d		$-N(CH_3)_2$	-Cl	-H	-Cl	57.07

Table 2: Incubation of cells with SLP VI compounds (from12.5 to 200uG/mL) at 48 hr

compound	Cell viability % at 48 hr				
	12.5(uG/mL)	**25(uG/mL)**	**50(uG/mL)**	**100(uG/mL)**	**200(uG/mL)**
SLP VI 2b	41.76	28.17	19.00	11.94	2.64
SLP VI 1c	88.35	74.29	61.41	24.52	14.11
SLP VI 2d	86.17	67.70	58.64	37.58	15.00
SLP VI 2a	80.70	76.11	62.00	41.35	14.52
SLP VI 1a	96.82	83.35	67.47	49.05	28.29
SLP VI 1b	96.76	90.35	83.76	67.17	49.58
Camptothecin	45.05 (15 uM)				

Table 3: Drug likeliness Properties of Compounds SLP II (1-2) and VI 1(a-d)-2(a-d).

Compound Property(desirable value)	SLP II 1	SLP II 2	SLP VI 1a	SLP VI 1b	SLP VI 1c	SLP VI 1d	SLP VI 2a	SLP VI 2b	SLPVI 2c	SLP VI 2d
MW (g/mol) (50-500)	192.17	205.21	534.94	550.94	547.99	573.81	547.99	563.99	561.03	586.85
iLogp(<5)	1.75	1.87	3.49	3.24	3.24	3.82	3.62	3.63	3.40	3.36
HBD(<5)	1	1	1	2	1	1	1	2	1	1
HBA(<10)	4	3	7	8	6	6	6	7	5	5
TPSA(<140)	59.67 $Å^2$	53.68	107.31	127.54	101.32	98.08	101.32	121.55	95.33	92.09
MR	58.71	58.71	143.81	145.83	151.52	147.33	151.52	153.54	159.24	155.05
nAH	10	10	22	22	22	22	22	22	22	22
nRB(<10)	1	1	9	9	9	8	9	9	9	8
Lipinsky's rule	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Number of violations	0	0	1(mw>500)	1(mw>500)	1(mw>500)	1(mw>500)	1(mw>500)	1(mw>500)	1(mw>500)	1(mw>500)
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Lead likelines	no	no	no	no	No	No	No	No	No	No

Number of violations	1(mw< 250)	1(mw< 250)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)	3 (MW>350, Rotors>7, XLOGP3>3.5)
Solubility Log s & soluble class	-2.86 & soluble	-3.00 & soluble	-5.57 & moderately soluble	-5.43 & Moderately soluble	-5.73 & moderately soluble	-6.68 & Poorly soluble	-5.73 & moderately soluble	-5.60 & moderately soluble	-5.90 & Moderately soluble	-6.85 & Poorly soluble
Synthetic accessibility	2.88	2.65	4.61	4.69	4.70	4.56	4.70	4.78	4.86	4.66
CYP1A2 inhibitor	Yes	Yes	No	No	No	Yes	No	No	No	No
CYP2C9 inhibitor	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No
CYP3A4 inhibitor	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
GI absorption & BBB permeation	High & yes	High & yes	High& No	High& No	High& No	High& No	High& No	High& No	High& No	High& No
Skin permeation (Log Kp)	-5.93cm/s	-5.90 cm/s	-6.66 cm/s	-7.01cm/s	-6.63 cm/s	-5.99 cm/s	-6.63cm/s	-6.98cm/s	-6.61cm/s	-5.95 cm/s

MW- molecular weight, iLogp- logp value of in-house physics based method, HBD- number of hydrogen bond donors, HBA-number of hydrogen bond acceptors, TPSA- topological polar surface area, MR-molar refractivity, nRB- number of rotatable bonds, nAH- number of Aromatic heavy atoms.

Table 4: Interaction between SLP II 1-2 and SLP VI 1 (a-d)-2(a-d) and the ER-α.

compound	binding (kcal/mol)	Estimated Inhibition constant (Ki)	Interacting amino acids, type of interaction
SLP II1	-7.57	2.71µM	GLY420-vanderwall,LEU384, LEU525, LEU387, ALA350, LEU391, LEU346-Pi-Alkyl,GLU353-ConventionalH-bond
SLP II2	-8.24	909 nM	MET343-Pi-Sulphur,GLU353-H-bond, LEU525, ALA350,LEU391,LEU346,LEU387-Pi-alkyl
SLP VI 1a	-8.69	429 nM	ALA350, MET343- Pi-Alkyl, MET522- Pi-Sulphur, CYS530-Conventional H-bond
SLP VI 1b	-7.19	5.34 µM	VAL533, LEU354, LEU536, LEU525-Pi-Alkyl, LEU539-Pi-Sigma, MET343-Halogen bond, ASP351-Conventional H-bond
SLP VI 1c	-10.22	32.52 nM	MET522-Sulphur-X, TRP383-Pi-Pi T-Shaped, MET-343-Pi-Cation, LEU387, PHE404Vanderval
SLP VI 1d	-9.62	89.15 nM	MET522-Pi-sulphur, LEU536- Pi-Sigma and Pi-Alkyl, LEU387, LEU384, ALA350, ASP351, TRP383, LEU525-

			Pi-alkyl
SLP VI 2a	-10.00	47.08 nM	MET528-Pi-sulphur and vanderwaals, ALA350, LEU346, MET522- Pi-Alkyl, LEU525- H-bond and Pi-alkyl, ALA350, LEU346, MET522- Pi-Alkyl
SLP VI 2b	-9.10	212.91nM	LYS362, ARG363, GLY366, ALA318-H-bond, LYS362, ALA318, PRO365- Pi-Alkyl, ARG363-Pi-cation
SLP VI 2c	-9.14	200 nM	TYR526- Pi-Pi T shaped, MET522-Pi-sulphur, LEU525, TYR526,VAL534-vanderwaal,LEU525, LEU536, VAL533- Pi-alkyl
SLP VI 2d	-10.71	14.07 nM	MET522-Pi-cation, MET421 - Pi-sulphur, GLY420-Cl, MET522-vanderwaal,TRP383, THR347, HIS524, ILE424, PHE404, LEU346, LEU525-Pi-alkyl

Table 5: Interaction between SLP II 1-2 and SLP VI 1 (a-d)-2(a-d) and the EGFR.

compound	Freeenergy of binding(kcal/mol)	EstimatedInhibition constant (Ki)	Interacting amino acids and type of interaction
SLP II 1	-7.40	3.75 µM	ARG705- H-bond and Pi alkyl, ILE1018- H-bond, ASP770-H-bond, Pi-anion
SLP II 2	-7.28	4.6 µM	NET766-Pi-sulphur, ASP855, LYS745- H-bond, LEU718, LEU844, ALA743-Pi-alkyl
SLP VI 1a	-7.53	3.01 µM	ASP984-Pi-anion, TYR813, GLY810, ASN 808- H-bond, ASN808, GLY983-amide-pi staked, Ile809-pi-alkyl,PRO795,ILE809-Vanderwaals
SLP VI 1b	-7.77	2.03 µM	ILE1018,ASN700- H-bond, ASP-770-Pi-anion,gln-701-Amide-pi staked,ARG-776, ALA767,ALA702-Pi-alkyl,VAL769-Vanderwaal
SLP VI 1c	-7.74	2.12 µM	LEU718, GLY796-Pi-Sigma, MET793-H-bond, ASN 855-Pi-anion, LYS728, LEU792, LEU844, VAL726-vanderwaals
SLP VI 1d	-8.49	601.06 nM	LYS757, LEU760- H-bond, ASN761-Pi anion, LYS 757, LEU760, TRY764, ALA 702, ALA767, LEU704, ALA763-Alkyl and Pi-alkyl.
SLP VI 2a	-8.15	1.06 µM	ILE706, LEU704- H-bond, LEU703, ILE 706- Pi-alkyl, LEU704-Pi lonepair, ALA 702-Pi-alkyl,TYR-764-Pi-Pi-staked, ALA 763-Vanderwaal
SLP VI 2b	-8.77	370.08 nM	ASN842, ASP855-vanderwaal, LEU844, LEU718-Pi-sigma, CYS797, LEU792-Pi-alkyl, MET793, PRO794, ASP800-H-bond.
SLP VI 2c	-8.55	536.7 nM	PRO-794, ASP855, ASP837-vanderwaal, LEU 718, LEU844-pi-sigma,ALA743, ARG 841-Pi-alkyl, ASP 855-Pi-anion, CYS797-halogen,MET793- H-bond, GLN791-unfavourable acceptor-acceptor
SLP VI 2d	-8.37	728.32 nM	ARG776, LEU703-Pi-donor hydrogen bond, ALA702-pi-alkyl, ALA702, GLN701, ASN700- H-bond, ARG831-Pi-cation.

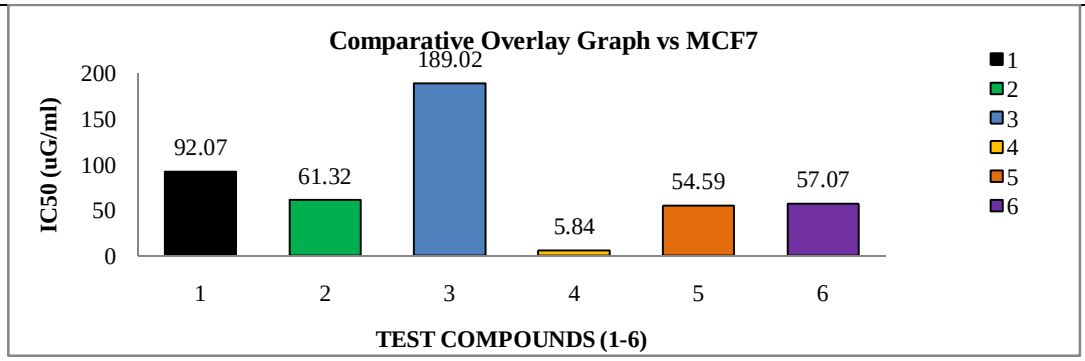


Fig 1: IC_{50} (µg/ml) of the Compounds SLP VI [1(a-c)-2(a, b and d)] against the MCF7 Cells.

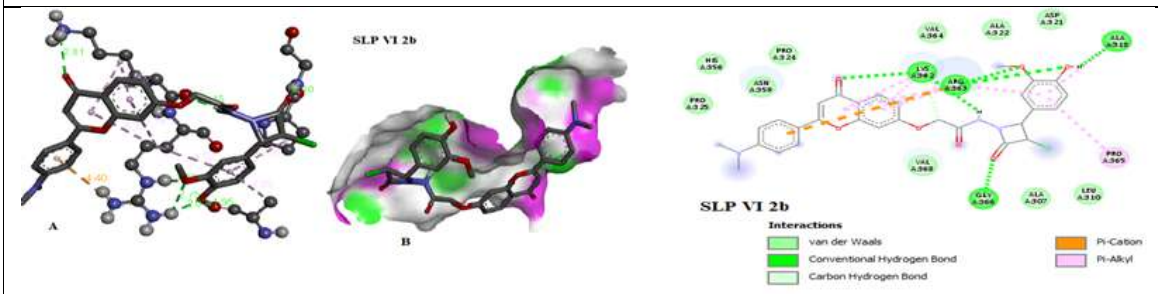


Fig 2: 3D and 2D interactions of SLP VI 2b at active pocket of HER-α [PDB id: 3ERT].

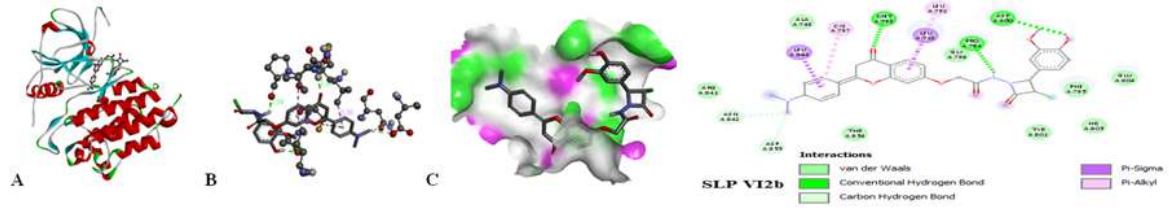


Fig 3: 3Dand 2D interactions of SLP VI 2b at active pocket of EGFR [PDB id: 2J6M].

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Experimental Study of Low Salinity Waterflooding in Secondary Recovery Mode in Geleki Oil Field of Assam-Arakan Basin, India

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Received: 02 Feb 2022 Revised: 16 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

The main objective of this work is to study the Low Salinity Effects (LSE) by conducting laboratory Low Salinity Water flooding (LSW) experiment in Secondary Recovery Mode in Geleki Oil Field of Assam-Arakan Basin, India. Set of core flooding experiments were performed in four numbers of core plugs using different low salinity brine samples where the oil recovery efficiency was 33.82% - 42.05% of Original Oil In Place(OOIP).Also, another two numbers of oil saturated core plugs were flooded by formation brine and high salinity brine where lower oil recovery efficiency was observed(33.12 % - 32.71% of OOIP). During LSW, the pH of the effluent brine was increased (by 0.29 to 0.73) from the injected brine. A reduction of oil-effluent brine IFT was observed (by 01.48 mN/m - 03.56 mN/m) compared to the oil-injection brine IFT after LSW. Also, wettability states of the low salinity brine flooded core plugs were more water-wet (Contact Angle: 35.55^0- 38.25^0) than the core plugs flooded by formation brine and high salinity brine (Contact Angle: 41.85^0 - 41.95^0). The study shows that, the LSEs are acting in the study area during the LSW which results in increased oil recovery efficiency through pH increase of bulk fluid, oil-brine IFT reduction and wettability alteration of the rock to more water-wet state.

Keywords: LSE, LSW, Secondary Recovery Mode, IFT, Wettability.

INTRODUCTION

Low Salinity Water flooding (LSW) is an emerging Enhanced Oil Recovery (EOR) technique which refers to the injection of brine into an oil reservoir with a lower ionic strength as compared to the formation brine [1].In recent years, LSW has received considerable attention as a new EOR method which has great potential to improve the oil recovery due to its low cost and less harmful environmental impact [2-10]. It is found that, LSW can increase the water flood performance from 5% to 38% [11]. Earlier study also shows that, depending on the brine chemistry, composition of crude oil and rock type; this method can enhance the oil recovery by 2% to 42% [12].It is observed that LSW affects the fluid/fluid interaction (IFT) and fluid/solid interaction (wettability) in such a way that it is favourable for higher oil recovery [13].Although, most sandstone reservoirs were originally water-wet by nature, during the migration and accumulation of oil in the reservoir, the wettability of the rock may shift to more oil-wet [14-16]. This makes the reservoir a suitable candidate for LSW. The alteration of wettability is due to the absorption of polar compounds (resin & asphaltene) of oil on the clay surfaces by Cation Exchange, Anion Exchange, Protonation, Ligand Bridging, Cation Bridging, Water Bridging, Hydrogen Bonding and Van der Waals Interaction mechanisms [17]. However, the mechanisms that strongly affects the wettability alteration are Cation Exchange, Ligand Bridging, Cation Bridging and Water Bridging [18].During LSW, the wettability state of the rock is altered from the earlier intermediate-wet or oil-wet to more water-wet condition through different mechanisms which results in higher oil recovery [4,19-21].

The first research works on the positive effects of low salinity brine on oil recovery was observed by Martin and Bernard [22,23]. Several earlier studies have put forward a number of mechanisms such as Fines Migration, pH increase, Multi-component Ion Exchange (MIE) and Expansion of Electrical Double Layer (EDL) as to how low salinity brine injection results in higher oil recovery through wettability modification [5].The effects of brine chemistry on oil recovery through Fine Migration was first observed in a series of laboratory LSW experiment on Berea Sandstone cores [20].In the experiment they observed Kaolinite and Illite clays (fines) in the effluent brine which was because of the EDL expansion. This migration of fines resulted in exposure of new rock surfaces that improved the water wetness of the rock and in turn oil recovery [24]. Earlier literature also shows that the migrated fines can result in enhancement of oil recovery through reduction of oil-water IFT and improvement of Sweep Efficiency [25,26]. It is found that the LSE is linearly proportional to the amount of clay minerals present in the rock [27].

A local increase in pH of the brine phase close to the clay surface was observed in a number of laboratory LSW experiments [4,5,8,28].The reasons behind the pH increase are 'Dissolution of Carbonate'and 'Cation Exchange' between the clay minerals and the invading brine [5].It is observed that, during injection of low salinity brine into a sandstone reservoir, the original Crude Oil/Brine/Rock (COBR) system equilibrium is disturbed and desorption of cations, especially Ca^{2+} occurs from the clay surfaces. As a result, re-stabilization of the COBR system occurs by the absorption of H^{+} on the clay surfaces from the brine resulting in increase of pH [28]. For this mechanism, the reservoir rock should have clay minerals having high Cation Exchange Capacity (CEC) like Montmorillonite, Illite and Kaolinite [28].Earlier studies have shown that, the pH increase of the brine results in enhancement of oil recovery through wettability alteration towards more water-wet state and reduction of oil-brine IFT [2-4,8,28-30].

The expansion of EDL between the clay surface and crude oil plays a crucial role on oil recovery through wettability alteration and improving Sweep Efficiency during LSW [21,31,32]. In a sandstone reservoir, both the silica surface and oil are negatively charged [15]. Therefore, EDL is formed between the negatively charged clay surface and the oil where divalent cations (magnesium & calcium) act as bridging agent [5]. With a sufficiently high salinity level in the bulk fluid, the EDL becomes significantly small enough where the crude oil can react with the clay surface forming an organo-metallic complex [21,33]. As a result, a local oil-wet surface is created [34]. But, during injection of low salinity brine, expansion of EDL takes place which increases the Zeta Potential and as a result the electrostatic

repulsion between the crude oil and clay surface increases [21]. This repulsive force in turn can forces the oil particles out from the clay surfaces making the rock more water-wet.

Multi-component Ion Exchange involves the competition of all the ions of the pore water for the mineral matrix exchange sites [18]. This LSW mechanism was observed earlier both in the laboratory core flooding experiment and field trial where the divalent cation (mainly calcium and magnesium) concentration in the produced brine were found lower than the invading brine [5,27,35,36].The reason behind this is the removal of organo-metallic complex from the clay surfaces and their replacement with some divalent cations due to MIE [19]. This removal of organo-metallic complex results in the wettability variation to more water-wet that improves the recovery of oil. It is observed that the elements required for the MIE mechanism to be successful for wettability modification are clay minerals (preferably kaolinite) in the rock, calcium ion in the reservoir brine and polar compounds in the oil [37].

Thus, alteration of the rock wettability in a direction of higher water-wet state is the key reason behind improvement of oil recovery during LSW. The prerequisites for a successful LSW in a sandstone reservoir are presence of polar compounds in crude oil,clay mineral in the reservoir rock and divalent cations in the formation brine. In Assam-Arakan Basin, the Geleki Oil Field is located towards the southern fringe of Upper Assam Valley. As there is no reported research work or systematic study on Secondary Recovery Mode of LSW in this oil field, this work has been undertaken.

MATERIALS AND METHODS

For the present study, a total of five numbers of Conventional Cores were collected from the Geleki Oil Field from a depth range of 2596-2861 meter. Also, Analytical Data on Formation Brine and 15 litres of Crude Oil were collected from that area. For further laboratory experiment, six numbers of Core Plugs(1.5-inch diameter)were plugged from the Conventional Cores vertically with respect to the whole core axis. The core plugs were then end faced to ensure that the ends of the plugs are normal to their axis and to obtain their required lengths.

Preparation of Brines

The formation and injection brines were prepared in the laboratory by mixing Potassium Chloride (KCl), Sodium Chloride (NaCl), Magnesium Chloride ($MgCl_2.6H_2O$) and Calcium Chloride ($CaCl_2.2H_2O$) with distilled water in such a way that the required salinity and the ion concentration can be obtained. The main purpose of using KCl was to reduce the clay swelling. As per the suggestion of Lager *et al.* [37],the concentration of magnesium and calcium ions were kept lower (4 ppm) in the Low Salinity Brinethen theFormation Brine which contains 6 ppm calcium and 8 ppm magnesium ion. For this study, Formation Brine (1404 ppm as NaCl), one High Salinity Brine (2500 ppm as NaCl) and four numbers of Low Salinity Brine (1100 ppm, 800 ppm, 500 ppm and 200 ppm as NaCl) samples were prepared. The salinity of each prepared brine samples was confirmed by Boyle's Method using Silver Nitrate ($AgNO_3$) and Potassium Chromate (K_2CrO_4).

Analysis of Crude Oil

The objective of this study is to do the quantitative analysis of Polar Organic Compounds (asphaltene and resin) and Acid Number as they play a pivotal role on the Low Salinity Effects. The other parameters determined are Water Cut, API Gravity, Pour Point, Wax Content, Yield Point and Plastic Viscosity.

The quantitative analysis of resin and asphaltene was determined as per IOC-AOD & IP143 standard respectively where N-Heptane, Benzene and Silica Gel were used.When N-Heptane and crude oil were mixed and heated to 50^0-60^0C, it dissolved the resin and the solution of resin + N-Heptane was separated outusing a filter paper. Silica Gel was then added with the solution after collecting it in a beaker which absorbed the resin fraction. The solid containing Silica Gel & resin was then separated from the N-Heptane using filter paper. After adding Benzene with the above solid on a filter paper, the solution of Benzene & resin was filtered out leaving behind the Silica Gel as a

residue. The filtrate was then boiled to about 80^{0}C to obtain theres in fraction as the boiling point of Benzene is 80.1^{0}C.As per the IP143 standard, Benzene was mixed with the solid which was left on the filter paper after the resin + N-Heptane filtrate was separated out. Benzene dissolved the asphaltene and the solution was then heated to about 80^{0}C so that the asphaltene fraction can be obtained by boiling out the Benzene.

The Acid Number of the Crude Oil sample was determined based on the IP standard (IP 001) for Petroleum and its Products (Part-I). Here, 10 gm of the oil sample was taken in a 250 ml conical flask. Also, 1 ml of Phenolphthalein solution was added to 50 ml of alcohol in another flask. Then the mixture was heated to 40^0-50^{0}C and neutralized it with Potassium Hydroxide solution. The neutralized alcohol was then added to the oil sample and heated the mixture for 5 minutes to boil and agitated it by swirling to ensure extraction of the acids by the alcohol. Further 1 ml phenolphthalein solution was added and titrate it immediately with the potassium hydroxide solution. The Acid Number was then calculated using the following equation-

$AN = 56.1NV/W$ ……..1

Where-

AN = Acid Number

N = Normality of the Potassium Hydroxide solution

V = Volume of the Potassium Hydroxide solution (ml)

W = Weight of the oil sample (gram)

In this study, the Wax Content, Water Cut and Pour Point were determined as per the UOP 46-85,IP358 and IP015 standard respectively. The Yield Point and Plastic Viscosity of the Crude Oil were determined using Fan Viscometer whereas the API Gravity was determined using Hydrometer. Here, the Yield Point and Plastic Viscosity were determined at, above and below the Pour Point of the oil.

Analysis of Formation Brine

As mentioned above, the divalent cations and salinity of formation brine plays a major role on oil recovery by LSW. Therefore, data on the ionic composition and salinity of the Formation Brine of Geleki Oil Field was studied in details [38]. The study shows that the brine salinity is 1404 ppm (as NaCl) which contains 8 ppm magnesium and 6 ppm calciumion.

Analysis of Reservoir Rock

The study of the mineralogical composition of the rock is very important as the presence of clays in the rock is a prerequisite for the successful implementation of LSW in a sandstone reservoir. For this study, the rock samples were analysed by Thin Section, X-Ray Diffractometer and Scanning Electron Microscope (SEM). Figure 1, 2 & 3 are showing Thin Section Photomicrographs, X-ray Diffractogram and SEM Photomicrographs respectively of some rock samples of the study area.

Core Plug Preparation

Prior to the measurement of different reservoir properties such as porosity & permeability, the original fluids & salts present inside the Core Plugs were completely removed. For this study, the cleaning of the Core Plugs was done by Distillation Extraction Method where 40% Chloroform, 40% Toluene &20% Methanol solvent mixture were used in the Soxhlet Extractor to extract oil, brine & salt. The extraction was arranged in a manifold so that the brine, oil & salt-laden solvent siphons from each extractor into a common still from where the fresh solvent mixture was continuously distilled, condensed and distributed again to all the extractors. The cleanliness of the plugs was determined using the colour of the solvent mixture that siphoned from the extractor periodically. Extraction was continued until the extract remains clear. For this study, the cleaning time of around 50-60 hours was used in order to fully clean the core plugs. The plugs were further cleaned using Acetone in Ultrasonic Cleaner which utilizes the ultrasonic wave energy to remove the debris. It ensures that the end faces are clean and no loose grains are blocking the pore spaces of the plugs. There after, the liquids present inside the pore spaces of the plugs were completely

removed using Humidity Cabinet.During drying, the Dry Bulb Temperature was set at 63ºC in the Humidity Cabinet whereas the Relative Humidity was set 40% due to the high amount of clay minerals (Smectite, Kaolinite and Illite) in the rock of the study area [39].

Core Flooding

After determining the Porosity and Gas Permeability using 'Coretest TPI-219 Instructional Helium Porosimeter'&'NDP-605 NanoDarcy Permeameter' respectively, the Core Flooding experiments were performed using the 'Ruska Positive Displacement Pump' which works on the principle of Volume Displacement. Here, a plunger of uniform diameter is forced into a liquid-filled cylinder by a measuring screw (spindle). The volume displaced by the movement of the plunger is expressed on a linear scale calibrated in cubic centimetre. The liquid injection rate can be adjusted by adjusting the Levers. It is equipped with motor drives and positive gear transmission so that the contents in the cylinders can be discharged at predetermined rates. The spindle is rotated by a worm and worm gear. A motor rotates the worm shaft through an appropriate transmission. The worm gear can be disengaged from the spindle using the hand wheel drive pin assembly which allows the manual volume adjustments by rotating the hand wheel that is mounted on the worm shaft to make volumetric fine adjustments.

The present core flooding experiments were conducted in Secondary Recovery Mode at an injection rate of 0.031 cc/sec at ambient temperature and pressure. The oil and brine were injected from the top of the core plugs which were oriented vertically in the 'Hassler Core Holder' with a confining pressure of 200 psi. All the plugs were first flooded by 20 Pore Volume (PV) 1404 ppm brine from cylinder-1 followed by 20 PV oil from cylinder-2.The oil saturated core plugs were then kept for an aging time in the core holder under the confining pressure to allow the Crude Oil/Brine/Rock (COBR)interaction. The aging time is affected by core plug lithology, temperature and the composition of crude oil & formation brine [40]. In this study, an aging time of 12-14 days was given to each oil saturated core plugs. Finally, the plugs were flooded by different salinity brines (2500 ppm, 1404 ppm,1100 ppm, 800 ppm, 500 ppm and 200 ppm). From each core flooding experiments; Connate Water Saturation, Remaining Oil Saturation, Displacement Efficiency and Oil Recovery Efficiency (% of OOIP) were determined.

Determination of Interfacial Tension (IFT)

The IFT between the Oil-Injection Brine and Oil-Effluent Brine were determined after the Core Flooding using the 'SVT 20N Spinning Drop Video Tensiometer' which measures the IFT by 'Spinning Drop' method. The technique for measuring the IFT between two immiscible fluids requires measuring the length and width of the oil drop, rotational speed and the density difference between the fluids [41]. In the present study, Smart Spin software used the following equation as thelength of the oil drop was less than 4 times it's width.

$\gamma = [\Delta\rho\omega^2] / 4C$ ……..…..2

Where-

γ = Interfacial Tension (mN/m)

$\Delta\rho$ = Difference in fluid densities (g/ml)

ω= Rotational velocity (rad./sec)

C = Constant

Determination of pH

During each Core Flooding, the Injection Brine samples and corresponding Effluent Brine samples were collected for determining their pH. Before measuring the pH value, the pH electrode of the pH Meter was first dip into distilled water and stirred for 30 second. Then the electrode was ringed with the brine to be calibrated for 30 second. The electrode was then dip into the sample being taken for calibration and the reading was taken when it was stable.

Determination of Contact Angle

As mentioned earlier, the different LSW mechanisms canshift the rock wettability to more water-wet state which was earlier shifted to more oil-wet state by the resin and asphaltene of crude oil [4,5,8,18,20,28]. Therefore, to see the Low

Salinity Effects on the wettability, the Contact Angles of the original reservoir rock and flooded core plugs were determined. For this study, 'DSA100 Drop Shape Analysis System' was used to measure the Contact Angle. The oil drop was placed on the sample located on a movable sample table. After adjusting the position of the sample, the oil drop was illuminated from one side and a camera recorded the drop image from the opposite side which was then transferred to a computer. The software of the system analysed the drop image and measured the contact angles.

RESULTS AND DISCUSSION

The Crude Oil analysis shows that 0.55 % (w/w) asphaltene and 15.49 % (w/w) resin are present in the oil. The Acid Number and Water Cut of the oil are 0.58 and 0.4% (v/v) respectively. The study also shows that the API Gravity, Pour Point and Wax Content of the oil are 29.8, 33^{0}C and 8.23% (w/w) respectively. The Plastic Viscosity and Yield Point were determined in three different temperatures as mentioned above. At the Pour Point temperature, Plastic Viscosity and Yield Point are 13 & 4 respectively whereas at 36^{0}C and 30^{0}C, these parameters are 8 & 2 and 30 & 52 respectively. It is already observed that, presence of resin & asphaltene and high Acid Number (AN>0.2)of crude oil is very important for wettability alterationin a direction of more water-wet state during LSW [37,42-44].Earlier literature also shows that, the resin and asphaltene ratio of greater than three indicates the asphaltene stabilization in crude oil [45].If the ratio is less than three, asphaltene deposition occurs which makes the rock more oil-wet, reduce the permeability and increase the residual oil saturation [46].In this study, theres in and asphaltene ratio is 28.16 which indicated that no asphaltene precipitation occurs in the study area.

As mentioned above,the magnesium and calcium ion concentration in the formation brine is 8 ppm and 6 ppm respectively whereas the brine salinity is 1404 ppm (as NaCl).According to Lager et al. [5], these divalent cations act as bridging agents (Ligand Bridging) between the clay surface and negatively charged oil. This Ligand Bridging makes the reservoir rock oil-wet which in turn makes it a favourable candidate for LSW. Also, as mentioned earlier, calcium and magnesium ion play a key role in 'Multicomponent Ion Exchange' LSW mechanism which alters the rock wettability in a favourable way for improving oil recovery [37,47].

The petrographic analysis (Thin Section, XRD and SEM) revealed thatQuartz, Smectite, Kaolinite, Illite, Plagioclase Feldspar and Mica are present in the rock of Geleki Oil Field (Figure 1, 2 & 3).During LSW in a sand stone reservoir, Kaolinite and Illite tend to detach from the rock surfaces and move with the injection brine [48]. The migration of those clay minerals is observed in the study are a during LSW [49].It is also seen that, swelling of Smectite and Mixed-layer clays occur during the injection of relatively fresher water that causes the fines to break which are attached with them. This causes Swelling-induced Migration where fines are migrated with the flowing fluid [48]. These migrated clay particles can enhance the Sweep Efficiency by diverting the direction of flow of injection brine into un-swept pores when they get trapped in the pore throats [26].Also, Mica may detach from the rock surface and moves along with the injection fluid which can further increase the recovery of oil by improving the Sweep Efficiency [50]. Earlier researchers also shows that these migrated fine particles enhance the oil production by reducing the oil-water Interfacial Tension and altering the rock wettability to more water-wet state as mentioned above [24,25].Further, the presence of Plagioclase Feldspar in the rock matrix strongly indicates that the study area is suitable for implementing LSW [51].

The routine core analysis in the area under study shows that the Effective porosity and Theoretical Liquid Permeability are in the range of 12.56 % -20.93 % and 34.60 md-116.79 md respectively. As mentioned above, Connate Water Saturation, Remaining Oil Saturation, Displacement Efficiency and Oil Recovery Efficiency are determined from the laboratory Low Salinity Water flooding experiment in the Secondary Mode. Additionally, the Core Plug 2 was flooded by brine having the same salinity as the formation brine (1404 ppm) and the Core Plug 1was flooded by the high salinity brine (2500 ppm) to compare their results with the LSW. Although a total of 20 PV brine was injected into each oil saturated core plugs, the oil recovery was observed only upto 6 PV brine injection. The

results of the core flooding experiments are given in Table 1.The Oil Recovery Efficiency against each PV brine injected for all the flooding experiment is represented in the Figure 4.

The core flooding experiments show that the oil recovery efficiency by flooding 2500 ppm and 1404 ppm brine are 33.12% and 32.71 % of OOIP respectively. The recovery efficiencies obtained by flooding core plugs using 1100 ppm, 800 ppm, 500 ppm and 200 ppm water are 33.82 %, 42.05 %, 37.69% and 40.47% respectively (Table 1). Thus, higher oil recovery efficiency is observed during LSW then the high salinity and formation brine flooding. Among all the flooding experiments, 800 ppm brine gives the highest oil recovery (42.05 %).

The study of the brine pH shows that the effluent brine pH is higher than the injection brine for all the LSW experiment (Table 2). The pH increase of bulk fluid during LSW was also observed earlier by different researchers as mentioned above [4,5,8,28]. However, the differences of the pH of injection and effluent brine during 2500 ppm and 1404 ppm core flooding are negligible. This indicates that the pH increase mechanism of LSW is acting in the low salinity core flooding experiment. As mentioned above, this pH increase of the brine is may be due to the cation exchange between Smectite, Illite& Kaolinite and the invaded low salinity brine. The highest increase of pH is observed during 800 ppm brine flooding (0.73).

The IFT analysis between the flooded oil and injection & effluent brine shows that the oil-effluent brine IFT is lower than the oil-injection brine IFT during LSW (Table 3).This reduction of IFT between oil-effluent brine is may be due to the generation of some surfactant because of the pH increase of the bulk fluid [52]. Also, high Acid Number (0.36) of the oil may be the other reason behind the reduction of oil-effluent brine IFT as oil having Acid Number more than 0.2 can generate enough surfactants during LSW [41,42].Further, it is found that the reduction of IFT is highest (3.5623 mN/m)during 800 ppm brine flooding. However, almost no variation of IFT is observed in the oil-high salinity brine & oil-formation brine before and after the core flooding.

The study shows that the Contact Angle of the reservoir rock of the Geleki Oil Field is 43.11^0.This indicates that the area is preferentially water-wet and suitable for implementing LSW [53]. The Contact Angles of the core plugs flooded by 2500 ppm and 1404 ppm brine are 41.95^0 and 41.85^0 respectively which are close to the original value (43.11^0).However, the Contact Angles of the other four core plugs used for LSW are low (Table 4).This Contact Angles reduction during LSW indicates that the wettability is shifted towards more water-wet state. The highest wettability modification is observed during 800 ppm brine flooding (Contact Angle: 35.55^0).

CONCLUSION

The potential for enhancing oil recovery by Low Salinity Water flooding in Secondary Recovery Mode has been investigated in the Geleki Oil Field of Assam-Arakan Basin, India through a series of laboratory Core Flooding experiment. The result shows that the LSW can improve the oil recovery in the study area through oil-brine IFT reduction, pH increase of brine and wettability alteration of rock towards more water-wet state. Out of the different low salinity brines, 800 ppm yields the highest recovery of oil during LSW.

ACKNOWLEDGEMENT

The authors have drawn this privilege to offer gratitude to the Basin Manager, Oil and Natural Gas Corporation Limited(ONGCL), Jorhat, Assam, India and Asset Manager & Surface Manager ONGCL, Nazira, Assam, India for providing conventional cores, crude oil and few unpublished data from the study area for this work. The authors also thankful to the authorities of Oil India Limited(OIL), Duliajan, Assam, India for providing laboratory facilities for crude oil analysis, core flooding and determining pH, IFT& Contact Angle.

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Table 1. Results of the Core Flooding Experiments

Core Plug	Effective Porosity (%)	Theoretical Liquid Permeability (md)	Brine Salinity (ppm as NaCl)	Connate Water Saturation (% of PV)	Remaining Oil Saturation (% of PV)	Displacement Efficiency (%)	Oil Recovery Efficiency (% of OOIP)
1	20.93	116.79	2500	34.36	43.89	33.14	33.12
2	12.56	43.82	1404	28.48	48.13	32.70	32.71
3	18.83	65.44	1100	34.86	43.10	33.83	33.82
4	19.99	55.19	800	24.53	43.74	42.04	42.05
5	17.68	34.60	500	35.32	40.29	37.71	37.69
6	16.24	61.85	200	32.84	39.98	40.47	40.47

Table 2. pH of the Brine before and after Core Flooding

Core Plug	Brine Salinity (ppm as NaCl)	pH before core flooding	pH after core flooding
1	2500	6.49	6.50
2	1404	6.52	6.51
3	1100	6.53	6.82
4	800	6.55	7.28
5	500	6.56	7.13
6	200	6.52	6.85

Table 3. Oil-Brine Interfacial Tension before and after Core Flooding

Core Plug	Brine Salinity (ppm as NaCl)	Oil-Injection Brine IFT (mN/m)	Oil-Effluent Brine IFT (mN/m)
1	2500	12.9839	12.9795
2	1404	12.9392	12.9389
3	1100	12.5296	11.0532
4	800	09.7616	06.1993
5	500	09.5518	06.0278
6	200	07.6606	05.4905

Table 4. Contact Angles of the Flooded Core Plugs

Core Plug	Brine Salinity (ppm as NaCl)	Contact Angle (Left)	Contact Angle (Right)	Contact Angle (Mean)
1	2500	42.1	41.8	41.95
2	1404	41.9^0	41.8^0	41.85^0
3	1100	38.1^0	38.4^0	38.25^0
4	800	35.5^0	35.6^0	35.55^0
5	500	37.6^0	37.5^0	37.55^0
6	200	35.6^0	35.7^0	35.65^0

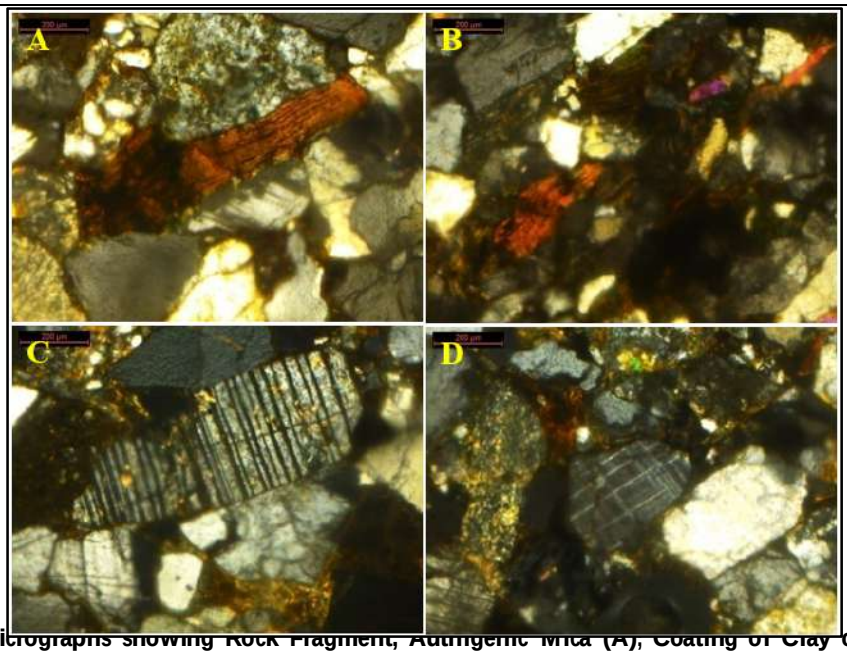

Figure 1: Photomicrographs showing Rock Fragment, Authigenic Mica (A), Coating of Clay over framework grains, Authigenesis of Mica (B); Plagioclase Feldspar, fractures in Quartz invaded by Ferruginous Cement, Argillaceous Matrix (C); Coarse Grained Quartz, Microcline Feldspar, Rock Fragment, Ferruginous Cement, Clay Coating, Argillaceous Matrix (D).

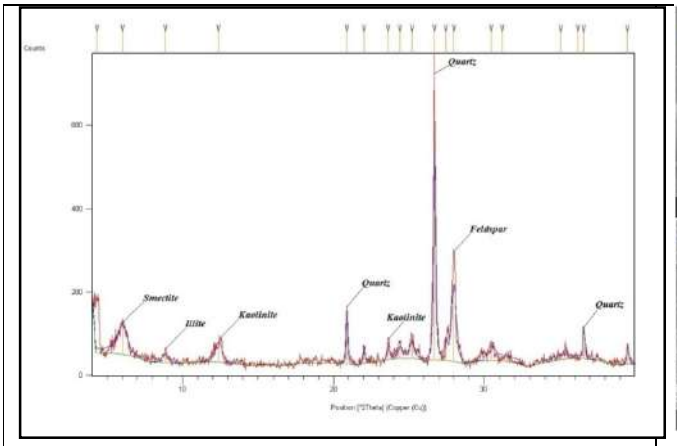

Figure 2: X-ray Diffractogram showing Smectite, Illite, Kaolinite, Quartz and Feldspar

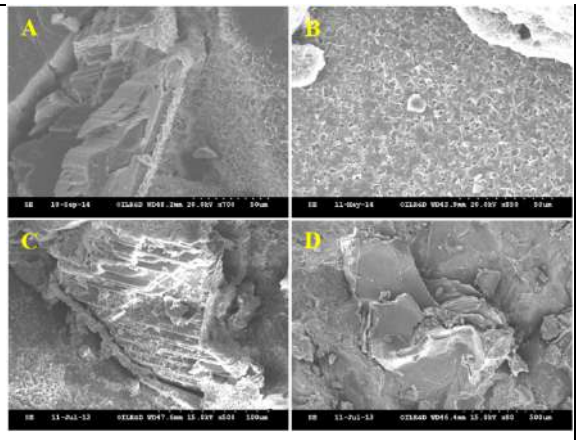

Figure 3: SEM Photomicrographs showing Smectite (A), Illite (B), Plagioclase Feldspar (C) and Mica (D).

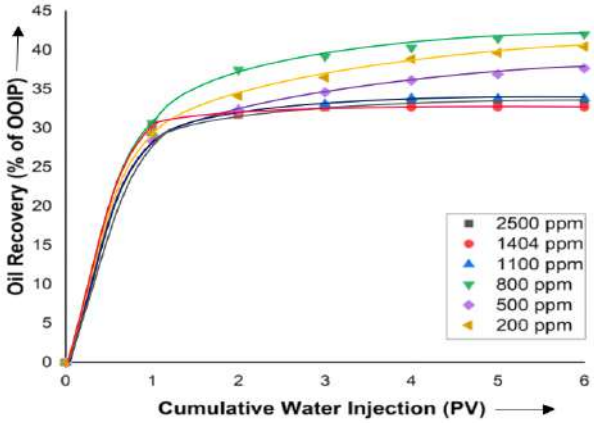

Figure 4: Correlation between Cumulative Water Injection and Oil Recovery

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Bioactive Compounds, Antioxidant Activity and FTIR Analysis of *Blumea eriantha* DC

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Received: 19 Jan 2022 Revised: 13 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

Phytochemicals play essential role in high-quality antioxidant effect, modulation of detoxification of enzymes and stimulation of the immune system in human body. *Blumea* genus includes various species, that are used in preparation of traditional and endemic medicines. Crud extract of leaf applied on cuts, wounds and also used in rheumatic pain.The purpose of this researchis to find bioactive compounds and evaluate the anti-oxidant potential of this weed species. The FT-IR spectrum analysis is used to identify the functional groups present in various plant parts with different solvents.FT-IR spectrum analysis was used to characterize bioactive components found in various extracts (roots, stem, leaves, and flowers) of *B.eriantha.* DPPH, reducing power, FRAP, and total antioxidant capacity were used to analyse the anti-oxidant activity of various extracts.The peaks of leaf and flower are similar to the absorption frequency of quercetin in ethanolic extracts. The results of this study confirmed that leaf of *B.eriantha* have possesses significant amount of phenolic (133.55±1.513 µg GAE/g DW), flavonoid component (82.79±0.223 µg Quercetin/g DW) and also shown noteworthy antioxidant activities with ethanol solvent. The regression analysis appears that phenolic compounds contribute to about 96% (r^2 = 0.9607, P < 0.05) and 92% (r^2 = 0.9224, P < 0.05) of ferric ions reducing and free radical scavenging antioxidant properties respectively. The result of FT-IR spectrum analysis might be helpful for isolation of polyphenolic compounds present in this weed.

Keywords: *Blumea eriantha* DC, weed, wounds, FT-IR spectrum analysis.

INTRODUCTION

Plants produce phytochemicals to protect themselves as well as they can be used to protect humans against infectious diseases in recent studies [1-2]. Phytochemicals can have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes and stimulation of the immune system [3]. *B. eriantha* belongs to Asteraceae family and commonly known as Nimurdi & Kukronda[4]. This plant has been traditionally used as a diuretic and also for the treatment of cholera and diarrhoea [5-6]. Crud extract of leaf applied on cuts, wounds and also used in rheumatic pain [7-8]. Ethanolic extract of plant is revealing good larvicides, repellent activity against *Musca domestica* and also showing significant *in vitro* antimicrobial activity[9-11]. The objectives of this study are screening of bioactive compounds and its antioxidant activities. The FTIR spectrum analysis is used to identify the functional groups present in various plant parts with different solvents.

MATERIAL AND METHODS

Reagents and chemicals

The HiMedia laboratory (India) supplied DPPH, Gallic acid, Quercetin, Folin-Ciocalteu reagent, $FeSO_4.7H_2O$, $FeCl_3 \cdot 6H_2O$ etc. Other reagents were provided by SRL(India).

Plant material collection and extraction

The whole plant sample of *B.eriantha* . was collected from Silvassa, Dadra Nagar Haveli, union territory, India, in Janury,2019. The authentication of plant was carried out by experts of Botany Department, Gujarat University, Ahmedabad. The plant materials (roots, stems, leaves and flowers) of *B.eriantha*. were collected, cleaned with water and air dried at room temperature. These dried plant parts were powdered using mechanical grinder and stored in airtight container till further use. Dried plant materials were extracted with two solvents -aqueous and ethanol using soxhlet extractor [12].

Determination of yield percentage and Phytochemical screening

The yields of evaporated dried extracts were obtained by gravimetric method. The percentage yield of extracts was calculated based on dry weight asper formula [13]

$$\text{Yield } \% = \frac{\text{W1 X 100}}{\text{W2}}$$

Where W1= weight of extract after solvent evaporation;
W2 = Weight of the grinded leaf powder
The dried extracts were subjected to qualitative analysis for various phytochemical constituents as described by Harborne, Adebayo & Ishola methods [14-15].

Infrared Spectroscopy

Identification of different bioactive compounds present in various extract of *B.eriantha*, FT-IR (Fourier transforms infrared) spectrum analysis was performed. FT- IR transmittance spectra were recorded in the Cary 630 FTIR spectrometer with ATR sampling module.

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The total phenolic content was measured by using Folin-Ciocalteu reagent with different extracts of *B.eriantha* , according to previously published method[13]. The total flavonoid content was quantified according to earlier describe protocol [12].

Antioxidant Assay

DPPH free radical scavenging activity

Free radical scavenging activity(RSA) of different extracts of *B.eriantha* was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH), according to the method described byChang [16]. Serial dilution of different concentration of aqueous and ethanolic extracts were taken. The equal amount of sample solution was mixed with an equal amount of 0.1mM ethanol solution of DPPH. The mixture was shaken vigorously and kept in dark for 20-30 minutes. After incubation, the absorbance of the mixture was read against a blank at 517 nm using UV-visible spectrophotometer. The average of each triplicate was taken for determination of percentage inhibition (%RSA) which is calculated as per the following equation: DPPH radical scavenging activity (%) = $A_{control}$- A_{sample} /$A_{control}$ x 100where, $A_{control}$ is the absorbance of the control (containing all reagents except the test compound), A_{sample} is the absorbance of the experimental sample with all reagents. The IC_{50} value was calculated from the plot of inhibition (%) against the concentration of the extract. Here, ascorbic acid was used as standard [17].

Reducing Power Assay

The reducing power assay was performed by the method of Oyaizu (1986) [18] with some modifications. One ml of different extracts (roots, stems, leaves and flowers) of *B.eriantha* with different concentration (100-1000µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated in water bath at 50ºC for 20 minutes. After that, 2.5 ml of trichloro acetic acid (10%)was added and centrifuged at 3000 rpm for 10 minutes. In supernatant solution, 2.5 ml deionized water was added and 0.5 ml freshly prepared ferric chloride solution (0.1%) also added. Then absorbance was measured at 700 nm against blank using UV spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power. This experiment was done three times and here, ascorbic acid used as standard [19].

Ferric ion reducing antioxidant power assay (FRAP assay)

The FRAP assay was performed using standard protocol given by Benzie andStrain[20] with some modification. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCL and 20 mM $FeCl_3 \cdot 6H_2O$ (10:1:1 v/v), warm up at 37 °C in water bath (10 minutes) prior to use. The freshly prepared FRAP reagent was added to different concentration (100-600µl) of standard solution of $FeSO_4.7H_2O$. The reaction mixture was incubated in dark (30 minutes) and the absorbance was measured at 593 nm using a spectrophotometer. This procedure was repeated 3 times for each extract. The result was quantified by the standard curve of $FeSO_4.7H_2O$ having regression formulas Y = 0.0018X+1.2837, R^2 = 0.9954. The result was expressed as mM Fe(II)/g dry weight of plant material.

Phosphomolybdenum Assay

The total antioxidant capacity (TAC) of various plant parts (roots, stems, leaves and flowers) of *B. eriantha* were performed by the green phosphomolybdenum method of Prieto [21] with different concentration (100- 700 µg/ml). Then, 1 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) was mixed in each aliquot which were capped, incubated in boiling water bath at 95°C for 90 minutes and measured the absorbance at 695 nm against a blank. The higher absorbance value is indicated higher antioxidant activity [22].

Statistical analysis

The result is expressed as mean ± standard deviation (n=3). In DPPH assay, observation is analysed using one way analysis of variance (ANOVA). IC_{50} value calculated using linear regression curve. The correlation between TPC, TFC, and antioxidant activity is presented by pearson correlation coefficient. Results are considered statistically

significant when *P*-values < 0.05. Spread sheet soft-ware (Microsoft Excel, version 2107) are used for all statistical analysis.

RESULT

Estimation of total yield percentage and phytochemical screening

The total yield percentage of *B.eriantha* was calculated by using formula. The aqueous extract of different plant parts (roots, stems, leaves and flowers) are contained 12.93 ,12.06, 12.27 and 12.11 % yield respectively. The leaf confines highest yield which is 13.60 % in ethanolic extract when compared with flower (12.39 %), root (7.87 %) and stem (6.69 %) of same extract. The dried extracts of root, stem, leaf, flower is used for phytochemical screening. The result shows that all plant parts having different phytochemicals such as flavonoids, phenolic compounds, proteins and cardiac glycosides are shown in table 1. Steroids is present in ethanolic extract of roots and flowers only.

FT-IR spectroscopy

Identification of different bioactive compounds present in various extracts of *B.eriantha* was performed using FT-IRwhich are shown in tables and figures. The FT-IR spectra of different plant parts compared with the standards gallic acid and quercetin as shown in figure 1 to 2. Gallic acid contained characteristic bands at 3265 cm^{-1}(O-H group), 2653 cm^{-1} (O-H stretching carboxylic acid) and 1699.7 cm^{-1} (C=O stretching conjugated acid). The bands at 1613 cm^{-1}(C=C stretching α-β-unsaturated ketone) and 1230 cm^{-1} (C-O stretching vinyl ether). Quercetin is contained bands at 3265.1 (O-H stretching vibration of phenol) and 1736.9 (C=O stretching δ lactone). The band at 1233.7 (C-OC-O stretching aryl ether) and 1159.2 is assigned to C-CO-C stretching and bending vibration of ketones. The absorption frequencies of aqueous extracts of stem and flower resembled the absorption frequency of quercetin while the absorption frequencies of aqueous extracts of root and leaf are similar to the absorption frequency of gallic acid (Table 2). The peaks of ethanolic extracts of leaf and flower are similar to the absorption frequency of quercetin while the peaks of ethanolic extracts of root and stem resembled the absorption frequency of gallic acid (Table 3).

Determination of TPC and TFC

In the present study, TPC of different plant part extracts varies from 36.14 µg to 130.55 µg of GAE/g of dry extract. The leaf shows highest phenolic content (130.55 µg GAE/g) in ethanolic extract while the stem is disclosed lowest phenolic content (36.14 and 41.24 µg GAE/g) in aqueous and ethanolic extracts respectively. The leaf (67.18 µg GAE/g) and flower (64.58 µg GAE/g) are contained moderate amount of phenolic content in aqueous and ethanolic extracts respectively. The TFC varies widely among both solvents which are ranged from 21.30 µg of Quercetin /g to 82.79 µg of Quercetin /g of dry weight. Stem discloses least flavonoid content which is 21.30 µg Quercetin /g but leaf indicated high amount of flavonoid content in ethanolic extract (82.79 µg Quercetin /g) while leaf (48.28 µg Quercetin /g) and flower (50.78 µg Quercetin /g) are contained moderate amount of flavonoid content in aqueous extract.

DPPH free radical scavenging activity

Free radical scavenging activity (RSA) of aqueous and ethanolic extracts of *Blumea eriantha* DC. are found to be increased asthe concentration of extracts increase. The percentage (%) inhibition of DPPH and IC_{50} values for samples with standard are displayed in table 4 and 5.The root and leaf show 72.81 %,72.61 % free radical scavenging activity with IC_{50} values 120.01 µg/ml and 111.28 µg/ml at the concentration of 200 µg/ml in aqueous extract. The root and leaf show 82.88 %,79.27 % free radical scavenging activity with IC_{50} values 127.42 µg/ml and 211.52 µg/ml at the concentration of 350 µg/ml in ethanolic extract. The percentage DPPH for samples and standard are depicted in figure 3 and 4.

Reducing Power Assay

In this study, it is noticed that as the concentration of extract increased (100 to 1000 µg /ml) there is an increase in absorbance with two different solvents. Ascorbic acid is used as positive control. The maximum absorbance for the aqueous extract is found to be 0.606, 0.530 at 500 µg /ml concentration in root, stem respectively and at same

concentration, the absorbance of standard is 1.024. The highest absorbance is observed to be 1.157 at 1000 µg /ml concentration for ascorbic acid, followed by leaf at same concentration which is 1.069 in ethanolic extract.

FRAP assay

The FRAP values of aqueous and ethanolic extracts are varied with different plant parts of *B. eriantha*. In this assay, $FeSO_4.7H_2O$ used as references standard. In aqueous extract, all plant parts of *B.eriantha* show high ferric ion reducing activity which is ranged from 104.42 to 143.68 µg of mM Fe(II) /g while ethanolic extract of same plant parts show least ferric ion reducing activity which is ranged from 77.46 to 96.68 µg of mM Fe(II) /g at 500 µg/ml concentration except stem (115.21 µg of mM Fe(II) /g).

Phosphomolybdenum Assay

In phosphomolybdenum assay, ascorbic acid used as references standard. In the present investigation, the total antioxidant capacity with various plant parts extract varied from 292.83 µg to 800.66 µg of AAE/g of dry extract.The root shows high total antioxidant capacity (800.66 µg AAE/g) in aqueous extract. The leaf (657.00 µg AAE/g DW) and flower (681.33 µg AAE/g DW) are contained moderate amount of total antioxidant capacity in same extract.

Statistical analysis

The DPPH radical scavenging activity showed a strong negative correlation with TPC (r=–0.907, P < 0.05) in aqueous extract (Table 6). DPPH radical scavenging activity is indicated by a negative correlation since the radical content decreases as activity increases. The FRAP assay is presented a strong negative correlation with TPC (r=–0.904, P < 0.05) in ethanolic extract. The total antioxidant capacity is reported a strong positive correlation with TFC (r= 0.85827) in ethanolic extract. The relationship between total phenolic content and antioxidant using DPPH assay is shown in figure 5. The regression analysis shows that phenolic compounds contribute to about 92% (r^2 = 0.9224, P < 0.05) of radical scavenging properties. The relationship between total phenolic content and antioxidant using FRAP assay is indicated in figure 5. The regression analysis appears that phenolic compounds contribute to about 96% (r^2 = 0.9607, P < 0.05) of ferric ions reducing antioxidant properties.

DISCUSSION

The C-CO-C stretching and bending vibration of ketones were observed at 1163 cm^{-1} confirms the absorption spectra of flavonoid quercetin [23]. The peaks at 3492 cm^{-1},3367 cm^{-1} and 3285 cm^{-1} correspond to different modes of the OH groups. The bands between 2850 to 2950 cm^{-1} assigned to stretching vibration of aliphatic CH, $CH_2$and CH_3 side chain of aromatic rings[24]. The gallic acid also indicated peaks at 1701 cm^{-1}(Carboxylic acid) ,1615 cm^{-1}(C=C stretching) and 1265 cm^{-1}(C=O stretching) [25]. The amount of phenolic content was lowest in aqueous (67.18 µg GAE/g) and ethanolic (130.55 µg GAE/g) extract of leaf than reported by Gore and Desai revealed that the total phenolic content of leaf was 0.935 mg GAE /g and 1.092 mg GAE /g in aqueous and ethanolic extracts respectively. Phenolic components present in plants is having powerful antioxidant activity [26].

The amount of flavonoids are lowest in aqueous (48.28 µg quercetin /g) and ethanolic (82.79 µg quercetin /g) extract of leaf than reported by Gore and Desai revealed that the total flavonoid content of leaf was 2.029 mg RE /g and 5.164 mg RE /g in aqueous and ethanolic extracts respectively. Flavonoid are major class of phenolic compounds present in plants are found to prevent various disease through their antioxidant activity [27]. The leaf tends to show high ferric ion reducing activity in aqueous extract. Oxygen is a highly reactive atom, possibly that is capable of becoming part of damaging molecules commonly referred as “free radicals” which is naturally controlled by various phytoconstituents known as antioxidants [28-29]. The reducing power of *B.eriantha* in aqueous extract with standard is appeared the following order: Ascorbic acid > Root >Stem > Leaf > Flower. The reducing power of *B.eriantha* in ethanolic extract with standard stat the following order: Ascorbic acid > Leaf > Root > Flower > Stem.

The present investigation reveals that leaf of *B.eriantha* extracts shows good amount of phenolic and flavonoid components because of that it shows higher antioxidant properties. The result of FT-IR spectrum analysis will be helpful for isolation and characterization of bio active components to protect humans against infectious diseases. Advanced research encouraged to understand the mechanism of weed's biological activities which might be helpful for isolation of polyphenolic compounds present in it for further investigation.

ACKNOWLEDGMENT

Authors are grateful to Department of Botany, Bioinformatics and Climate Change Management, School of Science, Gujarat university, Ahmedabad for providing the research facilities.

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Table 1: Qualitative Analysis of Different Parts of *Blumea eriantha* DC.

Sr No.	Phytochemical constituents	*Blumea eriantha* DC.							
		Root		Stem		Leaf		Flower	
		AE	EE	AE	EE	AE	EE	AE	EE
1	**Flavonoids**								
a)	Alkaline reagent test	+	+	+	+	+	+	+	+
b)	Lead Acetate test	+	+	-	+	+	+	+	+
2	**Phenolic/Tannin**								
a)	Ferric Chloride test	-	-	-	-	-	-	-	-
b)	Lead Acetate test	-	+	-	+	-	+	-	+
c)	Folin ciocalteu reagent	+	+	+	-	+	+	+	-
3	**Steroids**								
a)	Libermann Burchard's test	-	+	-	-	-	-	-	+
b)	Salkowski test	-	+	-	-	-	-	-	+
4	**Cardiac Glycosides**								
a)	Keller-Killiani test	-	-	-	-	-	+	-	+
b)	Legal's test	+	+	+	+	+	+	+	+
5	**Protein**								
a)	Millon's test	-	-	-	-	-	+	-	+
b)	Biuret test	-	-	-	-	-	-	-	-

AE=Aqueous Extract, EE=Ethanolic Extract,**+ :** Present ,- :Absent

Table 2: The FT-IR Spectra of Aqueous Extract of *Blumea eriantha* Dc

Aqueous (Wave number cm^{-1}).				Possible functional group
Root	Stem	Leaf	Flower	
3257.7	3291.2	3254.0	3261.4	Root & leaf: O-H stretching alcohol (Intermolecular bonded) Stem & flower: O-H stretching vibration of phenol
2929.7	2929.7	2929.7	2929.7	O-H stretching carboxylic acid (Centered on 3000 cm^{-1})
---	---	1625.1	1617.7	C=C stretching α-β-unsaturated ketone
1595.3	1595.3	1595.3	---	C=C stretching cyclic alkene
1401.5	1408.9	1401.5	1401.5	O-H bending carboxylic acid or O-H bending alcohol
---	---	---	1319.5	O-H bending alcohol
1259.8	1259.8	1259.8	1237.5	C-O stretching aryl ether
---	1192.7	1192.7	---	C-CO-C stretching and bending of ketones
1021.3	1021.3	1021.3	1021.3	C-O stretching Vinyl ether
935.6	---	---	---	C=C bending alkane
864.7	868.5	861.0	---	C-H bending 1,2,4-Trisubstituted
816.3	---	---	---	C-H bending 1,3-disubstituted

Table 3: The FT-IR Spectra of Ethanolic Extract of *Blumea eriantha* DC.

Ethanol (Wave number cm^{-1})				Possible functional group
Root	Stem	Leaf	Flower	
3302.4	3287.5	3347.1	3295.0	Root & stem: O-H stretching alcohol (Intermolecular bonded) Leaf & flower: O-H stretching vibration of phenol
---	---	3011.7	---	Aromatic C-H stretching alkene
2922.2	2922.2	2922.2	2918.5	O-H stretching carboxylic acid (Centered on 3000 cm^{-1})
2851.4	---	2851.4	2851.4	C-H stretching alkane
1707.1	---	1740.7	1736.9	Root: C=O stretching cyclohexanone orC=O stretching carboxylic acid dimer Leaf and Flower: C=O stretching δ lactone
---	1625.1	1602.8	---	Stem: C=C stretching conjugated alkene Leaf: C---C aromatic ring stretch
1599.0	---	---	1599.0	C=C stretching cyclic alkene
1513.3	---	---	---	C=O aromatic stretch
---	1408.9	1457.4	---	O-H bending carboxylic acid orO-H bending alcohol Leaf: C-H bending alkane (methyl group)
1375.4	---	1367.9	1371.7	O-H bending Phenol
1237.5	1252.4	1215.1	1233.7	C-O stretching aryl ether Leaf: C-O stretching Vinyl ether
---	---	1162.9	---	C-CO-C stretch and bending of ketone
1025.0	1054.8 1021.3	1028.7	1021.3	C-O stretching Vinyl ether
---	1054.8	---	---	C-O stretching primary alcohol
---	924.4	---	---	C=C bending alkane
864.7	864.7	849.1	861.0	C-H bending 1,2,4-Trisubstituted Leaf: C=C bending alkene trisubstituted
816.3	820	---	---	C-H bending 1,3-disubstituted

---	---	---	719.4	C-H bending 1,3-disubstituted or1,2,3 trisubstituted
---	663.5	663.5	---	C=C bending alkene disubstituted (cis)

Table 4: DPPH Scavenging Activity of Aqueous Extract of Various Plant Parts of *Blumea eriantha* DC

Concentration (µg/ml)	% Scavenging (Aqueous)				
	Ascorbic acid	Root	Stem	Leaf	Flower
25	10.97±1.14	19.83±2.25**	3.96±1.23**	23.21±2.14***	12.89±2.48#
50	23.10±1.38	32.13±1.78**	13.88±2.25**	34.32±2.25**	27.37±2.97*
100	43.09±1.71	47.41±3.27#	37.10±1.50*	48.80±2.14*	39.87±2.72#
150	72.45±1.30	56.34±1.81***	51.78±1.57#	62.29±4.80*	51.98±1.91***
200	88.01±1.86	72.81±0.34***	63.68±4.86**	72.61±2.72**	66.07±3.57***
IC_{50} Value (µg/ml)	110.48	120.01	150.88	111.28	141.12

The average value of triplicate experiments are represented as mean± standard deviation.
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, # - Not significant

Table 5: DPPH Scavenging Activity of Ethanolic Extract of Various Plant Parts of *Blumea eriantha* DC.

Concentration (µg/ml)	% Scavenging (Ethanol)				
	Ascorbic acid	Root	Stem	Leaf	Flower
100	47.39±3.00	42.34±1.13*	28.62±2.04***	18.61±0.1.58***	11.90±1.76#
150	52.40±6.69	54.05±0.60#	42.44±1.05*	32.43±1.30**	24.82±0.96**
200	59.72±5.78	63.46±0.55#	51.25±0.75*	49.14±1.25*	36.33±1.67**
250	73.79±6.18	74.97±0.75#	63.96±0.90*	68.26±0.75#	49.04±1.76**
300	79.18±6.66	80.17±0.62#	73.67±0.75#	72.77±0.62#	60.65±1.20**
350	84.19±6.01	82.88±0.34#	78.87±0.75#	79.27±1.08#	77.77±0.79#
IC_{50} Value (µg/ml)	123.70	127.42	193.31	211.52	250.59

The average value of triplicate experiments are represented as mean± standard deviation.
*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, # - Not significant

Table 6: Pearson's Correlations Between Different Antioxidant Activities and Total Phenolic / Flavonoid Content

Aqueous	TPC	TFC	DPPH	FRAP	TAC
TPC	1.000				
TFC	0.493859	1.000			
DPPH	-0.90726*	-0.08255	1.000		
FRAP	0.882971	0.831756	-0.60819	1.000	
TAC	0.230736	-0.45718	-0.49537	-0.17782	1.000
Ethanol	**TPC**	**TFC**	**DPPH**	**FRAP**	**TAC**
TPC	1.000				
TFC	0.499087	1.000			
DPPH	-0.15305	0.764458	1.000		
FRAP	-0.90459*	-0.80165	-0.22827	1.000	
TAC	0.249977	0.85827	0.69512	-0.63198	1.000

*$P \leq 0.05$

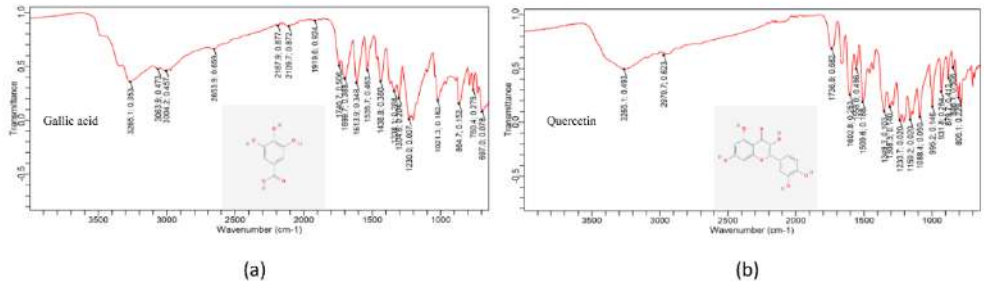

Figure 1: FT-IR spectra of (a) Gallic acid (b) Quercetin

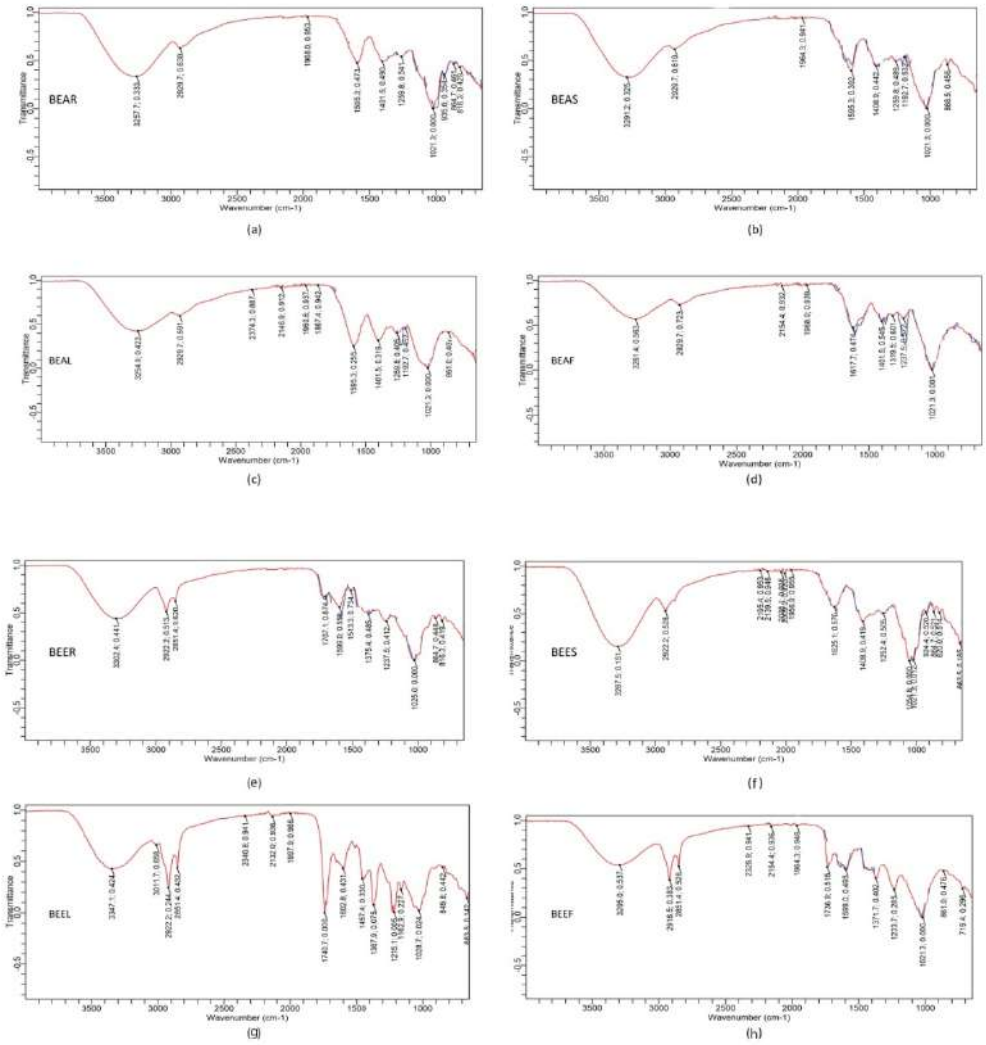

Figure 2: The FT-IR spectra of aqueous and ethanolic extract of *Blumea eriantha* DC.
(a) BEAR- *Blumea eriantha* aqueous root, (b) BEAS-Stem, (c) BEAL-Leaf, (d) BEAF-Flower
(e) BEER- *Blumea eriantha* Ethanol root, (f) BEES-Stem, (g) BEEL-Leaf, (h) BEEF-Flower

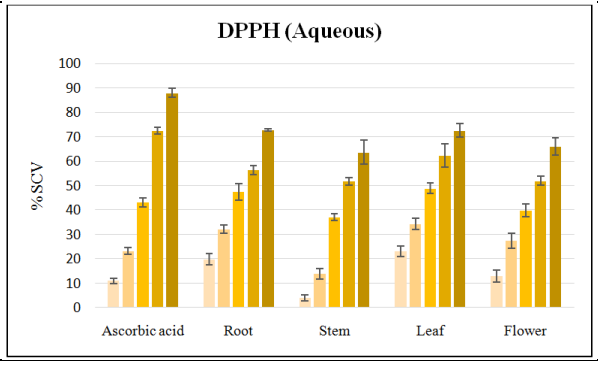

Figure 3: DPPH radical-scavenging activity of aqueous extract of *Blumea eriantha* DC.

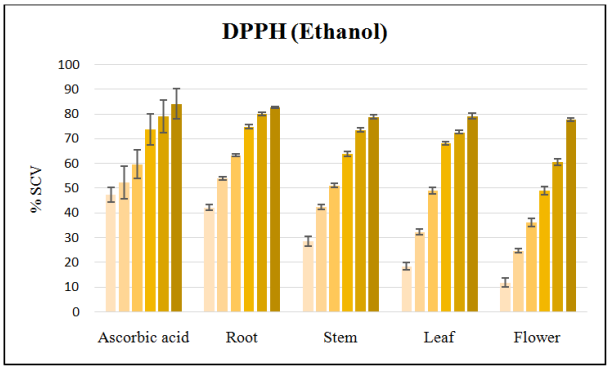

Figure 4: DPPH radical-scavenging activity of ethanolic extract of *Blumea eriantha* DC.

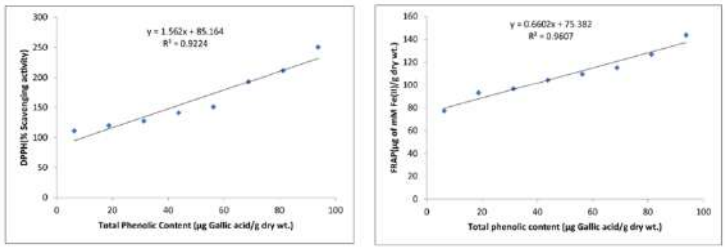

Figure 5: Relationship between TPC and antioxidant activity by (a) DPPH (b) FRAP assay

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Inhibition of CCl_4 induced Liver Fibrosis by *Boerhavia diffusa* Linn.

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Received: 10 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Antifibrotic effect of the ethanolic extract of the shade dried leaves of *Boerhavia diffusa* Linn. was studied in wister rats using the CCl_4 induced liver fibrosis. The extent of liver fibrosis was assessed by measuring the level of liver hydroxyl proline (HP) and by using biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin. Following CCl_4 administration HP was significantly increased and serum levels were elevated. Treatment with the ethanolic extract of *B. diffusa* at 200 and 400 mg/kg, p.o. body weight, reduced the HP content and also serum enzymes, which were comparable to the control in carbon tetrachloride induced liver fibrosis.

Keywords: *Boerhavia diffusa*; ethanolic extract; liver fibrosis; CCl_4 induced

INTRODUCTION

Boerhavia diffusa Linn. (Nyctaginaceae) is a small plant widely distributed throughout India. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions. The root is well known for its diuretic properties. It is also a good expectorant (1). Its anticancer (2, 3), immuno-modulatory (4), antidiabetic (5), antibacterial (6) and antiamoebic (7) activity has also been reported.

Liver fibrosis is the result of repeated repair of chronic liver damage (8). In chronic liver injury there is increasing deposition of collagens elastin and laminin (9). Thus fibrosis is characterized by excessive scarring, excessive production and deposition of collagen due to decreased collagenolytic activity (10). This collagen is stabilized by its

triple helix structure and this structure is given by an amino acid, hydroxyproline. Hence there is a major alteration in extracellular matrix-ECM, which is a major producer of fibrotic neomatrix (11, 12). The altered ECM is degraded by MMPs – Matrix metallic proteinase (13, 14). The MMPs are inhibited by TIMPS – Tissue inhibitors of matrix metallic proteinase. Hence expression of TIMP is increased in human and rat model fibrotic liver (15). In human liver the degree of TIMP expression correlates with the extent of fibrosis assessed by the content of hydroxyproline (16). So estimation of hydroxyproline content in an excellent biochemical marker to assess the degree of fibrosis. Though fibrosis is a common cause of death in human beings design of antifibrotic drug is still at an experimental stage (17). As antifibrotics are meant for chronic administration, they should be free from potential toxic effects. A review of literature afforded no information on the hepatoprotective aspects of this plant. So the present study is therefore an attempt to assess the efficacy of this indigenous herb in liver fibrosis

MATERIALS AND METHODS

Plant material

The leaves of the plant, *Boerhavia diffusa* were collected from the foothill of Yercaud, Salem, in the month of June 2004 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (BDK-1) has been kept in our museum for future reference. The leaves were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of leaves of *Boerhavia diffusa* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water (18). After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Test animals

Wistar rats of either sex and of approximately the same age, weighing about 150-175 g obtained from Perundurai Medical College, Perundurai, were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycles of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. Male mice weighing about 20-25 g each were used for acute toxicity studies. The experimental protocols confirmed with the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} (19) was calculated.

Liver fibrosis induced by CCl_4

CCl_4 was given to the rats orally (1ml/kg) mixed with an equal volume of liquid paraffin, twice a week for 28 days (20). Simultaneously the animals were treated with ethanol extract (200 mg/kg and 400 mg/kg) for the same period. One group of rats was just treated with the extract only (not pretreated with CCl_4) to find out the effect of the extract on biochemical parameters. On day 28th, the animals were sacrificed under ether anaesthesia; blood and liver samples were collected. The biochemical parameters such as ALT (21), AST (22), ALP (23), total bilirubin (24), were estimated as reported earlier and the HP content of the liver was determined separately (25). A small portion of liver was cut from each group and preserved in neutral buffered formalin and was processed for paraffin embedding, following the standard microtechnique (26). 5 μ sections of the livers stained with alum haemotoxylin and eosin and studied for degenerative and necrotic changes.

Statistical analysis

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple range test and differences below $p<0.05$ were considered significant.

RESULTS

The plant *B. diffusa* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of ethanolic extract of *B. diffusa* was found to be 3.6 % w/w and the LD_{50} was 1980 mg/kg. The ethanolic extract did not exhibit and toxic effects up to 400 mg/kg when administered to mice as a single i.p. dose. The results of biochemical parameters revealed to the elevation of enzyme level in CCl_4 treated group indicating that CCl_4 induces damage to the liver. Liver tissue rich in both transaminase increased in patients with acute and hepatic diseases. AST, which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease (27). A significant reduction was observed in AST, ALT, ALP, total bilirubin and HP levels in the animals treated with ethanolic extract of *B. diffusa* (Table 1). The enzyme levels were almost restored to the normal. So the animals treated with ethanolic extract of *B. diffusa* exhibited statistically significant ($p<0.05$) protection against CCl_4 induced hepatotoxicity in rats. This is further substantiated by the increase in liver weight which might be due to the deposition of collagen. The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl_4 manifested itself by the 28th d with the liver showing massive degeneration enveloping the not so visible necrotic areas as compared to the normal. The liver sections of rats treated with the ethanolic extract were similar to liver sections of normal and showed micro vesicular changes with mild congestion and widening of the sinusoids. There was no evidence of necrosis.

A considerable reduction in body weight was noted in the CCl_4 treated rats. Simultaneous treatment with the extract at two different doses (200 and 400 mg/kg) restored the body weight. The weight of the liver in the CCl_4 treated rats was increased which was reduced by the extract treatment at both doses (Table 2).

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical (28, 29). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl_4 (30). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin and HP content.

Estimation of serum transaminase levels gives a fairly good idea about the functional study of liver. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology, which has been disturbed by a hepatotoxin. The extracts decreased CCl_4 induced elevated levels of the enzymes in groups IV and V, indicates the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts.

Histopathological examination of the liver section of the rats treated with toxican showed intense centrilobular necrosis and vascuolisation. The rats treated with extracts alone with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vascuoles. Decrease in serum bilirubin after treatment with extract in liver damage indicated the effectiveness of the extracts in normal functional status of the liver. So, the result of present investigation indicates that the ethanolic extract of *B. diffusa* possess good hepatoprotective activity. Further investigation are required o characterize the active hepatoprotective principle and its mechanism of action.

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Table 1. Effect of ethanolic extract of *B. diffusa* on biochemical parameters

Treatment	Dose mg/kg, p.o.	AST U/L	ALT U/L	ALP U/L	Total bilirubin mg/dl	HP µg/g of liver
Normal	1 ml	112.17±4.49	40.17±1.88	174.33±6.53	0.47±0.003	48.17±2.11
Ethanolic extract of *B. diffusa* alone	200	117.83±3.51	40.83±2.20	172.17±5.47	0.49±0.005	44.33±1.31
Control (CCl_4)	1.25 ml/kg	185.50±4.98	110.67±3.27	268.67±5.22	0.98±0.02	136.0±2.78
Ethanolic extract of *B. diffusa*	200	130.33±5.72*	51.50±2.37*	196.83±8.49*	0.78±0.002*	92.50±2.86*
Ethanolic extract of *B. diffusa*	400	121.67±4.90*	45.33±1.79*	182.50±8.41*	0.67±0.003*	70.83±1.55*

Values are expressed as mean±SEM, $n = 6$, *$P<0.05$ when compared with control.

Table 2. Effect of ethanolic extract of *B. diffusa* on body and liver weight

Treatment	Dose mg/kg, p.o.	Body Weight		Liver weight on day 28
		Day 1	Day 28	
Normal	1 ml	162.67±4.41	175.17±1.19	3.83±0.02
Ethanolic extract of *B. diffusa* alone	200	165.33±4.90	172.50±1.22	3.78±0.01
Control (CCl_4)	1.25 ml/kg	168.50±4.98	144.67±1.63	5.93±0.06
Ethanolic extract of *B. diffusa*	200	156.0±2.78	154.33±5.72*	4.33±0.02*
Ethanolic extract of *B. diffusa*	400	172.50±2.86	166.50±3.51*	3.51±0.08*

Values are expressed as mean±SEM, $n = 6$, *$P<0.05$ when compared with control.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In silico Docking Approach of *Vitex negundo* (Nochi Plant) Leaves Chemical Constituents against Covid-19 Main Protease and 6LU7

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Received: 18 Feb 2022 Revised: 03 Mar 2022 Accepted: 19 Mar 2022

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ABSTRACT

In recent Covid-19 pandemic period, Homeopathy, Unani, Ayurveda, and Siddha like natural medicinal fields played a major role. Out of various plants, the rural people are using *Vitex negundo* (Nochi) medicinal plant leaves for steam trapping process to control the virus growth in respiration system. In India, *Vitex negundo* plant parts have been used for various health issues such as plethora of ailments, swelling, headache to migraine, and asthmatic pains. Hence, this work investigated the reported leaves chemical constituents of *Vitex negundo* theoretical in-silico outcomes using docking tools such as Covid-19 online server and offline iGemdock softwares for the future experimental biological activities. The online docking outcomes revealed that the Viridiflorol, Aromadendrene, ß –Caryophyllene, Globulolhave exhibited between -5.50 and -5.90 kcal/mol against Covid 19 main protease. Similarly, offline docking score exhibited between -50.33 and -26.09kcal/mol against Covid-19 protein 6LU7. These results showed

the good efficiency against the Covid 19 main protein when compared with Remdesivir. Remdesivir exposed the docking score against Covid 19 main protein is -7.00kcal/mol (online) and -16.64kcal/mol(offline) which is almost equal to the Aromadendrene, Viridiflorol and Globulol results.

Keywords: *Vitex negundo,* docking, 6LU7, Viridiflorol, Remdesivir

INTRODUCTION

Recent pandemic situation has changed the life of the people towards the natural medicines in India like developing countries and China like developed countries. Plants and their extracts are playing vital role in disease control and immunity enhancement [1].Apart from the various countries, Indian Ayurveda and siddha medicines have received more interest in Covid-19 period. Still, the extracts and leaveshot vapour steam of the plants are playing good role in sickness control. In addition, various chemical constituents of the plants are the important molecules in allopathic medicine development [2, 3]. Variety of plants and their products are curing various microbial based diseases like Whooping cough, Tuberculosis, Malaria, Chickenpox and Cold[4, 5]. Some of the plants like *Vitex negundo, Justicia adhatoda*, and *Azadirachta indica* are playing a vital role in Covid-19 pandemic as well as all time health issues [6-9].Particularly, *Vitex negundo* Linn (Figure.1) has countless medicinal values, which is used in natural traditional systems [10]. It contains major chemical constituents such as Viridiflorol, ß –Caryophyllene, Sabinene, Terpinen-4-ol, Gamma-terpinene, Oct-1-en-3-ol, Globulol, Linalool, α –Elemol, α –Farnesene, Aromadendrene and various terpenes which have exposed the good medicinal properties [11-13]. Recent research reports have reported the in-silico docking studies on the chemical compositions of *Vitex negundo* leaves extract against WNT-signalling proteins exposed the docking score between -6.7 kcal/mol and -8.7 kcal/mol which was compared with Silibinin drug [14-17]. Similarly, docking investigation has reported against enzyme EGFR tyrosine kinase and the compound exhibited good binding result. From the reports, this work observed the efficiency of the chemical constituents. Hence, this work selected above mentioned chemicals of *Vitex negundo* for the theoretical investigations against Covid-19 main protein and 6lu7 [18-20]. The outcomes were compared with temporary commercial drug Remdesivir.

MATERIALS AND METHODS

Chemical constituents of *Vitex negundo*

This research selected the chemical constituents from the report and chemicals were carried for the docking study after converted in to mol2 and pdbfiles using OPENBABEL. The chemicals such as Viridiflorol, ß –Caryophyllene, Sabinene, Terpinen-4-ol, Gamma-terpinene, Oct-1-en-3-ol, Globulol, Linalool, α –Elemol, α –Farnesene and Aromadendrene (Figure.2) were selected for the theoretical investigation against Covid-19 main protease.

Covid-19 main protease

Binding ability of the compounds are examined using both online and offline softwares. Online server (https://ncov.schanglab.org.cn/index.php) was used for Covid-19 main protease docking. Then, the compounds efficiency was cross checked by offline iGEMDOCK software by submitting the PDB formats of the target protein and ligand. The outcomes were recorded. Both proteins have presented in Figure.3.

Docking procedure

Initially, the compounds have drawn using chemdraw ultra and were converted to mol2, PDB files respectively. The converted mol2 files of the compounds were submitted for the docking after the selection of Covid 19 main protease in online server (Figure.4). The obtained job IDs were submitted after 30 min and recorded the values. Similarly, the converted PDB files of the molecules were submitted in iGEMDOCK and docked. The outcomes were recorded at active sites.

RESULTS AND DISCUSSION

From the report on *Vitex negundo* leaves chemical constituents, this work selected some chemicals which may be active while taking face steam. This process is useful in lungs infection during covid-19 viral disease. So, this work investigated the efficacy of the compounds against the Covid-19 main and 6LU7 proteins. The outcome results of both online and offline have shown in Table.1. The online results are showing moderate to good binding ability against the protein. When compare the results of the selected 11 compounds, four compounds have exhibited the docking score between 5-6 kcal/mol. The resultant docked poses are presented in Figure.5. Likewise to compare the docking results, the compounds were docked against the 6LU7 protein using iGEMDOCK software. The docked outcomes of a, b and c are presented in Figure.6. The docking poses and results were analyzed and compared with the temporary anti-covid drug Remdesivir. Also, both online and offline docking were conducted for Remdesivir against the same proteins (Figure.7). The graphical comparison of the simulation results are in Figure.8.

All the compounds have exhibited the negative docking score which showed the moderate to good binding ability against the covid 19 protein. Aromadendrene exhibited highest docking score or energy (-5.90 kcal/mol) in online result. When compared the offline result, Viridiflorol, Terpinen-4-ol, Globulol, Linalool and Aromadendrene have exhibited the docking score between -50.33 and -24.13 kcal/mol. Viridiflorol has the highest binding score. But, Remdesivir docking score is lower when comparing to the plant chemicals. When comparing Remdesivir, Aromadendrene, and Viridiflorol results, this research observed the minimum difference of 1.1 kcal/mol and these compounds are existing good drug binding character. All the chemicals are almost good in nature. From the outcomes, this work proposed the hypothetical multiple site binding mechanism of the steam with *Vitex negundo* leaves. When taking face-steam bath with leaves, the chemicals are entered in to the respiratory system and bind with different sites of the external microbes which become a confused complex state. That time, the cumulative docking score of the chemicals become -52.9kcal/mol (online) and -272.07kcal/mol (offline) respectively. This may be the reason for the effective control of the natural plant products in inhibition.

CONCLUSION

This research work successfully completed the docking against the Covid-19 proteins and we observed the compounds binding tendency with the target proteins when compare with the Remdesivir. This may be the reason for the face steaming with the help of *Vitex negundo* leaves. The compounds have the good binding and inhibitor against the Covid-19 target proteins. Finally, this work confirmed that the compounds are all good inhibitor which may minimize the Covid-19 infection in respiratory system at initial stage.

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Table 1: Docking Results of the major chemical constituents of the *Vitex negundo* leaves

Comp.ID	Compound Name	Cov-19 Server Job ID	Docking Score (kcal/mol)	Rf Value (pKd)	iGemdock Result (6LU7) (kcal/mol)
a	Viridiflorol	202202152109265309	-5.50	4.40	-50.33
b	ß–Caryophyllene	202202152110442774	-5.70	5.42	-19.65
c	Sabinene	202202152130092705	-4.00	3.72	-20.30
d	Terpinen-4-ol	202202152134079503	-4.90	4.04	-34.10
e	Gamma-terpinene	202202152205517346	-3.90	3.81	-7.99
f	Oct-1-en-3-ol	202202152242326149	-3.90	4.20	-16.55
g	Globulol	202202152248414754	-5.50	4.16	-44.07
h	Linalool	202202152256576328	-4.30	4.44	-26.09
i	α–Elemol	202202152305081882	-4.90	4.55	-15.89
j	α–Farnesene	202202152333396918	-4.40	5.56	-12.97
k	Aromadendrene	202202152344383985	-5.90	5.33	-24.13
Std	Remdesivir	202202161704024504	-7.00	6.29	-16.64

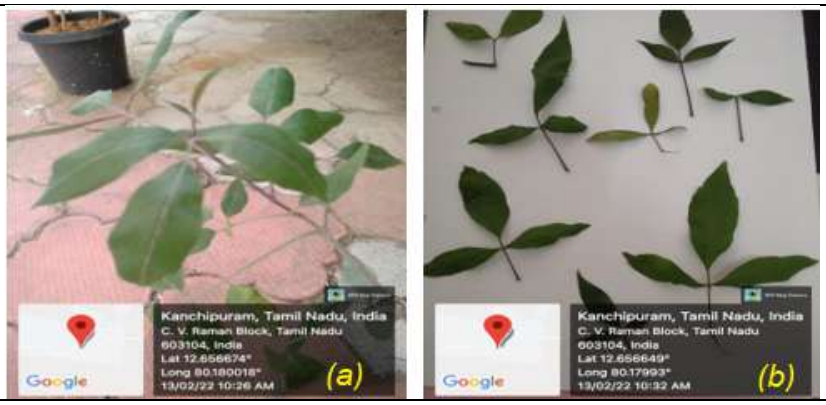

Figure 1. *Vitex negundo a) plant and b)* leaves

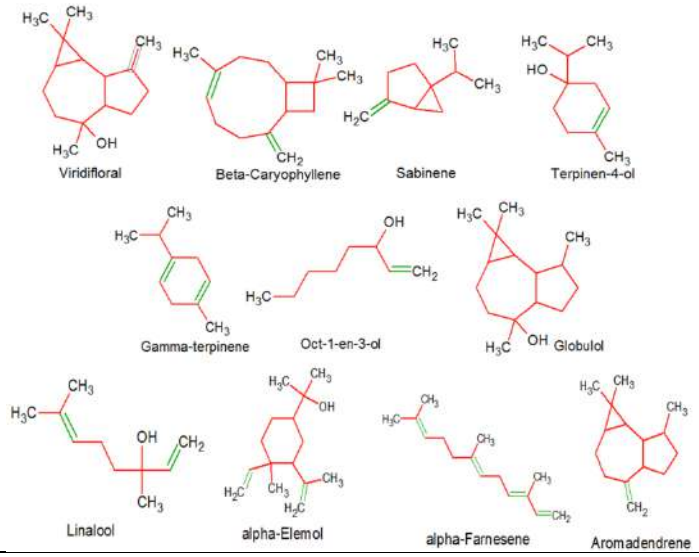

Figure 2. Some of the reported chemical constituents of *Vitex negundo* leaves

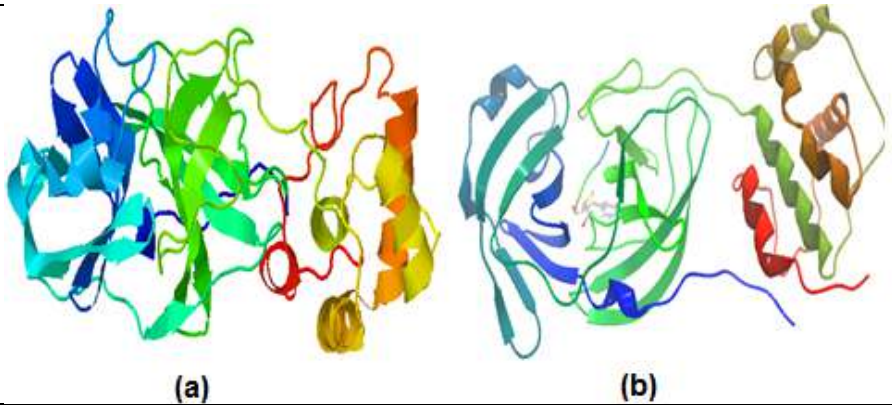

Figure 3. Covid-19 main protease(online) and 6LU7 (offline) proteins

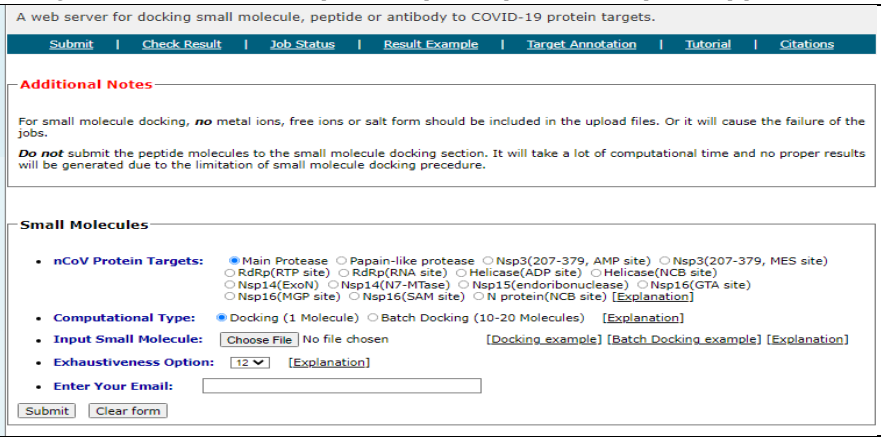

Figure 4. Covid-19 online docking server

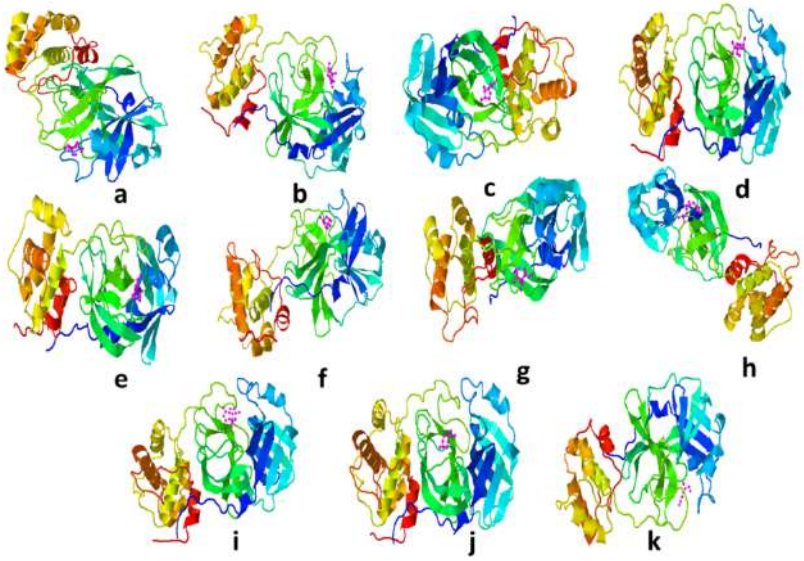

Figure 5. Online docking results of *Vitex negundo* chemical constituents(a-k) against Covid 19 main protease

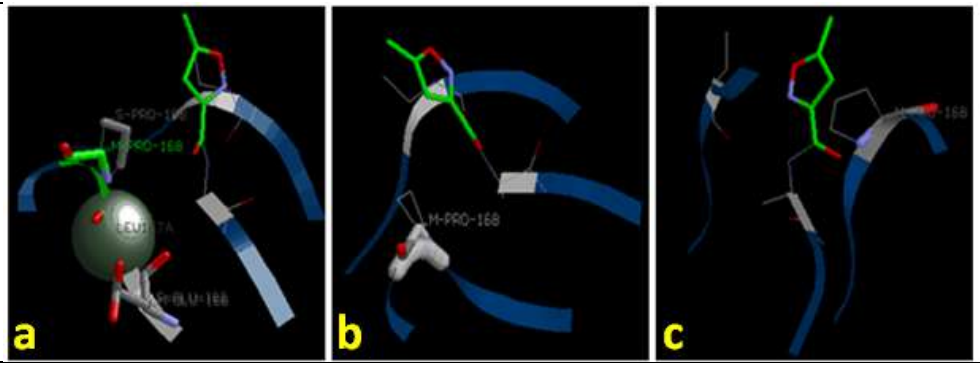

Figure 6. Offline iGemdock docking result of *Vitex negundo chemical constituents* a, b, c against Covid 19-6LU7 protein

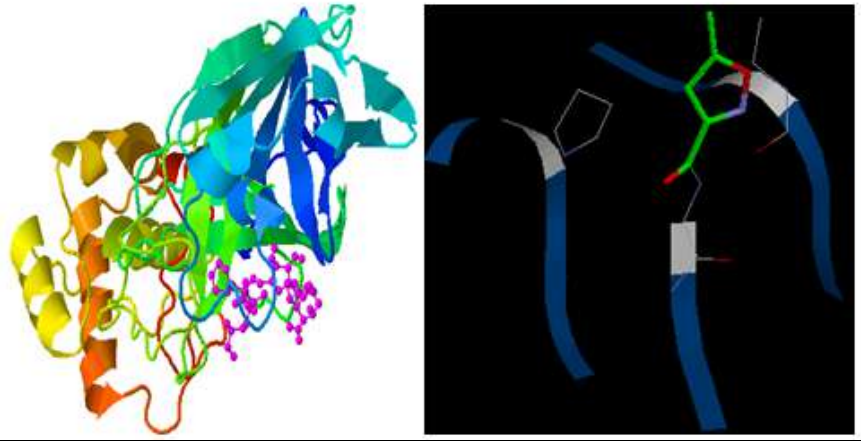

Figure 7. Both Online and Offline iGemdockdocking result of Remdesivir against Covid 19 main protease and 6LU7 protein

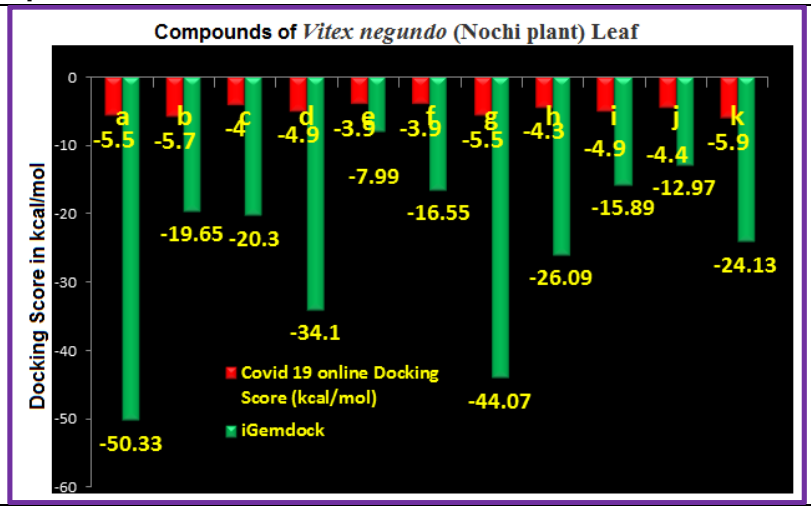

Figure 8. Graphical representation of docking results

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Smart Footwear for Patients with Diabetic Neuropathy

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Received: 24 Jan 2022 Revised: 20 Feb 2022 Accepted: 09 Mar 2022

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ABSTRACT

Diabetic Foot Ulceration (DFU) is the most common complication of Diabetic Peripheral Neuropathy (DPN), affecting 15% of diabetics. Plantar pressure modelling was chosen as a method for classifying a person with and without DPN and in this work an insole prototype was proposed. The smart footwear has a cost-effective foot pressure and blood flow monitoring system, embedded within smart footwear. The communication of the hand-held unit with the footwear unit is wireless and collects real time data for analysis by a doctor. Once the system detects the abnormal foot pressure distribution or foot motion, it enhances the blood flow on that zone of foot using a set of miniature vibratory motors. It stimulates the blood flow and controlled motion can be given using the hand-held unit. The technology uses actuators to regulate the stiffness of the shoe's sole, reducing the onset of diabetic foot ulcers and preventing them from worsening.

Keywords: Diabetes, Plantar pressure, vibratory motors, Blood flow stimulation

INTRODUCTION

India is the world's second-largest country in terms of the number of people having diabetics.Globally one in five people aged 65-99 years live with diabetes. Diabetes is one of the important reasons for illness and premature death worldwide. Prolonged demerits of diabetes may be micro vascular alteration as well as neuropathy. Neuropathy changes have a very high gradient in patients with diabetes. Most occurring conditions are 30-50% of diabetic patients commonly associated with diabetic neuropathy. Diabetic neuropathy is a type of nerve injury that develops in diabetic patients. Diabetes causes neurovascular complications; due to this it can increase pressure in foot and hand. Diabetic neuropathy causes nerve damage which can severely lead to amputations and ulcerations. More research led to the development of better and precise methodology from traditional methods. Developed methods show more precise results that benefit mankind in detecting diabetic neuropathy through foot. The present method used different factors as a threshold like temperatures, plantar pressure and hydration level. Monitoring the plantar pressure shows a predominantly good result in controlling diabetic neuropathy. Peak plantar pressure is obtained from the sole of the foot at different possible positions.

Plantar pressure monitoring method is chosen which aims to devise and construct a foot pressure monitoring system and blood flow activation system, inserted in the smart footwear. Once the sensor detects the abnormal user's plantar pressure distribution it enhances the blood flow on the zone of foot affected, as the smart footwear incorporates a set of miniature vibratory motors. As a remedy by sending imperceptible vibrations through the feet of diabetic neuropathy patients, significant improvement in the damaged nerves and stimulation of blood flow is done. The flex force sensors implanted in the shoes measure the foot pressure distribution in this system. The patients can wear these sensing shoes to check their foot pressure. Once the system detects abnormal foot pressure, it sends an alert to the app and stimulates the blood flow with the help of inbuilt vibrating motors.

LITERATURE SURVEY

The automatic recognition of various postures and activities like sitting, standing, walking/jogging, ascending stairs, descending stairs, and cycling using a wearable shoe-based device was developed in [1]. Monitoring posture assignments and activities allows for more precise energy expenditure calculation, which could help with obesity prevention and treatment. In the preliminary stage, accurate devices rely on multiple sensors distributed throughout the body and which may be too intrusive for regular use. This research describes a unique wearable sensor that can recognize common postures and activities with high accuracy. Despite the fact that just minimal pre-processing is required, the patterns of heel acceleration and plantar pressure uniquely identify postures and common activities, and no feature extraction is necessary. The shoe sensor was put to the test in nine adults who walked, ran, climbed and descended stairs, and cycled while sitting and standing. SVMs (Support Vector Machines) were employed to classify the data. The average accuracy of posture/activity categorization was 95.2 percent on a full sensor set and over 98 percent on an optimized sensor set after fourfold validation of a six-class subject-independent group model. The use of a combination of acceleration and pressure also allowed for a large reduction in sampling frequency without sacrificing accuracy (98 percent versus 93 percent). Subjects' shoe sizes (US) ranged from M9.5-11 to W7-9, and their BMI ranged from 18.1 to 39.4 kg/m^2, implying that the device can be used by people of various anthropometric features.

Benoit Mariani *et al.* in [2], developed a new system with in-sole wearable sensors and to evaluate primary motor symptoms through 9 Timed Up and Go (TUG) test and gait tests. If a person has foot ulcers, he or she must take care of them to avoid loss of sensation in the foot over time, which is a known neuropathy scenario that eventually leads to amputation. Diabetic shoe is a cure recommended by doctors to avoid foot ulcers. These shoes reduce the peak plantar force exerted on the foot, but temperature, humidity, and abnormal pressure are not controlled, which can be effective in preventing wound infection. The suggested concept "Dia shoe" in [3], is a revolutionary mobile base plug-and-play device that can be linked to any type of diabetic shoe to control foot ulcers and prevent them

from rotting, while also monitoring the patient's temperature, humidity, weight, and step count via a mobile application.

Jin Wang devised, constructed, and deployed a solution for fall detection system based on on-body smart sensors that effectively identified unintentional falls in a customer home application in [4], Ahuman's waist is fitted with a wearable gadget and the acceleration analysis allows the technology to detect older people falling. The system will then determine the location of the elderly person and send a quick alarm message to caretakers. As a result, elderly people who have fallen can receive prompt assistance, reducing the detrimental impact. Some forms of therapy are described in [5], that can produce minor improvements. Although therapy can provide minor gains, it is still a pricey solution, and patients remain a rehabilitation concern. As an alternative to this therapy, the wearable, wireless, portable health monitoring system has recently been introduced as a low-cost solution for supervision of foot health condition monitoring. Due to the possibility of quick and effective healthcare treatments, online telemedicine systems are beneficial. Advanced wireless and wearable sensor technologies are used in these systems. The scope of remote health monitoring systems has been greatly expanded thanks to rapid technological advancements.

In [6], a method for designing and building a low-cost foot pressure, foot movement analysis, blood flow stimulation, and heart rate monitoring system embedded within smart footwear was presented. The patient can wear the sensing shoes to monitor his or her foot pressure distribution and heart rate. The novel system is based on pressure sensors that are highly linear and have no hysteresis. In [7], a non-invasive health parameters acquisition is done to facilitate self-monitoring by the users. The suggestion in [8], is that when compared to the baseline evaluation, a minimum 25% reduction in mean peak pressure at the ROI was obtained (criterion A) or mean peak pressure was dropped below an absolute level of 200 kPa, the footwear was classed as successfully optimized (criterion B). Both criteria were chosen to imply a considerable reduction in plantar pressure that was likely clinically useful [9].

METHODOLOGY

The Diabetes - related foot numbness can be treated in a variety of ways. It deals with dynamic distribution of the pressure [10]. Diabetes affects several body areas when you have it for a long time. It affects the nervous system in the majority of instances [11]. The proportion of nerves that are affected is as high as 42% as per the study done in [12]. The flowchart depicts the main steps in the process in Figure 1, and the block diagram is shown in Figure 2.

Segmentation

Distribution of pressure and the value of plantar surface differ on body weight, Body Mass Index (BMI) etc. To make this common subdivision is made: static plantar pressure, dynamic plantar pressure. Pressure distribution also varies in mid and rare feet so in static condition midfoot with (100kpa) below and rare foot with (400kpa) below and BMI<35 is considered as abnormal pressure distribution. In the case of dynamic mid foot with (200 kPa) below and rarefoot with (879 kPa) below and BMI <35 is considered as abnormal [13].

Data Reception

Once the plantar pressure is sensed and segmented all these data are sent to the microcontrollerto make the correct decision. The communication unit of the system used in previous systems was the Bluetooth module which is a most sophisticated communication system for short distances. These entire sensor values are updated in the mobile application for the future reference using the ESP8266 which delivers a high Wireless Fidelity (WIFI) solution to meet the product.

Signalling

Once the system detects abnormal threshold conditions in the plantar pressure measured using a flexi force sensor it sends a signal for the operation of the vibrating motor. A vibrating motor is essentially an unbalanced motor that wobbles and vibrates as a result of the high- speed displacement. The position placing the vibration motor is according to F-scan system by tekscan (the gold standard for pressure mapping system) [14]. Plantar pressure refers to the pressure applied to specific plantar sites on the foot. There are two choices in this area. One is to choose the sensor itself, and the other is to choose the sensor's location. There are several options when it comes to sensor selection, and we chose flexi force sensors. This flexiforce sensor takes the plantar pressure and passes the collected data to the ATmega232-LCD(Liquid Crystal Display). Microcontroller process all the information gathered from the sensor and provides to important signal, one to the IOT module and second to the driver circuit to operate. Once the vibrating motor receives the signal from microcontroller, it starts to vibrate. Vibration can stimulate the blood flow. It continuously monitors foot plantar pressure of the diabetic patients. ATmega232 is used to communicate serially with other devices such as PCs, GPS modules, serial GSM. There are 32*8 general-purpose registers on it. The programme memory consists of a 512-byte EEPROM and a one-kilobyte internal SRAM. The operating voltage ranges from 0 to 5 volts. The speed grade is zero. The plantar pressure selected points, the threshold and the placement of vibrating sensors are shown in Figure 3.

RESULTS AND DISCUSSION

The foot neuropathy analyzer measures the pressure values in the foot and computes them. Once the plantar pressure is sensed and segmented all these data are sent to the microcontroller to make the correct decision. These entire sensor values are updated in the mobile application for the future reference using the ESP8266 delivers a high WIFI solutions to meet the project requirements continues demand for efficient and compact design and reliable performance in the IOT industry. The IOT module is shown in Figure 4 and the prototype model is shown in Figure 5. The prototype shows the snapshot of the footwear unit. This unit contains the insole of the shoe on which the FSR and vibrating motors are fixed. The FSR measures the pressure values and sends it to the handheld unit using IEEE 802.11. Flex force sensors are fixed on the insole in three places namely: rear foot, mid foot, medial rear foot.

The vibrating motors are fixed in the insole near the flux sensors accordingly. This system helps the diabetic neuropathy patients to stimulate blood flow and have a continuous monitoring of foot pressure values. The pressure values stored in the SD card can be sent to the consultant for reference. The shoe is easily portable and hence the diabetic patients can do the therapy easily at their convenience. The measurements for various sensor placements at different positions are as shown as a table in Table 1 and the Figure 6 represents the application environment outlook. In this work, the sensor has been fixed on the insole of the shoe. This work can be further processed by fixing all the components in the shoe and giving the shoe as a complete product. The foot neuropathy analyzer measures the pressure values in foot and computes them. The imperceptible vibrations are sent accordingly to the pressure values measured. This project helps the diabetic neuropathy patients to stimulate blood flow and have a continuous monitoringof foot pressure values. The pressure values are stored and can be sent to consultant for reference. The shoe is easily portable. The diabetic patients can do the therapy easily at their convenient time. The data management in the app is taken care of by saving the data in a cloud server; data will be erased after 6 months because it's a common platform for patients.

CONCLUSION

A smart monitoring system for diabetic neuropathy patients has been established through pressure sensing. Further the blood flow stimulation is also done. The adopted methodology performs well in pressure sensing, segmentation, and data handling by the microcontroller. The pressure measured is efficient since the placement of sensors is appropriate to detect the injuryin initial conditions itself. The plantar pressure is measured in 5 different

regions of the foot. Then the blood flow is stimulated by the vibrating motor if pressure is abnormal. Hence the methodology proposed helps in monitoring the pressure and consequently making it normal.

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Table 1. Measured Values Normalized With Pressure Range in kPa

Sensor_id	User_id	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 5
15841	91	699	0	0	0	136
15842	91	732	0	0	0	128
15843	91	743	0	0	0	109
15844	91	747	0	0	0	99
15845	91	738	0	0	0	88
15846	91	664	0	0	0	81
15847	91	722	0	0	0	74
15848	91	697	0	0	0	70
15849	91	0	0	110	0	699

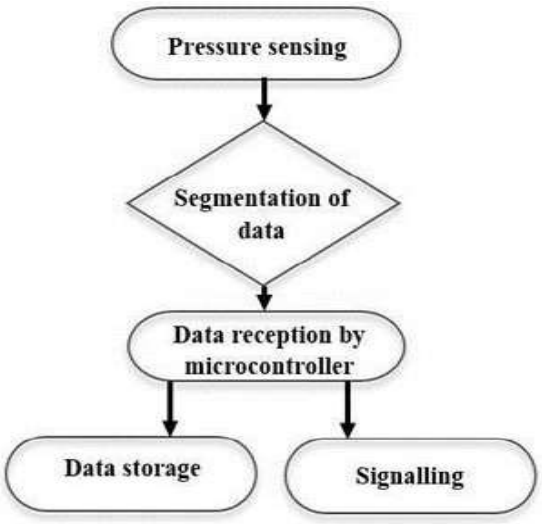

Figure 1. Flow Chart of The Proposed System

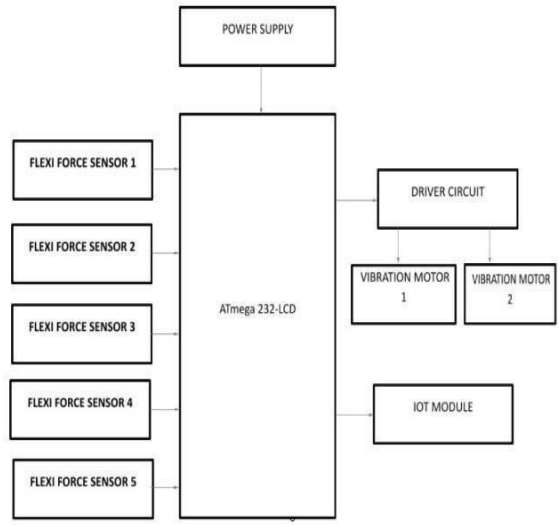

Figure 2. Block diagram

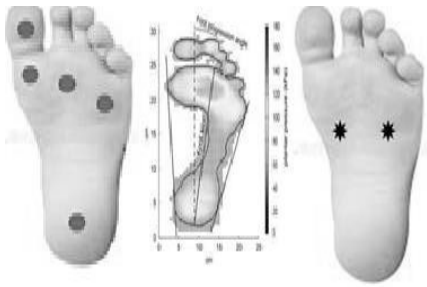

Figure 3. Plantar Pressure Selected Points, Threshold And Placement of Vibrating Motors

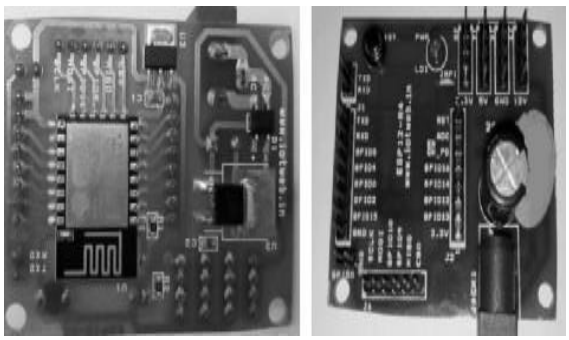

Figure 4. IOT Module

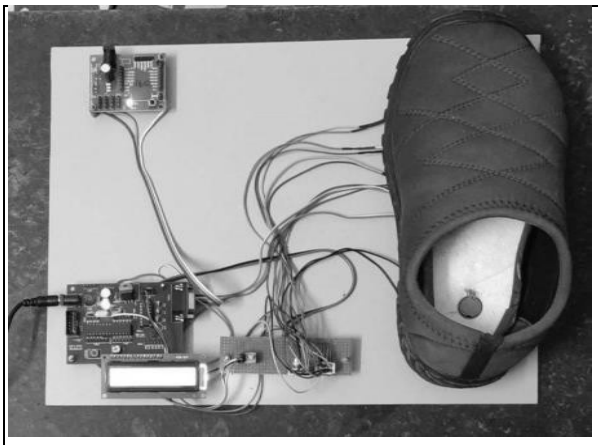

Figure 5. Prototype of Proposed System

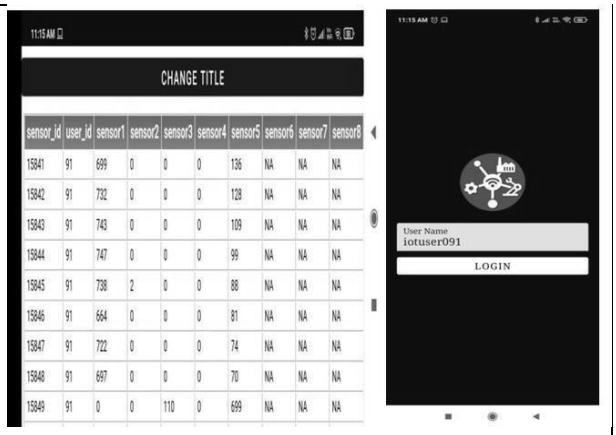

Figure 6. Application environment outlook

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Cardiometabolic Risk Factors in Polycystic Ovarian Disease

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Received: 24 Nov 2021 Revised: 23 Jan 2022 Accepted: 07 Mar 2022

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ABSTRACT

Polycystic ovarian disease is the most common endocrine disorder affecting women of reproductive age (PCOD). Although hyperinsulinemia and hyperandrogenemia are well-established pathologic pathways, there is mounting evidence that both conditions increase the risk of cardiovascular disease, dyslipidemia, and type 2 diabetes mellitus. Our study will examine the association between PCOD and cardiovascular risk factors. This study discovered a significant but distinct relationship between PCOD and major cardiovascular risk variables. This association has the potential to result in CVD outcomes, requiring early intervention programmes and preventative interventions to reduce cardiovascular event-related mortality. This study lays the foundation for future research on women with PCOD and the identification of novel cardiovascular risk factors.

Keywords: Polycystic ovarian disease, metabolic syndrome, Diabetes mellitus, cardiovascular disease, Risk markers, Hyperinsulinemia, Hyperandrogenemia

INTRODUCTION

Although polycystic ovarian disease (PCOD) is one of the most prevalent endocrine diseases affecting women, the cause remains unknown. Acne, amenorrhea or oligomenorrhea, hirsutism, infertility, and mood problems are common clinical signs of PCOD, and they are the main focus of therapeutic treatment [1]. The effect of PCOD on future cardiovascular disease (CVD) risk, on the other hand, should not be ignored, and chances to adopt CVD

preventive measures in these women should be prioritised [2]. PCOD etiology often includes insulin resistance, which leads to a variety of "cardio metabolic disorders (dyslipidemia, hypertension, glucose intolerance, diabetes, and metabolic syndrome), placing women at an elevated risk for CVD" [3]. Previous research has shown that subclinical CVD indicators "including coronary artery calcium scores, C-reactive protein, carotid intima-media thickness, and endothelial dysfunction are more likely to be elevated in women with PCOD" [4]. While the links between PCOD and cardio metabolic abnormalities are well known, it is unclear whether PCOD is linked with subclinical and clinical CVD in the absence of these CVD risk factors [5]. Lifestyle changes and weight control may help to reduce some of these future CVD risks and should be promoted [6].

MATERIALS AND METHODS

Glucose was determined using the Hexokinase method, and total cholesterol was determined using the CHOD-POD method. HDL cholesterol is determined using the modified CHOD-POD method. Triglycerides were determined using the Glycerol phosphate oxidase / PAP method, and anti-TPO antibodies were quantified using the Chemiluminescent Micro particle Immuno Assay. The above parameters are analysed using the following methods on a fully automated analyser located in the hospital. Following sample processing, the values of each parameter are captured and processed for analysis.

RESULTS AND DISCUSSION

The Mann-Whitney U statistic for comparing FBS between PCOD and non-PCOD women is 3849.000, the Z value is -0.577, and the p-value is 0.564. Because the p-value is greater than 0.05, we can conclude that there is no statistically significant difference in FBS between women with and without PCOD. The Mann-Whitney U statistic for comparing TG between PCOD and non-PCOD women is 3242.000, the Z value is -2.313, and the p-value is 0.021. Because the p-value is less than 0.05, we can conclude that there is a significant difference in TG between women with PCOD and those who do not have PCOD. The Mann-Whitney U statistic for comparing cholesterol in women with and without PCOD is 3846.500, the Z value is -0.583, and the p-value is 0.560. Because the p-value is greater than 0.05, we can conclude that there is no significant difference in cholesterol between women with PCOD and those who do not have PCOD. The Mann-Whitney U statistic for comparing HDL levels in women with and without PCOD is 2182.500, the Z value is -5.351, and the p-value is 0.000. Because the p-value is less than 0.05, we can conclude that there is a significant difference in HDL levels between women with and without PCOD. The Mann-Whitney U statistic for comparing LDL levels in women with and without PCOD is 2436.500, the Z value is -4.619, and the p-value is 0.000. Because the p-value is less than 0.05, we can conclude that there is a significant difference in LDL between women with PCOD and those who do not have PCOD. The Mann-Whitney U statistic for comparing Anti TPO levels in women with PCOD and those without is 9.000, the Z value is -11.578, and the p-value is 0.000. Because the p-value is less than 0.05, we can conclude that there is a significant difference between Anti TPO in women with PCOD and those who do not have PCOD. Primary infertility was the most commonly reported symptom of PCOD. In PCOD patients, mean arterial pressure, fasting hyperglycaemia, hypercholesterolemia, and anti-TPO levels were substantially greater than in controls. We discovered a typical atherosclerotic lipid profile in our research, with increased total cholesterol and low-density lipoprotein-C (LDL-C) levels and reduced serum high density lipoprotein-C levels (HDL-C).

CONCLUSION

Most of the CVD risk factors in women with PCOD are related to insulin resistance as well as hormonal and metabolic processes in their systems. A substantial but separate relationship between PCOD and major cardiovascular risk factors was found in this study, according to the authors. If this relationship persists, early treatment strategies and preventive measures will be necessary to decrease cardiovascular mortality. While investigating new cardiovascular risk variables, this study sets the groundwork for future research on

PCOD women. Dietary changes, regular exercise, and weight reduction have been shown to ameliorate PCOD-related cardio metabolic abnormalities, and should be promoted for all women in order to avoid CVD. To further understand the effect of CVD risk factors on women with PCOD, to really evaluate the impact of CVD risk factors and CVD outcomes, we need more population-based studies that last for a long time. As a consequence, cardiovascular disease risk is likely to be reduced in these women.

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Mann-Whitney U Tests between women with PCOD and without PCOD

	Mann-Whitney U	Z	p-value
FBS	3849.000	-.577	.564
TG	3242.000	-2.313	.021
CHOLESTEROL	3846.500	-.583	.560
HDL	2182.500	-5.351	.000
LDL	2436.500	-4.619	.000
Anti TPO	9.000	-11.578	.000

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Distribution of True Mangroves in Valapattanam, Kannur, Kerala

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Received: 15 Feb 2022 Revised: 24 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

The current work focuses on the distribution of true mangrove species of Valapattanam, Kannur District, Kerala. A total of four true mangroves namely *Rhizophora mucronata*, *Bruguiera cylindrica*, *Avicennia officinalis* and *Acanthus ilicifolius* are present at the study site. The frequency, density and abundance of the mangrove plants are found out using plot method. Relative frequency, relative density and relative dominance and Important Value Index were calculated. Shannon– Weiner diversity, Simpson index, species richness and species evenness were also measured and found that *Rhizophora mucronata* is the most dominant species in the study area.

Key words: Distribution, Mangroves, Quadrat Method, Valapattanam

INTRODUCTION

Mangroves are intermediate ecosystems, which connects the terrestrial and aquatic ecosystems. Until late 1800s, mangrove forests were considered as wasteland because there were no direct ecological values to the humans [1]. The ecological functions of Mangroves in India gained its attention after the tsunami incident of 2004. In the study conducted in the cyclone hit areas, the regions where mangroves were in relatively good condition were much less affected than the regions where mangroves were converted into shrimp farms and during the tsunami 2004 in Sri Lanka, apart from some uprooted *Excoecaria agallocha* plants the mangroves seemed unaffected [2]. The tsunami incident made hype in the researches in Mangroves. Nearly 3% of the world's Mangroves inhabit in India which has been estimated as more than 4700 sq.km [3], which indicates that the mangrove cover of India is increasing.

According to FSI (2005) [4], the mangrove forest cover of India was 4663 sq.km. Mangroves serve as breeding areas of many animals and birds including crabs, prawns, etc. The wide adaptations of mangroves help them to withstand tidal waves and they are the most important plant groups that make the tidal water nutrient rich which facilitates phytoplankton growth which in turn facilitates the growth of fishes, prawns and crabs. The root system of many mangroves provides breeding points for fishes and they also protects the infants from predators which assures a safe growth of the fishes into adults.

Kerala lies at the west side of India, its coast lying towards the Arabian Sea. Once the state was so abundant with more than 700 sq. km of Mangroves [5] but the current scenario is so much different as it contains only 9 sq. km of Mangroves [6]. Kannur has the greatest Mangrove vegetation followed by Ernakulam and Alappuzha and Thiruvananthapuram records the least [7].To understand the status of plants at a given area, to assess if the particular plants are degrading so that special consideration has to be taken, the distribution studies of the plant has to be carried out. Sadly the phytosociological studies of mangroves were not given so much importance even in the current era. Even the data presented in the IUCN Red Data List is aged more than a decade [8]. So the understanding of the current status of mangroves is important in order to conserve, protection and reclamation. Recent reports indicates that the destruction of mangroves have reached a rapid rate during the Covid–19 pandemic due to land filling and waste disposal. Due to the degradation process it is very important to assess and update the diversity and distribution of mangroves.

MATERIALS AND METHODS

Study Site

The most diverse mangrove vegetation in Kerala is present in Kannur district. Valapattanam is located at Kannur district in Kerala state, at the bank of Valapattanam River at 11° 54′ 0″ N and 75° 22′ 12″ E. Valapattanam is the smallest panchayat in Kerala with an area of 2.04 sq.km. It comes under the Valapattanam basin, one of the most mangrove rich basins of Kerala (Figure 1). About 80% of the mangroves in Valapattanam are under private ownership making it difficult for the conservation of Mangroves. The Valapattanam bank contains mud soil and is a place where high tide and low tide occurs.

Diversity and Structural Analysis

The study was conducted by making six plots of 5mx5m quadrats non–randomly in the mangrove inhabiting areas. Through the quadrant method, the frequency, relative frequency, density, relative density, abundance, relative abundance and Important Value Index were calculated. The data obtained from the plots were analyzed to calculate the diversity indices and species richness such as Shannon– Weiner diversity (H), Simpson index [9], species richness and evenness were measured [10]. Frequency, density, abundance, relative frequency, relative density and relative abundance were calculated using following formulae:

$$\text{Frequency (F)} = \frac{\text{Total no. of quadrats in which the species occured}}{\text{Total number of quadrats studied}} \text{x } 100$$

$$\text{Density(D)} = \frac{\text{Total no. of individuals of the species}}{\text{Total no. of quadrats studied}}$$

$$\text{Abundance (A)} = \frac{\text{Total no. of individuals of a species in all quadrats}}{\text{Total no. of quadrats in which the species occured}}$$

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of an individual species}}{\text{Freuency of all the species}} \text{x100}$$

$$\text{Relative Density (RD)} = \frac{\text{No.of individuals of the species in all quadrats}}{\text{No.of individuals of all species in all quadrats}} \text{x100}$$

$$\text{Relative Dominance(RDO)} = \frac{\text{Total basal area of a species}}{\text{Basal area of all species in the area}} \text{x100}$$

Importance Value Index = Relative Frequency + Relative Density + Relative Dominance

RESULT AND DISCUSSION

In the present study, it is found that four species of true mangroves belonging to two families are present at the study site, the details of which are given in Table 1 and Figures 2–5. All the four plants are Least Concerned in IUCN category but the status of the plants were last updated more than a decade ago which shows that the current status of the plants are currently unavailable [11]. In the data it is clear that *Rhizophora mucronata* is the most dominant species at Valapattanam followed by *Avicennia officinalis*, *Bruguiera cylindrica* and *Acanthus ilicifolius*.

Plant description

***Rhizophora mucronata* Lam.**

Trees up to 20m high, bark dark grey, rough, fissured with too many stilt roots. Leaves simple, opposite, dark green in colour, elliptic, tip mucronate, lower side with plenty of black dots, length 14.5–16.5×8.0–10.2 cm width (mean($\bar{X}$)= 15.26×8.97), mean length–width ratio 1.7, petiole green, 1.5–2.5 cm long. Inflorescence axillary cyme with 2–8 flowers, peduncle 1.3–1.6×0.3–0.5cm, calyx 4, yellowish green, petals 4, white, thin, hairy, stamens 8, brown, 0.7cm long, style terete with bilobed stigma, fruit pear shaped, calyx persistent.

***Bruguiera cylindrica* (L.) Blume.**

Trees up to 15m hight. Bark grey, fissured. Leaves simple, opposite, elliptic to obovate, margin entire, dark green, length 9.5–11.5×3.5–5.0 cm width (mean($\bar{X}$)= 10.18×4.05cm), mean length–width ratio 2.52, petiole green, 1.5–2.5 cm long. Inflorescence axillary cyme, 3 flowered, peduncle 0.5–1.5 cm. Sepals pale green, 8 in number, 0.5–0.7 cm long. Petals white, 0.5 cm long. Stamens 12, epipetalous, style 0.3–0.5 cm, stigma 3 lobed.

***Avicennia officinalis* L.**

Trees up to 20m hight. Bark dark grey to brown, finely fissured. Pencil like pneumatophores, 20–30×0.1–0.5cm. Leaves obovate to elliptical, apex rounded, length 8.5–10×3.5–5 width (mean($\bar{X}$)= 8.3×3.86cm), mean length/width ratio 2.18 cm, upper surface green, lower surface whitish green. Inflorescence capitate with 2–4 opposite decussate pairs. Calyx lobes 4, 0.5 cm, hairy. Petals 4, yellow–orange, 0.3–0.5×0.5–0.8 cm. Stamens 4, alternate with corolla lobes, 0.3 cm long. Style bilobed, 0.3 cm long.

***Acanthus ilicifolius* L.**

Shrub up to 3 m high. Stem thick, green, without any branches with 2–4 spines at each node. Leaves simple, opposite, sometimes in cluster, margin either entire or spiny,tip acute with or without spine, size highly variable according to the availability of sunlight, length/width ratio always greater than 2. Inflorescence axial or terminal up to 15 cm long. Bract 1, bracteoles 2. Sepals 4, 1.3–1.5×0.8–1.2 cm. Corolla purple, 3–4×2–2.5 cm. Stamens 4, 2–2.5 cm long. Style enclosed by stamens, 2.5–3 cm, stigma capitate or pointed. In the present investigation, four species of true mangroves belonging to two families are found at Valapattanam region of Kannur district of which *Rhizophora mucronata* is found to be most dominant. Many have investigated state wise and district wise distribution of mangroves [12],[13],[14] but locality based distribution of mangroves are very few [15]. conducted the distribution studies of mangrove forests at Ayiramthengu, Kollam district and found out that *Avicennia marina* was the most dominant species and *Sonneratia caseolaris* was the least dominant species. In the study conducted by [16], it was found that *Rhizophora apiculata* followed by *Sonneratia caseolaris* was the most dominant species at Asramam in Kollam district whereas *Avicennia officinalis* was the least dominant species in that area. [17] conducted study on the Thekkumbad region of Kannur district and reported that the dominant species were *Rhizophora mucronata*, *Bruguiera*

cylindrica, *Sonneratia alba* and *Excoecaria agallocha* but in Valapattanam region, the dominant species are *Rhizophora mucronata* and *Avicennia officinalis*.

ACKNOLEDGEMENT

The authors are very thankful for the Department of Botany, Annamalai University giving the opportunity for this study.

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Table 1: Plants currently available at Valapattanam and their IUCN status

SPECIES	FAMILY	IUCN STATUS	LAST ASSESSMENT OF IUCN STATUS
Rhizophora mucronata Lam.	Rhizophoraceae	Least Concerned	2008
Bruguiera cylindrica (L.) Blume	Rhizophoraceae	Least Concerned	2008
Avicennia officinalis L.	Acanthaceae	Least Concerned	2008
Acanthus ilicifolius L.	Acanthaceae	Least Concerned	2010

The distributions of true mangrove species are shown in Table 2 and Figure 6–7.

Table 2: Distribution status of true mangroves of Valapattanam.

	FREQUENCY (%)	RELATIVE FREQUENCY (%)	DENSITY (Individuals /m²)	RELATIVE DENSITY (%)	ABUNDANCE	RELATIVE DOMINANCE (%)	IMPORTANT VALUE INDEX (IVI)
Rhizophora mucronata Lam.	44	36.01	0.84	33.63	3.67	32.61	102.25
Bruguiera cylindrica (L.) Blume.	26	24.16	0.61	25.59	1.19	28.72	78.47
Avicennia officinalis L.	36	34.65	0.51	30.2	1.25	32.79	97.64
Acanthus ilicifolius L.	8.67	5.28	0.77	11.47	0.17	5.88	22.63

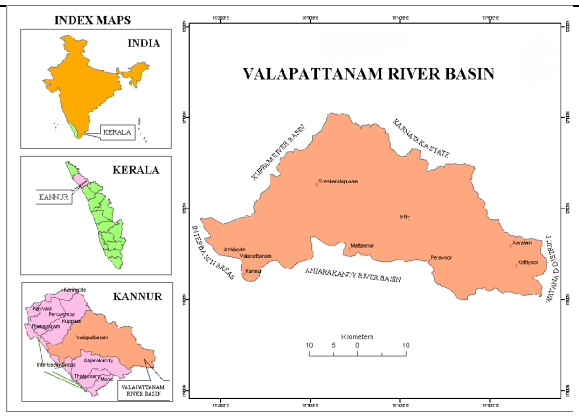

Figure 1: Study Area

Figure 2: *Rhizophora mucronata*

Figure 3: *Bruguiera cylindrica*

Figure 4: *Avicennia officinalis*

Figure 5: *Acanthus ilicifolius*

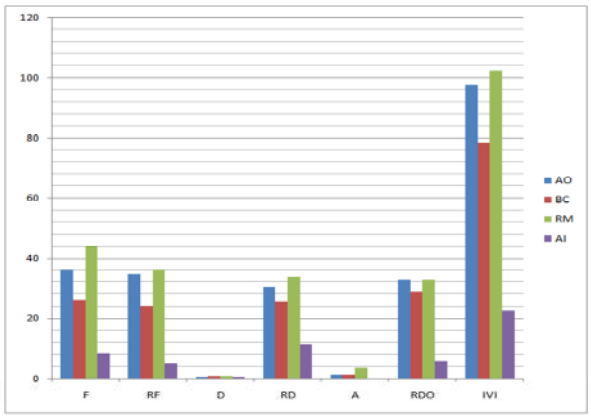

Figure 6: Distribution status of true mangroves at Valapattanam. (F= Frequency, RF= Relative frequency, D= Density, RD= Relative density, A= Abundance, RDO= Relative dominance, IVI= Important Value Index, AO= *Avicennia officinalis*, BC= *Bruguiera cylindrica*, RM= *Rhizophora mucronata* and AI= *Acanthus ilicifolius*.

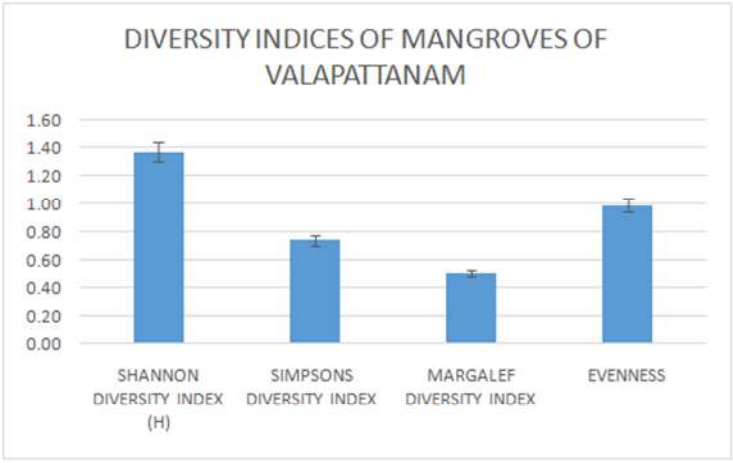

Figure 7: Diversity indices of mangroves at Valapattanam

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Acute Toxicity Study of Novel Synthetic Benzoxazole Derivatives

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Received: 06 Jan 2022 Revised: 08 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

Benzoxazole is one of the compound with various pharmacological activities. modifications of the benzoxazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. Addition of trialzole to benzoxazole helps in explore potential central nervous system (CNS) actions. Any synthetic drugs must be ensured to be safe before they could be used as medicines. The present study was carried out to evaluate the safety of the novel synthetic benzoxazole derivative as per OECD guidelines 423. In this study, single oral administration of 5, 50, 300 & 2000 mg/kg doses of 6,8-dichloro [1,2,4] triazolo [3,4-*b*][1,3] benzoxazole did not showed any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence, it was concluded from the results that the possible oral toxic doses of6,8-dichloro [1,2,4] triazolo[3,4-*b*][1,3]benzoxazoleare more than 2000mg/kg and found to be safer and non -toxic to rats and further chronic studies are required to confirm its therapeutic efficacy in animals and humans.

Keywords: Acute toxicity studies, Benzoxazole, Triazole, 6,8-dichloro [1,2,4] triazolo[3,4-*b*][1,3]benzoxazole.

INTRODUCTION

Benzoxazole derivatives possess diverse variety of pharmacological activities due to which they have occupied unique place in the field of medicinal chemistry. Benzoxazole finds its use in research as a lead compound for the synthesis of larger, usually bioactive structures. Modifications of the benzoxazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. The benzoxazole moiety is expanding its pharmaceutical importance and is being studied for its pharmacological assistance in varied circumstances. Literature survey indicates identification and isolation of several benzoxazole derivatives of importance in pharmacology as antibacterial and antifungal agents (1), HIV-1 reverse transcriptase inhibitors (2), topoisomerase-I inhibitors (3), anticancer agent (4) and in the treatment of Alzheimer's disease (5). Looking into the pharmacological importance of benzoxazole moiety, it is worthwhile to screen the derivatives for their biological activities in various animal models for potential medical indications. Addition of a triazole nucleus which has a planer five-membered heterocyclic system containing three nitrogen atoms, one pyrrole and two pyridine type (6), to the benzoxazole moiety could help explore potential central nervous system (CNS) actions of the substituted c6+ompounds.Two such structural isomeric forms of triazole are 1,2,3 and 1,2,4 (7).Of these two derivatives 1,2,4-triazole derivative has been explored for various pharmacological actions. On the basis of the pharmacological profiles of triazoles, their antidepressant properties have been well documented. Nefazodone has triazole nucleus and possesses antidepressant activity. It inhibits the binding of(3H) ketanserin to cortical serotonin 2 (5-HT2) binding sites, it antagonizes the 5-HT2 and also inhibits cortical serotonin uptake (8).

The following 1,2,4-triazole derivatives found application in medicine: alprazolam (tranquilizer) (9), etoperidone (antidepressant) (10), nefazodone (antidepressant, 5-HT_2 antagonist).Owing to the similarity in structure to alprazolam, the 1,2,4-triazole derivatives could have potential anti-anxiety properties too. As serotonin receptors may directly or indirectly depolarize or hyperpolarize neurons by changing the ionic conductance and/or concentration within the brain cells. 5-HT may be able to change the excitability in most networks involved in epilepsy (11). Based on the action of nefazodone (5-HT2 antagonist) (8) and other substituted 1,2,4-triazole derivatives it has anticonvulsant action (12). The substituted 1,2,4-triazole derivatives have been proved to possess analgesic activity in a recent study due to their central analgesic action (13).Hence, the said test compound 6,8-Dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole could potentially have the CNS action. Thus, the present study would aim at exploring the CNS actions in animal models. Synthetic drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used (14). The main aim of our study was to evaluate the novel synthetic benzoxazole derivative for their toxic effects before it can be used for applications that are of importance to the public. Hence, this study aims to evaluate its acute toxicity study as per OECD guidelines.

METHODS

Synthesis and Characterization of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

The research work was focused on the synthesis of biologically potent molecules. Triazoles are known for diverse pharmacological activities. In this study, 5,7-dichloro-2-hydrazino-1,3-benzoxazole reacts with formic acid and forms 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole (15).The structure of the product 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was confirmed by the spectral studies such as Infrared spectroscopy, NMR spectroscopy, and MASS spectroscopy.

Animals

Healthy adult female Swiss albino mice (25-30gm) were used for the acute toxicity studies. The animals were procured from CPCSEA listed suppliers of Sri venkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12 h

dark schedule at 25°C and 55–65% humidity levels. The animals had been given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of Animal

Healthy animals were randomly selected for the study and kept in their cages for at least one week prior to dosing to allow for acclimatization to the laboratory conditions. Before test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals

Toxicity Studies

Acute toxicity study was performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 423 (16).

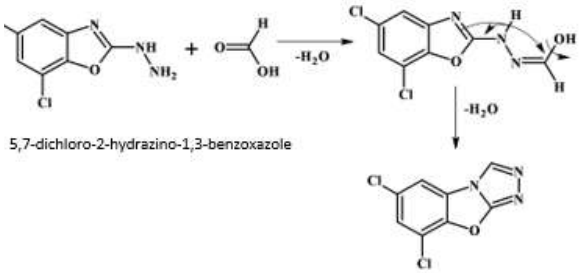
Acute Oral Toxicity Studies

The acute toxicity studies were performed as per OECD guidelines 423. A total of 12 mice weighing between 25-30g were randomly divided into four groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld overnight). Following the period of fasting, the bodyweight of the animals was measured and the 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was administered orally to each group at single doses of 5, 50,300, and 2000 mg/kg, respectively. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 14 days. All the animals were then allowed free access to food and water and observed for signs of acute toxicity. It includes changes in body weight, food and water intake, skin and fur, eyes and mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity, and behavior patterns. The number of deaths within this period was recorded. The urine analysis was performed to investigate any abnormalities in the excretion pattern after exposure to the test drug for 14 days.

RESULTS

Synthesis of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

The novel synthetic benzoxazole derivative 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazolewas synthesized by standard procedure and the reaction scheme was shown below.



5,7-dichloro-2-hydrazino-1,3-benzoxazole

6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazole

The structure of the product 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was confirmed by the spectral studies. In the IR spectrum, the stretching frequency at 3367 cm^{-1} and 3481 cm^{-1} disappeared (fig.1). A peak at 4.6 for two protons of $-NH_2$ and at 9.3 for a proton of -NH disappeared and a new peak was observed at 12.18 confirmed the

structure. The signal obtained for proton at 7.17 (s, 1H, Ar H) 7.35 (s, 1H, Ar H) and at12.18 (s, 1H, for -N=CH) (fig.2) confirmed the formation of compound. Mass spectrum at M^{+} (228), M^{+2}(230) and M^{+4}(232) corresponds to its molecular weight. It showed the absence of both -NH and -NH_2 groups in its IR spectrum, ^{1}H NMR and mass spectral studies.

Acute Toxicity Studies

Acute toxicity studies are performed to determine the short-term adverse effects of the drug when administered in a single dose orally. It also indicates the safety of the drug in-vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals. The LD50 determination was performed in mice as per OECD guidelines 423 and LD50 of the 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was found to be non-toxic upto 2000 mg/kg and the ED50 values were 200 mg/kg, respectively was shown in table.no.1.

DISCUSSION

For any therapeutic and cosmetic application, compounds used must be practically non-toxic. The aim of performing acute toxicity studies is for establishing the therapeutic index of a particular drug and to ensure the safety in-vivo. In the present study, the animals showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any of the four groups. Hence, it was safe up to 2000mg/kg.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Vinayaka Mission's Research Foundation (Deemed to be University), Salem for providing the facilities for carrying out this research.

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Table.1. LD_{50} value of the novel synthetic benzoxazole derivative.

S.No	Groups	No. of Animals/group	Dose mg/kg	No. of death animals
1	I	3	5	0
2	II	3	50	0
3	III	3	300	0
4	IV	3	2000	0
	LD_{50}		2000	
	ED_{50}		200	

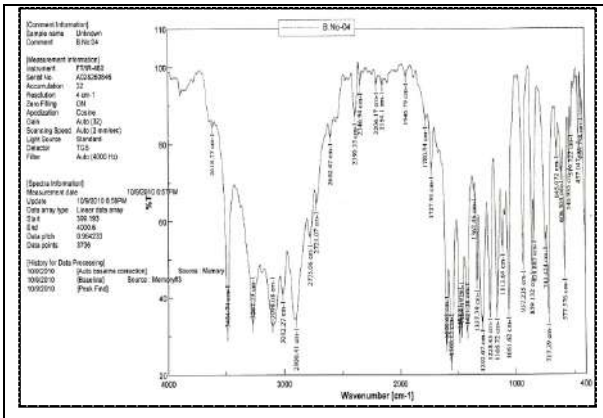

Figure 1. IR spectrum of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

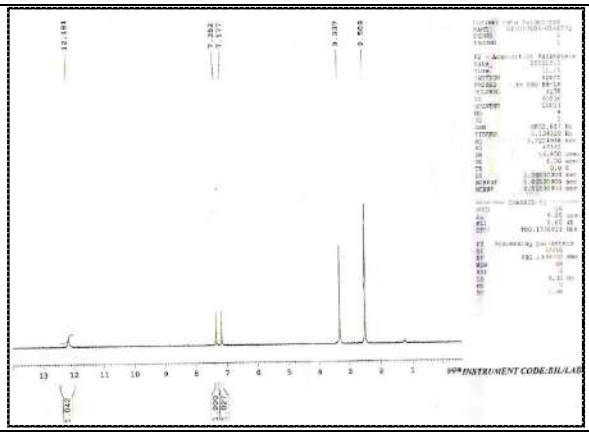

Fig.2: ^{1}H NMR spectrum of 6,8-dichloro[1,2,4] triazolo[3,4-*b*][1,3]benzoxazole

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Molecular Docking Studies of Potential Inhibitors against HPV 16 E6 Protein in *Eichhornia crassipes*

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Received: 19 Dec 2021 Revised: 13 Jan 2022 Accepted: 25 Feb 2022

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ABSTRACT

E6 proteins are those of the High- Human Papillomavirus (E.g.: HPV-16 and HPV 18 E6) which can provoke the ruin of the p53 tumour suppressor protein. E6 proteins interact with the cellular ubiquitin ligase E6AP, this interaction leads to the formation of the E6-E6AP complex. This form of the E6-E6AP complex degrades the p53, a tumour suppressor protein. Therefore, preventing the formation of the E6-E6AP complex which inhibits the viability and proliferation of infected cells (4). Here, *Eichhornia crassipes* (water hyacinth) risk compounds as the small molecules are used to inhibit the formation of E6-E6AP. Interrupting the E6-E6AP complex fails to target the p53 degradation. *In silico* process is used to find the small-molecule inhibitors, for the E6-E6AP interaction. Molecular Docking was carried out to find the potential inhibitors of E6 protein. Water hyacinth's fifty-four compounds are used and selected for docking according to Lipinski's rule. Among that, docked twenty-six ligands are bound more efficiently with target protein.

Keywords: HPV E6, Molecular Docking, *Eichhornia crassipes*

INTRODUCTION

HPV is a sexually transmitted virus and some of the HPV strains are identified epidemiologically where HPV-16 is the most prevalent type in cervical cancer arising from the cervix. It is due to the abnormal growth of cells that has

the ability to invade or spread to other parts of the body. Cervical cancer is the fourth most frequent cancer among among women in the world. The report from the WHO in 2018, shows an appraisal of 5,70,000 new cases and it concerns about representing 6.6% of all females. The WHO data in 2013 shows that developing countries are affected by more than 85% of these cases. India is the 2nd most populated country in the world where 80% of cervical cancers are present at an advanced stage. India's current appraisal shows that every year 1,22,844 women are diagnosed with cervical cancer and 67,477 people die due to the disease (From the Fact sheet 2017, HPV and related concerns). The development of cervical cancer is due to the infection with HR HPV which has been acknowledged as an essential reason. HPV can also cause the cancer in vagina, vulva, penis, and anus including some head and neck cancers, anogenital warts, and recurrent respiratory papillomatosis (6). HPV is a non-enveloped DNA virus with icosahedral capsid, it has double-stranded circular DNA 7900bp long. During the viral cycle the protein expressing the two functional genome regions have been identified. First one is the coding region, which contains the early genes, E1, E2, E4, E5, E6, and E7, and the second region contains two late genes, L1 and L2. L1 is major capsid protein and L2 is minor capsid protein. More than that, the HPV genome has a non-coding region, termed as long control region (LCR) which has most of the regulatory elements involved in viral DNA replication and transcription (8).

E1 acts as a viral inhibitor protein for the replication of viral genome, which includes functions such as helicase-ATPase activity. Whereas, E2 is a viral multifunctional protein, which helps in the specific recruitment of E1 to the e viral DNA. E1 accumulates as a double-hexamer and oligomerizes at the viral origin of DNA replication and it interacts among several host replication factors, such as polymerase alpha-primase, replication protein A, topoisomerase 1 , and cyclin E/cdk2. E2 functions as a transcription factor, capable of transactivation, repression, and supporting role in DNA replication. An intermediary of genome segregation is indispensable for viral persistence and required for genome maintenance in persistent infections. The infected cells undergo differentiation, late gene expression, and viral genome replication induced. E4 is ample in cytoplasmic protein in warts and disrupts keratin filaments. E5 functions in transformation, promoted from the down-regulation of activated receptors. E5 oncoproteins stimulate the activity of an enzyme stimulating the process of cellular apoptosis – COX-2. E4 and E5 have similar functions where both are required for viral amplification to maintain cellular replication machinery active. E6 binds with E6AP and promotes the E3AP ubiquitylation and degrades the p53. It also binds with other cellular proteins like Paxillin and IRF3. E6 stimulates the transcription of cellular telomerase. E7 inactivates the pRB and it forces the infected cells to stay in a proliferative state and cell cycle exit. While E6 repeals the p53 where E6 ensures cell survival by preventing apoptosis triggered by this aberrant growth signal. The virallife cycle of the productive phase is activated upon epithelial differentiation, therefore resulting in the amplification of viral genomes to thousands of viral copies per cell in suprabasal layers, which stimulates the increase of late gene expression. The amplified genomes are then packed into the infectious virions by the L1 and L2 proteins, which form the subunits of the icosahedral capsid. Finally, viral escape by the natural tissue desquamation and might be facilitated by the keratin network disrupting the competency of E4.

HPV E6 provokes the degradation of p53. The E1 ubiquitin-activating enzyme activates ubiquitin (UB), which is transferred by the E2 ubiquitin-conjugating enzyme to the E3 ubiquitin-protein ligase. HPV E6 binds to an E6-E6AP, and forms an E3 enzyme which specifically binds and ubiquitinates p53. And the 26s proteasome degrades the polyubiquitinated p53(7). E6 viral oncoprotein are key players in epithelial tumours provoked by Papillomavirus in vertebrates, including cervical cancer in humans. The E6 oncoprotein from high-risk HPV type 16 is a 151 amino acids residue protein which has two zinc binding domains and its function is to inactivate the P53 tumour suppressor protein. *Eichhornia crassipes* is commonly known as water hyacinth, a free-floating aquatic plant which is considered as an invasive species, since it grows rapidly and can form thick layers on the surface of the water which forms the mat-like coverage(2)(3)(10). The mat-like coverage blocks the sunlight from entering the bottom of the native submerged plants.Recently, substantiable concentration has been given to harvest the water hyacinth for the purpose of practical uses that is used as an economical source in many parts of the world. Despite that, in literature there are very few pharmacological studies on water hyacinth and are only used as some therapeutic properties in folkloric application. In contrast, water hyacinth, also used as a medicinal plant, has an anticancer activity, antimicrobial activity, and antioxidant activity. Many drugs are found in the plants, identified from the secondary

metabolites based on the active principle. The medicinally useful bioactive constituents belong to alkaloids, flavonoids, and phenolic (9). E6 protein interacts with the cellular ubiquitin ligase E6AP, this interaction forms the E6-E6AP complex. The E6-E6AP complex is responsible for the degradation of P53, a tumour suppressor protein through the ubiquitin proteasome pathway. E6 and E6AP alone cannot be bound with p53, as the E6-E6AP complex is crucial for p53 degradation. This complex binding induces conformational changes in E6AP that allows the link with p53. The E6AP binding relies on the LXXLL binding motif, and is a short leucine rich amino acid sequence(5). Interrupting the E6-E6AP complex formation, fails to target the P53 degradation. In this study, by using water hyacinth compounds to interrupt the E6-E6AP complex formation, an *insilico* analysis is performed through the Molecular Docking.

METHODOLOGY

Protein Preparation

HPV16 E6 Crystal Structure

HPV16 E6 (pdb: 4GIZ), the X-ray crystal structure is downloaded from the Protein Data Bank in PDB format and the protein is used for the docking experiments. 4GIZ E6 protein has 6 chains, that is A, B, C, D, E, F. Macromolecules Chain A and B is a chimeric protein: Maltose-binding periplasmic protein, UBIQUITIN LIGASE E6AP, and Chain C and D has only E6 protein. Oligosaccharides Chain E and F has alpha-D-glucopyranose-(1-4)-alpha –D-glucopyranose-(1-4)-alpha- D-glucopyranose-(1-4)-alpha-D-glucopyranose-(1-4)-alpha-D-glucopyranose-(1-4)-alpha-D-glucopyranose. Small molecule ligand is ZN, it has C and D chains, and Biologically Interesting Molecule chains are E and F. C and D chains alone are used for docking, which has only E6 protein.

Ligand Preparation

Fifty-four small molecules are identified from the Review (Secondary Metabolites of *Eichhornia crassipes* (water hyacinth): A Review (1949 to 2011)) (1). The fifty-four compounds are downloaded from PubChem in SDF format. (Fig 1) The downloaded ligands are in SDF format, using Open Babel the SDF format ligands are converted into PDBQT format.The fifty-four compound and Carcinol SMILIES is copied from the PubChem and it was pasted in the Molinspiration software, to find the molecular properties of compounds. Among the fifty-four compounds, based on Lipinski's rule twenty-six compounds are docked. Protein and Ligands were downloaded and selected ligands were analysed by using the Auto dock 4, based on the highest binding energy it is sorted.

RESULT AND DISCUSSION

Molecular docking was performed as a blind docking (blind docking refers to a use of a grid box that is large enough to encompass any possible ligand-receptor complex) Using Auto dock. These results revealed that all the predicted docking poses presented a root mean square deviation (RMSD) is $2A^0$. There was strong evidence that Auto dock 4 can predict docking poses accurately, a $2A^0$ usually considered a good threshold value for RMSD. Molecular docked ligands conformation is analysed in the terms of energy, hydrogen bonding and hydrophobic interaction between ligand and protein. The binding energy of the compound is calculated from docking scores of the ligands RMSD in dlg. File. Among the fifty-four compounds from water hyacinth were chosen based on Lipinski's rule along with ligands. Twenty-six ligands are docked into the 4GIZ protein using Auto Dock, and the results were in the graph. (Fig2). Molecular docking studies of twenty-six ligands with protein revealed that all the docked ligands bind more efficiently, in that Gibberellins have the highest binding energy. These results revealed that all the ligands approach towards the specific active site in the protein. Binding active sites of the protein is, LYS-65, GLU-41, GLN-36, LEU-38 and PHE-39. (table 2). *Eichhornia crassipes* solms is a protruding macrophyte, causing serious problems to the network of irrigation and drainage canals in the Nile Delta region (10). All over the world, water hyacinth causes serious problems, and is considered as an economical purpose only. It has also a pharmacological activity, harvesting the water hyacinth for medicinal properties, it clears all the problems. But this study results represent the new starting point for water hyacinth consider as a medicinal plant in future.

The highest binding energy ligands are Quinine, Codeine, 18,19- Secoyohimban19-oicacid, and Thebaine. Thus ligands binding active sites images are given below.

CONCLUSION

In the present study an in-*silico* methodology is conducted combining Molecular Docking to identify new compounds which are able to inhibit the E6-E6AP interaction in water hyacinth. First fifty-four compounds were selected from the review (secondary metabolites of *Eichhornia crassipes*: A Review (1949 to 2011) (1).Among the fifty-four compounds, based on Lipinski's rule, the ligands were selected for the docking. The docked twenty-six ligands are bound more efficiently, specifically scores of Gibberellins shows the highest binding energy than the other ligands in most of the binding active sites are the same. These results represent a new starting point for the water hyacinth as a potential inhibitor for the HPV16 E6 protein, and the twenty-six ligands of water hyacinth bind to the protein. All over the world, water hyacinth is considered as an economical purpose only, but it is also a pharmacological activity despite very few studies on water hyacinth. Hence this study was concluded that water hyacinth can be used to cure medicinal objectives also in future.

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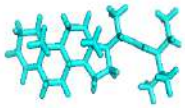
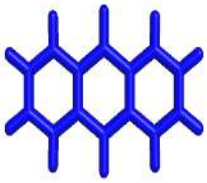
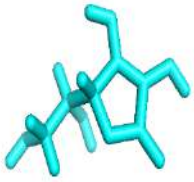
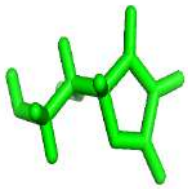
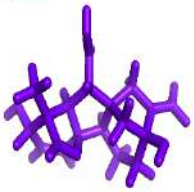
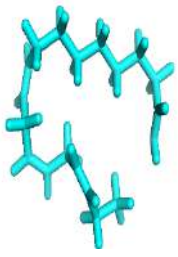
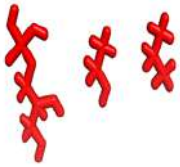
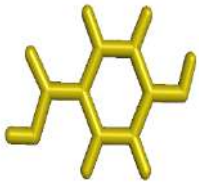
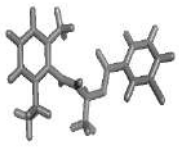
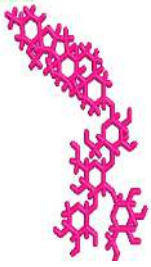
Table 1.The docked ligands RMSD, and estimated inhibition constants value

S.NO	Ligand	Lowest Binding Energy (kcal/mo)	Estimated Inhibition Constant (KI)	Inter molecular Energy (kcal/mol)	Internal Energy (kcal/mol)	Torsional Energy (kcal/mol)	Unbound Extended Energy (kcal/mol)	Cluster RMS	RMSD from Reference Structure
1.	Gibberellins	-7.15	5.77	-8.34	-0.07	+1.19	-0.07	0.00	70.388

2.	Codeine	-7.03	7.06	-7.62	-0.78	+0.60	-0.78	0.00	67.26
3..	Quinine	-6.94	8.14	-8.43	-0.80	+1.49	-0.80	0.00	70.74
4.	18,19 – Secoyohimban – 19 – oic acid	-6.84	9.74	-8.03	-0.55	+1.19	-0.55	0.00	70.02
5.	Thebaine	-6.82	10.0	-7.42	-0.51	0.60	-0.51	0.00	69.43
6.	16,17,20,21 – tetra hydro – 16 – (hydroxyl) methyl ester	-6.66	13.12	-8.15	-0.60	+1.49	-0.60	0.00	71.91
7.	Pipradrol	-6.40	20.43	-7.59	-1.69	+1.19	-1.69	0.00	70.72
8.	Rhein	-6.20	28.70	-7.39	-0.65	+1.19	-0.65	0.00	70.43
9.	Anthraquinine	-6.18	29.71	-6.18	+0.00	+0.00	+0.00	0.00	67.11
10.	Cytisine	-6.13	32.09	-6.13	+0.00	+0.00	+0.00	0.00	70.44
11.	Apigenin	-5.86	50.56	-7.05	-0.88	+1.19	-0.88	0.00	67.39
12.	Luteolin	-5.67	69.36	-7.17	-1.35	+1.49	-1.35	0.00	67.80
13.	Nicotine	-5.32	125.29	-5.62	-0.27	+0.30	-0.27	0.00	62.49
14.	Kaempferol	-5.22	148.18	-6.72	-1.40	+1.49	-1.40	0.00	71.15
15.	Quercetin	-5.05	198.88	-6.84	-2.40	+1.79	-2.40	0.00	70.97
16.	Anthocyanins	-5.02	208.31	-5.32	-0.28	+0.30	-0.28	0.00	66.69
17.	Tricin	-4.94	239.53	-6.73	-1.89	+1.79	-1.89	0.00	70.91
18.	Ferulic acid	-4.63	405.41	-6.12	-0.35	+1.49	-0.35	0.00	68.32
19.	Vanallic acid	-4.42	578.27	-5.61	-0.82	+1.19	-082	0.00	67.48
20.	Syringic acid	-4.25	769.19	-5.74	-1.05	+1.49	-1.05	0.00	69.46
21.	Ascorbic acid	-4.13	943.36	-5.92	-3.15	+1.79	-3.15	0.00	67.11
22.	2 – Methylresorcinol	-4.03	1.12mM	-4.62	+0.06	+0.60	+0.06	0.00	55.43
23.	Catechol	-3.78	1.70mM	-4.37	-0.99	+0.60	-0.99	0.00	71.28
24.	4H – Pyran – 4 – one	-3.69	1.96mM	-4.29	-0.84	+0.60	-0.84	0.00	69.48
25.	Pyrogallol	-3.52	2.64mM	-4.41	-1.89	+0.89	-1.89	0.00	72.34
26.	Phosphatidylcholine	-2.29	20.81Mm	-6.17	-1.59	+3.88	-1.59	0.00	70.21

 *(2,4-ihyroxyphenyl)2(4-methoxy-3-nitrophenyl)ethenone*	 *2-Hydroxy-8-(4-hydroxyphenyl)-phenalen-1-one*	 *2-Hydroxy-8-(3,4-hydroxyphenyl)-1H-phenalen-1-one*	 *2-Methylresorcinol*
 *4H-pyran-4-one*	 *16,17,20,21-tetradehydro-16-(hydroxymethyl)-methyl ester*	 *18,19-secoyohimban-19 oic acid*	 *22-dien-3-one*
 *Figure 9 Anthocyanins*	 *Figure 10 Anthraquinone*	 *Figure 11 Apigenin*	 *Figure 12 Ascorbic acid*
 *Figure 13 Beta sitosterol*	 *Figure 14 Carotene*	 *Figure 15 Catechol*	 *Figure 16 Chlorogenic acid*

 *Codeine*	 *Cytisine*	 *Dehydroascorbic acid*	 *Ferulic acid*
 *Galactomannan*	 *Gibberellins*	 *Gossypetin*	 *Indole*
 *Isoascorbic acid*	 *Isovitexin*	 *F Kaempferol*	 *Linoleic acid*
 *Linolenic acid*	 *Luteolin*	 *Nicotine*	 *Palmitic acid*

 *Phosphatidylcholine*	 *Phosphatidylglycerol*	 *Phosphatidylethanolamine*	 *p-Hydroxybenzoic*
 *Phytol*	 *Pipradrol*	 *Protocatechuic acid*	 *Pyrogallol*
 *Quercetin*	 *Orientin*	 *Quinine*	 *Resin*
 *Rhein*	 *Salicylic acid*	 *Saponin*	 *Stigmasterol*
 *Syringic acid*	 *Tannins*	 *Thebaine*	 *Tomatine*

Tricin

Vanallic acid

Fig 1;3D structure of the Ligands.

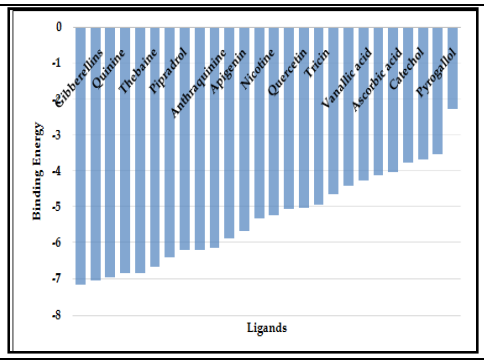


Fig 2, Ligands binding energy with E6 protein.

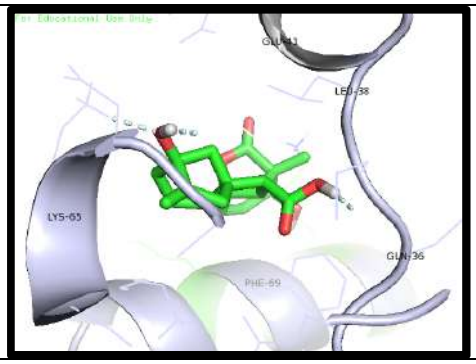
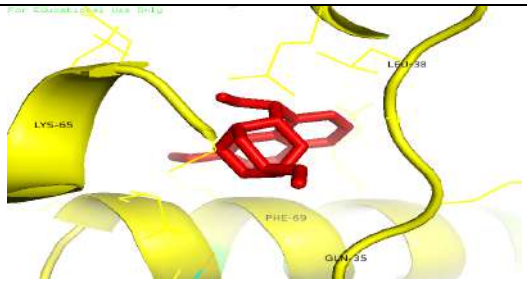
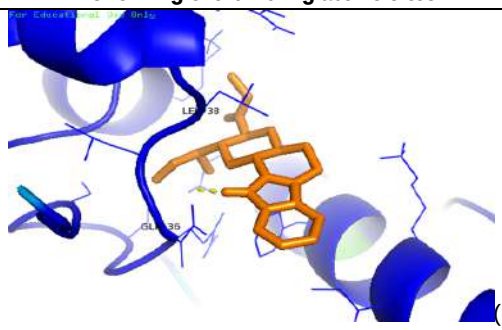


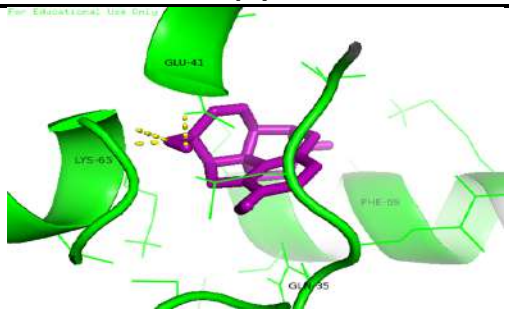
Fig 3 Gibberellins docked into the E6 protein, and showing the binding active sites



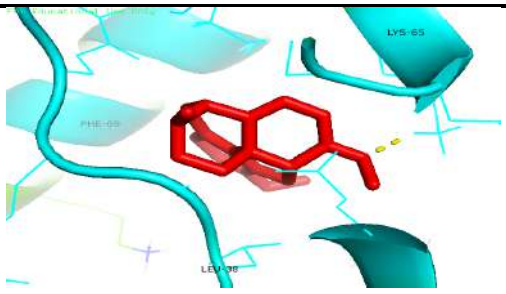
(A)



(B)



(C)



(D)

Fig 4. Showing where the ligands bind with the active residue sites of protein, (A) Quinine, (B) 18,19-Secoyohimban-19-oic acid, (C) Codeine, (D) Thebaine

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Technology and Language Development - A Review

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Received: 29 Dec 2021 Revised: 27 Jan 2022 Accepted: 21 Feb 2022

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ABSTRACT

The development of language is an essential part of human life. The significance of introducing technology into language acquisition at a young age is becoming more widely recognized. When used correctly and efficiently, technology can be one of the tools used to help young language learners create a realistic and stimulating environment. Technology also assists teachers and learners in developing their language and communicative skills. Researches in the past have indicated that, new technologies have greatly increased the opportunities for students to receive input outside of the classroom, they play an important role in the field of language development. But some researchers also have conflicting opinions regarding the impact of increased use of technology on language development in children. Hence there is a need for more research evidences in order to light with greater clarity both the positive and the negative aspects of the impact of technology on language development among the children. The aim of this study is to review the existing literatures highlighting the pros and cons of technology on language development and analysing how they can be balanced in a manner as to have a positive impact on language learning.

Keywords: Technology, Language, Development, Communicative skills

INTRODUCTION

Language development is a process which starts early in human life. Infants begin without knowing a language, yet by the time they are 10 months old they can distinguish between speech and sounds and learn to babble. Some researchers have concluded that the earliest learning begins in uteri when the foetus starts to recognize the sounds and speech patterns of its mother's voice and can differentiate from other sounds after birth. Receptive language abilities develop before verbal or expressive language abilities in children. Receptive language refers to internal language processing and comprehension. Expressive language emerges as receptive language becomes increasingly

widespread. It can also satisfy the visual and acoustic senses of the students. Pre-verbal communication, in which newborns express their intentions to others by gestures and vocalisations, is largely regarded to precede the formation of productive language. According to a general concept of development, new forms take over old functions, and a kid learns words to communicate the same communication functions that they had previously represented in proverbial ways. Normal learning processes are thought to occur throughout language development, in which children learn the shapes, meanings, and uses of words and utterances via linguistic input. Young (2013) believes that students can improve their language awareness by playing on-site games and participating in discussions in various social and cultural aspects. Ahmadi (2017), claimed that one of the most significant aspects of learning is the strategy that teachers employ in their lectures to aid language learning. Larsen-Freeman and Anderson (2011) affirmed that effective learning experiences can be gained by children by the use of technology. Gençlter (2015) states that computer technology can be used by teachers to motivate the learners for effective language learning.

Objectives

1. To review the research work done in past 18 years related to language development and integration of technology in language teaching.
2. To summarise the contribution of different researches and bring forth the gap existing in the researches related to language learning and technology.

Advent of Technology in language learning

The modern world is changing faster than ever before because of the new technological advancement. The impact is deep on all aspects of society and language learning is no exception. Even though technology dominates our daily lives in all aspects none the less the educational practitioners find ways and means to incorporate it even further and this holds good for language learning too. (Garrett, 2009). The benefit of using technology into language learning at a young age is increasingly gaining traction. Young learners require regular encouragement to avoid losing focus during the extensive language learning process. Technology has shown to be a valuable tool for creating a suitable learning environment that is also pleasant for the students. Even when they are not in their classrooms and interacting with others, technology aids young language learners in mastering their language abilities. Information and communication technology (ICT) has had a significant impact on our daily lives. It has been utilized all across the world to assist people in fields such as commerce, trade, marketing, science, and education. (2000) (Brian).

According to studies, 90% of our students have access to a computer or a mobile device at school, at work, or at home. Hence classrooms and teaching methods have witnessed a major evolution in the direction of technology. Technology has a significant role in increasing student activities and has a favourable impact on teachers' instructional approaches. Teachers would never be able to keep up with the expanding technological boom if they do not include technology into their classrooms. Solanki and Shyamlee (2012) and Pourhosein Gilakjani (2017) are of the view that language teaching has witnessed a major transformation due to the dominance of technology in it. It is investigated that in learning a new language, technological innovation is vital and effective. If used effectively, technology has the potential to favourably impact language learning by providing opportunities for language learners, providing them with endless resources, and improving cooperative learning. According to Pourhosein Gilakjani and Sabouri (2014), learners can profit greatly from the use of technology since they can control their own learning process and have access to a wealth of knowledge that their teachers do not have control over. Bennett, Culp, Honey, Tally, and Spielvogel (2000) explored that incorporating computer technology into the classroom increases teacher and student performance. Many studies have found that using technology to promote linguistic and cultural awareness in social and cultural situations can help children learn more quickly, and that intercultural dialogue can help children learn more quickly. Young learners can use technology to gain access to limitless resources and improve their language skills.

The use of technology can enhance the cooperation and healthy competition among learners which is a major tool for increasing learning among the students. (Keser, Huseyin, & Ozdamli, 2011). Bransford, Brown, and Cocking (2000),

explored that using computer technology, teachers and students can create local and worldwide societies that will help them connect with people and broaden their learning horizons. They went on to say that getting the most out of computer technology does not happen by accident; it is mostly dependent on how teachers use it in their language classrooms. Susikaran (2013) has opined that the transformation from traditional changes have been witnessed in classes other than the traditional methods of teaching because they were not sufficient to teach English effectively.

Technology Integrated Classrooms -Their role in language learning

Language learning has been more popular in recent years because to new technology-integrated teaching approaches. Blended learning, virtual classrooms, and learning management systems are some of the new era's educational tools. These organizations have embraced the use of technology in language learning to boost learner engagement, make the instructor's job easier, and provide a systematic, holistic learning experience to all participants in the teaching learning process. Various researchers have defined technology in a variety of ways. İŞMAN (2012) is of the view that use of technology in its the practical sense is the use of knowledge in a particular area to do a task especially using technical processes, methods, or knowledge. Dockstader (2008) defines technology integration as the use of technology to improve the educational environment. It helps in creating opportunities for learners in supporting the classroom teaching through by completing assignments by using the computer rather than the traditional way of using pen and paper. How teachers use technology to more efficiently execute familiar routines and how this usage can modify these activities is referred to as technology integration. Pourhosein Gilakjani Gilakjani Gilakjani Gilakjani Gilak (2017). As a result, it encompasses not only machines (computer hardware) and instruments, but also organized relationships between machines and their environments, as well as humans. Lam and Lawrence (2002) and Pourhosein Gilakjani (2017), technology helps students alter their own learning processes by providing them with access to a wealth of knowledge that their lecturers are unable to supply. Hennessy, Ruthven, and Brindley (2005) and Pourhosein Gilakjani (2017), technology integration is how teachers use technology to better conduct known activities and how this use can modify these activities.

Impact of Technology on meaningful and effective language learning

One of the most essential aspects influencing international communication is language. According to researchers, technology advancement is critical and beneficial in helping young learners learn a new language. If used correctly, technology, the internet, and some computer games can improve language learning. For proficiency and communication, students use various aspects of English language skills such as listening, speaking, reading, and writing. Swaffar (1985) stated that use of technology can help the language learners to explore the treasure of unlimited cultural knowledge and gain access. This can be interesting for students who have a chance to compare other cultures with their own. Child development and preschool learners enjoy playing with language through games such as rhymes, songs, and stories, according to Scott and Ytreberg (1990). The internet is one of the best venues to interact with language. Computers are a key teaching tool in language classes where teachers have simple access, are sufficiently educated, and have some curriculum freedom, Becker (2000). Many teachers believe that using computer technology is a crucial part of providing high-quality instruction.

Grabe & Stoller(2002) have stated that technology should be modeled by teachers in such a way that it complements the curriculum and enables the learners to promote the proper use of technology in enhancing the learning of their skills of language. Harmer (2007) and Genclter (2015) are of the view that the learners be encouraged and motivated by the teachers so that they are able to identify appropriate activities by using the computer technology which will positively impact their language learning. Cameron (2009) is of the view that motivation be given to young learners to choose course and topics from the internet. Clements and Sarama (2003) endorse this and are of the view that certainly learners are benefitted by the use of suitable technology. Tomlison (2009) and Gençlter (2015) are of the view that learning which is gelled with computer activities give the learners quick information and appropriate materials. According to Larsen-Freeman and Anderson (2011), teaching resources and technology aid in providing learners with a positive learning experience. Technology has a positive impact on language learning because it allows for the provision of authentic materials to learners, which can motivate them to learn the language.

Susikaran (2013) investigated and concluded that because the chalk and talk method was insufficient to meet the changing needs of the learner in language learning, changes in classes have occurred in addition to the traditional chalk and talk teaching methods. The use of technology has made teaching more interesting and productive, and it has significantly changed English teaching methods (Patel, 2013). Pourhose in Gilakjani (2014) Dawson, Cavanaugh, and Ritzhaupt (2008) are of the view that a positive learner centered environment is created in the classroom with the use of technology. They further added that with the use of computer technology more meaningful learning occurs because the learners become more responsible to their learning. Arifah (2014) opined that motivation of the learner is increased by use of internet. If film showing is used as a teaching aid the learners develop their knowledge and their higher order thinking skills receive a boost. Hence the use of multimedia and its related teaching methodology helps to attract the learner to English Language. Bull& Ma (2001) endorse this and add further that technology provides young learners unlimited resources and can be enhanced by intercultural communication.

The young learners are energetic and find difficulty in understanding a foreign language. Brewster, J. Ellis, G. Girard, D. (2004). The use of technology can be fruitful to them because then they can not only improve their knowledge through emails the internet, satellite and cable TV but will help them to comprehend and escalate the importance of English in their life. This will make their learning meaningful. The integration of technology in language learning also improves their language skills outside the classrooms too. According to Larsen-Freeman and Anderson (2011), technology enhances children's learning experiences. In a long language learning environment, technology also provides a wealth of authentic materials, and young learners can easily be motivated. They also stated that because children are primarily visual learners who enjoy seeing visuals while learning, online games can be beneficial to language learners. Larmer and Schleicher (1999) determined that Media and the internet can help children improve their language skills. Pensky, (2002) Young (2013) are of the view that online games improve various vocabulary fields and also gives language feedback. Harmer (2007), states that students shouldbe motivated by the teachers to explore suitable games and activities to make language learning more effective. He further stated that since computers are friends of students so computer-based language activities improve cooperative learning among the students. These can be used judiciously to bring the natural environment in the classroom.

According to Tomlison (2009), computer-assisted activities provide language learners with quick access to information and high-quality materials. The use of Multimedia encourages learners to learn more. Although it is true that children's concentration can be lost in a short period of time, providing engaging and authentic items can occasionally extend this time. Children have access to a wealth of authentic resources via the internet. But all said and done we cannot afford to overlook the factors that might affect students' acceptance of the new technology Razmah Mahmod (2005).

The American Council on the Teaching of Foreign Languages strongly recommended any language course that employs technology to facilitate language acquisition should have a language educator in charge of the planning, instruction, evaluation, and facilitation. Rather than being supplied solely through a computer programme or by a non-content specialist, language teaching is best supervised by language educators

- There is no evidence to support the isolated use of technology to learn a language.
- Working with a language instructor is critical for acquiring the kind of spontaneous interpersonal skills needed for real-world communication.
- Human connections and meaningful experiences, supported by a language instructor, are the most effective way to enhance multicultural capacity.
- Teachers enhance language learning with content expertise, research-based teaching practises, and effective technological applications.

Role of learners in technology-enhanced language learning

World-Readiness Standards for Learning Languages determined that language teachers can and should use technology to improve language instruction, practise, and evaluation. Through the strategic application of technology:

- Students read, listen to, and watch actual, interesting, and timely resources from the target culture.
- Students practise interpersonal skills by interacting with other speakers of the target language in real time via video, audio, or text.
- Students work on presentational activities with their peers or teacher at any time and from any location.
- At their own pace, students access internet content and/or use computer adaptive apps managed by their teacher.
- Students use interesting online games and applications to develop distinct skills.
- Differentiated education benefits kids because it allows teachers to assess students, assign a variety of tasks, track data, manage classrooms, provide real-time feedback, and lessons using several applications.

Language learners can use technology to help them use the target language in culturally relevant ways to do real-world tasks. It is not, in and of itself, a goal. Furthermore, all language learning opportunities should be standards-based, instructor-designed, learner-centered, and aimed at developing proficiency in the target language through interactive, meaningful, and cognitively engaging learning experiences, whether facilitated through technology or in a classroom setting. The American Council on the Teaching of Foreign Languages understands the benefits of hybrid, online, and distance learning teaching models that are aligned with state and national standards and supervised by language educators. Technology is best driven by the needs of language learners, allowing our students to have the kinds of interactions they need to be college, job, life, and world-ready.

Recommendations for Technology oriented language classes

Because technology is such a vital instrument for language learning, it undoubtedly aids learners in effectively improving all of their language learning skills.

- Facilitators, such as teachers, should be the driving force behind motivating students to use technology to improve their language skills.
- Teachers must be created more techno savvy by being trained in the use of technology in classroom language learning.
- Technical support must be provided to the teachers to implement this among the students.
- The effort for technology integration should be the collaborative efforts of all teachers irrespective of the subjects they teach.
- Teachers must be preparing their own technology-integrated lesson materials which should be student centric.

The technology plan should be tightly integrated with the pupils' academic standards. When integrating technology into the classroom, teachers should be aware of which pedagogical technique is the most beneficial (Pourhosse in Gilakjani, Leong, & Hairul, 2013).

CONCLUSION

The researchers have observed at some key aspects of the use of technology in the classroom in general, and language acquisition in particular, in this work. The study also discusses language development, some important concerns linked to the use of technology and its integration, and the role of educators and learners in enhancing language acquisition. The review indicated that before the students are guided and motivated to use the technology for language learning the teacher should himself be well versed with it the utility and ways and means by which it will enhance language learning should be ascertained thoroughly. The review brought about the changes that technology can bring in the students if it is used correctly and judiciously, the review also brought about how the use of technology can enhance cooperative and collaborative learning among the students and also make the teaching learning process enjoyable.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Lower Limb Muscles Explosive Training on Bio motor Abilities among Breaststroke Swimmers: A Randomized Control Trial

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 19 Mar 2022

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ABSTRACT

Among the four competitive swimming strokes, breaststroke is the one where the major source of propulsion is generated in cyclic and simultaneously movements of the lower limb during the kick. The purpose of the present study is that the breaststroke kick, especially the whip kick, makes a major contribution to the forward propulsion in breaststroke., in this study we are finding the effect of lower limb muscles explosive training on bio motor abilities among breaststroke swimmers. To study the effect of lower limb muscles explosive training on bio-motor abilities among breaststroke swimmers (power and speed). . 42 breaststroke swimmers will be selected based on inclusion and exclusion criteria with age of 12-18 years. They will be randomly assigned into two equal groups. The control group (Group-A). received no treatment. The intervention group (Group-B). will receive lower limb muscles explosive training. The treatment will be given for 3 days a week for 6 weeks continuously. Pre-test and post-test measurements of vertical jump test and 50-meter sprint for power and speed respectively will be conducted. There was significant difference found in the group A ($p>0.05$).. The data was assessed by SPSS 21.0 using paired t-test for intra and inter-group analysis. It is concluded that the explosive training of the lower limb muscles is beneficial for improving the bio-motor abilities of the breaststroke swimmers. Hence it can be included in the training of the swimmers.

Keywords: Breaststroke, Swimmers, Vertical jump test, speed, lower limb muscles, explosive training, Bio-motor ability, whip kick

INTRODUCTION

In the 19th century, competitive swimming got listed as an organized sport and it got internationally renowned(1,2). Swimmers are rewarded on the based on how quickly one has completed the distance in short span of time(1). Swimming is whole body movement in which along with upper and lower body, the trunk also moves in simultaneously in a complex manner(3). Swimming is considered as recreational activity and nowadays it is more popular as competitive sports which demands high level of training. (3). Many factors help the expert swim start to be well known. Such factors are (i). reaction time, (ii). glide time and (iii). transition time(4,5). Reaction time is the time taken from the start signal to action on the block along with the foot control, orientation and regulation of the foot force(4,6,7). Glide time is the time taken before the leg kicking begins. Transition time is the time needed to from leg kicking phase to full swimming with arm stroking. (4). Less skilled swimmers at start swim should practice all the above phases more so it will help to reduce the time taken to complete. (6,7,8). When the force is applied against the ground it generates an equivalent ground reaction force which helps the athletes to move and propel them forward(9). According to Newton's third law, foot contact on the ground (action). generates a resultant force that is equal in magnitude and opposite in direction(9). Running at slow speeds generates lesser ground reaction force when compared to faster running speeds. For the optimal movement control an appropriate amount of force is necessary in the desired direction which is applied by the lower body joints and muscles. (9). Repeated practice is needed of these movements by the athletes in various conditions in order to improve their performance. Improvement in the bio-motor abilities such as athlete's strength, speed and endurance is because of the muscular adaptation and proprioception which is due to the regular training(10). Example, in competitive situation it is necessary for the football player to play swiftly and without fatigue which can only be possible by the regular practicing their ball handling techniques.

Force created on the ground is ground reaction force which helps the athlete to move forward same way for the swimmers there is force created in water i.e. buoyancy and drag. An athlete propels forward only when the resistance of the water is less compared to the force applied by the athlete's body. (11,12). Foot is used as the propulsive tool in the kicking while swimming to increase the force applied against the resistance of water(13). There is passive movements of foot and ankle in the skilled swimmers while force is transferred by the muscles of hip and knee joints(13,14). This helps in the improvement of the kicking speed which indirectly improves the overall speed(1). For the swimmer high intensity program is more beneficial than high volume program. (15).in the field of scientific research, the most difficult task is to conduct research on competitive swimming(16). The researches on human kinematics is complex because human body have more complex biomechanical determinants when component to other while assessing the human body in the other habitat which is not then natural habitat of living such as water it become more difficulty as other physical principles have to be considered(16). There are four main styles in swimming: freestyle, backstroke, breaststroke and butterfly(40). Among the four styles, the breaststroke has the discontinuous motion (17). and it also produces the largest forces mainly by the legs(18,19,20). The breaststroke kick is also known as the whip kick and the forward propulsion is majorly contributed by the whip kick(21). Among the four competitive styles, the whip kick if done technically, is known to be the most propulsive one as well as breaststroke also has the lowest mean velocity(21). The breaststroke kick is subdivided into 4 phases or sweeps-1). propulsion, 2). in-sweep, 3). glide, 4). recovery (21,22).. The completion of all these 4 phases is characterised as one full breaststroke cycle. (21). The most important part of the whip kick is flexion -extension in sagittal plane(17,22,23). To generate forward propulsion in the breaststroke the upper and lower limbs as well as the trunk has to move in a dynamic and cyclic motion simultaneously and mainly this cyclic and simultaneous movement is carried out by the lower limb during the leg kick(22). Advanced kinematic analysis based on research proved that propulsive force of the stroke is the leg kick, and it occurs as the third propulsive force. (24). A powerful whip kick, propulsion is produced during the movement by the high rom, flexibility and above average musculoskeletal forces. As walking like a duck as described by others for becoming a good breaststroker, there is a major role of structure of leg and feet. (24). Due to which there is high risk of injury, in breaststroke swimming, the overuse injury as well as the injury to the knee is common compared to other styles. (25). It is known as breaststroker's knee, hence to minimize the risk of

injury it is necessary to strength the lower body and also improve their stroke skills along with their physical attributes. (25). Prevalence of injuries in breaststroke swimmers is 86% according to the survey of 36 competitive breaststroke swimmers. (25). The most crucial part of training series is dry land training for swimmers. (26). Explosive exercises are the exercise which require maximum or near maximum rates of force development. (27). They can be either isometric or dynamic. Factors that accord directly to the explosive exercise are rate of activation of muscles and synchronisation. (28). Explosive power output being the main determinant of performance produce a high velocity at release or impact activities requiring one movement sequence. (28,29). In throwing, jumping and kicking activities, explosive muscles actions is required. During various sports e.g. (football, baseball and gymnastics). when rapidly changing direction or accelerating, the sudden bursts of power are needed. (28).

Most of the studies focuses on recommending the general endurance, arm strength, core strength, swimming economy and flexibility training for enhancing the physical development and other motor abilities. (30). Bio-motor abilities plays an essential role in all the sports in achieving the goals of the athletes(31). Anthropometry, biomechanical, psychological and physiological all these factors will help the athlete to excel in performance(2,30,32). All the above factors go in hand in hand in sports as well as for normal person(31). There are several bio-motor abilities such as strength, power, flexibility, balance,(33).etc but according to the EMG studies in breaststroke, for the forward propulsion, the force is generated by the lower body more compared to upper body(23,34). and the force is assessed by power generated by the lower body as per the force-power relationship and the other important bio-motor ability is speed which is required in all the sports and is the common factor(35). speed is the important motor ability for the sports performance(56). The more powerful kick will help the swimmer to cover the distance in shorter period of time. For the powerful kick, the enhancement of explosive power in the legs is essential for achievement in various sports such as in basketball for re-bouncing, volleyball for attacking and in football for blocking(36). Not all the above activities rely on vertical jump but vertical jump test can be useful for measurement of power of legs indirectly(37,38). As vertical jump test can be done both on field and laboratory. The vertical jump test done in laboratory will provide with accurate measures but these are more expensive and less viable for use. Luckily, the field test does exist which are less expensive and more practical. (39).

Need of Study

Swimming use the bio-motor abilities of power and speed. It is recognized that the major contribution in forward propulsion in breaststroke is done by the breaststroke kick, known as whip kick. The breaststroke swimmers need to complete the four phases: 1). recovery (2). down sweep, (3). in sweep, and (4). glide (propulsion). but there is no specific protocol for lower limb muscles which improves the bio-motor abilities like power and speed.

So, the need of my study is to evaluate the effect of lower limb muscles explosive training in breaststroke swimmers.

Aims and Objectives

Aims

This study aims to know the effect of lower limb muscles explosive training in breaststroke swimmers in terms of power and speed.

Objectives

To know the effect of lower limb muscles explosive training on power in breaststroke swimmers.

To know the effect of lower limb muscles explosive training on speed in breaststroke swimmers.

To compare the interventional group with the control group

METHODOLOGY

Method

Study design- Experimental study
Study setting – RMC swimming pool, Race Course, Rajkot
Sampling technique – Purposive sampling
Study population – Elite swimmers
Sample size- 42 swimmers
Study duration- 6 months

Criteria For Selection

Inclusion Criteria

Elite swimmers
Age group- 12 to 18 years (males and females).
Doing swimming for at least 2 years with at least 10 hrs in a week

Exclusion Criteria

Any recent injury and undergoing any lower limb strength training.
Not involved in the Game from the last 6-8 week

Procedure

The study was registered under Clinical Trial Registry-India (CTRI). and the ethical approval was taken by the ethical committee of R.K. University prior to data collection. On the basis of NOC letter and ethical clearance approach to the swimmers was done at Shri Lokmanya Tilak Academy, Race Course, Rajkot.NOC in written was taken from the coach in return. The study process was briefly explained to the subjects and their parents. An informed consent was signed by the parents of the subjects who willingly participated. After the consent from the parents, 42 subjects were enrolled on the basis of inclusion and exclusion criteria. Two groups were assigned randomly with 21 subjects in each group by chit draw method. Group A was experimental group and Group B was control group. After the group allotment, the pre data was taken for power and speed. The lower limb muscles explosive training was started for the experimental group after the completion of pre data whereas no training was given to the control group. Training was given for 3 days/week for 6 weeks. Post data was assessed after the completion of 6 weeks training. Pre data and post data were analyzed by SPSS version 21 for both inter and intra group analysis.

Intervention

Lower limb muscles explosive training was given for 3 days/week for 6 weeks. The protocol included many exercises with body weight as well as free weights.Warm up-(10 mins).-Ankle rotation, kicks-forward, backward and sideways, marching, heel up and toe up, stretching (hamstring, quadriceps and calf). The exercises are enlisted below: Bended knee jump, Squat jump, jump to side with one leg, standing board jump, jump missile, Isometric squats, Lunges, step up, Calf raises-30 repetitions,3 sets with rest of 2 mins after each set.

RESULT

Table 1. Intra-group analysis of vertical jump test in GROUP A

GROUP A-VERTICAL JUMP TEST		MEAN	SD	T value	p value
	PRE	31.09	4.03	-17.93	0.001 (S)

Table 2 Intra-group analysis of 50-meter sprint test in GROUP A

		MEAN	SD	T value	p value
GROUP A-50-METER SPRINT	PRE	35.66	5.85	6.21	0.001 (S)
	POST	31.47	5.76		

Table 3:Intra-group analysis of vertical jump test in group B

		MEAN	SD	T value	p value
GROUP B-VERTICAL JUMP TEST	PRE	30.42	3.80	0.37	0.18
	POST	30.38	3.72		

Table 4: Intra-group analysis of 50-meter sprint test in group B

		MEAN	SD	T value	p value
GROUP B-50-METER SPRINT	PRE	34.71	4.84	-1.56	0.12
	POST	35.57	5.52		

Table5: Inter-group analysis of pre-vertical jump test in both groups

GROUP	VERTICAL JUMP TEST	MEAN	SD	T value	p value
A	PRE	31.09	4.03	0.55	0
B	PRE	30.42	3.80		

Table 6: Inter-group analysis of post-vertical jump test in both groups

GROUP	VERTICAL JUMP TEST	MEAN	SD	T value	p value
A	POST	33.71	3.74	2.89	0.006 (S)
B	POST	30.38	3.72		

Table 7: Inter-group analysis of pre 50-meter sprint test in both groups

GROUP	50-METER SPRINT	MEAN	SD	T value	p value
A	PRE	35.66	5.85	0.57	0.56
B	PRE	34.71	4.84		

Table 8: Inter-group analysis of post 50-meter sprint test in both groups

GROUP	50-METER SPRINT	MEAN	SD	T value	p value
A	POST	31.47	5.76	2.34	0.02 (S)
B	POST	35.57	5.52		

DISSCUSION

The objective of this study was to evaluate the effect of the lower limb muscles explosive training on bio-motor abilities in breaststroke swimmers in terms of power and speed. 42 subjects were enrolled between the age 12-18 years and were divided into two groups. Subjects of group A were treated with lower limb muscles explosive training and the group B subjects were given no training for 3 days/week for 6 weeks. The exercises were given for 60 mins. The vertical jump test and 50-meter sprint were assessed on 1st day and after 6 weeks. The results showed statistically significant improvement (p=0.001). in the group A after 6 weeks of intervention for the vertical jump test and 50-meter sprint by the intragroup comparison by using the paired t-test. But there was no statistical significance found in the group B (p=0.18). and (p=0.12). for vertical jump test and 50-meter sprint respectively. The intergroup comparison was done using independent t-test between groups for vertical jump test and 50-meter sprint also states that there was statistically significant improvement and the p value confirms it. (p=0.006 and p=0.02). Hence, the

result of the present study rejects the null hypothesis and supports the experimental hypothesis by its various analysis in the form of intra group and inter group. In the present it was seen that there was improvement in the interventional group who received explosive training and similar results are seen in study done by Daniel J. Dodd, *et al* (2007). who analysed various explosive training modalities to improve lower-limb power in baseball players and stated that acute explosive training alone or combined with other training offers increase in lower body speed and power due to neurological and muscular adaptations that occurs through the explosive training in single episodes in the baseball players(50).

Explosive exercises helps in increasing the power and speed as there is greater potential rise in the type II muscle fibres recruitment, secondly there is also increase in motor unit firing and the synchronicity because of which it provides the ability to use maximal or near maximal force as fast as possible by training the muscles to quickly switch from eccentric to concentric motion and reducing the delay time between the motion, thus less time is needed to finish more work(51,55). In swimming, the swimmer not only has to swim quickly but it also has to take start and turns in water as quickly as possible. In order to have powerful start and turn the swimmer should have power in the legs. In the study by Julian Jones *et al* (2017). who has stated that six-week training intervention where two dry-land training modalities were implemented with elite swimmers comparing traditional strength training to ballistic training, only the ballistic training group exhibited small positive changes in aspects of the push off stage of swim turn also supports the present study. During the isometric actions, there is increase in the neural input to agonist muscles due to the explosive strength training. As there is increase in the firing frequency of the motor unit it leads to increase in neural input. During, the explosive type strength training, the neural activation of muscles is high and the time required for muscle activation is very short which results in less hypertrophy of training induced muscles when compared to traditional resistance training. (51-54). According to Brice *et al* (2015). there are studies which shows the activation of the lower limb muscles such as tibialis anterior, biceps femoris, rectus femoris and gastrocnemius in the breaststroke swimmers and also studies that states whip kick i.e. breaststroke kick is the most propulsive kick among the other strokes and major propulsion occurs by the whip kick(53).

In the recovery phase as well as the kick phase rectus femoris has the major contribution in hip flexion and knee extension respectively. As rectus femoris is a 2- joint muscles, it plays an important role in both the phases. Biceps femoris does not have as much role as rectus femoris but it is actively in the kick phase. The other most important muscle is the tibialis anterior it is active during both the phases recovery as well as early kick phase. It has the main role of foot dorsi-flexion. So, if the strength training is done it is oblivious to have muscular as well as neural adaptation in the above muscles which will indirectly improve the power and speed of the swimmers. (3). Lower limb muscles explosive training helps the swimmers to perform the whip kick more dynamically and efficiently. It helps the swimmers to perform the kick in a synchronized manner.

HeydarSadeghi *et al.* (2014). concluded 6-week plyometric training program helps in enhancing the lower leg as well as arm power in the male swimmers aged 10-14 years(50). as seen in Z Jastrzebski (2014). et alstated in their study that 6-week explosive training helps to increase the power in the lower limb in volleyball players(52). These are the articles that supports the present study and it proves the experimental hypothesis by showing the improvement in power and speed by lower limb muscles explosive training. Swimming is considered as the competitive sport which requires a swimmer to speedily complete the given distance as well as there is ample importance of explosive power of lower body for breaststrokers. Hence to be a winner in competition, the swimmer should be trained for speed and lower body muscle power. According to the review of literature by Stewart Bruce-Low (2007). it concluded that there is pose to risk of injury by doing explosive exercises and it should be done with other slow, controlled weight training (55). but in this study, there were no injuries seen in the duration of 6 weeks. So explosive training can be considered alone for training when executed correctly. Power is equal to force multiplied by distance upon time. Here distance upon time can be considered as speed. So, power is directly proportional to speed and strength. As there is increase in power there will be increase in speed and strength. (28,54). Also, the present study shows a significant difference in the pre data and post data values of the interventional group which received lower limb muscles explosive training. So, this may be the reasons for the increase in speed and power among breaststroke

swimmers. In this study there was limited sample size, as the sample were taken from only Rajkot. Further study can be done on all the breaststroke swimmers of Saurashtra region with the same protocol. The power was measured by vertical jump test (Sargent test). due to lack of instrumentation can also be measured by force plates for more accurate result. In relation to this study, the training was given for only lower limb as it is seen that major forward propulsion in breaststroke is done by legs but more studies should be done by using combination of other training such as eccentric strength training on both upper and lower limb in breaststroke swimmers as well as other swimming styles.

CONCLUSION

According to the results of the current study, there is significant improvement in the interventional group and no improvement was seen in the control group. So, it is concluded that 6 weeks of explosive training of the lower limb muscles is beneficial for improving the bio-motor abilities i.e. power and speed in the breaststroke swimmers. Hence it can be included in the sports-specific training of the swimmers as it helps in the injury prevention and enhancement of the performance

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antidepressant Activity of Novel Synthetic Benzoxazole Derivative in Depressive Paradigms of Swiss Albino Mice.

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Received: 10 Jan 2022 Revised: 12 Feb 2022 Accepted: 13 Mar 2022

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ABSTRACT

Benzoxazole is one of the compound which have various of pharmacological activities, alteration in the benzoxazole nucleus have resulted in a large number of compounds which have varied pharmacological activities and addition of triazole to it helps in explore potential central nervous system (CNS) actions. The said test compound the triazole substituted benzoxazole derivative (TSBD)could potentially have antidepressant action. The test compound prepared by focusing on construction of biologically potent molecules. The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum, after obtaining ethical committee approval. Forced swimming test done using mice where mice were randomly divided into groups of 5 mice each and treated as follows for 5 days before the behavioral test; control (normal saline, 2 ml/kg p.o.), Fluoxetine (20 mg/kg,) and TSBD (50,100, and 200 mg/kg, I.P.). The CNS stimulatory or antidepressant effect of the TSBD was indicated by its potential to reduce immobility time of mice during force swimming test. Tail suspension test using mice where mice of either sex were randomly divided into groups of 5 mice each and treated as follows for 5 days before tail suspension test; control (normal saline, 2 ml/kg p.o.), Fluoxetine (20 mg/kg,, p.o.) and TSBD (50,100, and 200 mg/kg, I.P.). The CNS stimulatory or antidepressant effect of the TSBD was indicated by its potential to reduce immobility time of mice during tail suspension test. Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (New mannkeuls multiple range tests). Differences between means were considered significant at 5% level of significance ($P \leq 0.05$). The antidepressant activity tested TSBD using tail suspension test and force swimming test on swiss

albino mice, shows the significant reduction in the immobility time of the mice both the models and the effects were dose dependent dose used were 50,100 and 200mg/kg of TSBD.

Keywords: triazole, TSBD, benzoxazole, mice

INTRODUCTION

Depression shall become the second biggest illness in terms of morbidity by the next decade in the world, as for now out of every twelve men and five women have depression, according to the estimation of WHO. Apart from adults, school children (2%) and teenagers (5%) also suffer from depression, and these chiefly go unidentified. Most comments reason why people go to psychiatrists is because of depression, even though the layman's awareness is that all psychological problems are depression [1, 2]. Depression is a mental disorder associated with the interaction of social, psychological, and biological factors. Several etiological factors can trigger the development of this mood disorder which includes genetic, biochemical parameters, and personal and environmental conditions[3].Some of the depression related physical illness show an enigmatic mind body connection such as obesity, diabetes[4], cardiovascular diseases[5], cancer, lung diseases, stroke[6] and hearing and vision loss[7]. The main biomarkers for diagnosis of depression are the levels of monoamine neurotransmitters like serotonin, dopamine, nor-adrenaline, corticotrophin-releasing factor, cortisol, and adreno-corticotropic hormone, and also the actions of hypothalamic–pituitary–adrenal (HPA) axis, adenylyl cyclase, and monoamine oxidase (MAO) [8].

Tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors and specific serotonin-nor-adrenaline reuptake inhibitors are group of drugs currently available for the treatment of depression [9]. However, side profile effect profiles regarding libido, sleep, body weight, and cardiovascular system because of the use of these drugs in treatment for depression are still not at desired level [10-12]. Today, a large number of efficient treatment options are available for depression, but the fact is that no perfect solution exists that works quickly and is free of adverse reactions. Therefore, researchers are seeking alternatives to produce more specific, newer, safer, and cheaper medications. In the field of medicinal chemistry benzoxazole derivatives have occupied unique place because they possess diverse variety of pharmacological activities. Benzoxazole discover its utilization in research as a primary compound for the bioactive and larger structures. Alteration in the benzoxazole nucleus has yielded in a huge number of molecule having divergent pharmacological activities. The benzoxazole moiety is explored its pharmaceutical importance and is being studied for its pharmacological assistance in different circumstances. Literature survey indicates recognition and isolation of several benzoxazole derivatives of importance in pharmacology as antibacterial and antifungal agents[13], HIV-1 reverse transcriptase inhibitors[14], topoisomerase-I inhibitors[15], anticancer agents[16] and in the treatment of Alzheimer's disease[17]. Looking into the pharmacological importance of benzoxazole moiety, it is worthwhile to screen the derivatives for their biological activities in various animal models for potential medical indications.

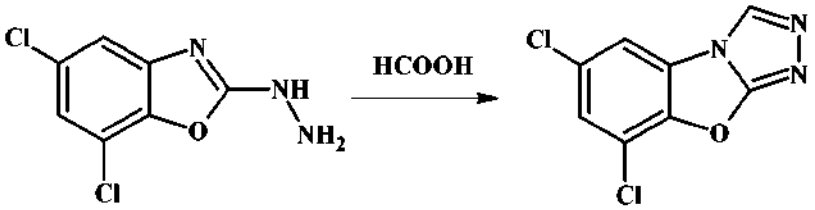
Addition of a triazole nucleus which has a planer five-membered heterocyclic system containing three nitrogen atoms, one pyrrole and two pyridine type [18], to the benzoxazole moiety could help explore potential central nervous system (CNS) actions of the substituted compounds.Two such structural isomeric forms of triazole are 1,2,3 and 1,2,4[19].Of these two derivatives 1,2,4-triazole derivative has been explored for various pharmacological actions. On the basis of the pharmacological profiles of triazoles, their antidepressant properties have been well documented. Nefazodone has triazole nucleus and possesses antidepressant activity. It inhibits the binding of [3H] ketanserin to cortical serotonin 2 (5-HT2) binding sites, it antagonizes the 5-HT2 and also inhibits cortical serotonin uptake[20]. The following 1,2,4-triazole derivatives found application in medicine: alprazolam (tranquilizer)[21], etoperidone (antidepressant)[22],nefazodone (antidepressant, 5-HT_2 antagonist)[20]. Owing to the similarity in structure to alprazolam, the 1,2,4-triazole derivatives could have potential anti-anxiety properties too. As serotonin receptors may directly or indirectly depolarize or hyperpolarize neurons by changing the ionic conductance and/or

concentration within the brain cells. Hence, the said test compound the triazole substituted benzoxazole derivative (TSBD) 6,8-Dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole could potentially have antidepressant action. Thus, the present study would aim at exploring the antidepressant activity of novel synthetic benzoxazole derivative in depressive paradigms of swiss albino mice.

MATERIALS AND METHODS

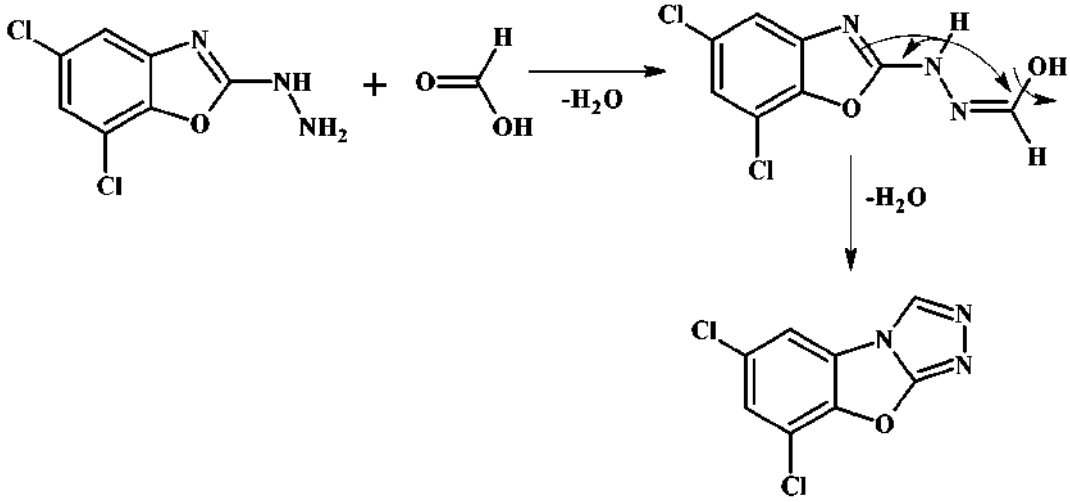
Preparation of 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole

The research work was focused on construction of biologically potent molecules. Triazoles are known for diverse pharmacological activities. Hence the intermediate was converted to triazole by reacting with formic acid. The reaction and its mechanism is mentioned in the scheme-1 and scheme-2respectively [23].



5,7-dichloro-2-hydrazino-1,3-benzoxazole **6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole**

Scheme-1



Scheme-2

The structure of the product 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole was confirmed by the spectral studies. It showed the absence of both -NH and -NH_2 groups in its IR spectrum, ^{1}H NMR and mass spectral studies. In the IR spectrum, the stretching frequency at 3367 cm^{-1} and 3481 cm^{-1} disappeared (fig.1). A peak at δ 4.6 for two protons of -NH_2 and at δ 9.3 for a proton of -NH disappeared and a new peak was observed at δ 12.18 confirmed the structure. The signal obtained for proton at δ 7.17 (s, 1H, Ar H) δ 7.35 (s, 1H, Ar H) and at δ 12.18 (s, 1H, for -N=CH) confirmed the formation of compound. Mass spectrum at M^+ (228), M^{+2}(230) and M^{+4}(232) corresponds to its molecular weight.

Animals

The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum. Permission and approval for animal studies were obtained from the K.M.College of Pharmacy, Madurai.

Evaluation of antidepressant activity

Forced swimming test

Mice were randomly divided into groups of 5 mice each and treated as follows for 5 days before the behavioural test; control (normal saline, 2 ml/kg p.o.), Fluoxetine (20 mg/kg,) and TSBD(50,100, and 200 mg/kg, I.P.). For assessing antidepressant activities, we employed the method described by Porsolt et al. (1977; 1978) [24]. The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice were individually placed in a circular tank (46 cm tall × 20 cm in diameter) filled with tap water (25 ± 1°C) to a depth of 20 cm and left there for 5 min. During this period, the behavior of the animals was recorded by an observer. Mice were considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water. The CNS stimulatory or antidepressant effect of the TSBDwas indicated by its potential to reduce immobility time of mice during force swimming test.

Tail suspension test (TST)

Mice of either sex were randomly divided into groups of 5 mice each and treated as follows for 5 days before tail suspension test; control (normal saline, 2 ml/kg p.o.), Fluoxetine (20 mg/kg,, p.o.) and TSBD(50,100, and 200 mg/kg, I.P.).. The total duration of immobility induced by tail suspension was measured according to the methods described by Steru et al, (1985) [25]. Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and were motionless. The CNS stimulatory or antidepressant effect of the TSBDwas indicated by its potential to reduce immobility time of mice during tail suspension test.

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (New mannkeuls multiple range tests). Differences between means were considered significant at 5% level of significance ($P \leq 0.05$).

RESULTS

Effect on Force Swimming Test

Treatment with TSBD (50,100, and 200 mg/kg, I.P.) to mice for five days significantly ($p<0.001$) reduced immobility duration though in dose-dependent fashion in mice during force swimming test when compared to control. The standard drug, Fluoxetine (20 mg/kg), similarly produced a significant ($p<0.001$) reduction in the immobility time of the mice when compared to control. . The reduced observed immobility time by the TSBD and Fluoxetine pretreated mice are demonstration of their antidepressant activities. The effect of the TSBD (50,100, and 200 mg/kg, I.P.) was higher than that of the standard drug, Fluoxetine portraying a superior antidepressant action (Table 1).

Effect on Tail Suspension Test

Treatment with TSBD (50,100, and 200 mg/kg, I.P.). to mice for five days significantly ($p<0.001$) reduced immobility duration in a non-dose-dependent fashion during tail suspension test when it was compared to control. The TSBD (50,100, and 200 mg/kg, I.P.) exerted prominent reductions in the immobility time which were higher than that of the standard drug. The standard drug, Fluoxetine (20 mg/kg) exerted a significant ($p<0.001$) reduction of the immobility time of the mice when compared to control. These reductions in observed immobility time by the TSBD and

Fluoxetine (20 mg/kg) pretreated mice are clear demonstration of antidepressant activities of the TSBD and drug. (Table 2).

DISCUSSION

In this study, evaluation of the effect of 1,2,4,Triazole derivative such as TSBD on central nervous system was carried out in mice using different models tail suspension test and force swimming test. The TSBD (50,100 and 200 mg/kg) was found to cause significant dose-dependent significantly reduced the immobility time of the mice in force swimming and tail suspension tests. The CNS stimulatory effect of the TSBD was further supported by its potential to reduce immobility time of mice during force swimming and tail suspension tests. Forced swimming and tail suspension tests are two of the most commonly used animal models of depression for antidepressant screening. In the forced swimming test, the development of immobility when mice are placed into an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior [26]. The tail suspension test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressants are able to reverse the immobility and promote the occurrence of escape related behavior.

Both models of depression are widely used to screen new antidepressants. These tests are quite sensitive to major antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, MAO inhibitors, and atypical antidepressant [27]. Forced swimming and tail suspension tests which represent the behavioral despair model, claimed to reproduce a condition similar to human depression. The tests are based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e. behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli [26]. It is well known that clinically effective antidepressants (such as fluoxitine) typically increase the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test. This was observed in this study. Similarly, the results of this study suggest that the TSBD exhibited significant antidepressant activity with a strong psychomotor stimulation.

CONCLUSION

The antidepressant activity tested TSBD using tail suspension test and force swimming test on swiss albino mice, shows the significant reduction in the immobility time of the mice both the models and the effects were dose dependent dose used were 50,100 and 200mg/kg of TSBD.

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Table1. The effects of TSBD on Forced swimming test

Groups	Treatment	Immobility period(min/Sec)
Group-1	Normal saline, 2 ml/kg p.o.	5.05 ±0.22
Group-2	Fluoxetine (20 mg/kg, p.o.	1.60 ±0.06*a
Group-3	TSBD -50mg/kg.I.P	2.02 ±0.12*a
Group-4	TSBD -100mg/kg.I.P	1.92±0.08*a
Group-5	TSBD -200mg/kg.I.P	1.83 ±0.11*a

Note: Percent inhibition expressed as mean ±SEM.

*a- values are significantly different from Normal control

P <.0001, considered as extremely significant

Table 2. The effects of TSBD on Tail Suspension Test.

Groups	Treatment	Immobility period (min/Sec)
Group-1	Normal saline, 2 ml/kg p.o.	4.54 ±0.20
Group-2	Fluoxetine (20 mg/kg, p.o	1.32 ±0.04*a
Group-3	TSBD –50mg/kg.I.P	1.68 ±0.09*a
Group-4	TSBD -100mg/kg.I.P	1.57±0.06*a
Group-5	TSBD -200mg/kg.I.P	1.49 ±0.05*a

Note: Percent inhibition expressed as mean ±SEM.

*a- values are significantly different from Normal control

P <.0001, considered as extremely significant

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Association of Classic and Emerging Cardiovascular Risk Variables with Micronutrient Levels in Patients with and without Metabolic Syndrome

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Received: 31 Jan 2022 Revised: 17 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

A metabolic syndrome (Mets) is a group of biochemical and hormonal changes that predispose to type 2 diabetes and heart disease. There has been a lot of research done globally to find more accurate diagnostic and prognostic indicators for metabolic syndrome. While global research is ongoing, there are few detailed studies. The current study aimed to link metabolic syndrome patients' physical, anthropometric, and socioeconomic status to biochemical and dietary changes. In this study, metabolic syndrome patients had significantly higher glucose, TC, LDL-C, TG, and Apo B levels than age- and sex-matched controls. However, compared to age and sex-matched control subjects, metabolic syndrome patients' HDL-C and Apo A1 levels were significantly lower. Finally, a lack of vitamin D and other micronutrients has been linked to the syndrome's components like obesity and atherogenic dyslipidemia, as well as cardiac health. These findings suggest that metabolic syndrome causes cardiovascular risk, some of which we can control and others we cannot. Rise in prevalence, morbidity and, mortality can be attributed to changes in lifestyle and eating habits, smoking, and/or drinking. Coronary artery disease is caused by diabetes and hypertension. Reducing oil consumption and quitting smoking and drinking may help reduce disease prevalence, morbidity, and mortality. Controlling diabetes, blood pressure, and hypertension all reduce the risk of coronary artery disease. An increase in natural antioxidant intake will act as a preventative measure.

Keywords: Metabolic syndrome, Micronutrients, Cardiovascular disease, Biomarkers, Conventional cardiac markers, emerging cardiac markers

INTRODUCTION

The metabolic syndrome is a collection of metabolic abnormalities that have been linked to an increased risk of obesity, type 2 diabetes, and heart disease [1]. Mets are brought on by a combination of metabolic, genetic, and environmental factors [2]. People with metabolic syndrome are more likely to develop heart disease, stroke, vascular dysfunction, and all-cause mortality [3]. Mets have risen in prominence in recent years as a result of the worldwide obesity epidemic. Anthropometric indicators used by MetS are BMI and waist circumference (WC). Hyperglycemia, dyslipidemia, and hypertension are all linked to MetS components. Despite the fact that BMI is the most widely used obesity indicator, it does not account for body shape. WC provides a more precise picture of body fat distribution. In MetS diagnostic criteria, high WC has been identified as a predictive factor. Mets are characterized by atherogenic dyslipidemia, which is characterized by elevated TG, decreased HDL, and elevated LDL. Each of these anomalies is a source of atherosclerosis in and of itself [4]. Traditional risk factors such as physical inactivity, smoking, obesity, hypercholesterolemia, hypertension, and diabetes are all linked to CVD [5]. This means that the primary risk factors for coronary artery disease have yet to be discovered. By altering risk factors through a healthy lifestyle, CVD can be avoided or delayed (primary prevention). In addition, lifestyle changes may improve health as a secondary prevention measure. Nutrition plays a major role in CVD genesis. They play a role in the pathogenesis, progression, and consequences of mortality and morbidity associated with a variety of chronic diseases, including cardiovascular disease. Micronutrient supplements are frequently used to help prevent chronic diseases like atherosclerosis. However, research on the relationship between dietary micronutrients and cardiovascular disease risk is still lacking [6]. The majority of related studies have failed to show that micronutrient supplementation improves subclinical atherosclerosis indicators. That is the question this study seeks to answer.

MATERIALS AND METHODS

Study participants with MetS were chosen for the test group. Adults between the ages of 25 and 60 with Metabolic Syndrome were included in the study. The modified NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) MetS criteria were used to classify all of the subjects. All participants were given a structured proforma to fill out in order to collect information about their behavioral, medical, personal, and familial history. A photometric analyzer was used to analyze the lipid profile. The Immuno turbidimetric method was used to perform the Apo A1 and Apo B assays.

RESULTS AND DISCUSSION

Table 1 describes conventional risk markers of CVD, results are expressed in mean and standard deviation. Serum levels of various risk markers in subjects with and without metabolic syndrome were compared in table 2. Table 2 compares serum levels of risk markers in patients with and without metabolic syndrome. BMI has a Z statistic of -4.330 and a p-value of 0.000. p-value less than 0.05 rejects the null hypothesis and shows a significant difference in serum BMI levels between subjects with and without metabolic syndrome. This is SBP's Z statistic: -10.266, 0.000. In this case, the p-value is less than 0.05, so we reject the null hypothesis. DBP has a Z statistic of -5.779 and a p-value of 0.000. p-value less than 0.05, we reject the null hypothesis and conclude that DBP serum levels differ between subjects with and without metabolic syndrome. This means that the p-value for FBS is 0.000. In this case, the p value is less than 0.05, so the null hypothesis is rejected. PPBS has a Z statistic of -13.247 and a p-value of 0.000. PPBS serum levels are significantly different between subjects with and without metabolic syndrome (p-value less than 0.05).TC's Z statistic is -12.470 and p = 0.000. In this case, the p value is less than 0.05, so the null hypothesis is rejected. TG's Z statistic is -6.646 and p = 0.000. In this case, the p value is less than 0.05, so we reject the null hypothesis. HDL's Z

statistic is -5.356 and p = 0.000. To conclude that there are significant differences in HDL serum levels between subjects with and without metabolic syndrome, we reject the null hypothesis. LDL has a Z statistic of -10.596 and a p-value of 0.000. In this case, the p value is less than 0.05, so we reject the null hypothesis. Its Z statistic is -3.191 with a 0.001 p-value. p value less than 0.05 rejects the null hypothesis and shows a significant difference in APO B serum levels between subjects with and without metabolic syndrome.APO A1 has a Z statistic of -0.528 and a p-value of 0.597. There is no significant difference in APO A1 serum levels between subjects with and without metabolic syndrome because the p-value is greater than 0.05.

Correlation results for the correlation between micronutrients and emerging markers are presented in table 3.Vitamin D, Vitamin C, Folate, Vitamin B6 PLP, Vitamin B12, Magnesium, Zinc, and Calcium have no significant correlation with APO B. The correlation coefficient between APO B and Phosphorus is -.197, indicating that the variables have a low negative correlation. Vitamin D, Vitamin C, Folate, Vitamin B6 PLP, Vitamin B12, Zinc, Calcium, and Phosphorus have no significant correlation with APO A1. The correlation coefficient between APO A1 and Magnesium is -.261, indicating that the variables have a low negative correlation. In the last 50 years, there have been a lot of changes in the human environment, habits, and way of life. Obesity rates have risen because of these changes. Vicarious obesity, as well as its role in the progression of cardiovascular disease, are linked to metabolic syndrome's clinical significance. Research shows that the WC is better than the BMI at predicting obesity-related health problems. It is important to note that the WC is linked to both subcutaneous and intra-abdominal fat. The data from this study show that WC is a better predictor of metabolic problems and cardiovascular risk than BMI is. A strong relationship was found between BMI and WC in this study, but not between FBG and TG. The metabolic syndrome is caused by high levels of TG, LDL-C, and TC, and low levels of HDL-C. FFA levels are higher in people with type 2 diabetes or insulin resistance because insulin doesn't work as well to stop fat from being broken down. Insulin resistance may also make it hard for insulin to stop VLDL production in the liver. Also, people who have metabolic syndrome or insulin resistance may make more Apo B-containing VLDL, IDL, and LDL. Insulin resistance may make lipoprotein lipase, an enzyme that helps break down VLDL, less active. Delaying the breakdown of VLDL particles leads to higher plasma triglyceride levels. VLDL particles break down less, which means there are less surface proteins and phospholipids that can be used to make HDL particles. TC, LDL-C, and Apo-B are all linked to insulin resistance in the same way as in previous studies. HDL-C, on the other hand, has a different relationship.

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Table 1: Descriptive Statistics of the conventional risk factors

		N	Mean	Std. Deviation
BMI	Experimental	120	26.6992	4.97266
	Control	120	24.0408	4.48946
SBP(mmHg)	Experimental	120	142.6333	23.93055
	Control	120	119.7083	5.90456
DBP(mmHg)	Experimental	120	86.0333	5.97042
	Control	120	81.8667	4.61625
FBS	Experimental	120	162.4500	54.16406
	Control	120	86.2500	6.71040
PPBS	Experimental	120	217.0667	57.53607
	Control	120	123.3667	7.93983
TC(mg/dl)	Experimental	120	263.9750	38.53460
	Control	120	185.2917	13.77575
TG(mg/dl)	Experimental	120	129.1300	32.57588
	Control	120	103.7250	17.18215
HDL(mg/dl)	Experimental	120	44.3583	5.47998
	Control	120	48.5833	6.12967
LDL (Mg/dl	Experimental	120	142.0500	22.74996
	Control	120	109.2500	13.12208

Table 2: Mann-Whitney test for comparison of serum levels of various risk markers in subjects with and without metabolic syndrome

Test Statistics	Mann-Whitney U	Wilcoxon W	Z	p-value
BMI	4871.500	12131.500	-4.330	0.000
SBP(mmHg)	1934.000	9194.000	-10.266	0.000
DBP(mmHg)	4374.500	11634.500	-5.779	0.000
FBS	404.500	7664.500	-12.648	0.000
PPBS	80.500	7340.500	-13.247	0.000
TC(mg/dl)	496.500	7756.500	-12.470	0.000
TG(mg/dl)	3627.000	10887.000	-6.646	0.000
HDL(mg/dl)	4324.500	11584.500	-5.356	0.000
LDL (Mg/dl	1505.500	8765.500	-10.596	0.000
APO B(g/L)	5486.500	12746.500	-3.191	0.001
APO A1(g/L)	6916.500	14176.500	-0.528	0.597

Table 3: Spearman's Rank Correlation results for the correlation between micronutrients and emerging markers

	APO B(g/L)		APO A1(g/L)	
	Correlation Coefficient	**Sig.(2-tailed)**	Correlation Coefficient	**Sig. (2-tailed)**
Vitamin D (ng/mL)	-0.127	0.166	0.17206	0.060
Vitamin C (mg/dl)	-0.00853	0.926	0.011922	0.897
Folate (5–20 ng/ml)	0.074	0.419	-0.05563	0.546
Vitamin B6 PLP (5 - 50 µg/L)	-0.159	0.083	0.017486	0.850
Vitamin B12 (20–80 ng/dl)	-0.11954	0.193	0.066	0.473
Magnesium (1.8–2.2 mg/dl)	-0.009	0.921	-.261**	0.004
Zinc (50–100 µg/dl)	0.068	0.460	-0.10896	0.236
Calcium (mg/dl)	0.069	0.455	-0.03484	0.706
Phosphorus (mg/dl)	-.197*	0.031	0.090	0.330

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Acute Toxicity and Histopathological Effect of Lambda Cyhalothrin and Pretilachlor on Intestine of Indian Earthworm *Lampito mauritii* (Kinberg)

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Received: 18 Feb 2022 Revised: 02 Mar 2022 Accepted: 25 Mar 2022

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ABSTRACT

Soil pollution has increased over time due to the widespread use of pesticides and chemical fertilizers in agriculture. Earthworms are important members of the soil ecosystem. In most ecological systems, earthworms play a crucial role in soil fertility and the formation of biogenic structures. Furthermore, earthworm gut mucus boosts the biological activities of beneficial soil microorganisms. The use of earthworms to enrich soil with essential nutrients is a cost-effective and environmentally friendly process. To control agricultural pests as well as weeds in India, the organophosphorus insecticide - lambda cyhalothrin and herbicide -pretilachlor are widely used. The present study the toxic effect of lambda cyhalothrin and pretilachlor of acute toxicity and histopathological effect reveals intestine of earthworm *L. mauritii* in a laboratory experiment. For acute study, mortality of *L. mauritii* was observed after 24, 48, 72, 96 and 120 h of the pesticides exposure. A sub-lethal concentration of lambda cyhalothrin and pretilachlor (1/5th of 96 h LC_{50} value 1.8 ppm kg^{-1} and 2.7 ppm kg^{-1}) was applied for 30 days. Histology of intestine was observed at 5th, 15th and 30th day of the pesticides exposure. On the 5th, 15th day of experiment, the skin showed significant pathological changes in epidermis layer, circular muscle, and longitudinal muscle. The changes such as vacuolization, degenerated nuclei, damaged epithelial lining of villi and congestion of blood sinuses was observed in 30th day, slight damages was observed. These results suggest that lambda cyhalothrin and pretilachlor could severely affect the intestine up to 15thday thereafter recovered of exposure when compared to 30th day. Histopathological study in *L. mauritii's* intestine is a suitable parameter for detection of soil contamination by application of pesticides in agricultural field.

Keywords: lambda cyhalothrin, pretilachlor, intestine, skin, soil contamination.

INTRODUCTION

Pesticide application has consistently increased over the years on a global scale to control pest and weed populations in agricultural fields. Earthworms are extremely sensitive to environmental changes, can uptake toxic substances through their permeable skin and those substances can accumulate in the body; therefore, earthworms are used as important indicators of ecosystem perturbations (Kavitha *et al.*, 2008; Nayak *et al.*, 2018; Samal *et al.*, 2019; Mishra *et al.*, 2020). Earthworms are standard test organism in soil toxicity testing. They have been broadly used to assess of environmental impact from heavy metal pollution; however, the knowledge on toxic effect from pesticides upon these organisms is still very limited (Castellanos and Hernandez, 2007). The widespread and global use of agropesticides is causing increasing concern about soil contamination. Since resident soil species can be affected, knowing the effect(s) of pesticides in the soil environment is important. Earthworms, which are essential for soil structuring and rising nutrient content, make up a significant portion (80%) of the biomass of terrestrial invertebrates. As a result, they're good bioindicators for chemical pollution of soil in terrestrial habitats, and they can be used to give an early warning of declining soil quality (Sorour *et al*., 2001; Bustos-Obregon and Goicochea 2002). This is critical for environmental protection and is becoming increasingly important for human health (Beedy., 2001), as well as other terrestrial vertebrates that eat earthworms. Since earthworms eat large amounts of decomposed litter, manure, and other organic matter accumulated on soil, they are good bioindicators of soil toxicity because they help in the conversion of decomposed litter, manure, and other organic matter into rich topsoil (Reinecke and Reinecke., 2007). Ingesting polluted soil or absorbing pesticides from soil and water may cause pesticide accumulation in worms. Reports are also available on ways herbicides could adversely impact the growth and reproduction of earthworms (Chen *et al.*, 2018; Niemar *et al.*, 2018). The negative impact of glyphosate on earthworms, *Octodrilus complanatus, Lumbricus terrestris* and *Aporrectodea caliginosa* in vineyards in Italy's northeast region has been reported (Stellin *et al.,* 2018). Significant histopathological and enzymatic changes in the earthworms *G. tuberosus* and *E. eugeniae* due to high concentration of phosphogypsum and pesticides in soil, respectively, have also been reported (Nayak *et al.*, 2018, Samal *et al.*, 2019a).

Lambda cyhalothrin (s(S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-choloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimthylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate) and Pretilachlor (2-choloro-N-2-6-diethyl-N-(2-propoxyethyl) acetanilide) are widely used pesticides to control wide range of insects such as sucking, chewing, and boring insects and spider mites and herbs cotton, olives, rice, maize, sorghum, soya bean, and tobacco, including. Because of its widespread occurrence in arable and pasture lands and resistance to surface-applied pesticides, *L. mauriti* was chosen as the indicator species in India; therefore, earthworms are ideal for assessing agro-ecosystem pollution. Histology is an excellent instrument for analysing the impact of agricultural pesticides, industrial contaminants, organic wastes, and other pollutants on the earth's tissue stage. . Information about the impact of pesticides on these earthworms is not adequate. Therefore, this study was undertaken to observe acute toxicity and histopathological changes in intestine of *L. mauritii* with 5th, 15th and 30th day of exposure to sublethal concentrations of lambda cyhalothrin and pretilachlor in soil under laboratory conditions.

MATERIALS AND METHODS

Materials

Clay loam soil and fresh cowdung were obtained from Annamalai University, agricultural Farm and Dairy-yard.

Chemicals

Lambda cyhalothrin and pretilachlor (made in India) was purchased from an agro-center in Thanjavur, Tamil Nadu, India.

Experimental Organism

In this study, the earthworm *L. mauritii* served as the test organism. The body of *L. mauritii* is long, cylindrical, and tubular. The ventral surface is pale in colour and the dorsal surface is light brown to yellow in colour with a purplish blackish band like tinge. Adult worms are 8–21 cm long, 3–5 mm in diameter, and weight 0.8–1.5 g with 165–190 segments. *L. mauritii* with a well-developed clitellum was obtained from Annamalai University's dairy yard in Annamalai Nagar, Tamil Nadu, and India. They were acclimatized to the laboratory conditions by growing in cow dung for one week.

METHODS

Experiment Media Preparation.

Sun-dried and powdered cow dung (a nitrogen-rich natural food for earthworms) was mixed with soil (low nitrogen) in a ratio of 1:3 (vol/ vol). It was termed the "soil substrate" and used throughout this study. The 96-hour LC_{50} value of lambda cyhalothrin and pretilachlor on *L. mauritii* was 8.93 ppm/kg and 13.46 ppm/kg. Using this value and as per multiple toxicology studies, we selected sublethal concentrations for both the pesticides 1.8 ppm kg^{-1} and 2.7 ppm kg^{-1} (1/5th of 96 h LC_{50} value). Three plastic troughs, each filled with one kg of soil substrate, were designated as C (control), T1, and T2 in triplicates. The control was mixed with only water. The sublethal concentrations of lambda cyhalothrin and pretilachlor were mixed with the soil substrate using 300 ml of water to ensure a homogenous mixture with the required moisture. Eight adult *L. mauritii* were introduced into each of the experimental setups. The troughs were covered with nylon nets and maintained at room temperature (28 ± 2 °C) with 60–70% moisture content.

Acute Toxicity Test

Acute toxicity tests by static bioassay method were conducted using artificial soil according to European Economic Community (EEC) and OECD guideline No. 207 with slight modification i.e., the clay loam soil with 20% cowdung was used as soil substrate. Ten concentrations of lambda cyhalothrin and pretilachlor were selected on the basis of exploratory test. Test concentrations are expressed as ppm/kg of soil substrate. For assessing acute toxicity, in plastic troughs (Diameter 25 cm x Height 6 cm) each of selected concentrations was mixed well with one kg of soil substrate using 300 ml water and 10 adult *L. mauritii* (clitellum well developed) were introduced into each treatment. The mortality of earthworms was observed and recorded after 24, 48, 72, 96 and 120 h of exposure. The lethal concentration (LC), lower confidence limit (LCL) and upper confidence limit (UCL) values were calculated by Probit analysis statistical method (Finney, 1971) Presumable harmless concentrations were derived according to Sprague (Sprague,1971) using the following formula:

Presumable harmless concentration = 48 h LC_{50} × (A)/S

Where, S = 24 h LC_{50}/48 h LC_{50}

A=0.03 (constant)

Histopathological Study

The earthworms were collected from control and treatments. Four earthworms were removed from each experimental period (5^{th}, 15^{th} and 30^{th} day) and kept in plain water overnight to clear their intestines. Then to minimize their movement while easily dissecting them, they were kept in a freezer for about 1 h before dissection. Later the animals were dissected and small pieces of the intestine (ranging from about 20 to 100 segments) were removed and fixed for 24 h in Bouin's fixative. After tissue processing, paraffin sections of intestine were cut at 5 μm thickness and stained with hematoxylin-eosin, and it was analysed for microscopic examination.

RESULTS

Acute toxicity of earthworm is an efficient tool in assessing ecological risks of contaminated soil. The result of the study showed that there was no mortality in the control group (Table 1). The mortality rate was least at the beginning of the study (24 h) and increased with exposure period. The values of median lethal concentrations (LC_{50}) and its lower confident limits (LCL) and upper confident limits (UCL) for earthworm *L. mauritii* exposed to lambda cyhalothrin and pretilachlor for different periods and given in Table 1. The Table 1 and 2 showed that LC_{50} values decreased with increased period of exposure time. The LC_{50} values of lambda cyhalothrin were 49.12, 26.46, 16.31, 8.93 and 6.67 ppm/kg for 24, 48, 72, 96 and 120 hours. Whereas the LC_{50} values of pretilachlor were 34.39, 25.00, 15.91, 13.46 and 8.92 ppm/kg for 24, 48, 72, 96 and 120 hours.

Histology of *L. mauritii* Intestine

The intestine is made up of four layers; an outer layer of visceral peritoneum which forms the outermost covering layer of intestine. The most of the cells of this layer around the stomach and intestine are modified and called chloragogen cells or chloragocytes. Chloragogen cells are believed to be of vital importance in the metabolism and they play a role similar to that of liver in vertebrates. These cells are excretory as well chief centre of synthesis and storage of glycogen and fat. Next to this, two muscle layer-outer layer of longitudinal muscle fibres and inner layer of circular muscle fibres are found. Both these muscle layers are well developed around the pharynx and oesophagus but poorly development around the stomach and intestine. The longitudinal muscle fibres are absent in gizzard. All the muscles of the gut wall are involuntary and unstriped. The last or fourth layer is the pharynx, the stomach and intestine. It is mostly glandular and ciliated to form Villi in the intestine. In the gizzard, it mostly secretes cuticle. The intestine blood sinus or plexus lies immediately outside the epithilum.

The untreated (control) earthworms have smooth dermal layers. Transverse sections of untreated earthworms indicated a thick and intact epithelial layer with closer circular and longitudinal muscle layers (Plate 1 and 2). After 5th day of lambda cyhalothrin exposure, the epithelial layer of intestine was ruptured. Vacuolated muscle layers with necrosis in the connective tissue were also evident. Damaged villi, fusion of chlorogogen cells, pyknotic nuclei in some regions, vacuoles in the cytoplasm, degenerated nuclei, damaged epithelial lining of the villi, space formation and congestion of blood sinuses were observed (Plate - 3). 15th day of exposure, intestinal epithelial layers were thin and damaged. Cell debris was accumulated in epithelial tissue. Connective tissues were also not intact. Damaged and vacuolated muscle layers, intestinal villi (V) and vacuolations in cells of Epithelial lining (EL) were observed. The extent of intestinal damage is more severe in 15th day exposure (Plate - 4). 30th day of exposure, no visible anomaly in the intestine was noticed. Renewed epithelial cells and more or less compact arrangement of the epithelial layer were observed (Plate - 5).

After 5th day of pretilachlor exposure, longitudinal and circular muscle layers were severely damaged. However, the fusion of muscle layers was observed. Damaged villi, fused chlorogogen cells, pyknotic nuclei in some regions, vacuoles in the cytoplasm, space formed between cells and congested blood sinuses was observed (plate-6).15th day of pretilachlor exposure, thin epithelial layers with loosely packed connective tissues were seen. The longitudinal muscle layer drifted away from the circular muscle layer. Necrosis was also noticed in the intestinal cells. Gaps were observed in the epithelial layer (Plate - 7). 30th day of pretilachlor experiment, no visible anomaly in the intestine was noticed. Renewed villi and muscle layers were observed (Plate - 8). No remarkable changes were observed in the intestine when compared to control at the end of 30th day of both the pesticides exposure. Minor displacement of circular and longitudinal muscle layers was observed. The results suggested that compared with 5th and 15th day of exposure, degrees of pathological changes was not observed in the 30th day of intestine.

DISCUSSION

Soil toxicity can be evaluated using indicator organisms such as earthworms. The LC_{50} values, LCL and UCL are useful measures of the acute toxicity of the insecticides. The acute toxicity results indicated that the rate of mortality was directly proportional to the concentration of pesticides. Declining trend of LC_{50} values with an increase in exposure period indicating the increasing toxic effect have been reported in earthworms exposed to chemicals and organic pesticides (Bakthavathsalam and Rajaraman., 2003; Bagchi *et al*., 1995; Ramalingam and Kavitha., 2006). In the present study also similar declining trend in LC_{50} values and increasing mortality with increasing periods of exposure (24, 48, 72, 96 and 120 h) due to increased toxic effect of lambda cyhalothrin and pretilachlor on *L. mauritii* was observed (Table 1 and 2).

In earthworms, contaminants may be absorbed through the skin and then transported throughout the body (Vijver *et al*., 2003). Various workers have noticed morphological aberrations such as swelling, lesions and skin discolouration in earthworms due to pesticide and heavy metal toxicity (Reddy and Rao, 2008; Singh *et al*., 2019). Reddy and Rao (2008) reported morphological and histological alterations in *E. fetida* exposed to the organophosphate profenofos. Identical effects have been observed in *E. fetida* with the herbicide glyphosate and 2, 4-D (Correia and Moreira, 2010). Mukherjee and Parida (2015) have reported lindane induced toxicity and significant biomass loss in *E. eugeniae*. Nunes *et al*. (2016) studied the effect of abamectin on *E. andrei* and observed thinning and discolouration of the skin, constriction of the body and fragmentation of the posterior segment. Singh *et al*. (2019) reported variable morphological changes in the earthworm *E. eugeniae* with sublethal concentrations of triazophos.

The histopathological changes such as damaged epithelial cells, longitudinal muscle layer, circular muscle layers, cell death or necrosis, vacuolizations in cytoplasm, damaged villi were observed in 5th and 15th day of lambda cyhalothrin and pretilachlor exposure. Reports are available on the effects of diverse groups of chemicals on the histology of earthworms, which are more or less similar to the results obtained in this study. The earthworms *P. sansibaricus* and *E. fetida* suffered from muscle degeneration and cuticular rupture when exposed to the weedicide butachlor (Muthukaruppan *et al*., 2005; Reddy and Rao, 2008; Gobi and Gunasekaran, 2009). Significant muscular aberrations were reported in *N. mbae* after exposure to sublethal doses of atrazine (Oluah *et al*., 2010). Effects of various weedicides, heavy metals and hydrocarbons on different earthworms have been tested, which indicated muscle anomalies (Eseigbe *et al*., 2013; Bangarusamy *et al*., 2014; Enuneku *et al*., 2014; Oluah and Ochulor, 2014). High concentrations of pesticides could cause significant damage to muscle and intestinal epithelium of tropical earthworms *G. tuberosus* and *E. eugeniae* (Nayak *et al*., 2018; Samal *et al*., 2019a). According to Bowen and Lochshin, (1981) cell death is not a single entity but it is heterogeneous in structure, mechanism and biological function. Cell death or necrosis is characterized by pycnotic nuclei, cytoplasmic swelling and mitochondrial damage, which is in keeping with the hypothesis that it results from failure in osmotic regulation caused by loss of cellular energy supplies. By the 30th day of exposure there was observed recovery of the epithelial lining. According to Stephensen, (1930) recovery could be brought by the chloragogen cells. These cells are known to migrate to the wound or lost tissue and regenerate them. It is the well known fact that earthworms have a great power of regeneration (Leblond and Walker, 1956; Hammana *et al*., 1995).

Muthukaruppan *et al*. (2005) have reported the glandular cell enlargement in the intestine of the earthworm exposed to sublethal toxicity of herbicide butachlor and they have further observed that changes in the intestinal region may massively affect food intake and which inturn may indirectly inhibit earthworm reproductive capacity. An extreme (2–fold) nuclear swelling has been reported in *E. fetida* exposed to herbicides under different experimental conditions (Fischer and Molnar, 1992). Gupta and Sundaraman, (1988) have reported the swollen nuclei and loss of chromatin material in carbaryl intoxicated *P. posthuma*. Morowati (2000) has reported that *P. elongata* exposed to a field dose of herbicide glyphosate showed loss of epithelial cell structure in intestine, lacking regeneration of the cells and total loss of chromatin from first week to the third week of exposure and a marked regeneration of the cells in the fourth

week of exposure. Bansiwal and Rai, (2010) observed that sublethal dose of organophosphate insecticide malathion has induced marked pathological changes in the body wall such as ruptured cuticle, with distortion of the shape of longitudinal muscle cells. Oluah *et al.* (2010) have been stated that after exposure to atrazine in the earthworm *Nsukkadeilusmbae*, damages were observed in chloragogenous layer, epithelial tissues and glandular enlargement of the epithelial tissues, prominent vacuolations and pylenotic cells.

Alternatively, Daane and Haggblom, (1999) have suggested that the microflora may influence the survival of earthworms exposed to toxic chemicals. The earthworms are known to have efficient detoxification capacity with a large number of aerobic and anaerobic bacteria (Karsten and Drake, 1995). During prolonged exposure (30 days) the increased microbial number and activity in the gut of *L. mauritii* might have resulted in accelerated degradation of pesticides as a consequence, the impact of pesticides on the gut of *L. mauritii* were minimized. Kavitha *et al*. (2008) observed that *L. mauritii's* gut bacterial and fungal species such as *K. pneumonia*, *E. aerogens*, *E*. cloacae, *B. subtilus*, *A. fumigatus*, A. *nigar* and *A*. flavus were able to survive and degrade endosulfan after 30 days of exposure, so the earthworms gut microbes might have played a major role in the biodegradation of pesticides. It is also strongly supported the results for recovery of intestinal epithelial lining in the 30thday of lambda cyhalothrin and pretilachlor exposure. Samal et al. (2020) have noticed that monocrotophos at each concentration and glyphosate at high concentration could bring about severe muscle and cuticular disintegration. Disorganization of the circular and longitudinal muscles, along with an accumulation of cell debris in the earthworms, could seriously impair their ecological functions. Clitellar damage could impair these animals' cocoon production and other reproductive functions. Rajashree *et al*. (2014) had found that the effect of Phorate and Methyl parathion on *E. Eugeniae* showed progressive signs and symptoms of toxicity such as coiling, curling and excessive mucous secretion with sluggish movements, swelling of the clitellum, extrusion of coelomic fluids resulting in bloody lesions within 48 hours of exposure, degenerative changes at the anterior part of nervous system, disappearance of metameric segmentations and loss of pigmentations within 48 hours of exposure. The present study results are also in accordance with the results of Kavitha *et al.* (2020) who reported that organophosphate insecticide, monocrotophos was affected microbial population and histology of intestine upto 15 days due to toxicity of monocrotophos. Thereafter, they were recovered by presence of some of the pesticide degrading bacteria and fungi in the gut.

CONCLUSION

In the present finding, the results of the study proved that lambda cyhalothrin and pretilachlor were highly damaged the intestine of *L. mauritii* up to 15th day thereafter the degrees of pathology was decreased. The changes such as disorganization and disintegration of the circular and longitudinal muscles, cell debris, vacuolization, degenerated nuclei, damaged epithelial lining of villi and congestion of blood sinuses were observed in the intestine of 5thand 15th day of both the pesticide exposure. In the 30th day of exposure, recovery was observed. The histopathological study in intestine of *L. mauritii* is a suitable parameter for detection of soil contamination. Therefore, to maintain the fertility of soil we should avoid application of inorganic pesticides and fertilizers in the agricultural field.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Department of Zoology, Annamalai University, Annamalai Nagar, 608 002, Tamil Nadu, India for providing the necessary facilities to carry out this work.

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Table : 1 Median lethal concentration (LC_{50}) and their 95% lower and upper confidence limits (LCL and UCL) of Lambda Cyhalothrin (5% EC) exposed *Lampito mauritii* (Values expressed as ppm)

Exposure period in hours	LCL ppm	LC_{50} ppm	UCL ppm	Log LC_{50}	Regression equations	Slope function(s)	Correlation coefficient square (r^2)	Calculated chi-square values (x^2)
24	-25.47	49.12	81.47	1.69	Y=-4+2X	1.018	0.856	112.4^S
48	2.53	26.46	109.47	1.42	Y=14+1.4X	1.890	0.922	127.7^S
72	14.53	16.31	121.47	1.21	Y= 50+1.4X	3.066	-0.62	114.2^S
96	-17.47	8.93	89.47	0.95	Y=60+2X	5.6	-2.6	203.2^S
120	-33.47	6.67	73.47	2.22	Y=100-3X	0.333	-0.28	300.1^S

Presumable harmless concentration = 2.249 ppm **Number of observations = 10**

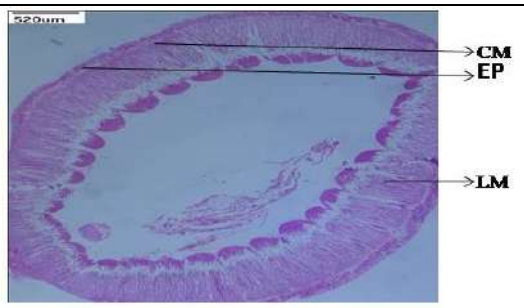
S = No Significant difference between observed and excepted mortality of 5 % level (p<0.05)

Table : 2 Median lethal concentration (LC_{50}) and their 95% lower and upper confidence limits (LCL and UCL) of Pretilachlor (50% EC) exposed *Lampito mauritii* (Values expressed as ppm)

Exposure period in hours	LCL ppm	LC_{50} ppm	UCL ppm	Log LC_{50}	Regression equations	Slope function(s)	Correlation coefficient square (r^2)	Calculated chi-square values (x^2)
24	-21.289	34.3879	39.289	1.536	Y=-42+2.6X	1.454	0.685	144.67^S
48	25.711	25.00	86.289	1.398	Y=2+1.9X	2.00	1	134.85^S
72	-16.289	15.9134	44.289	1.202	Y=-20+4X	3.142	0.946	149.86^S
96	-23.289	13.4626	37.289	1.129	Y=40+2X	3.714	0.142	178.86^S
120	-20.289	8.9286	40.289	0.951	Y=60+2X	5.6	2.6	280.01^S

Presumable harmless concentration = 0.0935 ppm **Number of observations = 10**

S = No Significant difference between observed and excepted mortality of 5 % level (p<0.05)



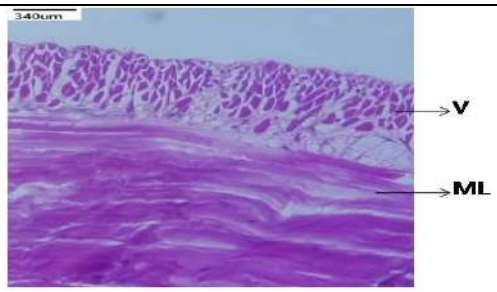


Plate 1 and 2. Control intestine showing normal tissue organization (x100)
EP=Epidermis, CM = Circular muscle, LM = Longitudinal muscle and V=Villi, ML=Muscle layer

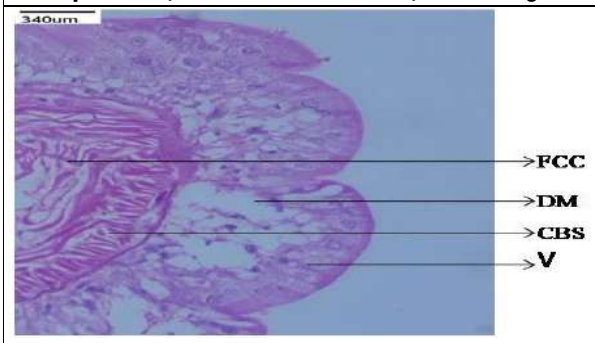


Plate 3. Section of intestine exposed to lambda cyhalothrin for 5th day (x100), DV=Damaged villi, V=Vacuols, CBS=Conjection of blood sinuses, FCH=Fusion of chlorogogen cells

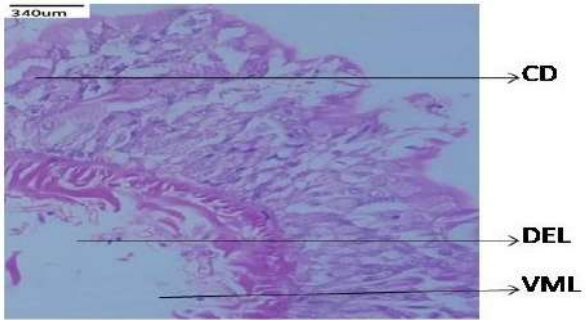


Plate 4. Section of intestine exposed to lambda cyhalothrin for 15th day (x100) CD=Cell debris, DEL=Damaged epithelial layer, VML=Vacuolated muscle layer

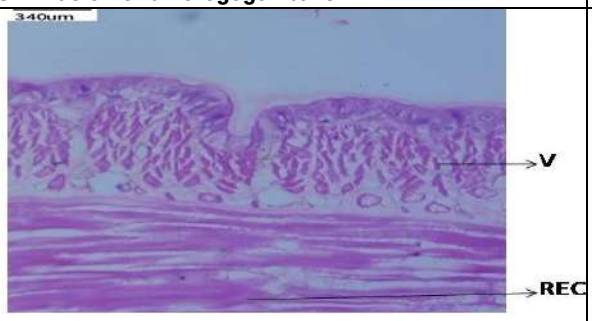


Plate 5. Section of intestine exposed to lambda cyhalothrin for 30th day (x100) V=Villi, REC= Renewed epithelial cells

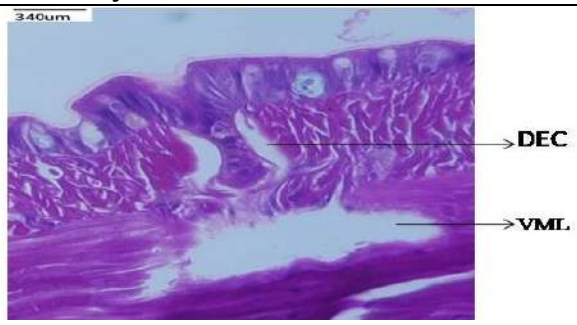


Plate 6. Section of intestine exposed to pretilachlor for 5th day (x100) DEL= Damaged epithelial cells, VML=Vacuolated muscle layer

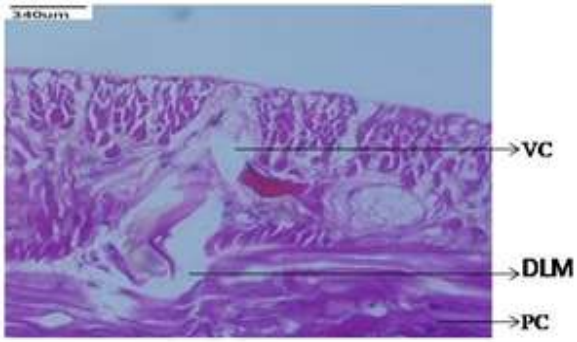


Plate 7. Section of intestine exposed to pretilachlor for 15th day (x100) VC=Vacuolated villi, DLM=Drifted longitudinal muscle, PC=Packed connective tissues

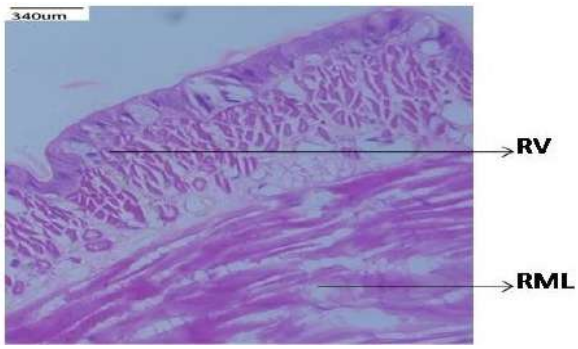


Plate 8. Section of intestine exposed to pretilachlor for 30th day (x100) RV=Recovered villi, RML= Recovered muscle layer

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Brain Specific Delivery - Various Approaches

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Received: 05 Feb 2022 Revised: 07 Feb 2022 Accepted: 22 Mar 2022

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ABSTRACT

In the central nervous system, targeted action can be achieved by direct administration of the drugs in to the CNS. Blood brain barrier can considerably impair the effect of the large number of drugs like antibiotics, antineoplastic agents and Neuropeptides-CNS stimulant drug)because of its obstinate hindrance affect . From some recent studies, it has been represented that the blood brain barrier is usually does not cross by almost 100% of large molecule drugs and 98% of small molecule drugs. Rate-limiting step in the BBB in brain drug development are, Blood brain barrier is the major confront toward brain targeted drug delivery and BBB have efficient ability to restrict and separate the human brain from circulatory network, and only allow the transportation of molecules that play vital role in functional activity of brain. It also limits the transport of water and lipid soluble substances from blood circulation into CNS. Advancement in the perception of the cell biology of blood brain barrier has started the innovative path or opportunities for better drug delivery to the brain. The main factors that affect passive transfer are: drug ionization, lipophilicity, molecular weight, and protein binding. Future aspects of brain targeting Technological challenges need are the Attainment of controlled release profiles particularly for sensitive drugs and the improvement of nanoparticles release from implantable devices. Also cytotoxicity of nanoparticles should be reduced to improve the biocompatibility

Keywords: nervous system, Blood, drugs, antibiotics, molecular, nanoparticles.

INTRODUCTION

The blood-brain barrier has been a great hurdle for brain drug delivery. The BBB in healthy brain is a diffusion barrier essential for protecting normal brain function by impeding most compounds from transiting from the blood to the brain; only small molecules can cross the BBB. Under certain pathological conditions of diseases such as stroke, diabetes, seizures, multiple sclerosis, Parkinson's disease and Alzheimer disease, the BBB is disrupted. The objective of this review is to provide a broad overview on current strategies for brain drug delivery and related subjects from the past five years. It is hoped that this review could inspire readers to discover possible approaches to deliver drugs into the brain. After an initial overview of the BBB structure and function in both healthy and pathological conditions, this review re-visits, according to recent publications, some questions that are controversial, such as whether nanoparticles by themselves could cross the BBB and whether drugs are specifically transferred to the brain by actively targeted nanoparticles. Finally, one particular area that is often neglected in brain drug delivery is the influence of aging on the BBB, which is captured in this review based on the limited studies in the literature.

ADVANTAGES

1. Side effect and toxicity reduces.
2. Dose of drug reduces by targeting organ.
3. Avoids degradation of drug (first pass metabolism).
4. Bioavailability increases.
5. Fluctuation in concentration decreases.
6. Permeability of proteins and peptide increases

DISADVANTAGES

1. Enhances clearance from target.
2. Difficult to target tumor cells.
3. Advanced techniques requirements.
4. Skill persons required.
5. Sometimes it may causes toxicity.
6. Difficult to maintain stability of dosage form, e.g. Resealed erythrocytes have to be stored at 40 c.

Blood-brain barrier in healthy brain

The BBB is a diffusion barrier essential for the normal function of the brain, which impedes the entrance of substances from the blood to the brain to maintain brain homeostasis. Brain microvascular endothelial cells (ECs), pericytes, astrocytes, tight junctions (TJs), neurons, and basal membrane construct physically tight brain capillaries in the BBB .The brain capillary ECs do not have fenestrations, which limits the diffusion of small molecules and proteins. Interendothelial junctions link the ECs to a continuous barrier, severely restricting the penetration of water-soluble substances. Pericytes, astrocytes and basal membrane surround the ECs and finally form the impermeable BBB. Additionally, efflux transporters are located in brain capillary ECs, which are further obstacles against substances entering the brain. The permeability of the BBB is mainly controlled by interendothelial junctions that are protein complexes such as adherens junctions, TJs, and gap junctions. Adherens junctions primarily regulate the permeability of the endothelial barrier. Tight junctions play a vital role in sustaining the permeability barrier of epithelial and ECs, which control tissue homeostasis [7]. Gap junctions, composed of 6 connexin molecules, direct electric and chemical communication between ECs. Instead of having a static structure, the components of the BBB continuously adapt in response to various physiological changes in the brain.

Molecules cross the BBB by a paracellular pathway (between adjacent cells) or a transcellular pathway (through the cells). For the paracellular pathway, ions and solutes utilize concentration gradients to pass the BBB by passive diffusion. The transcellular pathway includes different mechanisms such as passive diffusion, receptor-mediated

transport and transcytosis. Overall, passive diffusion is a non-saturable mechanism dependent on the physicochemical properties of the molecule. The physicochemical factors that influence BBB permeability include molecular weight, charge, lipid solubility, surface activity and relative size of the molecule [2]. Small lipophilic molecules such as carbon dioxide cross the BBB by passive diffusion through a transcellular pathway. BBB permeability can also be influenced by physiological factors such as efflux transporters (e.g., P-glycoprotein (P-gp)), enzymatic activity, plasma protein binding and cerebral blood flow. Hydrophilic molecules such as proteins and peptides enter the brain through specific and saturable receptor-mediated transport mechanisms such as glucose transporter-1 (GLUT-1), insulin transporter and transferrin transporter . These endogenous transporters are expressed at the luminal and abluminal EC membranes. Among these transport mechanisms, receptor-mediated transcytosis has been extensively studied to deliver drugs into the brain. A better understanding regarding the mechanisms of passage across the BBB will foster the development of new strategies for delivery of drugs into the brain.

Blood-brain barrier disruption in certain pathological conditions

The BBB is disrupted under various pathological conditions of diseases such as stroke, diabetes, seizures, hypertensive encephalopathy, acquired immunodeficiency syndrome, traumatic brain injuries, multiple sclerosis, Parkinson's disease (PD) and Alzheimer disease (AD) [11]. In certain pathological conditions, remodeling of the protein complex in interendothelial junctions is an important reason for the BBB breakdown . For example, the BBB becomes hyper-permeable to macromolecules during ischemic stroke. Albumin, a large protein molecule, is an indicator for studying BBB leakage since it rarely crosses the healthy BBB. FITC-albumin was observed in the brain in early and late disease stages of Huntington's disease in a R6/2 mouse model, indicating BBB disruption under these conditions . In multiple sclerosis, loss of organization of junctional molecules in cholesterol-rich cell membrane regions contributes to the increased BBB permeability. Moreover, BBB permeability can be significantly changed by disrupting adherens junctions. Thus, it has been confirmed that junctions are disrupted and, consequently, the BBB becomes permeable in some diseases. However, to date, the magnitude and time frame of BBB disruption in each disease is incompletely understood because of many limitations. For instance, the transient or chronic loss of BBB permeability is consistently observed in multiple sclerosis. Although BBB disruption could be visualized in vivo by injection of a Gd contrast agent, MRI scanning may underrepresent the overall extent of BBB disruption. On the other hand, BBB disruption is associated with disease complications. In the case of AD, vascular dementia and AD are normally comorbid. With many conflicting data, it is kind of agreed that the increase in BBB permeability in some AD patients is caused by vascular dementia, but not in pure AD alone. Taking into account the complexity of processes in CNS diseases, studies on BBB disruption in various diseases are still crude to this day.

Current strategies

Great efforts have been taken to deliver drugs and diagnostic agents into the brain. Combined with recent advances in BBB research, various new strategies have been exploited. This review summarizes the works published in the past five years. Some of them are still in a stage of proof-of-concept.

Viral vectors

Viral vectors have a natural ability to infect cells with nucleic acids. The application of viral vectors for gene delivery to patients with neurological disorders has been investigated for over two decades. In general, the transfection efficiency of viral vectors is high (e.g., 80%). Lentivirus, herpes simplex virus, adenovirus and adeno-associated virus (AAV) vectors have achieved gene transduction in the brain. The limitations of using viral vectors for drug delivery include difficulties in manufacturing, high cost of production, and, most importantly, the safety of viral vectors because of the death of patients in clinical trials . In order to use viral vectors for clinical applications, their safety must be confirmed. So far, AVV vectors have demonstrated exceptional safety profiles in humans as well as the ability for gene delivery in the brain, although a concern on immunogenicity still remains. Thus, AVV vector is a prominent vector used in current clinical trials of gene therapy for brain diseases. However, viruses normally cannot passively cross the BBB though viruses can transfect the gene into the targeted cells. Several administration routes

such as stereotaxic injection and injection into the cerebrospinal fluid (CSF) have been explored as means to either mechanically or biologically bypass the BBB [22]. Although these administration routes are very specific, risks from highly invasive neurosurgery are significant for patients. Systemic delivery is one area with a great unmet need when using viral vectors for CNS gene therapy. Advances in new vectors and delivery technologies in this area will accelerate the translation and application of viral vectors from preclinical to clinical studies.

Non-viral nanoparticles

With the advent of nanotechnologies, nanoparticles have been proposed as an intriguing tool to potentially enhance drug delivery across the BBB. Extensive reviews can be found elsewhere. This review focuses on new findings to redefine some concepts in this research area. When applying nanoparticles for brain drug delivery, the first question that has to be answered is whether nanoparticles, by themselves, could cross the BBB. Nanoparticles in general have the advantages on multifunctionalization, ability to carry drug payloads, control of drug release and modification of the pharmacokinetics of the drug. Moreover, nanoparticles, because of their nano size (< 200 nm), could penetrate into 'leaky' tumor tissue to facilitate drug delivery according to the enhanced permeability and retention (EPR) effect [32]. However, for brain drug delivery, observing increased drug concentration in the brain using nanoparticles does not necessarily imply that the small size of the nanoparticles makes them cross the healthy BBB. Nanoparticles could increase the drug concentration at the surface of BBB cells, or nanoparticles could provide more opportunities to the drug to cross the BBB by increasing their circulation time in the blood compared to conventional formulations. For instance, poly (ethylene glycol)-poly (lactic acid) block-copolymer (PEG-PLA)-protein complex nanoparticles cannot cross the healthy BBB.However, the complex nanoparticles delivered brain-derived neurotrophic factor to the brain and enhanced efficacy in a middle cerebral artery occlusion mouse model for stroke.

In addition, disease condition and progression have to be considered in the nanoparticle design when using actively targeted strategies. Ligands are selected to actively target an internalizing receptor on the apical side of brain ECs. However, expression of the receptor and transport mechanisms may change during the course of the disease. For example, transferrin receptors and insulin receptors are two common targeted receptors that have been used to develop actively targeted nanoparticles. Studies have evidenced that neuroinflammatory conditions and disease progression influence the expression of these receptors [44,45]. The iron regulatory protein system (IRPs) regulates the expression of transferrin receptor. Loss of IRPs plays a role in neurodegeneration causing a condition with neuronal iron deficiency. The genetic loss of IRPs results in reduced expression of transferrin receptor in IRP2-Null mice . Thus, the nanoparticle targeting transferrin receptors is not an effective system to deliver drugs to the brain in this specific disease condition. Moreover, the influence of disease conditions on the expression of receptors is very specific and should be evaluated case-by-case. Ho et al. demonstrated that the total content of insulin receptor had no significant change on the brains of nondiabetic sporadic AD, though they observed impaired signaling of insulin receptor . Nga Bien-Ly et al. measured levels of transferrin receptor in brain samples from both AD animal models and AD patients, and did not observe any decreases · Thus, regulation of the expression of transporters or receptors in the BBB during disease conditions is not completely understood. One needs to conduct detailed studies on this aspect prior to choosing the receptor for actively targeted drug delivery.

Recently, some new strategies on nanoparticles have been reported to specifically deliver drugs to the brain. In one study, propionylated amylose helix was used to form nanoclusters to encapsulate propofol, a hydrophobic drug for sedative effects in rabbits. Phosphatidylethanolamine has a higher content in the brain . The H-bonding between propionylated amylose and POPE induced binding of the complex to the surface of the POPE bilayer as well as triggered unfolding of the complex to release the drug, which generated a local high concentration gradient, facilitating the hydrophobic drug to cross the BBB. Molecular dynamics simulation was used to select the helix and simulate the encapsulation and release of the drug .Thus, this new approach utilizing a specific physiological condition to trigger drug release in the BBB focused on brain targeting rather than crossing the BBB.

Brain permeability enhancers

Many molecules have demonstrated the ability to transiently open the BBB and allow high concentrations of systemically administered chemotherapeutics to reach the brain. One of the rationales for these molecules to open the BBB is based on the transient disruption of the BBB by decreasing expression of TJ proteins such as claudin-1, occludin and tricellulin. Their early application was for intraarterial mannitol with chemotherapy agents to treat brain tumors. Currently, cereport (a bradykinin analog) has been shown to increase BBB permeability and consequently improve anti-cancer efficacy of co-administered anti-cancer drugs in animal models. However, clinical studies failed to show a benefit of the co-administration in glioma patients . Similarly, regadenoson (an adenosine receptor agonist) increased BBB permeability in animal studies, but did not influence the penetration of co-administered contrast agents in humans. An apparent mismatch between findings in rodents and humans existed for these molecules. The authors proposed further studies on doses, schedules and combination regimens. Recently, borneol has been studied to increase both oral absorption and brain penetration of drugs in animal models . Yi et al. compared four different oral formulations of puerarin with borneol. Among them, a self-microemulsifying drug delivery system containing both puerarin and borneol resulted in significantly higher AUCs both in plasma and in the braincompared to other formulations.It is very likely that co-administration of a drug and a permeability enhancer is insufficient to achieve the benefits of the enhancer in humans, as shown in the previous cereport and regadenoson studies. Since the interaction of the enhancers with the BBB is transient, co-delivery of both enhancer and drug by one carrier could be important to allow the drug to cross the BBB while the enhancer opens the BBB.

Delivery of drugs through the permeable blood-brain barrier under disease conditions

The BBB has been recognized as a great hurdle in brain drug delivery for a long time. Although the BBB leakiness is known to evolve with some disease conditions, detailed knowledge such as duration and size of the BBB opening is not well understood. With advanced studies, new mechanisms have been discovered. For example, glutamate release in ECs promotes BBB permeability. Recent studies based on new brain imaging techniques have also provided more detailed information on BBB leakiness. In this section, this “old” concept is revisited according to the recent findings on BBB permeability under disease conditions. BBB opening has been observed in diseases involving inflammatory, traumatic and degenerative situations. In many cases, BBB opening is the hallmark clinical symptom. Interendothelial junctions are key structures to maintain tissue-fluid homeostasis in the healthy brain. Under certain disease conditions, proteinaceous fluid enters the interstitium through the disrupted interendothelial junctions, consequently causing edema . On the other hand, brain injury further alters BBB permeability in the progression of the diseases.

Recently, MRI has become a common non-invasive tool to study BBB damage in patients. Inflammation is one of the root causes of BBB disruption. MRI was applied to patients with cardiopulmonary bypass, which induces a systemic inflammatory response. The BBB integrity was disrupted after the operation. Although BBB permeability recovered within several days, the short-time BBB disruption was correlated with postoperative neurocognitive dysfunction. Wong et al. demonstrated that DCE-MRI can be adjusted to provided moderate-to-excellent reproducibility on evaluating subtle BBB leakage in patients with cerebrovascular disease. Another major focus is to understand the dynamic change in BBB permeability and its mechanisms in ischemic stroke. When BBB disruption occurs after stroke is controversial. Some studies have suggested that stroke disrupts the BBB several hours after stroke onset [34]. However, a recent study based on enhanced MRI showed that BBB leakage continually increased in patients right after acute ischemic stroke

Non-invasive techniques to enhance brain delivery

Ultrasound has become an attractive technique to facilitate drugs to cross the BBB in recent years. Microbubble-enhanced diagnostic ultrasound (MEUS), a non-invasive technique, effectively helped drugs cross the BBTB by increasing BBTB permeability in glioma. Claudins, occludin and JAMs are major proteins in TJs in the BBB. The expression of these TJ proteins could be reduced by ultrasound irradiation and microbubbles, temporarily opening the BBB without damaging the normal brain tissue . In addition, Ningaraj et al. showed that MEUS increased the

expression of KCa channels in glioma, which promoted pinocytosis and consequently increased the BBTB permeability. Besides the BBTB, the BBB still remains a barrier for drug delivery in brain tumors. The combination of focused ultrasound (FUS) and microbubbles can enhance the permeability of the BBTB in brain tumors as well as disrupt the BBB in the surrounding tissue. Park et al. explored DCE-MRI to investigate the delivery of doxorubicin by using the combination of FUS and microbubbles. FUS and microbubbles were performed in both a rat brain tumor and the normal brain for doxorubicin delivery. They demonstrated that the combined technique increased the drug retention time in the tissue over 24 h while enhancing drug crossing of both the BBB and the BBTB. Moreover, it is interesting that MEUS was able to temporarily suppress P-gp expression. By using MEUS, P-gp was suppressed up to 48 h and restored by 72 h and the level of induced suppression could be controlled by adjusting instrument settings. To understand the physiological changes in the brain upon the BBB opening induced by FUS, non-human primates were treated with FUS at different acoustic pressures. The pharmacokinetic analysis confirmed that FUS locally and transiently opened the BBB and efficiently assisted drug delivery. The brain inhomogeneity and acoustic pressure determined the level of BBB opening and consequently the drug concentration in the brain. The basic principles and detailed discussion on the potentials of ultrasound-mediated drug delivery can be found.

Influence of age related factors

A neglected issue in the literature and research is the influence of aging on brain drug delivery. This section summarizes a few findings from the literature. The BBB is comprised of brain microvascular ECs, astrocytes, pericytes, neurons, and basement membrane. Aging could affect these components of the BBB. For example, studies showed that genes related to inflammation and scar formation were upregulated in aged astrocytes . Astrocytic functions critical for stroke recovery were influenced by aging in male and female rats . Moreover, with age, astrocytes decreased the secretion of trophic factors that prevented neural degeneration. In addition to astrocytes, studies have also shown that pericytes decrease with age, accompanying the increase in BBB permeability. Since aging influences the BBB structure, permeability to molecules is altered with age. One study tested the permeability to NGF in newborn rats with hypoxic-ischemic brain damage, neonatal and adult healthy rats. The results demonstrated that NGF penetration across the BBB was significantly higher in the newborn rats under hypoxic condition than in neonatal and adult rats; for the aging influence, NGF showed significantly higher permeation in neonatal rats compared to adult rats. In addition, common stresses in diseases will further alter the BBB function in old patients, although BBB dysfunction occurs early in the pathogenesis With limited research and complications, it is still unclear how aging influences the BBB, and to what degree. Certainly, it is critical to fully understand gene expression and permeability of the BBB in patients at different ages since many CNS disorders have high incidences in seniors. Drug delivery researchers need to consider aging influences when they design novel drug delivery systems for CNS diseases. The concept of adsorptive-mediated transcytosis through the BBB was originally suggested by the observation that cationic proteins can bind the endothelial cell surface but also cross the BBB . The mechanism, applied to NPs, is based on the proper functionalization of their surface allowing electrostatic interaction with the luminal surface of BBB. Given the presence of negative charges on endothelial cells this interaction can be promoted by conferring a positive charge to the NPs surface.

CONCLUSION

This review has covered recent strategies to deliver drugs to the brain in the past five years. To design effective drug delivery systems for brain diseases, detailed understanding of BBB disruption is necessary.. In addition to the common technologies including viral vectors and nanoparticles, novel non-invasive techniques such as MEUS and TMS have been studied to temporally open the BBB to enhance brain drug uptake. Innovative delivery systems should be expected to facilitate brain disease diagnostics. With understanding of the leaky BBB, previously developed nanoparticles that target tumors according to the EPR effect could be applied to brain diseases. Gliomas contain highly heterogeneous ranges in which permeability is normal in peripheral regions. Thus, a combination of strategies penetrating both permeable and normal BBB might have to be considered. Additionally, further studies on the dynamics of BBB disruption will come out, which will assist the design of sufficient delivery systems by taking

advantages of the leaky BBB. Another important area that deserves further investigation is the influence of aging on BBB dysfunctions. Brain drug delivery systems that have considered the influence of aging and were tested in animals of different ages are rarely found in the literature. In summary, the complexity of the BBB requires further detailed studies on delivery strategies, but on the other hand, it might offer unique opportunities to design efficient delivery systems to treat various brain diseases.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Ensemble Machine Learning Approach with Linguistic Analysis for Detecting Fake News

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Received:16 Feb 2022 Revised: 27 Mar 2022 Accepted: 15 Mar 2022

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ABSTRACT

In today's context, one of the main mediums of news consumption is through social media. It has become a common trend now to produce fake news for speedy propagation and popularity. Thus, it creates a sort of illusion and deception for readers. Amidst the umpty number of researches done, we could say that there is no research that could accurately predict fake news over online. In this study, the ensemble classifier is employed to develop a model for fake news identification in online social networks. This proposed method applies the stacked ensemble classification model, the proposed approach that learns the represented text model and classifies the textual data into real news and fake news. Then, the proposed approach takes the collaborative decision from the classified data that is generated from the multiple base learners using the weight-based ensemble method. The accuracy prediction and performance evaluation of time consumption of detection fake news are consecutively 80 Percentage and 11 ms respectively. Thus, it improves the efficiency and classification accuracy over large-scale social media messages through an efficient sentiment analysis model.

Keywords : Stacked Ensemble; NLP; Random Forest; Machine Learning; NBTree; Fake News detection

INTRODUCTION

With the increase of internet users nowadays, most of the people consume news through social media. The usage of social media for news updates is a double-edged sword [1]. Since it paves a broad road to freely express one's view, on the other hand, it widely opens its door for the dissemination of false news. Fake news is created as extremely significant and has the capability to spread extraordinarily fast through social media. The knowledge of fake

information on various topics is rapidly growing and is spreading faster, but overall, the more technical and complex a topic is, the harder it is to produce false claims and information for it [2]. Due to the increase in false news, incorrect information has become a crucial problem in today's society. Social media avails the exposure to new information and stories every day to its increasing numerous users. Thus, Misinformation could be difficult to counterbalance and may have lasting implications [3].

The advent of Web 2.0 technologies and social media enables users to express their own opinions and experiences over Internet. With the volatile growth of a vast amount of textual content in the Web 2.0 applications, news portals, and social networks, there is a huge need of analyzing and extracting knowledge from the posted textual content due to the richness of opinions and attitudes in the user-generated content [4]. The present technologies create easiness for social media users. Hence, the individual creates information, posts, news and promotes faster circulation. This adoption to the internet has been creating less quality with the rapid dissemination of fake news [5]. In this present scenario of online data with excessively with several competing origins of unpredictable quality, it becomes tough for readers to assess the trustworthiness and reliability of what they observe on the internet. [6]. Online social media platforms like YouTube, Twitter, and Facebook and have often challenged inspection for being unable to control the spread of false news. Today, social media websites are used as the most fascinated tools for seeing real-time knowledge pertaining to emerging threats, social effects, product styles, and epidemics [7].

Nations or groups have been using the news media to carry out propaganda or influence operations for hundreds, hence fake news itself is not a new problem. The rise of network-generated news on social media makes fake news a more powerful potency group that contests traditional journalistic ethics. In that respect, there are various features of this problem that make it uniquely challenging for automated detection [8]. In fake news detection, determining the truth from the fiction is a quite challenging task, which is in the form of deception detection. Social networks provide the platforms to share information and articles without moderation or fact-checking. Owing to the voluminous and variety of posted information on Social Media Sites, moderating user-generated content becomes an arduous task. Most of the fake news identification approaches transform the identification problems into classification problems using machine learning-based classifiers. Although, there is an essential need to contextually solve disambiguation problems created by the keyword matching methods. Most notably, to discern the veracity of the shared source information, the readers interact with both with the headlines and body of the text. Hence, it is essential to analyze both the headline and the body of the text to determine the fake news. Recently, ensemble learning methods have received greater attention in a variety of applications. Even though ensemble methods improve the classification accuracy in fake news detection, sequential decision making is a fundamental challenge while using the diverse multiple base learners. Also, the traditional sentiment analysis-based classification model lacks accuracy in predicting the intention of the content generated by the social media users due to the features in terms of words in the shared information is not always independent.

RELATED WORK

Fake news detection has become a central research topic in the news industry due to the need of assessing the veracity of digital content over the constant spread of false information [9]. The main aim of the sensationalism of inaccurate eye-catching and intriguing headlines is to retain the attention of audiences throughout all kinds of information broadcast [10].

- False News Detection
- Based on Ensemble Classifier

False News Detection

Chen et al [11], has explained the methods of the rapid spread of fake news using clickbait. The usage of textual and non-textual cues of click-baiting gives the authenticity of the web page regarding the accuracy of the information. In the subject matter and genre regarding the headlines, the structures of grammar and language flow are analyzed using lexical analysis to detect the accuracy of the data [12]. In the attention, certain news sources incorporate technology, for example, Twitter, Facebook, and other user submissions on BBC or ABC blog spots [13], This

encourages users to collaborate on on-spot footage and eyewitness interpretations. From some automated approaches, it is identified that list of stylometric features of text (grammatical and language flow) can be utilized to distinguish between two journalistic presentations of test magazines. This distinction was found to be existent regardless of topic, with a predictive accuracy of 77% [14].Also, the other researchers are analyzing the content based on the connections among news magazines, journalists, and news matters, using deep diffusive network technique has been subjected to integrate information with network structure towards model learning. Zhang et al, [15], have developed a novel diffusive component model, termed as Gated diffusive unit (GDC). This technique consents several inputs from various sources at a time, to efficiently combine this input for the creation of output with news matter as "forget" and "adjust" gates. Mohammad et al, [16] have considered the Stance Dataset tweets interpreted for stance towards trained targets and divergence of language. Thru a stance detection system that attained an F-score (70.3) greater than results attained for complex one, better-performing method in the competition. From the thorough analysis using an oracle system that has access to aureate sentiment and target of opinion annotations were capable to expect stance with an F-score of 59.6% only. Sriram et al, [17] have explained the classification of text from the Twitter text by using a small set of domain-specific features derived from the author's profile. The selected approach efficiently categorizes the text into a predefined set of common classes like Events, Opinions, News, Private Messages, and Deals. Developing a novel curse of dimensionality from the existing data sparseness enhances the accuracy of classification by using a small set of features present as a short text. This model presents a better accuracy when compared to the Bags of Words model [18].

Based on Ensemble Classifier

With the hasty development of the IT field, user-generated content can be expediently posted on the internet. Wang et al [19] has studied a comparatively of 3 various ensemble methods based on 5 base learners for evaluating the best performance of sentiment classification. From the entire 1200 comparative group investigations, empirical results describe those ensemble methods significantly enhance the effectiveness of distinct base learners for sentiment classification. Amongst the 3 ensemble methods, Random Subspace had shown better effective outcomes. Ensemble methods are used for the testing of the reliability of the text using the uniformly distributed weights and the data is filtered by employing the Bayesian paradigm. Fersini et al, [20] have optimized the ensemble model for obtaining 'N' no of possible solutions for acquiring the best accuracy results [21]. This optimized model gives a heuristic capability to evaluate the discriminative marginal improvement that each classifier affords with respect to an assumed ensemble. Xia et al, [22] have performed a comparatively studied ensemble technique for sentiment classification of the text with 2 features with grammatical and word relation features with Naïve Bayes, SVM, and entropy features. This classification is included with ensemble methods consisting of 'Fixed', 'Weighted', and 'Meta' classifier combinations to evaluate the effectiveness of the methods for sentiment analysis. Cornell movie-review corpora [23] is the dataset used for the analysis. In this overall sentiment polarity or subjective rating is derived to know the effectiveness. This ensemble system is an efficient way to associate diverse classification algorithms and feature sets for better classification performance.

RESEARCH GAP

The above discussed prior social media classification and fake news detection methods focus on classifying the fake news using machine learning algorithms. Several fake news detection research works have utilized ensemble classifiers to improve the classification accuracy in the social network further. However, there are several challenges in building accurate fake news predictive models, that includes limited availability of corpora, huge variations in the users' fake news messages when the user unintentionally generates fake news, and subjectivity in the ground truth labels. Hence the idea of combining context-based and Linguistic Inquiry and Word Count Dictionary to provide accuracy in the detection of fake news that can work without social signals. This eases the work of early detection of fake news and in turn, can limit the spread of fake news as a whole. In addition, context-based sentiment analysis is still in its infancy stage in the social network due to the dynamic change of the information context over time.

MATERIALS AND METHODS

In the previous section, it has been discussed about the Fake news detection and methods used to find out the fake news in any text or from any media that gives information. In the following section, we elaborate on our proposed framework, the algorithm description followed by the Performance Evaluation Metrics. Though there exist multiple challenges in predicting Fake news like a very limited amount of data available, a large amount of time spent by the user over the internet where sometimes un-intentionally or intentionally makes them generate and disseminate the false news This is very dangerous and sometimes times there occurs a chance of spreading a negative impact to society. In fact, defeating such situations there is enormous research work carried on to identify the Real news using Ensemble classification algorithms in order to predict with much accuracy. In addition, context-based sentiment analysis is also carried out but still, it is in its infancy stage in the social network due to the dynamic change of the information context over time. Also based on the Linguistic Inquiry and Word Count dictionary, there is no particular research that can assist in the decision-making of the Fake News detection.

This study aims to improve the classification accuracy of fake news detection and to reduce the computation time on a large scale. Hence comments this model is developed with combinations of the Ensemble classifiers using Linguistic Inquiry and Word Count (LIWC) dictionary. Fig.1. illustrates the outline process of the proposed fake news detection methodology in the social network. Initially, the proposed approach is targeted to find the discussed topic on a social network through the topic modeling method. After performing the topic modeling, it contextually applies the pre-processing method on the social media messages, including the headline and body of the text with the help of the LIWC dictionary. LIWC dictionary assists to extract the textual data contextually from cognitive, emotional, and spoken categories. The proposed approach extracts the features of the posted or shared content in a social network without modifying their original context.

Hence, it focuses to maintain the textual words in a sentence in its original structure until it is given as the input to the Ensemble learning classifiers. By applying the stacked ensemble classification model, the proposed approach learns the represented text model and classifies the textual data into real news and fake news. Then, the proposed approach takes the collaborative decision from the classified data that is generated from the multiple base learners using the weight-based Ensemble method. Finally, the proposed approach classifies the real and fake news from the shared news information by exploring both the headline and the body of the text in the content. The novelty of the proposed research is that the framework implemented has used various Ensemble techniques such as bagging, weight-based ensemble method to increase the performance of the model.

Ensemble Algorithms

Ensemble methods are meta-algorithms that associate numerous machine learning methods into one analytical modelin order to reduce variance (bagging), bias (boosting),and enhancepredictions (stacking).

There are 3 types of majorities choosing, where the ensemble selects the class were

- All the classifiers will agree (common elective);
- At least the half of number classifier may predict one (simple majority);
- The receiver will receive the highest number of votes if the vote is over 50%. The ensemble decision for plurality voting can be described as follows: Choose a class

$$\sum_{t=1}^{T} d_{t,J} = \max_{j=1}^{c} \sum_{t=1}^{T} d_{t,J} \quad (1)$$

Bagging

Bagging stands for bootstrap aggregation. One way to reduce the variance of an estimate is to average together multiple estimates. For example, data is trained by different trees on different subfields (randomly selected by replacement) and calculate the ensemble: Base learners use a boosting model to get data subjects for training. For acquiring the outcomes of base learners, bagging is used for classification and residuals for regression.

Boosting

Boosting is another kind of ensemble classifier that is widely used for messy datasets. In particular, these boosting ensemble classifiers are called Meta algorithms. These classifiers reduce the bias and variance. This method boosts the weak learner and makes them strong models. To get precise and accurate results the probabilistic results of ensemble classification are done. Boosting is done sequentially. The given problem uses initially equal-weighted coefficients for all data points which then allows weak learning to correctly classify the data points in an incremental approach. successively the weighted coefficients are decreased for data points that are correctly classified and increased for those which are misclassified. More weights are assigned to the weaker ones that have been identified in the earlier study. Finally, it produces the result by combining through weight-based majority vote or by regression. The only drawback that can be faced in boosting is that sometimes the model overfits the data and predicts the incorrect instances.

Stacking

Stacking is the technique used to combine multiple models like classification through a meta classifier. Every single classification in this model is trained based on the entire training set. Finally, the outputs are classified based on the fitting of the meta classifier. This meta classifier can be either trained well on a predicted label or the probability of the Ensemble classification. Along with this proposed Ensemble framework, the Learning algorithm NBTree, J48, Logistic Regression, and Random Forest are conjugated to explore the performance evaluation of classifying the fake news using the ensemble classifier

Learning Algorithms

NBTree

NBTree is an combination technique of hybridizing the naïve Bayes classifier [25] and decision Tree [26]. This can be implemented over large amount of data and expect the accurate prediction. The trained and learned knowledge is represented in the form of Tree. The construction of the tree is done recursively.

J48

The algorithm is used to create a decision tree which is most appropriately used for Classification and Prediction problems. The tree is generated with mutiple nodes and internodes. Decision tree classifiers are particularly used to make some decisions in critical situations. Tress are modeled by splitting up the input data based on previously trained splitting criteria. The representation of the Decision Tree is rather similar to flow charts denoting the instances. Classification is done based on the selected feature values. Nodes in the trees denote the input instance, wherein the outputs are termed as branches and the leaf nodes are the class labels. The J48 classifier is an application of the C4.5 decision tree algorithm[26]. From the given attribute values, the decision tree is developed and classifies the new instance. When a new training set is given, it immediately responds and takes the responsibility to accurately classify the various instances by eliminating the irrelevant and ambiguous data

Logistic Regression

This algorithm is used in classifying the given problem into multiple or binary classes as Yes/No, True/false, Fake/Real and predicts the output in a discrete/ categorical nature. Logistic Regression does not depend on a value to be in range. They use the Sigmoid function curve, where the Sigmoid curve converts any value from negative to infinity or to a discrete value, which actually Logistic Regression works. The sigmoid function curve works as a transition for Logistic Regression because it transforms an output to a probability value. The LR hypothesis function uses a threshold value

to indicate the probability of 0 or 1, where it means that the value 0 indicates the predicted output as true and 1 results as Fake.

Random Forest

The Enhanced Technique of Decision Tree is called Random Forest. It is a collection of Multiple random decision trees and it is less sensitive to the training datasets. They use multiple trees to randomly select the subset feature, hence it is known as random forest. Random forests are modeled to overcome the drawbacks of the Decision tree. The Decision tree has high variance hence tends to overfit the model. Multiple decision trees are built using the bootstrap technique. Where bootstrap technique ensures that they do not use the same data for every tree, in a way it helps the model to be less sensitive to the training data. The random feature selection helps to reduce the correlation between the trees. From the multiple decision trees, the predictions are noted. Finally, the predictions are combined. As it is a classification problem the majority voting is taken. The process of combining results from multiple models is called aggregation. Random forest are the most commonly used technique. As the researchers found the values close to log or the square root of the total number of features works well. For the regression problem, while combining the predictions the average is taken.

Performance Metrics

To evaluate the performance of the fake news detection algorithm, the experimental framework employs various evaluation metrics. The proposed approach considers the fake news problem as a classification problem that predicts whether a posted social media message is fake or real.

- Precision
- Recall
- F-measure
- Classification Accuracy

Precision

It is the ratio between the number of accurately predicted fake news and the total number of predicted data that are annotated as fake news.

Recall

It is the ratio between the number of accurately predicted fake news and the total number of data that are annotated as the fake news.

F-measure

F-measure or F-score is the harmonic mean of the precision and recall.

Classification Accuracy

It is the ratio between the number of accurately predicted fake news and real news and the total number of data that are in the social media messages.

RESULTS AND DISCUSSIONS

Initially, the data set is collected from the source in the string format and the data is read by the CSV file. The above-discussed algorithm is applied in the development using JDK, NetBeans with a Xamp server to detect the fake news from a data set from the Facebook news. From the input of the data, the Topic is analysed based on a probability model. In this analysis, the data is filtered using the Latent semantics of the text. This semantics is analysed using the vocabulary and words in the dictionary, from that analysis the word index is identified. The Latent semantics helps in identifying either the similar or the related terms to the target keywords. In the next phase, two options were given in order to collect the database. Primarily the benchmark dataset can be taken or else generating the source file

directly from the link. The input source is selected from the link and the text is read from the source. From the text source, the Pre-processing of the text is done through tokenization, NLP, Linguistic Dictionary and form the words based on vocabulary. The text is analysed in the next phase. The Processing is carried out to check the trueness of data. The data is analysed from the source and divided into sequences of the strings, framing them into words, keywords, phrases, symbols and, then the word count is calculated. In this pre-processing stage, the words analysed from the NLP Linguistic Dictionary are tokenized, thus converted to text and used for further extraction. From the pre-processing phase, the text is analysed to extract the features using the NLP technique with LIWC dictionary based on the POS model with whitespace Tokenizer.

The ensemble technique along with the Linguistic Inquiry Word Count dictionary is the novelty of this work. To generate the best feature from the corpus, LIWC 2015 is incorporated. The LIWC dictionary extracts various kinds of linguistic features. Here, the string is mapped with 5 different categories as Social, Affective, Cognitive, Perceptual, and Biological. Few to mention are positive emotions, words indicating Negative Emotions, stop words, Function words, Punctuations used, the informal language used, certain grammars used in the sentences such as articles, preposition, adjectives, and adverbs. From the extracted text the dependency probability is performed as textual analysis using the NLP technique. From this dependency probability, the text is classified into a set of data. The predicted features are then used to train the machine learning model, using the Ensemble classifier which is the second novelty of this work to find the performance and accuracy. Certain measures have been taken to ensure that the model does not overfit or underfit the data. In the classification phase, the data is sent to the classifier to give the Choice of the prediction using Ensemble methods. Here three different voting method has been carried on, where the first choice is to execute majority voting with replacing the missing Values. The second choice is Majority Voting by Replacing Missing Values (Mean & Mode Imputation) then using Synthetic Minority Oversampling Technique (SMOTE) Whereas the final or the third choice is with Majority Voting with Removing Missing Values. From the choice of algorithm, the ensemble classifier does the testing and predicts the result based on the majority of votes given by the models.

Thus, the proposed model yields the performance time, Accuracy, and Prediction from the respective fake news social site datasets. Using the confusion matrix with the given total number of instances, the classified data is analyzed and generates the results of the performance with the performance time and the state of accuracy. Then the data is predicted and presented as mostly false, mostly true and the mixture of true and false

Accuracy Prediction

From the entire classification and detection, the accuracy of the data sets is measured as per the number of records been scanned. The runtime of the same records is measured in number of times of scanning. So, the runtime is termed as choices 1, 2, and 3. The accuracy of scanning the data is shown as increase from the first time to the second time (i.e., ES_Choice I to ES_Choice II), and from second time to third-time accuracy is decreased (i.e., ES_Choice II to ES_Choice III) as number of records increases, the scanning of the records shows the best percentage of accurate prediction in ES_Choice II.

Performance Evaluation

As similar to the accuracy prediction, the performance of the algorithm is also analyzed. From Fig. 3. it is defined as that the performance of the choice II gives the best performance of the time consumption of the algorithm. Inference of Fig.4. As the number of the records, increases the accuracy prediction and performance of the algorithm increases, but it is limited to the 2 runtimes of the algorithm to get the best performance and high percentage of accurate prediction.

Comparison

The obtained result is compared with the single classifier to exhibit the performance and the error rate. Here, the Accuracy is validated between the Ensemble classification method and a single classifier Naive Bayes classification method. As per the expectation, our proposed Ensemble method outperforms more accurately than the Existing

Naïve Bayes shown in Fig.4. It has been proved that the combination of works done by the expertise sounds better compared with a single men army. It performs less when used as a single classifier, rather combining with the other classifier random forest, J48, Logistic Regression as an ensemble shows better performance. Finally, the Performance time was validated between Ensemble classification Method and Naive Bayes classification but the proposed Ensemble method is efficient compare to Existing Naïve Bayes. With the growth of the vast amount of user-generated data in the social network, the social network necessitates an accurate method to reduce the information overload as well as to avoid the fake news spreading over the world. Hence, this work presents a notion for a Fake news detection model in an online social network with the help of an Ensemble Classifier. To obtain the objective of fake news detection model, it targets to identify the topic model and exploit the LIWC dictionary to contextually classify the Fake news from the abundant social media messages. The proposed approach applies a Stacked Ensemble Classifier that consists of a set of base learners to recognize the exact stance based on the decision-making. The accuracy prediction and performance evaluation of time consumption of detecting Fake news is 80% and 11 ms respectively. Thus, it improves the efficiency and Classification accuracy over large-scale social media messages through an efficient Sentiment Analysis model.

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Table 1 Proposed Algorithm for stacking

Algorithm Stacking

Input: training data $D = \{x_i, y_i\}_{i=1}^{m}$
Ouput: ensemble classifier H
Step 1: learn base-level classifiers
for $t = 1$ to T **do**
    learn h_t based on D
end for
Step 2: construct new data set of predictions
for $i = 1$ to m **do**
    $D_h = \{x_i', y_i\}$, where $x_i' = \{h_1(x_i), ..., h_T(x_i)\}$
end for
Step 3: learn a meta-classifier
learn H based on D_h
return H

Table 2. Overall Confusion Matrix.

	Positive	Negative
True	11915	520
False	1397	2449

Table 3. Ensemble Classification

Time taken to build the model	11 Seconds
Correctly Classified Instances	14364
Incorrectly Classified Instances	1917
Kappa statistic	0.6458
Mean absolute error	0.1177
Root mean squared error	0.3431
Relative absolute error	32.6285%
Root relative squared error	80.7838%
Total Number of Instances	16281

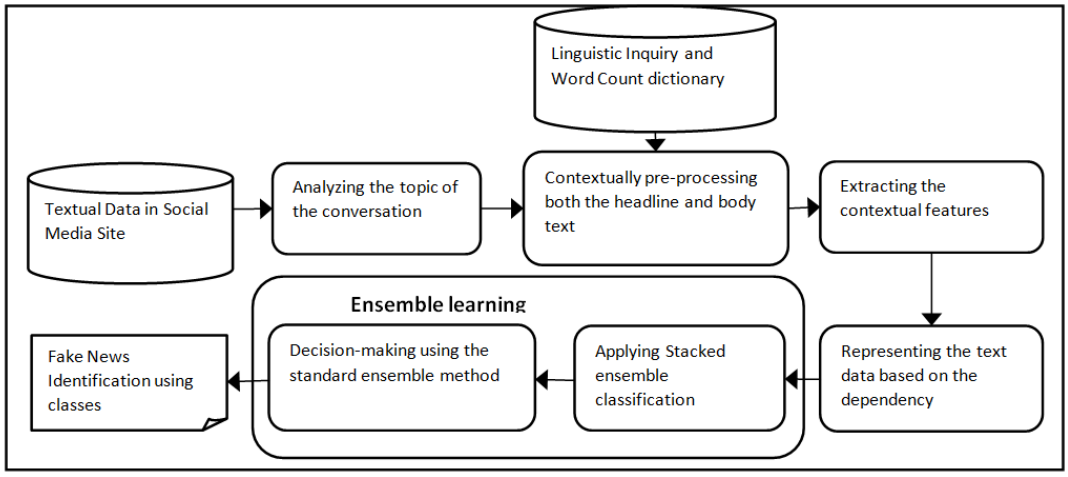

Figure 1. Proposed Architecture on Ensemble Classifier

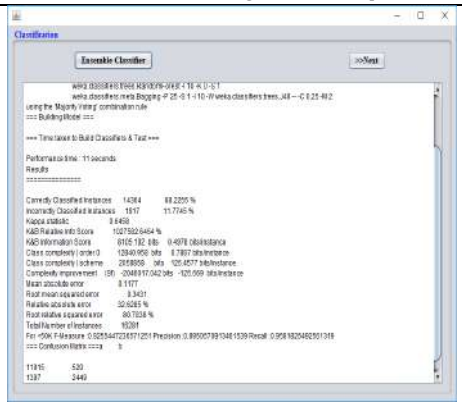

Figure 2. Performance Evaluation

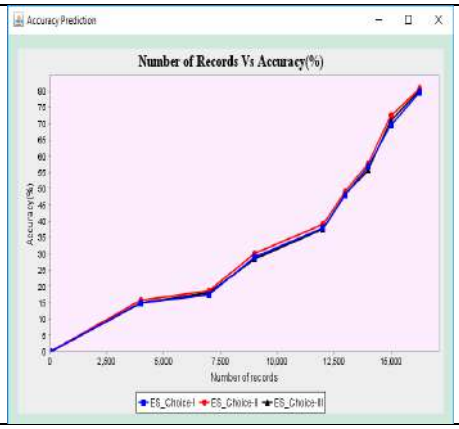

Figure 3. Accuracy Prediction

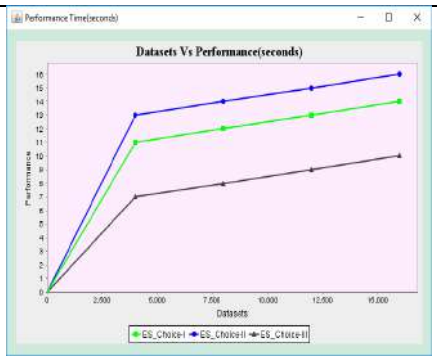

Figure 4. Performance Evaluation of Time(Seconds)

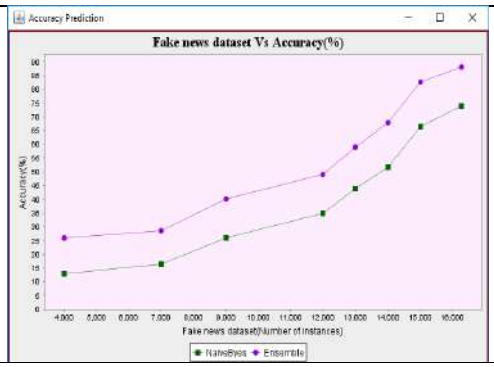

Figure 5. Accuracy Prediction

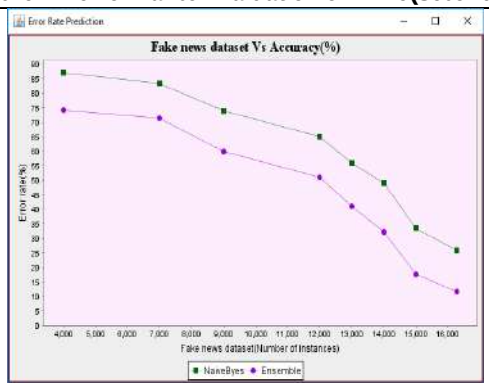

Figure 6. Error Rate between Ensemble Method and Naive Bayes

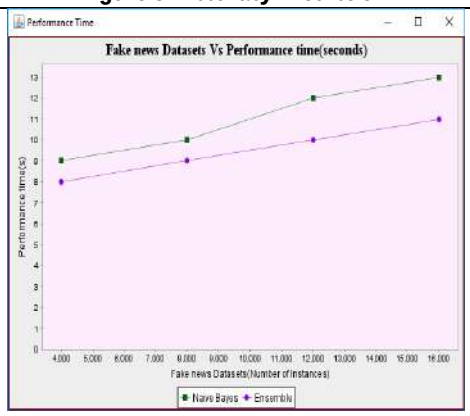

Figure 7. Performance Time Prediction

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Anti-inflammatory and Anti-ulcer Activity of *Morus indica* Linn

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Received: 06 Jan 2022 Revised: 08 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

Anti-inflammatory and anti-ulcer activities of the ethanol and aqueous extract of the shade dried aerial parts of *Morus indica* Linn. were studied in wister rats using the carrageenan induced left hind paw edema method and aspirin induced ulcer method. The ethanolic and aqueous extracts (100 and 250 mg/kg) produced the inhibition of carrageenan induced rat paw edema and also found to be effective in aspirin induced gastric ulcer. The results indicated that the ethanolic and aqueous extracts produced significant ($P<0.001$) anti-inflammatory and anti-ulcer activity when compared with the standard and untreated control.

Keywords: *Morus indica*; ethanolic and aqueous extracts; Anti-inflammatory; Anti-ulcer.

INTRODUCTION

Morus indica Linn. (Urticaceae) is a small plant widely distributed throughout India. Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation [1]. Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects are justified. There is much hope of finding active anti-rheumatic compounds from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs [2]. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions [3]. The enzyme, phospholipase A_2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A_2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cycloxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation [4 and 5]. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory and anti-ulcer activities in rats.

EXPERIMENTAL

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of June 2002 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (MIA-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *M. indica* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water [6]. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Wister rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies [7]

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} was calculated.

Carrageenan induced rat paw edema

Anti-inflammatory activity was assessed by the method described by Winter et al [8]. The rats were divided into four groups of six animals each. First group (negative control) received 1 ml of normal saline, second group (positive control) received 10 mg/kg p.o., indomethacin, third group received ethanolic extract (100 mg/kg) and fourth group received aqueous extract (250 mg/kg) of *M. indica*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St.Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Butte et al [9] as modified by Singh and Ghosh [10]. The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Aspirin induced ulcer model

Anti-ulcer activity was assessed by the aspirin-induced ulcer method described by Hegde et al [11]. The rats were divided into four groups each consisting of six animals. The first group served as a control group, the second group served as positive control, the third and fourth groups served as test groups. The second, third and fourth group were treated respectively with ranitidine (20 mg/kg), ethanolic extract (100 mg/kg) and aqueous extract (250 mg/kg) of *M. indica*, orally for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (200 mg/kg orally) on the day of sacrifice. The animals were sacrificed 4 h later and the stomach was opened to calculate the ulcer index by Kunchandy method [12].

Statistical Analysis

Statistical analysis was performed using student's t-test. The values are represented as mean±SEM. Level of significance was set at $P<0.001$.

RESULTS

The plant *M. indica* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of alcohol extract of *M. indica* was found to be 2.2 and for aqueous 3.7 % w/w. On preliminary phytochemical screening of the aerial parts of *M. indica* revealed the presence of alkaloids, flavanoids, saponins and tannins. The LD_{50} of the extracts were found to be 1149 and 2497 mg/kg for alcohol and aqueous extracts of *M. indica*.

Carrageenan induced rat paw edema

The effect of extracts of *M. indica* on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the ethanolic and aqueous extracts were found to have significant anti-inflammatory activity in rats. The ethanolic and aqueous extracts of *M.indica* reduced the edema induced by carrageenan by 59.46 and 51.35 % on oral administration of 100 and 250 mg/kg respectively, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 70.27 %.

Aspirin induced ulcer mode

The effect of ethanolic and aqueous extracts of *M. indica* on aspirin induced ulcer model is presented in Table 2. The results of the present study indicate that the ethanolic and aqueous extracts significantly reduce the ulcer score and also have activity against gastric ulcers in rats. The control animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extracts of *M. indica* there was significant reduction in ulcer index.

DISCUSSIONS AND CONCLUSION

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation without producing ulcer. The traditional medical practitioners of Kolli hills, Tamilnadu, are using this plant to cure inflammation and ulcer. To give a scientific validation to this plant, an attempt was made to study the anti-inflammatory and anti-ulcer activities. Carrageenan induced inflammation is a biphasic phenomenon [13]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances [14]. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and maintenance of the mucosal integrity through endogenous defence mechanisms [15]. The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as decrease in aggressive factors mainly acid and pepsin [16]. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage the cell membrane of mucosal, parietal and endothelial cells [17]. Thus it can be concluded that the aerial parts of the plant *M. indica* possess significant anti-inflammatory and anti-ulcer activity in rats. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory and anti-ulcer agent with a low toxicity and better therapeutic index.

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Table 1. Effect of ethanolic and aqueous extracts of *M. indica* on Carrageenan induced rat paw edema

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume (ml)	% Decrease in paw volume
Control (Normal saline)	1 ml	0.37±0.0073	-
Indomethacin	10	0.11±0.0057*	70.27
Ethanolic extract of M.indica	100	0.15±0.0026*	59.46
Aqueous extract of M.indica	250	0.18±0.0045*	51.35

**P*<0.001 when compared with control. Values are expressed as mean±SEM (n=6)

Table 2. Effect of ethanolic and aqueous extracts of *M. indica* on aspirin induced gastric ulcer in rats

Treatment	Dose (mg/kg, p.o.)	Ulcer score	% Protection
Control (Normal saline)	1 ml	4.1±0.02	-
Ranitidine	20	1.6±0.03*	58.54
Ethanolic extract of *M.indica*	100	2.6±0.05**	36.59
Aqueous extract of *M.indica*	250	2.1±0.05*	48.78

**P*<0.001, ***P*<0.01 when compared with control. Values are expressed as mean±SEM (n=6)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Immunoboosting Role of Herbs in Kitchen Garden: Garlic and Ginger

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Received: 29 Jan 2021 Revised: 14 Feb 2022 Accepted: 23 Mar 2022

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ABSTRACT

Ayurveda, an ancient science of life, recommends simplifying one's lifestyle and promoting awareness of boosting and maintaining one's immunity through the use of various herbs that are readily available in most people's kitchen gardens. The current study focused on review of immunoboosting activity of *Zingiber officinale* and *Allium sativum*. The study elaborates ginger is effective in viral infections and revitalizing the body at disease conditions, enhance immunity and re-boosting weakened physiological functions of the human body. Active ingredient in ginger 6-gingerole which on dehydration forms 6-shogaol and further on hydrolysis occurs 6-paradol, are responsible for these actions. Allicin present in the garlic shows immunoboosting activity. The review also includes the biosynthesis of allicin as well. Garlic and ginger both have components that aid in immune system support and inflammation reduction. These commonly available herbs in the kitchen garden are briefed in this review along with its chemistry and immunomodulating activity. Researchers will hopefully be encouraged to pursue more research on medicinal plants with immunomodulatory potential as a result of this study.

Keywords: Herbal plants, Immunoboosters, Traditional medicines, Ginger, Garlic, Immunomodulation.

INTRODUCTION

Herbs and traditional foods have been shown to be useful in the treatment of lifestyle diseases such as diabetes, heart disease, hypertension, arthritis, and cancer, as well as providing specific health advantages to the body's defence mechanisms by strengthening the immune system. Immunotonics are herbs that have been recognised as crucial for everyday immune system health and are categorised as immunostimulants, immunomodulators, or immunoadjuvants. These immunoagents are known to have a balanced impact on the immune system and are

expected to enhance or boost the defence system [1]. Immunostimulation, tonic, neurostimulation, anti-ageing, antibacterial, antiviral, antirheumatic, anticancer, adaptogenic, and other ethnopharmacological actions are included in the ayurvedic system of medicine, which is one of the oldest systems of medicine [2]. The novel corona virus illness (COVID-19) has spread over the world, infecting over 2 million people and causing over 100,000 fatalities. Patients with infectious and non-infectious disorders, such as diabetes, hypertension, and kidney disease, are thought to be more vulnerable to this viral infection due to lowered immunity. As a result, improving immunity (a natural bodily system) may be the most effective way to prevent many pathogenic illnesses while also preserving optimal health [3]. According to the World Health Organization (WHO), traditional remedies (mostly herbs) are used by nearly three-quarters of the world's population for health care. Herbs and plants are, in fact, mankind's oldest allies. They not only functioned as a source of food and shelter, but also as a remedy for a variety of maladies. As botanicals are chemically complex and diverse, they could be employed as immuno medication cocktail to restore immunostasis. Immunological and health benefits have been identified in herbs and traditional foods such as *Echinacea purpurea, Zigiber officinale, Solanum lycopersicum, Panax ginseng, Tinospora cardiofolia, Curcuma longa, Andrographis peniculata, Hypericum perforatum, Withania somnifera, and Allium sativum[*1]. Traditional medicine is currently being revalued all over the world as a result of considerable research on diverse plant species and their therapeutic capabilities.

Garlic

Garlic, *Allium sativum L*., is a *Liliaceae* family plant that has long been known as a wonderful spice and a popular treatment for a variety of diseases and physiological abnormalities. Garlic is one of the most popular bulb crops in India, where it is farmed and consumed as a spice or condiment. [4].

Constituents

It has higher levels of sulphur compounds, which give it its distinct flavour and taste and are also responsible for its health benefits. Garlic has 65 percent water, 28 percent carbohydrate (fructans), 2.3 percent organosulfur compounds, 2% protein (allinase), 1.2 percent free amino acid (arginine), and 1.5 percent fibre. The unpleasant odour and therapeutic capabilities of allicin (diallyl thiosulfate) are due to the active component allicin (diallyl thiosulfate).The chemical structure of allicin is shown in Figure 1. Garlic contains the sulfur-containing molecules gamma-glutamyl-s-allyl-cysteine and S-allyl-L-cysteine sulfoxides (alliin). These are also precursors of a variety of other chemicals. [5]. Alliin is a colour less, odour less, watersoluble amino acid present in the intact cells of garlic. Within the cell there are vacuoles that contain an enzyme known as allinase. When the cell is crushed/ injured/sliced, the enzyme allinase comes in contact with alliin and causes its breakdown into the sulphur containing product allicin. Allicin is the antibacterial substance of garlic and has the typical odour of fresh garlic. It is a chemically unstable, colour less liquid, which accounts for the odour of garlic. It breaks down into the strong smelling constituents of garlic oil[6]. Pharmacological activity of allicin is identified using PASS Way 2 Drug approach and mentioned in Table 2

Indications

Several garlic components have been shown to be effective in treating a variety of diseases, including cancer. Garlic and its constituents have been discovered to have significant antioxidant effects. Garlic's medicinal potential has also been investigated in the treatment of allergic-airway inflammation, inflammatory bowel illness, arthritic rheumatism, and atherosclerosis, among other inflammatory conditions. Garlic was also discovered to be able to maintain immune system homeostasis and to have positive impacts on immune cells, particularly through the regulation of proliferation and cytokine gene expression. [7]. The indications of garlic are illustrated in Figure 2. The antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antihypertensive, and immunommodulatory properties of garlic (Allium sativum L.) have long been recognised, and garlic has a reputation for its efficacy as an antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antihypertensive, and immunommodulatory agent[8].

Biosynthesis of Allicin

Stoll and Seebeck in 1948 established the structure of allicin, which is a thiosulfinate. Allicin is created in nature after an enzymatic process damages plant tissue. The non-proteinogenic amino acid alliin (S-allyl-L-cysteine sulfoxide) is allicin'sprecursor .Alliin and other S-alkyl-L-cysteine sulfoxides are hydrolyzed by the enzyme alliinase, which produces dehydroalanineand allyl sulfenicacid in the case of alliin. Two molecules of allyl sulfenic acid spontaneously condense into one molecule of allicin. Garlic (Allium sativum) and ramsons (Allium ursinum) contain alliin. Alliin (trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide) is synthesised by onion (Allium cepa) but not by its isomer isoalliin (trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide). The biosynthetic pathway to alliin is currently unknown. Granroth's pioneering work, in which he described two probable biosynthetic pathways based on radioactive labelling tests, has yet to be surpassed. [9].

Garlic as an Immunobooster (Allicin)

The predominant thiosulfinate in fresh garlic extract identified as allicin, has shown a number of health benefits like immunoboosting due to its anti-inflammatory, antioxidant and antiviral properties[10]. Allicin inhibits TNF- α induced expression of IL-1, IL-8, IP-10, and IFN- α, as well as the degradation of the NF κ-B inhibitory protein IB in intestinal epithelial cells, which lowers inflammation [11]. It inhibits the production of inducible NO nitric oxide synthase in activated macrophages [12]. Several garlic-related chemicals have been reported to have potent antiviral activity against a variety of viruses, including the human rhinovirus, herpes simplex virus (HSV)-1, HSV-2, and vesicular stomatitis virus (VSV). Ajoene, allicin, allyl, methyl thiosulfinate, and methyl allyl thiosulfinate are some of the garlic chemicals that have viricidal activity [13]. As a result, fresh garlic extract could be used as a COVID-19 preventive or as an immunobooster.

Garlic contains powerful antioxidants called organosulfur compounds, which assist to boost antioxidant enzymes in the liver[14]. Because antioxidative activity is mostly determined by the material's electron state, in vivo processes that occur in the body should be considered while evaluating garlic's active components. Fresh garlic extract is lower in antioxidants than aged garlic extract. Aged garlic extracts aids in the increase of cellular antioxidants, which aids in the maintenance of a healthy immune system [15]. It also helps to eliminate hazardous peroxides from the body by boosting peroxides, which helps to reduce medication toxicity. Garlic oil may potentially provide health benefits by acting as a free radical scavenger and providing protection against metal and chemical toxicity [16]. To prove garlic's positive effect, more long-term intervention trials are needed. As an all-natural immune booster with the emergence of terrifying viral infections such as HIV/AIDS, increasing one's immune system has become more important. Because there are no viable therapies or treatments for many disorders, bolstering the body's ability to resist infection has become even more critical. Garlic contains a lot of sulfur-containing amino acids and other chemicals that tend to boost immune system activation [17]. It is one of the most outstanding conductors of the immune system, stimulating immunological function by increasing the activity of macrophages or killer cells. Inadequate diet, cigarette smoke, physical damage, mental anxiety, and chemical pollution are all continuous enemies. [18,19]. The immunoboosting activity of garlic is given in Figure 3.

Ginger

Ginger consists of the rhizomes of *Zingiber officinale* belongs to family *Zingiberaceae*. It is a herbaceous plant with a cluster of lateral shoots that can reach a height of up to 90cm when fully developed. It also has a length of 15-30 cm and a width of 2-3 cm. Rizomnya is a yellowish brown aromatic fragrance that has a pleasant scent. It has leaves that are 17.8cm long and 1.9cm broad. The flowers are yellowish green in colour and are rarely seen. Ginger is a perennial creeping plant with a lengthy life span. [20]. Due to its diverse agro-climatic conditions, India's North East area has a rich ginger diversity. Although ginger is grown throughout the region, Meghalaya, Mizoram, Arunachal Pradesh, and Sikkim are the main producers of this cash crop. [21].

Chemistry of Ginger

The essential oil of the rhizome extract is made up of a variety of volatile and non-volatile chemicals that give ginger its distinct aroma and flavour. The unique pungency of ginger oil is due to the non-volatile chemicals glycerol, paradols, shogaols, and zingerone. [22,23]. The essential oil and oleoresin concentration of ginger cultivars from the north-eastern region was found to be higher than that of ginger cultivars from other parts of India[24]. Essential oil characteristics and yield vary by variety and are influenced by environmental factors. Ginger essential oil has been shown to have anti-inflammatory, antioxidant, antitumoral, anti-diabetic, hepatoprotective, gastroprotective, and antibacterial properties[25].The gingerol were discovered to be the most active components in fresh ginger rhizomes.Several studies have reported that the oil extracted from ginger had scavenging effects due to volatile oils[26]. The gingerol, a group of chemical homologs distinguished by the length of their unbranched alkyl chains, were discovered to be the primary active ingredients in the fresh rhizome. The shogaols, another homologous series, as well as the dehydrated form of the gingerols, are the most pungent elements in dried ginger. When shogoal is hydrogenated, paradol is generated, which is similar to gingerol. The chemical structure 6-gingerol, 6- shagoal and paradol are shown in Figure 4. Oleo resins are an additional component. Fats, waxes, carbs, vitamins, and minerals are all found in ginger. Zingibain, a powerful proteolytic enzyme found in ginger rhizomes, is also present[27]. Pharmacological activity of 6-gingerol, 6- shagoal and paradol are identified using PASS Way 2 Drug approach and mentioned in Table 2,3 and 4 respectively.

Indications

Ginger is a commonly used medicinal plant, primarily as an anti-emetic drug with a broad scope. Ginger (*Zingiber officinale*) has been studied for a variety of pharmacologic activities, including improving insulin sensitivity to reduce hyperglycemia and hyperlipidemia, antithrombotic and anti-inflammatory activities by inhibiting prostaglandin and/or cytokines/chemokine synthesis, direct and indirect anti-hypertensive effects, gastrointestinal protective effects against ulceration and emesis, antioxidant and radioprotective effects. Ginger also possesses antibacterial properties against a variety of bacteria, fungus, and nematodes[28].It has also been claimed that ginger has anti-inflammatory properties through processes that explain the role of suppression of pre-inflammatory factors such prostaglandin and leukotriene production, which may help to relieve pain from rheumatoid and osteoarthritis. It has been demonstrated to be effective in the treatment of rheumatic disorders throughout history[29]. Ginger's anti-inflammatory properties are beneficial in the treatment of respiratory infections, arthritis, allergic illnesses, and gout. In a nutshell, ginger and its components have been shown to have anti-inflammatory properties. They are useful in lowering the severity of chemical toxicity and are crucial in the treatment of inflammatory illness. [30]. The indications of ginger is illustrated in Figure 5.

Ginger and its Immunoboosting Activity

Polyphenol chemicals (6-gingerol and its derivatives) found in ginger root extracts have a significant antioxidant activity. Antioxidant chemicals are commonly utilised to combat free radicals, which cause oxidative stress in cells. Flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins, and isocatechins all have antioxidant action. The total phenolic content of the dried rhizome of ginger alcoholic extract was 870.1 mg/g extract. With an IC50 concentration of 0.64 g/ml, extract showed 90.1 percent DPPH radical scavenging activity [31]. Ginger's antioxidant activity is an important one that can be used to prevent a variety of ailments which have significant antioxidant and anti-inflammatory effects to protect the body and improve the immune system [32]. Ginger extracts have been found in multiple animal and test-tube experiments to help manage autoimmune illnesses including rheumatoid arthritis and boost immune system function [33]. The immunoboosting activity of ginger is illustrated in Figure 6.

CONCLUSION

Herbals have become increasingly popular for its immunoboosting activity in recent years in the United States and other parts of the world, but the mechanisms of action of most of these herbals have not been thoroughly

investigated. Herbs have therapeutic potential for preventing or curing specific diseases. Indeed, research revealed that these plants and its chemical constituents are efficient in balancing and correct immune system performance via various immune modulations such as activation and repression. Many common diseases are caused or progressed by changes in the immune system's equilibrium. Natural substances will assist in the delivery of optimal immune function and the regulation of undesired inflammatory responses in the body. Nature's Immune Stimulators are a combination of natural herbs that support and stimulate the immune system, fight infections, aid in the healing of infections-related injuries, and regulate other problems that can lead to disease. Immune enhancement is a primary concern of diet-based therapy for a variety of illnesses, and researchers are on the lookout for immune boosters. Garlic and ginger have a long history of both supporting the immune system and reducing inflammation. In a nutshell, these are thought to be one of the most promising choices for immunological boosting, improving the body's antioxidant state, and protecting the body against free radicals, inflammation, and cancer insurgence. The two herbs *Allium sativum* and *Zingiber officinale* discussed above have various chemicals components which stimulate the immune system in many ways. As a result, nature's immune stimulators will assist to strengthen the entire body, allowing you to live a higher quality of life.

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Table no.1: Pass Analysis of Allicin

Pa	Pi	Activity
0,959	0,003	Apoptosis agonist
0,884	0,001	Chemoprotective
0,852	0,020	Aspulvinone dimethylallyltransferase inhibitor
0,781	0,015	NADPH peroxidase inhibitor
0,756	0,005	Chloride peroxidase inhibitor
0,752	0,007	Fatty-acyl-CoA synthase inhibitor
0,764	0,030	Mucomembranous protector
0,726	0,009	Electron-transferring-flavoprotein dehydrogenase inhibitor
0,714	0,013	Cl--transporting ATPase inhibitor
0,719	0,022	TP53 expression enhancer
0,713	0,018	Alkylacetylglycerophosphatase inhibitor
0,703	0,014	Phosphatidylcholine-retinol O-acyltransferase inhibitor
0,706	0,022	GST A substrate

0,683	0,004	Glutathione S-transferase substrate
0,710	0,032	Sugar-phosphatase inhibitor

Pa: Probability of Activity Pi: Probability of Inactivity

Table no. 2: PASS Analysis of 6-gingerol

Pa	Pi	Activity
0,960	0,003	5 Hydroxytryptamine release stimulant
0,911	0,003	Linoleate diol synthase inhibitor
0,860	0,021	CYP2C12 substrate
0,817	0,010	Feruloyl esterase inhibitor
0,803	0,007	UDP-glucuronosyltransferase substrate
0,817	0,027	Ubiquinol-cytochrome-c reductase inhibitor
0,772	0,004	Preneoplastic conditions treatment
0,771	0,004	Steroid N-acetylglucosaminyltransferase inhibitor
0,762	0,007	Macrophage colony stimulating factor agonist
0,757	0,004	Mycothiol-S-conjugate amidase inhibitor
0,758	0,008	Fibrinolytic
0,765	0,027	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,757	0,027	Polyporopepsin inhibitor
0,730	0,007	Vasodilator, peripheral
0,707	0,005	Beta-carotene 15,15'-monooxygenase inhibitor
0,735	0,035	Chymosin inhibitor
0,735	0,035	Acrocylindropepsin inhibitor
0,735	0,035	Saccharopepsin inhibitor

Pa: Probability of Activity Pi: Probability of Inactivity

Table no 3: PASS Analysis of 6-shagoal

Pa	Pi	Activity
0,927	0,003	Linoleate diol synthase inhibitor
0,873	0,002	Preneoplastic conditions treatment
0,876	0,007	5 Hydroxytryptamine release stimulant
0,863	0,002	MMP9 expression inhibitor
0,855	0,002	Beta-carotene 15,15'-monooxygenase inhibitor
0,851	0,003	Antimutagenic
0,849	0,009	Mucomembranous protector
0,834	0,002	Steroid N-acetylglucosaminyltransferase inhibitor
0,842	0,011	Antieczematic
0,828	0,006	JAK2 expression inhibitor
0,812	0,011	HIF1A expression inhibitor
0,819	0,027	Ubiquinol-cytochrome-c reductase inhibitor
0,820	0,028	Aspulvinone dimethylallyltransferase inhibitor
0,803	0,013	Mucositis treatment
0,772	0,002	Vanillyl-alcohol oxidase inhibitor
0,772	0,009	UDP-glucuronosyltransferase substrate
0,767	0,004	HMOX1 expression enhancer

0,791	0,038	CYP2C12 substrate
0,754	0,004	TNF expression inhibitor
0,772	0,025	Chlordecone reductase inhibitor
0,757	0,016	TP53 expression enhancer
0,769	0,029	CYP2J substrate
0,743	0,003	Free radical scavenger
0,759	0,028	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,748	0,022	CYP2J2 substrate
0,728	0,005	Reductant
0,734	0,018	Feruloyl esterase inhibitor
0,730	0,019	GST A substrate
0,707	0,004	GST M substrate
0,717	0,014	Antiinflammatory
0,705	0,003	Peroxidase substrate
0,715	0,017	Fibrinolytic
0,711	0,014	Apoptosis agonist

Pa: Probability of Activity Pi: Probability of Inactivity

Table no. 4: PASS Analysis of Paradol

Pa	Pi	Activity
0,949	0,003	5 Hydroxytryptamine release stimulant
0,897	0,004	Linoleate diol synthase inhibitor
0,892	0,002	Preneoplastic conditions treatment
0,875	0,014	Aspulvinone dimethylallyltransferase inhibitor
0,874	0,018	CYP2C12 substrate
0,855	0,002	MMP9 expression inhibitor
0,854	0,002	Steroid N-acetylglucosaminyltransferase inhibitor
0,844	0,010	Mucomembranous protector
0,851	0,017	Ubiquinol-cytochrome-c reductase inhibitor
0,841	0,013	Chlordecone reductase inhibitor
0,811	0,003	Beta-carotene 15,15'-monooxygenase inhibitor
0,811	0,007	JAK2 expression inhibitor
0,798	0,013	Taurine dehydrogenase inhibitor
0,789	0,004	Antimutagenic
0,783	0,003	Mycothiol-S-conjugate amidase inhibitor
0,797	0,017	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,786	0,013	Feruloyl esterase inhibitor
0,787	0,015	Mucositis treatment
0,767	0,002	Catechol 2,3-dioxygenase inhibitor
0,764	0,007	Fibrinolytic
0,761	0,005	Cardiovascular analeptic
0,760	0,016	TP53 expression enhancer
0,757	0,027	Polyporopepsin inhibitor
0,729	0,002	Vanillyl-alcohol oxidase inhibitor

0,726	0,005	Lipid peroxidase inhibitor
0,724	0,029	Sugar-phosphatase inhibitor
0,701	0,014	Macrophage colony stimulating factor agonist
0,689	0,006	Cytoprotectant
0,681	0,004	Plastoquinol-plastocyanin reductase inhibitor
0,717	0,040	Chymosin inhibitor
0,717	0,040	Acrocylindropepsin inhibitor

Pa: Probability of Activity Pi: Probability of Inactivity

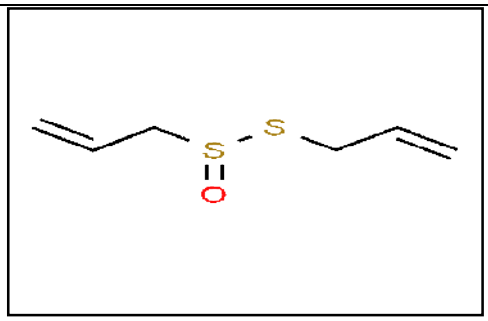

Figure 1: Structure of allicin

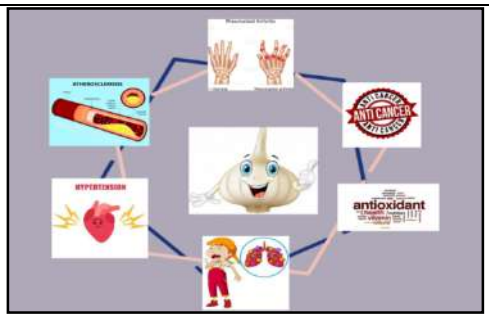

Figure 2: Indications of Garlic

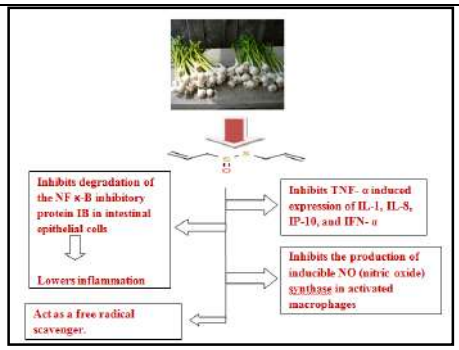

Figure 3: Immunoboosting activity of Allicin

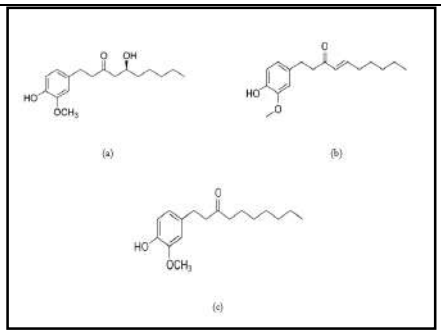

Figure 4: Chemical structure of: (a) 6-gingerol (b) 6-shagoal (c) Paradol

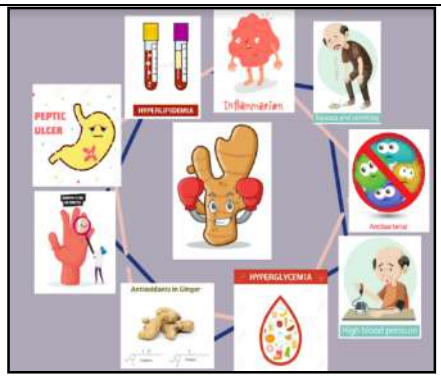

Figure 5: Indications of Ginger

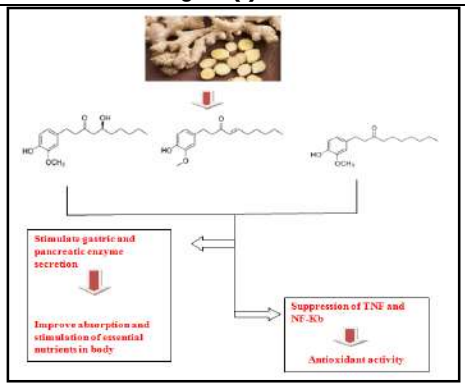

Figure 6: Immunoboosting activity of Ginger

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antibiofilm Efficacy of *Mangifera indica* Kernel Methanol Extract against *Staphylococcus aureus*

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Received: 25 Jan 2022 Revised: 20 Feb 2022 Accepted: 09 Mar 2022

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ABSTRACT

Staphylococcus aureus (SA) has shown to cause life-threatening nosocomial and community acquired infections. Biofilm formation is a significant virulence factor in staphylococcal associated infections. The concentration of tannin (0.4324mg/g TAE) and phenol (1.95 mg/g GAE) was significantly high in methanolic and acetone extracts of *Mangifera indica* kernel (MKE) when compared to its extraction in other solvents. Methanol extract of mango kernel was found to inhibit biofilm formation in dose dependent manner, upon 30-80% reduction observed with 4.52- 72.32µg/ml concentration, whereas it was only 35 to 40% in ciprofloxacin (10µg/ml). Methanol extract of mango kernel showed visual inhibition in biofilm architecture after 2hr, 4hr, 6hr, 8hr and 24hrs intervals, respectively when compared to its untreated controls. Bioactive compound analysis using GC-MS revealed presence of many important compounds such as Oleic acid (22.03%), n- Hexadecanoic acid (10.12%), Gamma-sitosterol (14.23%), Lathosterol (9.68%), and 24-Norursa- 3,12-diene (3.44%) etc. Bioactive compounds in MKE have contributed to its strong anti- biofilm function against Staphylococcus aureus. Hence, the present study demonstrates the anti-biofilm potential of methanol extract of mango kernel against *Staphylococcus aureus.*

Keywords: *Mangifera indica*, Mango, Staphylococcus aureus, kernel, antibiofilm.

INTRODUCTION

Staphylococcus aureus is a Gram-positive, ubiquitous bacterial species with 20-25% of colonization properties as the normal flora of the human population. *S.aureus* is perhaps the most important bacterial pathogen in the western world. These multidrug-resistant *S. aureus* isolated from clinical settings is likely to form biofilms in indwelling medical devices and increase the chances of developing into persistent, chronic and recurrent infections in humans causing osteomyelitis and endocarditis (1). *Staphylo cocci* are known to be the most common causes of biofilm-associated infections. Major challenges in treating *Staphylo coccal* infection is the increased resistance of the bacteria within the biofilm structures to antimicrobial agents and host defense mechanisms (2). Resistance to antimicrobial agents is mediated through a dormant phenotype caused by adaptation to an anoxic environment and nutrient deprivation. As a result, the metabolic levels of the bacterial cells are low and cell division occurs at radically down-regulated rates (3), producing many slow- growing cells and a subpopulation of dormant cells capable to withstand high doses of antimicrobial agents. Therefore, antibiotics such as β-lactams which are only active against dividing staphylococcal cells are not very efficient in eradicating biofilm infections (3). The exopolysaccharide matrix in biofilm may prevent the entry of antimicrobial agents thereby serving as diffusion barrier (4)(5). An effective treatment strategy in controlling these emerging and evolving multidrug-resistant *S.aureus* is important. Major three strategies in thwarting biofilm development is by preventing the adhesion of bacteria to living or non-living surfaces at the primary stage, disturbing biofilm matrix at the maturation process, and finally by interrupting the Quorum sensing (QS) signalling process (6). The organization of biofilm and its associated virulence ability is been regulated by complex QS phenomenon which increases its ability to survive at unfavourable situations (7). Thus, interrupting the QS system or quorum quenching (QQ) may decrease the expression of virulent factor determinants. Natural products are one of the most important sources of novel drugs since decade due to their unique structural diversity (8). Recent advanced state-of-the-art technologies were used in extracting bioactive molecules to discover lead compounds from plants which can be used eventually for treating persistent infections associated with biofilm formation in bacteria. Extracts from *Krameria, Aesculus hippocastanum*, and *Chelidonium majus* yielded four compounds, namely chelerythrine, sanguinarine, dihydroxybenzofuran, and proanthocyanidin, which demonstrated biofilm inhibition in *S. aureus* (9). Polyphenolic compounds tannic acid also inhibits *S.aureus* biofilm in multiple biofilm models without inhibiting bacterial growth (10). Ellagic acid derivatives from *Rubus ulmifolius* was found to decrease *S. aureus* biofilm formation and increase its susceptibility to daptomycin, clindamycin, and oxacillin without exerting toxic effects on normal mammalian cells (11).

Mango (*Mangifera indica*) is a fruit plant of family Anacardiaceae, widely grown all over the world, and is a very prevalent fruit in the world market. India is the world's largest producer of mangoes, with an annual production of 15 million metric tons (12). Large quantities of mango processing coproducts are generated (peels and seeds), which are discarded as waste, yet are a potential source of numerous bioactive compounds. Mango kernel is a remarkably rich source of phytochemicals such as tocopherols, phytosterols, carotenoids, polyphenols (gallotannins, flavonols, benzophenone derivatives, mangiferin, homomangiferin, isomangiferin, anthocyanins, kaempferol, and quercetin), and phenolic acids (4-caffeoylquinic acids, caffeic, coumaric, ellagic, gallic, and ferulic acid) are reported in mango kernel (13). Recently, mango peels and kernel have attracted attention of medical community because of their high content of potential compounds, such as polyphenols, carotenoids, enzymes, and antioxidants. Thus, in this study attempts were made to detect the phytochemical components and to identify the antibiofilm potential of mango kernel waste against *Staphylococcus aureus* (SA). So, the goal of this study is to explore the therapeutic potentials of mango kernel wastes and its metabolites.

MATERIALS AND METHODS

Preparation of mango kernel powder

The mango kernel samples were collected from Varadharajan mango pulp processing industry, Krishnagiri dt (Longitude -78°12′31.75″E (78.208817), latitude -12°31′43.43″N (12.528732)). The mango kernel powder were prepared by modified method of Mwaurah *et al.* (13)(14). Kernel stones were shadow dried and thoroughly washed to remove adhering particles and were dried in electric oven at 40°C for 48 hours and grind to a very fine powder. The kernel powder were stored in refrigerator for further use.

Extraction of Bioactive compounds

In order to extract bioactive compounds, mango kernel powder was extracted with different solvents such as, 80% methanol, hexane, water, chloroform, diethyl ether and acetone at 1:10 (w/v) ratio. This mixture was kept in shaker for 72 hours at 37°C and it was filtered with whatman filter paper. The filtrate was centrifuged for 10 minutes at 10000 rpm at 40°C). The supernatant was transferred in a clean beaker. The extract was kept in fumic cupboard until the residue gets dried. The percentage of extraction yield from the dried crude extract was determined using the formula:

$$\text{Percentage of Yield} = \frac{\text{Weight of the extract residue obtained after solvent removal} \times 100}{\text{Weight of the kernel powder}}$$

The dried residue was redissolved in methanol and the crude extract was stored and used for further anti-bacterial and anti biofilm activities and used for GC-MS analysis.

Estimation of total phenol content in dried MKE in different solvents:

The total phenolic content of dry extract was quantified using folin-ciocaltaeu assay. One ml of sample was mixed with 1 ml of folin ciocaltaeu 's phenol reagent (1mg/ml) (12). After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture and followed by the addition of 13 ml of deionized distilled water. The phenolic mixture was kept in the dark place for 2 hours at room temperature. After incubation absorbance was measured at 725 nm. Gallic acid standards were prepared and used for the comparison and quantification of total phenolic contentin MKE. The estimation of phenolic compounds was carried out in triplicates. The TPC was expressed in milligrams of gallic acid equivalents (GAE) /g of MKE (14).

Estimation of total tannin content in dried MKE in different solvents

Quantification of tannin in MKE was determined by folin-ciocaltaeu method. About 1 ml of the sample (1 mg/ml) was added to volumetric flask containing 75 ml of distilled water and 5 ml of folin-ciocaltaeu phenol reagent, one ml of sodium carbonate solution was added and the volume was made upto 100ml with distilled water. The content were shaken well incubated at room temperature for 30 minutes. A set of reference standard solutions of tannic acid (20,40,60,80,100µg/ml) was prepared and tannin estimation was done as described before in the same manner. The absorbance of standard solution was measured against the blank at 700nm with UV/ visible spectrophotometer. Prepared a standard graph using 0-100µg of tannic acid. The tannin content was expressed in mg of tannic acid equivalents TAE/g of dried sample (12).

Bacterial strains

Three different *Staphylococcus aureus* strains were obtained from SKS, clinical laboratory, Salem district, India and were named as SA-1, SA-2 and SA-3, These bacterial cultures were obtained from samples collected from urinary infected patients.

Determination of anti-bacterial activity of *MKE*

Muller Hinton Agar (MHA) plates were prepared and autoclaved. The overnight cultures of SA-1, SA-2 and SA-3 organisms were swabbed using sterile cotton swab. Using sterile well cutter of size 6mm the agar surface were cut aseptically. The wells are filled with 10, 20, 30, 40, and 50 µl of different concentration of crude MKE in 80% methanol. 80% methanol was kept as negative control. After 24hrs of incubation the zone of inhibition against SA were measured.

Determination of minimum inhibitory concentration

For the assay, bacterial strains were grown overnight in Muller- Hinton Broth (MHB) media at 37°C. Then 10 µl of the adjusted culture suspension (1×10^8 CFU/mL) was added to 190 µl of tryptic soy broth (TSB) medium in a 96 well flat bottomed microtitre plate and experiments were tested in triplicate. Wells containing sterile TSB was kept as controls. Purified MKE of 10 µl were added to the wells and kept for incubation in aerobic condition for 24 hours at 37˚C. After incubation, the absorbance were measured at 595 nm using Biorad, ELISA reader.

Biofilm inhibition assay

The culture suspensions were adjusted to 0.5McFarland scale (OD_{600}=0.08-0.13). Then 10 µl of the adjusted culture suspension was added to 190 µl of trypticase soy broth (TSB) medium in 96 wells flat bottomed microtitre plate. Each strains were tested in triplicate. Wells with sterile TSB alone served as controls. The plates were incubated aerobically for 24 hours at 37˚C. After incubation the culture was aspirated and the wells washed three times with 200µl of 0.85% phosphate buffered saline (pH 7.4) to remove non adherent cells and dried in an inverted position. Adherent biofilm was fixed with 200µl methanol for 15 minutes. Then methanol was discarded and wells were stained with 200ul of 0.1% (c/v) crystal violet and wait for 30 minutes. Then, unbound crystal violet was removed and then wells were washed three times with sterile distilled water. The water was then removed and the microtiter plate was air dried 30 minutes. Crystal violet solubilisation was done by adding 200ul of 95% ethanol and kept for 10 minutes and vigorous pipetting. The optical density (OD) of each well was measured at 570 nm and using Biorad, ELISA microplate reader (5).

Determination of minimum biofilm inhibitory concentration (MBIC)

For the assay, 90 µl of the adjusted culture suspension with OD_{600}=0.01-0.02 (1.0×106 CFU/mL) was added to each well. To this 10 µl of the mango kernel extract at varying concentrations (287 µg/ml to 17.8125 µg/ml) were added and mixed well by pipetting 2-3 times thoroughly. Ciprofloxacin antibiotic served as positive control. The culture was also added to the wells containing 10ul of 1% DMSO. The plates were kept under aerobic condition for 24h at 37°C. Following incubation biofilm was fixed with 200µl of methanol and kept for fixation for 15 minutes. Methanol was aspirated and wells were dried. Then wells were stained with 200µl of 0.1% (w/v) crystal violet for 30 minutes. The wells were washed with sterile distilled water**.** Crystal violet was solubilized by adding 200µl of 95% ethanol and kept for 10 minutes. The optical density (OD) of each well was measured at 570nm using Biorad, ELISA microplate reader(5).

Biofilm inhibition by methanol extract of MKE

Biofilm visualization of each bacterial strain was separately performed on cover glass slides placed in TSB medium. Mango kernel (extracts at MIC) were added in the medium and incubated for 24hrs. Following incubation, cover glass slides were fixed in methanol for 15 min, and air dried. Following methanol fixation, the cover glass slides were stained with 2% of acridine orange fluorescent stain (w/v) for 20 minutes at room temperature and washed and kept for air drying. Stained glass pieces were placed on slides with the biofilm facing on the top of the glass slide. Biofilms were evaluated and examined under Nikon, epifluorescence microscope, (USA) at different magnifications (17)(18).

Identification of bioactive compounds in MKE using GC-MS analysis

The Clarus 680 GC was used in the analysis and it employed a fused silica column, packed with elite-1 fused silica column (5% biphenyl 95% Dimethylpolysiloxane, 30m × 0.25mm ID ×250µm df) and the components were separated using Helium as a carrier gas at a constant flow of 1ml per minute. The injector temperature was set at 260°C during the chromatographic run. One µl of the mango extract sample was injected into the instrument. The oven temperature was as follows: by 300°C at the rate of 10°C min^{-1} : and 300°C, where it was held for 6 minutes. The mass detector conditions has temperature 240°C C: ion source temperature 240°C: and the ionization mode electron mode impact at 70e V, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrum of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library (12)(15).

Statistical analysis

All experiments were performed in replicates. Statistical analysis was performed using two-tailed paired Student's t test using MS-Excel tool. P value of ≤ 0.05 was considered to be significant.

RESULTS

Phytochemical Extraction of *Mangifera indica* in Different Solvents

The solvent extractive values of *M.indica* seed kernel was ranged between 1.3% to 4.9%. (Fig-1). Hexane extract showed maximum extractive value of 4.9%. Water and acetone fraction had least extractive values (1.3% and 2.1%).The total phenolics content was found to be higher in dried kernel powder extracted in mid-polar solvent, acetone compared to other solvents (1.95 mg/G GAE). However, kernel extracted using non-polar hexane solvent produced less phenolic amount (0.0164 mg/G GAE)and were in the order of acetone>water>diethylether> methanol>hexane (Fig 2). Furthermore, it was found that the concentration of tannin was more, (0.4324 mg/g TAE) in mango kernel extracted with high polar solvent methanol, and was lower in kernel extracted with hexane solvent (0.0073mg/g TAE) and presence of tannin in different solvents were found to be in the order of methanol>diethylether>acetone>water>hexane (Fig 3). It was inferred from the results that the polar solvent methanol is suitable for extracting tannin and the mid-polar solvent acetone is much better for polyphenol extraction.

Antibacterial Activity of Mango Kernel Extracts.

The antibacterial activity of mango kernel extract in different solvents (chloroform, hexane, acetone, 80- methanol, and water) against three different strains of SA were tested. It was observed that no zone of inhibition was found with chloroform, acetone, hexane and water extracts against three SA strains. Whereas significant antibacterial activity was observed with mango kernel in 80-methanol against all three SA strains [(12±3) mm] (Table -1). The results clearly showed that the secondary metabolites from mango kernel that are soluble in methanol would be effective in reducing the growth of *S.aureus* infections.

Effect of Mango Kernel Extract (Mke) on Bacterial Growth

Increasing concentration of mango kernel extracted in 80-methanol showed increased antibactericidal activity against SA. The MIC of mango kernel extract was found to be 17.8125 µg/ml against all three SA strains. Ciprofloxacin (10µg/mL) was used as a positive control. Among three SA strains used, SA-2 was highly sensitive than SA-1 and SA-3.

Biofilm Inhibitory Efficiency of Methanol Extracted Kernel against SA

Increasing concentration of mango kernel methanol extracted MK showed increased percentage of antibiofilm activity against SA. The biofilm inhibitory concentration (BIC) was fixed as 72.32µg/ml and MBIC is known to be the minimum concentration of drug required to inhibit more than 50% of biofilm without influencing the microbial growth. Percentage of biofilm inhibition against three individual SA strains was determined for BIC, ½ BIC, ¼ BIC, 1/6 BIC, 1/8 BIC and 1/16 BIC. And increased inhibition of microbial biofilm growth was observed with SA-2 when compared to SA-1, SA-3 and untreated control. The biofilm inhibition percentage of all three SA strains were in the

range of 30-80%. Cip used as positive control exhibited inhibition percentage of around 35 to 40%, whereas MKE was found to show biofilm inhibition of 40-70% in SA-1, 60-80% in SA-2 and 50-70% in SA-3, (Fig-5). In microtitre plate assay biofilm formed by SA treated with MKE showed decreased crystal violet staining compared with untreated cultures.

Inhibition of biofilm by MKE

SA cultures grown in TSB containing cover slips were treated with BIC concentration (72.32 µg/ml) of mango kernel for 2hr, 4hr, 6hr, 8hr and 24hrs, respectively. The coverslip was taken out and washed with sterile PBS and stained with 0.2% of Acridine orange and visualised under different magnification in epi-fluorescence microscope. With increasing incubation time a significant decrease in biofilm formation was seen, maximum inhibition of biofilm was observed after 24hrs when compared with untreated SA cultures (Fig 7).

Characterisation of Mango Kernel Extract

Extraction of secondary metabolites from mango kernel, and their identification using Gas chromatography-mass spectroscopy techniques was carried out and the mass spectrum was interpreted using the database of National institute of standards and technology. Chromatogram of mango kernel showed the presence of nearly 12 compounds among which five major compounds with higher abundance were found to be of therapeutic importance. A higher peak area of 22.03% at the retention time of 22.47min corresponds to the compound Oleic Acid, and 20.758 corresponds to n-Hexadecanoic acid phthalate (10.12%), 22.672 corresponds to Octadecanoic acid, and 28.391 corresponds to Lathosterol (9.68%), and24-Norursa-3,12-diene at RT of 27.330 (9.68%) etc. Other compounds with minimum peak percentage were Undecane (2.38%), Hexadecanoic acid, methyl ester (0.65%), 9-Octadecenoic Acid (Z)-Methyl Ester(0.70%), 9,12-Octadecadienoic Acid (Z,Z)-(2.76%), (.+/-.)-.alpha. -Tocopherol acetate (1.44%). It was reported previously that the compound n-Hexadecanoic acid was found to possess anti-inflammatory property and oleic acid was found to have antibacterial, and antioxidant properties and also found to inhibit atherosclerosis (Table 2; Fig 8).

DISCUSSION

Staphylococcus aureus is a most important human pathogen causing various nosocomial and community-acquired infections. Majority of SA infections are associated with highly invasive and potentially lethal infectionsof the skin and mucosa (19). Some *S.aureus* infections, such as endocarditis, osteomyelitis, and infections associated with indwelling medical devices, are associated with the formation of bacterial biofilms. Early detection of infection and management of pathogenic staphylococci is the essential step to prevent device-associated infections (19)(20). Bacterial attachment to surfaces and the succeeding biofilm formation are vital steps in the development of chronic infections and persistence in host tissues (21). Biofilms can delay wound healing significantly (22). Thus, it is very necessary to design a drug that will inhibit and prevent the microbial growth by targeting biofilm formation. Thus we investigated the underlying antibacterial and anti-biofilm efficacy of MKE against *S.aureus*. The present study is the first to shed focus on the anti-biofilm efficacy of MKE against *S.aureus*. In this study, different solvent extracts of mango kernel powder were investigated for its phytochemical composition and its ability to inhibit microbial growth and biofilm were evaluated. Phytochemicals from plants such as polyphenols are rich sources of anti-oxidants utilized for preventing various diseases caused by free radicals induced oxidative damage. The higher polyphenol content would then exhibit stronger inhibition and also higher antioxidant activity and antibacterial activities (23). Results in fig- 2 showed that the total crude content from mango seed extracts were significantly higher in hexane(non polar) extracts when compared to the highly polar and non-polar based solvents. In phytochemical quantification the concentration of tannin was higher in 80% methanol and were in the order of Methanol>chloroform>acetone>water>hexane and extract in acetone was found to be best suitable solvent for phenolic extraction (Acetone>water>chloroform>methanol>hexane). The total phenolic content of mango seed kernels in this study are in accordance with those of Thai mango which contained 11-28.62 mg gallic /g (24) and 28.33- 44.76mg gallic /g (25). The technique of phenolic isolation from plant material, including the methods and type

of extracting solvent, depends generally on the type of phenolic compound and the solvents (27). Mango kernel was found to contain various bioactive compounds such as phenolic acids and their derivatives, carotenoids, flavonoids, quercetin, and mangiferin,etc and phenolic acids (4-caffeoylquinic acids, caffeic, coumaric, ellagic, gallic, and ferulic acid) were reported in mango. Mango also found to have anti-microbial, anti-inflammatory, anti-oxidative and anti-cancer activities and also known to prevent coronary atherosclerosis and cancer cell growth in vitro. Using of synthetic antibiotics to control microbial infections led to post-antibiotic complications. So, to prevent the adverse effects caused by these antibiotics it is advisable to prefer natural organic compounds with the ability to target specific virulence molecule associated with chronic infections. Based on the results of bacterial growth curve assay, we concluded that MKE could inhibit the growth of *S. aureus* in a concentration-dependent manner. Meanwhile, the sub-inhibitory concentrations of MKE (36.16µg/ml) could reduce the biofilm formation ability of three *S. aureus* (60%-75%) strains and disrupt the biofilm architecture. The biofilm inhibition percentage observed with MKE was found to be more in SA-2 compared to SA-1 and SA-3. The above findings were further supported by microscopic observations. In this study the antibiofilm effect of mango kernel extract was clearly demonstrated. Furthermore, a high antimicrobial and antifungal activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli*, and *Candida albicans* has been reported in kernel powder of South African mango variety (28). The methanolic extracts of mango kernel powder were found to have greater inhibition against *S.aureus* bacterium at various concentrations than *E. coli* as reported by Somaris et al, 2021. And the anti-microbial results obtained from this study were in accordance to reports demonstrated by Somaris et al, 2021 (29).

So far, this work is the first attempt in identifying the anti-biofilm potential of MKE against *Staphylococcus Sp*. When compared with the untreated control, the samples treated with a subinhibitory concentration of the extracts showed absence of dense biofilm layers as observed with epifluorescence microscopic observations. Thus, the mango kernel extracts disintegrate the architecture of Staphylococcus Sp, microcolonies, suggesting a possible approach in reducing the resistance of sessile cells (which form a dense layer of biofilm) to antibiotics. From Fig.7, it is obvious that the mango kernel extracts disturb the architecture of biofilm by loosening the microcolonies. There are several reports regarding plant extracts interfering in the hydrophobicity against gram-negative bacteria and gram-positive bacteria (31) and thereby inhibiting biofilm formation. In the present study, it is also confirmed that the MKE reduce the biofilm hydrophobicity at their corresponding sub-BICs.

In the present study, upon GC-MS analysis presence of important bioactive molecules were revealed. Chromatogram of MKE showed the presence of nearly 21 compounds and compounds with higher peak area percentage were represented in table-3. Oleic Acidat the retention time (RT) of 22.47min with highest peak area of 22.03%, n-Hexadecanoic acid at RT of 20.758 min with peak area of 10.12%, Gamma-sitosterol at RT of 26.177 with peak area of 14.23%, Lathosterol with RT of 28.391 with peak area of 9.68%, and 24-Norursa-3,12-diene with RT of 27.330and peak area 3.44% etc. Oleic Acid is an octadec-9-enoic fatty acid which found to possess various therapeutic activity such as, anti-oxidant, anti-biofilm and anti-cancerous against chronic myeloid leukemia (33). Oleic acid has shown to inhibit primary adhesion and biofilm formation in various *S. aureus* strains (34). In accordance to this result Oleic Acid found in higher content in MKE also proved to show biofilm inhibition against *S. aureus* in this study. The fatty acid n-Hexadecanoic acid reported in this study was found to possess anti-Inflammatory property (35). Fatty acids such as caprylic acid (octanoic acid), lauric acid, myristic acid (tetradecanoic acid), palmitic acid (hexadecanoic acid), and stearic acid was found to inhibit biofilm formation in *S. aureus, K. pneumoniae, P. aeruginosa, Proteus mirabilis, Streptococcus pyogenes, E. coli* and the yeast *Candida albicans*(36). The compound 24-Norursa-3,12-diene reported in this study was also found in various ayurvedic plants such as in Panchvalkal kwath bark formulations comprising of *Ficus benghalensis*, *Ficus racemosa*, *Ficus religiosa*, *Thespesia populnea*, and *Ficus lacor*which is used for treating gynecological ailments, bacterial infections and heal wounds (37). Hence, it is evident from the present study that bioactive compounds in mango kernel confers anti-biofilm property against *S. aureus.* Mango kernel would be a future promising natural organic drug for controlling major gram-negative and gram-positive biofilm related bacterial infections. Such a natural compound has no adverse effect as found with synthetic antimicrobials and safe for human consumption.

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Table 1. Antibacterial Activity of Different Extracts of MKE against *Staphylococcus aureus*

S.NO	EXTRACT	BACTERIAL STRAINS	ZONE OF INHIBITION IN (mm)				
I-B	ACETONE	SA-1	NIL	NIL	NIL	NIL	NIL
		SA-2	NIL	NIL	NIL	NIL	NIL
		SA-3	NIL	NIL	NIL	NIL	NIL
II-A	CHLOROFORM	SA-1	NIL	NIL	NIL	NIL	NIL
		SA-2	NIL	NIL	NIL	NIL	NIL
		SA-3	NIL	NIL	NIL	NIL	NIL
II-B	WATER	SA-1	NIL	NIL	NIL	NIL	NIL
		SA-2	NIL	NIL	NIL	NIL	NIL
		SA-3	NIL	NIL	NIL	NIL	NIL
II-C	HEXANE	SA-1	NIL	NIL	NIL	NIL	NIL
		SA-2	NIL	NIL	NIL	NIL	NIL
		SA-3	NIL	NIL	NIL	NIL	NIL
II-D	80-METHANOL	SA-1	NA	12	13	14	15
		SA-2	12	12	13	14	15
		SA-3	NA	NA	11	14	14

Table 2. Phytochemical characterization of methanol extract of MKE

S.No	Mango Kernel-Chemical Compounds	Retention Time	Area%	Molecular Formula	Molecular Weight
1.	Hydrazinecarbothioamide	3.105	3.30	SSCH5N3S	91
2.	Undecane	9.945	2.38	$C_{11}H_{24}$	156
3.	Hexadecanoic acid, methyl ester	20.403	0.65	$C_{17}H_{34}O_2$	270
4.	n-Hexadecanoic acid	20.758	10.12	$C_{16}H_{32}O_2$	256
5.	9-Octadecenoic Acid (Z)-, Methyl Ester	22.110	0.70	$C_{19}H_{36}O_2$	296
6.	9,12-Octadecadienoic Acid (Z,Z)-	22.413	2.76	$C_{19}H_{34}O_2$	294
7.	Oleic Acid	22.471	22.03	$C_{18}H_{34}O_2$ or $C_8H_{17}CH=CH(CH_2)_7COOH$	282
8.	Octadecanoic acid	22.672	9.49	$C_{19}H_{38}O_2$	298
9.	Gamma.-Sitosterol	26.177	14.23	$C_{29}H_{50}O$	414

10.	(.+/-.)-.alpha.-Tocopherol acetate	27.330	1.44	$C_{31}H_{52}O_3$	472
11.	Lathosterol	28.391	9.68	$C_{27}H_{46}O$	386
12.	24-Norursa-3,12-diene	27.330	3.44	$C_{29}H_{46}$	394

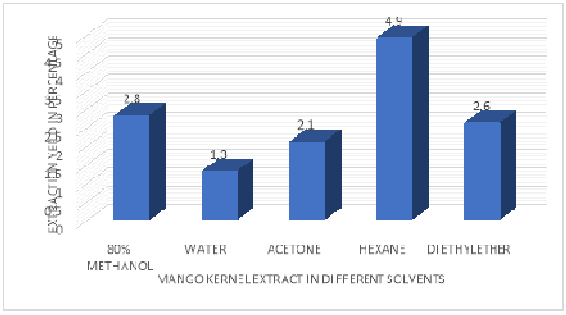

Fig-1: Efficiency of different solvents in mango kernel extraction

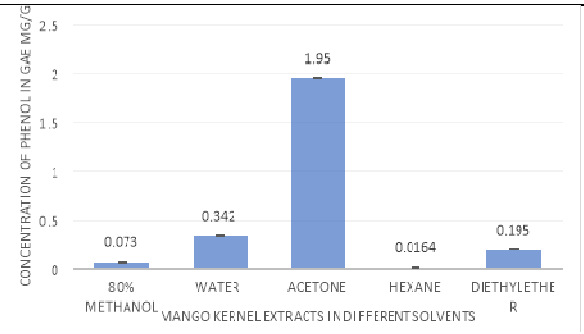

Fig-2: Estimation of polyphenol of mango kernel extracts

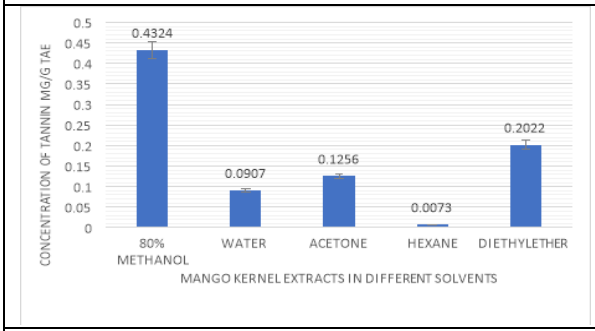

Fig-3: Total tannin estimation of mango kernel extracted in different solvents

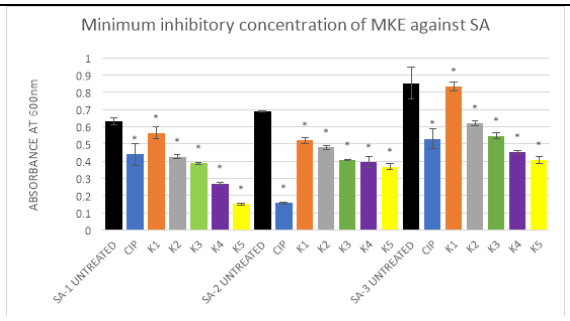

Fig-4 Effect of different concentration of mango kernel extract against *Staphylococcus aureus* growth. K1- 17.8125 µg/ml, K2- 36,625 µg/ml, K3-72.32 µg/ml, K4- 143.5 µg/ml, and K5- 287 µg/ml

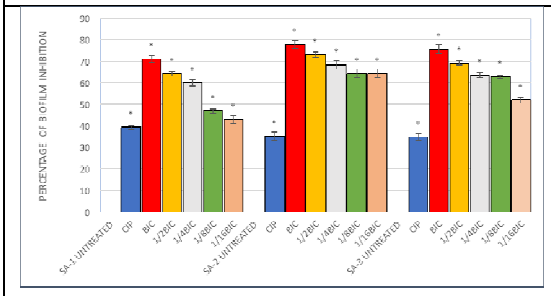

Fig-5 Effect of different concentration of mango kernel extract against *Staphylococcus aureus* biofilm

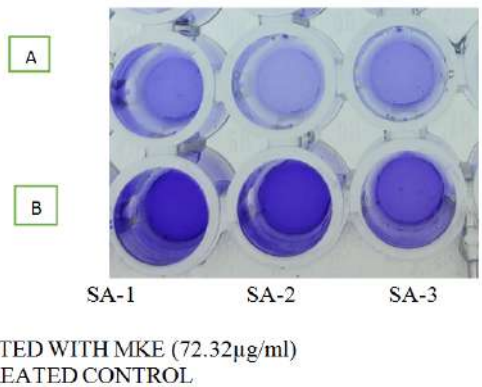

Fig-6, Effect of MKE on biofilm inhibition in *Staphylococcus aureus*

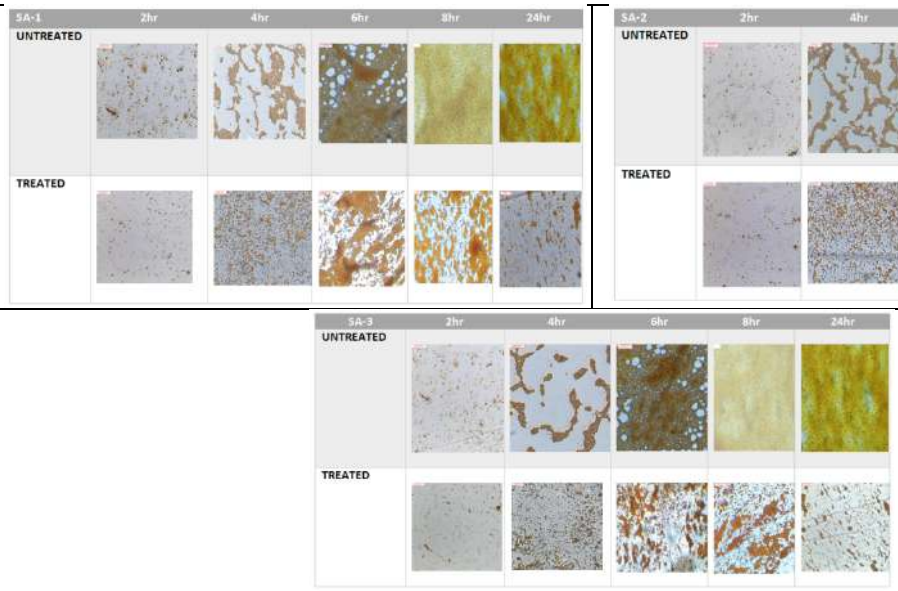

Fig: 7 With increasing concentration of mango kernel extract there is visible decrease in biofilm as evident by the light microscopic examination

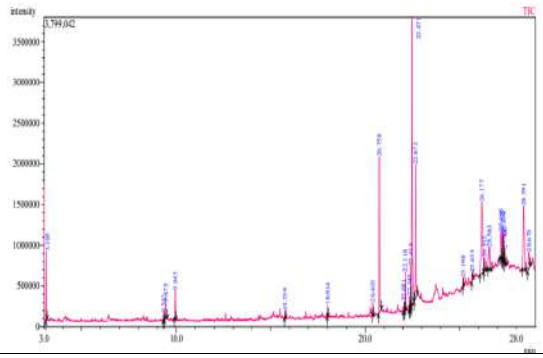

Fig: 8 GCMS Chromatograph of Mango Kernel Methanol Extract

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comprehension and Managing Strategies of Work - life Balance (Wlb) and Stress in Working Environment

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Received: 07 Jan 2022 Revised: 10 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

Work-life balance (WlB) is changing everyday exercises to accomplish a feeling of harmony between work life and individual life. A few advantages of a solid balance between serious and fun activities incorporate diminished feelings of anxiety, at work and at home, more noteworthy concentration and fixation, more significant levels of occupation fulfillment, the chance to partake all the more completely in family and public activity, more opportunity to seek after close to home objectives and side interests, further developed wellbeing. Everybody encounters pressure at some stage in their life. It is a way for us to realize that something in our life is causing us concern and is influencing how we are thinking and feeling. Stress unleashes destruction on passionate harmony, actual wellbeing. It limits the capacity to think obviously, work viably, and appreciate life. It might appear as though there is no way around stress. Powerful pressure the board assists with breaking the hold pressure has on life, it tends to be more joyful, better, and more useful. A definitive objective is a healthy lifestyle, with time for work, connections, unwinding, and fun and the strength to hold up under tension and figure out difficulties. In

any case, stress the board is not one-size-fits-all. In this paper the work life balance strategies, understanding and dealing with the work environment stress are talked about.

Keywords: WLB, stress, burnout, 4A, strategies, ABC, CBT

INTRODUCTION

Work-life balance (fig.1) is the condition of balance where an individual similarly focuses on the requests of one's profession and the requests of one's very own life. Balance between serious and fun activities is a significant part of a solid workplace. Keeping up with balance between serious and fun activities lessens pressure and forestalls burnout in the working environment. An excess of worry about an extensive stretch of time prompts work environment burnout. Representatives who work huge loads of additional time hours are at a high danger of burnout. Fulfilling time constraints at work while as yet possessing energy for companions and side interests. Having sufficient opportunity to rest appropriately and eat well.

Objectives

- Comprehend the advantages of accomplishing a sound balance between work and life.
- Carry out methodologies to further develop prosperity and accomplish better equilibrium.
- Perceive the signs and side effects of stress.
- Carry out methodologies to work on their strength and better adapt to stress.
- Use the ABC model to deal with their responses to unpleasant circumstances and challenge negative musings.
- Attract upon unwinding methods to further develop their adapting capacity.

Steps to a Work-Life Balance

Accomplishing a sound balance between serious and fun activities requires dealing with our expert and individual life in feasible ways that keep our energy streaming, our psyches and bodies solid and our entire selves glad and content. It implies focusing on everything that enhance and satisfy us including work and profession, wellbeing and wellness, family and connections, otherworldliness, local area administration, leisure activities and interests, scholarly feeling, rest and entertainment (fig.2).

Track Time

Examining current circumstance is the starting advance in accomplishing a healthy lifestyle. Keep a period log of all that accomplish for multi week, including business related and individual exercises.

Decide Priorities

Invest some energy truly pondering what is generally critical and make a rundown of main concerns at work and at home.

Specific Goals

Take rundown of needs and transform them into concrete and quantifiable objectives.

Plan Scrupulously

Effective individuals plan their work and afterward work their arrangement. Put away 15 to 20 minutes toward the start of every day to design undertakings and exercises for the afternoon and evening ahead.

Set up Boundaries

Put forth reasonable and practical lines accomplish both at work and at home. Obviously impart these limits to manager, collaborators, accomplice and family.

Deal with Health

Deal with wellbeing by eating quality suppers, practice no less than three times each week and rest at least seven hours of the evening. Have the opportunity to add exercise and additional rest to jam-pressed timetable, these practices soothe pressure, raise energy level, increment endurance, work on psychological clearness, support resistant framework, and make more joyful, more connected with, and more useful individual. Moreover, cease from the extreme utilization of liquor, tobacco, or medications to mitigate stress.

Support Family/Relationships

Associations with family, companions, and friends and family are, by a wide margin, the best wellspring of inward fulfillment. On the off chance that work or profession is harming own connections, the two regions will at last endure. By focusing on own connections, usefulness and viability hands on will really increment.

Set Aside A Few Minutes

As much as work, wellbeing, and connections take need in life, it is additionally essential to plan time for own reestablishment. Enjoy some little joy day by day. It will do ponders for prosperity, connections and profession will benefit as well. Interface with profound source. Faith in God, or a higher power, can be a profound well from which to draw motivation, direction, and strength.

Go Home at Work

Foster a psychological on-off switch among work and home. It assists with setting up a temporary movement between the two domains. This may comprise of paying attention to music or recorded books during evening drive, practicing at the wellness community, getting things done, or keeping individual arrangements.

Practice Options

Many ground breaking organizations today are making arrangements and projects that work with balance between serious and fun activities. Discover what choices business offers as far as flex hours, working from home, a compacted work week, work sharing, or low maintenance business. Observe a plan that permits to work all the more gainfully, while simultaneously cutting pressure and opening up significant individual time.

Work Smarter Not Harder

Utilizing time all the more effectively is a significant ability that everybody from the secretary to the CEO can learn. Embracing the right mix of time-usage practices can cut pressure and save as long as an hour daily. This can incorporate the utilization of innovation to turn out to be more coordinated, gathering messages and voice messages, keeping away from stalling and figuring out how to say "no."

Know When to Ask for Help

Indefensible work circumstances can typically be lightened, yet it will take some emphatic ness on part. Additionally, in the event that a healthy lifestyle keeps on escaping encountering ongoing pressure, talk with an expert an instructor, psychological well-being laborer, or priest. Exploit the administrations presented by worker help program. Attempt as we as a whole may, balance between fun and serious activities is certainly not a definite science. Every individual should track down their own particular manner of joining vocation, connections and individual consideration into a coordinated entirety.

Maintain Good Work-Life Balance

Adjusting the requests of a bustling way of life is certifiably not something simple to do, however is best overseen by consistently inspecting and evaluating the needs. The following are a couple of thoughts to assist with striking a decent balance between fun and serious activities.

- Put out objectives around esteem exceptionally.
- Deal with time adequately.
- Make a limit between adjusting work and individual time-go home

- Construct flexibility and have an uplifting perspective
- Stay away from stress, mental depletion and burnout.
- Keep a sound way of life.
- Enroll a decent emotionally supportive network.
- Partake in work stress

All things considered; stress was our companion. It went about as a defensive component that cautioned us of risk, a characteristic response that let us know when to run. This reaction is presently alluded to as the instinctive reaction or the stress reaction. Stress has remained part of the developmental drive due to its convenience in endurance. When utilized at the perfect opportunity, stress expands our mindfulness and works on actual execution in short explodes. Dull openness of the pressure reaction on our body is demonstrated to prompt dependable mental and actual medical problems; these incorporate cardiovascular sickness, diabetes, uneasiness and wretchedness (fig.3).

Signs and Manifestations of Stress

Mental

- Trouble thinking obviously
- Memory issues
- Cannot concentrate
- Low capacity to focus
- Poor judgment
- Constant stressing

Enthusiastic

- Moodiness
- Easily annoyed or hurt
- Irritability or touchiness
- Agitation, unfit to unwind or keep still
- Feeling overpowered
- Sense of depression and disconnection

Physical

- Tightness in muscles
- Aches and torments
- Headaches, shaking, perspiring
- Nausea, dazedness
- Chest torment, quick heartbeat
- Loss of hunger
- Lack of rest, dreams, bad dreams

Conduct

- Eating pretty much
- Sleeping to an extreme or excessively little
- Isolating from others
- Procrastinating or dismissing liabilities
- Using liquor, cigarettes, or medications to unwind
- Nervous propensities.

These signs and side effects of pressure can likewise be caused by other mental and clinical issues.

Reasons for Stress

The circumstances and tensions that cause pressure are known as stressors. There are two kinds of stressors:

1. Outer (where outside powers follow up on us)
2. Inside (self-created)

Outside Reasons for Stress

- Significant life changes
- Work
- Relationship challenges
- Monetary issues
- Being excessively occupied
- Kids and family.

Inner Reasons for Stress

- Powerlessness to acknowledge vulnerability
- Question
- Negative self-talk
- Ridiculous assumptions
- Compulsiveness
- Absence of decisiveness.

Manage Stress

Managing stress (fig.4) is tied in with making an arrangement to have the option to adapt viably to every day pressures. A definitive objective is to find some kind of harmony between life, work, connections, unwinding and fun. By doing more ready to manage day by day pressure triggers and deal with these difficulties. A few systems that can help care for psyche and body, and thus assist with bettering control practices that outcome from a lot of stress include.

Body

- Realize the stress triggers
- Perceive early admonition signs and manifestations
- Practice unwinding procedures or contemplation
- Eat an even, solid eating routine
- Practice routinely
- Get sufficient rest

Thinking

- Attempt to stress less over things
- Set little, sensible and feasible objectives
- Apply critical thinking methods
- Decide to have an uplifting perspective
- Contemplate and accomplishments
- Get some down time to envision a quiet and serene spot
- Contend with people around and focus on own best
- Create, keep and utilize comical inclination.

Practices

- Prepare to permit sufficient opportunity to finish assignments
- Use plans for the day and put forth boundaries to assist with accomplishing objectives
- Make a reasonable way of life and permit time for entertainment and unwinding
- Reward accomplishments and objectives
- Limit admission of liquor, caffeine and different medications.

Stress Versus Burnout

The term stress is characterized as an individual's reaction to an upsetting variable in the climate, prompting physical, mental or social uniqueness for authoritative members (fig.5). It is a significant piece of work life, which

incorporates the cooperation of individual and the climate. The elements from the climate which causes pressure are called stressors. The power of stress is not same for all people. As a rule, stress is appeared as negative, yet it has a positive aspect too. At the point when stress is positive, it is known as 'eustress' which is frequently considered to be an inspiration. Eustress gives a chance to a person to acquire something. The pressure is supposed to be negative when, it is identified with a heart illness, conjugal breakdown, illicit drug use, liquor addiction, and so forth. Burnout alludes to a psychological, passionate, or state of being, of constant fatigue happens because of delayed pressure. It is a perspective brought about by extreme openness to serious passionate pressure, shown through enthusiastic depletion and negative mentalities. A person who is burnout is hypertensive, faces mental melancholy and is critical about everything. It is the point at which feel overpowered and incapable to satisfy requests continually. There are three phases of burnout, enthusiastic weariness, depersonalization and sensation of incapability and absence of individual achievement. The added substance effect of these three phases is a large group of negative attitudinal and social outcomes.

Differences Between Stress and Burnout

- A person's reaction to an outside circumstance caused because of unfriendly conditions is called pressure. The condition of mental or passionate sleepiness happens out of constant openness to stretch is called burnout.
- In stress, the individual feels restless, grumpy, blameworthy, and so on Then again, in burnout, the individual feels hypertensive, intellectually discouraged, restless, touchy and so on.
- In stress, the singular experiences weakness while in burnout the individual appearances constant weariness.
- The individual loses trust and inspiration in pressure. Instead of burnout, in which the individual loses actual energy.
- Stress brings about disappointment with work yet burnout may prompt fatigue and skepticism towards work.
- Job responsibility is dropped in pressure. Dissimilar to burnout, wherein the individual feels intellectually disengaged from the association.
- In stress, the individual may think that it is hard to focus and will generally fail to remember things without any problem.
- The individual goes through physiological changes in pressure, as expanded circulatory strain or heartbeat. Actually, psychosomatic protests are experienced in burnout.

Different Stress Management Techniques & Strategies

Activity Orientated Approaches

It is utilized to make a move to change a distressing circumstance. Activity arranged methodologies permit to make a move and change the unpleasant circumstance.

Be Emphatic

Clear and successful correspondence is the way to being decisive. When self-assured, request what we need or need, and furthermore clarify what is pestering us. The key is doing this in a reasonable and firm way while as yet having sympathy for other people.

Lessen the Clamor

Turning off all the innovation, screen time, and steady upgrades can assist us with dialing back. It merits changing, for the good of own. Set aside a few minutes for some quietness every day.

Deal with Time

At the point when we focus on and arrange our errands, we make a not so much unpleasant but rather more charming life.

Making Limits

Limits are the interior arrangement of decides that we set up for ourselves. They diagram what practices we will and will not acknowledge, how long and space we want from others, and what needs we have. Solid limits are

fundamental for a peaceful life. At the point when we have sound limits, we regard ourselves and deal with our prosperity by plainly communicating our limits to other people.

Feeling Orientated Approaches

It is utilized to change the manner in which we see an unpleasant circumstance. Feeling focused methodologies are utilized to change the manner in which we see unpleasant circumstances.

Insistences and Symbolism

The force of positive symbolism and insistences is currently logically demonstrated to expand positive feeling. In this way, supplant those negative contemplations with positive explanations and challenge and change the manner in which see and experience the world.

Intellectual Restructuring

A method for understanding negative feelings and testing the occasionally wrong convictions that cause them. Intellectual rebuilding is a vital part of Cognitive Behavioral Therapy (CBT).

ABC Technique

The letters ABC represent A – adversity, or the stressful event. B – beliefs, C – consequences, the aftereffect of convictions prompts the activities and result of that occasion. Basically, the more hopeful convictions, the surer the result.

Acknowledgment Orientated Approaches

It is utilized for managing upsetting circumstances cannot handle. Acknowledgment arranged methodologies are helpful in unpleasant circumstances that cannot handle.

Diet and Exercise

Be aware of having a reasonable and sound eating regimen. Simplifying diet changes, for example, lessening liquor, caffeine and sugar admission is a demonstrated method of decreasing nervousness.

Contemplation and Actual Unwinding

Use procedures like profound breathing, directed perceptions, yoga, and directed body checks. These exercises assist with loosening up the body. A few models to give a shot are incorporated underneath.

Assemble flexibility

Strength is our capacity to ricochet back from upsetting or negative encounters. To streamline, tough individuals are talented at tolerating that the circumstance has happened, they gain from what unfolded and afterward they continue on.

Work it Out

Try not to hold everything inside. Converse with somebody near about concerns or the things getting down. Sharing concerns can slice them down the middle, and furthermore allow an opportunity to chuckle at possibly crazy circumstances.

Rest

Getting a decent night rest is principal for re-energizing and managing upsetting circumstances in the most ideal manner. While it fluctuates from one person to another, on the specific measure of rest required, a continuous rest of roughly 8 hours is for the most part suggested.

Side Effects of Working Environment Stress

Manifestations of work environment stress can show actually (migraines, stomach throbs, torments, weakness or eating, and dozing unsettling influences), intellectually (issue with concentrating, navigation, thinking or recalling), and inwardly (feeling down, tense and bothered).

Counteraction of Working Environment Stress

The anticipation of work environment stress is best when a blend of both authoritative change and individual pressure the executives is utilized.

- Advance leave, rest and breaks
- Empower exercise and contemplation, both inside and outside of work hours
- Guarantee the responsibility is in accordance with laborers' capacities and assets
- Give feeling and freedoms to laborers to utilize abilities
- Help working environment resolve by setting out open doors for social communications
- Obviously set out specialists' jobs and obligations
- Support investment in dynamic that influences people jobs
- Support open correspondence
- Build up no resistance strategy for working environment separation
- Connect with an outside advisor to recommend a new way to deal with any current issues
- Make family-accommodating strategies to support balance between serious and fun.
- Furthermore, give preparing to working environment stress the executives.

4A of Stress Management

Around 80% of Indians experience the ill effects of pressure, and 68% of them are awkward examining their concern with an emotional well-being proficient. Monetary imperatives and absence of social acknowledgment are the principle motivations behind for proficient assistance for dysfunctional behaviors like pressure. In spite of the fact that it is critical to be appropriately treated for any psychological maladjustment, stress is one issue that can be taken care of all alone as well (fig. 6).

Avoid

In spite of the fact that it is preposterous to expect to keep away from each upsetting circumstance like some are important forever and some are important to support presentation by keeping away from superfluous stressors can fundamentally affect emotional wellness. For instance, turn away pointless weight at the work environment by essentially denying additional work. Drop work that is less critical to focus on more significant errands. Additionally, stay away from the reason for pressure or the individual who is a persistent wellspring of stress by diminishing the time enjoy with them.

Alter

On the off chance that staying away from an unpleasant circumstance is absurd, the following most ideal choice is to modify it. It might expect either change the manner in which impart or work the things in everyday existence. Assuming a tight cutoff time is unavoidable, guarantee a quiet air to work quicker and all the more productively. Additionally, ready to modify too prior to requesting that the other individual change. Change timetable such that it obliges some family time, as working constantly can be very exhausting.

Adapt

Assuming there is a stressor that cannot be stayed away from or changed, it is ideal to adjust to it. This will assist with recapturing feeling of command over the stressor. Continuously feel worried while caught stuck, give a different take to the circumstance. Another variable that is a typical justification behind stress is perfectionism. The propensity for guaranteeing everything is simply awesome, it can really worry. Begin setting sensible guidelines and others and be content with great work. Try not to attempt to try too hard for flawlessness.

Accept

Ultimately, a few stressors are no doubt unavoidable, unalterable, or unadaptable. In such cases, the best way to adapt is by tolerating the circumstance and pushing forward. The passing of a friend or family member, actual maltreatment, serious disease, a characteristic cataclysm – these are largely circumstances that are not in our control, and can do very little with regards to them. They are wild, so it is ideal to acknowledge the circumstance and resolve to continue on. Pardoning is likewise a fundamental piece of tolerating the circumstance or stressor.

CONCLUSION

Work life balance can be distinctive to various individuals relying on the various phases of life. It essentially incorporates the issues of weariness and absence of time. Research shows that inability to accomplish an acceptable equilibrium of exertion is straightforwardly associated with absence of energy to satisfy individual responsibilities and absence of command over the responsibility. The unevenness here prompts outcomes like weakness, low quality of life and in particular lackluster showing. Balance between serious and fun activities is the connection between close to home responsibilities and work and its effect on each other. The current review looks at the creative and current balance between fun and serious activities arrangements and practices carried out and rehearsed by different Indian Companies. A report says stress has almost become a subculture in corporate India, resulting in physical and mental ailments. Following these simple steps of stress management can not only help to get rid of stress, but also control emotions in the worst situations. So, start avoiding, altering, adapting and accepting stressful situations and live a healthy and content life.

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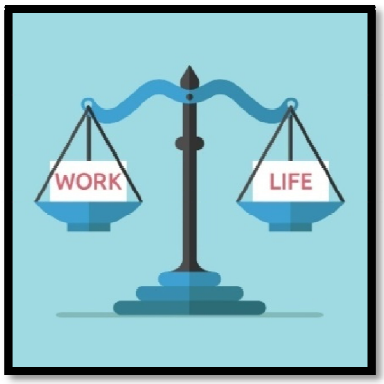

Fig. 1. Equality of work and life

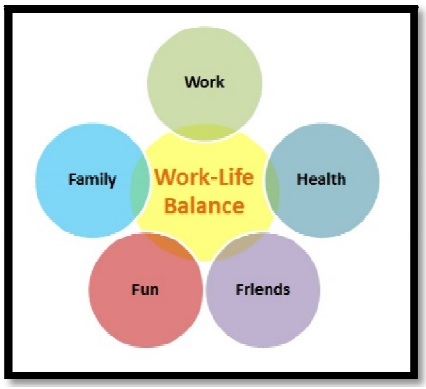

Fig. 2. Work life balance

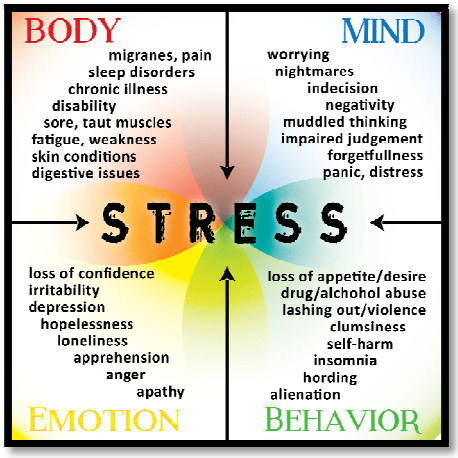

Fig. 3. Impact of stress

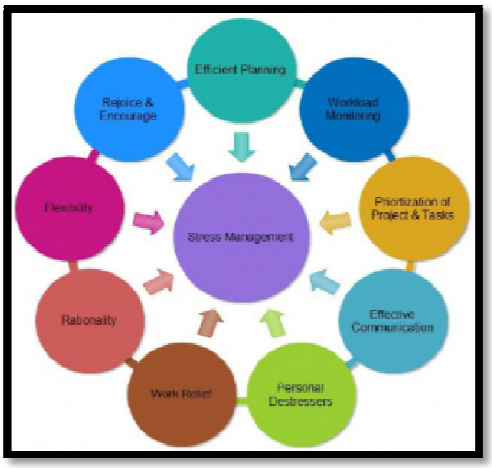

Fig. 4. Stress Management

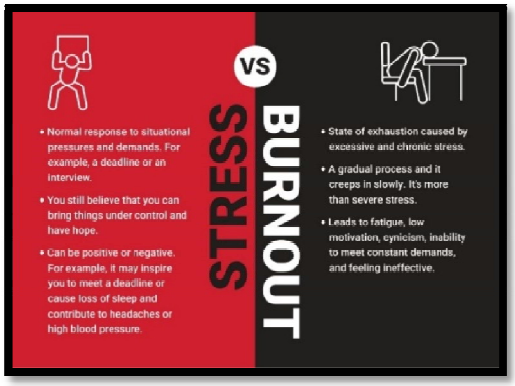

Fig. 5. Stress Vs Burnout

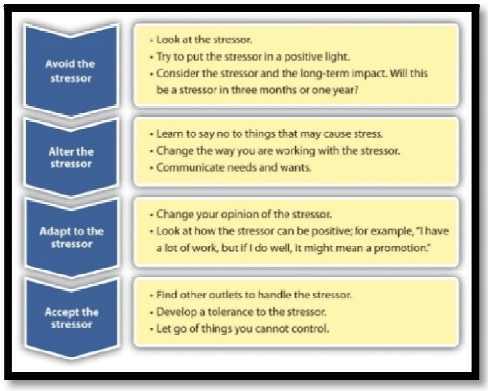

Fig. 6. 4A of stress management

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Efficacy of Electrical Stimulation (ES) in Post Stroke Rehabilitation– A Review

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Received: 08 Jan 2022 Revised: 10 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

A number of studies have been made to assess the usability and effectiveness of electrical stimulation in the rehabilitation of stroke survivors. Here we have classified studies under three heads: Modes of ES (Electrical Stimulation), ES with other interventions, and Electrode placement in ES for reduction of spasticity. We reviewed studies on low TENS (Transcutaneous Electrical Nerve Stimulation), EMG(Electro Myo Graphy)-triggered electrical stimulation, EEG(Electro Encephalo Graphy)-triggered NMES (Neuro Muscular Electrical Stimulation), mesh glove treatment, cyclic electrical stimulation, FES (Functional Electrical Stimulation), and contra laterally controlled FES under modes of ES. Studies on ES with functional hand orthosis, NMES with bilateral arm training, and ES incorporated into task-related exercise were included under ES with other interventions. Electrode placement in ES for reduction of spasticity includes stimulation of antagonist muscle to the spastic muscle and stimulation of spastic muscle. This review showed that mild paralytic patients have functional improvement, when weak NMES is applied at sensory threshold or below motor threshold. Moderately affected patients found EMG-triggered NMES as a potential rehabilitative treatment option to restore motor function and improve recovery. For severely affected patients, EEG triggered NMES might have therapeutic efficacy. Alternatively in case of patients with severe paresis, in whom there was no motor intent, NMES aimed at muscle strengthening might be required to acquire compensatory movement in the non-paralyzed side. The selected studies also suggested that when ES regimens were combined with mechanical strategies such as robotic training or neuroprosthetics or other types of orthoses, they had greater effect.

Key words: ES (Electrical Stimulation), FES (Functional Electrical Stimulation), NMES (Neuro Muscular Electrical Stimulation), TENS (Transcutaneous Electrical Nerve Stimulation), hand orthosis, spasticity.

INTRODUCTION

Different therapeutic methods are being used to prevent or decrease long-term impairments in stroke patients. Most of those affected by stroke survive, but the long-term impairment they undergo becomes a heavy burden for the patients as well as their families. Rehabilitation clinicians find it to be a major challenge to make the post stroke subjects perform their ADLs (Activities of Daily Living). In post-stroke rehabilitation, electrical stimulation is commonly used as a modality. Different modes of electrical stimulation like TENS (Transcutaneous Electrical Nerve Stimulation), EMG-triggered electrical stimulation, EEG-triggered NMES (Neuromuscular electrical stimulation), mesh glove treatment, cyclic electrical stimulation, FES (functional electrical stimulation), and contra laterally controlled FES have been experimented. Researches have suggested that if electrical stimulation (ES) is used along with other interventions it had the greatest effect. Specifically, when ES regimens were combined with mechanical strategies such as robotic training or neuroprosthetics or other types of orthoses better results were obtained. There had been quite a few studies on electrical stimulation used to reduce involuntary or spasticcontraction in muscles affected by spinal cord injury or stroke, to increase voluntary movement inparetic muscles after a stroke, spinal cord injury, or head injury and to increase strength in muscles without dysfunction.

METHODOLOGY

We carried out a search in Pubmed in the months of November and December 2021, with the search phrase 'Electrical stimulation in post stroke'. 69 studies were identified as relevant for the review. Out of the 69, 11 studies were excluded after analysis of summary. This review had been made on the remaining 58 studies related to electrical stimulation in post stroke subjects. A classification has been made based on the basis of modes of ES, ES with other interventions, and electrode placement in ES for reduction of spasticity.

DISCUSSION

Modes of ES

Low Transcutaneous Electrical Nerve Stimulation (TENS)

Sonde et al evaluated low-TENS in 44 stroke subjects who were divided into control and treatment groups. Both groups were given physiotherapy, while the treatment group was also given low-TENS. After three months, the treatment group exhibited significant increase in motor function, compared to the control group, without any decrease in pain or spasticity [1]. Weak current NMES (Neuromuscular electrical stimulation), administered at sensory threshold or below motor threshold, may be used to improve motor function in stroke subjects [2]. It has been reported that somato sensory input increased corticomoto neuronal excitability to the body parts which are stimulated [3, 4]. NMES is not used in isolation in this approach, but combined with rehabilitation training.

EMG – Triggered NMES

To stimulate the paralyzed hand in a bilateral simultaneous motion, Osu et al took up a study using a surface electromyogram (EMG) of the unaffected hand as a clue [5]. The walking ability of stroke subjects was found to improve with alternate stimulation of dorsiflexors and plantar flexors using reciprocal NMES [6, 7]. EMG-triggered NMES enabled adjustment of the NMES to the timing of motion, or, in other words, synchronizing to motor intention [8-10]. EMG could trigger electrical stimulation voluntarily to restore the residual activity of the affected muscle. Recently, EMG-triggered NMES combined with robot-aided rehabilitation, had been found to be effective in

improving motor function in stroke patients [11, 12]. EMG-modulated NMES devices controlled the timing as well as the intensity of electrical stimulation according to the quantity of residual voluntary EMG [13-15]. Francisco et al. administered EMG triggered neuromuscular stimulation to acute stroke survivors and found that it enhanced upper extremity motor recovery and functional recovery. The subjects were given wrist strengthening exercises for two 30-minute sessions per day either with EMG- triggered stimulation (experimental) or without (control). Items of the Functional Independence Measure (FIM) like feeding, grooming, and upper body dressing, and upper extremity Fugl-Meyer Motor Assessment were assessed at entry and at discharge. The subjects treated with EMG-triggered stimulation scored better in FIM and Fugl-Meyer scores compared with controls [16]. A similar study was taken up by Tekeoglu et al. who divided 60subjects into two equal groups. The functional changes were measured using the Barthel Index for daily living and the spasticity in the elbow was measured by the Ashworth Scale. Statistically significant improvements were reported for all parameters [17].

EEG – Triggered NMES

More recently, an electroencephalogram (EEG) is used instead of an EMG to detect the motor intention. To interpret the dynamics of brain oscillations, event-related desynchronization (ERD) and event-related synchronization (ERS) are used to decrease and increase EEG frequency band power respectively [18]. They are also associated with motor attempt, motor imagery, or voluntary movement [19]. There are clinical reports of stroke rehabilitation using a BCI system to trigger EEG-triggered NMES for finger function, [20] upper [21] and lower [22] limb training, and gait rehabilitation [23]. An EEG modulated NMES system had also been found to control the current intensity in a stepwise manner according to the appearance or disappearance of ERD [24]. NMES is used also for muscle reducing spasticity [25] and improving swallowing function [26] in stroke rehabilitation.

Cyclic ES

Several studies had been made on cyclic electrical stimulation or the sequential activation and relaxation of muscles. Chae et al. gave cyclic ES induced active repetitive exercise to wrist and finger extensors in sub-acute stroke patients. Self adhesive, pre-gelled electrodes were positioned over the extensor digitorumcommunis (EDC) and the extensor carpi radialis (ECR). The results showed enhanced motor recovery, which is sustained for up to 3 months after completion of treatment. [28].

ES with Implanted Electrodes

When implanted electrodes were used within the schema of EMG-triggered stimulation, the results were similar to applying ES with surface electrodes. J. Chae et al. and R. Turk et al. concluded from their studies with implanted electrodes that impairments like range of movement, strength and motor outcomes showed greater decrease in ES groups than controls [29, 30].

MESH Glove Treatment

MESH glove treatment is a technique, which has been found to improve arm and hand sensation. The anode is a flexible mesh glove made of wire conductor; a conductive gel serves as the interface between the glove and skin. 51 chronic post-stroke patients were selected for a study on this technique and their functional abilities were evaluated before and after the treatment. It was observed that the arm and hand sensation and voluntary motor control had improved, the hand temperature was normal, and the swelling and spasticity had decreased [31].

Cortical Stimulation

Transcranial magnetic stimulation (TMS), direct transcranial current stimulation (dTCS), and epidural cortical stimulation (ECS) are techniques which increase the cortical excitability of the affected hemisphere of brain and decrease the excitability of the non-affected side [32, 33, and 34]. Huang et al. studied the effect of an implanted cortical stimulator on hand and arm function in patients following ischemic stroke. Of the 24 stroke subjects selected, the investigational group was given target implanted cortical ES of the motor cortex along with upper limb rehabilitation therapy, while the control group was given rehabilitation therapy alone. During the 6-month follow-up period, the investigational group showed significantly greater mean improvement in Upper Extremity Fugl-Meyer

scores than the control group. The results suggested that cortical stimulation with rehabilitation therapy produces a lasting treatment effect in upper extremity motor control and is not associated with serious neurological complications [35].

Functional Electrical Stimulation (FES)

Spasticity is a common complication associated with stroke and studies had been made on the use of FES for a reduction of spasticity. At the same time, it had been observed that excessive spasticity might complicate successful electrical stimulation and therefore it is made as an exclusion criterion [36, 37, 38, and 39]. A few studies have observed that a treatment with FES brings about a decrease in spasticity [40, 41].Some other studies failed to find any significant reduction of spasticity compared to a control group [42, 43].

Contra laterally Controlled Functional Electrical Stimulation

J S Knutson et al. used contra laterally controlled functional electrical stimulation to improve hand function in stroke survivors. The subjects used an electrical stimulator to perform active repetitive hand opening exercises through the contraction of the paretic hand extensor muscles. There was a positive effect on motor impairment. Voluntary isometric finger extension moment, finger movement control, and Box and Block score increased [44].

ES with Other Interventions

NMES with Bilateral Arm Training

In the recovery of paretic limbs after stroke, NMES is administered particularly to increase muscle contraction for performing a specific motion or reciprocal NMES is performed with agonists and antagonists. A study was conducted by Rose william et al on stroke subjects with no upper limb function. They applied repetitive NMES for wrist extension, 30 minutes, and twice a day for 6 weeks. This was found to improve the wrist function of the subjects [45]. Another study experimented on NMES combined with bilateral arm training for the triceps brachii muscle and anterior deltoid muscle in the affected arm. Subjects were made to move both their paralyzed and non-paralyzed arms simultaneously in similar manner, and the difference in movement of both arms triggered the NMES to assist the motion [46].

Electrical Stimulation Incorporated into Task-related Exercise

When electrical stimulation was incorporated into task-related exercises, better and faster recovery was seen than cyclic and EMG triggered ES [47]. A clinical study was conducted on 21 acute stroke subjects randomized into 2 groups: Conventional therapy alone was given to the control group, while functional electrical stimulation (FES) was given along with conventional physiotherapy to the FES group. A third group of chronic stroke subjects was included and received only FES therapy (chronic group). According to the motor tasks FES was administered to proximal and distal muscle groups .The FES group showed better improvement than the control group in Upper Extremity Chedoke-McMaster Stages of Motor Recovery, object manipulation, Upper Extremity part of Fugl-Meyer scores, palmar grip torque, pinch grip, Barthel Index and pulling force. Though the chronic stroke subjects showed improvements in most categories, the changes were not statistically significant [48].

ES withFunctional Hand Orthosis

A study was under taken by Johan Anton Franck et al on the effect of electrical stimulation along with functional hand orthosis, in sub-acute stroke subjects with reference to the functional use of the impaired hand. Their finding was that there was a significant improvement in hand function from no dexterity to dexterity. The patients also displayed a high-intrinsic motivation and sense of self-regulation, creating opportunities for a nonfunctional hand towards task-oriented training [49].B M Doucet & J A Mettler made a study of EScombined with dynamic hand orthosis in a small sample of chronic stroke subjects. The range of motion, the grip strength, as well as activation-deactivation of the muscle while grip was released were measured. Ability to perform daily tasks was also assessed. The subjects exhibited improvement in all these aspects [50].

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Electrode Placement in ES for Reduction of Spasticity

Stimulation of Antagonistic Muscle to the Spastic Muscle

The most common application of electrical stimulation is to the antagonist, ornonspastic, muscle. Stimulation of the antagonist muscle makes use of the afferent nerve pathways, which, polysynaptic ally, inhibit the agonist or spastic muscle [51].When a muscle contracts, the activity of the antagonist muscle is reduced because of the inhibitory interneurones. This is called reciprocal inhibition effect. It can be exploited by stimulating the antagonist muscle to the spastic muscle. Stimulation will have a direct effect or through the mechanical changes of tension in the muscle itself. The period of reduced spasticity after such an exercise can befor a few minutes to hours. By repeating these exercises spasticity can be reduced for an extended time. Most studies had shown that in order to continue the therapeutic benefit, the treatment has to be continued for a long term [52].

Stimulation of Spastic Muscles

Studies had shown that stimulation of the spastic muscles themselves produce a relaxing effect. The effect of NMES on the reduction of spasticity might be explained by its actions on increasing Ibfiber activation via mechanisms that facilitate the Renshaw cell recurrent inhibition,on antagonist reciprocal inhibition and on increasing cutaneous sensory Stimuli [53,54]. Reciprocal stimulation of agonist and antagonist pairs can be an effective way of reducing spasticity. However, there are some concerns that by strengthening spasticmuscles, spasticity may be stronger when it returns [52]. The application of NMES combined with other interventions was associated with reductions on spasticity and improvements in range of motion when compared with a control group. Spasticity might impair functional activities in stroke survivors. Therefore, it has to be controlled before any motor control therapeutic protocol is applied [55].Cinara Stein et al. in their systematic review concluded that time after stroke, time of treatment, degree of spasticity, and degree of ability to voluntarily contract amuscle might affect response to electric stimulation [56].Chronic tissue changes because of immobilization, loss of sarcomeres, muscle conversion to Connective tissue, and decreased resting length of the muscle [57],and loss of motor units in the paretic arm, caused by secondarytrans-synaptic degeneration, might affect effective NMES performance [58].

CONCLUSION

This review of studies on application of ES to post stroke subjects showed its usability and efficacy. For patients with mild paralysis, weak NMES at sensory threshold or below motor threshold, combined with rehabilitation, might promote functional improvement. For moderate paralysis, EMG-triggered/modulated NMES might be a potential rehabilitative treatment option to restore motor function and improve recovery. For severely affected patients in whom surface EMG is not detectable, EEG triggered NMES might have therapeutic efficacy. Alternatively, in case of patients with severe paresis, in whom there was no motor intent, NMES aimed at muscle strengthening might be required to acquire compensatory movement in then on-paralyzed side. The selected studies also suggested that if electrical stimulation (ES) was used along with other interventions, it hada better effect. It was noted that when ES regimens were combined with mechanical strategies such as robotic training or neuroprostheticsor other types of orthoses, they had greater effect. Some studies on the use of electrical stimulation to reduce involuntary or spastic contraction in spinal cord injury/stroke affected muscles or paretic muscles, and to increase strength in muscles without dysfunction have shown positive results. But concern has also been raised that by strengthening spasticmuscles, spasticity may be stronger when it returns. Spasticity had to be controlled before any motor control therapeutic protocol is applied, because it might impair functional activities.The time after stroke, time of treatment, degree of spasticity, and degree of ability to voluntarily contract amuscle might affect response to electric stimulation. Factors like immobilization and loss ofmotor units in the paretic sidemayaffectthe effectiveness of electrical stimulation.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Relaxation Technique on Depression, Anxiety and Stress among Physiotherapy Students- A Pilot Study

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Received: 08 Jan 2022 Revised: 21 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

Stress, anxiety and depression are inevitable in Physiotherapy education. The objectives of the present study were to evaluate effect of relaxation technique on stress, anxiety, and depression, perceived academic stress and quality of life among physiotherapy students. Survey was done to evaluate level of stress, anxiety, and depression for 40 participants. 10 participants were found to have mild, moderate stress, anxiety, and depression. Pre-intervention DASS21, Perception of Academic Stress Scale (PAS) and WHOQOL-BREF Questionnaires were taken. Participants were divided into two groups.Group-1 Treatment group N=5.Group-2 Control group N=5.Group-1 was assigned Jacobson's progressive relaxation exercise and shavasana 3times/week for five weeks.Group2 was assigned shavasana for 10-15 mins for 3times/week for five weeks. Parameters like HR (Heart Rate), SBP (Systolic Blood Pressure), and DBP (Diastolic Blood Pressure), Temperature before and after treatment was checked for 15 sessions for both groups. Post-intervention DASS21, Perception of Academic Stress Scale and WHOQOL-BREF Questionnaires were taken. Significant decrease in HR, SBP was found in both the groups, PAS, DASS 21 scores decreased in both the groups and WHOQOL BREF score was found to be more significant in interventional group. Shavasana and Jacobson's Progressive relaxation exercise both were effective in reducing stress, anxiety and depression and Academic stress and to improve Quality of life, but Jacobson's progressive relaxation exercise was more effective in improving all parameters of QOL.

Keywords: Stress, Anxiety, Depression, PAS scale, DASS scale, Shavasana, WHOQOL BREF

INTRODUCTION

Mental health related issues represent significant problems for many college going students. Top three concerns are academic performance, pressure to succeed and post-graduation plans (1). Attending college can be a stressful time for many students. In addition to incorporate with educational pressure, some students must face the stressful task like separation from their family of origin and some may have to do numerous works and fulfill family responsibilities. Under such circumstances, numerous college students encounter early emergence of mental health and substance use problems or experience worsening of their symptoms. Given the uniqueness of college students, there is a need to outline critical issues to consider when working with this population(2).The previous study reported higher level of psychiatric morbidity depression 29.9% anxiety 41.1% and stresses 27% among undergraduate medical students, warrants need for strategic plans to alleviate depression, anxiety and the stressors right from the time they join medical school and has to be continued till they finish the course(3).With the propensity for mental health issues to hinder the success of college students, it is vital that colleges continually undertake evaluation of mental health of their students and customize treatment programs to expressly target their needs.[3]The results of the previous study highlighted the emotional vulnerability of a significant proportion of physiotherapy students, with educational and individual issues being the greatest concern. While personal causes of stress such as stressful events and mood are more difficult to control, manipulation of curricular factors may have positive effects on academic sources of stress (4). Early interventions are needed to improve the quality of life and reduce the stress among students (5). There are few studies evaluating the effect of anxiety reduction strategies on stress, anxiety and depression score and quality of life in Physiotherapy students.

METHODOLOGY

The present study was divided into two phases, Phase I was questionnaire-based survey and Phase 2 was quasi experimental study. Ethical clearance was obtained from the Ethics Committee of Nirmal Children's Hospital; Surat. Briefing about the study was done in the classroom. Survey was performed among 40 students from Shree Swaminarayan Physiotherapy College in phase 1 of study**.**10 undergraduate physiotherapy female students with age 18-25 years with mild and moderate category of DASS 21 Questionnaire score and willing to participate were recruited for the Phase 2 of the study (intervention). Participants with following findings were excluded from the study (participants taking medications which may influence mental health, with known cardio-respiratory, neurological, or Musculo-skeletal abnormalities, with a severe and extremely severe category of depression, anxiety and stress score on the DASS 21 questionnaire). Participants were divided among control group and intervention group by envelope method. For the present study materials used were Informed consent form, Data collection sheet, DASS-21Questionnaires, Perception of Academic Stress Scale, WHOQOL-BREF questionnaire. Instruments used were Omron's digital semi-automatic blood pressure monitor (machine is approved by British hypertension society having facility of comfort cuff which can be useful for arm of any size), Digital thermometer, Stopwatch. Demographic data were documented. All the participants were divided into 2 groups (treatment and control group) and were assessed pre- interventions using Questionnaires DASS21, PAS scale, WHOQOL-BREF. Intervention group was given 15sessions of Jacobson's progressive techniques and *shavasana* for 3sessions/week for 5 weeks. Control group was given 15 sessions of *shavasana* 3sessions/week for 5 weeks. All the participants were assessed post intervention using DASS21, PAS and WHOQOL-BREF and HR, SBP, DBP; Temperature was taken before and after intervention.

Procedure of Jacobson's Progressive muscle relaxation exercise: Progressive Muscle Relaxation Exercise was given in a comfortable supine position in Practical Lab Cardiopulmonary Physiotherapy Department where participants were undisturbed for 10-15 minutes. Participants were asked to give focus on each of the groups of muscles listed below and work through them one muscle group at a time. Participants were instructed to tense each muscle group and asked to notice how that muscle feels when it is tensed. Maintain this tension for five seconds while breathing in.Later, release and relax that muscle all at one time. Give attention and feel relaxed when releasing the contracted

muscle. Practicing tensing same muscle group one or two more times by less tension each time helped to build awareness of tension in the body and to improve the capacity to separate between tension and relaxation in muscle groups.

Step 1: Assume a comfortable position. Participants were asked to lie down in a comfortable chair, extricate any tight clothing and asked to close eyes and be quiet.
Step 2: Assume a passive attitude. Center on yourself and on accomplishing relaxation in specific body muscles. Tune out all other thoughts.
Step 3: Tense and relax each muscle group as mentioned:
Forehead -Take your eyebrows up to touch your hairline for five seconds. Relax.
Eyes and nose - Close your eyes as firmly as you can for five seconds. Relax.
Lips, cheeks, and jaw - Draw the centers of your mouth back and frown for five seconds. Relax. Feel the warmth and calmness in your comfort.
Hands - Extend your arms. Clench your fists firmly for five seconds. Relax.
Forearms –Straighten your arms out against an invisible wall and push with your hands for five seconds. Relax.
Upper arms - Bend your elbows. Tense your biceps for five seconds. Relax. Feel the tension leave your arms.
Shoulders - Elevate your shoulders up to your ears for five seconds. Relax.
Back-Take your back off the floor for five seconds. Relax. Feel the anxiety and tension disappearing.
Stomach - Fix your stomach muscles for five seconds. Relax.
Hips and buttocks - Fix your thigh muscles by squeezing your legs together as firmly for five seconds. Relax.
Thighs - Fix your thigh muscles by squeezing your legs together as firmly as you can for five seconds. Relax.
Feet - Take your ankles toward your body as distant as you can for five seconds. Relax.
Toes -Twist your toes as tightly as you can for five seconds. Relax.
Step 4: Give attention on muscles which are tensed. If you realize any muscle is tense, tighten and relax the specific muscle three or four times.
Step 5: Feel for relaxation in your mind. Human beings respond in different ways to diverse activities. Some feel refreshed, and others feel calm and relaxed after an activity like this. Some may notice little change the first time, but afterwards with practice, their control increases and benefits as well.
Group 2 was given *shavasana* for 15-20 mins, 3 sessions/week for 5 weeks. Where subjects were asked to lie down in the quiet room and asked to wear comfortable loose clothes and asked to relax with eyes closed. Before and after interventions to both the groups HR, SBP, DBP and temperature was taken as outcome measures (6).
Shavasana technique: The *Shavasana* technique seems simple to describe but not easy to practice. Technique involves lying on the back; legs are spread apart with comfortable distance. The arms are asked to be placed on the side of the trunk. The head ought tobe turned on one side. The back and shoulders stay in touch with the floor. The eyes are kept closed. All muscles of the body are then relaxed, there being no stretch or pull anywhere in the body. By keeping focus to each part of the body, one by one starting from the toes upward, one should see if any tension or uneasiness is felt anywhere and if so, one should try to avoid it. Care should be taken to avoid sleep (7).

RESULTS

Phase 1: Survey was done to evaluate level of stress, anxiety, and depression for 40 participants. Data analysis was done to know depression, anxiety, and stress score, perceived academic stress score and quality of life among physiotherapy students.10 participants were found to have mild, moderate stress, anxiety, and depression.

Basal Parameters: Mean height found was 154.60± 6.43 for control group and 154.10± 4.64 for experimental group. Mean weight found was 46.58±4.88 for control group and 50.80±7.96 for experimental group. Mean BMI found was 19.52±2.91 for control group and 21.40±3.78 for experimental group. Mann Whitney test was performed, and p value was considered, and no statistically significant difference was found among both groups. Homogeneity check was done.

Comparing across the duration: DBP, SBP, HR, Temp was compared across the duration from before 1st session and to all post session end, by using Friedman test and P value was considered, it shows statistically significant difference in HR in both the group, SBP in both the groups.

Perceptions of Academic Stress: Was assessed among both the groups before and after intervention. Wilcoxon signed rank test was used and p value was considered which shows statistically significant difference incontrol and experimental group before and after intervention but when compared between the group there was no statistically significant difference was found.

Depression, Anxiety and Stress Scale: was assessed among both the groups before and after the intervention. Wilcoxon signed rank test was used and p value was considered which shows statistically significant difference in control and experimental group before and after the intervention for stress, anxiety, depression components but when compared between the group, no statistically significant difference was found.

Quality Of Life: was assessed among both the groups before and after the intervention. Wilcoxon signed rank test was used and p value was considered which shows statistically significant difference in control and experimental group before and after the intervention for Environment, Physical health, psychological health component. For social relationships component only in experimental group there was statistically significant difference was foundbut when compared between the groups, no statistically significant difference was found.

DISCUSSION

The present study was done to evaluate the effect of Jacobson's progressive relaxation exercise on stress, anxiety, depression, and Quality of life among Physiotherapy Students. The findings of the study suggested that *Shavasana* and Jacobson's Progressive relaxation exercise both were effective in reducing stress, anxiety, depression, and Academic stress and showed improvement in Quality of life. Jacobson's progressive relaxation exercise was more effective in improving all parameters of QOL. Previous literature has found that both *Shavasana* and Jacobson's progressive relaxation exercise are significantly effective in inducing relaxation and in reducing anxiety (7,8). The present study found reduction in stress, anxiety, and depression score in each group. Stress could be a word derived from the Latin word "stringere", aiming to draw tight. Jacobson's progressive relaxation technique relies on the premise that muscle tension is that the body body's psychological response to anxiety provoking thoughts which muscle relaxation blocks'anxiety. This system involves learning to watch the strain in specific muscle groups by 1st tensing every muscle. This tension is then discharged as attention is directed towards the variations felt throughout tension and relaxation. Jacobson's progressive relaxation technique brings a couple of feeling of physical relaxation. Once the body is physically relaxed, anxiety is additionally reduced (9).

Present study found parameters SBP and HR were significantly reduced in both the groups. The finding of the present study was like previous study. Previous study found significant reduction in Heart rate in *shavasana* group compared Jacobson's progressive relaxation exercise group, but they could not however claim superiority of one modality of relaxation over the other due to a small sample size (7). The reduction of Heart rate and systolic blood pressure by *shavasana* may be due to cardiovascular adjustments and shift of autonomic equilibrium towards parasympathetic dominance (10). The reduction of Heart rate and systolic blood pressure by Jacobson's Progressive relaxation exercise may be due to break in recurring stressed-mind/tense-muscle cycle that is the fight and flight stress response, decreasing the amount of Cortisol which typically increases during the body's response to stress. It reduces the level of acetylcholine which leads to the reduction in sympathetic nervous system activity and increase in vagal activity (7).

There is an urgent need to establish prevention programs and to bring out evidence based psychological health promotion for Physiotherapy students to help Physiotherapy students to create smooth adaptations between

different learning environments with modified learning needs and a growing academic responsibility. Jacobson's progressive relaxation technique can be an effective means for reducing stress, anxiety, and depression and to improve Quality of life in Physiotherapy students too. Further study can be done to see the effectiveness of Jacobson's progressive relaxation exercise on the larger population.

CONCLUSION

Shavasana and Jacobson's Progressive relaxation exercise both were effective in reducing stress, anxiety and depression and Academic stress and to improve Quality of life. Jacobson's progressive relaxation exercise group was found to be having more effect in improving all parameters of QOL than *shavasana* group.

ACKNOWLEDGEMENT

The author acknowledges the scholars whose articles are cited and included in references of this manuscript and also to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been access and reviewed. The authors are extremely grateful to IJONS editorial board members and IJONS team of reviewers who have helped to bring quality to this manuscript. Authors are extremely thankful to all the participants for their participation in the study and for their co-operation.

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Table 1: Comparing across the duration (before 1st Session and all post session)

Parameter	Group	N	Freidman test value	df	p
DBP	Control	5	40.349	16	.001
	Experimental	5	10.632	16	.832
HR	Control	5	23.554	16	.100
	Experimental	5	36.964	16	.002
SBP	Control	5	33.037	16	.007
	Experimental	5	43.541	16	.000
Temp	Control	5	29.932	16	.018
	Experimental	5	15.494	16	.489

Table 2: Co-relation of Perceptions of Academic Stress Scale

Parameter			N	Mean	Std. Deviation	Median (IQR)	Mean difference	S.D of difference	Median (IQR)	comparison within the group		comparison between the group	
										wilcoxon signed rank test p value		Mann Whitney test p value	
Perception (90)	Control	Before	5	53.40	6.73	51(48.5--59.5)	16.2	1.64	17(14.5--17.5)	0.042	sig	0.119	NS
		After	5	37.20	5.63	37(32.5--42)							
	Experimental	Before	5	59.20	2.77	60(56.5--61.5)	17.6	0.54	18(17--18)	0.038	sig		
		After	5	41.60	2.97	42(39--44)							

Table 3:Co-relation of Depression, Anxiety and Stress Score

Parameter			N	Mean	Std. Deviation	Median (IQR)	Mean difference	S.D of difference	Median (IQR)	comparison within the group		comparison between the group	
										wilcoxon signed rank test p value		Mann Whitney test p value	
Stress	Control	Before	5	9.00	3.54	8(6--12.5)	3.2	1.3038	3(2--4.5)	0.042	sig	0.504	NS
		After	5	5.80	2.59	5(4--8)							
	Experimental	Before	5	8.00	3.16	7(5.5--11)	2.6	0.5477	3(2--3)	0.038	sig		
		After	5	5.40	2.88	5(3--8)							
Anxiety	Control	Before	5	10.00	3.54	11(7--12.5)	4.4	2.1909	5(2.5--6)	0.042	sig	0.334	NS
		After	5	5.60	1.52	6(4.5--6.5)							
	Experimental	Before	5	8.60	1.95	8(7--10.5)	3.8	0.8367	4(3--4.5)	0.041	sig		
		After	5	4.80	1.30	5(3.5--6)							
Depression	Control	Before	5	7.40	4.34	5(4--12)	2.8	2.5884	2(0.5--5.5)	0.068	NS	0.242	NS
		After	5	4.60	1.82	4(3--6.5)							
	Experimental	Before	5	10.20	2.28	11(8--12)	4.4	1.8166	5(2.5--6)	0.042	sig		
		After	5	5.80	0.84	6(5--6.5)							

Table 4: Co-relation of Quality of Life

Parameter			N	Mean	Std. Deviation	Median (IQR)	Mean difference	S.D of difference	Median (IQR)	comparison within the group		comparison between the group	
										wilcoxon signed rank test p value		Mann Whitney test p value	
Environment (0-100)	Control	Before	5	67.60	13.13	69(56--78.5)	-4.2	0.8367	-4(-5---3.5)	0.041	sig	1.000	NS
		After	5	71.80	12.30	73(61--82)							
	Experimental	Before	5	65.20	11.23	63(56.5--75)	-4.2	0.8367	-4(-5---3.5)	0.041	sig		
		After	5	69.40	11.35	68(60--79.5)							
Physical health (0-100)	Control	Before	5	71.60	10.43	69(63--81.5)	-4.2	0.8367	-4(-5---3.5)	0.041	sig	1.000	NS
		After	5	75.80	9.96	73(68--85)							
	Experimental	Before	5	60.00	14.95	56(47--75)	-4.2	0.8367	-4(-5---3.5)	0.041	sig		
		After	5	64.20	14.50	60(51.5--79)							
Psychological health (0 - 100)	Control	Before	5	62.40	9.02	56(56--72)	-4.2	0.8367	-4(-5---3.5)	0.041	sig	1.000	NS
		After	5	66.60	8.50	61(60.5--75.5)							
	Experimental	Before	5	48.80	14.34	44(37.5--62.5)	-4.2	0.8367	-4(-5---3.5)	0.041	sig		
		After	5	53.00	14.28	48(42--66.5)							
Social relationships (0-100)	Control	Before	5	57.60	28.43	50(34.5--84.5)	-3.6	2.0736	-4(-5---2)	0.063	NS	0.911	NS
		After	5	61.20	26.78	54(39--87)							
	Experimental	Before	5	40.20	12.13	44(31.5--47)	-4.2	0.8367	-4(-5---3.5)	0.041	sig		
		After	5	44.40	11.84	48(35.5--51.5)							

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Sterile Pharmaceutical Manufacture- A Regulatory Process in the United States (US)

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Received: 25 Jan 2022 Revised: 03 Mar 2022 Accepted: 26 Mar 2022

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ABSTRACT

The FDA has taken several steps to promote the development of sterile items for human health. Several recent initiatives aimed at improving medicine have been accomplished. Sophisticated contribution includes improved product and assembly measure development, along with safe use and item configuration. On the quality of sterile medications, we highlighted the administrative challenges. To produce high volumes of parenteral, the FDA has published CGMP guidelines. The current study manages a quick explanation of clean medication item production standards in line with global administrative needs. Pharmacies must adhere to a set of criteria about quality management. Parenteral commodities must be sterile and non-pyrogenic. Patients' health may be jeopardised if therapeutic prescription materials are not clean and non-pyrogenic as required. Determining the differences in rule requirements throughout global offices is crucial. It is important to know the distinctions in necessities to ensure quality items and supply for the targeted market. Read the requirements for creating clean drug goods first. Endotoxin control, research centre control, time constraint and approval are all considered administrative requirements.

Keywords: Food and Drug Administration [FDA], Sterile preparations, United Pharmacopeia [USP], Therapeutic medication, Current Good Manufacturing Practise [CGMP].

INTRODUCTION

The goal of this guidance is to help manufacturers comply with the FDA's current good manufacturing practise (CGMP) requirements (2I CFR sections 210 and 211) while producing sterile pharmaceuticals and biological products utilising aseptic methods [1]. They are crucial and delicate. These things must be devoid of pathogens, pyrogens, and undesirable particles. The technique induces microbial contamination of sterile products made in staffed cleanrooms. The existence of "strange" germs (defilement caused by the cycle) as well as tainting caused by the interaction may cause problems. Also, there is no plausible way to determine tainting in aseptic plants. You may become polluted [2]. All three components are sterilised separately before being assembled aseptically. Because the final container cannot be sterilised, it must be filled and shut carefully. Unlike terminal sterilisation, aseptic processing involves variables. Preparing individual components for aseptic assembly sometimes involves numerous sterilisations. Flexible terminations and fluid dosage structures are sterile sifted, while glass holders are cleaned with dry heat. They must be approved and supervised. Incorrect delivery of an item is possible with any method. Handling sterile components, containers, or closures before or during aseptic assembly raises the risk of cross-contamination. Disinfecting unsanitized medicines in a fixed compartment reduces errors. Non-sterile product health concerns should be known by manufacturers. Assembler industrial circumstances can risk patient safety [3].

Drug structure or how they are handled have influenced the aseptic assembly approaches for clinical medicine goods (for instance infusions, mixtures, drug structures for the eyes, for example, eye drops and so on) Parenteral products must be nonpyrogenic and sterile. Nonsterile or nonpyrogenic medications can cause substantial injury or jeopardise patient safety. In today's world, when global production network impacts are crucial, it is critical to understand the different needs of global single company sectors. Learn about aseptic assembly for the US market here. Knowing the requirements affects product quality and delivery to certain industries. First, sterility and aseptic creation are defined. Then a summary of the aseptic production requirements. With the viewpoint, the certificate measure will be consistent (rooms, gear, supply frameworks, materials, and approval draw near).

DISCUSSION

APPLICABILITY OF STERILITY SPECIFICATIONS TO PHARMACEUTICAL FORMS

Sterile medicine things must be sterile. It is described in the pharmacopoeias (doing the test, nutrient, and incubation conditions). The FDA Draft Guidance describes the sterility test's low sensitivity (statistical chance of recognising a positive unit). That means sterility cannot be guaranteed 100% of the time. For batch release, a sufficient number of samples must be examined, and those samples must be representative of the batch. (Begin, middle, and end samples, as well as process inputs). Other types besides pharmaceuticals are available in the US (21 CFR 200.51). Use a nebulizer to inhale inhalation solutions, suspensions, and sprays for local and/or systemic effects. Patients suffered serious side effects after ingesting contaminated pharmaceutical goods. In the USA, sterility for certain pharmaceutical formulations has been mandated [1,2,3].

CURRENT GUIDELINES AND RECOMMENDATIONS – OVERVIEW

The US Pharmacopeia (USP21) prescribes disinfection in the last compartment to make clean therapeutic items, although it is more usual to pick an assembling interaction (counting cleansing cycle) that is compatible with the item properties. The US Guidelines specify that if necessary, the holder closure structure must be altered. Unexpected benefits (e.g., double chamber needles) may be paid for by last-minute bundling. if terminal cleansing methods were used. In such instances, a producer can look into adding collaborator sterility assurance steps. It is implied in the FDA Draft Guidance12 and general necessities are communicated in segment 211.113 (b) of the Code of Federal Regulations (made strategies to hinder microbiological contamination of clean things, endorsement of any purging cooperation). The FDA's "Bearing for Industry Sterile Drug Products Produced by Aseptic Processing" illustrates the FDA's suspicions for a more organised endorsement of aseptic processing. This bearing revises 198724 primarily in terms of staff abilities, clean room design, isolators, air supply, holder end system quality, measure plan,

quality control, regular notice, and inspection of production records. Also covered is the use of isolators for aseptic prep. Also known as Blow-fill-seal advancement. The affirmation measures for evaluating media fill will be dropped. Each filthy unit of a media fill should be broken out. The microbial normal noticing (more repeat in testing) is agreed higher importance. The 'Rule for the Submission of Documentation for Sterilization' specifies the type of information and data that should be submitted with medication applications.

COMPONENTS REQUIRED TO BE CONSIDERED IN PROCESSING OF STERILE PRODUCTS [3]

- The following components must be addressed when creating clean drug goods.
- Only prepared people should prepare these.
- The designs and details of production facilities and structures should help create and support the item's quality, virtue, personality, and welfare.
- The microbiological (reasonable) and non-practical (unsuitable) conditions should be examined.
- The quality of the air, water, steam, and other gases used in the manufacturing process should be monitored.
- These include faculty, materials, material flow, gear, arrangement preparedness, natural observation and executives.
- Sanitation and sterilisation processes must be pre-validate.
- Perform regular system suitability studies (media fill).
- Only an authorised person may release production batches after a thorough examination of all manufacturing paperwork.
- The ultimate product should be "Quality Assurance" rather than "Quality Control".
- Wherever possible, use LAF units of suitable rating.
- Environmental monitoring is critical. Quality Requirements for clean medication creation should be documented in a manual. As well as the positions and commitments of the personnel at risk for leading body of Quality structures for clean medicine manufacture and chiefs of the specific systems should be outlined in the quality manual.
- The quality manual should also report the administrative, legal, and consistent pieces created by the business. The material should be instantly available to all employees. It should be constantly reassessed and re-energized to reflect new conditions. Diverse factors such as manpower, living conditions, test tactics, system approval, equipment, reference standards, examining and testing of test items all influence the outcome's correctness and reliability.

GENERAL CONSIDERATIONS

The following sections describe the general considerations and requirements for the manufacture of sterile items.

ENVIRONMENTAL PARAMETERS

It has limited air classification. Critical Area 100 Due to the risk of contamination and lack of quick sterilisation, this area is vital. Keeping aseptic procedures (e.g., equipment setup, filling) sterile requires constant monitoring and control of the surrounding environment. Promoting Clean Air Clean places are versatile. Many support sections prepare, hold, or move non-sterile components, formulated products, in-process materials, equipment, and containers/closures. Clean Areas Separation Separating work locations properly is critical to preventing contamination. To maintain air quality, it is necessary to maintain appropriate circulation from cleaner areas to dirty ones [1,3,4].

BUILDING AND PREMISES

USFDA Subjected goods, containers-closures, and the surrounding environment should not be exposed to unnecessary acts that could pollute them or the surrounding environment. People should be kept to a minimum in an aseptic processing environment. A aseptic processing plant should have several zones of activity that are suitably managed to satisfy varying air quality standards. Designers must adhere to microbiological and particle regulations as well as operational needs when creating a specific area.

The clean area control standards should be supported by microbiological and particle data from qualification investigations. The initial cleanroom qualifying process includes testing the air quality in static, as-built condition. When qualifying and classifying an area, it is vital to focus on data obtained in real-time (i.e., with personnel present, equipment in place, and operations ongoing). The effectiveness of an aseptic processing facility monitoring programme is evaluated regularly. [1,3]

- Part 211.42 of the 21 CFR (b) states that "the flow of components and drug product containers shall be planned to prevent contamination".
- In accordance with 21 CFR 211.42, "operations shall be conducted within clearly defined size limits (c). Separate or specified sites, as well as other control mechanisms, are required to avoid contamination.

Misunderstandings arising from the procedures

(i) Easy-to-clean smooth, hard surfaces on floors, walls, and ceilings;
(ii) Temperature and humidity controls;
(iii) An air supply filtered through high-efficiency particle air filters under positive pressure, regardless of flow direction;
(iv) A system for monitoring environmental conditions;
(v) A system for cleaning and disinfecting the room and equipment in order to achieve aseptic conditions;
(vi) A system for maintaining any equipment needed to maintain aseptic conditions.

- Air pressure, dust, humidity, and temperature must be controlled well when appropriate for medication product manufacturing and processing (b).
- Prefilters and particulate matter air filters are used in production areas as needed (c).
- 211.63 states that "equipment used in the manufacture, processing, packing or holding of a drug product shall be of acceptable design, suitable size, and ideally positioned for operations for its intended use, as well as cleaning and maintenance."
- "Equipment shall be built so that surfaces that contact components, in process materials, or drug products are neither reactive, additive, or absorptive," per 21 CFR 211.65. (a).
- To prevent malfunctions or contamination that might modify the drug product's identity, strength, quality or purity beyond the official or other set requirements, equipment and utensils must be cleaned, maintained and sanitised at appropriate intervals (a).
- In accordance with 21 CFR 211.113, "appropriate documented procedures" must be devised and followed to prevent microbiological contamination of sterile medicinal items (b). These methods necessitate validation of any sterilisation method.

PERSONNEL

Aseptic techniques, microbiology concepts, personal hygiene, gowning and any other related standard operating procedures should be taught to personnel in both didactic and practical settings (SOPs). Every employee must do one media fill a year. The operators' compliance with prescribed protocols and basic aseptic methods should be monitored regularly between media fills. An unclassified work space should have a maximum of two employees working there. Daily glove monitoring and regular sampling of one or more strategically selected areas of the gown are performed using surface sample plates. To avoid contamination, operators should wipe their gloves before surface sampling. The same level of training is required for microbiological testing of sterile objects as for manufacture. To guarantee that no errors have happened or have been adequately examined, you must be authorised to inspect production records. The quality control unit approves or rejects drugs manufactured, processed, packed, or contracted by third parties [3,4].

- Quality control unit must accept or reject any processes or standards impacting the identity, strength, quality or purity of a drug product (c).

- 21 CFR 211.25 states that "everyone involved in the manufacture, processing, packing or holding of a drug product shall have adequate education, training and experience to perform their assigned functions" (a). Training must be given to the employee in the procedures they perform.
- Manufacturing, processing, packing, or holding of each drug product requires an adequate number of qualified people (c).
- Staff involved in the manufacturing, processing, packing and holding of a drug product must wear clean clothing suited to their job (a). Anti-contamination clothes for medication items Head, face, hand, and arm coverings are required."
- Personal hygiene and health are required in 21 CFR 211.28. (b).
- Regulation 211.28 states that only supervisory personnel may enter restricted-access sections of structures and facilities (c).
- People with visible illnesses or open sores should not be allowed to touch components, drug product containers, closures, or other in-process materials, per 21 CFR 211.28. (d). until the issue is resolved or qualified medical professionals certify that the drug's safety or nature isn't compromised. All workers should inform administrative officials of any illnesses that may affect prescription goods.
- In accordance with 21 CFR 211.42, "operations shall be conducted within clearly defined size limits (c). In order to avoid contamination or mix-ups, the firm's operations must be divided into distinct areas.
- In accordance with 21 CFR 211.113, "appropriate documented procedures designed to preclude microbiological contamination of drug goods claiming sterility.." (b). These methods necessitate validation of any sterilising method." [1,4]

In order to ensure product sterility, managers must always use aseptic method. The aseptic assembly area requires appropriate training before anyone can enter. Patients' safety risks from non-sterile drug products should be included in basic training. Aside from initial training, representatives should constantly participate in a training programme. Authorities should regularly assess each administrator's adherence to written systems during actual activity.

COMPONENTS AND CONTAINER/CLOSURES

An aseptically prepared drug can be contaminated by at least one segment contaminated with bacteria or endotoxins. Components include active compounds, WFI, and excipients. The microbiological composition (e.g., bioburden, endotoxin) of each potentially contaminated component must be quantified. For nonpyrogenic parenteral products, endotoxin data is critical. Each lot of endotoxin-containing components should have specific approval or rejection criteria. Components that do not satisfy manufacturer endotoxin levels should be rejected. Components are either individually sterilised or combined and treated as a unit [1,4,5]. According to DA aseptic processing requirements, containers and closures must be washed with USP Water for Injection (WFI). Containers and closures must be depyrogenated to reduce endotoxin levels by 3 log units. Because endotoxin spikes on glass, plastic, and rubber are difficult to recover, validation of removal is the main issue.

- 21 CFR 210.3(b) defines component as any material used in the manufacture of a drug product, including those that do not appear in the drug product (3).
- Components, drug product containers, and closures must be approved or rejected according to written processes (21 CFR 211.80a).
- According to 21 CFR 211.80, "Components and drug product containers and closures shall be handled and stored to prevent contamination" (b).
- Before use, each lot of a component, drug product container, or closure that is prone to microbiological contamination that is undesirable given its intended purpose shall be subjected to microbiological tests.
- "Drug product containers and closures shall be clean, sterile, and treated to remove pyrogenic qualities," per 21 CFR 211.94. (c).
- In accordance with 21 CFR 211.94, "standards or specifications for drug product containers and closures shall be created and followed" (d).

- In accordance with 21 CFR 211.113, "Appropriate documented measures designed to avoid microbiological contamination of drug goods pretending to be sterile" (b). These guidelines demand validation of any sterilising technique. [6]

ENDOTOXIN CONTROL

Endotoxin-controlling formulation components, containers, closures, equipment, and storage time limits. All depyrogenation methods must be validated for endotoxin reduction/removal. Several depyrogenation technologies use clean-in-place approaches and final rinse procedures that require a 3 log reduction in an applied endotoxin challenge.

- Inadequate examination into the failure of media fill.
- Insufficient employee training after a botched media fill; media fills did not follow SOP. High particle counts interrupted medium loading, however the causes of the high counts were not investigated. After sterilisation, the media filling procedure did not begin.
- Failures are not counted if defective vials are discarded prior to incubation. The number of units filled was insufficient.
- The media fills did not correspond to the batch records. During the fill, some environmental data was not obtained.

TIME LIMITATION

Poor CGMP can lead to endotoxin contamination of injectables. Certain patient groups (e.g., newborns), multiple infusions, or parenteral infusions in very large volumes or dosages may be more prone to pyrogenic reaction than a normal solid adult. Such clinical considerations include implementing suitable CGMP measures to avoid endotoxins. Endotoxin control includes medicine items, holders, terminations, stockpile time cut-off points, and production hardware [3,4,5,6,]. Machines used in drug product manufacturing and processing must be of appropriate design, adequate size and well positioned for operations, cleaning, and maintenance. In accordance with 21CFR 211.65, "equipment shall be manufactured to ensure that surfaces that contact components, in-process materials, or drug products are not reactive, additive, or absorptive in a manner that alters the drug product's safety, identity, strength, quality, or purity" (a). 211.67 states that "Equipment and utensils shall be cleaned, maintained and sanitised to prevent malfunctions or contamination that might change the safety, identity, strength, quality, or purity of the drug product" (a). Drug product containers and closures must be clean, sterile, and free of pyrogenic properties before use (c). In accordance with 21 CFR 211.167, "appropriate laboratory testing shall be conducted on each batch of drug product professing to be sterile and/or pyrogen-free" (a). The test techniques must be documented." [7]

TIME LIMITATIONS

Time limits for completion of each phase of manufacturing shall be specified as required to ensure product quality, per 21 CFR 211.111. Exceptions to time constraints may be allowed if the drug's quality is not compromised. Exceptions must be justified and documented." Repeatable tests are required to establish maximum hold periods for filtration procedures. The time it takes to filter a non-sterilized bulk solution Is it safe to fill sterilised solutions? In order to use sterilised equipment, How long can sterilised containers and closures be kept? [6,8]

SANITATION

USFDA Evaluating disinfectant efficacy and limits should be done. It is important to assess the efficiency of these disinfectants and processes by removing possible contaminants from surfaces. [4,8,9]

EQUIPMENT

USFDA CGMP standards require that hardware be qualified, adjusted, cleaned, and maintained to avoid contamination and misconceptions. However, the CGMP guidelines also emphasise interaction and testing equipment.

MANUFACTURING PROCESS

Bio-weight is a common assumption. Bioburden should be measured before layer filtration, with a limit of 100 cfu/ml recommended. Preparation time for sanitization or filtration should be as quick as possible. It should be done in a single cycle. At the end of each activity, each cluster will be tested for sterility, and held until the findings are known. It is important to avoid contaminating the environment when filling powdered products.

- Blow-fill-seal technique
- Aseptic processing isolators

PROCESS VALIDATION AND EQUIPMENT QUALIFICATION IN ASEPTIC PROCESSING AND STERILIZATION-

Parts of aseptic process validation and equipment qualification include process simulation (media fills), filtering efficiency, and sterilisation of equipment and materials. Making an exact reproduction of the actual procedure involves many factors. In a media fill or process simulation test, sterile trypticase soy broth is prepared and sterilised (usually by filtering) before being filled into sterile containers under conditions that closely mirror actual filling.

The aim is to construct studies that mimic normal lot manufacturing conditions. On average, 4750 units are incubated for 14 days at temperatures validated to sustain microbial development, commonly alternating 20–25°C and 30–35°C storage. Test the media for microbiological growth. Contamination during processing can cause growth. Even if regulators forecast "approaching zero" growth, less than 0.1 percent of the challenged units must show growth. This test has also been used to establish an operator's skill level. [4,5,6,7,10]

- 21 CFR 211.63, 211.65, and 211.67 deal with "Equipment design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance," respectively.
- 21 CFR 211.84(c) - "Samples shall be collected according to the following procedures:
- 21 CFR 211.100(a) - "Written procedures for production and process control shall be in place to ensure that the drug products have the identity, strength, quality, and purity that they purport or are represented to have."
- "Appropriate documented methods designed to avoid microbiological contamination of medication goods claiming to be sterile" (21 CFR 211.113) (b). These procedures necessitate the validation of sterilising methods." [1,7]

This section deals with standard capability and approval checks. Contrary to popular belief, change management is not a one-size-fits-all approach to management. Revalidation or requalification is required whenever an office, equipment, interaction, or testing technique is changed.

LABORATORY CONTROLS

- Components, drug product containers, closures, packaging materials, in-process materials, and drug products must be approved (or rejected) by the quality control unit.
- 21 CFR 211.22(c) requires the quality control unit to accept or reject "any methods or specifications affecting the identity, strength, quality, or purity of the drug product."
- 21 CFR 211.42 states that "operations shall be done within specifically established limits of sufficient size" (c). Separate or specified areas, as well as other control measures, must be in place to prevent contamination or mix-ups.
- "Written procedures shall allocate responsibility for sanitation and specify in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities," states 21 CFR 211.56. (b).
- Rodenticides, insecticides, fungicides, disinfectants, and cleaning and cleaning specialists will be used in accordance with 21 CFR 211.56 (c). A written procedure must be established and followed to assure batch uniformity and integrity of medicinal products, according to 21 CFR 211.110(a). Control mechanisms must be built to monitor output and certify performance.
- In accordance with 21 CFR 211.113, "Appropriate documented measures designed to preclude microbiological contamination of drug goods pretending to be sterile" (b). These methods necessitate validation of any sterilising method.

- In accordance with 21 CFR 211.160, "Laboratory controls shall involve the formulation of scientifically sound and suitable specifications, standards, sampling plans," and test procedures (b). Laboratory controls must comprise the following:

(1) Acceptance of each lot within each shipment of components, drug product containers, closures, and labelling used in the production of drug products. The specifications must include a description of the sample and testing techniques. Representative and correctly labelled samples
(2) Determination of conformance to standards, including in-process material sampling and testing. We need representative samples with labels.
(3) Checking for compliance with defined sampling techniques and medication product specifications. We need representative samples with labels.
(4) In case the precision and exactness cut-off points are not met, the written programme includes specific headings, timeframes, and plans for remedial action. The use of non-compliant instruments, mechanical assemblies, measurements, and recording devices is prohibited."

- 21 CFR 211.165(e) - "The firm's test techniques' accuracy, sensitivity, specificity, and reproducibility shall be established and recorded." Validation and documentation in compliance with 211.194(a)" are possible.
- "Before a batch is issued or distributed, the quality control unit shall evaluate and approve all drug product manufacturing and control records, including those for packing and labelling, to determine conformity with all established, authorised written procedures," according to 21 CFR 211.192. [1,4,7,11]

Sterility testing

- A "representative sample" is defined as "a sample consisting of a number of units drawn based on reasonable criteria such as random sampling and designed to correctly characterise the item being sampled" in 21 CFR 210.3(b)(21).
- For each batch, formal processes must be devised and followed to ensure batch consistency and integrity of pharmaceutical products.
- Components, drug product containers, closures, in-process materials, labelling, and drug products must all meet applicable criteria of identification, strength quality, and purity, according to 21 CFR 211.160. (b). [1,4,8]

Laboratory controls shall include

(1) Acceptance of each lot within each shipment of components, drug product containers, closures, and labelling used in the production of drug products. The specifications must include a description of the sample and testing techniques. Representative and correctly labelled samples Retesting of any degraded component, drug product container, or closure is required.
(2) Determination of conformance to standards, including in-process material sampling and testing. We need representative samples with labels.
(3) Checking for compliance with defined sampling techniques and medication product specifications. We need representative samples with labels.
(4) A written programme must include specific bearings and schedules as well as precision and accuracy cut-off points as well as plans for remedial action if the precision and accuracy limitations are not fulfilled. Refusing to comply with stated norms is a violation of the law.' [8,9,10]

- In accordance with 21 CFR 211.165(a), "adequate laboratory assessment of satisfactory compliance with final standards for the drug product, including the identification and strength of each active ingredient,"
- (e) The firm's test procedures' accuracy, sensitivity, specificity, and repeatability must be established and reported. Validation and documentation are allowed under 211.194(a)(2). Under 21 CFR 211.167(a), "appropriate laboratory testing shall be performed on each batch of drug product claiming to be sterile and/or pyrogen-free". The test procedures must be in writing and followed."
- To determine the necessity for changes in drug item details, assembly, or control measures, 21 CFR 211.180 requires written records to be retained (e).

- Before a group is delivered or distributed, the quality control unit will review and endorse all medicine item assembling and control records, including those for bundling and labelling. any unexplained anomaly (counting a level of hypothetical yield surpassing the most extreme or least rates set up in ace creation and control records). Other prescription things that may be linked to the particular disappointment or disparity will be studied as well. [12]
- Sterility testing involves controlling the testing environment, recognising test constraints, and reviewing production systems after a positive test. The testing laboratory should have similar controls and facilities as aseptic filling processes. Poor or insufficient sterility test facilities or controls can cause sterility test failure.
- There is a risk of mistaking a positive sterility test result for a failed research facility, regardless of whether the object tested was nonsterile. It may so go unnoticed. Isolators are used in sterility testing to reduce false positive results.[13]

CONCLUSION

From the above study we have concluded that the sterility should be maintained according to the standards in the manufacturing unit and maintain good compatibility of the drug product so the drug should follow the quality guidelines. This article gives clear idea about regulatory requirements of current guidelines and their requirements include environmental parameters, building and facilities, personnel, trainings, components and container/closures, endotoxin control, laboratory control, time limitation and validation, sterility testing, batch record review and documentations. To reduce any type of contamination in various sterilization process this research has been conducted. For the manufacture of sterile product, it should follow the FDA norms. So, the main aim of the research to minimize the errors in the packaging areas, manufacturing unit, container closure system to produce high quality products that satisfies the customer according to their needs, here we discussed briefly about regulatory challenges in the sterile formulation.

ACKNOWLEDGMENTS

The Authors express sincere gratitude to JSS Academy of Higher Education and Research and JSS College of Pharmacy, Mysuru for the support in carrying out their work.

CONFLICT OF INTREST: None
FINANCIAL SUPPORT: None
ETHICS STATEMENT: None

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Table 1: Air Classification

Clean area classification (0.5 m particles/ft³)	≥0.5µm particles/m³	Microbiological active air action levels[c] (cfu/m³	Microbiological settling plates action levels[b,c] (diam.9mm; cfu/4 hours)
100	3520	1[c]	1[c]
1000	35200	7	3
10000	352000	10	5
100000	3520000	100	50

Table 2. Airbone Particulate Classification for Manufacture of Sterile Products

GRADE	AT REST		IN OPERATION	
	maximum no of permitted particles per m³ equal to or above			
	0.5µm	5µm	0.5µm	5µm
A	3520	29	3500	29
B	35200	293	352000	2930
C	352000	2930	3520000	29300
D	3520000	29300	NOT DEFINED	NOT DEFINED

Table 3: Comparison of Various Grades Described in Various Guidelines

SI NO	USFDA	GMP/WHO/MHRA
1	CLASS 100	A and B
2	CLASS 10,000	C
3	CLASS 1,00,000	D

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review of Artificial Intelligence (AI) Technologies and Education Relationship Management

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Received: 04 Jan 2022 Revised: 13 Feb 2022 Accepted: 31 Mar 2022

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ABSTRACT

As businesses prepare to change their focus from transactions to relationships, the Internet has provided new gateways between them and their customers. Today, it is commonly understood that how a company treats its customers has a significant impact on its future success, and businesses are increasingly investing in this area. In the digital economy's fiercely competitive, dynamic markets, companies who develop strategies and technologies for executing customer relationship management with the most profitable clients emerge as victors. Intelligent agents are a rapidly growing research field. It's a new technology with deep roots in artificial intelligence research. With the growth and expansion of the Internet, it has become a more prominent means of achieving customer happiness and joy through substantial e-CRM. However, it is still unclear how agents can be deployed in the e-CRM area. This paper begins by providing an overview of intelligent agents. The second section discusses how e-CRM might help businesses improve client acquisition and retention. Finally, we offer an integrated model in which intelligent agents are viewed as a potent instrument for achieving e-CRM over the Internet.

Keywords: Artificial Intelligence (AI), Education, Human resource management, and machine learning

INTRODUCTION

The use of Artificial Intelligence (AI) technologies has become the norm. Because AI is now used to power everything, it has changed our way of life.AI is being widely adopted by organisations and corporations, which is assisting them in streamlining operations, increasing production, enhancing efficiency, and lowering costs. Artificial intelligence combined with individual resource management approaches is transforming the way businesses hire, manage, and engage their staff. Artificial intelligence allows machines to make better decisions than people based on historical data and behavioural patterns. As a result of this shift, machines have taken over all of the manual labour, forcing HR professionals to take on more strategic duties. Understanding how this technology works and its role in various HRM tasks is critical for businesses and professionals. Artificial Intelligence (AI), a technology that enables machines to function intelligently and intelligently, is changing people's lives. Finance, agriculture, health care, production, marketing, e-commerce, and human resource management are just a few of the industries where this instrument is already in use. Artificial intelligence's potential to replace human cognitive ability is causing it to achieve widespread acceptance and favour in a variety of fields. Artificial Intelligence (AI) is a current and popular trend in HR (AI).Data mining, machine learning, deep learning, and neural networks, which were previously unknown terms, have now become popular buzzwords. Many firms have already begun to integrate and apply AI technology to their benefit, making it no more a futuristic idea [1].

In the literature, there are various definitions for AI, each reflecting its ability to think, learn, and perform like humans. Artificial intelligence is defined as "the study of how to make computers perform things that people are now better at" [2].According to another study, it is "a system's ability to correctly read external input, learn from that data, and apply that learning to fulfil specific goals and tasks through flexible adaptation" [3].AI techniques are defined as "machine-processable instructions to solve tasks that, if solved by humans, would need clear cognitive capabilities" [4].According to the findings of this study, the major groups are as follows: Knowledge discovery (extracting useful data), knowledge representation (representing relevant information in a way that machines can recognise and use it while solving complex problems), knowledge processing (creating new knowledge using represented knowledge), techniques for searching solutions, text processing, and speech processing are all examples of AI functions."Quick answers are required in the corporate environment due to rapid changes"[5].Many businesses have already begun to incorporate AI into their operations. The main reason why businesses are adopting modern technology and embracing the digital transformation is that they have grasped the huge potential of data and the critical role it plays in individual and organisational performance [6].This review paper aims to shed light on artificial intelligence's uses in the subject of human resources, as well as its benefits, problems, and future prospects. This is accomplished by the utilisation of secondary data from relevant studies.

Artificial Intelligence Applications

AI is gradually redefining all of HRM's numerous functions. Virtual assistants are now used in HRM services like as recruiting, selection, training and development, performance management, and employee engagement. Human resource planning is the first and most important step in human resource management. In this procedure, the Human Resource Information System (HRIS) is critical."A systematic technique for collecting, storing, maintaining, accessing, and validating data needed by an organisation concerning its human resources, personnel activities, and organisational unit characteristics" [7] is how HRIS is defined. HRIS can help with HR planning, job descriptions, performance evaluation, and training programme creation, among other things [8]. The fig 1 Computing is become an integral aspect of modern schooling. Information systems are increasingly supporting the generation of learning content and the management of education .Rapid changes in a complex environment, as well as rapid knowledge obsolescence necessitate quick reactions and innovative information system solutions. In these circumstances, traditional approaches to information system development via Turing machine programming should be replaced by the building of continuous self-adaptive systems with natural interfaces.

Recruiting and Hiring

By processing a huge number of applicant applications in a short amount of time, AI is assisting businesses and recruiting agencies in working more efficiently. Companies may now increase candidate engagement and implement both high volume and high touch strategies, resulting in a stable and long-term relationship with their candidates, thanks to AI.Bots powered by AI are used to communicate with applicants, answer their questions, and keep them linked and connected throughout the employment process [9].Natural language processing (NLP) capabilities of AI-powered assistants or bots enable them to play a key part in all forms of candidate communications.NLP can easily transform speech to text in microseconds, allowing recruiters to save time by eliminating the need to type [8].Recruiters can use AI assistants to aid them with duties such as candidate screening, making contact, organising meetings and interviews, and more. The AI assistants aid recruiters with duties such as candidate screening, contact establishment, meeting and interview scheduling, and candidate engagement. Organizations also profit from this by saving time and money, employing quality candidates, precisely mapping talents, minimising bias, and rapidly resolving candidate queries [10]. According to a reference cited in an article, using artificial intelligence-powered software in the recruitment process can help organisations cut their hiring costs by 71 percent per candidate, while also increasing recruitment efficiency by three times [11]. Pre-hire assessments are made much easier with online interviewing systems like HireVue. Recruiters can use HireVue to ask candidates pre-determined questions, which they must respond to in the form of a video. Recruiters then evaluate these pre-recorded films in order to choose the best prospects for a final interview. Recruiters then evaluate these pre-recorded films in order to choose the best prospects for a final interview.This technique allows a large number of prospects to be analysed in a short period of time. These kind of procedures aid in attracting candidates and simplifying the hiring process [12].

Orientation

The process of acclimating new employees to the organization's environment, policies, and work culture is known as onboarding.In most cases, companies hold induction seminars for this purpose. New employees, on the other hand, require more care, yet it is impossible to attend to each of them individually. Customized on boarding practises are now assisting with this, resulting in a high level of organisational adaption among new employees [13].Additionally, these days, HR workers are aided by bots that communicate with newly hired employees and keep them informed about their employment advantages, corporate laws, and policies, as well as assist them in answering their questions and doubts [11].

Education and Training

In today's fast changing economy, it's critical that employees remain up to date on the newest trends, advances, and changes affecting their jobs. As a result, in order to have a professional and technically qualified personnel, each firm must have a proper training facility. Through online learning platforms, new technologies in the field of training and development are assisting HR professionals in making their training programmes more effective and intelligent. It is critical to determine how fulfilling and effective these training sessions were for the employees, which is why they are asked to provide feedback and ideas based on their training experience.AI can also assist the HR department with similar feedback operations [14].

Employee Engagement

Organizations can now more easily forecast their employees' levels of engagement using various artificial intelligence-assisted prediction methodologies. These technologies and methodologies are capable of evaluating enormous data sets, generating useful outputs, and forecasting current and future employee engagement levels. Face recognition technology aids businesses in determining their employees' moods by scanning their facial expressions on any given day. This aids the firm in gaining a better knowledge of employee behaviour, making employees feel more important and valued. As a result, there is a higher level of employee involvement within the company [14].

Compensation Administration

Compensation management is a critical component of human resource management, and it is closely tied to employee performance at work. It is a procedure for establishing employee compensation in accordance with specified regulations and guidelines. An effective compensation management system in the workplace aids in the improvement of both individual and group performance [15].Artificial neural networks are a technique that can be used to provide a level of fairness in the compensation appraisal process. By simulating the operation of the human brain, the neural network system can recognise correlations in large data sets .

Managing Performance

Another essential HRM technique is performance management. This process has been made much faster and more efficient thanks to artificial intelligence-assisted systems.360-degree appraisals and other scientific assessment procedures are being carried out automatically. To achieve performance-based outcomes, the system is provided with employee assessment criteria as well as other important data [16].

Advantages

Artificial intelligence (AI) can be beneficial to a company's overall success. In human resource management, it serves as a support function. All of the actions connected to hiring, engaging, and keeping staff are now well-structured and organised. According to surveys conducted by companies such as IBM, roughly 66 percent of CEOs believe AI can make a substantial contribution to the field of human resources. Increased HRM automation has brought machines and humans closer together, allowing HR employees to focus on more human qualities like creative thinking, problem-solving, and empathy. Artificial intelligence (AI) and machine learning techniques have now made it possible to study and comprehend employee performance over time. Employees can improve their productivity by focusing on their weak areas with customised learning and development programmes. Employees can use intelligent bots to answer job-related questions, and the employment of modern technology in organisational operations has helped to increase openness and justice at work Intelligent AI systems can be quite useful in locating the best match for a specific job profile.AI can help HR managers by acting as both an assistant and a consultant. The "Virtual Assistant System" is in charge of communicating with both candidates and workers, as well as writing emails, organising meetings, reporting, and other time-consuming chores.

The "advisor system" can aid decision-making by intelligently forecasting future outcomes in key domains. Big data analysis can be used to forecast future outcomes for critical organisational issues. The information gathered can then be used to take the required precautions [8].AI can also aid in the resolution of compliance challenges. Any compliance issue can be recognised in advance by looking at the organization's network data

Difficulties

Despite the fact that artificial intelligence's new tools and techniques are proving to be a huge benefit to businesses, there are several issues that require specific attention. Artificial intelligence (AI) has the potential to eventually replace all forms of human occupations. Using mechanical and analytical intelligence, AI has already replaced professions at the task level, and it has the ability to execute even intuitive and compassionate duties. While this provides a chance for successful and innovative machine-human integration, it also poses a potential danger to human employment Another significant difficulty for businesses is determining the cost impact of implementing new AI technology in the workplace .

According to a study on AI adoption in the public sector, stakeholders believe that managers' primary attention should be on the integration, continuity, quality, and quantity of data used. This is because AI applications rely entirely on data to function, and if the data upon which these applications are based is not correctly integrated, no AI technology will be able to deliver the promised benefit [19].This report also suggested that the use of artificial intelligence can have a significant impact on crucial business decisions, and that a sound plan is needed to address AI transition challenges. Concerns about AI misuse, unethical and inappropriate use of shared data, and a lack of

trust in AI-powered judgments must all be addressed through the creation of a legal framework . Finding applicants that are well-versed in the latest technology-based tools and processes will be another big challenge for HR managers. Because most employees find it difficult to adapt to new AI technologies and tools and obtain knowledge on them, the advent of AI in organisations has resulted in an increasing demand for skilled candidates in the market .

Future Possibilities

Researchers anticipate that artificial intelligence technology will exceed humans in many tasks and activities in the next several years. According to scientists, AI has a 500 percent possibility of exceeding human work in 35 years and of automating human professions in the next 60years Many experts feel that AI will only serve as a supplement to humans and would never completely replace them.In the future, there will be a collaboration between machines and people .Another AI and deep learning study emphasises the importance of a collaborative relationship between computers and humans, in which machines forecast outcomes and humans decide and take appropriate responses

CONCLUSION

The field of human resources has done a good job of adapting to the technological revolution brought on by artificial intelligence, but there is still a lot of work to be done. Any new shift comes with its own set of challenges, but in order to fully benefit from it, we must always be on the lookout for ways to overcome them. In HR, a similar strategy is required as well. Based on the findings of the many research discussed in this paper, it is clear that data plays a critical role in the application of artificial intelligence inside organisational processes. As a result, the HR professional must pay close attention to guarantee that only high-quality data is used. The incorporation of artificial intelligence into HRM procedures has improved the accuracy and flexibility of planning and decision-making.AI-enabled applications have aided businesses in increasing employee productivity, increasing overall workplace efficiency, lowering costs, automating monotonous human jobs, providing helpful insights through data analytics, and forecasting future outcomes. Only those firms who understand the potential of AI early on and take full advantage of it will be able to survive and thrive in today's world of escalating competition and technological transformation. There haven't been many experimental and statistical studies in this field yet, which expands the possibilities for future research. and adaptable

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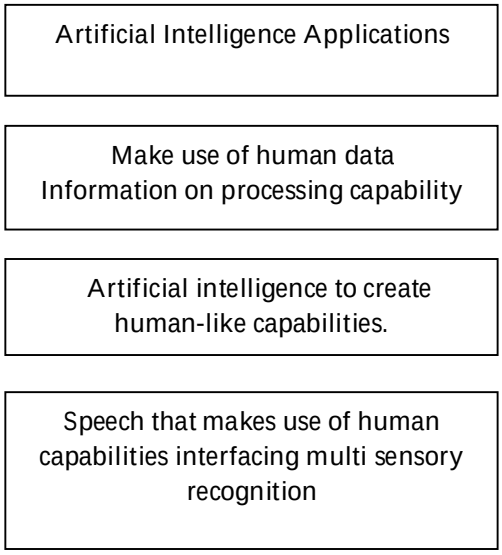

Fig 1 AI Application Description

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Next Agriculture Sensor Networks: Applications of System on Chip

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Received: 05 Feb 2022 Revised: 05 Mar 2022 Accepted: 31 Mar 2022

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ABSTRACT

The platform is being used to build an effective and low-cost tool that will aid in the development of sustainable agriculture. The System on a Chip architecture, on the other hand, is more application focused, with the goal of reducing power consumption and design costs. SoC is primarily expected to be used in the creation of Network on Chips. The different application-specific sensors that make wireless sensing platforms possible. We separate these sensors into three groups for easier classification: soil, environment, and plant-related sensors.

Keywords: Precision Agriculture ,Sensor, ZigBee, Soil Sensor , Climatic change ,Products

INTRODUCTION

Precision Agriculture

Precision agriculture refers to the concept of monitoring a farm's environmental factors in real time, such as temperature, humidity, and soil PH. Instead of sending the monitored parameters to a remote server so that suitable action can be taken, an actuator or an automated system can be utilised to take appropriate action depending on the observed parameters over time.

Sensor Networking

The universal networking sensor network is a novel type of information collecting and processing technology that, in some cases, outperforms existing technologies. This work finished the sensor node design and organised the network monitor system via wireless sensor. This type of wireless detection and control has several advantages over traditional agriculture: it is simple to set up the network, it is less expensive due to the one-time structure, it has a high expansibility and fine flexibility, it can effectively improve the mode of existing production and management,

and it can increase agricultural production efficiency.[3-5] WSNs (wireless sensor networks) are one of the most important technologies of the twenty-first century.WSN has become a reality in applications thanks to advancements in micro sensor technology and low-power circuits in recent years.

Agriculture-Related Products

It is critical to acquire data on the real-time impact of stress factors on the status of biological things Precision Agriculture in agriculture, ecological monitoring, and defence. The proposed wireless sensor network and multilevel sensor network can be used in industrial agriculture, environmental protection, and ecological monitoring to improve agricultural product efficiency and quality, save fertilisers. The development and creation of a WSN for monitoring human status parameters gives soldiers the capacity to boost their vitality in battle and extreme conditions by remotely monitoring fighting capabilities and providing timely medical help to wounded and injured soldiers. The device features a compact frame, is light in weight, performs consistently, and is simple to operate. It has a significant impact on agricultural production efficiency and automation. Implementing low-power, low-cost wireless sub-soil sensing systems to enable precision agriculture is becoming increasingly popular.

Changes In Climate

In industries such as agriculture, wireless sensor networks are commonly used. Continuous monitoring of agricultural fields is vital in terms of plant control and protection via sensors, as well as real-time monitoring of the agricultural system. Climate conditions are no longer consistent or predictable. Furthermore, there is a wireless sensor network. India's facility agricultural sector is growing and leading the world. Human beings, without a doubt, cannot exist without food and water[6-10].Human sustainable development has been jeopardised in recent years due to climatic change, water scarcity, and land resource depletion.

Traditional watering and fertilisation procedures are based on experience and have no scientific backing, resulting in water waste and soil damage.

Implementation.

Agriculture monitoring is frequently used to improve the quality and productivity of farming. Sensors in this application collect many forms of data in real-time circumstances, such as humidity, carbon dioxide level, and temperature. To avoid hostile adversaries, data gathering, transmission, and rapid response to new conditions all necessitate a secure data channel. As a result, this study focuses on data security from the data source to the end-user, and presents a comprehensive data security model that is independent of network topology and structure and can be broadly applied in farm monitoring applications.

Future Prospects

WSNs are currently gaining a lot of traction in agriculture. In agricultural production, the analysis of obtained data from the farming field is critical. Malicious competitors, on the other hand, can tamper with measured data and disrupt the production or testing process. As a result, this article developed a data security architecture that allows data to be protected from the sensor to management structures via the communications network, and vice versa. Wireless Sensor Networks have received a lot of attention in the industry and in our daily lives as a result of the advancements in sensor technology, MEMS, wireless communications, and the extensive use of wireless sensors. In order to achieve agricultural modernization while still protecting the environment, The devices, sensors, and communication techniques related with WSNs in agricultural applications are thoroughly examined in order to focus on the unique requirements. We present a number of case studies in order to thoroughly investigate the existing solutions proposed in the literature, categorising them according to design and execution factors.

CONCLUSION

In today's globe, agriculture faces a difficult scenario as a result of global warming and climate change. A variety of new procedures and technology are being introduced into agriculture in order to minimise costs and increase overall output. In agriculture, water is a valuable resource. Agriculture in a controlled environment is a new concept. Other

costs in agriculture, such as soil testing, temperature, humidity, and water supply control, are also dependent on the moisture content of the soil.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Numerical Solution to Tenth Order Linear Differential Equation using Decic Degree B-Spline Collocation Method

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Received: 28 Jan 2022 Revised: 18 Feb 2022 Accepted: 31 Mar 2022

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ABSTRACT

This paper describes the numerical method of decic degree B-spline collocation method. Present numerical method is formed by employing the B-spline functions as basis functions in approximating solution. Decic degree B-spline base function employed in collocation method gives the best approximate solution to the considered tenth order differential equation. It alternates of increasing the number of collocation points in the problem domain and also raising the degree of B- spline base function improves the numerical solution and to get best solution to the given differential equation. Numerical test results show that the present decic degree B-spline basis function which are employed in collocation method as basis are best choice to achieve appropriate solution to the differential equation.

Keywords: Decic, Linear differential equations with constant coefficients ,Collocation method, B-splines

INTRODUCTION

B-splines were first introduced by Schoenberg in [1, 2] who revealed that splines have powerful approximation properties. Subsequently, many approximation methods have been used [3].Most properties and efficient construction of B-splines can be found in [4].In particular, after de Boor's results [4], spline techniques became popular for a broad range of applications [5]. The efficiency of the method in explicit form has been proved by many researchers [6]-[12].In particular, recursive form of B-spline is employed in this paper to the higher order linear differential equations with constant coefficients. Considering the fifth order linear differential equations with constant coefficients

$k_1\frac{d^{10}U}{dx^{10}} + k_2\frac{d^9U}{dx^9} + k_3\frac{d^8U}{dx^8} + k_4\frac{d^7U}{dx^7} + k_5\frac{d^6U}{dx^6} + k_6\frac{d^5U}{dx^5} + k_7\frac{d^4U}{dx^4} + k_8\frac{d^3U}{dx^3} + k_9\frac{d^2U}{dx^2} + k_{10}\frac{dU}{dx} + k_{11}U = Q(x)$,

$a < x < b$ (1) with the boundary conditions

i) $U(a)=d1, U(b)=d2$ $\frac{dU(a)}{dx} = d3, \frac{dU(b)}{dx} = d4, \frac{d^2U(a)}{dx^2} = d5, \frac{d^2U(b)}{dx^2} = d6$

$\frac{d^3U(a)}{dx^3} = d7, \frac{d^3U(b)}{dx^3} = d8$ $\frac{d^4U(a)}{dx^4} = d9$, $\frac{dU(b)}{dx} = d10$

where $a, b, d_1, d_3, d_4, d_5, d_6, d_7, d_8, d_9, d_{10}, k_1, k_2, k_3, k_4, k_5\ \ k_6, k_7, k_8, k_9, k_{10}, k_{11}$ are the constants.

$Q(x)$ is a function of x

Let $U^h(x) = \sum_{i=-10}^{n+10} C_i N_{i,p}(x)$ ..... (2) ,

where C_i's are constants to be determined and $N_{i,p}(x)$ are B-spline functions , be the approximate global solution to the exact solution $U(x)$ of the considered tenth order linear differential equation with constant coefficients.

B-Splines

In this section, definition and properties of B-spline basis functions are given in detail. A zero degree and other than zero degree B-spline basis functions are defined at x_i recursively over the knot vector $X = \{x_1, x_2, x_3 ..... x_{n-1}, x_n\}$ as

$i)\ \ if\ \ p = 0$

$N_{i,p}(x) = 1\ if\ x \in (x_i, x_{i+i})\quad N_{i,p}(x) = 0\ if\ x \notin (x_i, x_{i+i})$

$ii)\ \ if\ \ p \geq 1\ N_{i,p}(x) = \frac{x - x_i}{x_{i+p} - x_i} N_{i,p-1}(x) + \frac{x_{i+p+1} - x}{x_{i+p+1} - x_{i+1}} N_{i+1,p-1}(x)$

where p is the degree of the B-spline basis function and x is the parameter belongs to X .When evaluating these functions, ratios of the form 0/0 are defined as zero

Derivatives of B-splines

If p=2, we have

$$N'_{i,p}(x) = \frac{x - x_i}{x_{i+p} - x_i} N'_{i,p-1}(x) + \frac{N_{i,p-1}(x)}{x_{i+p} - x_i} + \frac{x_{i+p+1} - x}{x_{i+p+1} - x_{i+1}} N'_{i+1,p-1}(x) - \frac{N_{i+1,p-1}(x)}{x_{i+p+1} - x_{i+1}}$$

$$N'_{i,p}(x) = 2\frac{N'_{i,p-1}(x)}{x_{i+p} - x_i} - 2\frac{N'_{i+1,p-1}(x)}{x_{i+p+1} - x_{i+1}}$$

In the above equations, the basis functions are defined as recursively in terms of previous degree basis function i.e. the p^{th} degree basis function is the combination of ratios of knots and (p-1) degree basis function. Again (p-1) th degree basis function is defined as the combination ratios of knots and (p-2) degree basis function. In a similar way every B-spline basis function of degree up to (p-(p-2)) is expressed as the combination of the ratios of knots and its previous B-spline basis functions.

The zero degree B-spline bases are C^0 –continuous. First degree B-spline bases are C^1-continuous and first derivative exist and second derivative exists for second degree B-spline basis functions. B-spline basis functions are defined on knot vectors. Knots are real quantities. Knot vector is a non decreasing set of knots. Knot vectors are classified as non-uniform knot vectors, uniform knot vector and open uniform knot vectors. Uniform knot vector in which difference of any two consecutive knots is constant is used for test problems in this paper. Two knots are required to define the zero degree basis function .In a similar way, the required number elements in a knot vector to define a p^{th} degree B-spline basis function at a knot is always more than the two of the degree of the basis function.B-spline basis functions of degree zero, degree one and degree two over uniform knot vector are shown graphically below in the following figures (i), (ii) and (iii)

B-Spline Collocation Method

Collocation method is used widely in approximation theory particularly to solve differential equation.In collocation method, the assumed approximate solution is exact solution at some nodal points. B-spline basis functions are used as the basis in B-spline collocation method whereas the base functions which are used in collocation method are the polynomials vanishes at the boundary values. Residue which is obtained by substituting equation (2) in equation (1) is made equal to zero at nodes in the given domain to determine unknowns in (2).Let $[a,b]$ be the domain of the governing differential equation and is partitioned as

$X = \{a = x_0, x_1, x_2 \ldots\ldots x_{n-1}, x_n = b\}$ with equal length $h = \frac{b-a}{n}$ of n sub domains. The $x_i's$ are known as nodes, the nodes are treated as knots in collocation B-spline method where the B-spline basis functions are defined and these nodes are used to make the residue equal to zero to determine unknowns $C_i's$ in (2).Ten extra knotsare taken intoconsideration beside the domain of problem both side when evaluating the decic degree B-spline basis functions at the nodes which are within the considered domain.

Numerical Experiments

Example 1

$\frac{d^{10}U}{dx^{10}} + U = -10(2x\sin(x) - 9\cos(x)$

$wit\square U(-1) = 0, U(1) = 0, U'(-1) = -2\cos(1),$

$U'(1) = 2\cos(1) and U''(-1) = 2\cos(1) - 4\sin(1)$

$U''(1) = 2\cos(1) - 4\sin(1) U'''(-1) = 6\cos(1) + 6\sin(1)$

$U'''(1) = -6\cos(1) - 6\sin(1) U''''(-1) = 12\cos(1) + 8\sin(1)$

$U''''(1) = -12\cos(1) + 8\sin(1)$

The analytical solution for the above problem is $y = \cos x\,(x^2 - 1)$

It is observed from the Table1that the values obtained by using the decic degree B-spline collocation solution are more close to analytic solution.

CONCLUSION

The decic degree B-spline collocation solution is tested by considering the tenth order linear differential equation with constant coefficients. The results obtained by using present decic degree B-spline collocation method is best fit with the exact solution. This is proved by taking the numerical example.

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Table1 : The decic degree B-spline collocation solutions and analytical solutions of Example1 at various considered domain points

X	*-1*	*-.8*	*-.6*	*-4.*	*-.2*	*0*	*.2*	*.4*	*.6*	*.8*	*1*
Numerical Solution by decic degree									-		

B-spline	0	-0.2530	-0.5427	-0.8097	-0.9976	-1.065	-0.9976	-0'8097	-0.5427	-0'2530	0
Analytical solution	0	-0.2508	-0.5282	-0.7737	-0.9409	-1.000	-0.9409	-0.7737	-0.5282	-0.2508	0

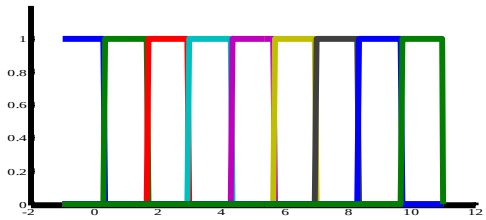

Fig (i), Zero degree B-spline basis function over uniform knot vector X= {- 1, 0 1, 2, 3, 4, 5, 6, 7, 8, 9, 10}

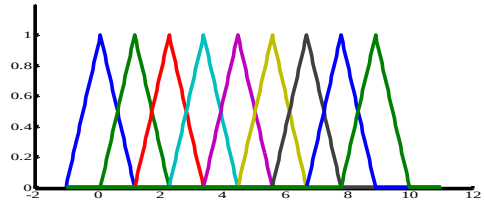

Fig (ii), First degree B-spline basis function with uniform Knot vector X= {- 1,0,1,2,3,4,5,6,7,8,9,10,11,12}

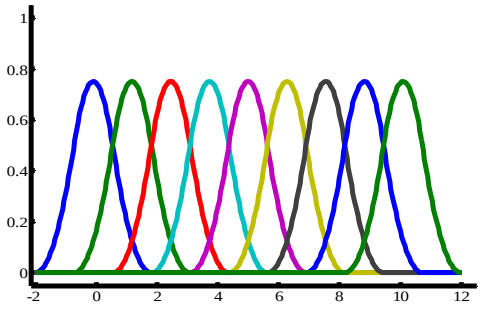

Fig (iii), Second degree B-spline basis function with uniform Knot vector X= {-2,-1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12}

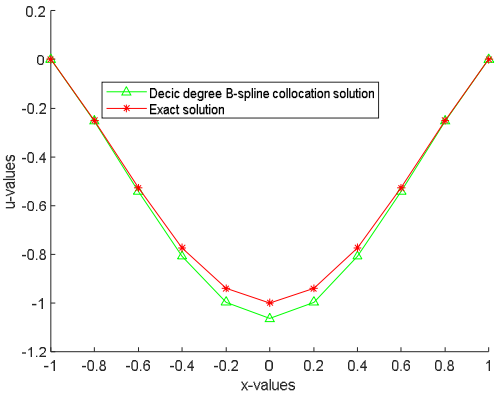

Fig (iv), Comparison of decic degree B-spline collocation solution with the analytical solution

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Utility of Different Biomarkers in the Management of Diabetes

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 20 Mar 2022

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ABSTRACT

The cornerstone of diabetes management is achieving and maintaining optimal glycemic control. Self-monitoring of blood glucose (SMBG) and laboratory testing for hemoglobin A1c (HbA1c), a surrogate biochemical measure of the average glycemia level over the previous 2-3 months, are now used to monitor glycemic management.HbA1c has been used to predict long-term outcomes related to morbidity and mortality in people with type 1 and type 2 diabetes in the vast majority of large clinical trials. Despite the fact that hyperglycemia is a critical biochemical signature of diabetes, the degree and exposure to high glucose, as well as glycemic variability, all play a role in the aetiology of diabetic complications in both type 1 and type 2 diabetes and follow distinct patterns.. HbA1c is a useful, standardized, and evidence-based metric for clinical decision-making, but it is limited in its ability to accurately reflect actual glycemia due to many biological and analytical confounders. Other glycated proteins, such as fructosamine, glycated albumin, and the nutritional monosaccharide 1,5-anhydroglucitol, as well as integrated measures from direct glucose testing by an SMBG/continuous glucose monitoring system, have emerged as valuable complementary data in recent years, notably when HbA1c results are unreliable or insufficient to assess the risk of adverse outcomes. To offer clinically meaningful cut-off values and prove their applicability in varied groups of diabetic patients, long-term relationships of these alternative glycemia biomarkers with the risk of complications must be examined. In this article, we'll go through the benefits and drawbacks of different glycemic control markers, along with options for evaluating glucose data.

Keywords: Diabetes control, CGMS, IDF, Diabetic complications, Glycemic indicators, American Diabetes Association, International Diabetes Federation.

INTRODUCTION

The cornerstone of diabetes management is achieving and maintaining optimal glycemic control [1].The landmark Diabetes Control and Problems Trial (DCCT) demonstrated conclusively that glycemic control is causally associated with micro vascular complications in type 1 diabetes [2]. The Epidemiology of Diabetes Interventions and Problems Study (EDIC) found that keeping glycemia as near to normal as feasible with enhanced insulin treatment reduced both microvascular and cardiovascular complications in the same cohort of patients over 30 years [3]. In type 2 diabetes patients, similar evidence of the favorable effect of rigorous glucose control methods in lowering the risk of diabetic complications, poor cardiovascular events, and death was seen in both the UKPDS intervention and follow-up trials [4,5]. In both the DCCT and UKPDS trials, HbA1c emerged as a critical driver of the risk cut-off for diabetic complications and as a set point for optimal glycemic control, and it is now regarded the gold standard of diabetes management in clinical practice[6]. Recent technical advancements in the field of continuous glucose monitoring systems (CGMS) have unveiled new clues into short-term glucose dynamics that are not reflected by HbA1c, even though HbA1c appears to be useful in predicting the risk of diabetes complications [7,8]. To avoid or postpone diabetic complications, tight glycemic management, which includes complete diabetes evaluation, is required, according to the American Diabetes Association (ADA) and International Diabetes Federation (IDF) standards. Nonetheless, the results of the ACCORD[9] and ADVANCE [10] studies have taught us that HbA1c levels should be adapted to the patients' health state, with lower goals for older patients and those with many comorbid diseases.

HbA1c has been used to predict long-term outcomes related to morbidity and mortality in people with type 1 and type 2 diabetes in the vast majority of large clinical trials, but the strength of the association with macrovascular endpoints was weaker than the strength of the association with microvascular endpoints. Furthermore, it is yet unknown how different glycemic measurements predict diabetic complications, or whether a mixture of indicators is even more strongly linked to negative outcomes than a single biomarker.Nathan et al11 recently published a research that supports the use of two glycemic markers to enhance risk prediction, based on data from the Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complication Study. As a result, it wouldn't be remarkable if, in the not-too-distant future, a combination of shorter and longer-term glycemic indicators could be utilized to more precisely predict cardiovascular outcomes. We now feel that the time has come to shift away from HbA1c testing and toward alternative markers that allow for the assessment of short- and intermediate-term changes in glycemia. To enhance the quality of clinical treatment across a diverse diabetic population, additional glycemic indicators that give accurate information regarding glycemic control in addition to and beyond HbA1c are needed [12,13].

MATERIALS AND METHODS

HBA1C

HbA1c is formed by non enzymatic glycation as an adduct of glucose and the hemoglobin molecule. The National Glycohemoglobin Standardization Program is the organization that evaluates, sets standards for accuracy, and certifies methods for measurement of HbA1c[14]. The measurement of glycated hemoglobin (HbA1c) in blood offers information on an individual's average blood glucose levels during the previous two to three months, which is the red blood cell half-life (RBCs)[15]. HbA1c is a measurement of average plasma glucose over the preceding eight to twelve weeks. It can be done at any time of day and does not necessitate any kind of specific preparation, such as fasting. Because of these characteristics, it is the primary test for determining glycemic control in diabetics. There has been a lot of interest in using it as a diabetes diagnostic test and as a diabetes screening test for people who are at high risk of developing diabetes[16].

When the conditions are physiologically favorable, proteins are regularly glycatedduring numerous enzymatic processes. In the case of hemoglobin, however, glycation happens via a non enzymatic interaction between glucose and the N-terminal end of the β-chain, resulting in the formation of a Schiff base [17,18]. The Schiff base is transformed into Amadori products during the rearrangement, the most well-known of which is HbA1c.

Hemoglobin and blood glucose interact in a reversible mechanism to create aldimine in the first phase of glycated hemoglobin synthesis. Aldimine is eventually transformed into the stable ketamine form in the irreversible secondary phase [19]. The most common locations of hemoglobin glycosylation are β-Val-1, β-Lys-66, and α-Lys-61, in that order. HbA (α2P2), HbA2 (α282), and HbF (α2y2), in proportions of 97 percent, 2.5 percent, and 0.5 percent, respectively, make up the majority of normal adult hemoglobin. HbA1 makes up around 6% of total HbA and is divided into HbA1a1, HbA1a2, HbA1b, and HbA1c fractions based on electrophoretic and chromatographic characteristics. HbA1c is the most prevalent of these fractions, accounting for around 5% of the total HbA fraction in health [20]. Depending on the glucose levels in the blood, the first stage of this process produces labile aldimine (a Schiff base), which can dissociate or undergo an Amadori rearrangement to become stable ketamine [18].

HbA1c was first identified as a small chromatographic fraction of adult hemoglobin in 1958 and termed after the chromatographic column elution sequence [21]. but its significance in diabetes was discovered in 1969 by Rahbar [22], who found considerably higher HbA1c readings in diabetic individuals. The law of mass action applies to glycation because it is a non enzymatic process. HbA1c shows the average glycemia level for one red blood cell life cycle (2-3 mo) [23], assuming normal erythropoiesis and steady hemoglobin content. Given the high biological variability, glucose dynamics, and the limitations of blood glucose monitoring technology at the time, the possibility of obtaining an integrated average glycemia value by measuring a single biomarker piqued everyone's interest and proved to be a powerful tool in diabetes research and clinical management. The introduction of a novel analytical technology suited for use in clinical laboratories aided the development of HbA1c testing [24]. HbA1c testing inherently suffers from a significant between-method variability due to differences in the use of different principles and a lack of integration, which has negatively affected its clinical accuracy in longitudinal monitoring of average glycemia with different methods and comparing the results of the DCCT- and UKPDS-derived targets The non enzymatic reaction involves not only the β-N-terminal valine but also other available amino groups within the α and β-globin chains, and the findings varied depending upon the analyte collected by the particular technique[25]. The heterogeneity of molecular entities that were measured by different methods significantly contributed to analytical variability.

The American Diabetes Association (ADA) and the American Association of Clinical Chemistry launched the National Glycohemoglobin Standardization Program (NGSP) to achieve clinical harmonization (AACC). The NGSP's purpose was to reconcile HbA1c findings acquired by various methodologies with the highly repeatable but insufficiently specific approach (ion-exchange chromatography) employed in the DCCT and UKPDS trials, allowing for data to be traced and compared to evidence-based clinical criteria [26]. The International Federation of Clinical Chemistry (IFCC) launched an HbA1c Standardization Program almost simultaneously with the NGSP, intending to develop a comprehensive reference system that included both reference methods and a primary reference standard for a structurally defined analyte [27, 28,29]. The comparison of the two reference systems demonstrated a high linear correlation between the DCCT and IFCC reference systems, however, the later, more specialized approach yielded much lower HbA1c readings. This data prompted concerns over the potential of deterioration of glycemic control with the implementation of the new reference system, which had been documented previously[30].

An international group including the ADA, European Association for the Study of Diabetes (EASD), International Diabetes Federation (IDF), IFCC, and International Society for Pediatric Diabetes (ISPAD) published a Global Consensus on HbA1c testing and reporting in 2010 [31]. In a nutshell, the Global Consensus established the IFCC reference as the sole legitimate anchor for commercial technique calibration, as well as reporting of HbA1c readings in percent (NGSP/DCCT-related units). For the inter conversion of the findings, a master equation explaining the relationship between the two reference systems should be used: HbA1c NGSP/DCCT (%) = 0.09148 × HbA1c IFCC (mmol/mol) + 2.152 Today, the analytical methodologies for measuring HbA1c have been standardized, and the differences across methods/laboratories have been continuously lowered toward a desirable objective of a coefficient of variation (CV) of less than 3.5 percent[25]. Except for point-of-care systems for HbA1c testing, which still require improvement[32], current recommendations demand a CV of less than 2% for NGSP-HbA1c equivalents[31]. This can be achieved with practically all commercially available laboratory procedures. However, despite worldwide

standardization and ongoing attempts to enhance analytical quality [33], the limits of HbA1c measurement owing to hemoglobin-related interferences cannot be eliminated. Hemoglobin variations have long been known to interfere with HbA1c synthesis and measurement, and the degree of this interference varies depending on the congenital condition affecting hemoglobin synthesis and the analytical method employed to determine HbA1c [32]. HbS, HbC, HbE, and HbF are among the most common hemoglobin-related interferences [34]. Thalassemia characteristics, HbC, HbE, and HbF are among the most common hemoglobin-related interferences. Other hemoglobin posttranslational changes, such as carbamylation by uremic toxins in end-stage renal illness, may also cause considerable interference with certain HbA1c assays [35]. It should be mentioned that developments in analytical procedures have minimized the preponderance of interferences, and the remaining interferences have been presented and thoroughly analyzed. On the NGSP website, there is a complete list of HbA1c techniques that have been described for their sensitivity to hemoglobin-related interferences[36]. Biological factors impacting the accuracy of HbA1c as a glycemic marker has surfaced as a major concern following analytical harmonization, although intra individual heterogeneity in HbA1c levels has long been acknowledged. Studies on the association between HbA1c tests and average glycemia levels found a significant linear correlation with considerable inter individual variability, for example, an HbA1c of 7% (53 mmol/mol) might correspond to an average glucose concentration of 6.8 to 10.3 mmol/L [37]. Age and ethnicity, as well as genetics, appear to be key drivers of this heterogeneity. Multiple chromosomal loci have been linked to HbA1c levels in recent genetic research, which might give a credible explanation for the physiological aspects that influence its variability and therapeutic use toward a more individualized approach[38].

There were 19 variations connected with glycemic pathways among the 60 genetic variants reported to impact HbA1c, and 22 erythrocytic variants among the remainder of the variants implicated in non glycemic pathways[37]. When compared to other ethnic groups, a variation on the X chromosome coding for glucose-6-phosphate dehydrogenase (G6PD) was linked to much greater HbA1c variability among African-Americans. This common mutation is linked to a shorter erythrocyte lifetime and, as a result, erroneously lower HbA1c values, which might have major consequences for diabetic patients' care[37]. A notable downside of HbA1c is its inability to anticipate hypoglycemia and identify short-term glycemic changes. Furthermore, we previously established that in well-controlled type 2 diabetes patients, HbA1c is mostly determined by chronic persistent hyperglycemia, with glycemic fluctuations going undetected [39]. Nonetheless, this is necessary for safe and timely insulin administration as well as clinical decision-making. The benefits of HbA1c over plasma glucose were low intra individual biological variability, stability of the analyte, and independence of findings from the prandial state, whereas the downsides were greater costs and restricted availability of the test[40]. However, several studies, as well as recent worldwide surveillance on the incidence and diagnosis of diabetes [41], have indicated that the diagnostic accuracy of HbA1c at a specific threshold is low, which is at least in part due to various biological confounders[42.43]. Image 2 shows a full list of biological, (patho) physiological, and pharmacological elements that may affect HbA1c production, measurement, and/or interpretation.

Fructosamine

All glycated plasma proteins are classified as fructosamine (1-amino-1-deoxy fructose). Glycation is a process that results in the permanent nonenzymatic binding of glucose to plasma proteins. Glycation is a nonenzymatic process in which a labile Schiff base (aldimine) is produced early in the process and then reorganized into a stable Amadori product (ketamine) due to the covalent binding of glucose to the amino-group residues of lysine, arginine, and cysteine in protein molecules [45]. In recent years, fructosamine and serum glycated proteins with shorter half-lives than hemoglobin (14-21 and 17-20 days, respectively) have been studied as glycemia indicators. Fructosamine is created through a nonenzymatic process that attaches the molecule to albumin. The fructosamine assay is a colorimetric technique that may be used to assess glycation of blood proteins, primarily albumin [45], and is quick, cheap, and specific. However, there is minimal standardization of this test. Several investigations have found strong associations between fructosamine, HbA1c, and glycated albumin[46]. Although the glycation rates of both proteins are equivalent, it is a more swiftly responsive indication than hemoglobin. Affinity chromatography and enzymatic tests are the most frequent techniques for quantifying GA; nevertheless, they have not been routinely standardized.

GA appears to be a superior measure of glycemic control than HbA1c, according to two cross-sectional investigations involving diabetic patients on hemodialysis, one Japanese and one American [47,48]. The consistently lower percent GA/HbA1c ratios in diabetic individuals without nephropathy compared to those on dialysis suggest that HbA1c understates glycemic management in these situations. Reduced red blood cell survival and transfusions are expected to contribute to decreased HbA1c levels in diabetes individuals on hemodialysis. GA has been demonstrated to be effective in detecting short-term changes in glycemia in neonates and pregnant women with diabetes [49,50]. Because glycated albumin is an independent variable of maximum glucose levels, it appears to be a more sensitive marker for glycemic excursions than HbA1c because they occur during postprandial times [51]. This is significant because postprandial glucose excursions are known to increase the risk of diabetic micro-and macrovascular complications. Recently, it was discovered that serum GA levels are higher in diabetes patients with reduced basal pancreatic β-cell function with HbA1c[52]. If serum concentrations were found to be increased in a state of postprandial hyperglycemia, indicating postprandial β-cell dysfunction, GA could be a useful surrogate marker for cardiovascular risk[53]. Clinical trials have yet to confirm this, though the finding of elevated GA but not HbA1c levels in patients with coronary artery stenosis suggests a link[54].

It's worth noting that measurements of fructosamine and GA aren't always accurate in physiological and pathological situations. Every clinical disease that affects protein and albumin metabolism (nephrotic syndrome, hyperthyroidism, glucocorticoid medication, liver cirrhosis, and so on) may have an impact on these data, which would necessitate cautious interpretation[55,56].

1,5-Anhydroglucitol

Another analyte, 1,5-anhydroglucitol (1,5-AG), has also been proposed as a glycemia intermediate marker to supplement HbA1c measurements [57]. It's an inert polyol that's found in nature and is a six-carbon chain monosaccharide with a structure comparable to glucose. GlycoMarkTM is a commercially accessible automated test. Because 1, 5-AG competes with glucose for tubular reabsorption, it cannot be used as a glycemic control marker in patients with kidney disease. It's also worth noting that glucose levels above the renal threshold for glycosuria, i.e. 10 mmol/L (180 mg/dL), cause a rapid drop in 1,5-AG serum concentration [58]. Low levels of 1, 5-AG are related to poor glycemic control, as demonstrated by high HbA1c readings (> 9.0 percent, > 75 mmol/mol). Although this measure responds sensitively and quickly to daily glucose excursions in individuals with HbA1c levels that are close to or at goal[59], it cannot detect hypoglycemia. Dungan *et al.*[60] found that despite having equal HbA1c, diabetes patients' 1,5-AG differed significantly, which they attributed to differing postprandial glucose excursions. As a marker for diagnosing postprandial hyperglycemia, 1,5-AG outperforms HbA1c and GA (serum fructosamine) tests. As a result, 1,5-AG has been utilized to test pharmacological strategies that affect postprandial glycemia.

Given the limitations of HbA1c and the growing body of information on the therapeutic value of unconventional glycemic indicators, their use in clinical practice seems likely. The most useful tool in enabling the translation of these indicators into ordinary clinical practice is the recently released reference intervals. The reference ranges for fructosamine, GA, and 1,5-AG in a healthy reference population of over 1800 people were 194.8-258.0 mol/L, 10.7%-15.1 percent, and 8.4-28.7 g/mL, respectively[61].

Direct Measures Of Glycemia

Fasting and postprandial plasma glucose (FPG and PPG, respectively) are apparent glycemic measurements that provide "snapshot" glucose levels for main use in setting treatment objectives, which are presently established at 4.4-7.2 mmol/L for FPG and 10.0 mmol/L for PPG[1]. The contributions of these parameters to HbA1c have been assessed [62]. and a substantial relationship between PPG and cardiovascular risk has been established [63]. Patients who use SMBG as part of their normal diabetic care have access to daily plasma glucose readings, however, analyzing and understanding the cumulative SMBG findings might be difficult for healthcare professionals[64]. Advances in both analytical accuracy and software supporting SMBG, as well as the development of continuous glucose monitoring sensors and, most recently, flash-glucose sensing technology, have prompted the development and validation of

new, metrics-derived surrogate markers of glycemia, which have improved our understanding of the complex glucose dynamics and provided new tools for patients and healthcare providers in achieving optimal diabetes control and reducing the occurrence of complications[65,55]. The glycemic risk assessment diabetes equation (GRADE) and average daily risk range (ADDR) were found to best correspond with the degrees of risk of hypo- and hyperglycemia associated with the glucose profile[66], and they had positive correlations with HbA1c and negative correlations with c-peptide levels[67]. Unlike SMBG-derived profiles, which are based on a limited number of static plasma glucose measurements throughout the day, CGMS allows for a continuous view of daily glycemia, allowing for a more personalized approach and a powerful tool for patients in achieving their glycemic targets and avoiding glycemic excursions. Glycemic variability, average glucose exposure, duration in range, hypo- and hyperglycemia, and glycemic variability are all now possible thanks to CGMS (glucose excursions). Glycemic variability was regarded to be a substantial risk factor for problems that weren't represented in HbA1c levels[65]. The benefits of using SMBG to improve patient outcomes have been well documented in studies involving a variety of vulnerable populations of diabetic patients, including children[68], pregnant women[69], the elderly[70], and patients with diabetic kidney disease[71] or hypoglycemic episodes[72]. However, high prices, insurance-related constraints, and attitudes about CGMS among patients and healthcare providers continue to limit its use. The recently published International Consensus on Use of Continuous Glucose Monitoring is a positive step forward in that it aims to provide technical and clinical recommendations on the use of CGMS in conjunction with HbA1c, as well as a comprehensive overview of the current evidence supporting CGMS-derived metrics to improve patient care and clinical outcomes [73].

CONCLUSION

To reduce the risk of diabetic complications, hyperglycemia, a fundamental biochemical hallmark of diabetes, should be strictly managed and kept in a range as close to normal as feasible. Glycemic variability, as well as the amount and exposure to hyperglycemia, all have a role in the etiology of diabetic complications, with differing patterns of disease pathogenesis in individuals with type 1 and type 2 diabetes. HbA1c remains the most important biomarker for long-term glycemic management, despite its analytical and biological limitations. Other glycated proteins, 1,5-AG, and integrated measurements from direct glucose testing by SMBG/CGMS have emerged as significant data complements to HbA1c in recent years, particularly in situations where HbA1c values may be incorrect or inadequate to estimate the risk of bad consequences. Long-term relationships between these alternative glycemia biomarkers and the risk of diabetic complications must be investigated to provide clinically meaningful cut-off values and demonstrate their applicability in a variety of diabetic patient groups. To attain optimal glycemic control, it's important to understand that diabetic complication are caused by more than just high blood glucose levels. The capacity to estimate the risk of complications and tailor treatment of diabetes will improve with the development of novel markers as reliable surrogates for clinical outcomes rather than just glucose control.

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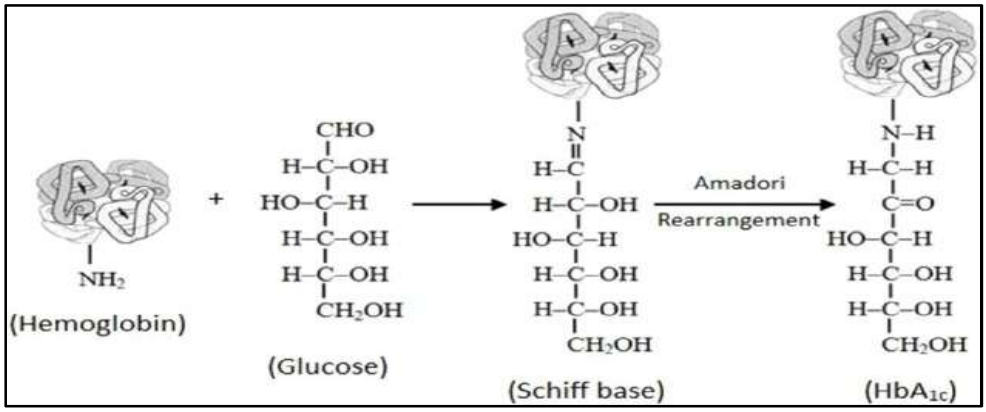

Image 1:The binding of glucose to hemoglobin results in the formation of glycated hemoglobin (HbA1c).

Biological, (patho)physiological and pharmacological factors influencing hemoglobin A1c

Factor influencing HbA1c synthesis/measurement/interpretation
Age, ethnicity
Genetic factors (e.g. Glucose-6-phosphate dehydrogenase variants)
Pregnancy
Red blood cell lifespan
Haemolytic anaemia
Iron deficiency anaemia
Haemoglobin variants
Accute haemorrhage
Splenomegaly
Splenectomy
Transfusion
Chronic liver disease
End-stage renal disease
Rheumatoid arthritis
Vitamin C
Drugs (aspirin, erytropoietin, dapsone, antiretroviral agents)
Endogenous interferents (high levels of bilirubin/triglycerides)

Image 2

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Perception of GST Amongst the MSMEs in Assam – A Study Using Logistic Regression

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Received: 08 Feb 2022 Revised: 03 Mar 2022 Accepted: 28 Mar 2022

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ABSTRACT

While the GST implementation is a well thought policy implementation and considered a stepping stone towards the tax reforms in the country, considering the fact that it streamlines all the specific types of oblique taxes by bringing them under a "single taxation" system, little is known about how the implementation of GST has affected the enterprises in small and medium sector .Micro, Small and medium enterprises are the vehicles for sustainable and inclusive growth of any economy. It is a founding pillar for the socioeconomic framework. While many referred to GST as the next 'Great Step towards Transformation' or a 'Great Step towards Transparency' the influence of GST among the states is not uniform throughout and not much is known on how the GST implementation has affected the north eastern part of India. To get an insight on this issue a few factors have been identified which can explain the perceptions that MSMEs held regarding GST – whether they consider it beneficial or not. The study is concentrated in Kamrup district of Assam which is one of the foremost districts of the North Eastern Region and housing the city of Guwahati, the commercial hub and largest amongst the cities of the region. A sample of 100 registered MSME units operating in Kamrup district has been considered for study purpose. The statistical tool used for analysis here is Logistic regression. The results of the study do not point towards a specific trend –rather MSMEs have a mixed perception on the utility of GST. The piece of work is novel in its way as a very few studies has been conducted along this perspective.

Keywords: GST implementation, challenges, MSME, economy

INTRODUCTION

The Micro, Small & Medium Enterprises (MSMEs) which are the engine of economic growth are responsible for promoting development all over the world. They play a pivotal role in the economy as they are the contributors in

industrial outputs, exports and last but not the least employment generation that too at low capital cost. The MSMEs contribution in the industrial economy of our country is highly remarkable. The recent years has shown that MSME sector has time and again registered higher growth rate compared to the overall industrial sector. But due to the increasing Chinese imports the Indian MSMEs are facing a dynamic and complex business scenario; they are under immense pressure to respond on cost efficiencies, quality parameters, and technology up gradation. Pareek (1978) in his work delved on the role of financial institutions and state agencies who played a fruitful role in extending credit to small-scale units . He further pinpointed how these institutes were indifferent towards the need of the tiny units. He further suggested that these financial institutions should tune their policies as per the needs of small-scale sector in general. Roy and Zegveld (1983) has shown that SMEs were technologically innovative. This innovation was significant and played the role of a harbinger in economic growth and employment creation. Sandesara (1988) stressed on the concept of institutional framework which forms the basis for the functioning of the small and medium industries in India. Khanka (1998) also laid proof for inadequate MSME development due to infrastructural facilities, problems of finance, marketing and insurgency. Therefore on a priority basis ; for industrial development, it is imperative for the Government to create infrastructural facilities like transport, communication, power, energy etc. which could initiate the process of economic development in the State. Later Koshy and Joseph (2000) in their study showed the growth in the no. of women entrepreneurs as a result of the various support measures extended by governmental and non- governmental agencies and the changing attitude towards women entrepreneurship. Bagchi (2000) also reported in his paper the contribution of small-scale sector towards industrial output, employment and foreign exchange earnings but he also observed that they suffers from severe technological obsolescence and lack of economies of scale. Although considerable efforts for the promotion of technology were taken up by the industries development organisation under the ministry of SSI and ARI but in a vast country like India these facilities are inadequate. All these papers discussed on MSME had not given importance on the tax issues that affect the overall growth , however Palil (2010) in his study had studied the issue of GST and he revealed that low public awareness towards GST can happen in the early years due to lack of familiarity with the new system. Again in 2012 and 2014 Lahiri(2012) and Garg (2014) explored the opportunities and constraints faced by the MSMEs during the pre and post liberalisation period . Also Bidin and Marimuthu (2014) in their paper discussed the perception and probable area of difficulties that companies face towards the implementation of the GST in Malaysia. Also Yesegat and Fjeldstad (2016) argued on the taxpayer's satisfaction or dissatisfaction with the government's provision of goods and services is expected to have an impact on tax compliance. A study of four African countries also shows a significant association of satisfaction with the tax system and positive tax compliance attitudes. Nayyarand Singh (2018) considered the importance of Goods and Services Tax (GST), implemented on July 1, 2017, as a major tax reform in India since independence in 1947. Though GST implementation was scheduled on April 2010, but was postponed due to political issues and conflicting interest of stakeholders. According to Nayyar and Singh (2018) the GST was introduced to subsume all sorts of indirect taxes and was expected to bring more transparency in taxation system. The objective was to increase GDP rate from 1% to 2% and also reduce tax theft and corruption in the country. The basic theme observed in the above literature is depicted in the word cloud, Fig 1 using Nvivo software which brings forward the most prominent phrases and words which found a place in these studies. None of these studies have dealt in detail regarding the tax implications on MSMEs .This gap is identified through literature and a detailed analysis on this aspect is taken in this paper.

Present Work

The NE Region (NER) of India which comprises of eight states-Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Tripura, Manipur and Sikkim States is blessed with a plenty of natural resources like forest and oil, tea, water etc . However this region could not achieve the position which it deserved due to some main stream constraints like infrastructure deficiency, insurgency, imperfection in factor and product market and indifferent governance(Sarma 2006). Also some other contributing factors which are responsible for industrial backwardness of NE include low per capita income, inadequate infrastructural facilities, low capital formation, communication bottleneck ,inadequate exploitation of the natural and human resources, high unemployment and last but not the least is the geographical isolation from the mainstream . Against this backdrop many researchers argued upon the

potential of MSMEs for the speedy development of NE region through its meaningful involvement in India's Look East Policy and Globalisation process(Maisnam 2015). With the government's emphasis on the Act East Policy which is a diplomatic initiative to promote economic, strategic and cultural ties with the vast Asia Pacific region , there could be considerable expansion of markets and it is expected that MSMEs could grab this opportunity and contribute towards the economic development of the state. Adeep insight into the status of MSMEs in NE gives the picture of a slow but steady growth of the MSME sector but there has been a high incidence of sickness. Therefore, it is important to highlight the factors that have held back this engine of growth. Literature suggests the problems of the region include problems related to availability of credit, high cost of operation, erratic power supply, lack of infrastructure, inadequate market linkage etc. (Biswas 2014). Amidst such challenges the dawn of GST in this sector is scripting a different story altogether. Whether the new tax system brought in disruption or brought a new life into these enterprises is the problem this paper delves into. Some fresh angles of investigation will be persued. The objectives of the study pertains to understanding the utility of the recent roll-out of the GST implementation in MSME sector in Kamrup which is one of the thirty districts in Assam. It aims to examine the awareness and perception regarding the benefits/no benefits that GST has brought on the MSME units.

The Settings Of The Study

The study is set in the largest urban city of the north eastern part of the county . Here the registered MSMEs of Kamrup District are considered. As of 2014-15, there are altogether 377(registered MSMEs in Kamrup District (Kamrup and Kamrup Metro). The selection of the sample of MSMEs is based on random sampling technique. Out of a total of 377registered MSME units, 100 units are selected randomly by using lottery method. As reported in the table 2 and 3out of the different MSMEs 68 belong to service sectors and 32 are manufacturing units .It also reflect the source of fund available to these sectors ,the amount of investment incurred by these MSMEs. A chi-square test of contingency table was used to test for the significance of differences among the various categories. To determine whether GST was beneficial, binary logistic regressions was carried out. For this analysis, the dependent variable is assumed to take binary values, 1 for beneficial and 0 for not beneficial. We used SPSS, version 11.0, for analysis. Similar works on logistic regression were carried out by Choudhury (2019) and Bharati et.al (2009)

ANALYSIS AND DISCUSSION

The variable of interest is binary denoting whether the implementation of GST is beneficial or not. Data for the study was collected through personal interviews using a structured questionnaire from a sample of 100 MSMEs.The survey was conducted mainly in the city of Guwahati covering a wide spectrum of sectors like, food retailers, jewellers, metal works, textiles, and book shops.The objective is to analyze the odds of an MSME perceiving the implementation of GST as being not beneficial. Consequently logistic regression technique is adopted for analysis taking into consideration the following explanatory variables.These are derived from literature and explained in brief in the subsequent paragraph.

(i) Category (msme_classification) of the MSMEs
(ii) Type (msme_sector) of the MSMEs
(iii) Annual investment (msme_investment) of the MSMEs
(iv) Source of fund (msme _fund) of the MSMEs

Finance is an important component for success of an MSME. But getting timely access to finance (Biswas, 2014) is the main constraint for an MSMEs. In spite of its significant contribution to Indian economy, this sector faces hindrance in terms of finance. Access to timely and adequate credit at a reasonable cost is essential for growth of MSME(Mund,2020). The Bankstoo have a negative perspective towards MSMEs as they are high risk borrowers due to insufficient assets and low capital. Not only there is a fear of sicknesss of MSME units , they are is highly vulnerable to market and economic fluctuations. The RBI provisional data of September, 2016, suggests that there are 480280 number of sick Micro & Small enterprises with Rs. 326.74 billion outstanding credit. As the MSME units donot reveal complete and transparent information therefore it becomes difficult for them to avail formal credit. This also

compels the banks to impose credit rationing on the loans being sanctioned to the MSMEs.(Biswas et.al). The enactment of Micro, Small & Medium Enterprises Development (MSMED) Act, in 2006 by the Government of India (GOI) gave a legal basis and framework to the micro, small and medium enterprises by defining and classifying these enterpriserson a uniform basis. This classification covers industries both under manufacturing and service sector and the above limits are excluding the cost of land, building and other specified items. In this study we considered small and medium enterprises under one category to understand the influence of this variable on the beneficial aspect of GST. In addition the terms of investment below 10 lakh is considered one category and above 10 lakh another category. Also loan from self and other sources is one category and loan obtained from Government sources is clubbed under a separate category. This is shown in Table 3.

Now to understand the association of various variables Chi-square test is conducted which confirms of the association of the various factors. Table 4 shows the cross tabulation between the type of enterprise and MSME sector. The Fisher's test of association is not significant (p value=0.387>0.05) which indicates that the two variables are not related. Similar interpretation holds for Table 5 and 6as well which shows the presence of a significant relation between the type of MSME and MSME sector and the loan they obtained from different agencies. Same applies in case of MSME sector and the source of loan . This is depicted in Table 7.Again Table 8again shows the presence of relationship between MSME sector and investment is the amount invested is related to the type of MSME. Table 9 also shows a significant relation between source of loan and investment .

To satisfy the objective of whether the MSME units consider the GST as beneficial or not we apply binary logistic regression in the data obtained using different groups of explanatory variables and response variable. When the dependent variable is binary and there are several independent variables that are metric, we use the logit models for estimation. The binary logit model commonly deals with the issue of how likely an observation are to belong to each group. It estimates the probability of an observation belonging to a particular group. In the given model two logistic regression is run taking type of sector and investment as control variables in the first case and in the other case regression is run controlling for type and sector of the MSME. Since the binary variable has a value of 1 for GST being beneficial a positive value of the coefficient signifies a higher probability of being beneficial as compared with the reference group. The results of coefficient estimates and the change in odds with 95% confidence intervals (CI) corresponding to unit increase in the dependent variable is given in the Table 10.

In model 1 of Table 10, micro enterprises are observed to have significantly higher odds of being beneficial as compared to the small and medium enterprises. We calculate an odds ratio as 3.385 meaning that the chance of a micro enterprise considering GST beneficial is 3.385 times the chance of the reference group not considering it beneficial. Manufacturing sector firms are about 1.2 times more likely to report that GST is beneficial to them compared to service sector firms. Similar results are observed after controlling for sectors and type of MSME in model 2. The odds ratio of 3.341 shows that the chance of an enterprise which consider GST beneficial are the ones whose investment is below 10 lakh. The overall correct prediction of the models is found to be 65%.This prediction is also shown separately in Table 11.

CONCLUSION

The analysis depicted above clearly shows the perspective of different enterprises. It reveals that in general the smaller firm (the micro sector as well as the firms whose investment is below 10 lakh) is the one which seem to have reaped the benefits of GST more than the small and medium enterprises. This is justified by the fact that the multiple VAT legislation which was in place since a long time created confusion and complexities for small traders. Through this goods and services taxation system, the micro enterprises have been able to tap the benefits of increased output, employment opportunities, economic development etc which it intended to enhance . It is further observed that all the 100 MSMEs are engaged in two types of activities – manufacturing and service and all of them are in a state of progress. Though, in the initial phase of its implementation it is likely to increase the administrative difficulties and

the compliance cost, MSME's would be able to reap the benefits of GST, if they are able to revamp their business processes and systems in line with compliances as required under this law, as it would not be operationally feasible for an MSME's to operate out of credit chain. The shift from the traditional tax regime to GST is considered as a 'behavioural change' more than a tax change because its successful implementation depends to a great extent on how quickly businesses adapt to the digital format of taxation. Although the GST implementation aims to upsurge the taxpayer base, largely SMEs into its opportunity, it presents a problem of compliance and related charges for them. Nevertheless, GST will make the MSMEs more competitive in the long run and will make the playing arena level between big enterprises and them. Additionally, the Indian MSMEs would be able to compete with the international market goods and competition coming from cheap price epicentres such as China, Philippines, and Bangladesh and actually thrive in the world market scenario. Logistic regression being a type of multivariable analyses is used in such domain because it can model the relationship between dichotomous dependent variable and one or more independent variables.

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Table 1: Table showing the number of MSME units in Kamrup and Kamrup Metro

District (year 2014-15)	No of units	Investment in plant and machinery	Total no of people employed
Kamrup and Kamrup Metro	377	8925.14	2921

Source: Compiled from Economic Survey Assam and Statistical Handbook Assam for various years

Table 2: Investment limits in Micro, Small and Medium Enterprises

Category of MSME	**Manufacturing Sector** (Investment in Plant & Machinery)	**Service Sector** (Investment in Equipment)
MICRO	Doesn't exceed 25 lakh rupees.	Doesn't exceed 10 lakh rupees.
SMALL	More than 25 lakh rupees but doesn't exceed 5 crore rupees.	More than 10 lakh rupees but doesn't exceed 2 crore rupees.
MEDIUM	More than 5 crore rupees but doesn't exceed 10 crore rupees.	More than 2 crore rupees but doesn't exceed 5 crore rupees

Source: Annual report of Ministry of Small Scale Industries(2006-07)

Table 3: Table showing the distribution of the enterprises

Type of Industry	Frequency	Percent
Micro	59	59.0
Small and medium	41	41.0
Sector		
Service	67	67.0
Manufacturing	33	33.0
Investment		
Below 10 lakh	59	59.0
Above 10lakh	41	41.0
Source of Loan		
Self and other sources	43	43.0
From Govt.	57	57.0

Table 4: Cross tabulation between the sector and type of enterprise MSME

		Sector		Total
		Service	manufacturing	
Type	Micro	42	17	59
	Small and Medium	25	16	41
Total		67	33	100

Table 5: Cross tabulation between type of MSME and the source of loan

		Source of loan		Total
		Self and other sources	From Govt	
Type	Micro	35	24	59
	Small and Medium	8	33	41
Total		43	57	100

Table 6: Cross tabulation between investment and type of loan

		Investment		Total
		Below 10 Lakhs	Above 10 lakhs	
Type	Micro	58	1	59
	Small and Medium	1	40	41
Total		59	41	100

Table 7: Cross tabulation between sector and source of loan

		Source of loan		Total
		Self and other sources	From Govt	
Sector	Service	37	30	67
	manufacturing	6	27	33
Total		43	57	100

Table 8: Crosstabulation between investment and MSME sector

		Investment		Total
		Below 10 lakh	Above 10 lakh	
Sector	Service	41	26	67
	manufacturing	18	15	33
Total		59	41	100

Table 9: Cross tabulation between Source of loan and Investment

		Investment		Total
		Below 10 lakh	Above 10 lakh	
Source of loan	Self and other sources	34	9	43
	From Govt	25	32	57
Total		59	41	100

Table 10: Showing odds ratio of Model 1 and Model 2

Independent variables	Odds ratio of Logistic regression [Model1]	Odds ratio of Logistic regression [Model2]
Type (reference category is Small and Medium enterprises)	3.385*	-
Sector (reference category is service industries)	1.242	1.171
Investment (reference category is above 10 lakhs)	--	3.341*
Overall correct prediction	65%	

Significant at 5% level of significance

Table 11: Table showing the overall prediction of the model

Classification Table[a]

Observed			Predicted		
			GST (Beneficial/Not beneficial)		Percentage Correct
			GST is not beneficial	GST is beneficial	
Step 1	GST (Beneficial/Not beneficial)	GST is not beneficial	14	8	63.6
		GST is beneficial	27	51	65.4
	Overall Percentage				65.0

a. The cut value is .780

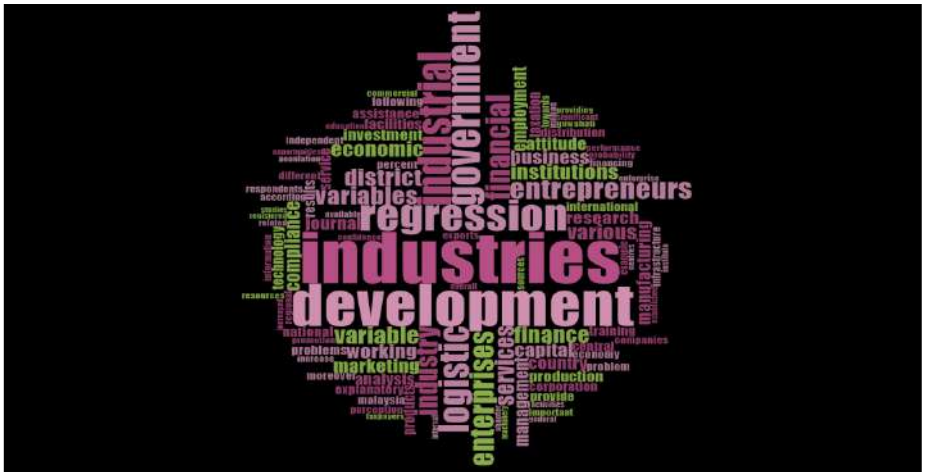

Fig 1: The word cloud

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Investigational Study on Pre-Filled Syringe Formulation Registration in the US and EU

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Received: 12 Jan 2022 Revised: 21 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

It is possible to create more effective and efficient medicines and tests through the use of a combination of therapeutic and diagnostic reagents, diagnostic tools, devices, and biological products. Because of the increasing convergence of pharmaceuticals and devices in the most recent generation of combination goods, regulatory agencies have introduced new competencies and laws over the last decade. Manufacturers must be aware of the specific requirements in each region in order to ensure timely and reliable market access for new combination products. The regulatory clearance for the production of combination products requires a very specific pattern of interactions between manufacturers and regulatory agencies. With the increasing complexity of commodities on the market in the last few decades, the demand for joint production of medications and devices employing capital from both sectors has highlighted, encouraging commercial partnerships and technology licencing.

Keywords: Combination product, regulation, Drug-device combination, Mode of action, Pre-filled syringe.

INTRODUCTION

Every year, the use of combination products to administer medicines (pharmaceutical formulations) is increasing in popularity. This can be seen in the rise in the number of requests for scientific assistance and marketing approval for combination goods. Patients and healthcare providers benefit from the latter. Combination products with automated functions that assist patients in administering their own medications are more prevalent [1]. A combination product is one that combines a medicine with a device; a biological product with a device; a pharmaceutical product with a biological product; or a medicinal product, a device, and a biological product [2]. Prefilled syringes are projected to become increasingly popular in the future decades. Prefilled syringes have many advantages for the pharmaceutical industry, including reducing waste, extending product life span, and increasing market share. It is widely accepted that prefilled syringes are a safe way to administer medications to patients, especially in the hands of healthcare providers. Prefilled syringes of healthcare therapy can now be used at home instead of the hospital due to the ease with which many injectable pharmaceuticals can be self-administered by the patient [3, 4] .

Prefilled syringes will face a new competitor in the pharmaceutical business in the near future. Prefilled syringes have gained widespread appeal because of their many advantages, including their ease of use, their ability to be used at home, and their ability to deliver precise doses. Pre-filled items are convenient and expedite the administrative process. [5] In this way, the patient does not have to be concerned about accidentally discarding a small bit of their medication while switching from a vial to a syringe [6]. A combination product is characterized under 21 CFR 3.2 (e) to include a combination product:

1. As a single entity combination product, it is defined as a product containing two or more controlled components (i.e. drugs, biologics, or devices) that are combined or mixed physically, chemically or otherwise and made as a single entity;
2. Packaging comprising two or more independent items sold as a single unit, with the majority of the products being either pharmaceutical and/or biopharmaceutical or biopharmaceutical and/or pharmaceutical products;
3. In accordance with its investigation plan or proposed labelling, a pharmaceutical, device, or biological product packaged separately intended for use only with an approved individually specified drug, device, or biological product, where both are required to achieve the intended use (e.g., In order to reflect a change in the intended usage, type of dosage, intensity, route of administration or substantial dose change,) [Sometimes referred to as "cross-labeled"]; or
4. Investigational novel medicine, device or biological product that is packaged separately and intended for use only with another independently decided research medicine, device, or bio-drug under his/her proposed labelling, where both are required by the intended use, indication, or effect. An further sort of "cross-labeled" combination product is described below [2].

Examples of combination products according to US:

1. Combination products for a particular organization (21CFR 3.2(e)(1))
   - Monoclonal antibody paired with a preventive treatment
   - Prefilled methods of drug delivery (syringes, metered dose inhaler)
2. Combination commodities co-packaged (21CFR 3.2(e)(2))
   - Drug or vaccine vial with a delivery unit packaged
   - First-aid kits containing instruments and medications (bandages, gauze) (antibiotic ointments, pain relievers)
3. A commodity which could be cross-label mix product (21 CFR 3.2(e)(3) or 21 CFR 3.2(e)(4))

As a medicinal product or medical device, Combination Products are regulated by the EU. For these products, the European Union does not have a standard definition. As stated by the EMA, this combination is best described as:

"The medical device may be supplied as an integral component of the medicinal product, or separately, as a non-integral combination with the medicinal product, or independently marketed devices meets the requirements for the necessary delivery system stated in the Summary of Product Characteristics (SmPC) For the medicinal product" [1].

Examples of combination products according to EU:

1. Combination products for Drug & Device
   - Pre-filled syringes
   - Pre-filled pens
   - Nebulizers
2. Combination products for Device & Drug
   - Drug-eluting stents
   - Bone cement containing an antibiotic
   - Catheters coated with heparin
3. Combination products for Device & Drug (cross labelled)
   - Transdermal patches
   - Bone void fillers with drugs

Classification of combination products in USA & EU:

The approach of a regulatory agency to a group of products and/or the related regulatory pathways as well as decision tree will be known from the way it defines, classifies and assigns scope. The classification of combination products by the regulatory agencies of USA & EU have been studied and summarized in the below paragraphs. We have found a fundamental difference in their approach. The US regulator as classified combination products based on the nature of drug delivery system and the origin of the drug. However, the EU approach to the classification is guided by the way by which a drug and a device are combined. The US regulator as provided a structured approach to help the applicants to go through the right regulatory pathway. This has been termed as 'Assignment Algorithm' and presented as Figure 2. The deciding factor is the 'Primary Mode of Action' (PMOA). The PMOA will guide the applicant to approach either Center for Drug Evaluation and Research (CDER) or Center for Devices and Radiological Health (CDRH) for the purpose of registration.

The EU regulator has provided a structured approach for identifying the appropriate regulatory pathway based on PMOA. The latter is applied to understand how a drug & a device are integrated with each other and which of the two should be credited with the major role for therapeutic action. It has become clear in assessing the current process that the significance and complexity of the issues may depend on the type of combination product involved. Combination products are generally categorized into four separate classes on the basis of their specific components:

1. Novel drug delivery systems
2. Traditional drug delivery systems
3. Drug-enhanced devices
4. Regenerative medicinal products

1. Novel drug delivery systems: It is hoped that these products will make it easier for patients to take their medication and increase the effectiveness of the treatment by allowing it to be administered locally rather than by mouth, subcutaneous injections or intramuscular injections. Since the major mode of action in these circumstances is drug-related, the CDER would be responsible for regulating these items.
2. Traditional drug delivery systems: To facilitate administration, these products combine or bundle of drugs with injectable devices. A pen-based delivery device, prefilled syringes, medication pumps, and auto injectors are all examples. These components may also be regulated individually under the existing regulatory systems for medicines and devices.

3. Drug-enhanced devices: In many cases, these things integrate already-existing technology and medications. In many of these circumstances, the drug-device interface is unique. As a result, producing these devices is more challenging than producing comparable device-only items. In these instances, the devices contribute significantly to the total therapeutic action through their support. Thus, device regulators (CDRH) will primarily supervise these items, with secondary monitoring provided by drug-related regulatory organisations (CDER).
4. Regenerative medicinal products: These are products that combine devices and biologically active ingredients to aid in the repair and regeneration of injured tissue. These are some of the most difficult-to-combine products available. The regulatory bodies will scrutinize these combination products on a case-by-case basis, given the primary mechanism of action for these combination medications varies depending to the specific combinations. [7]

Combination products are treated at par with medical devices in EU and referred to in the sense of the regulation of medical devices as:

- Device integrating a medicinal product
- Medicinal product integrating a device
- Devices intended to administer a medicinal product
- When presented as a single, integral unit, not reusable [8]

There are three types of medical devices incorporated into combination products

1. Procedural techniques for dispensing pharmaceuticals (e g., empty single-use syringes and reusable spoons or droppers). Medical Devices Regulations apply to these devices.
2. Devices that combine a medicinal product with a device to make a single, integral product, such as prefilled syringes. These are non-recyclable commodities, and in the EU, they are governed by Directive 65/65/EEC. Additionally, the Directive 93/42/EEC's significant criteria for medical devices apply to the protection and performance features of such devices. This requires that the mixture be tested by the drug regulatory authorities and that the product also adhere to the Directive's fundamental standards for medical devices. Generally, the usage of a CE mark satisfies this need.
3. Devices that incorporate a substance that, when used alone, may qualify as a medical product. Additionally, the substance (medication) may exert an effect on the body that is distinct from that of the device—for example, a heparin-coated catheter. In this situation, the medical device assessment authority (notified body) examines the combination product, and the notified body's findings are transmitted to the drug regulatory authority for review of that particular section. The regulatory authority for medications is responsible for ensuring the drug's safety, efficacy, and usefulness. [9]

Regulatory considerations of combination products

- How the commodity will be used and by whom
- Additive effects of other elements
- The whole product life cycle should be considered
- The most relevant therapeutic benefit of the product helps decide how to control
- Direction of action: This implies that the desired therapeutic effects or acts are accomplished by a product.
- Primary mode of action (PMOA): One of the primary therapeutic actions of a combination product is its ability to combine multiple therapeutic actions into one.

Regulatory objectives of Combination product's

- Efficient combination control, taking account of each contributing attribute
- Risk-based and fair checks
  1. Patient Security
  2. Encourage Creativity
- Clinically acceptable tests

- Stop unwanted or repeated feedback
- Transparency of determinations of jurisdiction, criteria, and pathways of analysis
- Timely access to these basic essentials for patients and clinicians [10]

Applicable regulations

Title 21 of the Code of Federal Regulations (CFR), subpart A

Part 3 - Substance Authority

Organizational elements

- Designated agency protocol

For pre-market product analysis and control, the PMOA of the product must be defined by a component of the Agency that has primary jurisdiction over the product.

The goal of the therapy is to help the patient

The categorization letter must identify the primary authority for a combination product.

Part 4 - Regulation of Combination Products

- Identify and discuss the current GMP and QMS requirements in the industry (part 820)
- Post-marketing security tracking for commodities that have been blended
- General specifications for each operating mode are listed below.

Sec. 4.4: (b) (1)CGMP compliance has been demonstrated for the new good manufacturing practise operating system, hence it is critical to demonstrate the following standards of the device quality system regulation:

(i) Section 820.20. Management responsibility.

(ii) Section 820.30. Design controls.

(iii) Section 820.50. Purchasing controls.

(iv) Section 820.100. Corrective and preventive action.

(v) Section 820.170. Installation.

(vi) Section 820.200. Servicing.

Sec. 4.4: (b)(2) The new operating system of good manufacturing practice has shown that the following provisions of the drug CGMPs must also be shown to comply with the device quality system regulation:

1. Section 211.84. Testing and approval or rejection of components, drug product containers, and closures.
2. Section 211.103. Calculation of yield.
3. Section 211.132. Tamper-evident packaging requirements for over-the-counter (OTC) human drug products.
4. Section 211.137. Expiration dating.
5. Section 211.165. Testing and release for distribution.
6. Section 211.166. Stability testing.
7. Section 211.167. Special testing requirements.
8. Section 211.170. Reserve samples. [11]

Syringes filled with a therapeutic substance are called prefilled syringes because they have been preloaded with a predetermined amount. There are many advantages to using this method over old drug delivery methods in which pharmaceutical drugs were stored in vials that were difficult to obtain. As opposed to prefilled syringes, these vials were more prone to contamination and dosage mistakes. It is becoming more common for healthcare facilities to use this type of parenteral medication delivery system format [12] . The approach required to obtain registration of a new prefilled syringe product with US & EU regulatory bodies differs from each other.

There have been prefilled syringes on the market for more than 20 years. Compared to the United States, the prefilled syringe business in Europe is older and more established. Aside from this recent growth in the market for prefilled syringes, the pharmaceutical sector is expanding and becoming more complex. With a value of $300 million, the European pre-filled syringe business grows by 8 to 10 percent per year. Pre-filled syringe makers have had to

adapt to rising demand, new regulations, and more advanced methods of medication delivery. Syringes have advanced greatly over the years. Syringes are now being filled with new products. Injectable medications and diluents are packaged in prefilled syringes. Vaccinations, blood stimulants, therapeutic proteins, erythropoiesis, interferon and rheumatoid arthritis are some of the types of medications supplied in pre-filled syringes. Injectables that need to be prefilled for security reasons are becoming more common. [13]

Factors responsible for growth of prefilled syringes

Convenience for healthcare professionals and end-users, ease of use at home and in emergency situations, reduction of drug errors, misidentification and better dose accuracy; increased sterility assurance; better use of controlled drugs such as narcotics; lower injection costs for less preparation, fever material; easy storage and disposal; and elimination of vials are some of the benefits of this technology. [14]

Various steps to involve prefilled syringes:

Step 1: Verify the label on the prefilled syringe, as there could be catastrophic consequences if the syringe is injected incorrectly.

Step 2: In order to avoid contamination of the needle tip, remove the needle cap and syringe cap without touching them.

Step 3: The needle has been inserted. Injecting a needle into the skin by hand can be the most difficult part of self-injection. Our survival has depended on avoiding damage, therefore avoiding self-inflicted wounds is in our nature. This is why it's expected. The needle is inserted into the syringe automatically in the latest generation of auto injectors to avoid this difficult procedure. Step 4: In order to dispose of the used syringe, the patient must first complete the injection procedure. [15]

Regulations for Pre-filled syringes (PFS) in USA:

- PFS is a blend of goods in the United States of America (USA) {21 CFR 3.2ee (i). Historically, pre-filled syringes for the medicinal substance found in them have usually been registered as a jar closure device.
- Pre-filled syringes are a "single brand commodity combining two or more controllable, marketed as a single company"
- 21 CFR part 4 elucidated intentions to authorize Prefilled syringes as combination products subject to current Good Manufacturing Practices (cGMPs).

“Syringe is a device used to deliver medical product. Accordingly, a pre-filled syringe is a combination product and subject to this rule” (preamble)

In the Secure Medical Devices Act of 1990, Congress previously acknowledged the need for specific guidelines on hybrid goods (SMDA).

As part of the implementation of the Medical Device Consumer Fee and Modernization Act of 2002 by the Food and Drug Administration, the Office of Combination Products (OCP) was set up in 2002. (FDA). The OCP is liable for:

- Assignment of a centre of primary expertise for mixed goods
- Ensuring prompt pre-market assessment of combination goods
- Ensuring enforcement of mix goods with post-market regulations
- Analysis and alteration of guidance documents and legislation relating to combination goods. [16]

On 22 January 2013, the FDA released the Final Rule of CGMPs for combination products, which was issued in compliance with 21CFR Part 4 of the Convention. The concept of a 'streamlined approach' to the application of good manufacturing practices or quality system regulation was part of the new guidance (QSR) [17]

A Marketing Authorization Process

Due to the primary mode of operation, mixed items such as pre-filled syringes are assigned to CDER or CBER as the lead core (PMOA). OCP is in charge of timely assignment of a new mix product that may be CDER, CBER or CDRH to lead the FDA review centre.

Three step processes to obtain marketing approval for pre-filled syringes:

i. Lead centre assignment:

The PMOA protocol determines the Lead Centre. The method of assigning the lead centre is shown in the figure. If necessary, give the FDA Combination Products Office a request for classification. [18]

Pathway availability and related considerations

Device –led combination products

In Section 3038 of the Cures Act, numerous aspects of combination drug regulation were addressed. For example, the De Novo classification, PMA, and 510(k) pathways for device-led combination products are all taken into consideration by the law. Discussions like this one try to shed light on the FDA's thoughts on whether or not a device-led combination product is eligible for a 510(k) or a PMA.

Application for Premarket Approval (PMA)

FDA approval of a premarket approval application is required before nearly all class III devices can be legally marketed. The FDA approves a PMA if it contains sufficient valid scientific data to establish that the device or device-led combination product is safe and effective for the intended application (s). Sponsors should ensure that their PMA applications for device-led combination goods include sufficient data to demonstrate the combination product's safety and effectiveness as a whole, including data on each constituent portion (s). The PMA contains sections devoted to technical data, non-clinical laboratory tests, and clinical investigations, among others. Before FDA approves or denies a PMA, the appropriate FDA advisory committee may conduct a public assessment of the proposal and make a recommendation to FDA on whether the submission should be approved.

De novo classification requests

Class III is automatically assigned to devices of a new type that have not previously been classified or reclassified by the FDA. Classification by De Novo might place device-led combination items in either class I or class II. A De Novo classification request can be made by a sponsor if it believes their product is acceptable for class I or class II classification. General controls are put in place if the product falls into the class II category. To be classed as class I or II, the product cannot be rejected and must remain in class III for approval by the PMA. 510(k) applications can use the product as evidence that it has been legally promoted. De Novo requests can be submitted without the need for a 510(k) (k). A predicate product that could reasonably be used as a basis for a substantive equivalency analysis may not be taken on by the FDA. Several products, such as infectious diseases and the difficulty of replication, raise unique concerns.

Premarket notification (510(k)) submission

The 510(k)-review standard (substantial equivalence of a new product to a predicate product) differs from the PMA and De Novo review standards. The 510(k)-review standard is comparative, whereas the PMA and De Novo review standards rely on an independent demonstration of safety and effectiveness. Nonetheless, the principles of safety and effectiveness underlie the substantial equivalence determination in every 510(k) review.

The standard for a determination of substantial equivalence in a 510(k) review is set out in section 513(i) of the FD&C Act. A product is substantially equivalent to a predicate product if it:

- has the same intended use as the predicate product; and
- has the same technological characteristics as the predicate product; or
- has the same primary objective as the predicate component;

- features that are unique to each technology; and
- The information submitted to the FDA, along with any applicable clinical or scientific data, indicates that the product is safe and effective:
  - Did not raise any additional safety or efficacy concerns in comparison to the predicate product; and
  - Showing it is safe and effective when compared to a reference product.

Combination products based on drugs.

Generally, an NDA or ANDA is the most appropriate route for marketing authorization of a drug-drug combination product.

New Drug Application (NDA)

An NDA is often the ideal approach for drug-led combination medicines that are not generic versions of already-approved drug-led combination products. Among other things, an NDA for a drug-led combination product must demonstrate the product's safety and efficacy for the circumstances specified, suggested, or indicated in the proposed labelling. There are two types of NDAs in section 505 of the FD&C Act. A 505(b)(1) application, commonly referred to as a "stand-alone" NDA, needs complete records of safety and effectiveness investigations conducted by or for the applicant, or for which the applicant has a right of reference or use. A 505(b)(2) application similarly includes complete records of safety and effectiveness inquiries, but at least some of the data required for approval originates from studies that were not conducted by or for the applicant and for which the applicant did not receive a right of reference or use. Both 505(b)(1) and 505(b)(2) applications are made pursuant to Section 505(b)(1) of the FD&C Act and are authorised pursuant to Section 505(c).

Abbreviated New Drug Application

An ANDA is typically the ideal path for a drug-led combination product that shares the same active ingredient(s), dose type, strength, route of administration, conditions of use, and (with certain permissible variations) labelling as a previously licenced product (i.e., a reference listed drug (RLD)). An ANDA applicant is not required to submit independent proof demonstrating the proposed product's safety and efficacy in order to obtain approval, as an NDA applicant is. By contrast, an ANDA is based on the FDA's prior judgement of the RLD's safety and reliability. Additionally to the foregoing, an ANDA must include sufficient details to demonstrate that the proposed product is bioequivalent to the RLD and to ensure the product's identity, strength, consistency, and purity. [19]

Regulations for pre-filled syringes in Europe

The publication of the new Medical Devices Regulation (MDR, Regulation (EU) 2017/745) introduces a major addition to the legislation on medicinal drugs, Article 117 of which provides for the concept of a single integral product in Article 1.9 for integral drug-device combinations: “If the device intended to administer a medicinal product and the medicinal product are placed on the market in such a way that they form a single integral product which is intended exclusively for use in the given combination and which is not reusable, that single integral product shall be governed by directive 2001/83/EC or regulation (EC) no 726/2004, as applicable” [20].

Medicines can be sold for use in conjunction with a medical device, typically to facilitate the delivery of the drug, as per new guidance documents published by the EMA on 28 February 2019. The medicinal product as a whole shall be regulated as a medicinal product pursuant to Directive 2001/83/EC or Regulation (EC) No 726/2004 if the principal intended action of the combination product is accomplished by the medicinal product. So, PFS is regulated by CHMP as a medicinal product.

Process for marketing authorization

The Pharmaceutical Goods for Human Use Directive (MPD) (Directive 2001/83/EC) is amended by Article 117 of Regulation (EU) 2017/745 on Medical Devices (MDR). This would have a huge effect on pharmaceutical producers that supply drug delivery systems in conjunction with their medicinal drugs (such as pre-filled syringes). It will also affect device manufacturers that supply pharmaceutical manufacturers with these drug delivery devices for use in

medicinal products (such as empty syringes). Notified bodies will be impacted as well. This alteration affects medical kits that, if supplied separately, contain a device that would be protected by the MDR. The rationale of the system part's compliance with the relevant general safety and efficiency specifications in Annex to the MDR shall be included in the dossier of the MPD business authorization. It could be either:

- An EU compliance declaration or certificate issued by a notified authority allowing a CE mark to be appended or attached to the equipment. An opinion on the conformity of the device with the general security and efficiency requirements of the MDR. The adequately appointed notified body shall include this if the device's enforcement assessment requires the involvement of a notified body while it is used separately. Under the existing regulatory landscape, guidance addressing pharmaceutical registration criteria such as ICH M4Q does not resolve the considerations of Module 3 adequately to ensure the registration of an integral drug-device combination product. It is understood that the location of information related to the system part, as well as precisely what level of details are submitted, is variable across businesses in the absence of certain key guidelines.

Position of the safety and performance quality information and data of the PFS system portion for eCTD Module 3:
3.2 P All quality data pertaining to the system part and the protection and performance of combination products
3.2 R Compliance with MDD Annex referring to relevant studies conducted to show compliance with compliance
3.2.P sections the cross-reference studies treated in the QMS may not be included in the dossier.
With regard to the strategy for Module 3 dossier content, it is the industry position that Module 3 is designed around a high-level package for the manufacture and control of the part of the medical device in Module 3 that focuses on:

- Production and controls
- Compatibility/interaction between the result of the drug and the instrument
- Integrity of Container Closure
- Dosing accuracy
- Functional quality
- The product's usability

If device parts may be used for a variety of different injectable items (e.g., PFS and pre-filled pens), if a Notified Body review is needed, a method similar to the US Device Master File may be suggested, e.g. where a Notified Body review is required, e.g.

- Holding a general technical file (with the optional support of the component suppliers).
- For individual combination items, a special section is created. [21]

Regulatory review process

There are 4 regulatory pathways to obtain product registration in EU namely:

1. Centralized procedure
2. De-centralized procedure
3. National procedure
4. Mutual Recognition procedure

We have focused on the centralized procedure. Because, registration through this regulatory pathway enables the applicant to launch the product all over the EU region

The review process for combinations products such as pre-filled syringes, follows a centralized EU protocol. [22]

- Next, the applicant submits the Marketing Authorization Application (MAA) to the CHMP for the combination of drug devices (e.g., PFS) and the device file to the NB (Notified Body) for evaluation.
- The assessment of the pharmaceutical substance and the delivery system takes place at the same time.
- CHMP requires approximately one day to validate the submission.
- NB offers an opinion on the approval or rejection of the delivery device within 80 days
- CHMP submits a medicinal product analysis with NB opinion to CA after 120 days (Competent Authority).

- A query response process is then carried out between the CHMP and the applicant.
- Eventually, after a period of 210 days, the CHMP gives an opinion/decision on the approval of an MAA in the European Union for a pre-filled syringe.

CONCLUSION

This article examined in detail the regulations and submission procedures governing pre-filled syringes in the United States and the European Union. Several significant facts emerged from this article, including the following: Prefilled syringes offer various benefits, including versatility, availability, accuracy, sterility, protection, marketing, production, and marketing. On the market, various types of prefilled syringes are available. Compliance with regulatory criteria and a product's safety profile that includes syringe components will prove to be critical in driving the prefilled syringe market's growth. The pre-filled syringe is gaining widespread adoption in the pharmaceutical sector as a result of the advantages it offers over traditional pharmaceutical containers such as vials and ampoules. On the other hand, regulators are encountering difficulties when it comes to submitting applications for licencing of pre-filled syringes, particularly in developing regions, where no single application is appropriate, as is the case in the United States and Europe. As a result, there is a lack of transparency in the Indian market's evaluation of pre-filled syringes.

ACKNOWLEDGEMENT

I sincerely thank my guide for his support throughout in completing the article.

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Table 1: Components of the Pre-filled syringe

Components	Material
Barrel	Glass/ Plastic
Piston	Elastomer
Tip cap	Elastomer
Plunger Rod	Plastic
Lubricant	Silicone oil
Needle	Stainless steel
Needle	Elastomer
Needle shield cover	Plastic
Lock adapter	Plastic
Temper evident	Plastic
Finger Grip extender / Back stop	Plastic

Table 2: List of drugs that are used via Pre-filled syringe

Ardeparin	Hospiral lidocaine 1%w/v
Choriogonadotropin Alfa	Lidocaine Hydrochloride 2%
Dalteparin injection	Nadroparin
Erythromycin injection	Reviparin
Vasopressin injection	Sodium hyaluronate 1% w/v
Enoxaparin sodium injection	Tinzaparin
Diclofenac sodium	Amiodarone

Table 3: ISO Standards

Quality management	ISO 13485- Medical Device Quality Management System ISO 14989- Guidance on application of ISO 13485
Risk management	ISO 14971- Application of risk management to medical devices ISO 24971- Guidance on application of ISO 14971
Biological evaluation Clinical trials	ISO 10993 Part 1-20 –Biological Evaluation of Medical devices ISO 14155- Clinical investigation of medical devices for human subjects-Good clinical practice

Table 4: Comparison of regulatory aspects of Drug and Devices

	Drugs	Devices
Regulated by:	**CDER**	**CDRH**
Relevant sections	**Drug cGMPs (21CFR 210-211)**	**Device cGMPs (21CFR 820**
Emphasis	• **Quality systems** • **Corrective and preventive action** • **Individual outputs** • **Large clinical trials** • **Risk assessment (but not really…)**	• **Quality systems** • **Design controls** • **Quality by design** • **Risk assessment** • **Process as a continuum** • **Small, focused clinical trials (maybe)**
Timeframes	**Long (6-10 years)**	**Short-medium (1-5 years)**

Table 5: Applications for combination products are evaluated in accordance with the PMOA and the evaluation timeframe

LEAD CENTER	APPLICATION TYPE	REVIEW CLOCK
CDER/CBER LEAD	**New Drug Application or Biologic License Application**	**6 Month (priority review) or 10 Month (standard review)**
	Post Market Approval **510K premarket notification** **HDE (Humanitarian device exemption**	**180 Days** **90 Days** **75 Days**

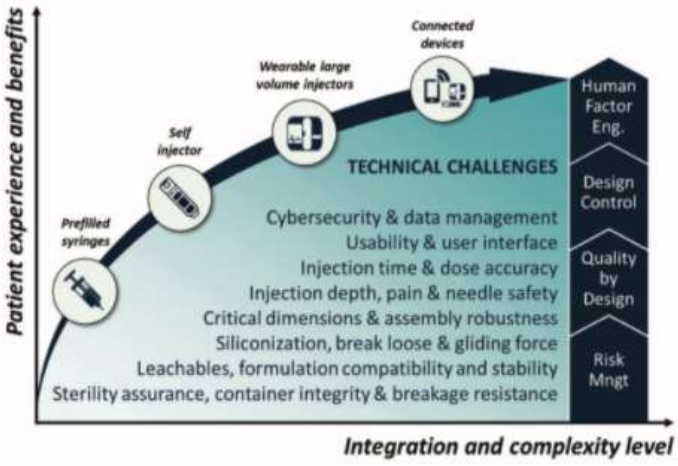

Figure 1: Technical Challenges

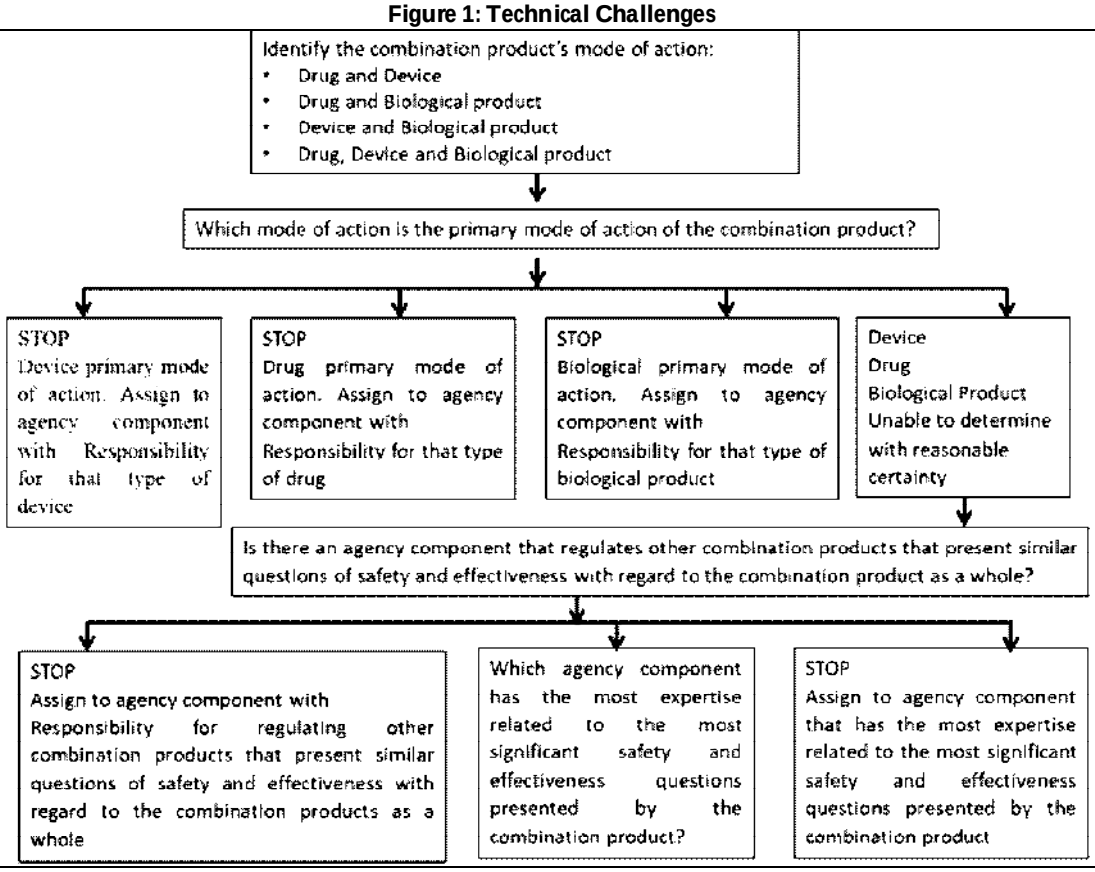

Figure 2: Primary Mode of Action as per USFDA

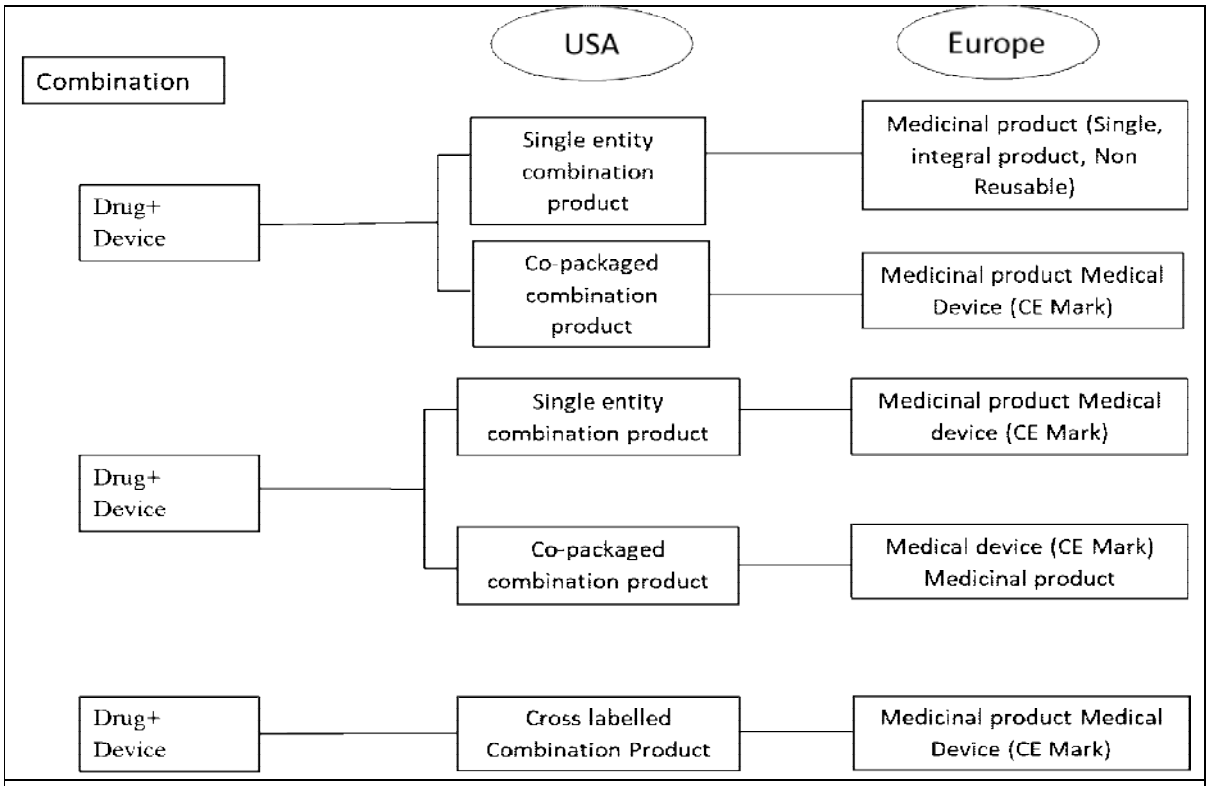

Figure 3: The USA and EU have quite different methods for evaluating medication (or biologic) and device combinations.

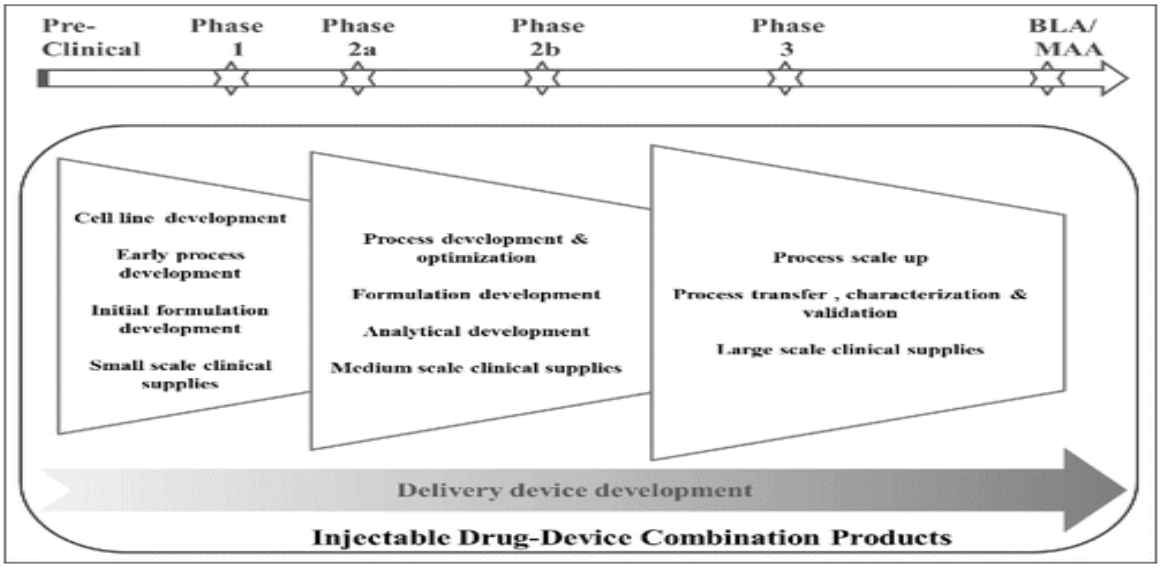

Figure 4: Overview of Injectable DDC Product Development Process and Typical Timelines in Relation to the Clinical Development Timeline

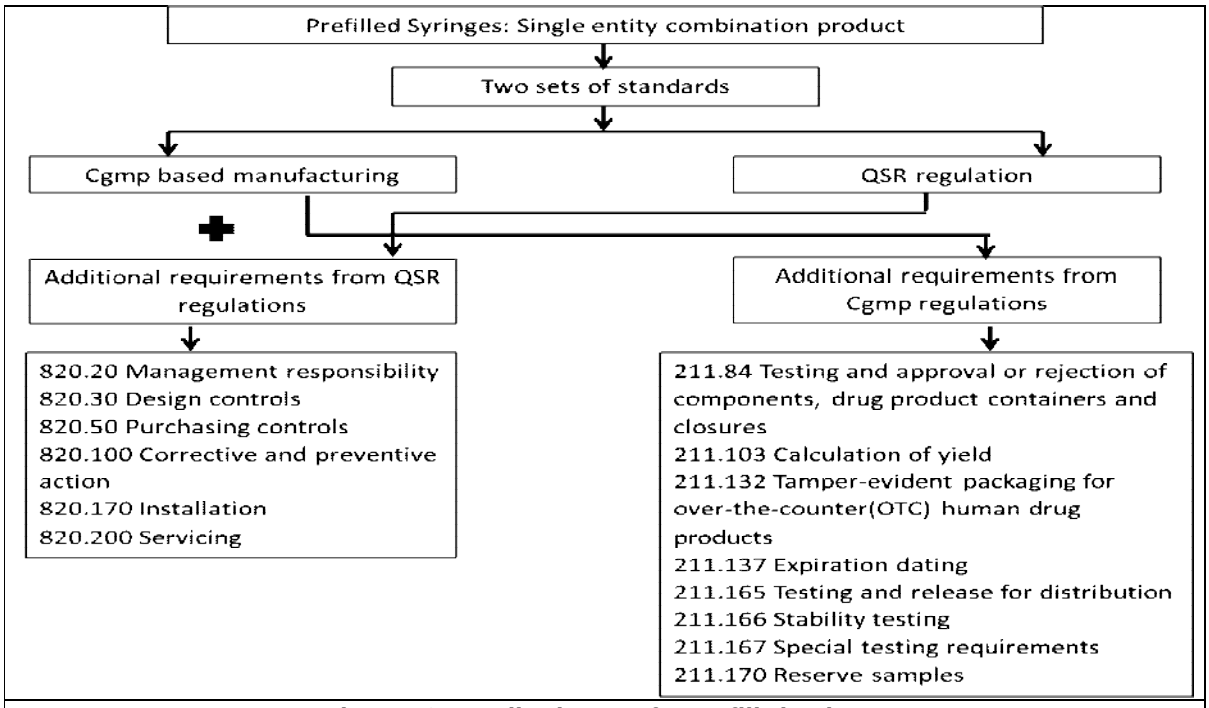

Figure 5: Stream-lined system for pre-filled syringes

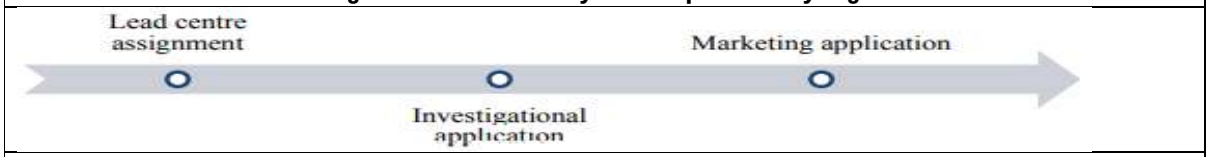

Figure 6: Process to obtain a marketing approval for pre-filled syringe

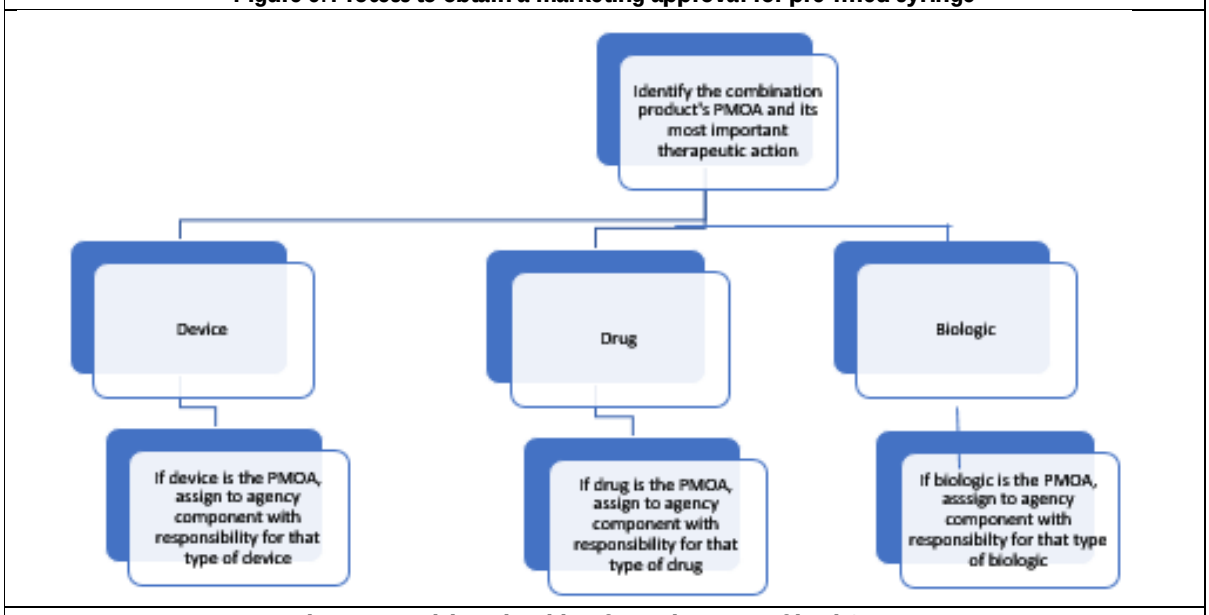

Figure 7: Decision algorithm for assignment of lead Centre

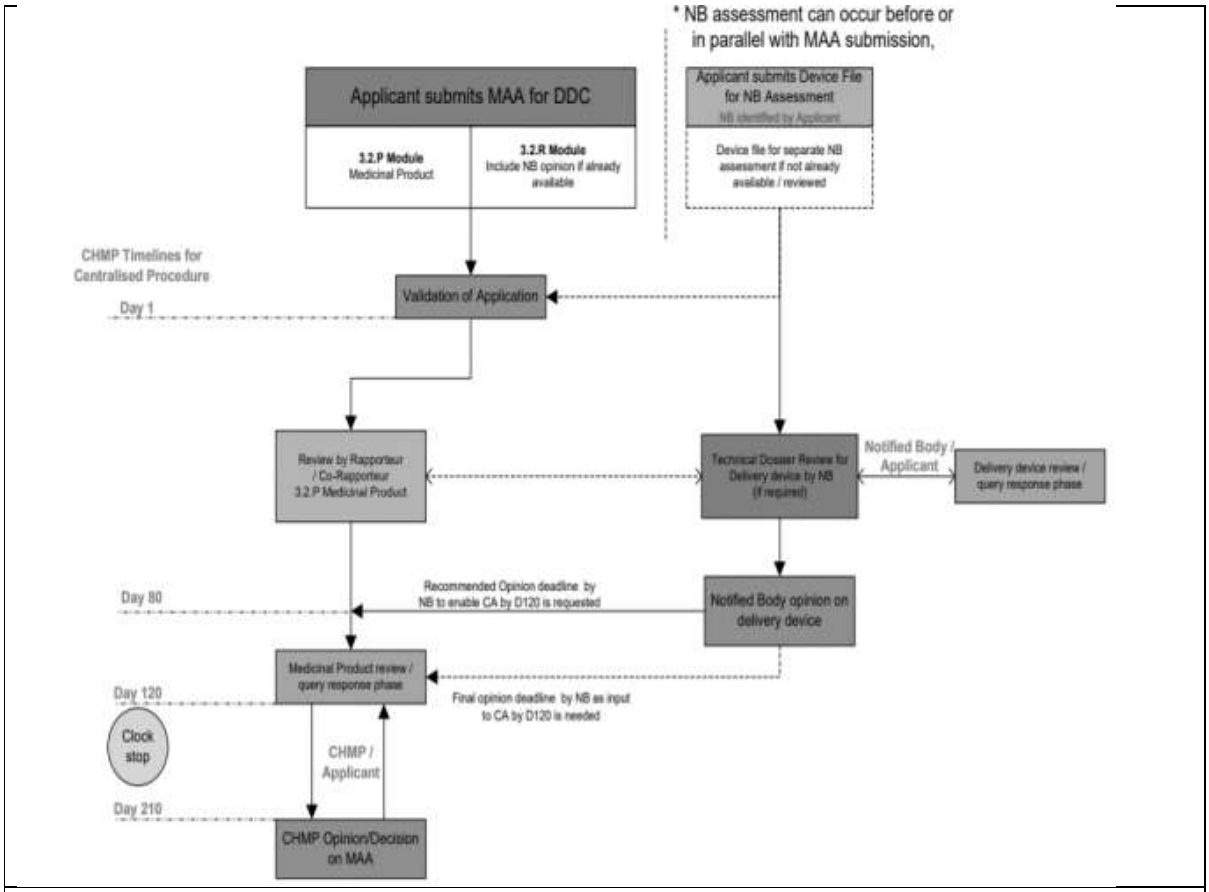

Figure 8: Marketing authorization application review and approval of integral drug-device combination

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of *Cycas circinalis* L. and Ionidium *Suffruticosum* Ging. on Fertility of Male Wister Albino Rats

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Received: 18 Jan 2022 Revised: 23 Feb 2022 Accepted: 28 Mar 2022

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ABSTRACT

In this study *Cycas circinalis L*. and *Ionidium suffruticosum* extract (200mg/kg body weight) were administered orally to the experimental albino rats of Wister lineage and compared to the control albino rats using various parameters such as weight of animals, dimension of testes, hormonal analysis, semen analysis, histological analysis of testes, diameter of seminiferous tubules and the drug's efficacy was investigated. The administration of the drug showed significant improvement of all the parameters in experimental rats when compared to control rats. The data's were analyzed using one way ANOVA and found to be statistically significant. The herb was found to be effective on the gonads of male albino rats and also it's a safe drug without adverse effect for enhancing the male sexual activity.

Keywords: Fertility, Male Wistor Rats, Gonads, Stress, Alcohol, Diabetes.

INTRODUCTION

Infertility is a major public health concern. Infertility imposes a strain on any marriage. Due to social stigma, emotional involvements etc., it cannot be treated like other illnesses, as it often represents to the couple the very essence of maleness and femaleness [1]. As many as 15% of couples have difficulty in conceiving and the male factor is implicated as the main cause up to 50% of cases [2]. In case of sub fertility and improper spermatogenesis, the use

of hormonal preparation has been well appreciated but only with warnings of grave side-effects on prolonged treatment [1]. In man, injection of testosterone caused disappearance of the Leydig cells, atrophy of the tubules, arrest of spermatogenesis and pronounced hyalinization of the basement membrane [3]. In humans stress and strains of modern living may make an individual sexually neurasthenic and functionally impotent. Spermatogenesis was suppressed at all the stages of cell division and maturity due to stress [4]. Alcohol abuse was well known to impair reproductive performances due to impaired testosterone production and testicular atrophy [5,6]. Ageing is associated with the diminished function of various tissues in the body. The gonadal function declines with age. In male, there is progressive atrophy of the sperm- producing elements of the testis, resulting in diminished spermatogenesis [7]. There is a remarkable increase in the use of herbs over the past few years and research interests have focused on various herbs. In Indian system of medicine many herbs were used for treating male sexual disorders since ancient times.

Cycas circinalis L. is a gymnosperm tree (Family-Cycadaceae), the male cone has aphrodisiac activity and commonly known as Madana Kaman in Tamilnadu [8]. *Ionidium suffruticosum Ging*. is a perennial herb widely distributed in India (Family –Violaceae) and has aphrodisiac activity and commonly known as orithazthamarai in Tamilnadu [9]. Both the plant has tremendous medicinal value particularly in improving male fertility. Scientific study on fertility effect of *C.circinalis* and *I.suffruticosum* were not yet done so the present research was undertaken.

AIM OF THE STUDY

- To study the fertility effect of *Cycas circinalis* (Cc) and *Ionidium suffruticosum* (Is) on the young, senile, and sterile male Wister rats.

OBJECTIVES OF THE STUDY

- To compare the effect of synthetic testosterone hormone, Cc and Is on gonads of young, senile, and stress, alcohol and diabetes induced sterility of male Wister rats.
- To find out the mode of action of Cc and Is on gonads of young, senile, and stress, alcohol and diabetes induced sterility of male Wister rats.
- To find out the most effective herb in improving fertility of young, senile, and stress, alcohol and diabetes induced sterility of male Wister rats.

MATERIALS AND METHODS

Study Design: Experimental study
Study Centre: Biomedical Research Unit & Laboratory Animal Centre (BRULAC), Saveetha University, Laboratory Animal Centre and research lab – Vinayaka Mission's Research Foundation (Deemed to be University).

Extraction of *Cycas circinalis* and *Ionidium suffruticosum*

The *Cycas circinalis* (Cc) male cones and the *Ionidium suffruticosum* (Is) were identified and authenticated, collected, washed with distilled water and air dried for 10 days. The dried male cones of Cc and whole plant of Is were powdered using pulveriser and passed through sieve to get fine powder. 50 grams of powder of plant material of Cc and Is were successively extracted with 500ml of ethanol by hot continuous percolation method in Soxhlet apparatus for 24 hrs [10]. The extracts were concentrated by using hot water bath and subjected to drying in hot air oven [11]. The percentage yield of Cc and Is extracts was found to be 7.14 and 8.42 respectively.

Experimental protocol for Inducing Sterility
Animal selection and drug administration
Sampling: simple Random Sampling

120 Healthy adult male Wister rats were house–bred in polypropylene cages with paddy husk bedding, standard rat pellets and fresh drinking water was provided, acclimatized on a 12 hour light & 12 hour dark schedule. The cages are labeled with group, weight of the animal and dosage of the drug.

Inducing sterility on young rats by Stress

Stress was induced on young rats of group C1 (Table 1) by Immobilization for 4 hrs /day for 14 days. The stress induced was confirmed by estimating serum cortisol [4].

Inducing sterility on young rats by Alcohol

Sterility was induced on young rats of group C2 (Table 2) by administering 3 gm of ethanol/ kg body weight/ day orally for 4 weeks for experimental rats and distilled water for control [5,6].

Inducing sterility on young rats by Diabetes

Diabetes was induced on young rats of group C3 (Table 2)by administration of streptozotocin 50 mg / kg body weight intraperitoneally to experimental rats and control rats received 0.1M of Sodium citrate buffer [12]. Animals were fed with glycosylated water to avoid hypoglycemia. On 4th day blood glucose level was estimated. Blood glucose level above 250mg/dl considered as diabetic and the procedure was continued for 6 weeks.

Confirmation of sterility induced on young rats

The sterility induced in all groups (C1, C2, and C3) was confirmed by testicular biopsy, sexual behavior and testosterone hormone analysis.

Extract of *C.circinalis* and *I.suffruticosum* administration:

The Cc and Is extracts were administered orally to the experimental group using oral gavage tube (200 mg/kg bodyweight) and simultaneously sterile water were given orally to control rats for 30 days. The positive control rats were given synthetic testosterone (Aquaviron, Nicholas India Ltd.) 10µg/kg body weight subcutaneously, biweekly on alternate days [4]. After the drug administration a rest period of about 10 days were given to all the group of animals.

Sexual behavior of male rats:

Adult healthy young female rats weighing about 130 – 140 gm were selected and administered benzoate oestradiol 10µg/100g body weight to bring the female rats for oestrous phase 48 hrs before copulatory study and progesterone 500µg/100g body weight was administered through subcutaneous route 4 hours before the copulatory studies. The experiment was carried out in a specially designed box. One male rat randomly selected marked and kept initially in the box and two female rats which were prepared as explained above were introduced into the box (Javeed Ahmed Wani et al 2011). After a 15 minutes acclimatization period, the sexual behavior was observed for one hour till ejaculation of semen in any one of the female [4].

Sample collection

The rats were anaesthetized using Xylazine & ketamine. The jugular vein was traced out; about 2ml of blood was taken for estimation of testosterone hormone using ELISA analyzer. The heart was perfused with buffered formalin followed by a midline incision made on scrotum and the testicles were removed. A fine dissection was done to remove the epididymis from the testes [13,14].

Measuring dimension of testes

The length, breadth, height of the testes were measured using Vernier caliper and the volume of the testis were calculated using the Lambert's formula [15].

(Volume = Length x Breadth x Height x 0.71 cu.cm)

weight of testes were taken. The relative weight of testes i.e., gonado-somatic index (GSI) were calculated [16,17] with the help of following formula **(GSI = Weight of testes in grams / Body Weight in grams x 100).**

Statistical Analysis

- Mean, Standard deviation, Standard error mean were calculated and tabulated.
- Student t test for comparison between two groups
- One way ANOVA to compare the data's within the groups
- Chi square test for sperm morphology analysis
- Post HOC pair wise comparison test for comparison among the groups.

RESULTS AND DISCUSSION

PHASE – III

Confirmation of sterility Induced in Young Rats group (C1, C2 & C3)

The parameters were compared between the normal and sterile rats and found to be highly significant except mounting index (Table 2). The testicular biopsy showed degeneration of seminiferous tubules, luminal diameter increased desquamation of the epithelium in the tubule were observe in the alcohol administered rats and the normal rats did not show any histological changes in the tubules. This confirms that sterility was induced in experimental rats. The parameters were compared between the normal and sterile rats and found to be highly significant (Table 3). The blood glucose level was increased in experimental rats. The testicular biopsy showed degeneration of seminiferous tubules, desquamations of the epithelium in the tubule were observed in the diabetic rats and the normal rats did not show any histological changes in the tubules. This confirms the sterility was induced in experimental rats.

The parameters were compared between the normal and sterile rats and found to be highly significant except mounting index (Table 4). The serum cortisol was found to be increased in stressed rats than normal control rats this confirms that stress was induced in experimental rats. The testicular biopsy showed degeneration of seminiferous tubules, desquamation of the epithelium in the tubule were observed in the stress induced rats and the normal rats did not show any histological changes in the tubules. This confirms the sterility was induced in experimental rats. The *Cc* and is both have aphrodisiac activity which was compared with a positive control administered with testosterone. The positive control showed increase in sexual performance followed by is extract administered group. The total sexual behavior was more in the Is administered rat when compared to positive control. *I.suffruticosum* was proved to be more effective for sexual performance than the *C.circinalis*. The sexual behavior was found to be increased in PC and EII (Is infused group) followed by EI (Cc infused group). had done a trial with some indigenous drugs and showed a significant hypertrophy of the seminiferous tubules, increase in weight of the gonads and increase in the sperm count [18]. Arora et al., (1973) observed the changes in gonads of albino rats after administering Speman and found that there was an increase in size of seminiferous tubules, thus in turn an increase in the weight of the gonads [1]. Elevated testosterone level increases the diameter of the seminiferous tubules, which induces spermatogenesis resulting in an increase in sperm count [19]. Arora et al 1973 also added the condition of increased spermatogenesis in rats treated with speman.[1] Shivraj et al (1971) and Mitra et al (1996) studied the effect of 2 patent drugs (Speman and Tentex forte) and reported increased sperm counts upto 81.50±2.70 million\ml [20,19]. The present study showed an increase in the sperm count in PC and E II than NC and E I. The testosterone hormone was more in EII when compared to other groups (Table 5).

Shivraj et al (1971) studies involving Speman in rats brought out the increase in tubular volume and increased weight of gonads [20]. Rabia Latif et al (2008) Qamar Hamid et al (2010) has done a study using GSI as one of their parameters to correlate the increase in body weight and gonadal weight of the rats. The present research showed an increase in GSI in PC when compared to other groups [16,17]. Oyeyemi et al (2008) and saba et al (2009) studied the morphology of spermatozoa after treating the rats with an extract of Vernonia and Lagenaria (herbs) and categorized

the abnormal and normal spermatozoa [14,21]. In this study abnormal spermatozoa were found to be more in control, whereas much less in PC, EII then EI and the data's analyzed by Chi-Square test proved to be highly significant (0.001). The spermatozoa completely filled the lumen of seminal tubules of E-II (80-87%); whereas in the E-I (75-80%); when correlated with control (54-60%) were filled with sperm cells and the remaining were found to be empty. The histomorphometry of the testes showed an increase in all the nuclear diameter of EII when compared to other groups (Table 15). All the parameters were analyzed by One way ANOVA proved to be highly statistically significant (P<0.001) except mounting index which was not significant. The post hoc pairwise comparison shows the significance among the groups.

Effect of *Cycas circinalis* and *Ionidium suffruticosum* on Fertility of Senile Rats (Group B)

The present study clearly shows the effect of Cc and Is on the hypothalamo-pituitary gonadal axis which increases the secretion of testosterone by acting on the leydig cells [22]. Testosterone has been proven to have anabolic effects (Subaro et al 1973) thus causing an increase in the general body weight [23]. The aged rat's (Senile) gonadal function declines, with progressive atrophy of the sperm-producing elements of testes resulting in diminished spermatogenesis [7]. When all other parameters were comparatively high in senile rats, the gradual increase in the body weight was equal in all groups, a positive finding in this study, because a sudden and remarkable increase in weight in senile rats, will also lead to the deleterious effects of obesity in these aged rats. The Weight and Volume of the Testes is one of the markers of a possible alteration in Androgen status, increase in weight of gonads along with the increase in size of seminiferous tubules [17,18,1] and the present study also shows an increase in the gonadal weight of the rats treated with is followed by Cc (Table 6). The GSI is a better way to assess the damage of the testes in relation to the body. The increase in GSI was due to increased level of Serum testosterone, as androgen exerts its major role in sex organs [17]. Sekar Suresh et al, 2009 have worked on aged rat's sperm by orally_infusing some herbal drug on senile rats and observed that it increased the sperm count to a higher level [23]. Shivraj et al (1971) and Mitra etal (1996) also added to the above finding, but they used a patent drug. The concentration of the spermatozoa in the Cauda epididymis of the senile wistar rat was found to be increased in PC and EII because of increase testosterone that plays a vital role in spermatogenesis [20,19]. The abnormal sperms were found to be less in EII and EI , further the data were analyzed using Chi-Square test and found to be highly significant (0.001).

The progressive atrophy of the gonads results in decreased secretion of testosterone and this trial has proved to be having a great efficacy in improving testicular and other accessory sexual functions of senile rats [7]. The hypertrophy of seminiferous tubules found in E-II could be androgenic effect of the drug Is on testes in general and was responsible for increased production of testosterone which in turn was responsible for the increased size of tubules and the spermatozoal count [1]. The spermatozoa completely filled the lumen of seminal tubules of E-I (75-80%); whereas in the E-II (82-87%) when correlated with control (50-60%) tubules filled with sperm cells. The histomorphometry of the testes showed an increase in all the nuclear diameter of EII when compared to other groups. All the parameters were analyzed by One way ANOVA proved to be highly statistically significant (P<0.001) except mounting index, body weight and volume of testes which was not significant. The post hoc pairwise comparison shows the significance among the groups.

In humans chronic alcohol consumption results in disorders of spermatogenesis.[24] Male alcoholics undergo erectile dysfunction and infertility due to reduction in testosterone [25]. Ethanol is a primary testicular toxin [27]. Apoptosis was induced in ethanol treated animals indicating the tissue injury of testicles followed by testicular DNA fragmentation, and increased number of apoptosis of spermatogonia and spermatocytes [27,28]. Alcohol causes an adverse effect on the secretory function of Sertoli cells [27]. Testicular atrophy occurs due to loss of sperm cells and decreased diameter of the seminiferous tubules. The present study showed desquamated seminiferous tubules and pronounced changes in the nuclear diameter of the germinal epithelium. Ethanol ingestion in mice revealed degenerative changes of epithelial component of the seminiferous tubules [29] and alcohol-treated rats showed testicular lesions with decrease in the diameter of the seminiferous tubules, decrease of Leydig cell's number [30]. The sexual behavior was observed to be more in testosterone, Cc and is infused rats. There was an increase of body weight, testes weight and GSI, testosterone hormone in experimental animal when compared to sterile rats. The

sperm count was found to be increased almost twice when compared to sterile controls (Table 7). There was much reduction in abnormal sperms in experimental groups. The histomorphometry of the testes showed increase in all the nuclear diameter of EII when compared to other groups. All the parameters were analyzed by One way ANOVA proved to be highly statistically significant (P<0.001). The post hoc pairwise comparison shows the significance among the groups.

Fertility Effect of *Cycas circinalis* and *Ionidium suffruticosum* on Stress Induced Sterility of Male Wister Rats (Group C2)

In men stress interferes with the reproductive capacity adversely, resulting in inhibition of male reproductive functions due to depression in the hypothalamic-pituitary-testicular axis [31,32] Immobilization stress can decrease testosterone secretion in males, resulting in deficit of sexual performance and sexual act [31]. Many research findings stated stress related decrease in semen quality, sperm concentration, morphology and sperm motility [33]. Exposure to stress affects the endocrine system thus activating hypothalamo-hypophyseal-gonadal system as well as hypothalamo-hypophyseal-adrenocortical along with neuroendocrine axis, which affects male reproductive function and sexual act by decreasing testosterone secretion, spermatogenesis and libido by increasing serum cortisol as in the present study [34]. In this study abnormal spermatozoa were found to be more in control, whereas much less in PC, EII then EI and the data's analyzed by Chi-Square test proved to be highly significant (0.001). The histological examinations of testis in stressed rats showed a marked decrease in seminiferous tubular diameter, reduction in spermatogenesis, increased interstitial spaces and germinal epithelium nuclear diameter was reduced much when compared with normal control group. An increase in nuclear diameter of spermatogonium, spermatocytes and spermatids in testosterone treated groups (PC) followed by EII and EI indicates that testosterone restores male reproductive system. All the parameters were analyzed by One way ANOVA proved to be highly statistically significant (P<0.001) (Table 8). The post hoc pairwise comparison shows the significance among the groups.

Fertility Effect of *Cycas circinalis* and *Ionidium suffruticosum* on Diabetes Induced Sterility of Male Wister Rats (Group C3)

The incidence of diabetes was high among the men attending fertility clinics and was found to be subfertile [35]. *Cycas* and *Ionidium* do not have any deleterious effect on the testes of diabetic rats, but resulted in improvement of its cytoarchitecture. Regeneration of testicular tissue suggests that these plants have antioxidant properties that can mop up free radicals produced by streptozotocin. This is in conformity with work carried out by Cc and Is antioxidant properties were demonstrated which may be due to the high level of alkaloids and flavonoids found in the herbs.[36] Alkaloids were used as precursors in the manufacture of steroidal drugs [37]. Flavonoids are functional as disease resistant; Is also contains tannins that inhibit oxidation [38]. The alkaloids may have triggered the production of testosterone, flavonoids may have acted by resisting the effect of diabetes and tannins may have inhibited oxidation by acting as an antioxidant [39]. These potent properties of Cc and Is may have likely influenced the regenerative effects of testicular tissue observed in the groups given Cc and Is.

The sexual behavior was observed to be more in PC, followed by EII and EI. There was an increase in body weight, testes weight, GSI and testosterone hormone in experimental animal when compared to diabetes induced sterile rats. The sperm count was found to be increased almost twice when compared to sterile controls. There was much reduction in abnormal sperms in experimental groups. The histomorphometry of the testes showed increase in all the nuclear diameter of EII when compared to other groups. All the parameters were analyzed by One way ANOVA proved to be highly statistically significant (P<0.001) (Table 9). The post hoc pairwise comparison shows the significance among the groups.

CONCLUSION

Cycas circinalis and Ionidium suffruticosum both have fertility effect and was compared to that of a synthetic hormonal preparation and was found to be having almost an equal effect for *I.suffruticosum* than *c.circinalis*. Since the synthetic

hormonal preparation have grave side effects it's better to go with herbal aphrodisiac for better results without any side effects. Even though *herbs* may take a long period to show its effect on improving the fertility, drastic side effects can be prevented by avoiding synthetic hormonal preparations. The *C.circinalis* and *I.suffruticosum* administration has shown significant positive results in improving various parameters involved in maintaining maleness. The phytoconstituents such as alkaloids of *C.circinalis* and *I.suffruticosum* may have triggered the production of testosterone, flavonoids may have acted by resisting the effect of stress, diabetes and alcohol on gonads, tannins might have inhibited oxidation by acting as an antioxidant This study has given us a definite hope about the efficacy of the drug. The alkaloid specificity of the drug has to be further studied with more parameters with the aids of advanced technology. *I.suffruticosum* was found to be more effective than *C.circinalis*. The study done in animal if extended in humans and if found to be equally effective; will turn out to be a boon for infertile couples who were anxious to conceive.

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Table 1: Distribution of Rats for Experimental Study

S.no	Groups	Group-A (young) (10-12 weeks)	Group-B (Senile) (12-14 months)	Group-C (sterile rats)(10-12 weeks)		
				C1	C2	C3
1	Normal Control	6	6	6	6	6
2	Positive Control	6	6	6	6	6
3	Experimental I	6	6	6	6	6
4	Experimental II	6	6	6	6	6
		Total no. of rats used for main study				120

Normal Control – administered sterile water, Positive control – administered testosterone hormone, Experimental I – administered C.circinalis extract, Experimental II – administered I.suffruticosum extract, C1 – Alcohol induced sterility, C2 stress induced sterility, C3 Diabetes induced sterility

Table 2: Confirmation of Alcohol induced sterility in Young Rats

Parameter	Control Mean ± SEM	Sterile rats Mean ± SEM
Mounting Index (MI)	6.95 ±1.68	4.55 ± 1.10$^{\#}$
Total sexual behavior (TSB)	202 ± 0.967	134 ± 0.61***
Serum Testosterone Hormone (ng\ml)	2.1 ± 0.21	1.2 ± 0.12**

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, *$P<0.05$, **P<0.01 & ***P< 0.001 control groups compared to Sterile group, Statistical analysis – Students t test.

Table 3: Confirmation of Diabetes induced sterility in Young Rats

Parameter	Control Mean ± SEM	Sterile rats Mean ± SEM
Blood Glucose Level (mg/dl)	96.23± 2.5	380 ± 3.21***
Mounting Index (MI)	6.61 ± 1.80	3.8±0.2**
Total sexual behavior (TSB)	196.22 ± 0.87	94.2±2.8***
Serum Testosterone Hormone (ng\ml)	2.86 ± 0.10	1.2±0.05***

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, *$P<0.0,5$, **P<0.01 & ***P< 0.001 control groups compared to Sterile group, Statistical analysis – Students t test.

Table 4: Confirmation of Stress induced sterility in Young Rats

Parameter	Control Mean ± SEM	Sterile rats Mean ± SEM
Mounting Index (MI)	6.95 ± 1.68	5.84 ± 1.59$^{\#}$
Total sexual behavior (TSB)	190± 0.97	140 ± 0.98***
Serum Testosterone Hormone (ng\ml)	2.1 ± 0.21	1.6 ± 0.13$^{\#}$
Serum Cortisol (µg\dl)	0.9 ± 0.04	1.8 ± 0.11***

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, *$P<0.05$, **P<0.01 & ***P< 0.001 control groups compared to Sterile group, Statistical analysis – Students t test.

Table 5: Various Parameters of the Young rats (Group A)

S.No	Parameters	Normal Control	Positive Control	Experimental 1	Experimental 11
1	MI	6.58 ± 1.80	7.34 ±1.24	7.21±1.41$^{\#}$	7.31±0.45$^{\#}$
2	TSB	186.20 ± 0.67	223.4 ± 0.85	210±0.87 ^{a***}	221.4±0.9 ^{b***}
3	BW (gm)	181.17± 1.90	220± 1.88	219.33 ± 1.7^{a***}	228 ±1.88 ^{b***}
4	VT (cu.cm)	0.83± 0.05	1.36 ± 0.12	1.28 ± 0.05^{a***}	1.31±0.126 ^{b***}
5	WT (gm)	0.95 ± 0.01	1.42 ± 0.02	1.13 ± 0.02^{a***}	1.11±0.03 ^{b***}
6	GSI	0.48 ± 0.01	0.62 ± 0.01	0.56 ± 0.01^{a***}	0.52±0.01 ^{b***}
7	SC (millions/ ml)	30.86 ± 0.40	56.4 ± 0.81	50.41± 0.72^{a***}	58.38±0.97 ^{b***}
8	TH (ng/ml)	2.26 ± 0.05	5.26 ± 0.22	3.01± 0.05^{a***}	5.83±0.19 ^{b***}

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, ^{a}*$P<0.0,5$, ^{a}**P<0.01 & ^{a}***P< 0.001 control groups compared to Experimental I group, ^{b}*$P<0.05$, ^{b}**P<0.01& ^{b}***P< 0.001 control groups compared to Experimental II group, Positive control – administered testosterone hormone, Experimental I – administered *C.circinalis* extract, Experimental II – administered *I.suffruticosum* extract, Statistical analysis –One Way ANOVA.

Table 6: Various Parameters of the Senile Rats (Group B)

S.no	Parameters	Normal Control	Positive Control	Experimental 1	Experimental 11
1	MI	7.85 ± 1.68	8.72 ± 1.72	7.42±1.24$^{\#}$	8.43 ±1.59$^{\#}$
2	TSB	198.2 ± 0.97	207±0.91	204±0.91 ^{a***}	210.30 ± 0.98 ^{b***}
3	BW (gm)	361.17 ± 1.53	362±1.81	359.17 ± 1.22$^{\#}$	365.33 ± 1.78$^{\#}$
4	VT (cu.cm)	1.21 ± 0.09	1.62±0.14	1.59±0.09$^{\#}$	1.38 ± 0.10$^{\#}$
5	WT (gm)	1.06 ± 0.02	1.45±0.02	1.28±0.01 ^{a***}	1.42 ± 0.02 ^{b***}
6	GSI	0.29 ± 0.01	0.41±0.01	0.33±0.01 ^{a***}	0.39 ± 0.01 ^{b***}
7	SC (millions/ ml)	27.9 ± 0.33	48.2±0.71	38.13±0.71 ^{a***}	46.91 ± 0.64 ^{b***}
8	TH (ng/ml)	1.83 ± 0.16	3.8±0.26	2.4±0.21 ^{a***}	3.26 ± 0.22 ^{b***}

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, ^{a}*$P<0.0,5$, ^{a}**P<0.01 & ^{a}***P< 0.001 control groups compared to Experimental I group, ^{b}*$P<0.05$, ^{b}**P<0.01& ^{b}***P< 0.001 control groups compared to Experimental II group, Statistical analysis –One Way ANOVA.

Table 7: Various Parameters of the Alcohol Induced Sterile Rats (Group C1)

S.no	Parameters	Normal Control	Sterile Control	Positive Control	E 1	E 11
1	MI	6.58 ± 1.80	5.83±0.31	8.20±0.37	7.17±0.24 ^{a***}	7.92±0.13 ^{b***}
2	TSB	186.20 ± 0.7	133.17±3.2	195±3.85	193±1.69 ^{a***}	199.8±1.5 ^{b***}
3	BW (gm)	181.17± 1.9	159.7±3.4	208.3±4.7	194.8±1.6 ^{a***}	208±3.73 ^{b***}
4	VT (cu.cm)	0.83± 0.05	0.61±0.03	0.89±0.13	0.84±0.01 ^{a***}	1.28±0.09 ^{b***}
5	WT (gm)	0.95 ± 0.01	0.73±0.03	1.12±0.06	1.04±0.05 ^{a***}	1.18±0.05 ^{b***}
6	GSI	0.48 ± 0.01	0.46±0.02	0.54±0.02	0.53±0.25 ^{a**}	0.56±0.02 ^{b**}
7	SC (millions/ ml)	30.86 ± 0.40	25.55±1.44	57.31±2.1	43.26±1.6 ^{a***}	54.3±2.16 ^{b***}
8	TH (ng/ml)	2.26 ± 0.05	1.37±0.08	4.02±0.22	2.87±0.12 ^{a***}	3.48±0.14 ^{b***}

Values are expressed as Mean ± SEM, n = 6, $^{\#}$ - non significant, * -significant, ^{a}*$P<0.05$, ^{a}**P<0.01 & ^{a}***P< 0.001 control groups compared to Experimental I group, ^{b}*$P<0.05$, ^{b}**P<0.01& ^{b}***P< 0.001 control groups compared to Experimental II group, Statistical analysis –One Way ANOVA.

Table 8: Various Parameters of the Stress Induced Sterile Rats (Group C2)

S.no	Parameters	Normal Control	Sterile Control	Positive Control	E 1	E 11
1	MI	6.58 ± 1.80	5.5±0.22	7.67±0.42	7.05±0.3 a***	8.82±0.36 b***
2	TSB	186.2 ± 0.67	104.3±4.9	196.7±3.4	188.5±2.2 a***	191.8±1.8 b***
3	BW (gm)	181.17± 1.90	156.17±1.6	221.7±7.0	205±3.4 a***	211.7±2.7 b***
4	VT (cu.cm)	0.83± 0.05	0.58±0.14	0.93±0.02	0.90±0.01 a***	1.17±0.08 b***
5	WT (gm)	0.95 ± 0.01	0.71±0.04	1.19±0.06	1.14±0.1 a***	1.26±0.07 b***
6	GSI	0.48 ± 0.01	0.46±0.03	0.54±0.02	0.57±0.3 a**	0.59±0.03 b***
7	SC (millions/ ml)	30.86 ± 0.40	25.15±0.85	55.78±2.1	46.03±0.8 a***	54.7±2.63 b***
8	TH (ng/ml)	2.26 ± 0.05	1.47±0.09	4.20±0.3	3.37±0.08 a***	4.02±0.18 b***

Values are expressed as Mean ± SEM, n = 6, # - non significant, * -significant, a**P<0.0,5,* a**P<0.01 & a***P< 0.001 control groups compared to Experimental I group, b**P<0.05,* b**P<0.01& b***P< 0.001 control groups compared to Experimental II group, Statistical analysis –One Way ANOVA

Table 9: Various Parameters of the Diabetes Induced Sterile Rats (Group C3)

S.no	Parameters	Normal Control	Sterile Control	Positive Control	E 1	E 11
1	MI	6.58 ± 1.80	4.50±0.22	8.5±0.02	8±0.07 a***	9.37±0.44 b***
2	TSB	186.20 ± 0.67	97.8±3.06	202.3±5.3	192.7±1.4 a***	197.3±1.2 b***
3	BW (gm)	181.17± 1.90	149.5±1.4	218.3±5.4	198.8±2.7 a***	213.3±5.6 b***
4	VT (cu.cm)	0.83± 0.05	0.54±0.01	1.05±0.09	1.14±0.08 a***	1.37±0.14 b***
5	WT (gm)	0.95 ± 0.01	0.72±0.03	1.08±0.01	1.23±0.07 a***	1.29±0.09 b***
6	GSI	0.48 ± 0.01	0.48±0.02	0.50±0.03	0.62±0.03 a***	0.60±0.04 b**
7	SC (millions/ ml)	30.86 ± 0.40	28.32±0.9	63.96±0.8	46.2±1.02 a***	62.25±0.6 b***
8	TH (ng/ml)	2.26 ± 0.05	1.47±0.07	4.42±0.09	3.23±0.1 a***	4.37±0.09 b***

Values are expressed as Mean ± SEM, n = 6, # - non significant, * -significant, a**P<0.0,5,* a**P<0.01 & a***P< 0.001 control groups compared to Experimental I group, b**P<0.05,* b**P<0.01& b***P< 0.001 control groups compared to Experimental II group, Statistical analysis –One Way ANOVA.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Smart Navigation Gadget for Visually Impaired People

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Received: 13 Feb 2022 Revised: 27 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

Eyes are one of the vital sense organs in human beings. Unfortunately, 80 percent of blind people are deprived of vision. The proposed electronic navigation gadget for the visually impaired helps to navigate comfortably and efficiently without facing any difficulties during their movements. It revolves around Internet of Things (IoT) in which all the equipment are interconnected and communication is facilitated via internet. The different types of sensors such as Ultrasonic sensor, Infra Red (IR) sensor and heartbeat sensor are embedded in the proposed gadget which helps the visually impaired people (ViP) to overcome overheads in their navigation. Also, the traffic alert signal is given to the impaired people as to whether they have to cross or wait. A buzzer in the gadget tells the person to locate the misplaced cane, i.e., walking stick. In this paper, we have discussed the proposed product workflow in detail and its usefulness to the visually impaired people.

Keywords: IoT, Navigation gadget, Sensors, walking stick, ViP

INTRODUCTION

A comfortable system which guides or assists people with loss of vision, ranging from partially blind to totally blind, through audio commands is referred to as Navigation Assistance for Visually Impaired (NAVI). Many new and vigorous researches are being conducted to develop and build a navigation system for the ViP. Most of these technologies have their own limitations as the challenges involve accuracy, usability, interoperability, coverage which is not easy to overcome with current technology for both indoor and outdoor navigation. The Internet of Things (IoT) is the interconnection via the internet of computing devices embedded in everyday objects, enabling them to send and receive data. IoT plays an important role in communication among networks and it is ubiquitous. Electronic Travel Aids (ETAs) are the major technical devices that are used to send an auditory or/and tactile signals as a warning, when an obstacle is in front of the person and helps the user to avoid it. This system is intended to provide overall measures for real-time assistance. The ViP face several constraints while moving independently or performing their navigation without anyone's help. Mobility is defined as being possible of moving liberally, without the guidance of any human assistance, in both indoor and outdoor environments. The stick used (white cane) by the ViP performs scanning the floor surface and this cannot detect the obstacles in front of the user. The scope of our proposed work is to provide a sensor based efficient electronic aid for navigation, audio command in the case of obstacle, potholes and traffic signals and for the blind people location tracking and notification about them to the care taker.

This enhances the safety and the confidence which helps them move on an obstacle-free path in different environmental situations. The blind people face difficulties in managing traffic signals. Their need for care taker has increased in today's world. This electronic aid helps them to perform their movements in and out of their surroundings. Hence, our implementation was focused on developing a reliable low cost user-friendly navigation gadget for visually impaired people, thereby ensuring that the ViP are safer and less dependent on others while they are moving from one place to another. We developed an electronic aid prototype model for visually impaired people, which is more reliable and affordable by them. Also, the proposed product would replace the caretaker's time and availability for ViP. Our work is organized as follows in this paper. The brief literature survey is presented in Section II. Section III explains the proposed system. Section IV describes the experimental set-up and procedures and along with the results obtained. Finally, conclusion and future works are discussed in Section V and VI.

LITERATURE SURVEY

People who are visually impaired tackle enormous limitations in terms of mobility. Lack of disabled friendly infrastructure and transportation facilities are main causes of blind death. Blindness causes considerable social challenges, usually in relation to the activities in which a blind person cannot participate. The following is the literature survey of available electronic gadgets for the visually impaired people.An electronic aid for the visually impaired has been proposed in [1] which is a low power embedded system. It consists of vibration motors, ultrasonic sensors and also a battery. The vibratory sensor generates voltage and is sensitive to high frequency noise which is hazardous for the impaired user's health. In [2], a survey paper for home automation system has been discussed. In this work the integration of cloud networking using Internet of Things enhances home security using Passive InfraRed sensor (PIR), digital door locks, Light Dependent Resistor (LDR). The other sensors have to be used for the impaired people to move independently. Navigation gadget for the ViP has been proposed in [3]. This gadget detects the obstacles which are processed using ultrasonic sensors and are controlled by the microcontroller. The GPS is used in this gadget to identify the location and also the way to the destination the person concerned has to move along are received via headset. The gadget detects only the obstacle and does not inform the user of what object it is and it cannot be used if it is misplaced anywhere. Cost efficient navigation E-cane system has been designed in [4], based on the robotics in which they use an algorithm for detecting the objects with the help of an ultrasonic sensor. When an object comes in front of a person, a piezoelectric beeper used here produces an alarm to be aware of his/her movement. This cane supports only Bengali language and it cannot be used by the people around the world.In [5], a smart

guiding glass has been developed for the ViP. This system consists of display glasses and several low-cost sensors being developed, and its efficiency and accuracy are tested by a number of users. This is developed for helping the visually impaired people to move in indoor environment. The ViP need much assistance to move in the outdoor environment without guidance. But this system is proposed only for indoor environment. In [6], Smart walking cane with voice assistance has been designed. This system uses ultrasonic sensor for detecting the obstacles, detecting the location using GPS, and crossing guidance is provided using RIR sensor. The stick detects only the obstacle in front of the user but does not tell what the object is and does not detect the potholes. A smart guide extension for blind cane has been proposed in [7]. The smart extension gives information regarding the detected obstacles, hitches, holes and also the compass wind position direction to guide the user. This cane supports only in detecting obstacles, holes, and wind direction. But these are not sufficient to help the ViP to make their movement.

A smart stick for obstacle detection and navigation has been implemented in [8]. This is a smart stick which helps to detect the obstacles by means of infrared, ultrasonic and water sensors. It also uses GPS module to guide the ViP to reach their destination. This stick is not comfortable for the blind people to move freely without any guidance and does not offer any kind of assistance for traffic signal detection. The smart cane with range notification for blind cane has been proposed in [9]. The aim of this work is to develop a smart cane with distance measurement system. The system comprises of an ultrasonic sensor as input and earphone as the output. This does not provide effective guidance for independent navigation. Further detections have to be implemented to move freely. Smart guidance system for the visually challenged people was proposed in [10]. This design is advantageous as it uses both camera and ultrasonic module to determine obstacle in real time, as well as give information about the object ahead of the user. But it does not support features like potholes detection, traffic signal detection, light detection and water detection. In [11], Obstacle present at the head level is detected in WeWalk cane which is an international commercial product available with multi support language. Also, Smart phones may pair with cane using Bluetooth.

METHODOLOGY

The aim of the proposed work is to provide a sensor based electronic aid for the safe navigation of ViP. The main objective is to assist them in detecting and identifying the obstacles that lie in their path and to alert them through audio commands. The proposed smart cane is developed in such a way that it was more reliable, can be used independently, comfortable with mobility, and easy movement in both indoor and outdoor environments. The proposed electronic aid was designed economically and its system architecture is shown in Fig.1. The major components utilized in the proposed system are Raspberry pi 3, Webcam, Ultrasonic sensor, Infrared sensor, and Heart beat sensor, Light emitting diodes (LEDs), GSM modem and head set. Raspberry pi is the low cost data storage, computing capabilities and communication among things or devices which are connected in the internet via Internet Gateway (IG). Raspberry pi with IoT sensors are get connected in the IG network. In the proposed system, obstacle is detected by Webcam and Ultrasonic sensor and both are connected with Raspberry pi for monitoring and processing signals. The webcam in the proposed system uses Open Source Computer Vision (Open CV) is to recognize the object image and to perform image processing. The frame rate in webcam is defined as the number of images captured per second. For streaming video, high speed internet connection is needed with the lower threshold value of 15 frames per second (fps) and the upper threshold value of 30fps. The camera attached in the gadget is used to capture the object image and is compared with the training dataset (objects) which is already stored in it. Then the detected object name will be intimated to the user through the headphones as a voice command.

The ultrasonic sensor is used to measure distance of the obstacles and buzzer in the cane alert the ViP by generating signal. If the detected object range is found to be nearer to the cane, then the strength of the vibration (measured in terms of frequency) will be higher. Otherwise, frequency of the signals returned back is lower indicating obstacle is far away from the ViP. The distance between the object and the gadget is calculated by measuring the time difference between the generation of sound wave once the object is detected and return back to the gadget. The InfraRed sensor (IR) is another sensor, which is embedded in our proposed gadget is to detect any pit or pot hole or staircase by emission of infrared radiations during navigation of the ViP. The heart beat sensor implant in the gadget, is used to

detect abnormal heart beat of the impaired people based on their blood circulation. The abnormality of the impaired people immediately intimated to their care taker as an alert text message and also alerts the surrounding people through buzzer. The Global Positioning System (GPS) present in the gadget is used for location tracking and helps the impaired people to reach their destination by intimating through headphones. All these components and sensors are connected to the Raspberry Pi. It has 32 GB SD card to store the information. Its USB port is connected with the head set which sends the output in the form of voice message. The text message is sent to the user through GSM with the help of Raspberry Pi. The traffic signal can also be identified by using the images captured from the traffic light of the raspberry-pi kit. The intimation is given to the user in the form of audio, such as whether they have to wait for the signal or to move across it. The buzzer implanted in the gadget alert the impaired people, when they misplaced gadget. The product makes use of widely available components which are easy to handle and of light weight so that the blind people can easily carry them. The electronic kit is integrated with the cane they are using in everyday life. Thus, the proposed work is organized into six modules such as object detection module, pit and staircase detection module, traffic signal detection module, heart beat detection module, emergency alert module and finally buzzer alert module. Each of these six modules has its own functionality to perform and they are different in their own way. The product workflow is illustrated in Fig.2.

The headset/ear phone helps them to navigate around nearby locations, through audio commands. These commands helped them to identify the content of the received text message and the phone call received by them. Buzzer alert is also used to find the misplaced cane. The rechargeable lithium-ion battery is used in the proposed cane. This system provides different innovative solutions rather than proceeding with the conventional methods of guiding ViP. Thus, the proposed system was showcased with different types of IoT sensors for navigation of ViP. The innovative design of smart walking stick prototype is showcased in Fig.3

RESULTS AND DISCUSSION

The working model of the electronic navigation gadget is shown in Fig.4. The prototype was implemented using Raspbian Jessie Operating System, Tensor flow and python language along with the embedded components. MNIST dataset in tensor flow is used for object detection.

Obstacle Detection

The obstacles faced by the blind people can be detected in two ways either by web camera or by ultrasonic sensors. The object detection module uses a webcam to capture the object which is controlled by raspberry pi. In our work, twenty different images are used for object detection. The Feature-Based Object detection algorithm is used to compare the captured object with the training dataset (objects) which is already stored in it. Training data set is done using machine learning supervised algorithm. If the detected object (test data) is matched with trained data, the object name is given as a voice message. Thus, the output such as detected object name is intimated to the person through voice command using head phones connected to the cane. The Obstacle Distance for camera (ODC) from the gadget is calculated as

ODC=(WD X FL)/Pix ..... (1)

Where WD is the width of an object, FL is the focal length of the camera and Pix is the camera pixel rate. The object detected using webcam is shown in Fig.6. The detected object is intimated to the ViP through voice as Bottle

The ultrasonic sensor along with the buzzer is another method to detect an object distance in front of the ViP. The detected object is intimated as a voice message and alert sound. The obstacles at a range of 4 meters can be identified by the ultrasonic sensors. Ultrasonic sensors calculate the distance of the obstacle based on the time of reflection waves transmitted and received. The formula to calculate Obstacle Distance for ultrasonsic sensor (ODS) using the formula as

ODS=((T) X S)/2 .....(2)

S is speed of sound in Air is 340m/sec and T is the time taken by the signal to travel from gadget to object and vice versa. The working of ultrasonic sensor is shown in Fig.6. The output of object detection using ultrasonic sensor is shown in Fig.7. The distance of the object in front of the user is measured and intimated through head phones. When it is very near to the user, the buzzer alert is produced.

Pothole Detection

Infrared sensors are used to detect the pits, potholes and staircases based on the reflections. An infrared sensor is an electronic instrument which can detect the objects by emitting infrared radiation. This sensor is capable of measuring the heat being emitted by an object. The comparison with and without object detection using IR sensor is shown in Fig.8. The IR sensor always needs reflection from the object so that it always receives signals. If there is no object to be hit by the waves from the IR sensors, then it is considered as the pot hole and indicated to the user. The potholes in their way during navigation can be easily detected with help of signals reflected from the infrared IR sensors. If there is a miss in the reflection, then it is considered as the pothole. The pothole detection output is showcased in the Fig.9. If the IR sensor received a reflection from the object, then it confirms no potholes or staircase is there. If it does not receive any reflection from the object, then sensor confirms that a pothole is there on the surface. Hence, if there is no hole, light will be ON. Otherwise, light off.

Traffic Signal Recognition

The basic software tool required for Raspberry pi is OpenCV with any of the programming languages like C/C++, python, Java, Ruby, and Pearl. The Raspberry pi video controller port gives the high resolution HD images but it does not have the real time clock. So it cannot display the current day and time. Raspberry pi captures the road signs using web camera module. The captured signs are processed by a JPEG encoder the result of which gives the resized signs suitable for image processing results. The result produced is sent to the sphinx software through the UART protocol which is voice converted through speaker software. The working of traffic signal detection is shown in Fig.10. The Traffic Signal Recognition output is shown in Fig.11. The detected signal is intimated to the ViP through voice

Heartbeat Detection

The heartbeat sensor is used to detect any fluctuation in the heartbeat of the user. The heartbeat sensor usually consists of a light emitting diode and a light detecting resistor. When light is emitted by the LED, it either reflects or transmits the light. The blood volume in the tissue is responsible for the amount of light that a person can absorb and it also produces an electrical signal as an output. If any abnormality is noticed, it is intimated to the caretaker about their condition through a text message using GSM. This also sends an alert to the people around them using a buzzer. The heartbeat detection is shown in Fig.12. The visually impaired person's heartbeat is detected and is shown in Fig.13. Also, an alert message is sent to the caretaker about the heartbeat abnormality of the ViP which is shown in Fig.14.

Adversity Notification

The electronic gadget also aims to provide a full time assistance to the blind people without the help of caretaker. The need for the caretaker to guide them has been increased in today's world. In order to avoid the need for caretaker and for their self motivation an electronic gadget is needed to move independently. This module is used to send an alert message during the emergency situations of the ViP. If an emergency help is needed for the user, then a text message is sent using GSM to their caretaker. Once the visually impaired person is in danger, notification / message is sent to their caretaker's registered mobile number which is illustrated in Fig.15.

Misplaced Cane Notification

The features of the gadget cannot be used if the ViP have misplaced their cane. Thus a buzzer is embedded in the gadget which produces a beep sound in order to find the misplaced cane. The buzzer alert of the misplaced cane is shown in Fig.16. The end product of the prototype is showcased in the Fig. 17. The size of the cane ranges from 122-125 cm. It consists of all the components which are embedded in the prototype and is used for visually impaired people navigation. The cost of proposed cane is estimated to the amount of 150 US dollars. The estimated cost is

cheaper when compared with the existing cane cost which is 500 US dollars. The smart canes which are available in the market are to detect obstacles only and do not alert the user about the detected object types. Similarly, misplaced cane cannot alert the ViP. The proposed cane overcome all those challenges and in addition to that if there is any abnormality in the heartbeat of end user, then it alert the well wisher of the ViP about their health condition.

CONCLUSION

An electronic aid for the ViP has great social related benefits as it aids easy movement. Rather than only detecting the object, this system also recognizes the object and sends information about the object to the user through headset. They can derive much benefit from this innovation at a very low cost as the sensors used are of good quality and of low price. The proposed product's prototype suits manufacturer's requirement so that it will be received well in the market. It is hoped that there will be a boost in the entrepreneurial prospects.

FUTURE DEVELOPMENT

Future scope involves the development of product aid for both deaf and blind people. In future, the characteristics of detected obstacles such as shape, size and colour of the object will detect using machine leaning techniques. Location tracking feature and decision making capabilities can be added along with sensors to help the impaired people in all situations. The gadget can be modularized to notify people regarding the distance they have to travel or the mode of transport they could use to reach their destination. Also, our future work will concentrate on security issues related with IoT devices. In this work, we consider the working concept of IoT modules with the limited number of objects detection and concentrated only on the prototype design. In future, we will extend our work in the realistic situation (i.e.) real ViP to receive realistic response. The different types of machine learning algorithm such as regression and classification algorithm were implemented in our work with large number of objects. The statistical analysis will perform after the number of iterations with the different test data and train data.

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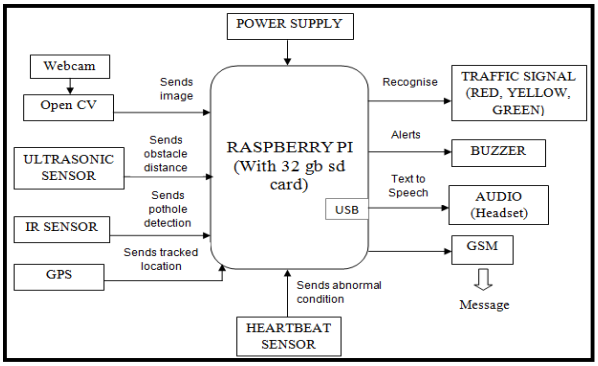

Figure 1. Flow chart of the proposed system

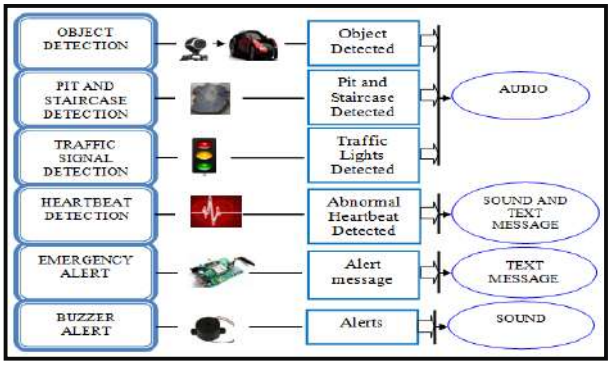

Fig.2. Product Work flow

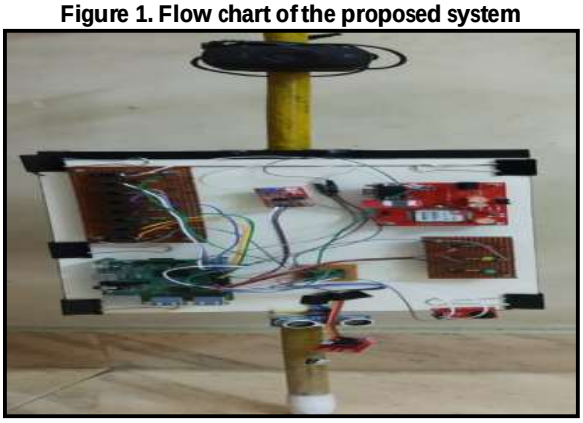

Fig.3. Smart Walking Stick Prototype

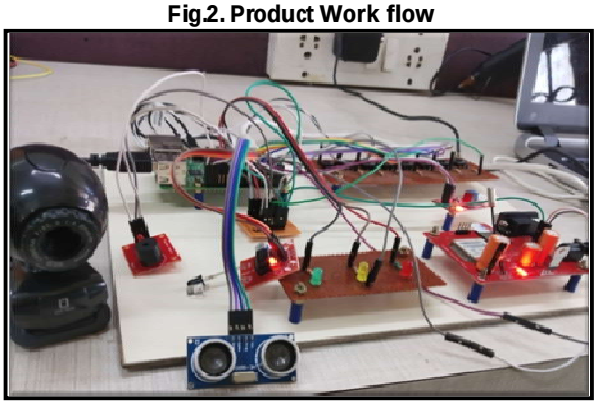

Fig.4. Working Model of Navigation Gadget

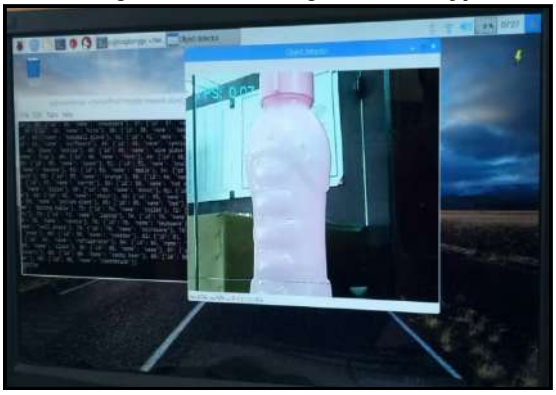

Fig.5. Object Detection Using Webcam

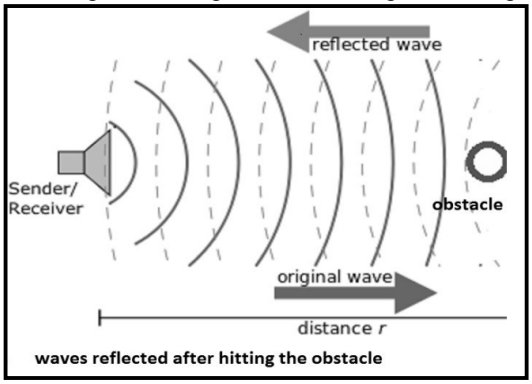

Fig.6. Working of Ultrasonic Sensor

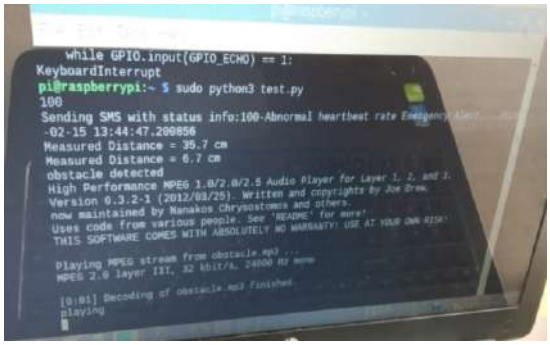

Fig.7. Object Detection Output Using Ultrasonic Sensor

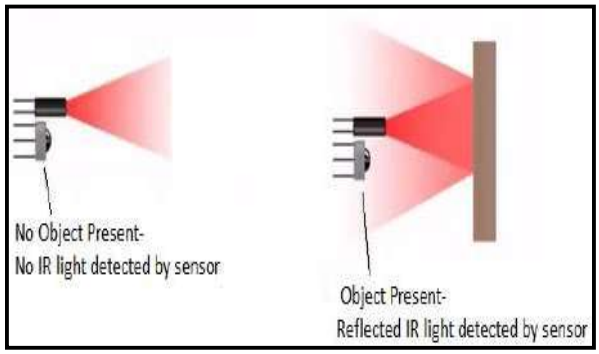

Fig.8. Comparison with and without Object using IR sensor

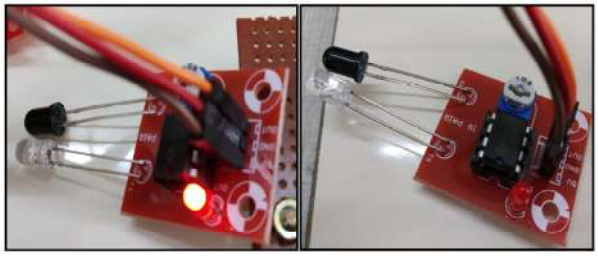

Fig.9 Pothole Detection Output

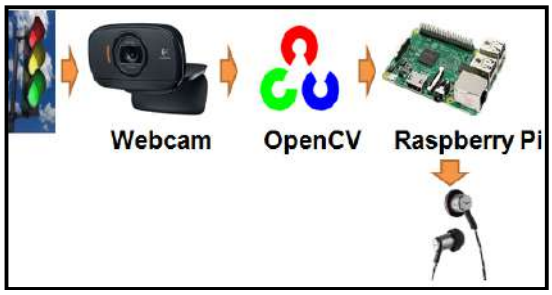

Fig.10. Traffic Signal Detection

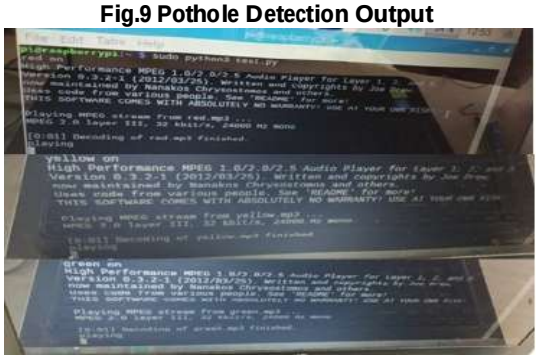

Fig.11. Traffic Signal Detection Output

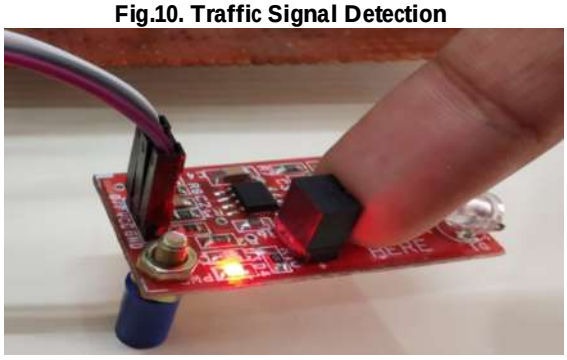

Fig.12 Heartbeat detection

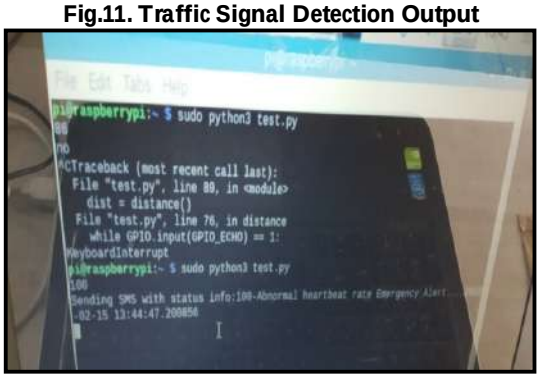

Fig.13. Heartbeat Detection Output

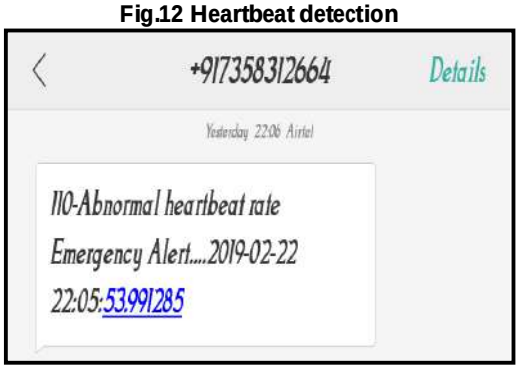

Fig.14. Alert Message about abnormality

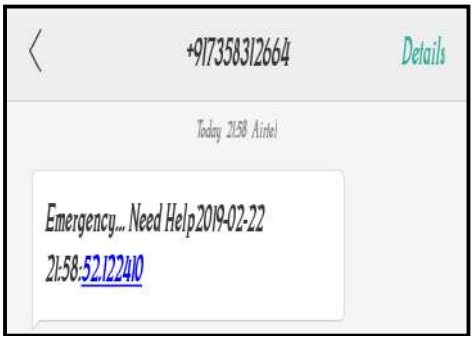

Fig.15. Emergency Message about ViP

Fig.16. Buzzer Alert for misplaced cane

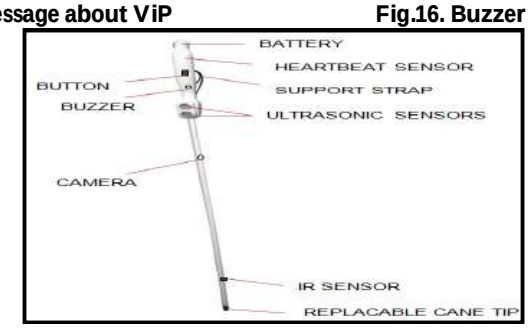

Fig. 17 Smart Cane Design

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

IoT Enabled Smart Energy Management System

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Received: 12 Feb 2022 Revised: 04 Mar 2022 Accepted: 28 Mar 2022

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ABSTRACT

Power saving can be done only when consumption of energy by the load is monitored. Only when the energy consumption of the load is monitored can power savings be made. Once the energy usage has been monitored, a suitable control mechanism can be used to optimise the use of energy, resulting in energy savings. Traditional energy-saving and energy-monitoring approaches are ineffective in terms of conserving a considerable quantity of energy. To overcome these challenges, the proposed solution is done using the Internet of Things (IoT), which monitors the power utilised by loads and saves energy in a cost-effective manner. The Internet of Things (IoT) framework is being used in a variety of applications ranging from home automation to industries where physical objects are connected to a network from anywhere. This paper discusses using an Arduino controller to monitor solar energy and an energy metre, as well as using IoT with a Esp8266 Wifi module to automate the home. This module is utilised to regulate data as well as monitor energy levels on the consumer side. The data from the solar panel and the energy metre is sent to the cloud via the Wi-Fi module, and the user can monitor the data via a web page. This initiative also keeps an eye on the system for any signs of theft. A network of sensors is linked to a controller to detect energy theft and send the data to a GSM module, which then sends the data to the IoT.

Keywords: IoT, Energy Management System, Arduino, Energy meter, Energy Monitor, Energy Theft.

INTRODUCTION

Energy consumption is quickly increasing over the world as a result of fast population increase and industrialization. This encompasses the energy requirements of industrial processes as well as domestic gadgets. As traditional power generation sources become depleted, energy management becomes increasingly important. The residential sector is one of the primary areas where energy demand is constantly increasing. Household energy management has two benefits: it can improve energy efficiency while also contributing to demand side management. For optimal energy management of household appliances, continuous monitoring and sophisticated control are necessary [1] and [2]. The Internet of Things (IoT) has made it possible to connect household appliances, monitor their behaviour, and control them successfully in recent years [3]– [5]. As a result, we may be able to save energy by utilising technology, which is one of the most efficient ways to fulfil the ever-increasing need for energy. Smart house monitoring is growing more common; there were less than 2 million smart homes in 2012, but there were 22 million by 2017 [6]. The Internet of Things (IoT) is a smart network that links a variety of devices. A standard residential building might theoretically be turned into a smart environment with energy awareness by merging smart metering technologies with the IoT framework [7]. Since 2004, the growth of the Internet of Things (IoT) has been entirely dependent on the surge in interest in smart home systems; in 2016, the IoT took a major step forward, propelling the smart home energy management system [8]– [10].

Due to insufficient monitoring, certain household appliances are kept on standby during off-peak hours when they should be utilised, while others are used during peak hours when they should be used during off-peak hours. Unmonitored power consumption wastes a significant amount of energy. A large amount of energy may be saved if properly monitored and regulated. The Internet of Things (IoT) can assist us in more effectively monitoring and operating our assets. The Internet of Things (IoT) is a cloud-based architecture that connects "things" to a cloud-based architecture that gets data from other smart devices via a gateway and is managed by multifunctional smart home monitoring tools on mobile and computer platforms. This technology's main benefit is that it allows for intelligent switching and scheduling of shiftable appliances, resulting in increased energy efficiency.The fundamental problem with this technology is communication, which is caused by the use of a large number of sensor units from different manufacturers. The phrases [11–13] are used to denote a group of people. When a system has a large number of input devices (sensors), the increased communication traffic, together with the increased stress on the system, might lead it to fail. Furthermore, the danger of losing original data increases as the number of linked devices increases [14].

Effective gateway control may be able to eliminate this threat. A smart monitoring system may offer a secure environment for a person by keeping a continual connection with all sensing equipment such as smoke and fire alarms, intrusion warnings, and facial identification utilising an image processing system. If any of the system's components are damaged or malfunctioning, a sensor device will notify the user [15–17]. Industrial clients, on the other hand, place a lower priority on active automation, data security, and dependability [18]. Control, affordability, and accessibility are the three most important qualities of SHTs. Despite the fact that both industrial and residential users had a good impression of the company, consumer trust in data privacy and security problems was low. A study curriculum has been created [19] that focuses on data and privacy, design development and operational standards.

To provide users with consumption information and optimization suggestions, a smart mobile application design system is used. Based on the data obtained and observed power usage patterns, the programme employs artificial learning to manage the appliances in various modes of operation in real time [20]. As a result, household appliances must be used effectively in order to preserve energy. Furthermore, for both energy savings and demand control, exact scheduling of shift-able appliances is required. Both goals may be met by creating a data collecting and control system for appliances that includes an intelligent algorithm for optimal performance. The next sections of the article go over the design, development, and operation of an IoT-based energy management system. This technology will

improve energy efficiency and demand-side management of home load by monitoring energy usage in real time and intelligently scheduling appliances to reduce consumption while preserving the highest level of user comfort. For optimal appliance scheduling and demand side control, the proposed IoT-EMS employs data analysis and optimization approaches. A typical household's load profile was compared to that of an IoT-EMS enabled home.

METHODOLOGY

The IoT-EMS will read data from sensors and relays, as shown in Fig. 1. The data collected by the sensors will be uploaded to the cloud. The energy price, client preferences, load status, and appliance operating time are all included in the cloud data. The Raspberry Pi application can anticipate human actions by analysing the above data.
The most important characteristics of this system are listed below.

- An ESP8266 NodeMCU, which has a built-in internet connection and supports a variety of peripheral communication protocols, serves as the project's brain.
- A voltage and current sensor module is linked to a load and has its own power source independent from the measurement circuit's 5V supply.
- An I2C LCD adapter module drives a 16 x 2 display with real-time voltage, current, and power data.
- The NodeMCU is connected to the internet and delivers the data it collects to a server, which saves it for further analysis.
- Real-time data may be seen on a PC, laptop, or smartphone using any browser.

Advantages of IoT based energy monitoring system are

- It can be monitored in real time from anywhere on the planet.
- Data can be read in real time from anywhere as long as you have an internet connection because the project is connected to the internet.
- The power plant receives real-time energy use data, allowing it to generate power more efficiently.
- Power plants must adjust their energy production in real time to suit consumer demand in the real world.
- If the electricity supplier providers produce less electricity than demand, grid voltage will rise to unsafe levels, triggering protective devices and resulting in a blackout; In turn if more electricity is produced than demand, grid voltage will increse to unsafe levels, triggering protective devices and resulting in a blackout.
- With real-time data, power firms can better control power generation, resulting in fewer or no outages.
- Collecting monthly power use data for individual residences or industries requires no human work, and electricity producers may bill customers right away.
- Individual consumers can monitor their energy consumption and perhaps reduce it to lower their expenses.

Disadvantages of IoT based energy monitoring system are

- Concerns regarding data and privacy. New technologies benefit society, but they also provide tech fraudsters, scammers, and hackers new opportunities. They may use the information they illegally gather against you for financial benefit.
- The expense of maintaining the software and hardware will be borne by the businesses.
- Both customers and power providers will be responsible for the initial costs of replacing old energy metres with new ones.
- The components shown in the block diagram make up the proposed circuit design. Let's take a closer look at each of the components.

Node MCU

As illustrated in Fig. 2, ESP8266 Node MCU is the important part of the project which controls the LCD display, interacts with the INA219 (current and voltage sensor), which connects to the internet through Wi-Fi. The NodeMCU is powered by a micro USB port, while the INA219 and LCD module are powered by 5V DC from the NodeMCU. On

the NodeMCU's I2C peripheral, the D1 (SCL) and D2 (SDA) pins are utilised to operate the LCD display and obtain data from the INA219 module. The flash and reset on-board buttons on NodeMCU is utilixed to programme the device. The 5V output for INA219 and LCD is available at VU pin and GND on Lolin NodeMCU. Pin diagram of NodeMCU version can be referred to locate the 5V output pin.

INA219 Voltage And Current Sensor Module

To measure the voltage and current, INA219 module can be used. The voltage and current sensor module is given in Fig.3.

The INA219 is a voltage and current measuring module. Let's look at the module's technical specifications:

- Input supply ranges from 3.0 to 5.5 volts.
- Measuring terminals Vin+ and Vin-
- Measures voltages up to 26V.
- Current measuring in both directions up to 3.2A.
- 0.1 ohm shunt resistor

I2C is a supported communication protocol.D1 and D2 pins on the module link to NodeMCU. This module receives 5V from the corresponding output pin of NodeMCU. The Vin+ and Vin- pins are used to detect both current and voltage across a load. Please carefully observe the circuit schematic and do not reverse the polarity of the load or the external supply. If a breadboard is used in the system, the load is connected to the Vin+ and Vin- pins that is next to the SDA and SCL pins, or use the screw terminals if your load uses more than 500mA.

I2C Adapter and the LCD Display

The suggested project employs an I2C adapter for a 162 LCD display, reducing the amount of wires connecting the NodeMCU to the LCD to four: Vcc, SCL, GND and SDA. The I2C adapter module uses the same SDA and SCL bus as the INA219 module and is powered by 5V. The module's accompanying trim pot may be used to adjust the display's contrast.

The Appliance Scheduling & Control algorithm is used for data accusation and appliance monitoring. Remote monitoring, energy efficiency optimization, appliance control, appliance automation, and a complete intake statistics document primarily based totally on analysis displaying the appliance status report are just a few of the many benefits of IoT-EMS. The timing switches and Wi-Fi relay are connected to the controller, which manages the appliance's operating length while also documenting the load state for future development. Users may view real-time energy use information and receive a full picture of energy savings with remote monitoring. Raspberry Pi will offer the best scheduling options based on the data obtained for maximum power savings to the user using the peak and off-peak rates as a comparison.

The IoT-EMS controller's algorithms are employed to achieve energy efficiency. The algorithm's working logic is set up to loop for low-cost power appliance shifting, as well as peak clipping and valley filling for demand side control. It may maintain track of the condition of the appliance and provide a push notification to the user. By using this low-cost effective method, every home's efficiency might be boosted by 15-20%. This will aid in the reduction of energy costs, greenhouse gas emissions, and demand control. The Internet of Things Energy Management System may also arrange time-shiftable loads for off-peak or low-energy-price hours (IoT-EMS). IoT-EMS has scheduled the dishwasher to run during off-peak hours rather than peak hours, as can be observed. Even during off-peak hours, the rescheduling was done at a low-demand period.

Proposed System

The suggested IoT-EMS, as shown in fig.7, uses electrical switches to control household appliances in a systematic manner, improving energy efficiency and lowering carbon footprint while ensuring user safety and comfort. Before transferring the data to the IoT cloud and guiding the appliance control relay, the controller evaluates the input from the sensor and relay and analyses it using a pre-programmed algorithm. Customers will receive weekly and monthly energy consumption statistics from the IoT-EMS, allowing them to have a comprehensive understanding

and awareness of their household's energy use. Because it is linked to a website, the entire system may be seen and operated from any internet-connected personal device. The data will be analysed using a smart algorithm for appliance scheduling and control of networked devices.

An Ardunio uno controller with IoT capabilities and a Wifi 8266 module are utilised in this setup. Solar panels and energy metres provide data to the cloud, which is controlled by a microcontroller. Finally, the sun and the energy metre provide steady energy to the user. The Internet of Things (IoT) uses a wireless network to track energy metre readings and theft associations throughout the world. Under a range of load conditions, the complete system is successful in reducing power theft and lowering the consumer's electricity cost. In terms of control architecture, individual control, centrally controlled systems, and distributed-controlled systems, there are many different types of energy management topologies.

Separate control devices employ controllers and timers, but a distributed controller system uses a connected network to communicate inside. A central controller has the advantage of making it easier to correlate all of the machines with their sensors in a single system. It also features a feature that makes interacting with the cloud server a breeze. In case of traditional appliances, Parallel manual control has been kept to mitigate the danger of single-point failure. Arduino, the cloud with a network of linked appliances were used to construct the IoT-EMS. A wireless connection connects the sensor network with the cloud. The cloud service aids with database, algorithm execution and data analysis, while the controller acts as a data accusation and control unit. Based on the cloud-based scheduling logic and the condition of the environmental sensor relay, the appliance control relay was employed as intelligent switching.

An efficient strategy to manage energy is developed based on the user's needs and lifestyle. The current controller is unable to govern new digital data inputs in the context of home automation. As a result, we employ an Arduino controller in the proposed system, which has more digital inputs and can control more data in parallel. Figure 6 depicts the usage of IoT technology to monitor the energy metre and solar energy. Modifying the LDR value affects solar energy, and the ardunio uno controller is controlled, with both values shown on the LCD.

RESULTS

Small-scale implementation using low-voltage equipment has been done to assure safety in the laboratory setting. Figure 7 depicts the completed system, which includes all of the necessary connections and appliances. Solar power is used to generate electricity, and the output is shown in fig.8. Using clever sensing, it guarantees that the amount of energy generated by the panel meets the demand. It uses sensors to automate the load. Relay modules control the loads, which are automated via IoT.

It calculates the total energy produced by the solar panel as well as the total energy consumed by the system's loads. It also detects energy theft by analysing sensor outputs. As illustrated in fig.9, a desktop application has been developed to monitor the energy management system

Nodemcu receives the sensor output, which is subsequently processed by the microcontroller. The functioning of loads will be automated by relays based on the inputs to the microcontroller. As data is transferred through cloud storage, the quantity of solar panel generation and energy consumption by loads from smart sensing devices is shown on the web page. Consumers can access real-time data from a faraway location thanks to the Internet of Things. The produced web page shows the amount of power spent by the load as a graph, together with the average and peak demand of the loads over time. Figures 10a&b demonstrate the different output parameters of the proposed IoT-Ems system

The data report collection of the particular date can be generated from the system as shown in fig.11.

CONCLUSION

This study recommends combining Internet of Things (IoT), optimization approaches and data analytics to maximise energy savings while retaining the highest degree of user comfort, dependability and safety. It was expected to help with demand-side management as well. The most systematic method to improve energy efficiency in household

energy consumption is through intelligent appliance scheduling and switching. IoT and machine learning can assist reach this aim by providing real-time online monitoring of energy behaviour, intelligently scheduling equipment and data analysis to cut energy use. When compared with fixed scheduling and passive energy saving approaches, IoT-based data collection and management of home appliances has dramatically boosted energy efficiency. The efficiency of the smart home power management system has increased by 10% when compared to the conventional technique of using with the use of occupancy sensors.

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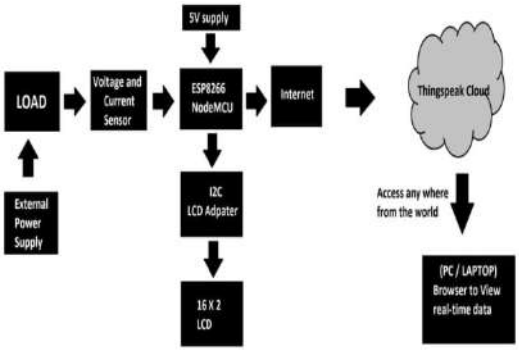

Fig.1 iot based energy management system

Fig.2 nodemcu

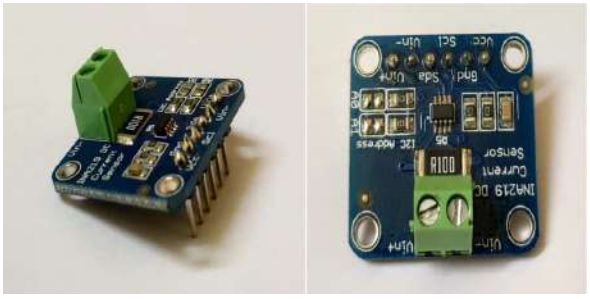

Fig.3 ina219 voltage & current sensor

Fig.4 i2c and lcd

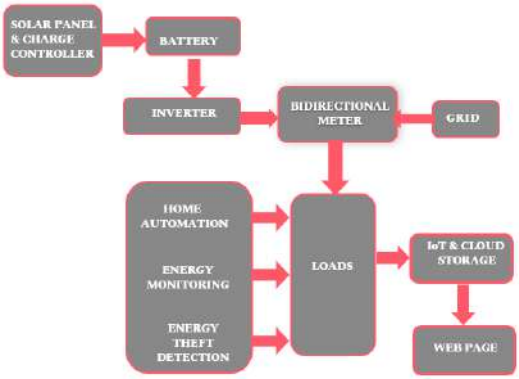

Fig. 5. Functional block diagram of proposed iot-ems.

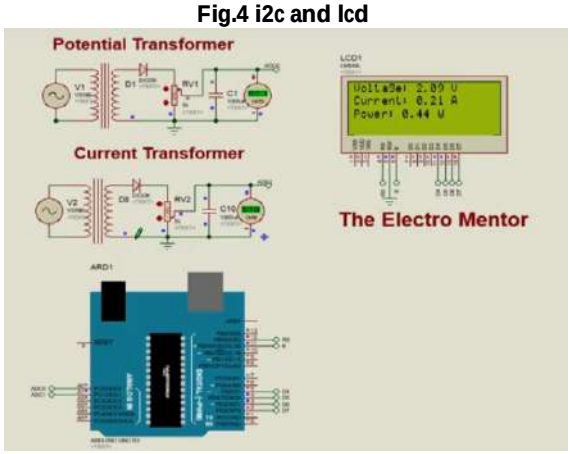

Fig. 6. Circuit diagram of the proposed iot-ems.

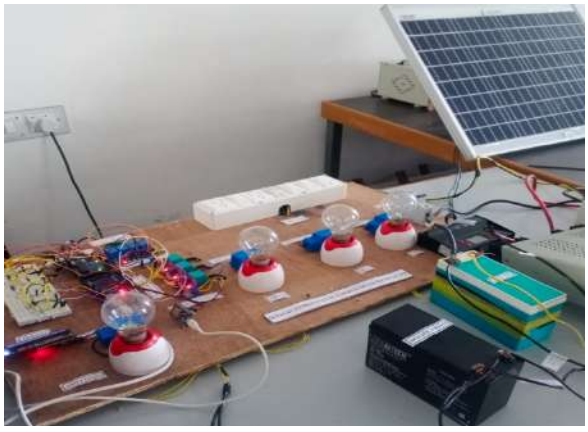

Fig. 7. Hardware setup of the proposed iot-ems.

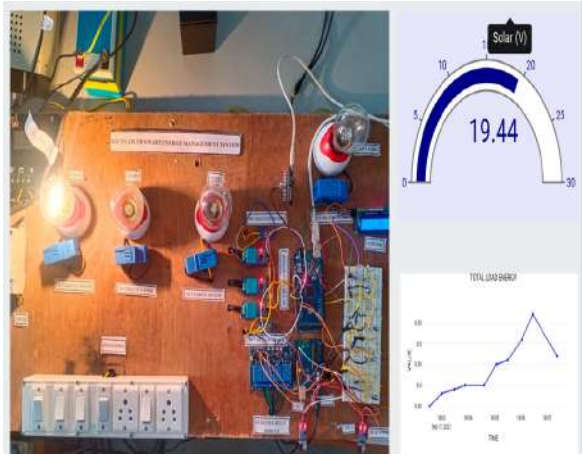

Fig. 8. Solar output

Fig. 9. Dashboard for iot-ems.

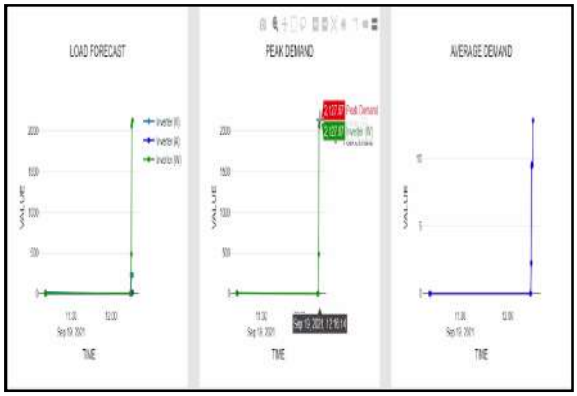

Fig. 10.a output parameters of the suggested iot-ems.

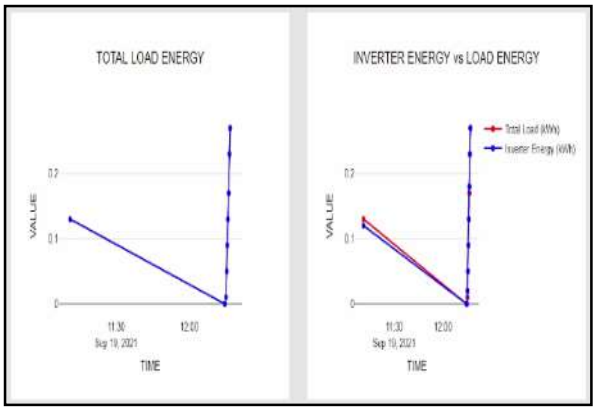

Fig. 10.b output parameters of the proposed iot-ems.

SNO	SOLAR	INVERTER				LOAD 1	LOAD 2	LOAD 3	BATTERY		TIME
	(V)	(V)	(A)	(W)	(kWh)	(kWh)	(kWh)	(kWh)	(V)	(A)	(YYYY-MM-DD HH:mm:ss)
92	12.5	0.04	0	0	0	0	0	0	12.43	0	2021-09-19 10:46:29
93	12.5	0.05	0	0	0	0	0	0	12.48	0	2021-09-19 10:46:49
94	12.5	0.05	0	0	0	0	0	0	12.48	0	2021-09-19 10:47:07
95	12.16	36.85	2.24	0	0.01	0	0	0	12.45	0	2021-09-19 10:47:24
96	12.13	42.13	2.31	84.66	0.01	0	0	0.01	12.48	0	2021-09-19 10:47:40
97	12.23	231.18	2.09	0	0.11	0	0	0.11	12.48	0	2021-09-19 10:48:18
98	12.21	230.7	2.29	464.16	0.13	0.01	0	0.12	12.48	0	2021-09-19 10:48:34
99	12.18	231.4	2.15	536.11	0.14	0.01	0	0.13	12.45	0	2021-09-19 10:48:51
100	12.31	234.57	2.26	481.64	0.15	0.01	0	0.13	12.48	0	2021-09-19 10:49:08
101	12.16	231.94	2.57	505.35	0.16	0.02	0	0.14	12.5	0	2021-09-19 10:49:25
102	11.74	229.94	2.06	572.41	0.17	0.02	0	0.15	12.48	0	2021-09-19 10:49:43
103	12.11	229.32	2.1	0	0.12	0	0	0.11	12.52	0	2021-09-19 10:50:04
104	11.67	227.51	2.23	461.73	0.13	0.01	0	0.12	12.48	0	2021-09-19 10:50:20

Fig. 11. Output report

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

New Era of Smarter Food Safety: Insights from FDA's Blueprint

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Received: 25 Jan 2022 Revised: 24 Feb 2022 Accepted: 23 Mar 2022

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ABSTRACT

The food system is expected to undergo greater changes in the next ten years than it has in the previous several decades. Food is being prepared, new foods verities and production techniques are being improved, and the food chain is becoming digital. The COVID-19has presented some challenges and emphasized the need for modern methods. It has shown the requirement for all the more continuous, information-driven, agile ways to deal with assistance guarantee a solid food framework, and guard all individuals during an emergency. The New Era of Smarter Food Safety is another way to deal with sanitation that joins innovation and different apparatuses to create a more secure, more advanced, and discernible food framework. It aims to reduce the number of illnesses associated with food borne illness in the United States. This article represents the achievable goals of the FDA to build recognizability, work on the prescient investigation, react quicker to outbreaks, address new plans of action, decrease food defilement, and empower the foundation of more grounded food handling societies. The article also highlighted the core pillars that make up the FDA's approach to fighting foodborne illness in the country.

Keywords: Food, Safety, FDA, FSMA, Outbreak, Modernization.

INTRODUCTION

Despite many developments in food safety over years, foodborne diseases in the United States have remained relatively unchanged. As pert he Center for Disease Control and Prevention, foodborne infections affect 48 million Americans (1 in 6) each year, resulting in 128,000 hospitalizations and 3,000 deaths. This is a major public health problem that is mostly avoidable [1]. This regulation comes after many illness outbreaks and recalls involving fresh-

cut lettuce in 2020 alone. In June 2020, 90 products from the same facility were recalled due to a Cyclospora illness outbreak. At least 206 persons were affected by the incident, which was connected to a single manufacturing company. Outbreaks like this will be reduced by the FDA's new regulation, as each step of the supply chain will have detailed data to track and manage [2]. The main objective is to reduce the number of foodborne illnesses in the country. Essentially, FDA is attempting to develop a stronger legal environment for food standards while simultaneously employing new developing technologies and methodologies to improve our foresight skills, faster outbreak response, and improve abilities to quickly respond to food supply problems. While technology is a key element of the new era of smarter food safety. It's all about using more current, simpler, and more precise techniques and procedures. It's all about being inventive and leadership and establishing a culture of food security that transcends the public and private sectors. This new era of Smarter Food Safety is people-led, FSMA-based, and technology-enabled [3,4].

The Food Safety Modernization Act (FSMA)is extensive regulation intended to safeguard the people by encouraging food manufacturers to focus on avoiding sickness instead of reacting to outbreaks after people have become ill. FSMA's tools and authority help to develop an adaptable structure that can react to altering food quality conditions. The FDA has embraced seven significant principles to execute FSMA, recognizing that sanitation is a common obligation across a wide range of focuses in the overall store network for both human and animal food. The FSMA rules are intended to clarify explicit moves that should be made at every one of these focuses to forestall contamination [1,5].

FSMA has the following seven rules [6]:

1. Produce a Safety Law: A law that governs the growth, harvesting, packaging, and storage of a product.
2. Regulations for Human Food Prevention and Control: Implementation of a food risk-based approach to food safety management, such as HACCP.
3. Animal Feed Control Prevention: The need for animal feed manufacturers including animal feed to follow a risk-based management system and good manufacturing practices (GMPs) such as human food production without allergen control.
4. Export Supplier Verification Program: Importers are required to ensure that imported goods are produced safely. This may include site-based testing
5. Authorization of Third-Party Auditors: Allows importers to rely on third-party auditors who are accredited or FDA-certified to certify that their imported items are safe to eat.
6. Intentional Adulteration of Food: Requirement to build up food protection frameworks to avoid food contamination
7. Sanitary Transportation of Human and Animal Food: Setting up sanitary controls and inspecting vehicles to ensure that they follow clean procedures.

FDA keeps on gaining ground in executing this basic principle that are the FSMA building blocks, which form the standards for human and animal food production, transit, and imports. Complete execution of the leftover FSMA commanded standards will support further pollution counteraction. The FDA's defense strategy, on the other hand, must continue to emerge. Because of advancements in detection technology more epidemics are being discovered than previously possible. Recognizing this fact, the FDA intends to focus on improving prevention, recognizing contaminated food immediately, and assisting in its removal from the market. Our world moves at a dizzying speed. New technologies, beginning with digital innovation to new sources of food, have emerged as a result of this change. It also introduces innovative business models, such as e-commerce and omni channel food supply, including online shopping, mobile, mobile, and store shopping. These developments offer new techniques and methods for addressing food safety concerns, but also raise new questions about how to govern food safety. The FDA has decided that it is the moment to reconsider food safety's future, with a process that builds on the FSMA's making headway in establishing a risk-based regulatory structure while protecting the use of innovations to build a secure and easy-to-follow digital system [3,4]. According to the blueprint, the FDA intends to engage firms, academic institutions, business organizations, civic groups, and regulators, as well as agencies and entities with which it has never

collaborated previously. The overall purpose is to reduce the number of diseases associated with foodborne illnesses in thecountry [4,7].

DISCUSSION

Principles of smarter food safety [3,4]

People-focused and led

Consumer confidence in the diet plan will be enhanced by a greater focus on safety, improved food tracking, and ongoing attempts to offer customers effective and precise data on the products they eat. We need activities educated by the best aptitude to address the developing intricacy of the food framework. Our general wellbeing mission depends on logical and administrative information, and FDA workers and partners are indispensable to its future achievement. The FDA will unite assorted gatherings to discover shared views and foster imaginative arrangements.

FSMA-based

The FSMA created prevention-based system will serve as the foundation for future development. The future of food and food security is being re-invented by digital technology and a more complex, powerful global supply chain. The FDA's collective vision and dedication will be required to realize the opportunities that come with transformation. To further increase food safety, this methodology will stack instructive and specialized progressions onto the FSMA base.

Technology-enabled

The entire world is going digital at a breakneck speed. Artificial intelligence, the internet, technology for sensors, and blockchain are all helping to improve business measures. New computerized advances can support the forecast and counteraction of sanitation issues, just as the discovery and reaction to issues that do emerge. FDA will help out partners to research low-or no-cost choices sooner rather than later, guaranteeing that drives are comprehensive of and feasible for food organizations, all things considered. With regards to ensuring an assorted food framework, there is nothing of the sort as one-size-fits-all, as we discovered with the FSMA regulations.

Process for developing the blueprint [3,4]

FDA revealed the New Era of Smarter Food Safety project in April 2019. In July, FDA's Foods Program administration ventured out, collecting a group of specialists from the organization to offer their viewpoints on the most proficient method to make this a reality, starting with thoughts and advancing to a dream of how those thoughts may be executed. The Office of Food Policy and Response, the Center for Food Safety and Applied Nutrition, the Center for Veterinary Medicine, the Office of Regulatory Affairs, and the Office of the Commissioner were addressed by the specialists. During brainstorming sessions on these fundamental themes, almost 100 experts took part. Delegates were questioned about releasing their thoughts in the initial round of brainstorming sessions, without regard for practical factors such as staffing and resources. The time for pragmatism arrived in the second set of sessions, when these experts re-examined their thoughts, considered viability, and proposed genuine targets for the next ten years.

The FDA hosted an open conference in October 2019 to engage stakeholders and encourage communication with local and legal allies, manufacturers, consumer activists, and other stakeholders. Over 1300 persons have attended the event, physically or on the streaming. In addition, the FDA has established a docket for the Federal Register for community input. After the end of the response time on the 5th of December, FDA staff met and assessed all responses to use in the draft. The Administration then published the New Era of Smarter Food Safety Blueprint on July 13, 2020 (delayed from Spring 2020 due to COVID-19-related issues). This blueprint highlights the strategy the FDA will take to build a New Era of Smarter Food Safety that advances in tandem with food sciences and systems, as well as the consequences these changes will have on the food industry and consumers.

This document incorporates responses from a variety of stakeholders and provides an overview of the next decade, including short-term and long-term objectives that will change as we make progress with the application. The key will guide resource allocation and can be adjusted according to food safety metrics and participant feedback.

The Four Core Elements

The New Era of Smarter Food Safety is based on an establishment of advances, investigation, plans of action, modernization, and qualities. There are a lot of synergies here; an idea from one angle could be identified with at least one other. For example, Analytical procedures can be applied to detect ability and creative plans of action. The significance of food handling society is entwined with the subjects of shared dependence among government and state accomplices. Measurements and solid outsider reviews are normal necessities. Together, these components will usher the authority into the New Era of Smarter Food Safety. The Four Core Elements are shown in figure 1.

Core Element 1: Tech-Enabled Traceability

Most of the documents utilized in the food store network are still on paper. These outcomes in a framework where one stage forward is needed to figure out where the food has reached and one stage return is needed to decide the earlier source. This, together with an absence of information distinguishing the item across the store network, makes it hard to monitor and follow food rapidly. Throughout the spread, this could lead to loss of life, a large sum of money in unavoidable goods losses, and loss of citizen confidence. The anonymity and unavailability of the dietary system are the major weaknesses that have hampered the progress made in the rapid recovery efforts, as evidenced by the emergence of fresh leafy greens and other products throughout the last ten years [4].

The FDA works to keep track of consumer protection from goods that are affected by creating immediate issues, locating certain resources, and assisting in the immediate removal of products from the market where appropriate. The new era is largely focused on enabling a sophisticated and traceable food supply chain.According to the plan, the first phase will be to finalize the implementation of the FSMA Section 204 to align the data priorities with the tracking events required to achieve improved compliance. Establishing this basis for tracking will enable commodity participants to adopt and use digital-enabled technologies, allow sharing of information, and present ways to significantly lower the amount of time required to determine the source of food that has been tainted. In the case of an emergency of population health, such as an epidemic, this will also provide the necessary precautionary measures and help prevent the disruption of the supply chain [3,4].

A definitive objective is to have started to finish detect ability across the whole food handling framework. The FDA will investigate approaches to urge firms to deliberately take on tracking advancements and approaches to fit tracking services, which will uphold interoperability across an assortment of innovation arrangements, pursuing results that are feasible for all areas.
It includes three primary goals [12,13].

Develop Foundational Components

The FDA seeks to promote the development of "basic components" (e.g., acceptance of existing compliance standards). This includes organizations across the food system that speak the same "follow-up language" by setting up key monitoring events and data items. The FDA seeks to increase its ability to process data faster on all food items by supporting voluntary development of adherence compliance to meet the most comprehensive inventory. In the blueprint, the FDA mentions standards bodies like GS1 and Codex and advocates for the adoption of food business standards to assure interoperability.

Encourage and Incentivize Industry Adoption of New Technologies

The FDA emphasizes the benefits of follow-up access to the food sector and non-traditional partners such as the industry of financing and tech industries to boost adoption of new technologies in the industry. The agency intends to find ways to incorporate strong traceability systems into its food safety activities of supervision, such as risk-based inspection designing. FDA empowers food traceability technology suppliers to create inventive monetary low-to-no-

cost models arrangements, corresponding to rewards got from taking part and empowering food makers, everything being equal, to partake in a versatile, savvy way.

Leveraging the Digital Transformation

The Agency plans to evaluate its existing pandemic and recall policies, featuring how it asks to trace backfirms' requests and gets data in digital form, as well as cooperate with the province and regional partners to develop techniques for doing expedited tracebacks and trace forwards. The Agency also plans to run a pilot on concepts for scaling up traceability. Industries that have recently been the focus of outbreaks would be given first consideration. Another goal of the FDA is to create a digital technology system for internal use, such as blockchain, to obtain important monitoring activities and facts from industries and to synchronize its efforts with other FDA-regulated product traceability efforts.

Core Element 2: Smarter Tools and Approaches for Prevention and Outbreak Response

FDA tries to investigate the value for prevention of modern food safety methods that produce new information streams just as apparatuses for quickly breaking down enormous information. FDA is hoping to improve and reinforce Root Cause Analysis (RCA) and prescient examination. RCA discoveries can be a helpful device for changing cycles to forestall perceived dangers and giving more hearty information to the prescient investigation. It's additionally basic for the FDA to work together with others in fresh and imaginative ways. FDA's internal mutual dependence project, for example, intends to expand on existing endeavors to work together with states that have comparative administrative and general wellbeing frameworks, utilizing each other's information and examination to guarantee the most productive utilization of assets and extend our food safety reach. They likewise incorporate depending on solid outsider reviews to advance food handling and having reinforcement designs if customary techniques come up short. The FDA and its regulatory partners will use these tools and approaches to improve inspections, outbreak response, and recall modernization [8].

It includes six primarygoals: [13,14]

Invigorate Root Cause Analyses

Root Cause Analysis (RCA) is one of the most important steps in reducing food safety hazards after an incident. This goal includes improving communication tools as well as reinforcing and expanding root cause analysis techniques and reporting patterns. To improve the chances of anticipating and reducing future contamination episodes, the FDA aims to include RCA data into the agency's threat level and anticipatory analytical tools. All stakeholders, including governments, merchants, and producers, will prioritize collaboration and information exchange relating to this process.

Strengthen Predictive Analytics Capabilities

This objective includes spreading the utilization of Artificial Intelligence (AI) and learning tools, just as extending the FDA's confirmation of the idea of utilizing AI for food screening. FDA is investigating approaches to make public-private "information trusts," or a lot of information created by an industry that can be open for logical work to upgrade anticipation methodologies. FDA needs to start by attempting to make a " leafy greens data trust." FDA wishes to team up with partners to make interaction for breaking down large information and non-customary information sources that may be used to improve foodborne forecast capacities and create better risk management choices.

Domestic Mutual Reliance

The FDA takes note that "domestic mutualreliance" is a drive to work with states to use one another's information and examination to guarantee that assets are utilized productively and that their food safety approach is augmented. The organization plans to cooperate with state and city governments on challenges like prioritization of risk and classification, as well as the general guidelines for testing sample collection. This objective likewise incorporates reinforcing government state quick reaction groups to elevate a coordinated way to deal with crisis and occurrence reaction coordination.

Inspection, Training, and Compliance Tools

FDA tries to assess the achievability of utilizing distant, virtual, and additionally part examinations of external and local industries with an exhibited background of consistency for office prioritization objectives, just as to utilize mobile investigation innovations and computerized reporting tools to smooth out the organization's inspectional and announcing measure. Industry's utilization of sensor innovation to build observing of basic and preventive control focuses is likewise being upheld and assessed. FDA likewise needs to support the utilization of "reliable" outsider reviews.

Outbreak Response

FDA investigates the hindrances and components that could make it simpler to utilize industry food evaluating outcomes to spot breakouts. FDA desires to investigate choices for state offices to submit foodborne infection data to government specialists all the more rapidly. FDA additionally needs to grow the utilization and limit of existing instruments like Genome Trakr, Pulse Net, and Artificial Intelligence (AI) to extract non-conventional information sources (e.g., client online surveys) to identify breakouts.

Recall Modernization

FDA's objectives for recall modernization concentrate generally around information correspondence, including blending how FDA and United States Department of Agriculture (USDA) impart review data to customers, growing accepted procedures on shopper warning of reviews, and investigating various innovations to upgrade outside interchanges and the adequacy of recall. (e.g., building up an administration app for cautioning buyers about recalls).

Core Element 3: New Business Models and Retail Modernization

The third core component emphasizes shielding food sources from tainting as modern plans of action arise and shift to address the issues of the new purchaser. The advancement of how food is delivered from the ranch to the table proceeds with the development of internet business and new conveyance designs. With the introduction of new plans of action that advance leap forwards in novel ingredients, new food varieties, and production of new foods frameworks, the difference in how food is delivered proceeds. These new models incorporate an internet-based supper and basic food item purchasing, which has become progressively well known since the COVID-19 pandemic. The FDA is looking into methods to modernize and assist with guaranteeing the wellbeing of goods sold in restaurants and other retail foundations by exploring more traditional business models. Restaurants and other retail facilities continue to be the most common source of foodborne illness outbreaks, according to the Centers for Disease Control and Prevention (CDC). To improve behaviors and practices, the FDA will focus on the known risk factor. [13]. Itincludes two primary goals: [13]

Ensure Safety of Food Produced or Delivered Using New Business Models

FDA tries to team up with administrative accomplices to deal with new plans of action that may not be covered by FSMA to confront the element that "owns" the last-mile food. It additionally needs to work with food conveyance organizations to teach individuals about the significance of appropriate food taking care of, featuring an effort to firms like UPS, Uber, Door Dash, and Lyft. FDA needs to advance the utilization of innovation that screens item hazard factors like time and temperature. Working with the protected improvement of new food fixings and assembling innovation is likewise important for this objective.

Modernize Traditional Retail Food Safety Approaches

The FDA is thinking about a few initiatives to modernize retail food safety, for example, advancing the improvement of business smart kitchen hardware equipped for checking time and temperature measures, advancing the utilization of new computerized instruments that trigger wanted practices (e.g., handwashing), underlining the significance of office and gear plan for retail food handling the board, and recognizing mediation techniques known to be compelling at decreasing the foodborne ailment hazard factor. FDA additionally needs to work together with the Conference for Food Protection to increment steady reception of the FDA Food Code and further include a retail

food safety management method for outlets inside the Food Code. Also, FDA plans to upgrade preparing educational programs for government, state, nearby, and regional retail controllers, just as advance exploration on retail food handling and work together with accomplices to further develop existing administration affirmation, food overseer preparing, and instruction.

Core Element 4: Food Safety Culture

The Blueprint centers on developing, supporting, and strengthening food safety cultures on ranches, food processing plants, and households. The blueprint states that without behavioral changes, the burden of foodborne illness cannot be decreased dramatically. Dramatic improvements in the weight of the foodborne infection will need everyone to do more to impact people's convictions, perspectives, and, most crucially, their practices and the activities of associations. Effective food safety management requires a strong food safety culture. [8]

It includes three primarygoals: [13]

Promote Food Safety Culture Throughout the Food System

This objective incorporates making a social advertising plan for food handling, empowering powerhouses like gourmet experts, bloggers, cooking shows, VIPs, and industry pioneers to demonstrate wanted safe-food practices, and giving preparing, schooling, and inspectional apparatuses that consolidate set up conduct science standards to encourage a food handling society. The agency looks to perform and uphold research on challenges, boundaries, and freedoms to impact and further develop food handling society practices, just as help the creation and harmonization of measures that organizations can use to survey their food handling society.

Further, Promote Food Safety Culture Throughout the Agency

The FDA's work in this space would involve deciding what an organization's decent food handling society means for assessment recurrence decreases and showing agents on the characteristics that signal a solid sanitation culture. The agency's objective likewise incorporates guaranteeing that the focal job of food safety culture is considered as a center precept in propelling the organization's food handling task, creating systems to additionally fortify and quantify interior comprehension of food handling society

Develop and Promote a Smarter Food Safety Consumer Education Campaign

FDA tries to frame an alliance with new accomplices to advance food handling and influence new, tech-empowered famous mediums and devices to arrive at buyers with food safety informing as a feature of the shopper instruction campaign. FDA additionally plans to foster measures to help shoppers in accessing new food safety innovation and working with their reception of new food handling instruments, for example, apps.

Monitoring Process

This blueprint lays out a perspective in the coming ten years, including both long- and short-term actions that would change as the FDA works toward the objectives it targets. It is a dynamic document that will be updated as new food technology, food production, delivery techniques, and monitoring instruments become available. Each Core Element has been designated a lead, and the FDA will screen progress toward the anticipated results. FDA as of late launched the Food Safety Dashboard, a reporting tool for performance evaluation that tracks the effect of the FDA Food Safety Modernization Act's (FSMA) seven essential standards and helps in their proceeded with refinement. FDA expects this dashboard to be one of the approaches to follow the progress as a component of the New Era of Smarter Food Safety effort, which is innovation empowered and means to expand concerning the work completed under the FSMA rules. [8].

COVID and the new era of smarter food safety [8].

Significance of the new era relative to the Covid-19 pandemic

The epidemic highlights the need for advanced methods as the FDA responds to different needs in the food system, from unprecedented market inequalities to changing consumer behavior and the increase in online business, to the challenges of conducting traditional testing and compliance. The epidemic has underlined the requirement for all the more continuous, information-driven, and adaptable measures to assist with guaranteeing a solid and tough food

framework and guard all individuals during an emergency, regardless of whether government staff, food industry laborers or clients.

Food safety and security needs highlighted during the pandemic

Improved tracking, as well as advanced analytics technology, can offer a more extensive supply network awareness and can assist the FDA and firm in anticipating the types of market inequalities identified during an epidemic, as the epidemic has shown. Coronavirus has facilitated the necessity for the most effective methods to guarantee the safety of foods requested on the web and conveyed to buyers straightforwardly. The trend has been steadily growing in recent years but has erupted in recent years as families have been flocking home and beginning to order foods from the restaurant on the web and via telephone, frequently for the very first time. It turned out to be more significant than any time in recent memory for the FDA to sort out some way to guarantee that these food products are manufactured, packed, and shipped securely to customers. Furthermore, the pandemic uncovered having solid food handling societies, which incorporates individuals who are employed on farms and in establishments tolerating liability regarding creating safe food varieties and protecting colleagues when they become sick. Food handling society additionally incorporates showing purchasers the best sanitation measures for home cooking, which has become progressively famous since the outbreak.

CONCLUSION

The blueprint lays out the FDA's strategy for bringing in the New Era of Smarter Food Safety over the next decade. It builds up clear objectives to further develop recognizability, work on prescient examination, react all the more rapidly to outbreaks, address new plans of action, lessen food pollution, and cultivate the advancement of more grounded food handling societies, with the cooperation of government, industry, and public health advocates. A challenge experienced by past food safety innovations is the siloed idea of the data and the failure to incorporate information into existing projects for big business-wide use and investigation. Quality groups should assess not exactly what they require or require a mechanical answer for having the option to perform, yet additionally where the data will dwell and the necessities of different offices with the goal that it adds value beyond a solitary departmental group all along.

It is important to remember that the Blueprint is for everybody in the food area. All partners, including end-clients, customer associations, makers, wholesalers, buying specialists, innovation organizations, and others, will possibly impact the last program throughout the following two years, from the biggest restaurant network to the littlest mother and-pop food business. FDA also outlined important steps it expects to take over the next two years to advance the New Era Blueprint. These are some of them [17]

- Finishing FDA's Food Traceability Rule.
- Fostering the FDA stage for getting electronic discernibility information.
- Investigating approaches to harmonize internal and worldwide detectability endeavors.
- Growing recognizability pilot exercises beyondleafy greens.
- Facilitating workshop to draw in partners on Root Cause Analysis and data sharing tools.
- Modernizing reviews by researching the reasonability of building a versatile application for government reviews and inspecting systems to blend informed with the US Department of Agriculture.
- Arranging a New Business Model Summit in 2021, at which partners can distinguish and talk about food handling issues applicable to new food retail plans of action.
- Proceeding to draw in with partners including industry, government, the scholarly community, and customer bunches on different parts of the New Era Blueprint.

On the off chance that the FDA prevails with regards to satisfying this command in the years to come, the advantages to the US food framework will have extensive impacts on general wellbeing and the economy, and the US might be at the cutting edge of worldwide administration and oversight.

FDA stressed that it will keep on working together and look for input from different partners, including industry, as it carries out the New Era Blueprint. Therefore, firms should keep on surveying how their short-and long-haul objectives interface with Blueprint's objectives and search for approaches to collaborate with the office on these issues later on. Contributing forward the ideas in this plan, the joint effort and cooperating and considering some fresh possibilities, will make a more computerized, detectable, and more secure food framework that propels sanitation, works on the personal satisfaction for buyers in this nation and everywhere, and better sets up for surprising occasions that could affect the food supply.

ACKNOWLEDGEMENT

The authors thank JSS Academy of Higher Education and Research and JSS College of Pharmacy, Mysuru for providing the necessary facilities to carry out this work.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest for the publication of this manuscript.

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Figure 1: The Four Core Elements of New Era of Smarter Food Safety

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Intrusion Detection System using ML Techniques

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Received: 14 Jan 2022 Revised: 23 Feb 2022 Accepted: 22 Mar 2022

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ABSTRACT

In the era of unaccountable advanced smart services, each person is surrounded by plenty of smart devices and comes in contact with these on a daily basis. The enhancement of IoT leads to more security challenges as compared to before as it also introduces different types of attacks and its severity is concerned for combating the cybersecurity attacks and threats that quarry them, including malware and privacy breaches, denial of service attacks, are included among others. For target and analyze such challenges, we introduce a model that uses machine learning algorithms and can be integrated with edge devices to detect such anomalies. The proposed model has been applied to the KDD Cup 1999 Data to validate its ability and accuracy to detect attacks. The satisfactory objectives can be demonstrated through the result obtained from the proposed model.

Keywords: Intrusion detection, KDD Cup 1999 datasets, Machine Learning, security, ML classifiers

INTRODUCTION

The Internet of Things is diversifying in all places, offering a variety of benefits to almost every single aspect in our lives, such as healthcare, Media and entertainment, commerce, industry, intelligent institutions, and workplaces. Every device can connect to the worldwide Internet and connect to the web or mobile devices we handle , or share data (Atzori, L., Iera, A., & Morabito, G., #). This developing IoT technologies deal with various security attacks and Denial of Service (DOS) attacks, security threats to resources, and disruption of IoT networks and devices as well as the connections between them, must be protected: massive amounts of data can be shared between devices, as well as between customers, and also the compromised availability and confidentiality of the information which can have a significant impact. Along with all the other benefits, the Internet has also developed many ways to jeopardize the instability and insecurity of the systems and servers connected to it. Although static security measures such as security walls and software development can provide a decent level of security, more robust security measures such as intrusion detection systems should also be used. Machine learning

algorithms offer a viable alternative for securing IoT devices. ML is a powerful artificial intelligence technology that can outperform dynamic networks and does not require precise coding. Machine Learning and deep learning approaches may be used to train the machine that can detect various anomalies and provide appropriate defence measures and insights. Hence, the attacks and threats can be detected at a very early stage. Moreover, ML techniques turn out to be promising in detecting new types of attacks using learning skills and effectively handling them intelligently. We propose a combination of already built architecture and ML strategies that provides real time threat detection and protection of IoT security attacks, which generates an improved architecture. This improved architecture can be attached with any model-driven solution to provide graphical definition of the defensive threats. This can be done using ESB, a middleware. Its function is to create data connections between IoT networks and ML algorithms. Not only limited to this, we can even connect to third parties such as servers, cloud, and alarming sources using ESB. We're working on adding the ability for the predictor to automatically recognize which package properties are most important in our domain context.

The revised architecture for an IoT network prototype was built using KDD Cup 1999 data and machine learning techniques. A standard collection of data to be studied is included in the data, which comprises a range of simulated interventions in a military network environment. Dataset contains 41 features, 5 different attacks (DOS, normal, probe ,r2l ,u2r), and 3 protocol types(ICMP, TCP, UDP). The other way which can be used to generate the dataset to study and design a model is by simulating the data using the help of NodeRed Mqtt. Different machine learning algorithms can be effectively used and then verified to categorize the data as normal and abnormal threat data. Evaluation of the classifiers (ml models) is done on the accuracy, error rate, recall, precision, score rate, and F1 score. The K fold method is used for data sampling which generates 5 independent sets: training (80% of data) and testing classifiers (20% of data), where each set contains all kinds of attacks. Confusion Matrix is generated for every classifier implemented. The result generated provides the potential capability of our classifiers models being tested. E.g.: SVM, naive Bayes, decision tree, Logistic Regression. The paper is arranged study the different attacks on the IoT Networks and present an overview of the security of IoT and why it is important is also illustrated. The analysis of the dataset on ML-based security is also discussed and going to study and try to build a better model to detect anomalies in the IoT-Network.

LITERATURE REVIEW

Rüdiger Gad [1] Various uses of events in network research and surveillance were investigated, as well-as four actual use-cases and how they addressed utilizing C E P and event pattern matching. A model driven strategy for real time decision making in SOA 2.0. The Knowledge Based Systems research calculates the standard deviations in relation to the detection rate of each assault type to assess the methods' performance and resilience. Different predictive machine learning algorithms were examined, including Decision Trees and Random Forests, as well as probabilistic processes like Naive Bayes and Gaussian. Farnaz Gharibian and Ali A. Ghorbani discovered that probabilistic processes are more robust than predictive ones when trained utilizing a variety of training datasets [2]. Machine learning-based security solutions for the Internet of Things are examined in depth in this paper. The study's major purpose was to look at a variety of security risks, attack surfaces with repercussions, ML techniques, and security solutions. There is also a comparison of numerous supervised and unsupervised learning approaches [3]. Creating an IDS for an IoT Environment Using ML method Mr. R. Karthi and his colleague's designed adversarial systems to produce attacks using Node MCU and DHT11, and devised and built a machine-learning approach to recognize and categorize network assaults (humidity and temperature sensor). They were able to develop the most accurate decision tree model possible [4]. In the paper, they propose a hybrid approach and a unique framework model to solve Bot-IoT attacks and IoT traffic detection in a smart city. They investigated five known ML classifiers and used a shoddy data mining tool to do so. All of the chosen ML classifiers are run on the Weka application using ten-fold cross validation. For identifying abnormalities and intrusions in IoT networks, the Naive Bayes ML algorithm was demonstrated to be far better than the other ML methods [5]. Mohamed Faisal Elrawy did a comparative analysis of the most current IDSs developed for the IoT paradigm, concentrating on the methodology, features, and processes that were relevant. This research looked at a number of articles. The purpose

of this research is to develop and create IDSs for use in smart environments using the Internet of Things (IoT). This article discusses the IoT architecture, as well as security vulnerabilities and their interactions with the layers of the IoT architecture [6].

In order to have a better knowledge of strategic investigations, Nadia Chaabouni and Mohamed Mosbah conducted a poll on IoT risk classification. Based on learning approaches and state of-the art intrusion detection findings, a complete review of NIDS for IoT is presented [7]. The recent trends involved in IoT research, which are driven by different applications and the need for convergence in a number of interdisciplinary technologies, as well as the overall IoT vision and technologies, are outlined in Rajkumar Buyya's study [8]. This paper presents a study of linear regression poisoning attacks and countermeasures, which includes a design for the statistical type attack , it required knowledge of the learning process, as well as extensively evaluated attacks and defenses on the four types regression models (OLS , ridge , LASSO, and elastic net) and variety of datasets from different parts [9]. For intrusion detection systems, examine various forms, repercussions, and surface attacks on IoT networks. The major focus is on machine learning classification algorithms used to IoT system networks to increase the efficacy of identifying threats. This paper focuses on the many types of assaults and attack classes. The accuracy of the classification model is 94.57 percent. The classification report also includes classification based accuracy and F1--score on the result (which ranges from 0 to 1), as well as applying the model to training and test datasets, which yields significantly more accurate results based on avg. macro and avg. TCP packet weight. The selection of threshold points, as well as the optimization of anomaly detection, should be precise to the dataset. As demonstrated in the table above, the model accuracy was projected using Machine Learning throughout the IoT network, with a comprehensive classification chart based on data obtained from an IDS.[10]

DATA SOURCE

The selected Data Set for our study is KDD Cup 1999 Data. The data has a generalized collection of analysed data that generates a variety of network attacks in a military context. The intrusion detection datasets developed by KDD 99 are taken from a 1998 DARPA initiative. It provides a baseline for intrusion detection system (IDS) builders to compare different approaches. A virtual military network, consisting of three 'targeted' PCs running a range of operating systems and apps, is used for simulation. Three additional machines are then used to generate traffic by manipulating various IP addresses. And then, a sniffer uses the TCP dump format to record all network communication. The estimated total time in seven weeks. Normal communication is built on the expected profile of the military network and the attack falls into one of four categories. The 4 types of attacks are User to Root, Remote to Local, Denial of Service, and Probe. There are 3 parts of the selected dataset, namely “10% KDD”, “Corrected KDD”, and “Whole KDD”.The 10% of KDD dataset contains only 22 types of attack types which is a comparatively much shorter version of the “Whole KDD” database. It has a higher number of intrusion instances than traditional connections, and the types of assaults are not evenly distributed. Rejection of service attacks accounts for the majority of database attacks due to their nature. The "KDD Fixed" data set contains data with a statistical distribution that is different from "10% KDD" or "KDD Total" and it contains 14 additional attacks. The list of class labels and their corresponding categories present in the dataset used for our analysis for detecting types of attacks are described in Table 1. We have done our analysis on the KDD dataset described here.

DIFFERENT LAYERS IN IOT

IoT uses a wide range of internet connections to send data from very small devices such as switches and sensors to the cloud, local feed farms, or large data platforms to make the world model more accurate. IoT Architecture is a gateway to a variety of hardware applications, which helps to establish links and make life much easier. Various communication systems, like Bluetooth, WiFi, LPWAN, compact and Zig Bee, RFID, are adopted by different categories of IoT architectures in order to transmit and receive various data. The physical layer, application layer, and network layer are three layers that form the standard IoT structure (Fig 1)

Application layer

Mobile and web-based applications are used by the application layer to give services to users. The application layer works as an intermediary between the IoT device and the network it will communicate with. It controls data formatting and presentation and acts as a visual link between what an IoT device does and the data it generates is transmitted over a network. IoT applications can be smart homes, smart cities, smart health, animal tracking, etc. It is responsible for providing services to applications.

Perception / physical layer

The perception layer is the first layer in the IoT architecture, and it functions similarly to the human eyes, hearing, and nose. It's in charge of identifying items and getting data from them. RFID, 2-D barcodes and sensors are only some of the sorts of sensors that may be attached to things to gather data, which includes the physical (PHY) and Medium-Access-Control (MAC) layers. The MAC layer creates a connection between physical devices and networks so that they may communicate properly.

Network layer

The network layer is also known as the transmission layer. The network layer serves as a bridge between the perception layer and the application layer. Information is transmitted through this layer, which collects data from sensory material and distributes it to various levels as needed. The communication device may be wireless or phone-based. Responsibility for connecting smart devices, network devices, and networks to each other is also required. As a result, it is very susceptible to attacks from adversaries. The integrity and validity of data sent over the network have major security issues

METHODOLOGY

The major aim of our paper is to compute, compare and analyze the performance of different supervised machine learning techniques involved intrusion detection. Our goal is to compute the sensitivity and performance of supervised techniques such as Decision Tree, Naive Bayes, Logistic Regression, SVR and by using different distributions of training datasets. We are also trying to investigate and tabulate different accuracy of the techniques used to detect the different types of threats and attacks in the dataset.

Description of the KDD99 data set

There are mainly four types of attacks that were used in our simulations.

• **Probe**: Attacks that are deliberately crafted and automatically scan a network of computers and hardware to gather information or find known vulnerabilities i.e., the probable weak point in the computer system.
• **Denial of Service (DoS):** These attacks are intended to bringing a system or network to a standstill, making it inaccessible to its intended users. This attack inhibits legal requests from lawful users of the system by consuming excessive resources, such as flooding the target with traffic.
• **User to Root (U2R):** Beginning attacker tries to access the normal user account then obtain entry to the root by exploiting the weakness of the systems these attacks would be a threat to superuser privileges from a normal user privilege.

• **Remote to Local (R2L):** The attacks which actually results in a local user account launching a remote exploit.
These four attack types were ordered sequentially. In each turn, a different attack was randomly picked up from the next category. Since the count of the Probe, R2L, and U2R attacks are very few compared to the DoS attack.

Normalization

Normalization is an important step in preparing a training dataset. Because the qualities of KDD are of various natures and sizes, and their scales vary widely. Standardization of the training dataset is a basic requirement

for many ML and deep learning algorithms; they might be have badly if there exists a class imbalance. minority sampling can be performed to avoid higher class imbalance. The standard normal distribution is an example of a normal distribution. A normal random variable is considered to be normal if it has a mean of 0 and a standard deviation of 1. The random variable in the standard normal distribution is the standard score (z score). To convert any normal random variable X into a z score, apply the formula below:

$Z = (X–\mu) /\sigma$

Here, Xii a normal random variable, μ is the mean of X, and σ is the standard deviation of X. In this study, we have only considered four major supervised ML techniques: Decision Tree, Naïve Bayes, Logistic Regression, SVR. Following isia brief explanation of each technique. Decision Trees are powerful and popular techniques for classification and prediction problems. Classification is done in aherarchcal order in the form of a tree. It follows a top-down approach Each internal node actsion a particular attribute and the leaf node represents the value of the target attribute. Building the decision tree: Decision trees are generally based on a set of training data. In this case, a particular set of attribute will be elected for eachinode. Also, the leaf nodes would be also labeled according to the appropriate class. Classification: To generally classify a new event, an attribute for each node (top to bottom) is considered. Based on the value of the attribute, the tree branch is selected. This process is repeated until the algorithm reaches the leaf node. Naïve Bayes Classifier Algorithm is a simple and strong probabilistic classifier algorithm based on the Bayes rule. The main aim of the Naïve Bayes classification is to frame a rule which will allow as signing future objects to a class when only provided with the vectors of varibles that describe the future objects.

Logistic Regression is a regression algorithm that can be used for classification and segmentation. Logistic R egression is a statistical method that allows for the analysis and prediction of many events, especially dichot omous events. With respect to the number of values in the dependent variable, a logistic regression model is divided into binomial regression analysis and multinomial regression analysis. Support Vector Machine (SVM) is a robust and flexible machine learning model which possesses remarkable robust performance with respect to sparse and noisy data. It is mainly used in classification problems. SVM can perform both regress ion and classification tasks and can also handle continuous and categorical data. SVM creates an optimal hyperplane, a hyperplane between two classes. SVM follows an iterative training algorithm. The purpose of the hyperplane is, maximize the distance from each of the available classes and try in a way to distinguish each class with a minimum error obtained at the maximum margin

DATA PREPARATION

In 42 features present the dataset, protocol type, service, and status flags are categorical features and the rest 38 features are continuous in nature. These categorical variables are converted to continuous variables by using cat. codes function. The value counts of various types attacks are shown in Table 2. On treating the duplicate and null values in KDD99 data set, we performed a series of feature extraction and standardization procedures. Some of the features were coupled together and replaced with specific variables for easy computation. Certain features had to be converted from categorical to numerical. Well-formatted and checked data protects against empty values, unexpected duplication, erroneous identification, and inconsistent formats, which improves data quality and protects programmes against encryption. Basic Exploratory data analysis was performed to visualize different patterns and important features. To test the sensitivity of various strategies in the distribution of training data, data sets with variable relative amounts of attack recordings were created based on specified demographic parameters. Data is then selected using K-fold from the training set. The selection is done in such a way that each training set contains all the attack categories.

EXPERIMENTAL RESULT

The outcome of our model is tested using K-fold verification and this is done by the use of scikit-learn. This is a re-sample method that will provide a measure of model performance. It does this by dividing the data into k

segments, training the model into all components except one held as a test set to test model performance. This process is repeated k-times and the score scale for all built-in models is used as a solid performance measure. Techniques were used in each training set. The same test data was used in different training sets for each phase. The results of each of the 5 different set strategies (in each phase) were combined to evaluate the method. We have summarized the result for different methods in table 2. The results from different models i.e., Decision Tree and SVR show an accuracy rate more than 90% for the detection of attacks. Naive Bayes has possess detection accuracy rate of 61%. Classification reports for different attacks were also summarized in the table. We have compared our work with the latest work done in the same and the reports generated by other papers are summarized in Table 3.

CONCLUSION

In the paper, datasets containing a variety of assault types and percentages are used to assess supervised intrusion detection systems. In this article, training data sets with variable numbers and percentages of attacks are utilised to evaluate intervention strategies for discovering intervention. The final simulation results shows that the highest acquisition rates of all three groups are the similar at each stage of attacks. Due to their high detection rate, Decision Trees and SVR are useful in detecting DoS. In comparison to the other models tested in this research, Naive Bayes produces superior results for identifying further stages of the assault. We also considered a general deviation from the level of acquisition of different strategies in each class of people. Strategic effectiveness is assessed based on databases with different percentages of attacks. Based on the results obtained from this paper, the strategies are likely to show stronger than predictability when training using different training data sets. It was also noted that the strategies likely to reflect different detection rates in the sample data that have few samples such as R2L, U2R, and Probe.

As part of the future work, we aim to find the right combination of these accessibility features. We will work on forming a model with appropriate integration with CEP and ml models to try to build a better model to detect anomalies in the IoT Network.

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Table 1. Data Preparation

Attack Types	Value Counts
dos	391458
normal	97278
probe	4107
r2l	1126
u2r	52

Table 2. Summarized the result for different methods

Models	Accuracy	Precision
Decision Tree	0.9202	0.9867
Naïve Bayes	0.6171	0.9664
Logistic Regression	0.9609	0.9578
SVR	0.9746	0.9691

Table 3. Generated by other papers

	Date of pub-lish-ing	Detection methodology	Treated threats	Algorithms used	Accuracy
Analysis of KDD-Cup'99, NSL-KDD and UNSW-NB15 Datasets using Deep Learning in IoT.	2019	Deep learning neural networks	DoS, R2L, U2R, Probe..	DNN, ANN	97.5%
A Detailed Analysis of the KDD CUP 99 Data Set.	2009	Feature extraction and building new data set (NSL-KDD)	-	-	-
Benchmarking datasets for Anomaly-based Network Intrusion Detection: KDD CUP 99 alternatives.	2018	-	DoS, R2L, U2R, Probe, Normal	Naive Bayes, SVM, Decision Tree, RF, ANN, K-Means	Naive Bayes-86 % SVM-94% Decision Tree-94% RF-94% ANN-94% K-Means-93%
Intrusion Detection System using KDD Cup 99 Dataset.	2020	J48, Naive bayes, RF	DoS, R2L, U2R, Probe, Normal	J48, Naive bayes, RF	Naïve-92.90% RF- 99.99% J48-99.98%
Integrating complex event processing and machine learning: An intelligent architecture for detecting IoT security attacks	2020	Complex Event Processing (CEP) technology and the Machine Learning(ML)	TCP, UDP and Xmas port scans, and DoS	-	
Building a Intrusion Detection System for IoT Environment using Machine Learning Techniques	2020	Machine Learning		Naïve Bayes, SVM, decision tree, Adaboost	Naïve Bayes -97% SVM-98% Decision tree-100% Adaboost- 98%

Table 4. Classification Report

Model	Attack types	precision	recall	F1 score	support
Decision tree	**dos**	1.00	0.31	0.48	14500
	Normal.	0.99	1.00	0.99	14439
	probe	0.02	0.99	0.03	160
	r2l	0.06	1.00	0.11	1
	u2r	1.00	0.35	0.52	17
Naive Bayes	**dos**	0.92	0.31	0.46	14500
	Normal.	1.00	0.72	0.83	14439
	probe	0.01	0.94	0.02	160
	r2l	0.00	0.00	0.00	1
	u2r	0.02	0.71	0.03	17
Logistic regression	**dos**	0.95	0.98	0.97	14500
	Normal.	0.98	0.95	0.96	14439
	probe	0.61	0.21	0.31	160
	r2l	0.00	0.00	0.00	1
	u2r	0.00	0.00	0.00	17
SVM	**dos**	0.99	0.99	0.99	14500
	Normal.	0.99	1.00	0.99	14439
	probe	0.88	0.53	0.66	160
	r2l	0.00	0.00	0.00	1
	u2r	0.00	0.00	0.00	17

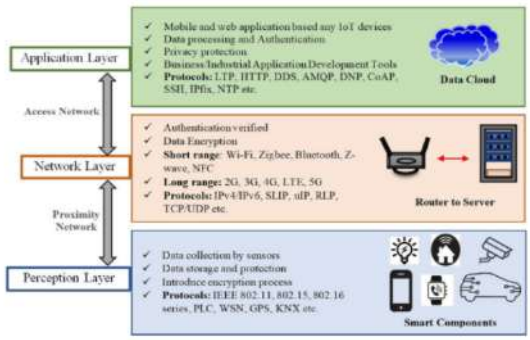

Fig 1. Network layer

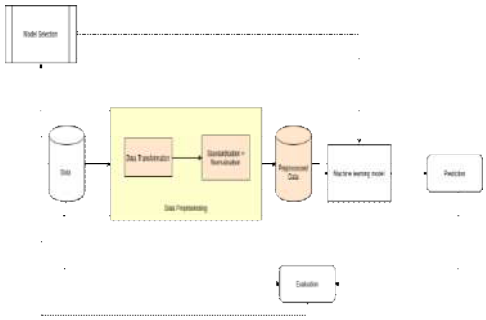

Fig 2. Normalization

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Productivity Improvement of Rear Axle Carrier Assembly using Motion and Time Study– A Case Study for Tractor Manufacturing Company

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Received: 06 Jan 2022 Revised: 18 Feb 2022 Accepted: 24 Mar 2022

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ABSTRACT

In the present work, assembly task of Rear axel carrier (RAC) at one of the leading tractor manufacturing company in India at Nagpur is studied.The study regarding the workplace layout, number of components involved, movement of workers, available tools and their location etc. were analysed. After critically analysing the scope of productivity improvement is identified. The time for each the activity is measured using stop watch and material flow process chart is prepared. Then critical analysis of each activity is carried out in order to identify the movement/actions which are unwanted or needed improvement. Accordingly modified layout is proposed. Further the Energy consumption measurement is also carried out for existing and proposed layout. It was found that with the proposed modifications time of 48 sec was saved for one assembly of RAC. Considering the 8 hours shift with 70 Assemblies, total 56 minutes per shift can be saved.

Keywords: Rear Axel carrier, productivity, flow process chart, stopwatch, energy consumption.

INTRODUCTION

To satisfy the ever increasing market demand, the efficiency of Man, machine and methodology is very crucial. If the workstation of operator is properly designed then the efficiency of man i.e operator will be high. Machine will be more efficient if its utilization is more. However the efficiency of both man and machine is highly influenced by adopted procedure in the manufacturing system. The unwanted and unproductive activity, operations will result into fatigue to operator and under utilization of machine. For this reason it is important to have proper production scheduling. In Time Study the activity or task is observed and the time taken to execute a task is recorded, by using devices such as stopwatch, digital stopwatch and video camera, etc [1]. Time Study investigates all the activities being carried out in the operations and eliminate delay, waiting time, etc. Proper utilization of space helps to create an optimum plant layout which aim at reducing unnecessary motion[2]. Unproductive activity and motion of the reduces the productivity of the task and also increases the worker fatigue. This is a kind of wastage of valuable resources which in todays competitive environment can be a problem for the business. Time study is part of work

study which analyses the operations and suggest corrective action plan to increase the productivity [3,4]. In the below presented work, task of assembly of rear axel case (RAC) for one of the leading tractor manufacturing company in India at Nagpur is examined with the objective of performance evaluation of the productivity. During the study initially the layout of workplace, no of components to be assembled, available tools with their location, workers movement, etc., were analysed. The stopwatch method was used to record the time required for execution of activity. The flow of various components, parts of assembly and the movements of the workers were also examined and noted. The flow process chart for entire activity is plotted and later on the all these activities are critically examined by finding answers to the Primary and Secondary questions such as what is achieved through that activity, is that activity is necessary, can it be eliminated, what else might be done etc. from this critically analysis unnecessary and unproductive activities for the assembly operation at each of the workstation is determined. Accordingly the critical analysis charts for unnecessary and unproductive activities are developed.

Review of Literature

Raghunath Kulkarni etal used the technique of work study for productivity improvement of motor winding unit work station. He observed that improvement of 44% in cycle time as compared to existing layout [5]. S Ramkumar etal applied the method of time and motion study at Toyota production system for productivity improvement and reduction of cycle time. They proposed 3 solution in existing layout to improve the productivity [6]. Gurunath & Jadhav used methods of motion study to identify factors which are time consuming and causing fatigue to workers of welding shop assembly work station [7]. In another research of Gurunath and Jadhav they used novel computer based approach to study ergonomics problems[8]. Battani & Faciao developed methodological framework for better productivity and ergonomics. This framework provides valuable information to companies looking for linking assembly and ergonomics [9]. Adi sapatri et al comparatively investigated effect of two different design sets of work station, assembly line, and jig design on the productivity. He found that amongst these three factor jig design is most affecting factor on productivity of plugs[10].

Methodology Adopted

The study was conducted at workstation of Rear axel Carrier consisting of assembly operation of 27 components performed manually. The total assembly process was executed at three different processing station. The station wise components along with their numbers are as below. All these parts are kept in different bins around the processing workstations. Four operators, 1 at station no 1, 2 operators at station no 2, and 1 operator at station no 3 are engaged in this assembly operation. During initial observation of the task, many unwanted and unproductive process and movements were noted and accordingly we found the scope for reducing the cycle time require to assemble one unit. Therefore all the operations were examined critically using time and motion study. Stopwatch technique was used to record the time required for each activity.

Analysis of Existing Layout: Fig no1 shows the existing layout for rear axel carrier assembly in which position of 3 processing stations, different storage bins around them, Roller conveyor for transportation of material, etc can be seen clearly. At station 1, the 6 components as mentioned in table no1 are assembled. The retainers are stored in the retainer rack located just behind the operator as shown in fig. Similarly axel, oil seals, gaskets, ball bearings are stored in different bins surrounding the station 1. The distance of all these storage locations from assembly station is also shown in figure. While making assembly operator has to move a distance as mentioned in figure to reach to that location, collect the required parts for assembly and again move back to station and then finish the task of assembly. Similarly the for assembly station 2 and station 3, operators has to move around the location to collect and assemble the components.

Flow Process Chart: For the existing workplace layout and each station, the material flow chart is prepared for all three stations. Flow chart contains the information like nature of activities, total number of activity, time required to complete the activity at each station etc. The sample material chart is as shown in figure. For each station readings were recorded for 8 times and average value is calculated. The number activity to be performed and time required to complete it for each of the station is as below.

Critical Analysis of Flow Process Chart

The flow process chart for all the activities at 3 stations as developed were critically analysed for the identification of unnecessary, unwanted, time consuming activities. This is achieved by finding the answers to the to the Primary and Secondary questions such as what is achieved through that activity, is that activity is necessary, can it be eliminated, what else might be done, etc. In this way critical analysis chart is prepared. The sample critical analysis chart for station no is as shown in fig 2.

Suggested Modification in Process & Existing Layout

From the critical analysis chart of station 1, it was observed that activity no 2,3,4,5,6,39 and 40 were unnecessary and can be modified by making certain arrangements in workplace layout. For example, activity no 2 to6 involves the movement of worker from workstation and storage location for picking and transportation of retainers and oil seals to station 1. This activity was consuming the time of 33.85 secs. This can be modified by using gravity conveyors for constant supply of retainers near station 1. Similarly the storage bins for oil seals can be placed within the reach of the operator so that unnecessary transportation can be eliminated and will result in saving of time. Similarly for activity 39 and 40 requires the movement and pick up if the bearing by the worker and for this he requires the time of 14.4 sec. This activity can also be modified by use of storage bins within the reach of the operator at station 1. In the same way unwanted activity from station 2 and 3 were modified by providing the suitable alteration in workplace layout. The identified unnecessary activity and suggested modification is as shown in following table no 4.The time required for above identified unwanted activities is as below. From critical analysis of activities at all three station mean time of 1.48 mins were found to be wasting in performing unnecessary activities. The proposed layout with suggested modification is as shown in fig 3.

Productivity Improvement with Modified Layout

Mean Total time recorded by stop watch=14.37 Min
Time for activity as recorded by company=13.37 min
Total unproductive time found after critical analysis=1.48 min
Expected time to complete the activity with modified layout=13.37-1.48=12.49 min
Time savings for one RAC assembly after implementation of modified layout=13.37-12.49=48 sec

CONCLUSION

In the presented work, the existing methods and layout for assembly of rear axel carrier (RAC) is examined using material flow process chart. The time for each activity is recorded and unwanted and unnecessary activities are identified using critical analysis chart. By making simple changes in process and modification in existing layout, the time taken to finish the activity is reduced by 48 sec per assembly. This simple research conducted has shown that how a simple techniques can be used for improving the material flow process and the productivity.

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Table No1: Station-wise details of parts to be assembled

Station No	Name of Part	Quantity	Total Parts
1	Retainer	1	6
	Oil seal	2	
	Gasket	1	
	Axel	1	
	Ball Bearing (Axel)	1	
2	Circlip (Rear Axel)	1	13
	Pr. Bearing (carrier)	1	
	Spacer (Carrier)	1	
	Carrier	1	
	Collar	1	
	Bolts	4	
	Washer	4	
3	Long D – Headed Wheel Bolt	8	8
Total Parts to be assembled at all three stations			**27**

Table No 2.Sample Material flow process chart for existing process.

FLOW PROCESS CHART – MATERIAL TYPE (STATION 2)

FLOW PROCESS CHART		MATERIAL TYPE			
CHART NO. SHEET NO.		SUMMARY			
Subject charted Used bus engines		ACTIVITY	PRESENT	PROPOSED	SAVING
ACTIVITY Stripping cleaning and degreasing prior to inspection METHOD: PRESENT		OPERATION ○ TRANSPORT ⇨ DELAY D INSPECTION □ STORAGE △			
		DISTANCE (m)			
LOCATION: Degreasing Shop		TIME (man-min)	-	-	-
OPERATIVE(S): CLOCK Nos.		COST LABOUR MATERIAL	- -		
CHARTED BY: APPROVED BY: DATE		TOTAL			

	DESCRIPTION	QTY	DISTANCE (m)	TIME (sec)	SYMBOL	REMARKS
					○ ⇨ D □ △	
52.	Carrier stored in trolley near assembly station 2			00		
53.	Fixed the hook of pneumatic hoist into carrier (labour 1)			8.48		
54.	Lift the carrier near to assembly station 2 (labour 1)			8.67		
55.	Move the carrier over the pressing fixture (labour 1)			8.68		

Table No 3: No of activities and time required in existing layout

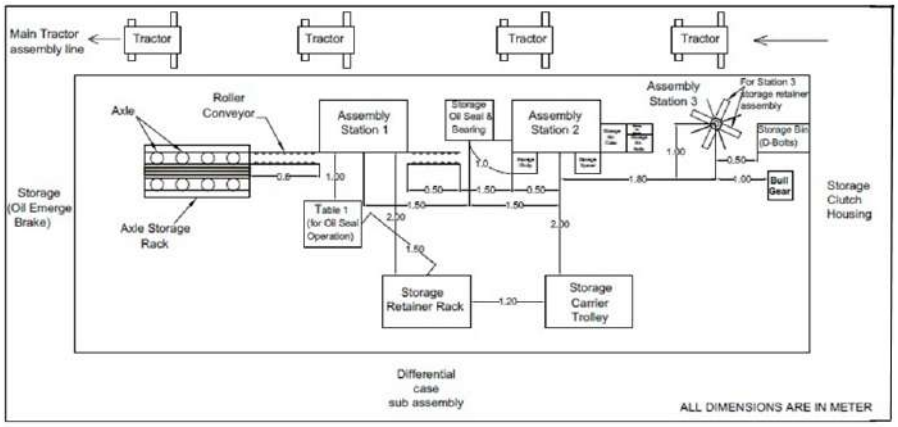
Station No	Number of activities	Total time
1	51	5.633
2	38	4.825
3	24	3.513

Table No4: Unnecessary operations and suggested modifications.

StationNo	Operation No.	Activity (Existing layout)	Suggested Modification
1	Op 2	Pickup the retainer from retainer rack	By providing the gravity conveyor system near assembly station 1 instead of retainer rack to avoid operation 2 and 3.
	Op 3	Move to table 1	
	Op 4	Pickup the oil seal from table 1	This operation is canceled out by providing bin system near assembly station 1. So as to avoid the operation on table 1.
	Op 5	Move retainer and oil seal to assembly station 1	
	Op 6	Placed the oil seal on station 1	
	Op 39	Pick up the bearing on table 1	By providing bin for storage of bearing near assembly station 1. So as to avoid operation 39 and 40.
	Op 40	Move bearing to station 1	
2	Op 58	Pick up the bearing from storage (labour 2)	By providing bin near assembly station 2 to store bearing and subsequently to avoid the operation 58 and 59.
	Op 59	Move bearing to assembly station 2 (labour 2)	

Table No5: Time required by identified unnecessary activity.

	Reading							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Op 2	6.82	8.68	6.79	7.90	8.74	6.88	8.53	7.81
Op 3	11.64	12.37	11.88	11.58	12.72	11.49	11.53	12.73
Op 4	6.48	8.61	7.56	7.89	8.95	6.70	6.94	8.70
Op 5	9.81	10.86	10.71	9.58	9.92	10.49	11.05	10.77
Op 6	7.79	8.47	7.73	9.46	8.95	7.68	9.05	8.80
Op 39	9.60	10.80	11.04	9.07	11.71	9.84	11.65	11.26
Op 40	10.47	11.40	11.77	10.79	9.81	11.86	10.78	11.60
Op 58	9.68	9.52	10.62	11.06	10.65	10.71	10.49	10.88
Op 59	9.85	10.38	10.74	9.71	10.82	10.95	10.81	9.74
Total time	**82.14**	**91.09**	**88.84**	**87.04**	**92.27**	**86.6**	**90.83**	**92.29**
Total time in min	**1.369**	**1.518**	**1.480**	**1.450**	**1.53**	**1.443**	**1.513**	**1.53**



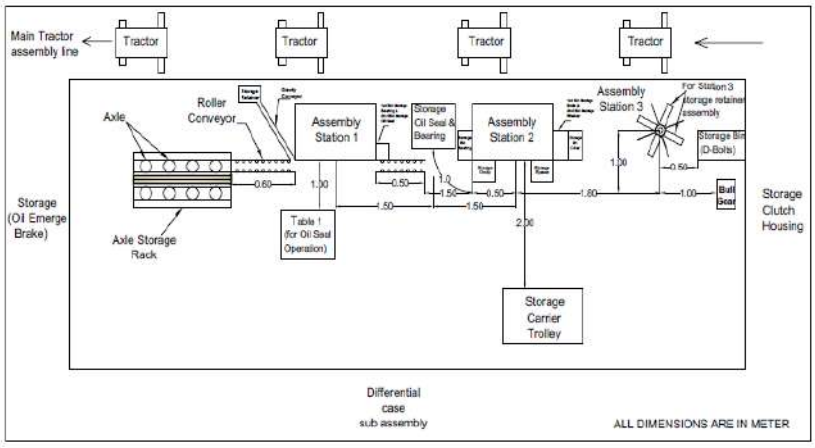
LAYOUT :- REAR AXLE CARRIER ASSEMBLY OF TRACTOR (Existing)

Fig1: Existing layout of RAC Assembly

Subject of Chart – Rear Axle Carrier (RAC) assembly station 1, 2 and 3.

Activity	Primary Questions	Secondary Questions	Remarks
Purpose 2) Pickup the retainer from retainer rack 3) Move to Table 1 4) Pickup the oil seal from Table 1 5) Move retainer and oil seal to assembly station 1 6) Placed the oil seal on station 1	What is achieved? Retainer is pickup by the operator from retainer rack and then move up to table 1 from where he pickup the oil seal and move up to station 1 where he place the oil seal on station 1. Is the activity necessary? Yes, this activity is necessary for performing the operation for a human operator.	What else might be done? The retainer is stored in a bin which is mounted above side of gravity conveyor. This gravity conveyor can be mounting on the left side of assembly station 1 for incoming of retainer on a platform of assembly station 1. Similarly the bin for oil seal can be stored on a right side of assembly station 1 within maximum working area so that operator can pick the incoming retainer with his left hand and oil seal with his right hand simultaneously.	For eliminating the unwanted movement of operator, here we used the gravity conveyor for incoming of retainer and place bin for storing oil seal within maximum area. So we can eliminate the unnecessary motion of the operator and save the production time and can increase productivity.

Fig 2: Critical Analysis Chart



LAYOUT :- REAR AXLE CARRIER ASSEMBLY OF TRACTOR (Proposed)

Fig 3: Modified Layout

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Development and Licensure of Vaccines to Prevent COVID-19

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Received: 09 Dec 2021 Revised: 14 Jan 2022 Accepted: 23 Feb 2022

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ABSTRACT

COVID-19 affects various people in different ways. The majority of infected persons may experience mild to severe disease, however there are presently FDA-approved COVID-19 vaccinations. Vaccine development and licencing for COVID-19 prevention are reviewed, with the objective of giving considerations and a path-way for the creation of COVID-19 vaccines, and chemistry, manufacturing, and controls are discussed. Non-clinical data, clinical data, post-licensure safety review, diagnostic and serological tests, and a mechanism for COVID-19 vaccine emergency usage, based on our conversations, we determined that, despite concerns regarding vaccine safety, there was strong rivalry for and approval of COVID-19 immunisation during the pandemic era.

Keywords: Vaccines, COVID-19, SARS-CoV-2, OVRR, BLA, ERD, FDA, Emergency use, Authorisation

INTRODUCTION

The Food and Drug Administration (FDA) is basic in shielding the United States against risks like irresistible illness flare-ups, for example, the Coronavirus Disease 2019 (COVID-19) pandemic brought about by the extreme intense respiratory disorder Covid [2]. (SARS-CoV-2). The FDA is devoted to delivering convenient direction to further develop pandemic reaction endeavors. This direction is being distributed by the FDA to help supports in the clinical

turn of events and enrolment of COVID-19 immunizations. This exhort is expected to stay in power until the Secretary of Health and Human Services (HHS) proclaims a general wellbeing crisis identifying with COVID-19 on January 31, 2020, with impact from January 27, 2020, including any expansions allowed by the HHS. The direction's suggestions are intended to help the Agency and supporters in their endeavours. clinical turn of events and authorizing of COVID-19 antibodies, just as mirror the Agency's present perspectives on the issue.

So the FDA surveyed that earlier open support for this direction is neither achievable nor proper (see area 701(h)(1)(C) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), (21 U.S.C. 371(h)(1)(C)), and 21 CFR 10.115(g)(2)), this counsel is being executed without earlier open remark.

Objectives

- To provide knowledge on COVID-19 vaccines.
- To provide considerations and the path-way for the development of COVID-19 vaccines.

DISCUSSION

Background

A novel Covid is causing a respiratory infection episode. SARS-CoV-2 is the name of the infection, while COVID-19 is the name of the ailment brought about by it. The Secretary of Health and Human Services will resign on January 31, 2020[1]. Because of COVID-19, the President proclaimed a general wellbeing crisis and conveyed the HHS Operating Divisions. Moreover, because of COVID-19, the President proclaimed a public crisis on March 13, 2020. This guidance gives a diagram of essential angles for meeting administrative prerequisites in the investigational new medication application (IND) guidelines in 21 CFR Part 312 and permitting guidelines in 21 CFR Part 601 for science, assembling, and controls (CMC), just as non-clinical and clinical information through advancement and licensure, and for COVID-19 post-licensure wellbeing assessment. The FDA is focused on supporting any experimentally strong techniques for diminishing COVID-19's [2].

Chemistry, Manufacturing, And Controls (CMC)– Key Considerations

General considerations

The immunization item should be adequately depicted and fabricated as per pertinent norms, including current great assembling practice (cGMP) (area 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and 21 CFR Parts 210, 211, and 610). Segment 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and 21 CFR Parts 210, 211, and 610) necessitates that the immunization item be appropriately portrayed and produced in consistence with material principles, including current great assembling practice (cGMP) [3].COVID-19 immunization improvement might be sped up to the degree lawfully and experimentally allowed dependent on information obtained from comparable items fabricated utilizing a similar very much described stage innovation. The FDA suggests that antibody engineers talk with OVRR from the get-go to decide the sort and amount of CMC data required for the turn of events and permitting of their COVID-19 immunization.

Manufacturing of Drug Substance and Drug Product

Data exhibiting that all source material utilized in assembling is sufficiently controlled, for example, the set of experiences and capability of cell banks, the set of experiences and capability of infection banks, and the distinguishing proof of all creature determined materials utilized for cell culture and infection development, ought to be given. Thus, supports should give information and data illustrating significant cycle boundaries, basic quality provisions, cluster records, predefined hold times, and the in-measure testing strategy. Each basic boundary ought to have its own arrangement of prerequisites. Approval information from stage related item creation might give valuable extra data, especially in recognizing basic boundaries. In-measure control tests should be set up to permit quality to be checked for each part at all phases of creation (area 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and, if material, 21 CFR 211.110(a)). The FD&C Act's Section 501(a)(2)(B) (21 U.S.C. 351(a)(2)(B) and, if material, 21

CFR 211.100(a) and 211.110) require sufficient approval of the assembling system. Approval would ordinarily comprise of an enormous number of business scale clusters that can be delivered consistently while meeting characterized in-measure controls, basic cycle boundaries, and part discharge prerequisites. To approve the assembling system, information from no less than three business scale clusters is regularly fundamental [3]. Storage conditions, especially the trustworthiness of holder terminations, should be fastidiously checked (21 CFR 211.166).

Facilities and Inspections

Facilities should be of adequate size and development to permit exercises while staying away from tainting, cross-pollution, and mistakes (segment 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and, where relevant, 21 CFR 211.42(a)). Approval is needed for all conveniences (counting plumbing and disinfection), and HVAC frameworks should give satisfactory power over pneumatic force, microorganisms, residue, dampness, and temperature, just as sufficient security or control (area 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and, as appropriate, 21 CFR 211.46). (c) [4]. Cleaning and support techniques for offices and gear should be made and approved (segment 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B) and, if appropriate, 21 CFR 211.56(c) and 211.67). (b). Manufacturing hardware should be qualified, and cleaning and filtration strategies should be approved. Staff ought to be all around prepared and skilful for their jobs, and aseptic cycles ought to be painstakingly tried utilizing media reproductions. The FDA suggests that antibody makers contact CBER's Office of Compliance and Biologics Quality, Division of Manufacturing and Product Quality, and Division of Manufacturing and Product Quality when achievable to examine office planning and review timing. Pre-permit examinations of assembling offices are important for the BLA audit measure and are generally performed once a BLA record has been acknowledged (21 CFR 601.20).

Non-Clinical Data: Key Considerations

General Considerations

Non-clinical examination on a COVID-19 antibody competitor's immunogenicity and security attributes in vitro and in vivo is the point. The amount of non-clinical proof needed to continue to First in Human (FIH) clinical preliminaries is chosen by the immunization configuration, supporting information for the form, and information from antibodies that are intently equivalent. In these examinations, animal models were managed vaccination works against a couple of Covid and thusly tried with the fitting wild sort disease. These investigations discovered signs of immunological pathologic lung reactions equivalent to ERD in infants and creatures given a formalin-inactivated respiratory syncytial infection (RSV) inoculation and consequently tested with RSV infection through normal openness or in the lab, individually [5-10].

Toxicity studies [11-15]

Non-clinical security studies will be needed prior to continuing to FIH clinical preliminaries for a COVID-19 antibody competitor that is an original item type with no earlier non-clinical and clinical information 21 CFR 312.23(a) (8). In certain cases, directing non-clinical wellbeing examines before FIH clinical preliminaries may not be important since adequate information to portray item security might be accessible from different sources. For instance, if the COVID-19 immunization applicant is appropriately characterized and made utilizing a stage innovation recently used to produce an authorized antibody or other recently explored trial immunizations, toxicological information (e.g., information from rehash portion poisonousness examines, bio dispersion contemplates) and clinical information gained. Non-clinical security assessments, including poisonousness and neighbourhood resistance testing, should be done as per the guidelines overseeing great lab rehearses for non-clinical lab research (GLP), as needed to help movement to FIH clinical preliminaries (21 CFR Part 58). Data from harmfulness testing might be given as unaudited last drafts toxicologic reports to rush the inception of FIH clinical preliminaries utilizing COVID-19 immunization up-and-comers. The last, absolutely quality-guaranteed reports ought to be submitted to the FDA inside 120 days of the initiation date of the FIH clinical review. The utilization of COVID-19 preventive antibodies during pregnancy and in ladies of regenerative potential will be basic in inoculation programs. Thus, the FDA encouraged supporters to direct DART examines with every specific COVID-

19 immunization competitor prior to selecting pregnant ladies and ladies of conceptive potential who are not effectively keeping away from pregnancy in clinical preliminaries.

Characterization of the Immune Response in Animal Models

Immunogenicity examines in creature models receptive to the COVID-19 antibody antigen of decision ought to be completed to assess the immunologic components of the COVID-19 immunization up-and-comer and to help FIH clinical preliminaries. Each of the COVID-19 antigens ought to be assessed as far as humoral, cell, and useful invulnerable reactions. Antigen-explicit Enzyme Linked Immunosorbent Assays (ELISA) ought to be contemplated to decide the humoral reaction. Cell reactions, especially CD8+ and CD4+ T cell reactions, ought to be researched utilizing touchy and explicit measures.

Studies to Address the potential for Vaccine-associated Enhanced Respiratory Disease

Current information and cognizance of the possible risk of COVID-19 immunization related ERD are restricted, as is comprehension of the worth of current creature models in extending the likelihood of such advancement in people. Regardless, creature research (e.g., rodents and nonhuman primates) is viewed as fundamental in tending to the probability of immunization related ERD. In creatures inoculated with clinically pertinent dosages of the COVID-19 immunization applicant, utilitarian invulnerable reactions (e.g., killing counter acting agent) against all out immunizer reactions, just as Th1/Th2 balance, ought to be evaluated. COVID-19 immunization competitors with immunogenicity information showing high killing counter acting agent titers and Th1-type T cell polarization might be permitted to continue to FIH preliminaries without first finishing post inoculation challenge examines in proper creature models, as long as sufficient danger relief procedures are carried out in the FIH preliminaries. Post-immunization challenge studies will be directed in mix with FIH preliminaries in equivalent conditions to guarantee that the danger of antibody related ERD is tended to prior to selecting enormous quantities of human subjects in Phase 2 and 3 clinical preliminaries. Without even a trace of evidence of post-immunization challenge to address the danger of ERD, the prompted by the antibody will be considered in deciding if Phase 3 preliminaries might continue.

Clinical Trials: Key Considerations

General Considerations

The current situation with information on SARS-CoV-2 immunology, particularly immunization insusceptible reactions that might foresee security against COVID-19, is inadequate and evolving. While immunogenicity testing is a fundamental part of COVID-19 antibody improvement, the point of advancement projects ought to be to seek after ordinary licensure through direct evidence of immunization adequacy in shielding individuals from SARS-CoV-2 contamination as well as sickness as of now. As clinical improvement advances, there is a requirement for an adequate collection of information, remembering information for the danger of antibody related ERD, to help the security of inoculating the proposed concentrate on populaces and number of members, and, for later stage improvement, to help the wellbeing of immunizing the proposed concentrate on populaces and number of members. Proposals for assisted/consistent clinical advancement might get early direction and a speculative arrangement from the FDA [16].

Trial Populations

To start, sound grown-up volunteers with an okay of extreme COVID-19 ought to be remembered for FIH and other beginning stage considers (which normally uncover 10–100 people to every antibody up-and-comer being assessed) [17].Early clinical fundamentals should, if reasonable, keep away from people who are at high risk of SARS-CoV-2 receptiveness (e.g., clinical consideration worker's). The support should gather and assess fundamental clinical security and immunogenicity information for each portion level and age bunch (e.g., more youthful versus more established grown-ups) to help the movement of clinical improvement to incorporate a bigger number of members and those at higher danger for extreme COVID-19.Because COVID-19 is bound to affect racial and ethnic minorities, the FDA unequivocally urges them to take part in the examination. It is important that the old and those

with clinical issues be appropriately addressed in late-stage clinical investigations on immunization security and adequacy in grown-ups [18].

Trial Design

In beginning stage considers, randomization of members to various treatment bunches is frequently used to decrease the field of antibody up-and-comers as well as portion regimens. This isn't needed for beginning stage preliminaries, despite the fact that it can help with the assessment of primer security information.Procedures for consistent preliminaries, like those for versatile preliminaries, ought to contain predefined rules for adding or erasing inoculation applicants or measurements regimens. When an inoculation that is both protected and successful opens up, adequacy studies ought to incorporate emergency courses of action for expanded development and an audit of security and viability information (e.g., as exhibited in an arranged break investigation or as shown in another clinical preliminary).

Efficacy Considerations

Fever or chills, hack, windedness or inconvenience breathing, exhaustion, strong torments, migraine, new taste or smell misfortune; sore throat; clog; runny nose; queasiness or retching; and looseness of the bowels are for the most part side effects of SARS-CoV-2 contamination. Coronavirus immunization patrons ought to consider doing formal speculation testing on an extreme COVID-19 endpoint since a COVID-19 antibody is expected to be definitely more powerful in forestalling serious COVID-19 disease than gentle COVID-19 contamination

Statistical Considerations

The essential viability endpoint point gauge for a fake treatment-controlled adequacy preliminary ought to be basically half to guarantee the adequacy of a broadly circulated COVID-19 antibody, and the measurable achievement standard ought to be that the lower bound of the properly alpha-changed certainty stretch around the essential viability endpoint point gauge is more prominent than 30%.A lower bound ≤30% but >0% may be acceptable as a statistical success criterion for a secondary efficacy endpoint, provided that secondary endpoint hypothesis testing is dependent on success on the primary endpoint.When contrasting non-mediocrity with a formerly exhibited fruitful COVID-19 antibody, the measurable achievement standards ought to be that the lower bound of the fittingly alpha-changed certainty span around the essential relative adequacy point gauge is more than - 10%.

Safety Considerations

The general wellbeing assessment of COVID-19 antibodies, including the size of the security information base fundamental for immunization licensure, ought to be like that of other prophylactic inoculations for irresistible sicknesses. A critical number of exploration members had neighbourhood and fundamental sick impacts for somewhere around 7 days following each study inoculation to decide reactogenicity (counting no less than a subset of members in late-stage adequacy preliminaries). All research volunteers had undesirable incidental effects for something like 21–28 days after each study inoculation. Serious and other therapeutically went to unfriendly occasions in all examination members for no less than a half year following the finish of all review inoculations. Pregnancy results like pregnancy adversity, stillbirth, and inborn abnormalities should be followed for all assessment individuals with beginning dates before to or inside 30 days of inoculation.

Post-Licensure Safety Evaluation: Key Considerations

General Considerations

As with other authorized immunizations, there might be restrictions in the wellbeing information base incorporated from a COVID-19 antibody's pre-licensure clinical examinations. The number of patients getting a COVID-19 immunization in pre-licensure clinical examinations might be lacking to distinguish some uncommon antagonistic occasions. COVID-19 immunizations are authorized, the security follow-up an ideal opportunity to screen for potential antibody related ERD and other antagonistic occasions might not have been finished for all people associated with pre-licensure clinical examinations. Manufacturers could consider making novel Current

Procedural Terminology (CPT) numbers and using standardized tags to assign the prompt holder to support the exact enrolment and distinguishing proof of immunizations in wellbeing records.

Pharmacovigilance Activities for COVID-19 Vaccines

Routine pharmacovigilance for approved organic items incorporate announcing genuine and startling unfavourable occasions as quickly as time permits, just as submitting intermittent wellbeing reports as per 21 CFR 600.80. (Post showcasing announcing of unfriendly encounters). The FDA suggests that candidates present a Pharmacovigilance Plan (PVP) for a COVID-19 inoculation along with their BLA, as determined in the FDA Guidance for Industry; E2E Pharmacovigilance Planning [20]. All generous known dangers, critical future dangers, and huge missing data ought to be tended to in the PVP. A pre-birth openness vault that effectively gathers information on pre-birth inoculation just as pregnancy and baby outcomes [21].

Required Post marketing safety Studies [22]

Section 505(o)(3) of the FD&C Act (21 U.S.C. 355(o)(3)) grants the FDA to require certain post marketing reads or clinical preliminaries for doctor prescribed prescriptions endorsed under segment 505(b) of the FD&C Act and organic items supported under area 351 of the PHS Act (42 U.S.C. 262)22. Under area 505(o)(3), the FDA might require such investigations or preliminaries at the hour of endorsement to survey a realized genuine danger related with the medication's utilization, to evaluate signs of genuine danger related with the medication's utilization.

Emergency Use Authorization (EUA)

Only when numerous lawful conditions have been met may an EUA be allowed (area 564 of the FD&C Act (21 U.S.C. 360bbb-2)). 23. Among these prerequisites is the FDA's assurance that an item's known and planned advantages offset the item's known and potential dangers when used to analyse, forestall, or treat genuine or hazardous sicknesses for a COVID-19 antibody might be proper for a COVID-19 immunization before the finishing of huge randomized clinical adequacy preliminaries might restrict the investigational antibody's capacity to show viability in a clinical sickness endpoint viability preliminary to help licensure, and such clinical illness endpoint.

CONCLUSION

When contrasted with run of the mill immunization advancement, the improvement of an antibody for Corona infection ailment is moving quickly (in no time). The quicker turn of events, then again, has no effect on clinical or non-clinical wellbeing audit. The organization's monetary danger, just as the need to lead numerous activities simultaneously to foster an immunization that can lessen the awful results of the COVID-19 pandemic as fast as could really be expected, is driving the speed increase. The FIH research depended on non-clinical wellbeing information from stage harmfulness testing, just as true security information from earlier poisonousness preliminaries with immunizations utilizing novel stages. Non-clinical creature models and clinical investigations will be utilized to test immunization viability and upgrade infection assessments. Understanding the basic needs for SARS-CoV-2 antibody improvement, for example, quickly setting up immunization testing models and discovering implies for effectively disseminating antibodies to billions of individuals around the world.

ACKNOWLEDGMENTS

The Authors express sincere gratitude to JSS Academy of Higher Education and Research and JSS College of Pharmacy, Mysuru for the support in carrying out their work.

Conflict of Interest: None
Financial support: None

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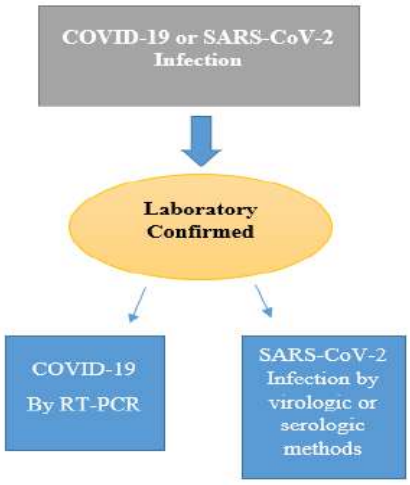

Figure 1: Efficacy Considerations

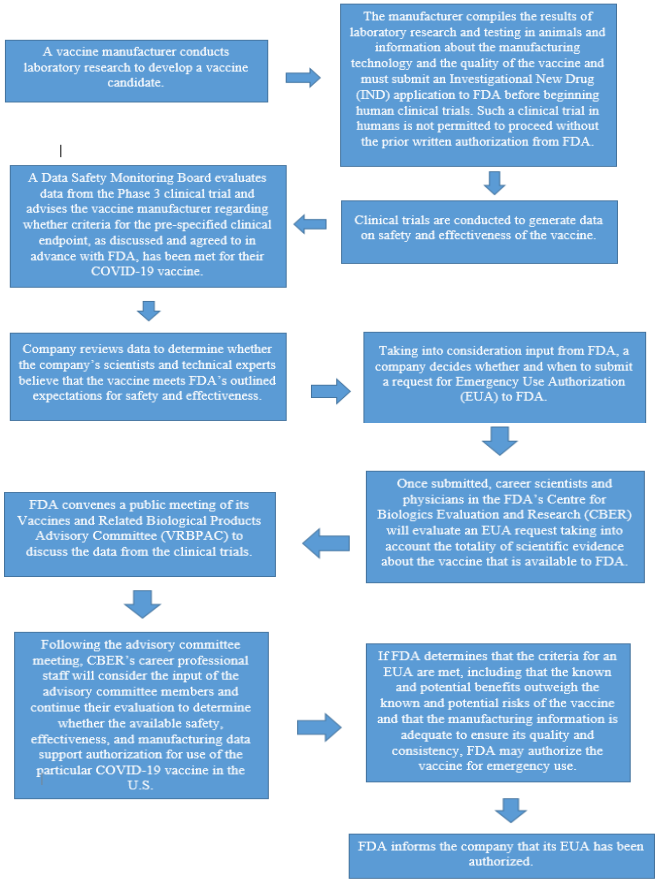

Figure 2: Pathway for Emergency Use Authorization of Vaccines in US

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Nanostructured Lipid Carriers: As Phytomedicine Deliverable

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Received: 19 Jan 2022 Revised: 14 Feb 2022 Accepted: 16 Mar 2022

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ABSTRACT

Since ancient times phytomedicines are extensively used as a treatment approach. Herbal medicines not only cure or treat disease, they also strengthen the immune system . No or less adverse effect is the major reason for the wide acceptability of phyto constituents. More than 20 percent of pharmaceutical active ingredients are derived from plants. But the poor absorption of the herbal constituents is a major challenge that has led to its abridged usage. Numerous technologies are explored to improve the efficiency of the plant-based drugs. Nano structured lipid carriers are recently gaining fame due to the presence of lipids and its nano size. Nano structured lipid carriers possess site specific activity which is explored of late. These nano carriers are proficient to enclose lipophilic as well as hydrophilic drugs, also their imperfect structure increases the drug loading and entrapment. The employment of nano structured lipid carriers as an agent to carry herbal drugs are broadly discussed in literature. It is also found that encapsulating herbal drugs in nano carriers increases their stability, produce sustain and controlled release thus improve the efficacy. The present review focuses on the usage and application of NLC as phytomedicine deliverable.

Keywords: Herbal, phytochemical, nano structured lipid carriers, nanotechnology

INTRODUCTION

Herbal or phytochemical based medicines are considered to be one of the earliest means of treatment. In contrast to the allopathic system, herbal medicament has multiple elements with multiple activities. The popularity of phyto medicines is increasing due to its reduced adverse effect and higher therapeutic efficacy. Integration of modern

medicine system and traditional phytomedicines with a holistic approach has paved way to many treatment vistas [1]. The major drawback associated with the herbal formulation is the *in vivo* reproducibility owing to its poor solubility, instability, and absorption issues, leading to reduced bioavailability and effectiveness. Also many herbal constituents get destroyed in the highly acidic pH of the stomach before reaching the blood, and few other constituents may indeed be metabolized by the liver. As a result, the sufficient amount of active medicament may not reach the blood. Adding to this is the compatibility issues of the herbal constituents with the many synthetic pharmaceutical excipients in the formulation [2].

Many technologies have been developed to overcome these drawbacks like using solubilizing technique, formulating them into injectables, or by means of nanotechnology. Among all nanotechnology is considered to be more acceptable and one of the novel strategies to deal the limitations associated with the traditional systems [3].
Advantages of using nano technology.

- High entrapment efficacy and site-specific delivery of drug
- Rapid dissolution of these formulations in body fluids accounting to its nano size
- The nano size promotes its penetration through various physiological barriers and also retention owing to deprived lymphatic drainage
- Incorporation of bio compatible lipid
- All the above factors lead to reduction in dose and side effects

Numerous nano technology-based drug delivery systems are attempted to deliver the phyto chemicals to the body. The common ones are liposome, dendrimer, nanoemulsion, polymeric nanoparticles, solid lipid nanoparticle, nanostructured lipid carrier (NLC), liquid crystal (LC) systems, precursors systems for liquid crystals (PSLCs) etc [4]. Among them nanostructured lipid carriers are well-received for the fact that these carriers are capable of encapsulating and delivering both lipophilic and hydrophilic drug. NLCs have also proven to show site and organ specific disposition of drug. Additionally, the presence of distorted core due to the use of liquid and solid lipid in NLCs empowers better drug loading. Thus, avoiding drug eviction during storage. Because of their small size, nanocarriers applied to herbal remedies will carry the optimum amount of the drug to their site of action, bypassing all barriers such as acidic pH of the stomach, liver metabolism, and increasing the drug's prolonged circulation into the blood. Nanostructured lipid carriers are extensively studied and cited in literature for its promising approach in delivering phytomedicines so as to support sustain release, reduce the required dose, lessen adverse effects and enhance curative activity [5]. Present review focuses on the use of NLC as a conveyor of phytochemicals in various disease conditions and improvement of its activity.

METHOD OF PREPARATION OF NLCS

High pressure homogenization (HPH)

This technique includes both hot and cold homogenization methods which utilize high energy in the preparation of NLCs. In hot HPH the lipid soluble drug is completely dissolved in a solid lipid melt at a particular temperature. The hot lipid melt is directly added to the aqueous phase maintained at the same temperature. High shear stress is applied to enhance the mixing of the two phases, which results in a pre-emulsion formation followed by homogenization to produce stable NLCs. In cold homogenization, drug is dispersed in the lipid melt and allowed to solidify by rapidly cooling the mixture in dry ice or liquid nitrogen and subjected to milling for producing particles of the desired size range followed by addition of aqueous phase produces a pre-emulsion which is subjected to 3-5 cycles of homogenization resulting in the formation of NLCs [6].

Solvent emulsification-evaporation

A volatile organic solvent such as chloroform, cyclohexane which is water immiscible is used to dissolve the lipids. This solvent is dispersed in an aqueous surfactant to formulate an o/w emulsion. As a low concentration of lipids was dissolved in organic solvents, nanoparticles were formed by evaporating the organic solvent [7].

Membrane emulsification method

In this method, a conventional emulsion is prepared by extrusion of a lipid melt through a porous membrane into an aqueous phase with or without use of a surfactant. The NLCs are formed as a lipid globule solidifies upon cooling the emulsion [8].

Electro-spraying

Nano-spheres are produced by using electrodynamic atomization technology. A fine spray of lipid melt is introduced into a chamber which forms lipid powder. To enhance the production of nanoparticles, lipids are primarily dissolved in organic solvent to reduce the viscosity of the feed prior to spraying in the chamber [9].

Supercritical fluid technique

This technique utilizes the appropriate supercritical fluid (SCF) which is beneficial in preventing extreme temperature. A water immiscible organic solvent is used to dissolve the drug and carrier lipid, o/w emulsion is prepared. Fine droplets of lipid are formed by subjecting the o/w emulsion to HPH. The fine emulsion is sprayed into a chamber and the organic solvent is extracted by a counter flow supercritical carbon dioxide. SCF is used to dissolve the lipid and the drug. A fine spray of this solution is introduced into a chamber below the supercritical temperature and pressure condition. The lower temperature and pressure decrease the lipid solubility and causes its precipitation and consecutively leading to lipid encapsulation of the drug [10].

Phase inversion technique

A primary emulsion of w/o is prepared by dissolving the lipophilic bioactive compound (LBC) in the organic phase. Further changing the temperature above phase inversion temperature induces phase inversion which converts w/o to o/w emulsion, followed by diluting the emulsion with water (2^0C) causes restructuring of this mixture to form NLCs [11].

Micro emulsion method

An aqueous solution of drug is added to the lipid melt. Surfactant is added above the melting point of the lipid which leads to the formation of w/o micro emulsion . This micro emulsion is added to the water-surfactant mixture with continuous stirring which leads to the formation of lipid suspension. Ultrafiltration, freeze drying, can be applied to concentrate the NLCs from the large amounts of surfactant [12].

Anti-solvent precipitation

Physical properties of lipids such as solubility can be used in formulation of nanoparticles. An organic solvent miscible with water is chosen for dissolving the lipid. This mixture is dispersed in water, the droplets of organic phase diffuse into the continuous aqueous phase causing precipitation of lipids which forms the nanoparticles. Thermo labile LBCs can be formulated with the aid of this technique [13]

Double emulsion technique

This method involves the formation of multiple emulsions of the type w/o/w and is best suited for water soluble drugs. The aqueous drug solution is dispersed in lipophilic organic continuous phase. This primary emulsion is subjected to homogenization or sonication before addition of aqueous phase to form w/o/w double emulsion, which is followed by solvent removal or solvent extraction process [14].

APPLICATION

Solubility and permeation enhancement

Phyto pharmaceuticals exhibits bioavailability issues either due to low water solubility or due to low permeation. Inclusion of these phyto pharmaceuticals into lipid nanoparticles is one of the best strategies to overcome bioavailability problems. Both solid lipid nanoparticles (SLNs) and nanostructured lipid carriers are considered to be useful as nano carriers. The presence of biodegradable and biocompatible lipids in the formulation is the major advantage.

NLCs possess greater advantage when compared to SLNs due to the presence of liquid lipids along with solid lipids. The structure of NLC possess oily nano-chamber encompassed by a solid matrix and the solid matrix of the lipid nanoparticle contains liquid nano-chamber of oil; this irregular lipid association enhances drug solubility, leading to an improvement of the drug loading ability. Hallan *et al* developed NLCs of ellagic acid (EA) an effective antioxidant of natural origin which is outlined by poor biopharmaceutical character and low water solubility. The goal of the work was to formulate nano carrier to encapsulate EA for dermal delivery. Two different combinations of solid and liquid lipids were investigated. First case included caprylic/capric triglycerides (Miglyol) as liquid lipid and the tristearin as solid lipid. In the second instance, a combination of caprylocaproyl macrogol-8 glyceride (Labrasol) and tristearin was used to produce NLCs. Release study was done for NLC-EA1 and NLC-EA2 using Franz-cells with nylon membrane, the results indicated a better release of EA from the two formulations compared to the EA solution. To summarize, the inclusion of EA within NLCs could improve the water solubility hence reduce the dose and promote better bioavaibility [15].

Lacatusu *et al* investigated an anti-obesity therapy in which capsaicin (Cap) an herbal medicament along with an endogenous lipid appetite regulator oleoylethanolamide (OEA) and phenylalaninol oleamide OEA (PAO) were simultaneously encapsulated into nanostructured lipid carriers (NLC). The results indicated weight reduction of about 15% in obesity induced albino swiss mice when treated with NLC-POA, after 10-days of treatment. Blood glucose was reduced to 117.38 ± 12.04 mg/dL and 111.20 ± 20.18 mg/dL for NLC-OEA/PAO-Cap treated animals when compared to 213.94 ± 50.90 mg/ dL glucose in obese mice batch. Apart from this these formulations also showed notable antioxidant activity and reduction in cholesterol and triglyceride level. The constructive results can be attributed to the enhanced gastric tolerability of the capsaicin due to its confinement within NLC [16]. Piazzini V *et al* studied the possibility of enclosing lipophilic silymarin (SLM) inside NLCs to obtain a successful oral dosage form. The usefulness of silymarin in type 2 diabetes was identified lately. The use of (SLM) was limited due to its low solubility and low permeability. The permeation study was done with Caco-2 cells which simulates intestinal epithelial cell monolayers and the results indicated that the transport of the NLCs across the cell monolayer was energy-dependent and mediated by clathrin- and caveolae (or lipid raft)-related routes [17].

Diabetes and associated conditions

Diabetes mellitus (DM) management requires collaborative techniques. Progress of diabetes mellitus may be either due to the inability of β-cells of the pancreas to secrete a sufficient quantity of insulin or insensitivity of the body to the secreted insulin. In the long run diabetes may lead to other complications which may affect the kidneys, heart, brain, eyes, nerves and feet. The only remedy to overcome this is to control the blood sugar level. The conventional synthetic drugs used in the treatment of diabetes management lead to serious consequences, hence herbal drugs are emphasized [18]. The accurate and targeted delivery of nanotechnology has popularized nanotized herbal delivery Baicalin-loaded nanostructured lipid carriers (B-NLCs) was developed using high-pressure homogenization by Shi *et al.* to improve its antidiabetic property. Precirol and Miglyol were used as the solid lipid and liquid lipid. The *in vivo* results showed improved hypoglycemic and hypolipidemic activity in B-NLCs compared to the reference baicalin in type 2 diabetes mellitus rats [19]. Silymarin extracted from the milk thistle (*Silybum marianum*) is a phytochemical with hepatoprotective, antioxidant, anti-inflammatory, anticancer, and cardioprotective activities. As mentioned previously hypoglycemic activity of the silymarin is recently spotted through many clinical trials. SLM loaded NLC showed downturn in blood glucose and and triglyceride levels in STZ-induced diabetic mouse model [20].

Berberine an isoquinoline alkaloid isolated from Coptis chinensis. Its hypoglycemic activity is due to inhibition of mitochondrial function, glycolysis stimulation and activation of AMPK pathway [21]. To overcome poor intestinal absorption berberine, was enfolded with in nanostructured lipid carriers laminated with selenium (BB-SeNLCs). BB-SeNLCs showed effective hypoglycemic activity with less variation of blood glucose level [22].

Anti-cancer therapy

Effective cancer therapy is one of the greatest challenges faced globally. Though there is a continuing development in the field, there is a demand for constructive and affordable remedy with less strain and adverse effect to the patients

.[23] The site-specific targeting nature of NLCs has made them noteworthy and popular as a conveyor of anticancer drugs. Use of NLC also reduces the cytotoxic effect of the anti-cancer drugs on non-cancerous tissues and cells [24]. Inclusion of herbal drugs in nano carriers has fascinated the research field. Many nanotized herbal formulation are found to be promising and justifiable in anti-cancer therapy [25]. There are proposals for the use of these herbal formulations for prophylaxis purpose to prevent tumor development in an individual at risk [26]. A natural phenol and a phytoalexin, resveratrol (RSV) was studied by Poonia et al as an effective treatment for breast cancer. Resveratrol is produced by several plants in response to injury or when the plant is attacked by pathogen. Nanostructured lipid carriers were developed in conjugation with folic acid and stearic acid (RSV-FA-NLCs). Pharmacokinetic profile was studied in female wistar rats, there was nine times enhancement in AUC values in RSV-FA-NLCs (57.92 ± 4.65 μg h/mLh) when compared to free RSV (6.37 ± 1.165 μg h/mLh) [27]. Curcumin (CUR) an active constituent of curcuma longa belonging to the ginger family is used globally for its multifarious activity. The therapeutic benefit of curcumin is mainly due to its anti-oxidant and anti-inflammatory mechanisms [28]. In a study conducted by Kamel et al, curcumin was loaded into NLC (CUR-NLCs) for increasing its potential of infiltrating into the cancer cells. MTT reduction assay was utilized to study cytotoxicity of CUR-NLCs on MCF-7 breast cancer. The results fascinated the suitability of curcumin to stimulate apoptosis in assorted cancer cell lines [29]. Enhancement of anticancer activity by concurrent therapy is one of the most researched treatment options. Herbal constituents have often proved to show synergistic effect when used in combination with synthetic drugs. But the herb–drug interactions upon concurrent administration must be taken into consideration [30]. Hyaluronic acid coated baicalein (BCL) and doxorubicin (DOX) loaded NLCs were prepared for obtaining synergistic anticancer effects. The *in vivo* anti-tumor effectiveness of HA-BCL/DOX-NLCs, was examined in mice in which human breast cancer was induced. The magnitude of the solid tumors was evaluated [31].

Psoriasis

Psoriasis is a persistence long term condition with inflamed and rapidly multiplying skin conditions which scale up on the skin [32]. In the field of dermatology psoriasis is the most researched topic due to the lack of reasonable treatment available [33] Systemic embodiment is also reported which affects the victim's standard of living. Clinical studies on curcumin for its anti- psoriatic activity demonstrated that topical application was more efficacious compared to the oral [34]. Combining curcumin with caffeine possessing anti-inflammatory activity was studied by Iriventi, *et al* to explore the synergistic affect [35]. A topical therapy for psoriasis with combination of *Smilax china* (Family: Liliaceae) and *Smilax alba* (Family: Salicaceae) was developed by Qadir *et al*. The nanostructured lipid carriers were developed by melt emulsification method combined with ultra-sonication. The psoriatic score of optimum NLC formulation loaded with smilax china and smilax alba confirmed a decrease in all visible factors like erythema (1.0), scaling (1.1) and thickening (0.85) as compared to positive control and marketed formulation [37]. *Azadirachta indica leaves extract (AE), Lawsonia inermis leaves extract (LE)* and fruit extract of *Mallotus philippensis (ME)* are known to posses antipsoriatic activity. Topical NLC ladened cream showed definite anti-proliferative activity against the HaCat cell lines [37].

Arthritis

Arthritis is one of the most common hassled autoimmune inflammatory disorders affecting all ages [38]. Recently phyto pharmacotherapeutics has been utilized as a surrogate treatment tactics in arthritis [39]. Tulsi or Ocimum sanctum the herbal queen is a popular traditional medicine. Phenolic actives of tulsi are known to possess antioxidant and anti-inflammatory activity. A crude tulsi extract based NLC was used in arthritis management by Ahmad *et al*. Percentage inhibition of the protein denaturation was used as a parameter to measure the anti-arthritic activity. The results were similar to the reference diclofenac gel. The analgesic activity was also better in OLE-NLC-gel attributed to superior drug penetration into skin [40]. Tetrandrine a Stephania tetrandra extract possesses anti-arrhythmic activity similar to quinidine. A study suggested incorporation of tetrandrine into solid lipid nanoparticle to avoid the drawbacks associated with conventional dosage form, which can be extended to nano structured lipid carriers [41].

CONCLUSION

Phytomedicines has notable benefit of being considered safe and less toxic. Formulating herbal extract into a nano formulation improvises its activity by overcoming the associated drawbacks. The presence of biocompatible lipids in the formulation has conquered attention especially for poorly soluble phyto constituents [42]. Further nano structured lipid carriers act as a shield and protect the drug from degradation. Many *in vitro* and *in vivo* studies demonstrated the capability of the NLCs to enhance the bioavailability [43]. Along with these merits, issues like specific drug targeting and their interaction with the bio surfaces needs to be addressed. Hence an extensive investigation with this regard is the need of the hour.

ACKNOWLEDGEMENT

The authors place their sincere thanks to Nitte College of Pharmaceutical Sciences and Krupanidhi College of Pharmacy for providing the facilities to carry out this review.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

ABBREVIATIONS

NLC: Nanostructured lipid carrier, LC: liquid crystal, PSLCs: Precursors systems for liquid crystals, HPH : High pressure homogenization

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Different Levels and Source of Silicon Fertilizers on the Growth and Yield of Groundnut in Coastal Saline Soil

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Received: 29 Dec 2021 Revised: 22 Jan 2022 Accepted: 19 Feb 2022

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ABSTRACT

The field experiment was carried out in a farmer's field at singarakuppam Coastal village during December - April, 2020 to study the effect of different levels and source of silicon fertilizers on the growth and yield of groundnut in coastal saline soil. Texturally, the experimental soil was sandy and taxonomically classified as *Typic Usticpsaments* with initial soil characteristics of the experimental site were, pH–8.23 and EC– 4.14 dS m^{-1}. The soil registered low organic carbon status of 2.32 g kg^{-1}, 139.50 kg ha^{-1} of alkaline $KMnO_4$ –N, 9.57 kg ha^{-1} of Olsen–P, 164.78 kg ha^{-1} of NH_4OAc-K and available silicon content of 31.0 mg kg^{-1}. The treatments consisted of five levels of silicon *viz.,* 0, 20, 40, 60 and 80 kg Si ha^{-1}as factor-L and three different sources of silicon fertilizers viz., S_1 – Calcium silicate(Csi); S_2 – Silixol granules(SG) and S_3–Diatomaceous earth(DE) as factor-S. The experiment was laid out in a randomized block design with factorial arrangement (FRBD) and replicated thrice using groundnut variety VRI 2 as test crop. The results revealed that the combined application of Si @ 60 kg ha^{-1} through Diatomaceous earth (DE)(L_3S_3) significantly increased the growth characters, yield characters and yield of groundnut.

Keywords: Calcium silicate, Silixol granules, Diatomaceous earth, Growth, Yield, Groundnut, Coastal Saline Soil.

INTRODUCTION

The Indian coastal region stretching over a long strip of 8129 km over the eastern and western border are severely degraded and pose serious problems for agricultural production in coastal ecosystem. The coastal agriculture faces a host of problems related to seawater, poor quality water, cyclones and flood. The low lying agriculture lands frequently suffer from severe drainage and soil salinity problem. Salinity is the most brutal environmental factors limiting the productivity of crop plants because most of the crops are sensitive to salinity caused by high concentration of salts in the soil and the area of land affected by it is increasing day by day (Singaravel *et al.,* 2019). Soil salinity is assuming menacing proportions for production of agricultural and horticultural crops in South India. Soil salinity hampers crop production in the coastal ecosystem to a greater extent (Elayaraja and Jawahar. 2020).In India, salt affected soils occupies 25.01 million hectare, out of which around 18.28 M ha are saline soils including 6.73 M ha alkali soils (constitute 30% of the total salt affected soils of the country). This area extends to about 3.18 L ha salt affected soils in Tamilnadu (Subhasis mandal *et al.,* 2018).Coastal saline soil is a significant soil with low status of organic carbon and poor exchange property, which results in nutrient deficiency in plants and yield loss. Even the nutrients are applied, the poor physical properties associated with poor exchange and low organic carbon status of soil leads to leaching of nutrients, further extent of these problem of nutrient deficiency. The coastal farmers are exploiting these lands with unscientific management practices and realizing very low yield of crops as compared to other regions (Arulmathi and Porkodi, 2020). In India, groundnut (*Arachishypogaea*.L) is one of the important oilseed crops; it occupies an area of 4.89 M ha with the production of 10.10 M tons and yield of 2065 kg ha^{-1}.India ranks first in the worldwide in area and second in production after China and the productivity of groundnut is quite low as compared to world average. It is grown in 0.35 M ha in tamilnadu with a production of 0.98 M tons and yield of 2840 kg ha^{-1}(Agriculture statistics at a glance. 2020).

The productivity of state and national level is less as compared to USA and China due to poor fertilizer management and high nutrient leaching losses in coastal soil as compared inland areas. In recent years, more research has been performed with regards to soil and foliar nutrition using silicon fertilizers, which brings unequivocal production benefits and at the same time is much cheaper and more convenient to use than soil fertilization especially in coastal salt affected soils. Silicon is second most abundant element in the lithosphere and it is the most abundant element in soil next to oxygen and comprises 28 per cent of its weight and 3 - 7 per cent in soil solution and alleviates biotic and abiotic stresses and to enhances crop productivity (Mehdi Sadeghi *et al.,* 2021).Si is the only element not detrimental to plants even excess application. Silicon has a number of well recognized beneficial effects on plant growth such as less cuticular water loss due to epidermial accumulation of silica and also affects lignin biosynthesis and provides resistivity to plants against pest attack. The essentiality of silicon for higher plants has been reported by Parthiban *et al*. (2017) and Packirisamyparthiban *et al.* (2019). Silicon is most commonly found in soils in the form of solution as orthosilicic acid (H_4SiO_4) and plants take up directly for their growth and development(Sajadmajeedzargar *et al*., 2019). The role of exogenous silicon in enhancing plant resistance to various biotic and abiotic stresses: salinity, drought, pest attack, metal toxicity and ultra violet radiation were earlier reported by several researchers (Jawahar *et al*., 2019 and Gokulapriya natarajan *et al*., 2021). Further, silicon is a beneficial or quasi element to alleviate the salinity stress and enhance the nutrient availability and also increased the available beneficial cations (Adamfrew *et al*., 2018). Hence, inclusion of silicon as beneficial nutrient in the fertilization programme becomes an imperative need to improve the yield of groundnut. Hence, the present investigation was conducted to optimize the level and source of silicon application to groundnut as well as to identify the best optimized dose of Si for improving the growth and yield of groundnut in coastal saline soil.

MATERIALS AND METHODS

A field experiment was carried out in a farmer's field during Dec – March, 2020 at singarakuppam coastal village, near Chidambaram taluk in cuddalore district, Tamilnadu. The field experiment was conducted to find out the effect of different levels and source of silicon fertilizers on the growth and yield of groundnut in coastal saline soil. The

experimental soil was sandy loam texture with pH–8.23, EC–4.14 d sm^{-1} and 2.32 g kg^{-1}.The initial experimental soil status of alkaline $KMnO_4$-N, Olsen-P and NH_4OAC-K were 139.50, 9.57 and 164.78 kg ha^{-1}, respectively and the available silicon content was 31.0 mg kg^{-1} in soil. The treatments consisted of different levels of Siliconviz.,L_0-control, L_1-20 kg Si ha^{-1}, L_2 – 40 kg Si ha^{-1}, L_3 – 60 kg Si ha^{-1} and L_4 – 80 kg Si ha^{-1} as factor- L (Si Levels) and different sources of silicon fertilizers *viz.*,S_1-Calcium silicate (Csi), S_2– Silixol granules (SG) and S_3–Diatomaceous earth (DE) as factor- S (Si sources). The experiment was laid out in a randomized block design with factorial arrangement (FRBD) and replicated thrice. A uniform NPK dose of 17:34:54 kg ha^{-1} was supplied through urea, super phosphate and muriate of potash to all plots. Half of the N and entire P and K were applied as basal and the remaining half N was applied in two splits at flowering and peg formation stage. The biofertilizer, *Rhizobium* @ 2 kg ha^{-1} were incorporated to all the experimental plots. Gypsum @ 400 kg ha^{-1} was applied at flowering stage to each plot. Calculated amount of Si was applied through Calcium silicate (Csi), Silixol granules (SG) and Diatomaceous earth (DE) as per the treatment schedule just before sowing. Various growth components like plant height, number of branches $plant^{-1}$, dry matter production (DMP) and yield components *viz.,* number of pods $plant^{-1}$,100 pod weight, 100 kernel weight and shelling percentage. The yield of pod, kernel and haulm yield were recorded separately and expressed in kg ha^{-1}.

RESULTS AND DISCUSSION

Growth Components of Groundnut (Table 1)

Growth components of groundnut such as plant height, number of branches $plant^{-1}$ and dry matter production were significantly increased due to different levels and source of silicon fertilizers. Among the different levels of silicon (Si) studied, application of Si @ 80 kg ha^{-1}(L_4) recorded the maximum plant height (46.95 cm), number of branches $plant^{-1}$(9.34) and dry matter production (4521kg ha^{-1}) of groundnut. However, it was found to be on par with application of Si @ 60 kg ha^{-1}(L_3), which recorded a comparable plant height (46.71cm), number of branches $plant^{-1}$(9.29) and dry matter production(4503 kg ha^{-1})of groundnut, respectively. Among the three different sources of silicon nutrition fertilizer, Diatomaceous earth (DE) (S_3) excelled the other two sources Silixol granules (SG) and Calcium silicate (Csi) of silicon fertilizers in improving the growth components of groundnut. The interaction effect between levels and source of silicon on growth characters of groundnut was significant. The treatment (L_4S_3), which received Si @ 80 kg ha^{-1} through Diatomaceous earth (DE),recorded a maximum plant height (48.82 cm), number of branches $plant^{-1}$ (9.75) and dry matter production(4652kg ha^{-1}). However, it was found to comparable with Diatomaceous earth (DE) @ 60 kg Si ha^{-1}(L_3S_3). This was followed by treatment pairs of L_4S_2 and L_3S_2. The lowest plant height, number of branches $plant^{-1}$ and dry matter production was noticed in control (RDF alone).

In coastal saline soil, the groundnut responded to a level of 80kgSiha^{-1} of silicon as Diatomaceous earth (DE) through soil application was established as the best treatment in increasing plant height, number of branches $plant^{-1}$ and dry matter production. However, it was comparable with Diatomaceous earth (DE) @ 60 kg Si ha^{-1}. Increased growth characters might be due to application of silicon as Diatomaceous earth (DE) which improved the plant growth through enhancement of total chlorophyll contents in leaves as well as increased in the photosynthetic rate by supplying more amount plant available silicon of Si O_2which resultant in higher dry matter production of groundnut. This observation was in accordance with Jawahar *et al*., (2019). Further, the improvement in growth characters with the different levels and sources of silicon fertilizers, application of Diatomaceous earth (DE) @ 60 kg Si ha^{-1} (L_3S_3) along with recommended dose of NPK registered highest plant growth characters. This might be due to the greater availability of macro and micronutrients in silicon applied plots which might have enhanced photosynthetic and other metabolic activity. This led to an increase in various plant metabolites responsible for cell division and elongation (Jawahar *et al.,* 2020). The increase in number of branches may be due to higher nutrient use efficiency, physiological efficiency and photosynthetic rates (Zhaoxia dong *et al*., 2018), whereas, dry matter accumulation increased due to increase in plant height, number of branches and greater nutrient availability and increase in photosynthetic rate. The result obtained was in accordance with the findings of Thais Chagas barros *et al*., (2018).

Yield Components of Groundnut (Table 2 and 3)

Application of different levels of silicon (Si) through different sources favorably increased the yield components of groundnut *viz.,* number of pods plant^{-1}, 100 Pod weight, 100 kernel weight and shelling percentage. Irrespective of the sources of silicon, groundnut responded to Si application up to 80 kg Si ha^{-1} in coastal saline soil. Among the different levels of silicon evaluated, application of Si @ 80 kg ha^{-1} recorded the maximum number of podsplant^{-1}(26.30),100 Pod weight (81.80 g),100 kernel weight (42.93 g) and shelling percentage(68.22)of groundnut. However, it was found to be equally efficacious with Si @ 60 kg ha^{-1}, which recorded a value of 26.22 numbers of pods plant^{-1}, 81.46 g of 100 Pod weight,42.66 g of 100 kernel weight and 67.78% of shelling percentage, respectively. Among the three sources of silicon fertilizers tried, application of silicon through Diatomaceous earth (DE)(S_3) was found to be superior in increasing the yield characters *viz.,* number of pods plant^{-1} (25.76), 100 Pod weight (79.97 g),100 kernel weight (41.91 g) and shelling percentage(66.68), respectively. This was followed by the application of silicon through Silixol granules (SG) (S_2) and Calcium silicate (Csi)(S_1). The interaction effect due to different levels and source of Si fertilizers on the yield components of groundnut was significant. The treatment (L_4S_3), which received Si as Diatomaceous earth (DE) @ 80 kg Si ha^{-1} recorded a highest number of pods plant^{-1} (27.12), 100 Pod weight (84.13 g), 100 kernel weight (45.12 g) and shelling percentage (71.21 %) of groundnut. However, it was found to be on par with Diatomaceous earth (DE) @ 60 kg Si ha^{-1} (L_3S_3). This was followed by the treatment pairs L_4S_2 and L_3S_2. The control treatment L_0S_1, registered the lowest yield components of groundnut (without silicon). All the yield characters of groundnut increased significantly with Diatomaceous earth (DE) application. Among the various levels of silicon treatments, the application of Diatomaceous earth (DE) @ 60 kg Si ha^{-1} recorded the higher number of pods plant^{-1},100 Pod weight, 100 kernel weight and shelling percentage of groundnut. This fact was attributed to the increased availability of silicon in the soil. The increased yield characters of the groundnut plants can be attributed to the beneficial effects of silicon in the plants resulted in improvement of architecture with more biomass, which intercept high solar luminosity, increasing the photosynthetic efficiency and higher chlorophyll content. These findings are in conformity with those of Mikhina *et al*., (2019); Bukhari *et al*., (2021)

Yield of Groundnut (Table 4)

Groundnut responded well for the different levels and source of silicon fertilizer application. The effect was very clearly reflected in pod, kernel and haulm yield of groundnut. The effect of different levels of silicon (Si) in increasing the groundnut yield was well evidenced in the present study. Increase in the level of Si from 0 to 80 kg ha^{-1} increased the pod, kernel and haulm yield of1473 to 1696 kg ha^{-1}, 902 to 1135 kg ha^{-1} and2114 to 2357 kg ha^{-1} respectively. Among the various levels of silicon, application of Si @ 60 kg ha^{-1} was excelled the other four levels. Application of Si @ 80 kg ha^{-1} (L_4) registered a higherpod, kernel and haulm yield of 1696, 1135 and 2357 kg ha^{-1} respectively. This was on par with application of Si @ 60 kg ha^{-1} (L_3) by registering 1688, 1126 and 2348kg ha^{-1} of pod, kernel and haulm yield of groundnut, respectively. This was followed by the treatments L_2 (Si@ 40 kg ha^{-1}), L_1 (Si @ 20 kg ha^{-1}) and control (L_0). Among the different sources of silicon fertilizers, application of silicon through Diatomaceous earth (DE)(S_3) recorded the highest mean pod (1650 kg ha^{-1}), kernel (1113 kg ha^{-1}) and haulm yield (2314kg ha^{-1}) of groundnut. This was followed by the treatments S_2Silixol granules (SG) and S_1Calcium silicate(Csi).The interaction effect due to levels and source of silicon fertilizers significantly increased the pod, kernel and haulm yield of groundnut. Application of silicon through Diatomaceous earth (DE) @ 80 kg Si ha^{-1} (L_4S_3) recorded the highest pod, kernel and haulm yield of 1743 kg ha^{-1}1240 kg ha^{-1}, and 2424 kg ha^{-1} which was 15.54, 27.33and 12.51 per cent increase over control. This treatment was closely on par with the treatment which received Diatomaceous earth (DE) @ 60 kg Si ha^{-1} (L_3S_3). Application of Diatomaceous earth (DE)@ 60 kg Si ha^{-1} registered a pod, kernel and haulm yield of 1733, 1231 and 2413 kg ha^{-1} which were15.06, 26.80 and 12.47 per cent increase over control (RDF alone). The application of Diatomaceous earth (DE) @ 80 kg Si ha^{-1} (L_4S_3) excelled all other treatments in improving the yield of groundnut. However, it was found to be on par with treatment L_3S_3, which supplied with Diatomaceous earth (DE) @ 60 kg Si ha^{-1}. This might be due to increased nutrient availability, which resulted in better vegetative growth coupled with better partitioning of photosynthates in vegetative and reproductive parts. Silicon plays a key role in retaining the water capacity in plant cells and also corrects the level of endogenous growthhormone *viz*. auxins, gibberellins and cytokinins under stress conditions. The earlier report of Laxmanarayanan *et al*., (2020) and Ahmed Khalaf *et al*., (2020) confirm the present findings.

CONCLUSION

The results of the present study clearly revealed that the beneficial effect of silicon fertilizers for increasing groundnut production in coastal saline soil. Among the silicon fertilizers, the best level and source of silicon were selected based on the yield performance of groundnut. Soil application of silicon as Diatomaceous earth (DE) @ 60 kg Si ha^{-1} was the optimum dose of silicon identified to maximize the yield of groundnut in coastal saline soil.

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Table 1. Effect of different levels and sources of silicon fertilizers on the growth characters of groundnut

L / S	Plant height (cm)						No. of branches plant^{-1}						Dry matter production (Kg ha^{-1})					
	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean
S_1	40.20	41.46	43.35	44.87	45.07	**42.99**	7.04	7.91	8.43	8.91	8.95	**8.24**	3991	4117	4239	4367	4389	**4220**
S_2	40.24	43.23	45.09	46.75	46.98	**44.45**	7.07	8.31	8.86	9.29	9.34	**8.57**	3994	4244	4370	4504	4523	**4327**
S_3	40.47	45.04	46.55	48.53	48.82	**45.88**	7.02	8.79	9.24	9.69	9.75	**8.89**	3995	4378	4499	4640	4652	**4432**
Mean	**40.30**	**43.24**	**44.99**	**46.71**	**46.95**		**7.04**	**8.33**	**8.84**	**9.29**	**9.34**		**3993**	**4246**	**4369**	**4503**	**4521**	
		SE_D			CD (p=0.05)			SE_D			CD (p=0.05)			SE_D			CD (p=0.05)	
L		0.28			0.58			0.04			0.09			12			25	
S		0.59			1.22			0.09			0.19			32			67	
L × S		0.65			1.34			0.14			0.29			47			98	

L- Levels; L_0-control, L_1-20kg Si ha^{-1}, L_2 – 40 kg Si ha^{-1}, L_3 – 60 kg Si ha^{-1} and L_4 – 80 kg Si ha^{-1}
S- Sources; S_1-Calcium silicate (Csi), S_2– Silixol granules (SG) and S_3–Diatomaceous earth (DE).

Table 2. Effect of different levels and sources of silicon fertilizers on the yield characters of groundnut

L / S	Number of pods plant^{-1}						100 Pod Weight(g)					
	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean
S_1	23.17	23.82	24.61	25.43	25.51	**24.50**	71.93	74.26	76.53	79.27	79.49	**76.29**
S_2	23.15	24.63	25.38	26.22	26.29	**25.13**	71.94	76.56	78.86	81.52	81.79	**78.13**
S_3	23.11	25.41	26.17	27.01	27.12	**25.76**	71.98	78.91	81.25	83.61	84.13	**79.97**
Mean	**23.14**	**24.62**	**25.38**	**26.22**	**26.30**		**71.95**	**76.57**	**78.88**	**81.46**	**81.80**	
		SE_D			CD (p=0.05)			SE_D			CD (p=0.05)	
L		0.06			0.13			0.35			0.73	
S		0.14			0.29			0.54			1.12	
L × S		0.27			0.57			0.81			1.67	

L- Levels; L_0-control, L_1-20kg Si ha^{-1}, L_2 – 40 kg Si ha^{-1}, L_3 – 60 kg Si ha^{-1} and L_4 – 80 kg Si ha^{-1}
S- Sources; S_1-Calcium silicate (Csi), S_2– Silixol granules (SG) and S_3–Diatomaceous earth (DE).

Table 3. Effect of different levels and sources of silicon fertilizers on the yield characters of groundnut

L / S	100 kernel weight (g)						Shelling %					
	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean
S_1	36.91	38.12	39.33	40.89	41.18	**39.28**	57.71	60.09	62.63	64.24	64.68	**61.87**
S_2	36.92	39.34	40.67	42.14	42.49	**40.31**	57.69	63.08	65.56	68.18	68.79	**64.66**
S_3	36.89	40.67	41.94	44.97	45.12	**41.91**	57.72	65.52	68.03	70.92	71.21	**66.68**
Mean	**36.90**	**39.37**	**40.64**	**42.66**	**42.93**		**57.70**	**62.89**	**65.40**	**67.78**	**68.22**	
		SE_D			CD (p=0.05)			SE_D			CD (p=0.05)	
L		0.17			0.35			0.25			0.52	
S		0.27			0.57			0.57			1.17	
L × S		0.45			0.94			0.86			1.78	

L- Levels; L_0-control, L_1-20kg Si ha^{-1}, L_2 – 40 kg Si ha^{-1}, L_3 – 60 kg Si ha^{-1} and L_4 – 80 kg Si ha^{-1}

S- Sources; S_1-Calcium silicate (Csi), S_2– Silixol granules (SG) and S_3–Diatomaceous earth (DE).

Table 4. Effect of levels and sources of silicon on the yield (kg ha^{-1}) of groundnut

L / S	Pod yield						Kernel yield						Haulm yield					
	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean	L_0	L_1	L_2	L_3	L_4	Mean
S_1	1473	1517	1584	1644	1648	**1573**	904	985	1034	1022	1033	**995**	2112	2156	2196	2278	2285	**2205**
S_2	1472	1572	1631	1689	1697	**1612**	903	1033	1078	1126	1134	**1054**	2116	2217	2267	2354	2362	**2263**
S_3	1476	1625	1676	1733	1743	**1650**	901	1074	1120	1231	1240	**1113**	2114	2279	2341	2413	2424	**2314**
Mean	**1473**	**1571**	**1630**	**1688**	**1696**		**902**	**1030**	**1077**	**1126**	**1135**		**2114**	**2217**	**2268**	**2348**	**2357**	
		SE_D			CD (p=0.05)			SE_D			CD (p=0.05)			SE_D			CD (p=0.05)	
L		5			12			8			17			6			14	
S		10			22			13			28			13			28	
L × S		17			35			20			41			21			43	

L- Levels; L_0-control, L_1-20kg Si ha^{-1}, L_2 – 40 kg Si ha^{-1}, L_3 – 60 kg Si ha^{-1} and L_4 – 80kg Si ha^{-1}

S- Sources; S_1-Calcium silicate (Csi), S_2– Silixol granules (SG) and S_3–Diatomaceous earth (DE).

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Investigation over Antibacterial, Thermodynamic, and Photocatalytic Activities of Herbal Synthesized Iron Oxide Nanoparticles

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Received: 19 Feb 2022 Revised: 06 Mar 2022 Accepted: 28 Mar 2022

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ABSTRACT

The present work described an eco-friendly approach of sol-gel method for synthesizing ferric oxide nanoparticles utilizingferric chloride hexahydrate($FeCl_3.6H_2O$) as a precursor material. The biosynthesized Fe_2O_3 nanoparticles were characterized using X-ray diffraction spectroscopy to analyses the crystallinity, SEM was used to study the morphology, FTIR was used to determine the functional groups present in the synthesized nanoparticles, and UV-vis spectroscopy was used to determine the optical band gap of the material. The antibacterial activity of the produced nanoparticles is investigated as well asthe acoustical properties of the iron oxide nanofluid is evaluated using ultrasonic technique. The synthesized Fe_2O_3 nanoparticle is tested as catalysts for the photo catalytic decomposition of Congo red dye and methylene blue dye under the radiation of UV- light and the maximum degradation achieve with 60 min of reaction time.

Keywords: sol-gel, precursor, crystallinity, morphology, band gap, antibacterial, degradation.

INTRODUCTION

Exploiting natural resources for metal nanoparticle production has proven to be a reliable and environmentally friendly strategy. Metallic nanoparticles are currently a hotspot of interdisciplinary research due to their inherent potential for diverse nanotechnological applications [1]. Plant mediated NPs synthesis is currently the most efficient method to produce large scale NPs in a short time. The bioactive components contained in the plant extract act as reducing and capping agents in the synthesis process and reduce the metal ions to NPs [2, 3] Therefore, no additional surfactants or capping agents are required for the synthesis [4]. Green synthesis of metal or metal oxide NPs using plant extracts as biogenic agents has numerous advantages such as cost-effectiveness, atom economy, simplicity, benign, nontoxic, elimination of toxic and dangerous materials, and easy availability [5].

Iron oxide has been one of the extensively investigated transition metal oxides because of its significant variable oxidation states, crystal structures, low cost, magnetic properties, and environmentally friendly nature [6,7]. Nanoscale materials such as nanoadsorbents, nanocatalysts, nanofiltration, and nanobiocides such as metal and metal oxide nanoparticles are currently being employed for remediation of water and wastewater pollutants. Among these metallic nanoparticles, iron nanoparticles (FeNPs) have promising advantages that can combat environmental pollution. The production of iron nanomaterials, such as metallic iron and oxide of iron via a more convenient greener route, is a great step forward in the development of nanomaterials [8]. Metal oxide NPs can have stronger bactericidal or bacteriostatic effects than sole metal ions [9-11]. Because of their small size, large surface area, and high degree of dispersion, NPs are able to penetrate bacterial membranes, resulting in bactericidal activity.An active ingredient thymoquinone present in nigella sativa oil extract possess an anti-inflammatory, anti-tussive, anti-hypertensive, anti-diabetic, antibacterial and anti-cancer properties.

The present study focused on synthesis iron oxide nanoparticles by using Nigella sativa extract as reducing/stabilizing agent and assess their antibacterial efficacy. Thermoacoustical properties of iron oxide nanofluid prepared using polyethylene glycol as a base fluid investigated using ultrasonic technique with the results are interpreted in terms of intermolecular interactions between solute and solvent. Aside from these the photocatalytic efficiency of Fe_2O_3 – nanoparticle is analyzed by degrading both congo red dye and methylene blue dyesand the absorbance at 664 nm/498 nm with a UV-vis spectrophotometer.

MATERIALS AND METHODS

Sample Collections

Ferric chloride hexahydrate of AR grade was purchased from Avantor Performance Materials India Limited and seeds of Nigella sativa were obtained from a local market and brought to the laboratory.

Preparation of Plant Extract

The following approach was used to make an aqueous extract of nigella sativa. 25 g nigella sativa seeds were washed and immersed in 200 ml of distilled water and boiled for 20 minutes at 300º C. The nigella sativa extract was then filtered through Whatman filter paper. For the synthesis of iron oxide nanoparticles, the resultant solution will operate as a reduction and capping agent.

Herbal Synthesis of Iron Oxide Nanoparticles

1 mM of ferric chloride hexahydrate ($FeCl_3.6H_2O$) was dissolved in 100 ml of nigella sativa seed extract and mixed thoroughly. The prepared solution under stirring for 20 hours in magnetic stirrer. The color of the reactant solution turned to yellowish brown tobrownish black which confirmed the formation of iron oxide nanoparticles. The reacted solution was further dried at 180º C before being calcined at 500º C for 2 hours. The biosynthesized iron oxide nanoparticles are shown in fig.1. The XRD, UV, FTIR, and SEM analyses were performed for the resultant nanoparticles. Further antibacterial activity of biosynthesized iron oxide nanoparticles was carried out.

Characterization

The XRD pattern of the prepared iron oxide nanoparticles was recorded by an X-ray diffractometer by using Cu kα radiation ofwavelength of 1.54060 Å. The morphology of the nanoparticles was studied by scanning electron microscope run at a voltage of 10.0 KV. FT-IR spectra of the sample was recorded in the wavenumber range of 4000 – 500 cm^{-1}. The UV- Visible spectra of the sample to measure the absorbance and optical band gap which was done by using UV series spectrophotometer.

RESULTS AND DISCUSSION

XRD Analysis

The phase identification and crystalline structures of the nanoparticles was characterized by X-ray powder diffraction [12]. The XRD pattern of prepared iron oxide nanoparticles were shown in fig. 2. The presence of diffraction peaks at 25.16˚, 35.12˚, 36.63˚, 40.64˚, 49.97˚, 57.08˚, 59.42˚, 62.6˚ and 64.1˚ are corresponding to the crystal planes of (012), (104), (110), (113), (024), (116), (018), (214) and (300) confirms the formation of Fe_2O_3 nanoparticles. The intense and sharp peaks revealed that Fe2O3 nanoparticles formed by the reduction method using Nigella sativa seed extract were crystalline in nature. The crystallite size of synthesized nigella sativa iron oxide nanoparticles can be calculated using the Debye-Scherrer equation [13], which reveals a relationship between X-ray diffraction peak broadening and crystallite size. The Debye Scherrer equation is given as, $d = \frac{k\lambda}{\beta \cos\theta}$

Where d is the average crystallite size of synthesized iron oxide nanoparticles, k is the Scherrer constant with a value, λ is the X-ray wavelength (0.15406 nm), β is the line broadening in radians, and θ is the Bragg diffraction angle. The average crystallite size as determined using the Debye–Scherrer equation was found to be 7.62 nm.

SEM Analysis

Morphological screening of synthesized nanoparticles is important in terms of composition and size analysis [14] and this study was made use of SEM analysis. SEM images of the synthesized Fe_2O_3 nanoparticles are shown in fig. 3 (a) with magnification of 35 K and fig. 3 (b) with magnification of 45 K revealed the rod shape of prepared particles. The SEM images indicate that the prepared iron oxide nanoparticles get agglomerated due to the accumulation of tiny building blocks of various bioactive reducing agents of plant extract or this might be due to the lower capping ability of the plant extract and agglomeration tendency of the iron-based nanoparticles due to magnetic interactions [15].

FTIR Analysis

The main functional group of magnetic iron oxide nanoparticles was identified using FTIR measurements. The transmittance band ranging from 4000 cm^{-1} to 500 cm^{-1} was recorded for the FTIR spectra as depicted in figure 3. The –OH bond stretching from the aqueous phase is shown by the peak at 3527.35 cm^{-1}, whereas the N-H stretching is represented by the peak at 2350 cm^{-1}. The band in 2085 cm^{-1} could be assigned to C≡C. The peak in 1510 cm^{-1} represents C=C stretching vibration. The C-O stretching and O-H stretching vibrations are denoted by the peak present at 1123 cm^{-1} and 1053 cm^{-1} respectively. The peak at 872 cm^{-1} reflects the aromatic C-H bending vibration. Moreover, a band at 571 cm^{-1} belongs to Fe-O (iron oxide) group.

UV- Visible Spectroscopy Analysis

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region [16]. Using UV-visible spectroscopy theoptical band gap of iron oxide Fe_2O_3 nanoparticles annealed at 500°C were estimated. Fig. 5(a) shows the variation of absorption coefficient (α), as a function of wavelength. The maximum absorption observed at the wavelengths of 280 nm and 552 nm. The optical band gap was extracted according to the following relation, $\alpha = \frac{B(h\nu - E_g)^n}{h\nu}$

where hν is the incident photon energy, α is the absorption coefficient, B is a materials dependent constant and E_g is the optical band gap. The value of n depends on the nature of transition. Depending on whether the transition is

direct allowed, direct forbidden, indirect allowed or indirect forbidden, n takes the value 1/2, 3/2, 2 or 3 respectively [17]. Tauc plot shown in fig. 5(b) illustrates the relation between $(\alpha h\nu)^2$ vs $h\nu$ for direct band gap while (5c) shows the relation between $(\alpha h\nu)^{1/2}$ vs $h\nu$ for indirect band gap. As demonstrated in figs. 5(b) and 5(c), the direct band gap of the synthesized iron oxide nanoparticle is 2.02 eV and the indirect band gap is 3.62 eV.

Applications

Antibacterial Efficacy

The pathogenic bacteria *were used for antibacterial studies.* The bacterial strains were sub cultured in nutrient agar medium (g/l) was used for bacterial strains. Bacterial cultures were injected onto agar plates and incubated for 30 minutes at 37°C. The nutrition agar plates were pierced with 6 mm diameter holes. 20 mg/ml of iron-oxide nanoparticles were poured into the holes and incubated at 37°C for 24 hours. The zone of inhibition assessed the antibacterial activity. The antibacterial activity of iron oxide nanoparticles against the pathogenic bacteria such as, *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa is shown in table 1. The zone of inhibition at 0.02 mg/ml of Fe_2O_3 nanoparticles againstStaphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosaare 10, 11 and 12 respectively*. The zone of inhibition of iron-oxide nanoparticles was directly proportional to the increase in concentration of iron-oxide nanoparticles [18].

Thermoacoustical Properties Of Iron Oxide (Fe_2O_3) Nanofluid

Thermodynamic, acoustic, and transport aspects of fluids are all important in understanding the nature of their molecular interactions, which influence the physico-chemical properties of liquids. In the present work,the ultrasonic velocity of prepared iron oxide nanofluid of five different concentrations (0.01 wt(%), 0.02wt(%), 0.03wt(%), 0.04wt(%) and 0.05wt(%)) is measured at four different temperatures using an ultrasonic interferometer with the precision of ±0.001 ms^{-1} working at the fixed frequency of 2 MHz supplied by the Mittal enterprises, New Delhi. The density of the nanofluid is measured using a 5 ml specific gravity bottle and the viscosity is by using an Ostwald's viscometer. Aside from these, the adiabatic compressibility, free length, acoustic impedance and relaxation time of the nanofluid are also estimated in order to explore the solute – solvent interactions. As shown in Fig. 7(a), ultrasonic velocity (U) exhibits non-linear behaviour as temperature and nanoparticle concentration in the base fluid increase.This indicates the qualitative measure of particle fluid interactions. It ensures that the intermolecular interaction is dominating over the intramolecular interaction or in other words it shows the predominance of particle–fluids interaction over particle–particle interaction [19].

Internal resistance to fluid flow is described by viscosity. The viscosity (**η**) of the nanofluid falls at lower concentrations of nanoparticles, then increases, as depicted in Fig. 7(b) which reveals that at lower concentrations, the particle–fluid interaction predominates over the intraparticle interactions. The viscosity of the nanofluid reduced as the temperature was raised, indicating that intermolecular interactions were deteriorating at higher temperatures.
Compression waves can propagate in most fluids since all real fluids are compressible. According to fig. 7(d), adiabatic compressibility (β_{ad}) of nanofluid get increases in 0.02 wt. (%) afterwards it gets decreases as the concentration of nanoparticle increased. This decrement in compressibility suggests the strong intermolecular interactions between the solute and solvent. Fig. 7(d), shows the plot for free length (L_f) which exhibits the same trend as that of adiabatic compressibility. In the view of temperature, both compressibility and free length are increase. It demonstrates that the particle-fluid interaction is substantially greater at lower temperatures than at higher temperatures. Acoustic impedance (Z) determines the resistance offered to the propagation of ultrasonic wave in a material [20]. It can be seen from the fig. 7(e), acoustic impedance has non- linear behaviour which reveals the significant intermolecular interactions. At higher temperatures, interactions become weaker is responsible for the decrease of acoustic impedance. The relaxation time (τ) can be used to assess the heat conductivity of nanofluids.As shown infig. 7(f), relaxation time of nanofluid increases at higher concentration of the Fe- nanoparticles. The long relaxation time of the Brownian movement of nanoparticles extensively influences to enhance the heat transporting properties of nanofluids [21].

Photocatalytic Degradation Of Synthetic Dyes By Fe_2O_3 Nanoparticles

The photocatalytic activity of theFe_2O_3 nanoparticles was evaluated by the degradation efficiency of methylene blue/ congo red (MB/CR) under visible light (Philips, 40 W). In each experiment, 5, 10 mg of the catalyst was suspended in 10 ml of the aqueous solution of MB (10 mg L^{-1})/CR (10 mg L^{-1}), and the suspension was magnetically stirred in the dark for 30 min to establish adsorption/desorption equilibrium of MB molecules on the surface of the catalyst. Subsequently, the mixture was transferred to a test tube and exposed to visible light. At a given interval of time (10-60min), 2 ml of the suspension was taken out and the concentration of MB/CR was analysed by measuring the absorbance at 664 nm/498 nm using a UV-vis spectrophotometer. Fig. 8 depicts the degradation of methylene blue and congo red dyes in the presence of synthesized iron oxide nanoparticles under UV- light which assures that, the Fe_2O_3 nanoparticles have excellent degradation efficiency for both the dyes.

It exhibits the enhanced photocatalytic degradation even at lower concentration (0.005 g) of iron oxide nanoparticles. Both methylene blue and congo red dyes were completely decomposed under the irradiation of UV- light with the reaction time of 60 minutes. As a result, it was concluded that, the green synthesized iron oxide nanoparticles with the average crystallite size of 7.62 nm are stable and hold the high decolorization property.

CONCLUSION

Utilizing the seed extract of *nigella sativa*, the iron oxide nanoparticles (Fe_2O_3) were produced effectively employing a green synthesis approach. Synthesized nanoparticles were further characterized by XRD, SEM, FT-IR and UV-Visible spectroscopy. Using the Debye–Scherrer equation, the average crystallite size of Fe_2O_3 nanoparticles is found to be 7.62 nm. The rod structure of produced nanoparticles was revealed by SEM pictures. The nanoparticles' antibacterial activity was investigated as well. The thermodynamic characteristics of iron oxide nanofluid of five different concentrations at the temperatures of 308 K, 313 K, 318 K and 323 K were analysed and it was exhibited a good particle- fluid interactions. After that the degradation efficacy of Fe_2O_3 – nanoparticles were investigated against methylene blue and congo red dyes which exhibits enhanced photocatalytic activity even at the lower concentration.

ACKNOWLEDGEMENTS

One of the authors (C. Duraivathi) would like to express her heartfelt thanks to the Director of Collegiate Education, Chennai - 600006 for the stipend provided under the full time Research Scholar Scheme, since 2018.

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Table 1. Antibacterial activity of fe_2O_3 nanoparticles

Microorganism	Zone of inhibition (mm)
S. aureus	10
K. Pneumonia	11
Pseudomonas aeruginosa	12

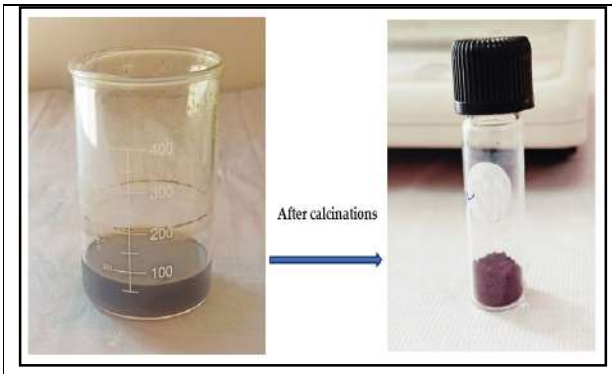


Fig. 1 Prepared Fe_2O_3 nanoparticles.

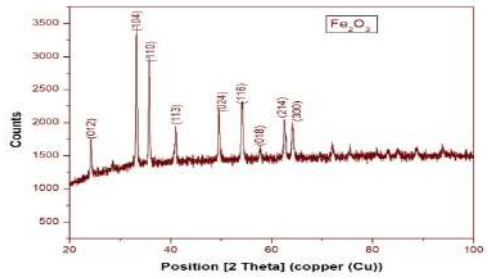


Fig.2 XRD pattern of synthesized Fe_2O_3 Nanoparticles

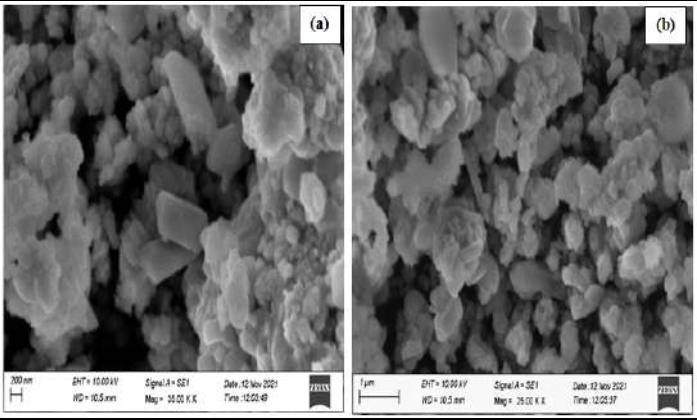


Fig. 3 Morphology of iron oxide nanoparticles (a) with magnification of 35 K (b) with magnification of 25 K.

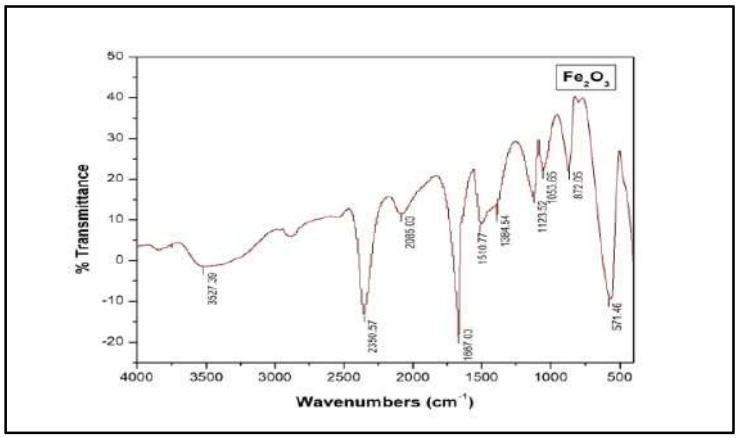


Fig. 4 FT-IR spectra of synthesized Fe_2O_3 Nanoparticles

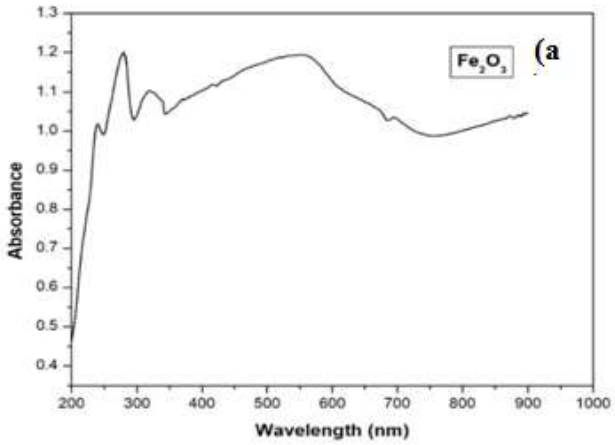


Fig. 5 (a) variation of absorption coefficient (α) with wavelength.

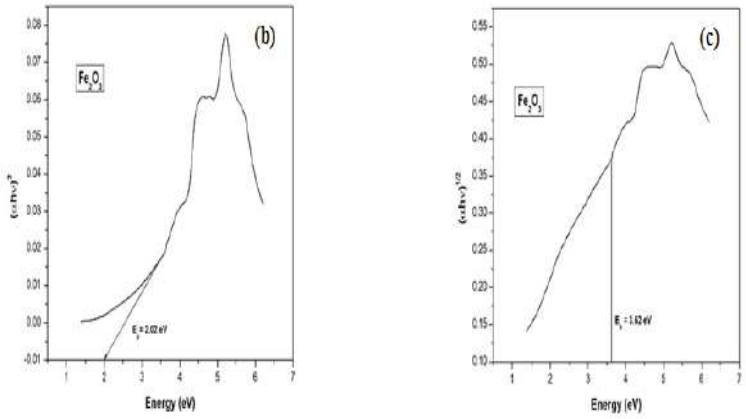


Fig. 5 (b) direct band gap and (c) indirect band gap of Fe_2O_3 nanoparticles.

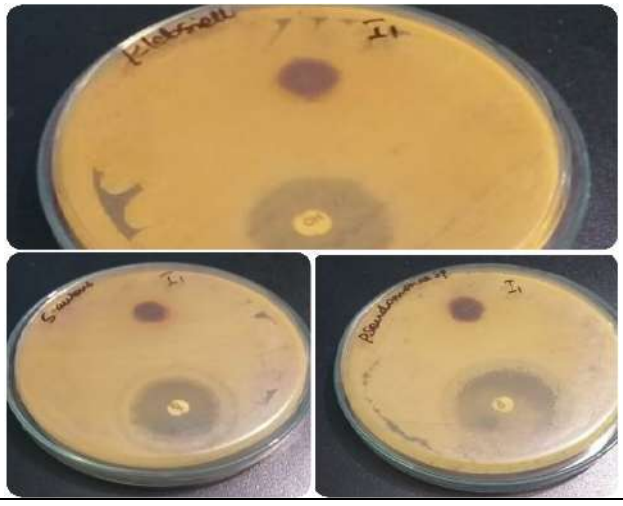


Fig. 6 Antibacterial activity of Fe_2O_3 nanoparticles

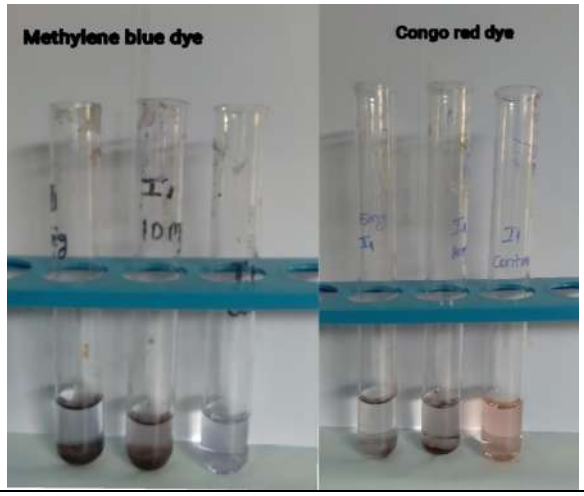
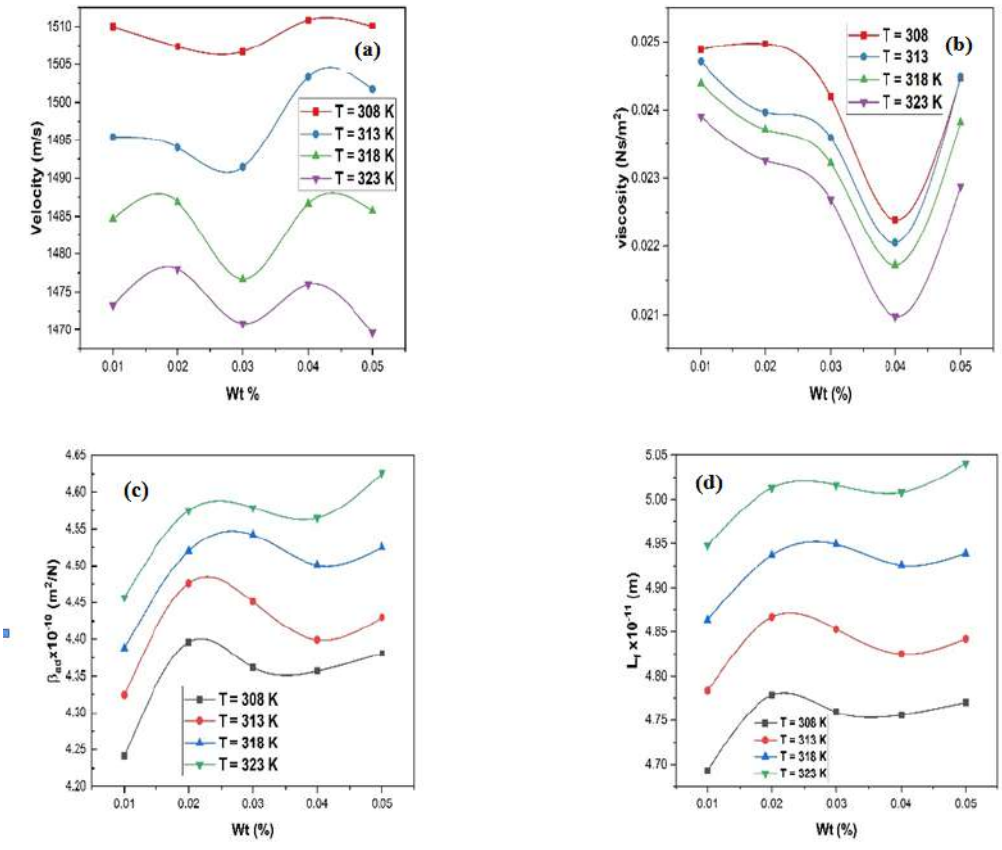


Fig. 8 dye degradation of synthesized iron oxide nanoparticles.



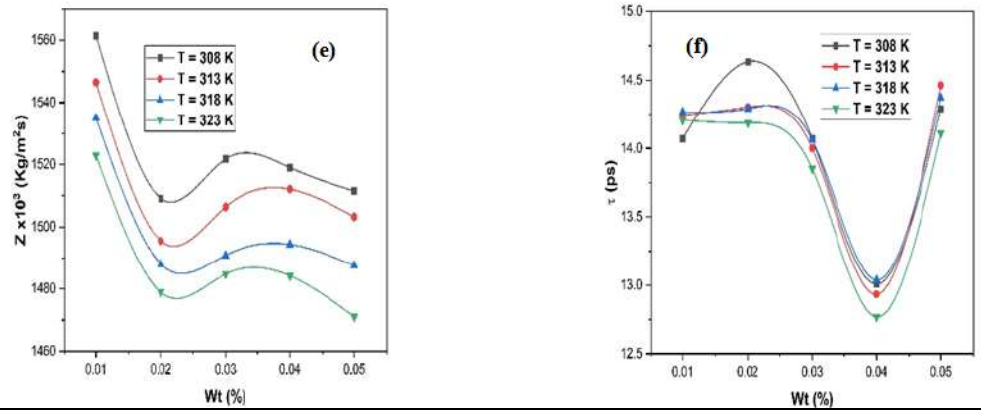


Fig. 7 shows variation (a) velocity, (b) viscosity, (c) adiabatic compressibility, (d) free length, (e) acoustic impedance and (f) relaxation time with mole fraction.

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Intergenerational Transfer of Awareness on Environment - A Child Oriented Awareness Program

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Received: 11 Jan 2022 Revised: 30 Jan 2022 Accepted: 21 Feb 2022

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ABSTRACT

Environmental awareness is the ultimate driving force that stimulates on environmental matters. Children may take an active role in conserving the environment and its resources. To assess the effectiveness of intergenerational transfer of awareness on environment – A Child oriented Awareness program. It was a quantitative pre- experimental two group pretest-posttest design with no control group. The eligible participants were selected by Nonprobability Purposive Sampling technique. The significant improvement was found on awareness in post interventional phase as compared to the pre interventional phase. It means that the Mean & SD 14.6±4.07; 16.26±4.68 and 22.98±4.47; 23.62±4.05 children and their mothers pre and posttest knowledge score respectively. The study concludes that the Intergenerational approach is a cost- effective method to disseminate the health awareness to the community.

Keywords: Intergenerational, Awareness, Environment, Child Oriented.

INTRODUCTION

Environment the word originated from French is that "Environ" means to surround in which organisms live. It comprised both a-biotic, biotic means respectively physical or non-living and living organism. The two dynamic and complex component of nature are Environment and the organisms [1,2]. Environment is the sum total of all conditions and influences of the development of the human being life and other organisms. These are divided in to

three such physical, social or cultural conditions and the surroundings of an inanimate object of intrinsic social value. It's are influencing and affecting the growth and development of an individual as well as community. So the concept of environment is a complex one, far ranging in its implications and challenging to our understanding [3,4]. Healthy ecosystems and environments provide services and protect the humans and other living organisms. Humans are inability to fit their activities for conserves the environment and saves energy. To rising consumption of energy depleting the natural resources and degrading the environment. To meet the need of present generations without compromising and the ability of future generations to meet their needs, sustainable development is fundamental. It is essential to using renewable energies and energy efficiency measures that ultimately will reduce energy consumption and air pollution [5,6]. Education helps to bring any desired change in society. It not only helps in the development of child personality also determine their future. The necessity of environmental education is to educate the all ages of people regarding environment for making decisions which are compatible to environment. Even educate the students about environmental issues beginning from pre-school to higher education. Environmental Education is an important tool for taking appropriate action for prevention of environmental problems [7,8,9]. Environmental awareness is the ultimate driving force that stimulates knowledge on environmental matters. Environmental education (EE) has the potential to facilitate awareness that leads to this connection. Exposure to nature, either through structured EE programs or unstructured play, has many benefits [10,11]. Awareness on Environmental provides a good understanding of the set of beliefs, interests, or rules that influence environmentalism or pro-environmental action". Nurses as a health educator should inculcate the children positive values and attitudes towards environmental conservation. Children may take an active role in conserving the environment and its resources and hence preserve them for the future generation also passes the awareness among near peers, family members and society [12,13,14]. Many environmental issues are arising in India. Air pollution, water pollution, soil pollution and wildlife natural habitat pollution its makes challenge. The Health care personnel, social worker has a vital role in environmental issues and creating awareness among public. The priority goes mainly for generating awareness among the children through mass media, campaign, competitions, voluntary work etc. Environmental issues are becoming more and more complex and the need to take urgent action. Children are needed to be equipping with appropriate skills that can help them to make better decisions and choices [15,16,17]. So the best ways of environmental preservation is creating awareness among society especially in Young generation [18]. The success of environmental protection depends on the collective efforts and every member of the community shares the responsibility. So the present study was aimed to assess the environmental awareness among the children and their mother and enhance their knowledge on Environmental Education by intergeneration approach.

STATEMENT OF THE PROBLEM

Intergenerational transfer of awareness on environment - A child oriented awareness program

MATERIALS AND METHODS

The present study was a pilot study that examined the feasibility of an Environmental educational program for children attending primary school in a rural community at Karaikal. Investigator planned to evaluate the effect of awareness program among children on Environment, and explored the intergenerational transmission of that awareness to their mothers. The Accessible population for this study was all the students studying the grade 3rd to 5th standard and aged in 8to 10 years. The Eligible 100 study participants (50 children and 50 mothers) were selected by non probability purposive sampling. Children who provided assent and signed parental informed consent were able to participate the study. The children willingness to communicate the information was asked by using open-ended questions. Baseline data were collected. The pre test level of awareness was assessed by using same self structured interview questionnaires for both children and their mothers. The awareness program consisted of three 45-minute sessions and was based on the Environmental Education for Children developed by the investigator. Awareness programme was multidimensional approach. 7 days after the program was implemented the investigator administered the same questionnaire to participating children and their mother. Children who did not present on the day were considered lost to follow-up. Descriptive and inferential statistics both analyses were done.

RESULTS

The above table .1 revealed that before the multidimensional approach followed by Intergenerational transfer of awareness on environment from children to mother's majority of the children (36%) as well as their mothers (21%) had moderately adequate knowledge on Environmental education in pre interventional phase. The significant improvement was found on awareness in post interventional phase. This study result was proved that this innovative approach was most effective in spreading the useful information to the society. Table.2 showed that the intergenerational approach was helped to transfer the knowledge on Environmental Education as evidenced by pretest, post test mean percentage among children were48.66 %, 76.6 % and the mean percentage difference as 27.94 % where as the mothers pretest, post test mean percentage were 54.2 %, 78.73 % and the mean percentage difference as 24.53 %.The significant difference was found in both children and their mothers level of knowledge. Regarding Association between pretest level of knowledge on environmental education among primary school children and their mothers, the significant association was found with age of the mother, mother educational status also with age of the child and no significant association was found with sex of the child.

DISCUSSION

Implementing the child oriented Environment educational program for primary school children were feasible and acceptable. The intergenerational program significantly increased awareness on environment among the children and their mother. The current study found that during the pre assessment phase Majority of the children as well as their mothers had moderately adequate knowledge on Environmental education. The results obtained from the pre- and post-tests, the success rate rose from 59.96 out of 100 in the pre-test to 70.72 in the post-test among women. It was identified that t-test difference was statistically significant [19]. One more study result was supported that more than two thirds of mothers had inadequate knowledge practice on indoor pollution and the majority of children had health problems due to indoor pollution. The author recommended that implementing of educational intervention for mothers about indoor pollution in the home may help to prevent or comes this type of problem [20]. Another one study was aimed to identify the most important health problem which infant suffer from as results of environment pollution and assessed mother knowledge about mode of disease transmission. The higher percentage of infant's health problems are respiratory system (acute bronchitis, asthma pneumonia) and food poisoning. Educating the people regarding taking care of accommodation, giving up smoking inside rooms, facilitate good ventilation and getting rid of [21]. Shriya A et al., conducted study on maternal knowledge and practices towards sanitation and their relationships with occurrence of diarrhea in Children. The study revealed that most of the mothers 71.3 % had good knowledge score but regarding practice 50 % of the mothers had poor score. Improvement in the environmental and personal hygiene practices of the mothers can contribute largely in decreasing the prevalence of diarrhoea among children [22]. P. Damerell et al conducted study and supported that environmental education can be transferred between generally and induce behavior changes in their family members [23]. Many research findings were suggested and recommended that improvement in the Awareness on environmental and personal hygiene practices of the mothers can contribute largely in reducing the prevalence of common illness among children.

CONCLUSION

The study result supported that the intergenerational transmission of awareness as a cost effective, innovative approach in environmental education. It is valuable measure for increasing environmental awareness among community people. Children are learning about their environment and are transfer this information to their mothers. It influencing and reflecting in their daily household activities.

ACKNOWLEDGEMENT

The author has grateful for the Teachers, children and parents for their cooperation and participation.

Financial support and sponsorship: Nil
Conflicts of interest: There are no conflicts of interest

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Table.1. Comparison of pretest and post level of knowledge on Environmental education among primary school children and their mothers

Level of Awareness	Pre test		Post test		Pre test		Post test	
	CHILDREN				MOTHER			
	F	%	F	%	F	%	F	%
Adequate	03	06%	32	64 %	09	18 %	35	70 %
Moderately Adequate	18	36 %	12	24 %	21	42 %	11	22 %
Inadequate	29	58 %	06	12 %	20	40 %	04	08 %

Table.2. Comparison of pretest and post level of Mean, SD, Mean percentage and Mean percentage difference on Environmental education among primary school children and their mothers

Group	Test	Mean	SD	Mean Difference	Mean %	Mean % Difference	t-Test value
Children	Pre test	14.6	4.07	8.38	48.66 %	27.94 %	13.98
	posttest	22.98	4.47		76.6 %		S*
Mothers	Pre test	16.26	4.68	7.36	54.2 %	24.53 %	12.06
	posttest	23.62	4.05		78.73 %		S*

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Air Pollution Tolerance Index (APTI) of Selected Plants Growing Around Stone Cutting Workshops in Vazhukamparai, Kanyakumari District, Tamil Nadu, India.

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Received: 01 Feb 2022 Revised: 17 Feb 2022 Accepted: 05 Mar 2022

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ABSTRACT

Air pollution due to anthropogenic activity threatens the environment and health of organisms. Stone cutting is an industry which generates a lot of dust. Air Pollution Tolerance among selected plants growing around the stone cutting workshops by using the Air Pollution Tolerance Index (APTI) was performed in this study. The study measured four physico-biochemical parameters such as ascorbic acid content, total chlorophyll content, pH of plant tissue and the relative water content of leaf tissue to calculate the APTI for ten species of plants at the study site. The ascorbic acid was present in the range of 6.20 mg/g FW to 39.84 mg/g FW with *Calotropis procera* having the highest value. The total chlorophyll values ranged from 0.16 mg/g FW to 0.43 mg/g FW. All the plants studied had an acidic pH in the range 5.54 to 6.80. High relative water content values were observed for *Calotropis gigantea* followed by *Terminalia catappa*. The shrubs registered higher APTI values than trees. *C. procera* was more tolerant to air pollution while *Ficus religiosa*, *Morinda tinctoria* and *Azadirachta indica* were sensitive. Screening of plants with low APTI values could be useful since such plants may serve as biomonitors of air pollution.

Keywords: Air pollution, impact, monitoring, parameters and stone work areas.

INTRODUCTION

Air pollution is harmful to living organisms. Plants are continuously exposed to air pollutants. Pollutants descending on plant foliar surfaces are absorbed, accumulated and integrated into plants. Trees function as biological filters removing airborne pollutants and improve air quality [1]. On exposure to air pollutants, most plants experience

physiological change first and later show visible damage on the leaves depending on their sensitivity [2]. The intensity of the pollution determines the air pollution injury to plants. Plant leaves respond to pollution by undergoing changes in varying degrees [3].The ascorbic acid content, chlorophyll content [4], leaf extract pH [5] and relative water content [6] are affected by air pollution. The susceptibility of plants to air pollution is evaluated by measuring the quantities of ascorbic acid [7], chlorophyll [8], relative water content [9] and pH of leaf extracts [10]. Using the above physiological parameters, the Air Pollution Tolerance Index is calculated. APTI is an inherent quality of plants. Plants with higher APTI are more tolerant to air pollution while sensitive plants have lower APTI. Plants with lower APTI may act as bioindicators. Based on the APTI indicators, plants may be categorised into tolerant, moderately tolerant, intermediate and sensitive [11]. According to Mashita and Pise [12], plants with APTI values in the range 30-100 are pollution tolerant; with APTI values in the range 17 - 29 as intermittently tolerant and sensitive plants have APTI value in the range of 1-16. Plants with APTI value lower than '1' is "highly sensitive". Pollution tolerant plants have higher APTI values [13]. Species having lower APTI may act as bioindicators of pollution. The identification and selection of air pollution resistant plants can be useful for the air pollution control. The current study is conducted to evaluate the impact of granite dust pollution from stone carving and cutting workshops on plants in the study area.

MATERIALS AND METHODS

Study Area

The present study was carried out in Vazhukamparai area of Agastheesvaram Taluk of Kanyakumari District. It constitutes the southernmost tip of India with the state of Kerala to its North - west. The area is famous for different types of sculpture work done using granite.

Sampling Techniques

The common species which were available in and around Vazhukamparai were selected. The leaf samples were collected early in the morning between 6.30 a.m. to 8.00 a.m. The leaves were collected from the plants with a height between two to five feet. Leaf samples were taken from plants present both in sunlight and shaded areas. After sampling, the fresh weight was measured in the field itself and all the leaf samples were packed in separate plastic pouches to be taken to the lab for further processing. All the plant samples were analysed within 3 to 4 hours of sampling. The studies were conducted between November – December 2019.

Relative Water Content (RWC)

With the method described by Singh [14], fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water overnight, blotted dry and weighed to get turgid weight. The leaves were dried over night in an oven at 70°C and reweighed to get dry weight.

RWC= (FW-DW/TW-DW) ×100

FW = Fresh Weight

DW = Dry Weight

TW = Turgid Weight.

Leaf extract pH – The leaf extract pH was measured as per the method of Singh and Rao [15].

Total chlorophyll content (TCH): The total chlorophyll content was measured as per the method described by Arnon[16].

Ascorbic acid content – the ascorbic acid content was measured as per the method of Abida and Harikrishna, [9].

Air Pollution Tolerance Index (APTI) Determination

This was done following method of Singh and Rao [15].

The formulae of APTI is given as APTI = [A+ (T+P) R]/ 10.

A = Ascorbic acid content (mg/g FW)

T = Total chlorophyll (mg/g FW)

P = pH of leaf extract
R = Relative water content of leaf (%)

APTI Calculation

The APTI value is calculated referring to the formula given below,
APTI = [A (T+P) R]/10

Graduation of APTI:

The spectrum of APTI was divided into four grades of air pollution tolerance as per Kalyani and Singhacharya[17]: tolerant (T or Grade 1). Moderately tolerant (MT or Grade2), intermediate (I or Grade 3) and sensitive(s). The tolerance grades were defined as follows.
1. Tolerance APTI > Mean APTI + SD;
2. Moderately tolerant: Mean APTI < APTI< Mean APTI + SD;
3. Intermediate: Mean APTI – SD<APTI<Mean APT;
4. Sensitive: APTI < Mean APTI-SD

RESULTS

The results obtained by measuring the four physico – biochemical parameters of the plants namely ascorbic acid, total chlorophyll, pH and relative water content were used to calculate the value of air pollution tolerance index (APTI) which is provided in Table 1 and Figure 1. The generated APTI represents the parameters of ten plants each at the study sites.

APTI index Range :< 1=>Very Sensitive; 1-16=>sensitive; 17-29=>Intermediate; 30-100=>tolerant
The ascorbic acid content in the plants was within the range of 6.20mg/g FW to 39.84mg/g FW; the highest value was for *Calotropis gigantea* (39.84mg/g FW) and the lowest value for *Muntingia calabura* L. (6.20mg/g FW). All the plants showed lower total chlorophyll values at the polluted site. The ranges were 0.16mg/g FW to 0.43mg/g FW in the site and A*zadirachta indica* and *Muntingia calabura* scored low and high values respectively. All the plants showed acidic pH ranges from 5.54 to 6.80. High relative water content values were observed for *Calotropis gigantea* (66.22) followed by *Terminalia catappa* (61.53) and the lowest was recorded for *Mangifera indica* L.(28.43). The APTI values were in the range of 12.07 to 33.31 in the study site. Shrubs have registered higher APTI values than the trees. Among the selected plants, *Calotropis gigantea* has the highest APTI (33.31) value and it is tolerant in nature. Plants like *Ficus religiosa, Morinda tinctoria and Azadirachta indica* are sensitive in nature.

The APTI determination provides a reliable method for screening large number of plants with respect to their susceptibility to air pollutants. This is a simple method that can be applied in all type of field conditions. The sensitive species can be used as a sink for air pollutants. The APTI was found to be above 16 in *Calotropis gigantea, Muntingia calabura*, *Millingtonia hortensis*, *Terminalia catappa*, *Mangifera indica* and *Annona reticulate* whereas the APTI value was found to be very low in *Ficus religiosa* and *Azadirachta indica.*

DISCUSSION

Plants having low index values were generally sensitive to air pollutants. According to Lakshmi *et al*. [18] plants having APTI value in the range of 17 to 29 were intermediate to pollution and below 16 and up to 1 are sensitive to pollution. As per this classification, *Calotropis gigantea* and *Muntingia calabura* are the two plants that have APTI value above 30 and are tolerant to pollution. Moreover, plants like *Ficus religiosa, Morinda tinctoria, and Azadirachta indica* are having the APTI value 1-16 and are sensitive to pollution. Plants like *Ficus benghalensis*, *Millingtonia hortensis*, *Terminalia catappa, Mangifera indica* and *Annona reticulata* L. are having the APTI value 17-29 and thus serve as plants intermediate to pollution. No plants were found to be very sensitive (less than one value) to pollution.

Plants having high index value are tolerant to air pollution and can be used as sinks to mitigate pollution, while plants having low index value show less tolerance and can be used as bio-indicators of air pollution [19]. It is suggested that for some species, the biggest effects of pollution exposure are associated with the early stages of the life cycle [20]. The long term, low concentration exposure of air pollution produces harmful effects on plant leaves without visible injury [21]. Plant adaption to changing environmental factors involves both short-term physiological responses and long-term physiological structural and morphological modifications. These changes help plants minimizes stress and maximize use of internal and external resources [22]. At higher concentrations, plants are reported to exhibit certain physiological and biochemical changes (invisible injury). The pH of the leaf extract plays an important role in deciding the tolerance level of the plants against the pollution. Higher pH provides better tolerance to plants against pollutants. The values presented in Table No. 1 shows range of pH that could provide tolerance in plants against pollutants as observed by [23]. In the presence of acidic pollutants, the leaf pH is reduced. The rate of reduction is more in sensitive plants when compared to tolerant plant species [24]. Thus the level of pH in leaf extract of plants under polluted condition is strongly associated with their tolerance level to air pollutants. High pH may increase the efficiency of conversion from hexose sugar to ascorbic acid [25]. Low leaf pH extract showed good correlation with sensitivity to air pollution. It also reduces photosynthetic efficiency [26]. The pH range between 5.54-6.80 lies in both intermediately tolerance and sensitive plant species [17] and thus all plant species are both intermediately tolerant and sensitive to air pollutants.

The presence of SO_2 and NO_2 in the ambient air causes a change in pH of the leaf sap towards acidic range [15]. Upon diffusion of SO_2 through stomata, gaseous SO_2 dissolves in water to form sulphites, bisulphate and their ionic species with generation of protons influencing the cellular pH [8]. The pH change towards acidic range observed in most species is due to entry of SO_2 into leaf mesophyll tissue [27]. Hanna *et al*. [28] reported that the heavy metals emitted from automobiles can affect the pH of the road side soil. The photosynthetic efficiency of plant species strongly depends upon the leaf pH. The decrease in the pH indicates the development of a detoxification mechanism in plants which is necessary for tolerance [29]. In highly acidic conditions, the metabolic processes in plants including glycolysis are disrupted probably due to imbalance of pH gradient within the cell structure [30]. Photosynthetic efficiency is strongly dependent on leaf pH and photosynthesis is reduced in plants with low leaf pH [31].The decrease in chlorophyll content with decrease in pH may be due to chloroplast damage in acidic conditions. Relative water content of a leaf is the water present in it relative to its full turgidity. It is associated with protoplasmic permeability in cells [32].Plants have a high water content to maintain their physiological balance under stress conditions when the transpiration rates are high thus favouring drought resistance in plants [33]. Relative water content is useful for the state of water balance in a drought resistant plant [34]. Reduction in relative water content of plant species is due to the impact of pollutants on transpiration rate in leaves [35]. The reduced relative water content indicates disturbed physiological status in the plants due to pollution [36]. Plants with high relative water content under polluted condition are tolerant to pollution [11].

Ascorbic acid is a strong reductant and it activates many physiological and defense mechanisms. Its reducing power is directly proportional to its concentration [37]. Ascorbic acid also plays a vital role in cell wall synthesis, defense and cell division [38]. It is a natural detoxicant, which may prevent the effects of air pollutants in the plant tissues [39]. Ascorbic acid is increased in plants under stress and it is a main factor for increasing APTI in stressed plants [40]. The higher ascorbic acid content in plants may indicate its tolerance to sulphur dioxide pollution [41]. Plants with lower ascorbic acid contents may be sensitive to pollution from automobile exhausts. At high concentrations, the pollutants present in exhaust gases could damage plants [42]. Ascorbic acid may prevent the damaging effect of air pollutants in plant tissues. The presence of high amount of ascorbic acid favours pollution tolerance in plants. A definite correlation between ascorbic acid content and resistance to pollution exists in plants [8]. Plants resistant to pollution contain high amounts of ascorbic acid while sensitive plants have it in low levels. High content of ascorbic acid in plant leaves provides for specific physiological defense mechanisms. Its reducing power is directly proportional to its concentrations [38].The level of ascorbic acid in leaves declines on pollutant exposure. Plants tolerant to air pollution have high ascorbic acid levels [39]. In the present study the ascorbic acid content varied between 6.20 to 39.84 mg/gm FW with *Calotropis gigantea* having highest and *Muntingia calabura* having lowest

content of ascorbic acid. The chlorophyll content of plants determines its photosynthetic activity and biomass formation. Its measurement is important to evaluate the effects of air pollutants on plants. Any reduction in chlorophyll content directly affects plant growth [43]. The dissolution of alkaline chemicals present in the dust particles may lead to reduction of chlorophyll. Chlorophyll degradation may also be attributed to action of SO_2 and NO_2 on chlorophyll [44]. Both of these gases are the constituents of vehicular emission [45]. Increase in the quantity of chlorophyllase enzyme may reduce the concentration of chlorophyll in plants [46].Gaseous sulphur dioxide destroys chlorophyll by replacement of the Mg^{++} by two hydrogen atoms. It also degrades chlorophyll molecules to phaeophytin [47]. The loss of chlorophyll in plants exposed to pollutants supports the argument that chloroplasts are attacked by pollutants such as SO_2 and NO_2. Plants act as sinks in the environment, so they are useful in reducing dust concentration and other particulate matters in air. Plants can reduce air pollution at a particular place by absorption, deposition, accumulation and detoxification of harmful pollutants [48]. The dust deposition on plant leaves depends on many factors. The leaf orientation, surface geometry, phyllotaxy, epidermal and cuticular features, the height affect dust deposition [49]. The response of the plant to dust accumulation may vary according to different species. Dust deposition fluctuates with plant species due to leaf orientation [50], leaf surface geometry [51], phyllotaxy [52], epidermal and cuticular features [53], leaf pubescence, height and canopy of roadside plants [49]. This accumulation mainly depends on vegetation type.

Krajickova and Mejstrik [54] found that particulate matters from a coal-fired power plant affected photosynthesis of *Calamagrostis epigejos* L (Roth.) and *Hypericum perforatum* L. but the stomata were rarely blocked. They suggested that the dust might act directly on the guard cells, though the mechanisms for this effect remain uncertain until now. After dust deposition on the leaves, *Rhododendron catawbiense* exhibited an increased absorption in the infrared spectrum and a reduced reflection and transmission of radiation [55]. It has been established that aerial parts of a plant especially leaves act as persistent absorbers in a polluted environment [56]. Dulal and Pratap [57] have studied the effects of stone crushing industry on *Shorea robusta* and *Madhuca indica* foliage. According to Rai *et al*. [33] the foliar surface was an excellent receptor of atmospheric pollutants leading to a number of structural and functional changes. Leaves of trees dusted with particulate matter showed significant reduction in protein and nitrogen content with corresponding leaf fall in deciduous species [58]. Dust deposition on leaves results in reduction in the photosynthetic leaf area resulting in reduced photosynthesis. Reduction in number of flower and yield in black gram [59] and other flowering plants have been noticed due to dust pollution [60]. Continuous exposure to dust on leaf surface can lead to the formation of dense dust layers. Such layers reduce light capturing capacity of plants and finally hamper plant photosynthetic activity [61]. Decreased rate of photosynthesis and alteration of stomatal conductance are responsible for the reduction of the stomatal index as well as dry matter content of leaf [62]. Reduction of the stomatal index of the roadside plant may be due to shading effect of dust layers, which may block the stomata and reduce the photosynthesis rate of roadside plants [63].

CONCLUSION

Of the various plants considered in the present study, *C. gigantea* and *M. calabura* are tolerant to pollution. Plants such as *F. religiosa*, *M. tinctoria* and *A. indica* are sensitive to air pollution. Hence, *C. gigantea* and *M. calabura* can be planted around such workshops to reduce air pollution. The sensitive plants could be as biomonitoring agents to combat air pollution. Further studies need to be conducted to screen more number of plants for their APTI tolerance levels.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Management Authorities, Principal, S. T. Hindu College, and HOD, Department of Botany and Research Centre, S. T. Hindu College, Nagercoil, Kanyakumari District (Tamil Nadu), India, for providing necessary facilities and encouragement.

Abbreviations: APTI, Air Pollution Tolerance Index; FW, Fresh Weight; DW, Dry Weight; TW, Turgid Weight; RWC, Relative water content; TCH, Total chlorophyll content.

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Table No 1. Air Pollution Tolerance Index of Certain Plant Species from Study Area.

S. No.	Plant species	Ascorbic acid (mg/g)	Total chlorophyll (mg/g)	pH	Relative water content (%)	APTI	APTI Grade
1.	*Calotropis gigantea* L.(S)	39.84	0.3005	6.40	66.228	33.31	T
2.	*Ficus religiosa* L.(T)	9.96	0.171	6.45	54.794	12.07	S
3.	*Muntingia calabura* L.(S)	6.20	0.4325	6.20	38.18	31.03	T
4.	*Ficus benghalensis* L.(T)	18.30	0.173	6.80	56.12	18.34	I
5.	*Millingtonia hortensis* L.	24.9	0.30	6.65	56.66	22.97	I
6.	*Morinda tinctoria* Roxb. (T)	16.6	0.387	6.34	50.877	16.25	S
7.	*Azadirachta indica* A. Juss. (T)	9.96	0.168	6.45	54.80	12.07	S
8.	*Terminalia catappa* L. (S)	21.58	0.411	5.54	61.538	18.99	I
9.	*Mangifera indica* L. (T)	23.24	0.266	6.20	28.43	17.87	I
10.	*Annona reticulata* L. (S)	19.92	0.243	5.80	50.485	17.08	I

T = Tree; S = Shrub (S=Sensitive; I-Intermediate; T-Tolerant)

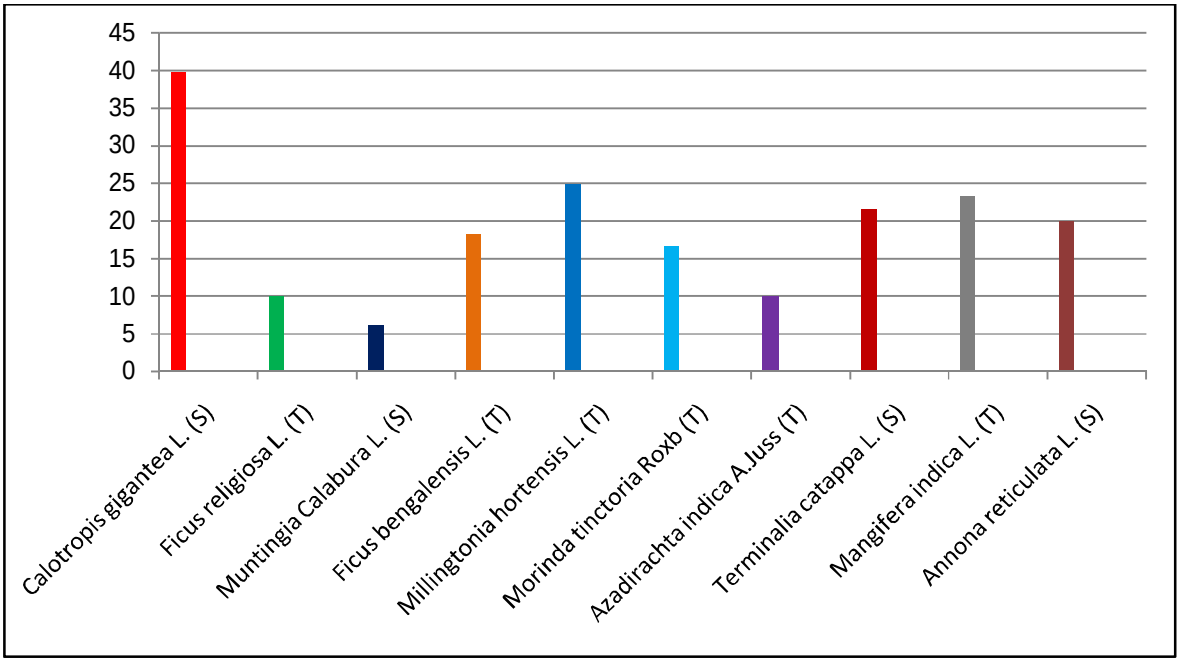

Figure No. 1.Air Pollution Tolerance Index of Plant Species from Study Area

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Antiadhesive Action of Marine Biosurfacant: A Review

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Received: 17 Jan 2022 Revised: 16 Feb 2022 Accepted: 15 Mar 2022

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ABSTRACT

Biosurfactants or microbial surfacant have been the subject of intense investigation during the past few decades, relatively small numbers of microorganisms and research output have focused on their production from marine microorganisms. Nevertheless, some marine microbial communities including Pseudomonas, Halomonas, Bacillus, and Lactobacillus have been explored for the production of surface-active molecules. The market for producers are increasingly becoming more aware and interested in biosurfactants due to their excellent properties of biodegradability, low toxicity, and surface activity. This article emphazise on antiadhesive action of biosurfacant and also research findings on specific biosurfactants such as high surface activity rhamnolipids, sophorolipids, lipopeptides, and trehalose lipids that have potential for environmental, industrial, and medical uses .

Keywords: Marinebiosurfacant, anti-adhesive, surface treatment, disrupting,biofilm, biosurfacant

INTRODUCTION

Marine biosurfacant is a surface-active chemical produced by microorganisms.[1] Biosurfactants are microbial molecules that have a high degree of surface activity[2].Surface active compounds produced by microorganisms are of two main types; first, that reduce surface tension at the air water interface (biosurfactants) and second, that reduce interfacial tension between immiscible liquids, or at the solid-liquid interface (bioemulsifier)[2] .Some marine microorganisms create a marine biosurfactant that is used in bioremediation of crude oil-polluted seas[1]. Because their complex structures are made up of a hydrophilic and hydrophobic half, biosurfactants have unique amphiphatic characteristics [1]. Biosurfactants are biodegradable, have low toxicity, and are environmentally friendly[1]. There isn't much known about either biosurfactant produced by sea microorganisms [1]. Adsorption of biosurfactant to a substratum surface alters hydrophobicity of the surface and causes interference in microbial adhesion and desorption processes [5]. Biosurfactant have been found to possess several properties of restorative as

well as biomedical importance and also inhibit fibrin clot formation and anti-adhesive action against several pathogenic microorganisms[6,7]. Biosurfactants have been these usually display anti-adhesive and anti-biofilm activities, making them useful to reduce the adhesion and colonization by pathogenic microorganisms, as well as to remove pre-formed biofilms [8]. Biosurfactant isolation and purification are processed through precipitated crude biosurfactant molecules were purifiedby gel filtration chromatography and after lyophilization the pure product that was used for testing the antiadhesive activity [3] . The microbial surfactants or biosurfactants have been found to inhibit the adhesion of pathogenic organisms to the surgical instruments or to infection sites thus might constitute a new alternative and effective means of combating colonization of pathogenic microorganisms[2].

Classification of Marine biosurfacant

Biosurfactants are classified primarily based on their chemical makeup and microbiological origin[1]. Glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric biosurfactants, and particulate iosurfactants are the most common types of biosurfactants[1,2]. Some marine biosurfactants and application are shown in the table I. Some of the marketed marine biosurfacant are given in the figure [i]

Glycolipids

Glycolipids are carbohydrates containing long-chain aliphatic acids or hydroxy aliphatic acids[1,2]. Marine bacteria such as *Alcaligenes* sp., are able to produce glucose lipid.

Rhamnolipids

Glycolipids composed of one or two molecules of rhamnose linked with one or two molecules of β-hydroxy-decanoic acid are called rhamnolipids[2]. Rhamnolipid biosurfactants have low CMCs and can reduce the surface tension of water to as little as 25 mN/m. [14]. Another crucial component influencing behaviour is pH. Rhamnolipids are likely to be anionic at higher pH (>4) due to the presence of carboxylic acid groups [13,14]. The solubility of these biosurfactants in aqueous solutions is also directly affected by pH, increasing the pH causes rhamnolipid solubility to rise. There are two known mechanisms involved are

i) Solubilizing the otherwise insoluble substrate, making them more usable for cells.
ii) Introducing changes into the membrane of cells

Trehalolipids

Trehalolipids are disaccharides trehalose linked at C-6 and C-6' to mycolic acids which is associated with most species of Mycobacterium, Nocardia and Corynebacterium [2]. Antiadhesive property of trehalolipids are trehalose lipid was studied for its antiadhesive activity against Gram positive and Gram-negative bacterial strains, as well as fungal strains, such as C. albicans[16]. They exhibited good antiadhesive activity and this effect was concentration-dependent. The antiadhesive activity strongly depends on the type of microorganisms [16].

Sophorolipids

Sophorolipids are glycolipids that are made up of a dimeric carbohydrate called sophorose that is glycosidically connected to a long chain hydroxyl fatty acid[2]. Sophorolipids are usually found in a mixture of macrolactones and free acid form[2]. Cosmetics Example 1: Hydroxypropyl-etherified glycolipid ester [15].

Phospholipids and fatty acids

When Myroides sp.SM1 was grown in Marine Broth, it produced bile acids such as cholic acid, deoxycholic acid, and its glycine conjugate[1]. Rhodococcus erythropolis cultured on n-alkane produces phosphatidylethanolamine, which lowers the interfacial tension between water and hexadecane to less than 1mN/m and a CMC of 30 mg/l[2].

Polymeric biosurfactants

Polymeric surfactants can also be amphiphilic proteins[1]. [Microorganism : Pseudomonas nautica]. Emulsan, liposan, alasan, lipomanan, and other polysaccharide-protein complexes are the most common polymeric

biosurfactants. Alasan is an anionic covalently bound alanine-containing hetero- polysaccharide protein biosurfactant with a molecular weight of about 1MDa derived from Acinetobacter radioresistens KA-53[2].

Particulate biosurfactants

Extracellular membrane vesicles partitioned hydrocarbons to form a microemulsion, which plays an important role in microbial cell alkane uptake[1]. Pseudomonas nautica cellular lipid content increased up to 3.2 fold in eicosane-grown cells compared to acetate-grown cells[1,2]. Polymeric biosurfactants / bioemulsifiers with high molecular weight: Many bacteria of various species produce high molecular weight polymeric (HMW) biosurfactants, which have been suggested to be referred to as bioemulsifiers to distinguish them from low molecular weight metabolites[9].

Low Molecular Weight Biosurfactants

LMW biosurfactants range from simple free fatty acids and phospholipids to amino acids linked to lipids, lipopeptides, and glycolipids[9].

i. Fatty acid and phospholipid derivatives: -It is one of the most basic amphiphilic metabolites[9]. It has the potential to act as a surface-active substance. For example is marine yeast aureobasidium pullulans YTP6-14 has been shown to produce massoia lactones as surface-active compounds, which are lactonised hydroxy fatty acids that can be used to make perfume[9].
ii. Lipoamino acid :- A few marine bacteria have been found to produce lipoamino acid. A few marine microorganisms have also been found to produce lipoamino acids. A crude oil emulsifying metabolite produced by the flavobacterium Myroides sp. [29].
iii. Glycolipids

Lipopeptide

Lipopeptides consist of short linear chains or cyclic structures of amino acids, linked to a fatty acid via ester or amide bonds or both[16].

i) Surfactin is a powerful biosurfactant produced by (Bacillus subtilis ATCC 21332)[2]. Surfactin was discovered in the culture broth of B. subtilis by as a potent inhibitor of blood clotting, but it was also discovered to be a powerful surface active agent[10].
ii) Lichenysin: Bacillus subtilis also produces two additional families of lipopeptide biosurfactants, iturins and fengycins, both of which have excellent surface and biological activities[12]. Serrawettin from Serratia marcescens [12], viscosin from P. luorescens and P. viscose, and arthrofactin from Arthrobacter sp. strain MIS38 are all effective surface active lipopeptides. Some of the application of marine biosurfacant illustrated in table i

Biosurfacant surface treatment: biofilm control

Biofilm formation occurs gradually, dynamically, and sequentially, and includes adhesion, maturation, and dispersion [22]. The steps involved in the procedure.

Planktonic microorganisms' proximity to a surface [19]

⇩

Inhibiting microbial adhesion (Surface aqueous medium exposure)[23]

⇩

Surface adherence becomes irreversible

⇩

Growth, microcolony production, and adherence of supplementary microorganisms in the local environment[19,23,22].

Anti-adhesive and disrupting biosurfactant

Adhesion is a complex event influenced by factors such as the microorganism and surface involved, nutritional and environmental conditions, and the presence of cell appendages, among other

1. BS isolated from Lactobacillus plantarum in rice fermented food demonstrated anti-adhesive activity against the food pathogens E. coli (ETEC), *Yersinia enterocolitica, Salmonella Typhi*, and *S. aureus*, as well as antimicrobial activity against the same pathogens[25].
2. Incorporating biosurfactants into packaging materials is a new way to investigate their anti-adhesive properties. Example are Polyvinyl alcohol (PVA) mixtures with a BS produced by *Lactobacillus rhamnosus* inhibited *S. aureus* and *P. aeruginosa* biofilm formation[25,19].

Marine microbial surfactant's : antiadhesive action

Marine microorganisms have distinct metabolic and physiological properties, and they are a significant source of novel biomolecules such as biosurfactants. Microbial adhesion to surfaces can be caused by a number of factors involves the interaction of two components: a receptor and an adhesin. [4]. It was tested against a variety of microbes, and the activity was found to be dependent on both the biosurfactant concentration and the microorganism under test.

Marine Microbial Biosurfacant Application

Degradation/remediation of hydrocarbons

Marine Biosurfacant were more stable in a variety of settings and broad-spectrum emulsification activities, implying that they could be used against a wide range of hydrocarbons and in a wide range of habitats [29].

Adhesion of microorganisms

Inhibition of microbial adhesion to silicone rubber treated with BS from *Streptococcus thermophilus* A. inhibition of microbial adhesion to silicone rubber treated with BS from *Streptococcus thermophilus, Rothia dentocariosa* GBJ 52/2B and *Staphylococcus aureus* GB 2/1 (bacterial strains) were used in this study, as well as *Candida albicans* GBJ 13/4A and *Candida tropicalis* GB 9/9. (yeast strains) [29,24].

Antimicrobial cell adhesion activity of rhamnolipid BSs on silicone rubber voice prostheses; *S. salivarius* GB 24/9 and *C. tropicalis* GB 9/9 had an average antiadhesion activity of 66 percent [21,29].

Biosurfactant in nanotechnology

Synthesis of silver nanoparticles using glycolipid BS isolated from the marine *B. casei* MSA19[26]

Capping Agents

Chemically synthesized amphiphilic molecules were initially used as templates for nanoparticle synthesis . Capping agents such as oleic acid, linoleic acid, and their derivatives were also used. Examples are Surfactin-stabilized supermagnetic ironoxide nanoparticles (SPION) that can serve as a contrast agent for magnetic resonance imaging [29].

Biosurfacant used in food

Food processing industries can utilize BS in two distinct manners: a) indirect utilization to the treatment /cleaning of food contact surfaces

Direct use as food additive/ingredient[22,29].

Food additive

Food additives are substances that are intentionally added to foods to perform specific technological functions[23].

Emulsifiers in Food

High-mass surfactants, such as proteins (gelatin, casein, and whey proteins) and polysaccharides (alginate, gum arabic pectin, xanthan, dextran, starch, and cellulose derivatives), are involved in the formation and stabilisation of emulsions despite their low surface activity. Here is a list of biosurfactants with antiadhesive properties[29]. Example Biosurfactant : Surfactin and rhamnolipids
Target : *L. monocytogenes* , *P. fluorescens* [29].

Biofilm preventing and disruption

A biofilm is a sessile community of microbes embedded in a matrix of extracellular polymeric material that are irreversibly associated with a surface. [23,24].

Reversible adhesion ⟹ Irreversible adhesion ⟹ Development of biofilm architecture ⟹
Biofilm maturation ⟹ Detachment and dispersion

CONCLUSION

The Commercial applications are limited due to the cost of producing biosurfactants. Improved yields, rates, and recovery, as well as the use of crude preparations and low-cost or waste substrates, can all help to cut costs.. As a result, research is required to advance knowledge in this area, as it may improve the biosurfactant's applications.. Surface conditioning with biosurfactants resulted in a significant reduction in bacterial adhesion at much lower concentrations than previously reported. The biosurfactant's antiadhesive capacity was tested against a variety of pathogenic opportunistic microorganisms, including E. coli, M. flavus, and P. vulgaris, to name a few.. Biosurfactants made from environmentally renewable resources are preferred because they are made with low-temperature, cost-effective methods, are environmentally friendly, and produce little waste.

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Table 1. Some types of biosurfactants and their application

CLASSIFICATION	BIOSURFACANT	APPLICATION
GLYCOLIPID	Rhamnolipid	Flushing Studies PAH, Pb, Zn, Ni using medium [sediments and clay] [27] Biodegradation Studies using Medium: Liquid
	Sophorolipids	Microorganism:Consortium Contaminant :Diesel[28]
	Trehalolipid	Trigger cell differentiation instead of cell proliferation biological activities of seven glycolipids, including succinyl-trehalose lipids STL-1 and STL-3.[17]
Polymeric Surfacant	Emulsan	Polymeric extracellular emulsifying agent produced by Acbrrrobacrer RAG-1. [19] Emulsifier
	Mannosyl erythritol lipid	Microrganism : Candida lipolytica [stable oil-in-water emulsion with variety of commercial vegetable oils] [20]. The resting cells of Microorganism : Candida antarctica strain T-34 Medium : only carbon source. [29]
Particulate biosurfacant	Vesicle	Good emulsification activity Microrganism: Acinetobacter sp. HO1-N [extracellular membrane] [18].

i) sophorolipids ii) Surfactin biosurfacant iii) Rhamnolipid biosurfacant

Figure 1. Marketed Marine Biosurfacant

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Yogic Practices with Psychoneurobics on Cortisol and LDL Among Adolescent Stressed Girls

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 31 Mar 2022

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ABSTRACT

The goal of experimental study through random selection was to see how yoga practises with Psycho Neurobics affected adolescent stressed girls' Cortisol and LDL levels. Thirty stressed teenage girls aging 14 to 18 yrs old were randomly taken from Chennai and grouped into two sets of 15 participants. It was expected to have substantial disparities in Cortisol and LDL among stressed adolescent girls. Before the training programme execution, two group's preliminary test scores on Cortisol and LDL were noted. For a total of 12 weeks, the training was on Yoga with Psychoneurobics to Group I subjects onehour for six days in a week. Group II was given no instruction and was expected to lead the routine. Scores after training on two groups were also recorded for the same variables. The significant differences between the experimental group practising yoga with Psycho Neurobics and the control group were determined using ANCOVA. Confidence levels for the significance test was set at 0.05. Due to Yogic practises with Psychoneurobics among teenage stressed girls, the Experimental Group revealed substantial variations in Cortisol and LDL compared to the Control Group. At a confidence level of 0.05, the hypothesis was accepted. As a result, it can be stated that combining Yogic practises with Psycho Neurobics helps adolescent girls maintain a Cortisol and LDL in a healthy way.

Keywords: Cortisol, LDL, PsychoNeurobics, Stress, Yoga, Yogicpractices.

INTRODUCTION

Adolescence is the time between 13 and 19 age group. During this period, adolescents suffer many psychosocial and physiological changes. Adolescence accounts for approximately17% size of world human population. Largest population of adolescence of the world is witnessed in India.

Key Facts Of Adolescent Population

- Globally, 16 percent of the population is between the ages of ten and 19, and India accounting for 21% of the world's teen population. Adolescents of age 10 to 19 are having 16% of mental health disorders account to the world's count of diseases and is contributing to 50 per cent among adolescents in the age of 14, majority of which are unnoticed and untreated.

-Statistics reveal Depression as most common root of sickness and impairment among adolescents round the globe.

-Four this the rank of causes for mortality in teenagers.

-Ignoringteenunseenmentalhealthwillgolong-terminjuringtotalwellbeingandlimiting possibilities to live healthy and happy as adults.

Common problems faced during the adolescent period

- A change in the financial situation of the parents
- A severe illness or injury to a family member
- An increase in disputes with the parents
- Issues with a sibling or a sibling's sibling
- Conflicts with parents
- Conflicts with classmates
- Break up with a male/female friend

These issues are centred at home and at school. The fear of losing friends or parents is at the root of the majority of clashing situations. They become ensnared in insecurity's clutches. Most of the time, they are afraid that if they are unable to match the expectations of their loved ones, they would lose them. The added pressure of general expectations from parents, friends, teachers, and others stresses the youngster, and failure affects their self-esteem. Stress is exacerbated when children establish unreasonable expectations for themselves.

Stress

Stress is an inescapable fact of life that affects everyone at some time. It might be caused by any occurrence or thought that provokes annoyance, rage, or worry. At some time in human history, it has occurred to everyone. One among life's distinctive characteristics is stress, and it is acknowledged in creative arts and literature witnessed in human history. The complexity of human social, personal, and ecological surroundings, the diverse and concurrent interactions of individuals with their settings, and the variety of expression of strain contribute to stress's increasing prevalence and inclusion in human communities. One's response to challenge/demand is stress. Briefly, stress is beneficial when it assists in avoiding danger/meeting a deadline. Flip of the coin, Stress can be adding to one's health negatively if it lasting long. The primary cause of stress is the shortening of telomere length in DNA. Stress is caused due to various factors, that include faulty genes, excessive ghrelin hormone secretion in the brain, excessive adreno cortico tropic, adrenaline, non-adrenaline, and cortisol hormone secretion in the adrenal gland, excessive prolactin hormone secretion in the brain, less neuropeptide chemical production in the brain as well as adaptation and reaction to the surrounding environment. Stress in extremely state, on the other hand, can cause catastrophic injuries to many apart from the routine effects experienced. Stress may be induced externally, such as the environment, or internally, by an individual's views. The latter kind, in turn, may result in anxiety and other unpleasant feelings and sentiments such as sorrow, sadness, and others, eventually leading to significant psychiatric disorders such as p0st-traumatic stress dis0rder (PTSD)

Stress and Adolectives

Anystressrelatedwithschoolisreferredtoasacademicstress.Ateveryeducationallevel, stress that occurs in an academic atmosphere can influence students as well as teachers. Academic environments can be intensely competitive, so students must rely on their coping skills to properly manage school-related stress. Stress affects high school pupils in a variety of ways. Adolescence is a period of rapid change that must be managed carefully if teens are to develop properly. Although some adolescent difficulties do not originate in the school setting, they can nonetheless have an impact on students' academic achievement. To avoid the detrimental impacts of adolescent stress on health and academic performance, Williams believes that we must attention to the social, physical, and psychological requirements of teenagers. Exams, homework, and work outside of school, making presentations, competitiveness with peers, cognitive overload, and animal models have all been identified as major sources of stress in the academic setting. However, academic stress is not the only source of stress; family issues, concern about impending school terms, difficulties adapting to change, and body image perception have all been highlighted as significant stressors.

The Body And Stress

Stress causes the body to release hormones. These hormones cause the brain to become more alert, muscles to stiffen, and the heart rate to rise. In short, these reflexes are beneficial since they canassist the human in dealing with stressful situations and safeguarding itself. Children's amygdala volumes diminish as a result of stress, which has an impact on their learning, memory, and emotions later in life. These flaws are remedied by yogic practises.

Prolonged Stress Risk Factors

- Hypertension
- Cadio-vascular disease
- Diabetes
- Obesity
- Anxietyor depression
- Acne and eczema are examples of skin conditions
- Menstrual irregularities

Symptoms Of Stress

- Nausea or indigestion
- Forgetfulness
- Aches and pains on a regular basis
- Headaches A lack of energy or concentration
- Sexual issues
- Tense jaw or neck
- Tiredness
- Inability to sleep or excessive sleeping
- Stomach ache
- Using drink or drugs to unwind
- Weight increase or loss

To prevent negative stress-related consequences in teenagers, such as depression, anxiety, and suicide, more emphasis should be paid to measuring stress in order to assist them improve their coping skills and, as a result, enhance their quality of life. Yoga is the most effective method for dealing with stress.

Yoga For Stress Relief

Yoga the body to mind discipline incorporates postures, conscious breathing, and complete presence of awareness through meditation or relaxation. Yoga can help you relax, lower your blood pressure, and slow down heart rate.

Organize yourself - It's all about planning when it comes to dealing with stress. According to studies, yoga practitioners may complete tasks much faster than non-yogic practitioners, which aids individuals who are well structured and live in the present now. Creating schedules for work, family, hobbies, spiritual time, time with friends and alone, exercise and leisure. Visualize the best outcome - Coping with stress entails anticipating and dealing with a difficult event. Yoga can help you deal with it by teaching you to be present and positive all of the time. Non-Postponement of Actions - One of the finest ways to cope with stress is to avoid putting things off. Yoga will assist you in overcoming this mindset. Being realistic - Yoga encourages people to develop realistic life goals by emphasising quality over quantity and working at a relaxed pace.

Timeliness in all actions - Coping with stress entails correctly treating our bodies. Swami Vishnu Devananda states that proper exercise, breathing, diet, relaxation, and meditation will aid in stress relief.

Psychoneurobics For Stress Relief

Neurobic exercises are a fantastic brain-training programme based on the most recent findings of scientific research. Hearing, vision, smell, taste, and contact are among the genuine faculties presented in the brain practise programme. It strengthens and creates brain cells by reviving neuronal activity. Neurobics engage neuronal frameworks and assemble blood to the brain in a flexible manner .Neurobic practise integrates truly with the cortex and hippocampus, and is set up at atypical transitory projection that serves as a temporary store for new knowledge before progressively moving to long-term accumulating in the cortex. The sense of smell is linked to emotional memory and helps to strengthen memory by storing information. Psycho Neurobic Practices are excellent for improving mental attention and memory. The cerebral cortex and psychoneurobics are responsible for the brain's diverse relationships with the body. Psycho Neurobics exercises cause nerve cells to imitate and animate neuron cells, resulting in the production of normal brain nutrition. Neutrophils are the name for these nutrients. The practise of Psycho Neurobics allows the brain and body to stay young, alert, and concentrated. Energy therapy is Psycho Neurobics. Psychoneurobics involves inhaling cosmic energy and then transferring it to one's neuro system through the power of thought. Light Neurobics, Sound Neurobics, and Easy Neurobics are all included. It provides a direct link between the activities of the soul, mind, and psychical well-being. Psycho-Neurobics is a three-part healing approach that includes Easy Neurobics, Sound Neurobics and Light Neurobics. The balance restoration in human body's seven energy centres (chakras) and five elements (Panch Mahabhootas) is considered a crucial prerequisite for holistic health. When the vibrations of body organs and their related functions do not harmonise with the energy of their respective styles, or if there is an imbalance in the energy flow among the five components that makeup the human body, they diverge from their optimal programme. Combination of above mentioned neurobic techniques were also applied to engage in a series of treatments that include mudras, guided imagery, monochromatic healing cabins, and mantra-induced hypnosis.

The Study's Objective

The study's goal was to see if yogic practises combined with Psycho Neurobics resulted in any significant differences in Cortisol and LDL in stressed adolescent girls.

Objective Of The Research

The goal of the study was to see how yogic practises combined with Psycho Neurobics affected adolescent stressed girls' Cortisol and LDL.

Hypothesis

It is expected that yogic practises combined with Psycho Neurobics will result in substantial variations in Cortisol and LDL among teenage stressed girls compared to the control group.

Delimitations

Only teenage Stressed girls from Chennai City, India are in the study. Participant's age was limitedto 14 to 18 years old. The research was limited to yogic practises, with Psycho Neurobics serving as the sole independent variable. Cortisol and LDL were the only dependent variables in the study.

METHODOLOGY

The Random Group Sampling Design was used to randomly pick 30 people for a random group experimental investigation. The individuals were separated into two groups, each with 15 participants. With the Psycho Neurobics group, the first group was offered yogic practises, whereas the second group was not given any. The two groups were given start and end testing on selected dependant variables, with statistical methods used to compare the results. The effect of yoga practises with Psycho Neurobics on individuals was determined bythe variations between beginning and final scores on dependant measures. I. Exercising for Loosening II. Suryanamaskar . III. Vrkshasan, Veerabadrasan, Trikonasan, Vipareethakarani, Ushtrasan, Shasangasan, Matsyasan, Pachimottasana, Setubandasana, and Shavasana are some of the asanas. Kapalbathi, Nadishodhana, and Brahmari are the types of pranayama. V. Sound Neurobics

During the trial, Group II (Control Group) volunteers were allowed to go about their daily lives as usual, with no special instruction. Thetwo groups were retestedafter12 weeks on the same dependent variables, such as Cortisol and LDL, and their results were recorded. The significant differences between the experimental and control groups were determined using Analysis of Covariance (ANCOVA). The level of confidence for the significance test was set at 0.05.

RESULTS AND DISCUSSIONS

Data on the variable were gathered from the two groups before to and after the training period and evaluated statistically using Analysis of Covariance (ANCOVA) to assess the significant difference, with the hypothesis tested at the 0.05 level of confidence.

These are detailed in the following tables.

The obtained F value of 0.124 on pre-test scores was lower than the required F value of 4.2 to be statistically significant at the 0.05 level. This demonstrated that there was no substantial difference between the pretest and posttest groups, and that the pretest randomization was equal. The study of post-test results revealed a substantial difference between the groups, with a F value of 216.362 above the required Fvalueof4.20. This demonstrated that the differences between the subjects'posttest means were substantial. Adjusted mean scores were calculated and statistically treated after taking into account the pre and post test scores among the groups. The F value of 211.801 obtained was higher than the required F value of 4.21. According to the study conducted by Ma X et al., (2017) expressed significant difference in the means due to 12 weeks of yogic practices with Psychoneurobics on Cortisol. The above table on Cortisol was presented down graphicallyinFigure-1.

The obtained F value of 0.662 on pre-test scores was less than the required Fvalueof4.2 to be statistically significant at the 0.05 level. This demonstrated that there was no substantial difference between the pretest and posttest groups, and that the pretest randomization was equal. The study of post-test results revealed a substantial difference between the groups, with a F value of 310.987 above the required F value of 4.20. This demonstrated that the differences between the subjects' post-test means were substantial. Adjusted mean scores were calculated and statistically treated after taking into account the pre and post test scores among the groups. The F value of 313.519 obtained was higher than the required F value of 4.21. According to the study conducted by Shilpa Pratinidhi (2019) there was a substantial difference in the means due to 12 weeks of yogic practises with Psychoneurobics on

LDL. The ordered adjusted means on LDL was presented through bar diagram for better understanding of the results of this study in Figure - 2. The table of the study's findings revealed that Yogic practises with Psycho Neurobics significantly reduced Cortisol and LDL in Group I compared to Group II. As a result, the hypothesis was accepted with a confidence level of 0.05. Experts such as Shilpa Pratinidhi (2019) and Ma X et al., (2017) backed up the aforesaid conclusions

DISCUSSION ON HYPOTHESIS

The hypothesis stated there will be substantial variations in chosen dependant variables such as Cortisol and LDL between stressed teenage females and the control group because of yoga practises with Psycho Neurobics. The findings shown that yoga practises with Psycho Neurobics had a substantial effect on Cortisol and LDL (Decreased) in stressed teenage females as compared to the control group.

CONCLUSION

It was determined that yoga practises using Psycho Neurobics considerably lowered Cortisol and LDL in stressed teenage females. Thus, yoga practises using Psycho Neurobics benefit teenage females by helping them manage their Cortisol and LDL.

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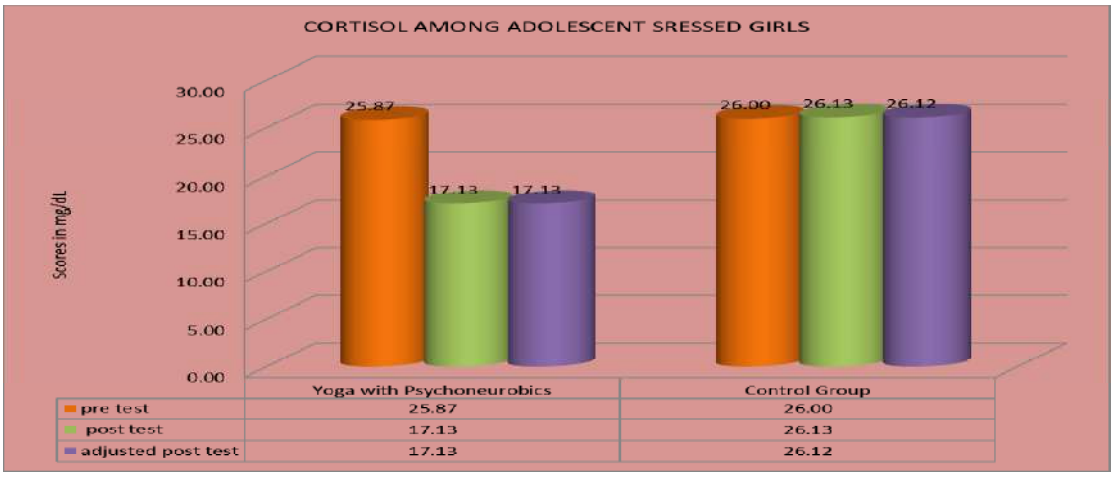
TableI: Analysis Of Covariance Of The Means Of Two Experimental Group And The Control Group On Cortisol (Scores In Mg/Dl)

Tests/ Groups	EX. GR-I	CG –II	SV	Sum of Squares	Df	Mean Squares	"F" Ratio
PreTest	25.867	26.0	**B**	0.533	1	0.267	**0.124**
			W	90.667	28	2.159	
PostTest	17.133	26.133	**B**	663.511	1	331.756	**216.362***
			W	64.400	28	1.533	
Adjusted PostTest	17.133	26.123	**B**	659.238	1	329.619	**211.801***
			W	63.807	27	1.556	
Significant at 0.05 level of confidence. (Table F ratio at 0.05 level, of confidence for df 1 and 28= 4.2, 1and27= 4.21)							

Table II: analys is of covariance of the means of two experimental group and the control group on ldl (scores in mg/dl)

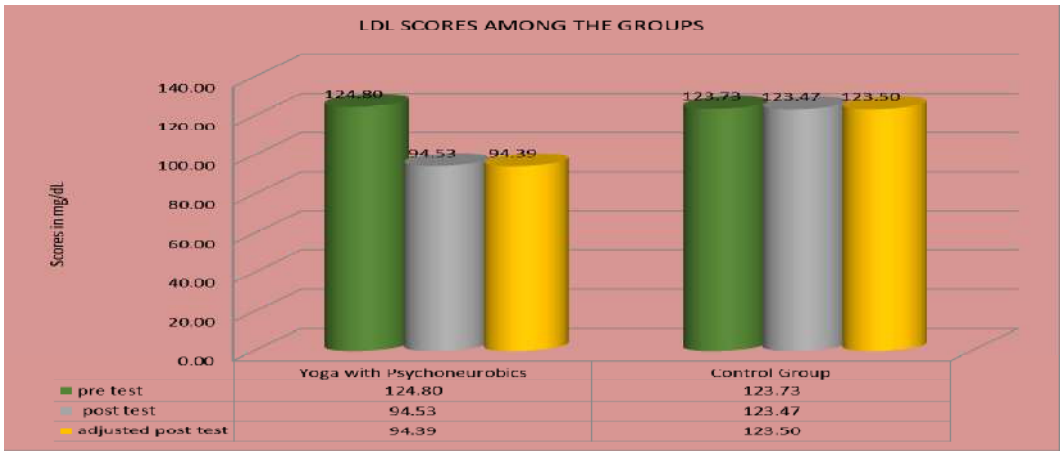
Tests/ Groups	EX. GR-I	CG –II	SV	Sum of Squares	Df	Mean Squares	"F" Ratio
PreTest	124.80	123.733	B	17.244	1	8.622	0.662
			W	546.667	28	13.016	
PostTest	94.533	123.467	B	7416.311	1	3708.156	310.987
			W	500.800	28	11.924	
Adjusted PostTest	94.393	123.504	B	7428.396	1	3714.198	313.519
			W	485.718	27	11.847	

***Significant at 0.05 level of confidence.(Table F ratio at 0.05 level, of confidence for df 1 and 28= 4.2, 1and27= 4.21)**



*** Significantat0.05 level of confidence.(Table Fratioat 0.05 level, of confidencefor df 1 and 28= 4.2, 1and27= 4.21)**

Figure–1 Bar diagram on cortisol (Scores In Mg/Dl)



***Significant at 0.05 level of confidence.(Table F ratio at 0.05 level, of confidence for df 1 and 28= 4.2, 1and27= 4.21)**

Figure–2 Bar Diagram On LDL(SCORESIN mg/dL)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In silico Analysis and Cluster Validation of Potential Breast Cancer nsSNPs of Serological Tumor Marker CA27.29

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Received: 12 Jan 2022 Revised: 09 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

In the present manuscript, an *in silico* analysis of the genetic variations in MUC1 gene associated with CA27.29 was done that possibly alter its stability. The function predictability tools SIFT, Provean and Polyphen2 were used to uncover variations in the analyzed nsSNPs and their functionality. A total of 16, 20 and 10 non-synonymous SNPs (nsSNPs) were predicted to be damaging or deleterious by SIFT, Polyphen, and Provean tools respectively. The substitutions C/G->T and G->A/T were observed to be dominant in the analyzed nsSNPs that probably have damaging role to CA27.29 glycoprotein. Moreover, the validation of results using ClinVar tool revealed that all the analyzed possibly, probably and highly damaging nsSNPs are yet to be reported and studied. Besides this, We found the Global minor allele frequency (Global MAF) for only 11 nsSNPs, the values of which were observed to be <0.1% that further confirmed the novelty of the analyzed variants. Subsequently, among the analyzed nsSNPs, 3 nsSNPs rs145691584, rs148332231 and rs191544901 were found to be located in 3'UTR region of CA27.29 gene that were assumed to have the possible functional roles in altering the protein stability.

Keywords: *In silico* analysis, Breast cancer, MUC-1, CA27.29 protein, tumor marker, nsSNPs

INTRODUCTION

About one in 4 of all new cancer cases (24.2%) diagnosed worldwide in women is breast cancer [1]. According to the press release from the International Agency for Research on Cancer (IARC), breast cancer is the leading cause of cancer death in women (15.0%), followed by lung cancer (13.8%) and colorectal cancer (9.5%) [2]. The disease caused 627000 deaths in 2017-18 which is 6.6% of the total mortality rate that year [3]. It is possible to treat early-stage breast

carcinoma successfully but once this melanoma enters in the advanced or metastatic stage, it will become incurable. Consequently, it is important to recognize the symptoms of metastasis in breast cancer patients as early as possible. The truquant BR radioimmunoassay test or CA27.29 test is the most commonly used metastatic breast cancer detection test that helps medical practitioners to diagnose the reappearance of carcinoma cells after its successful treatment. The normal measurement of CA27.29 antigen in the blood is less than 40U/ml. The higher the measurement of CA27.29 antigen, the greater the likelihood of the presence of breast cancer cells in the body. This test is based on the breast cancer-associated antigen known as CA27.29 (MUC-1; mucin gene product), a special glycoprotein present on the breast cancer epithelial cells. Breast cancer cells shed copies of CA27.29 protein into the bloodstream in case of metastatic breast cancer or in advanced stages of breast cancer [4,5]. The other tumor markers are CA15-3, carcinoembryonic antigen CEA, and HER2 extracellular domain (ECD) [6].

The guidelines of the American Society of Clinical Oncology (ASCO) recommends the use of breast cancer tumor markers such as CA15.3, CA27.29, and CEA in the breast cancer diagnosis [6,7]. Among these, CA15.3 and CA27.29 tumor markers have received also US Food and Drug Administration (FDA) approval in 1996 for monitoring of the disease recurrence or metastasis in patients with stage II/III breast cancer [8]. However, ASCO also recommends that these tumor markers should not be used alone for the surveillance of disease during active therapy and be used in conjunction with diagnostic imaging, history, and physical examination. Conversely, some studies recognize MUC-1 tumor markers CA15.3 and CA27.29 as independent predictors for the primary diagnosis of the disease in addition to the traditional markers such as tumor size and nodal status [9]. The studies also indicate that after primary treatment, the level of these markers can predict the disease recurrence about six months in advance than the other available methods [10, 11]. However, according to the current guidelines, the tumor markers CA15-3 and CA27.29 are not recommended as the prognostic markers for routine clinical use. Despite these limitations, these tumor markers are widely used in disease surveillance and treatment monitoring [12]. In a study, medical practitioners attempted a trial to evaluate the role of the tumor marker CA27.29 after surgery before taxane-based adjuvant chemotherapy in 2669 primary breast cancer patients and they found that CA27.29 levels were above the cut-off level in 8% of the analyzed patients [12]. The study provided information on the prognostic relevance of CA27.29 before the systemic treatment and demonstrated the role of this tumor marker in adjuvant chemotherapy, endocrine, bis-phosphonate, and treatment monitoring [12]. Thus the role of CA27.29 has been observed to be inestimably useful in identifying the response of the patients to the breast cancer treatment and re-occurrence of the disease after treatment. Importantly, other than breast cancer, lung cancer, liver cancer, pancreatic cancer, colon cancer, ovarian cancer, prostate cancer also show elevated levels of CA27.29 antigen [41].

The functionality of a gene cannot be fully understood without knowledge of the potential variability within a gene [13]. Genetic variants can have a major impact on gene function. Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variations. It has been estimated that one SNP exists every 290 base-pairs in the human genome [14]. Evidence showed that a wide range of human diseases including cancer can be triggered through SNPs [15, 16]. SNPs might also affect the pharmacokinetics and pharmacodynamics of certain drugs in cancer therapy [17]. There are two groups of SNPs: synonymous (sSNP) and non- synonymous SNPs (nsSNP), the latter one results in changes of the translated amino acid sequence [18]. With the increasing number of known human nsSNPs, the studies pertaining about the identification of a subset of nsSNPs that affect protein function/s have also increased. Various types of features can be used to predict the functional impact of nsSNPs such as physical and chemical properties of the affected amino acids, structural properties of the encoded protein, and evolutionary properties. Sequence alignment of homologous proteins [19]. SIFT (Sorting Intolerant from Tolerant) [20], PROVEAN (Protein Variation Effect Analyzer) [21] and PolyPhen-2 (Polymorphism Phenotyping v2) [22] are some reliable computational prediction tools that consider these features to predict whether a given nsSNP has a functional impact or not. To understand the biological function and regulation of CA27.29 and its potential for its use as a marker, in the present research, an *in silico* analysis of the genetic variations in CA27.29 gene was performed to identify possible functional nsSNPs . The study involved, CA27.29 associated 2205 SNPs that were downloaded from the NCBI dbSNP database. These nsSNPs were investigated and validated using various computational tools to assess their functional impact on CA27.29. The observations from the present study led to the identification of a

small number of nsSNPs that presumed to affect the CA27.29 protein function adversely. The present study provides more insights to mark the functional nsSNPS that mediate the genetic variations in CA27.29 protein. The analyzed variations may perhaps alter the functionality of the protein and play a critical role in determining susceptibility to breast cancer.

MATERIALS AND METHODS

SNP Database

The dbSNP database, the largest repository of SNPs with over 140 million submitted variations [23], hosted by the National Center for Biotechnology Information (NCBI) [27] was used for SNP mining.

Prediction of Functionally Important nsSNPs

SIFT

The prediction tool SIFT [20,34,35] comprehends the sequence homologies of SNPs and then evaluates the functional impact. The prediction of SIFT tool is based on the degree of conservation of each amino acid residue in the query sequence. SIFT scans the dataset of functionally related protein sequences to evaluate the degree of conservation. This is done by searching the protein databases, UniProt and TrEMBL, using PSI-BLAST algorithm. Here, in the first step, the identified sequences are aligned with the query sequences for the function prediction [18]. Thereafter, in the second step, each substitution at each position in the aligned sequences is assessed and the probability of each substitution is calculated and recorded in a probability matrix. The calculated probability of sequence substitution is called the SIFT Score. A sequence substitution is considered to be tolerated by the SIFT tool if the SIFT Score is above 0.05. The sequence substitution with SIFT Score under 0.05 is considered to be deleterious. SIFT presumes that the sequence substitution at the highly conserved position cannot be tolerated than the sequence substitution at poorly conserved positions [24].

PROVEAN

PROVEAN [21,36,37] uses an alignment approach to evaluate the functional impact of SNPs similar to the SIFT tool. It consists of two main steps. In step 1, BLASTP is performed and similar sequences are collected from NCBI NR Protein database. The sequences with 80% or more similarity are clustered and termed as supporting sequences. In the second step, the delta score is calculated for each cluster of supporting sequences using the BLOSUM62 substitution matrix. This average delta score is the final PROVEAN score [18]. The PROVEAN approach assumes that a variation, that reduces the similarity of a protein to its homologous protein can cause a damaging effect. Thus, the impact of a variation or substitution, on the protein function can be measured as a change in the alignment score that is termed as the delta score for PROVEAN. The low delta scores lead to an increase in the possibility of a deleterious effect on the protein function while the high delta scores are interpreted as variations with neutral effect [25].

PolyPhen-2

PolyPhen-2 [22,38,39] tool coalesce the information of the query sequence, its multiple alignments with homologous proteins and structural parameters to predict the effect of the SNP on the protein function. PolyPhen-2 scans the query sequence in the UniProtKB/Swiss-Prot database using the feature table of the corresponding entry and checks if a given SNP occurs at the functional relevant site i.e. within transmembrane or signal peptide or binding region or not. Similar to SIFT tool, PolyPhen-2 also evaluates the degree of conversion of each substitution position by performing multiple sequence alignment with the homologous sequences. PolyPhen-2 calculates a position-specific independent count (PSIC) score. PolyPhen-2 assumes that a difference of PSIC score between the two variants determines the impact of substitution. Higher PSIC score difference leads to the higher functional impact of the substitution. PolyPhen-2 also performs a BLAST query within the protein structure databases to consider 3D protein

structure. Once the 3D structure is found, it is used to investigate the impact of SNP on the structure of the protein (destruction of the hydrophobic core, interaction with legends etc). Eventually, PolyPhen-2 combines all the parameters to apply prediction rules to make the final decision to find whether the SNP has a damaging or benign effect. The SIFT, PROVEAN and PolyPhen-2 tools are available online at http://sift.jcvi.org/, http://provean.jcvi.org/, and http://genetics.bwh.harvard. edu/pph2/ dbsearch.shtml respectively.

RESULTS AND DISCUSSION

SNP Mining

A total of 2205 records CA 27.29 associated SNPs were found at NCBI SNP database. Among these SNPs, 7 at 3' splice site, 6 at 5' splice site, 133 at 3' UTR, 26 at 5' UTR, and 1351 SNPs in introns were observed. Moreover, 420 SNPs were observed to be missense relative to 13 nonsense SNPs. Consequently, these 2205 SNPs were submitted to dbSNP dataset that categorized 213 (9.66%) SNPs as sSNPs and 24 (1.09%) SNPs as nsSNPs (Fig. 1). These 24 nsSNPs were selected for further investigation. The genomic coordinates and variants of thus filtered 24 nsSNPs were submitted at PROVEAN portal to 'Human Genome Variants' tool that provided PROVEAN and SIFT predictions. The default threshold of delta score <= −2.5 was selected to detect the deleterious variations. The list of genomic coordinates and variants of 24 nsSNPs was also submitted at Polyphen 2 portal, by using the option 'Batch query'. The selected 24 nsSNPs with their nucleotide and amino acid substitutions have been enlisted in table I. Besides this, overall predictions made by SIFT, PROVEAN, and PloyPhen tools, have been provided for the analyzed 24 nsSNPs. The detail of each variation in nsSNPs was considered to filter the nsSNPs that have a deleterious effect on the protein.

Damaging nsSNPs Predicted by SIFT Tool

Among 24 analyzed nsSNPs, 9 nsSNPs rs141460657, rs144273480, rs144273480, rs145691584, rs146141676, rs146950322, rs149173724, rs183700327 and rs191544901 were predicted by SIFT tool to be damaging to CA27.29 with a tolerance index score >= 0.5 (Table I). While, seven nsSNPS namely rs35819649, rs139437006, rs140871200, rs145108039, rs145667707, rs148332231 and rs146950322 were predicted to be highly damaging to CA27.29 with a tolerance index score of 0.00 (Table I). The detailed analysis of each damaging allelic variation in the tested nsSNPs, predicted as functionally significant by SIFT tool has been also provided in Appendix A.

Deleterious nsSNPs Predicted by PROVEAN Tool

PROVEAN tool predicted 10 nsSNPs rs148332231, rs183700327, rs146950322, rs191544901, rs145224844, rs145667707, rs145108039, rs139437006 and rs145691584 to be highly deleterious to CA27.29 with a delta score of <− 4.00 (Table I). The detailed analysis of each damaging allelic variation in all the analyzed nsSNPs, predicted as functionally significant by PROVEAN tool has been also provided in Appendix B.

Damaging nsSNPs Predicted by PolyPhen2 Tool

Out of 24 nsSNPs submitted to PolyPhen-2 server, 18 nsSNPs were predicted to be damaging wherein 15 nsSNPs were observed to be probably damaging and 3 nsSNPs were possibly damaging. The remaining 6 nsSNPs were predicted to be benign (Table I).

Deleterious/Damaging nsSNPs Predicted By At Least Two Or Three Tools

As summarized in Fig. 2, nsSNPs rs144273480, rs191544901, rs183700327, rs148332231, rs145108039, rs145667707, rs145691584 and rs146950322 were predicted to have negative effect on the CA27.29 protein by all the three tools used in the present work (Table I). While SIFT and PROVEAN tools predicted rs139437006, rs145108039, rs145244844, rs145691584, rs146950322, rs148332231, rs183700327, rs191544901 nsSNPs to have adverse effects on the protein (Fig. 2). On the other hand, SIFT and POLYPHEN2 tools predicted rs1611770, rs145667707, rs35819649, rs141460657, rs145224844, rs145691584, rs148332231, rs183700327, rs191544901, rs141460657, rs144273480, rs146950322 nsSNPs to have negative effects on CA27.29 (Fig. 2).

Functional Consequences Of Targeted nsSNPs

Functional Consequences of all 24 nsSNPs were identified and out of 24, 23 nsSNPs (95.83%) were found to be missense, wherein 21 nsSNPs (87.5%) and 3 nsSNPs (12.5%) were observed to be located in the intronic region, and 3'UTR region respectively.

Frequency Distribution Of Allelic Variations in nsSNPs

An attempt was also made in the present research to find the frequency distribution of allelic variations in the analyzed 24 nsSNPs by using SIFT, PROVEAN and PolyPhen-2 prediction tools. It was observed that G|T substitution was the most common type of allelic variation present in the analyzed 24 nsSNPs. A G|T substitution was observed in 26.67% nsSNPs whereas 10%, 20%, 23.33%, 6.67%, 6.67%, 6.67% nsSNPS were observed with C|A, C|T, G|A, A|G, T|A and C|G substitutions respectively (Fig. 3).

The nsSNPs that were predicted by SIFT as damaging exhibited 10.53%, 21.05%, 15.79%, 36.84%, 10.53%, 5.26% allelic variations for C|A, C|T, G|A, G|T, A|G, T|A and C|G substitutions respectively. SIFT tool did not predict any nsSNP with C|G substitution as damaging. While deleterious nsSNPs, as predicted by PROVEAN tool were observed to have 11.11%, 16.67%, 5.56%, 2.22%, 11.11% allelic variations for C|A, C|T, G|A, G|T and A|G substitutions respectively. PROVEAN did not predict any nsSNP with T|A, and C|G substitutions as damaging (Fig. 3).

Frequency Distribution Of Amino Acid To Amino Acid Variations in nsSNPs

It was observed that the analyzed nsSNPs resulting in 100% damage to CA27.29 had one hydrophobic amino acid to another hydrophobic amino acid variation. The details of the amino acid to amino acid variation (AA change) in all the selected nsSNPs have been provided in Table I. The status of the polarity of amino acid variations was also analyzed (Fig. 4).It was found that the polarity of amino acid in 15 (62.5%) nsSNPs was not affected even if the observed variation was damaging. Wherein 8 (33.33%) and 7 (29.17%) nsSNPs were analyzed with the polar amino acid to polar amino acid (P to P) and non-polar amino acid to non-polar amino acid (NP to NP) variations respectively (Fig. 4). However, non-polar to polar (NP to P) and polar to non-polar (P to NP) amino acid variations were observed to occur in 6 (25%) and 3 (12.5%) nsSNPs respectively. The variations of polarity distribution in nsSNPs, predicted by SIFT tool to be damaging, suggested that NP to P amino acid variations had the maximum frequency of 33.33% followed by NP to NP (27.78%), P to P (27.78%) and P to NP (11.11%) amino acid variations. While polarity distribution of variations in nsSNPs, predicted deleterious by PROVEAN tool, suggested that the frequency (36.36%) of P to P amino acid variations was observed to be maximum. However, the frequency of NP to P and P to NP amino acid variations in deleterious nsSNPs were assessed to be 27.27% and 9.09% respectively. The damaging variation as predicted by PolyPhen2 exhibited the frequency of 31.58% for NP to NP and P to P amino acid variations. However, other NP to P and P to NP variations in polarity distributions were noted to have the frequency of 10.53% and 26.32% respectively (Fig. 4).

Benchmarking of the results provided by SIFT and PROVEAN tools using clustering methods

Four clustering methods KMeans [28], MakeDensityBasedClustrer [29], FarthestFirst [30a, 30b] and ACERAM [31] were used to benchmark the number of damaging or deleterious nsSNPs identified by SIFT, POLYPHEN2, and PROVEAN tools. The method k-means clustered 80% nsSNPs and 66% nsSNPs correctly, that were predicted as damaging by SIFT and PROVEAN tools respectively (fig.5). Fig.5 Benchmarking of predictions made by SIFT, PolyPhen2 and PROVEAN tools using clustering methods a. K_Means b. Make_Density_Based_Clustrer c. Farthest_First d. ACERAM While Make_Density_Based_Clustrer clustered 98% and 73% nsSNPs correctly that were predicted as damaging or deleterious by SIFT and PROVEAN tools respectively. On the other hand, FarthestFirst clustered 99% and 83% nsSNPs correctly for the predictions made by SIFT and PROVEAN tools respectively (Fig. 5). As compared to that, ACERAM clustered 99% and 98% nsSNPs correctly, predicted as deleterious by SIFT and PROVEAN tools respectively. Overall results from this experiment indicated that the clustering method ACERAM provided more precise results than the other clusterers used in the present study (Fig. 5).

Comparison of the predictions made by SIFT and PROVEAN tools with SNPs database

The predictions made by computational tools were compared using four clustering methods with SNP database (Table 2). The overall comparisons identified 215 SNPs as damaging whereas 147 SNPs were found to be tolerated by SIFT tool. While the PROVEAN tool predicted 199 SNPs as neutral and 163 SNPs as deleterious. While clustering method K-means revealed that there were 172 damaging, 189 tolerated and 80 SNPs clustered incorrectly (false positive + false negative) predicted by SIFT tool. Similarly, the K-means clustering method identified 168 SNPs as neutral and 193 SNPs as deleterious for the predictions made by the PROVEAN tool, wherein, 66 SNPs were recognized as incorrectly clustered (Table 2).

ACERAM clusterer identified 201 and 122 SNPs as damaging and tolerated respectively. A total of 69 SNPs were marked as incorrectly clustered for the predictions made by the SIFT tool. While for the predictions made by the PROVEAN tool, ACERAM identified 167 SNPs as neutral and 156 as deleterious SNPs. ACERAM clusterer observed, 69 SNPs as incorrectly clustered for the predictions made by the PROVEAN tool (Table 2). On the other side, Make_Density_Based_Clusterer method clustered 274 SNPs as damaging and 87 SNPs as tolerated whereas 98 SNPs were found to be incorrectly clustered as predicted by SIFT tool. The predictions made by the PROVEAN tool provided 161 SNPs as neutral, 200 deleterious while 73 SNPs as incorrectly clustered. On the other hand, FarthestFirst algorithm clustered 285 SNPs as damaging, 76 tolerated and 99 SNPs as incorrectly clustered for the predictions made by SIFT tools. While the predictions made by PROVEAN tool presented 159 SNPs as neutral and 202 SNPs deleterious and 83 SNPs as incorrectly clustered (Table 2).

Validation of Polymorphisms in nsSNPS using SNP Database

It is important to check the true status of polymorphism and confirm validation status for the analyzed nsSNPs. A polymorphism can be validated by independent submissions of the frequency or genotypic data to the dbSNP database [18]. The genotypes of each of the 24 nsSNPs were submitted one by one to dbSNP server to observe their validation. The functional consequences for each of the nsSNPs were checked and validated by 1000G clusterer, Heapmap and frequency cluster validation techniques. Global MAF and clinical significance of the analyzed nsSNPs were also observed in the present study. The observations derived from these analyses have been provided in Table 3.

DISCUSSION

To distinguish the critical regions of a gene, it is important to be familiar with its genetic variation. SNPs are genetic variations that have been linked and reported with various complex diseases such as sickle-cell anemia, β-thalassemia, cystic fibrosis and breast cancer etc [18]. For example, SNPs in BRCA-1 gene are not just involved in the onset of the disease but are also involved in promoting the disease progression [32, 33]. In the present study, we analyzed the MUC1 gene product CA27.29 protein to identify possible nsSNPs that modify its functional properties. There are various features used by computational tools to predict the functional influences of nsSNPs on the gene product. In the present work, three contemporary online computational tools, SIFT [20, 34, 35], Provean [21, 36, 37] and PolyPhen2 [22,38,39] were used, to predict the effects of different allele variations in nsSNPs on amino acid substitution and protein function. Using SIFT, PROVEAN and POLYPHEN2 tools, out of 2205 analyzed nsSNPs, a total of 24 (1.08%) malicious nsSNPs were predicted. These 24 nsSNPs were analyzed further wherein 9 (37.5%) out of 24 nsSNPs were predicted as damaging or deleterious. SIFT tool predicted 16 (66.66%) nsSNPs as damaging while PROVEAN tool predicted 10 (41.66%) nsSNPs as deleterious. Where as PolyPhen2 tool predicted 20 (83.33%) nsSNPs as damaging. A total of 14 (58.33%) nsSNPs were predicted to have a damaging effect when at least two predictions tools were used. Intriguingly, 12 (50%) nsSNPs were predicted in common to be deleterious by SIFT and PolyPhen2 tools. This might be due to the common steps of assessing the degree of the conversation by both the tools that utilize multiple sequence alignment of the homologous sequences [18]. Similarly, a total of 6 (25%) nsSNPs were predicted in common by SIFT and PROVEAN tools. Ankyrin (ANK) repeat motifs are unique motif mediating protein-protein interaction, the structure of which has been observed to be conserved and known to occur in many functionally

assorted proteins. But in the present study, out of 24 analyzed nsSNPs, none of nsSNPs was found to contain the ANK repeat motif.

There are many misclassified SNPs in the public databases, therefore it was important to validate the nsSNPs under present investigation. On submitting the selected 24 nsSNPs under investigation to dbSNP, importantly, the allele frequencies were provided for only 11 nsSNPs. SNPs can be classified according to their minor allele frequencies wherein the variants with minor allele frequencies (MAF) between 0.1% and 3% are defined as rare variants while the variants with minor allele frequencies of less than 0.1% are defined as novel variants. While the variants with high allele frequencies greater than 5% are defined as common variants [27, 28]. It is important to note that the minor allele frequency of all the analyzed nsSNPs in the present study was observed to be below 0.1% which establish these variants as novel variants that are yet to be studied. The identification of the functional consequences of 24 nsSNPs revealed that out of 24 analyzed nsSNPs, 23 (95.83%) nsSNPs are missense. It has been reported that the cancer-associated missense mutations can lead to the drastic destabilization of the resulting protein [40]. Besides this, it was also assessed in the present study, that 21 (87.5%) nsSNPs were located in the intronic region and 3 (12.5%) nsSNPs were found to be located in 3'UTR region. The introns are removed in the process known as RNA splicing in mature RNA, that suggests that these will not be expressed in the final messenger RNA (mRNA) product. Thus 21 (87.5%) nsSNPs located in the intronic region might be having no functional consequence on CA27.29 protein but probably the rest of 3 (12.5%) nsSNPs rs145691584, rs148332231 and rs191544901 might have the functional role.

To check the clinical significance of the selected 24 nsSNPs, ClinVar tool was used and the observations showed that all of the analyzed nsSNPs are yet to be reported. While assessing the frequency distribution of allelic variations, G to T allelic variation was found to occur in 8 (33.33%) out of the 24 nsSNPs tested followed by G to A substitution, frequency of which was observed in 7 (29.16%) nsSNPs under investigation. Similarly, SIFT and PROVEAN tools also predicted allelic variation in 7 (36.84%) and 4 (33.33%) nsSNPs with G->T substitutions while C->T substitutions were observed in 4 (21.05%) and 3 (25%) deleterious nsSNPs. Polyphen2 tool predicted allelic variation in 6 (30%) nsSNPs with G ->T substitution while G->A substitution was observed in 5 (25%) nsSNPs. In light of these observations, it was assumed that probably G->T/A and C/G->T allelic variations are responsible for the damaging effect of the analyzed nsSNPs.

Four different clustering algorithms k-means, Make_Density_Based_Clustrer, Farthest_First and ACERAM were used to compare the predictions made by SIFT and PROVEAN tools for each allelic variation. SIFT tool made incorrect predictions for 80 nsSNPs while it was observed to be 66 with PROVEAN tool when assessed with k-means clustering algorithm. However, using ACERAM clustering algorithm, SIFT and PROVEAN tools were found to made 92 and 69 incorrect predictions respectively. Similar to this, Make_Density_Based_Clusterer identified 98 and 73 incorrect predictions in nsSNPs variations made by SIFT and PROVEAN tools respectively. On the other hand, as per FarthestFirst clustering algorithm, 99 and 83 nsSNPs variations were differently predicted by SIFT and PROVEAN tools respectively. The present analysis suggest that some of the allelic variations were misclassified and the predictions made by these tools are not 100% accurate. Consequently, investigating the prediction accuracy of these tools is an area of further research. Or it could be also possible that there are few incorrect or incomplete sequences submitted in the public databases that have led these tools to made predictions in the wrong direction. To find out these incomplete and incorrect sequences in SNP database is another prospective research area.

CONCLUSIONS

After primary treatment of breast cancer, the level of MUC1 gene product CA27.29 in the bloodstream can predict the disease recurrence about six months in advance. For this and many other reasons, it was important to know the factors affecting its stability, particularly the functionality of MUC 1 gene and its associated CA27.29 glycoprotein. Besides this, few reports directly relate nsSNPs to the increased risk of breast cancer. To keep these in mind an attempt was made in the present study to identify nsSNPs that have malicious effect on CA27.29 protein. From a

total of 2205 SNPs recovered from dbSNP database, 24 nsSNPs were found to be related to the target. SIFT, PROVEAN and Polyphen2 tools predicted 16, 10 and 20 numbers of nsSNPs as deleterious or damaging respectively. Subsequent to this analysis, in the C/G->T and G->A/T allelic variations were prominently observed in the deleterious nsSNPs under investigation. The clinical significance assessed by ClinVar tool confirmed that all the analyzed nsSNPs are yet to be reported and are not studied till date. The results were validated after submitting nsSNPs individually to the dbSNP to find their Global minor allele frequency (Global MAF) that revealed allele frequencies for only 11 nsSNPs. In addition, the allelic frequency of all nsSNPs was observed to be below 0.1% that might establish to consider these nsSNPs as the novel variants. Moreover, 3 nsSNPs rs145691584, rs148332231 and rs191544901 were found to be located in 3'UTR region of CA27.29 gene that might have the functional role in altering its protein levels in the blood stream of breast cancer patients.

ACKNOWLEDGEMENTS

We are grateful to Prof. Vinod Kumar Singh, Vice-Chancellor, Maharaja Ganga Singh University, Bikaner, Rajasthan, India for providing the departmental research grant.

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Table I Nucleotide and Amino acid (hydrophobic to hydrophobic) variations in nsSNPs predicted by SIFT, PROVEAN and PloyPhen2 tools

S.No.	SNP ID	Nucleotide variation	Amino acid variation	*SIFT Prediction	*PROVEAN Prediction	#PolyPhen 2 Prediction
				Tolerance Value (Cutoff Range)		
1.	rs1611770	G|A, G|T	V|M, V|L	-	-	Probably Damaging (0.999)
2.	rs11465205	G|A, G|T	G|S, G|C	-	-	Possibly Damaging (0.052)
3.	rs11465207	G|A	S|N	-	-	Possibly Damaging (0.862)
4.	rs35819649	T|A	S|T	**Highly Damaging (0.000 to 0.019)**	-	Probably Damaging (0.999)
5.	rs412649913	T|A	N|K	-	-	Probably Damaging (0.946)
6.	rs112431868	G|C	T|S	-	-	Probably Damaging (0.947)
7.	rs113445744	G|C	P|A	-	-	Probably Damaging (0.966)
8.	rs139437006	C|A, C|T	T|K, T|M	**Highly Damaging (0.000 to 0.009)**	Deleterious (-2.66 to -5.39)	-
9.	rs140871200	G|A	G|G	**Highly Damaging (0.000)**	-	-
10.	rs141460657	G|T	L|L	Damaging (0.003)	-	Probably Damaging (0.997)
11.	rs143297835	C|G	P|A	-	-	Probably Damaging (0.998)
12.	rs144273480	G|A, G|T	D|N, D|Y	Damaging (0.006 to 0.041)	Deleterious (-2.54 to -2.94)	Probably Damaging (0.999)
13.	rs145108039	G|T	S|I	**Highly Damaging (0.000 to 0.003)**	Deleterious (-2.79 to 5.56)	Probably Damaging (0.988)
14.	rs145224844	G|T	G|V	Damaging (0.001)	Deleterious (-5.41 to -6.32)	-
15.	rs145667707	C|T	S|F	**Highly Damaging (0.000 to 0.001)**	Deleterious (-2.67 to -5.64)	Probably Damaging (0.998)
16.	rs145691584	C|A, C|T	S|S	Damaging (0.001-0.010)	Deleterious (-2.70 to -4.80)	Probably Damaging (0.997)

17.	rs146141676	C|T	T|M	Damaging (0.001-0.006)	-	-
18.	rs146950322	C|T	P|S	Damaging (0.000)	Deleterious (-3.54 to 6.47)	Highly Damaging (1.000)
19.	rs147388624	C|G	A|G	-	-	Possibly Damaging (0.745)
20.	rs148332231	A|G	Y|C	**Highly Damaging (0.000)**	Deleterious (-7.37 to -8.01)	-
21.	rs149173724	G|A, G|T	G|A, G|T	Damaging (0.014)		Highly Damaging (1.000)
22.	rs183700327	A|G	A|G	Damaging (0.046)	Deleterious (-6.46 to -6.69)	Probably Damaging (0.999)
23.	rs191544901	G|A, G|T	G|A, G|T	Damaging (0.001to 0.022)	Deleterious (-3.89 to -6.27)	Probably Damaging (0.999)
24.	rs149945265	C|G, C|T	C|G, C|T	-	-	-

***PROVEAN and SIFT tools with default prediction cutoff of -2.5 and 0.05, respectively**
#PolyPhen2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite meanings. A variant with a PolyPhen2 score of 0.0 was predicted to be benign. A variant with a SIFT score of 1.0 was predicted to be benign

Table 2 Comparison of the prediction made by SIFT and PROVEAN tools using various clusterers

Method	**SIFT**			**PROVEAN**		
	Damaging	**Tolerated**	**Incorrectly Clustered**	**Neutral**	**Deleterious**	**Incorrectly Clustered**
	SNPs					
K-Means	172	189	80	168	193	66
ACERAM	201	122	92	167	156	69
Make_Density_ Based_Clusterer	274	87	98	161	200	73
FarthestFirst	285	76	99	159	202	83

Table 3 Functional consequences, validation, global MAF and clinical significance of the observed nsSNPs

S. No.	nsSNPs	Functional Consequence				Validation				Global MAF	Alleles	Clinical Significance (as per ClinVar tool)
		Missense	Intronic Variant	Synonymous codon	UTR variant 3 prime	1000G	Cluster	Frequency	Hapmap			
1	rs1611770	y	y			y	y	y	y	T=0.0116/58	C>T	Not reported
2	rs11465205	y	y			y	y	y		T=0.0054/27	C>A / C>T	Not reported
3	rs11465207	y	y			y	y	y		T=0.0016/8	C>T	Not reported
4	rs35819649	y	y			y	y	y		T=0.0002/1	A>T	Not reported
5	rs41264913	y	y			y	y	y		T=0.0002/1	A>T	Not reported
6	rs112431868	y	y				y			-	G>C	Not reported
7	rs113445744		y				y	y		-	G>C / G>T	Not reported
8	rs139437006	y	y				y			-	G>A / G>T	Not reported
9	rs140871200	y	y	y			y	y		-	C>T	Not reported
10	rs141460657	y	y	y		y	y	y		A=0.0004/2	C>A	Not reported
11	rs143297835	y	y			y	y	y		C=0.0022/11	G>C	Not reported
12	rs144273480	y	y				y	y		-	C>A / C>T	Not reported
13	rs145108039	y	y				y	y		-	C>A	Not reported
14	rs145224844	y	y				y	y		-	C>A	Not reported
15	rs145667707	y	y				y			-	G>A	Not reported
16	rs145691584	y		y			y	y		-	G>A / G>T	Not reported
17	rs146141676	y	y				y	y		-	G>A	Not reported
18	rs146950322	y	y	y			y			-	G>A	Not reported
19	rs147388624	y	y				y	y		-	G>C	Not reported

20	**rs148332231**	y			y		y	y		-	T>C	Not reported
21	**rs149173724**	y	y			y	y	y		T=0.0004/2	C>A / C>T	Not reported
22	**rs183700327**	y	y	y		y	y	y		C=0.0002/1	T>C	Not reported
23	**rs191544901**	y			y	y	y			A=0.0002/1	C>A / C>T	Not reported
24	**rs149945265**	y	y	y		y	y	y		C=0.0016/8	G>A / G>C	Not reported

Appendix A : Summary of deleterious nsSNPs predicted by SIFT tool

SNP ID	Variation in Nucleotide			Variation in Amino Acid			Tolerance Value Cutoff - 0.05
	Accession Number	Allele variation	Location of Allele Variation	Accession Number	Residue variation	Location of Residue Variation	
rs1611770	NM_001018016.2	G|A	448	NP_001018016.1	V|M	126	0.026
	NM_001018017.2		421	NP_001018017.1		117	0.031
	NM_001204285.1		1081	NP_001191214.1		337	0.013
	NM_001204286.1		1108	NP_001191215.1		346	0.012
	NM_001204287.1		502	NP_001191216.1		144	0.026
	NM_001204288.1		448	NP_001191217.1		126	0.021
	NM_001204289.1		370	NP_001191218.1		100	0.029
	NM_001204290.1		307	NP_001191219.1		79	0.015
	NM_001204291.1		397	NP_001191220.1		103	0.025
	NM_001204292.1		373	NP_001191221.1		101	0.026
	NM_001204293.1		475	NP_001191222.1		135	0.018
	NM_001204294.1		346	NP_001191223.1		92	0.032
	NM-002456.5		475	NP_002449.4		135	0.024
	NM_001204285.1	G|T	1081	NP_001191214.1	V|L	337	0.030
	NM_001204286.1		1108	NP_001191215.1		346	0.026
	NM_001204287.1		502	NP_001191216.1		144	0.046
	NM_001204288.1		448	NP_001191217.1		126	0.044
	NM_001204293.1		475	NP_001191222.1		135	0.044
rs11465205	NM_001204285.1	G|T	658	NP_001191214.1	G|C	196	0.006
	NM_001204286.1		685	NP_001191215.1		205	0.005
rs11465207	NM_001037856.1	G|A	241	NP_001037856.1	S|N	57	0.019
	NM_001204285.1		901	NP_001191214.1		277	**0.000**
	NM_001204286.1		928	NP_001191215.1		286	0.002
	NM_001204287.1		322	NP_001191216.1		84	**0.000**
	NM_001204288.1		268	NP_001191217.1		66	0.002
	NM_001204293.1		295	NP_001191222.1		75	**0.000**
	NM_002456.5		295	NP_002447.4		75	**0.000**
rs35819649	NM_001044391.2	T|A	241	NP_001037856.1	S|T	57	0.019
	NM_001204285.1		901	NP_001191214.1		277	**0.000**
	NM_001204286.1		928	NP_001191215.1		286	0.002
	NM_001204287.1		322	NP_001191216.1		84	**0.000**
	NM_001204288.1		268	NP_001191217.1		66	0.002
	NM_001204293.1		295	NP_001191222.1		75	**0.000**
	NM_002456.5		295	NP_002447.4		75	**0.000**
rs139437006	NM_001018016.2	C|A	134	NP_001018016.1	T|K	31	**0.000**
	NM_001018017.2		137	NP_001018017.2		22	0.002

	NM_001044390.2		137	NP_001037855.1		22	**0.000**
	NM_001044391.2		137	NP_001037856.1		22	**0.000**
	NM_001044392.2		164	NP_001037857.1		31	**0.000**
	NM_001044393.2		137	NP_001037858.1		22	**0.000**
	NM_001204285.1		137	NP_001191214.1		22	0.009
	NM_001204286.1		164	NP_001191215.1		31	0.002
	NM_001204287.1		164	NP_001191216.1		31	0.001
	NM_001204288.1		164	NP_001191217.1		31	**0.000**
	NM_001204289.1		164	NP_001191218.1		31	**0.000**
	NM_001204291.1		164	NP_001191220.1		31	**0.000**
	NM_001204292.1		164	NP_001191221.1		31	**0.000**
	NM_001204293.1		164	NP_001191222.1		22	0.001
	NM_001204295.1		164	NP_001191224.1		31	**0.000**
	NM_001204296.1		164	NP_001191225.1		31	**0.000**
	NM_001204294.1		164	NP_001191226.1		31	**0.000**
	NM_002456.5		137	NP_002447.4		22	0.001
	NM_001018016.2	C|T	134	NP_001018016.1	T|M	31	**0.000**
	NM_001018017.2		137	NP_001018017.2		22	0.002
	NM_001044390.2		137	NP_001037855.1		22	0.001
	NM_001044391.2		137	NP_001037856.1		22	**0.000**
	NM_001044392.2		164	NP_001037857.1		31	**0.000**
	NM_001044393.2		137	NP_001037858.1		22	**0.000**
	NM_001204285.1		137	NP_001191214.1		22	0.049
	NM_001204286.1		164	NP_001191215.1		31	0.001
	NM_001204287.1		164	NP_001191216.1		31	**0.000**
	NM_001204288.1		164	NP_001191217.1		31	**0.000**
	NM_001204289.1		164	NP_001191218.1		31	**0.000**
	NM_001204291.1		164	NP_001191220.1		31	**0.000**
	NM_001204292.1		164	NP_001191221.1		31	**0.000**
	NM_001204293.1		164	NP_001191222.1		22	0.001
	NM_001204295.1		164	NP_001191224.1		31	**0.000**
	NM_001204296.1		164	NP_001191225.1		31	**0.000**
	NM_001204294.1		164	NP_001191226.1		31	**0.000**
	NM_002456.5		137	NP_002447.4		22	0.001
rs140871200	NM_001044393.2	G|A	305	NP_001037858.1	G|G	78	**0.000**
rs141460657	NM_001044393.2	G|T	403	NP_001037858.1	G|G	111	0.003
rs144273480	NM_001018016.1	G|T	445	NP_001018016.1	D|Y	125	0.009
	NM_001018017.2		418	NP_001018017.1		116	0.012
	NM_001204285.1		1078	NP_001191214.1		336	0.010
	NM_001204286.1		1105	NP_001191215.1		345	0.012
	NM_001204287.1		499	NP_001191216.1		143	0.018
	NM_001204288.1		445	NP_001191217.1		125	0.004
	NM_001204289.1		367	NP_001191218.1		99	0.028
	NM_001204290.1		304	NP_001191219.1		78	0.041
	NM_001204291.1		376	NP_001191220.1		102	0.007
	NM_001204292.1		370	NP_001191221.1		100	0.022
	NM_001204293.1		472	NP_001191222.1		134	0.006
	NM_001204294.1		343	NP_001191223.1		91	0.027
	NM_002456.5		472	NP_002447.4		134	0.011
rs145108039	NM_001018016.2	G|T	182	NP_001018016.1	S|I	37	0.001
	NM_001018017.2		155	NP_001018017.1		28	0.001
	NM_001044390.2		155	NP_001037855.1		28	0.001
	NM_001044391.2		155	NP_001037856.1		28	0.001

	NM_001044392.1		182	NP_001037857.1		37	0.001
	NM_001044393.2		155	NP_001037858.1		28	0.001
	NM_001204285.1		155	NP_001191214.1		28	0.003
	NM_001204286.1		182	NP_001191215.1		37	0.001
	NM_001204287.1		182	NP_001191216.1		37	0.001
	NM_001204288.1		182	NP_001191217.1		37	0.001
	NM_001204289.1		182	NP_001191218.1		37	0.001
	NM_001204291.1		182	NP_001191220.1		37	**0.000**
	NM_001204292.1		182	NP_001191221.1		37	**0.000**
	NM_001204293.1		155	NP_001191222.1		28	0.001
	NM_001204294.1		155	NP_001191223.1		28	0.001
	NM_001204295.1		110	NP_001191224.1		28	0.001
rs145224844	NM_001018016.2	G|T	389	NP_001018016.1	G|V	106	0.001
	NM_001018017.2		362	NP_001018017.1		97	0.001
	NM_001204285.1		1022	NP_001191214.1		317	0.001
	NM_001204286.1		1049	NP_001191215.1		326	0.001
	NM_001204287.1		443	NP_001191216.1		124	0.001
	NM_001204288.1		389	NP_001191217.1		106	0.001
	NM_001204289.1		311	NP_001191218.1		80	0.001
	NM_001204290.1		248	NP_001191219.1		59	0.001
	NM_001204291.1		320	NP_001191220.1		83	0.001
	NM_001204292.1		314	NP_001191221.1		81	0.001
	NM_001204293.1		416	NP_001191222.1		115	0.001
	NM_001204294.1		287	NP_001191223.1		72	0.001
	NM_002456.5		416	NP_002447.4		115	0.001
rs145667707	NM_001018016.2	C|T	185	NP_001018016.1	S|F	38	0.001
	NM_001018017.2		158	NP_001018017.1		29	0.001
	NM_001044390.2		158	NP_001037855.1		29	0.001
	NM_001044391.2		158	NP_001037856.1		29	0.001
	NM_001044392.2		185	NP_001037857.1		38	0.001
	NM_001044393.2		158	NP_001037858.1		29	0.001
	NM_001204285.1		158	NP_001191214.1		29	0.001
	NM_001204286.1		185	NP_001191215.1		38	**0.000**
	NM_001204287.1		185	NP_001191216.1		38	**0.000**
	NM_001204288.1		185	NP_001191217.1		38	0.001
	NM_001204289.1		185	NP_001191218.1		38	0.001
	NM_001204291.1		185	NP_001191220.1		38	**0.000**
	NM_001204292.1		185	NP_001191221.1		38	**0.000**
	NM_001204293.1		158	NP_001191222.1		29	0.001
	NM_001204294.1		158	NP_001191223.1		29	**0.000**
	NM_001204295.1		185	NP_001191224.1		38	0.001
	NM_001204296.1		185	NP_001191225.1		38	**0.000**
	NM_001204297.1		185	NP_001191226.1		38	0.001
	NM_002456.5		158	NP_002447.4		29	0.003
rs145691584	NM_001018016.2	C|A	240	NP_001018016.1	S|R	56	0.001
	NM_001018017.2		213	NP_001018017.1		47	0.004
	NM_001044390.2		213	NP_001037855.1		47	0.001
	NM_001044391.2		213	NP_001037856.1		47	0.001
	NM_001044392.2		240	NP_001037857.1		56	0.001
	NM_001044393.2		213	NP_001037858.1		47	0.001
	NM_001204286.1		240	NP_001191215.1		56	0.004
	NM_001204287.1		240	NP_001191216.1		56	0.010
	NM_001204288.1		240	NP_001191217.1		56	0.001

	NM_001204289.1		240	NP_001191218.1		56	0.003
	NM_001204290.1		177	NP_001191219.1		35	0.001
	NM_001204291.1		240	NP_001191220.1		56	0.002
	NM_001204292.1		240	NP_001191221.1		56	0.002
	NM_001204293.1		213	NP_001191223.1		47	0.003
	NM_001204294.1		213	NP_001191223.1		47	0.003
	NM_001204295.1		240	NP_001191224.1		56	0.002
	NM_001204296.1		240	NP_001191225.1		56	0.001
	NM_001204297.1		240	NP_001191226.1		56	0.004
	NM_002456.5		213	NP_002447.4		47	0.015
rs146141676	NM_001018016.2	C|T	503	NP_001018016.1	T|M	144	0.002
	NM_001018017.2		476	NP_001018017.1		135	0.002
	NM_001204285.1		1136	NP_001191214.1		355	0.002
	NM_001204286.1		1163	NP_001191215.1		364	0.002
	NM_001204287.1		557	NP_001191216.1		162	0.001
	NM_001204288.1		503	NP_001191217.1		144	0.001
	NM_001204289.1		425	NP_001191218.1		118	0.004
	NM_001204290.1		362	NP_001191219.1		97	0.006
	NM_001204291.1		434	NP_001191220.1		121	0.002
	NM_001204292.1		428	NP_001191221.1		119	0.003
	NM_001204293.1		530	NP_001191222.1		153	0.004
	NM_001204294.1		401	NP_001191223.1		110	0.003
	NM_002456.5		530	NP_002447.4		153	0.001
rs146950322	NM_001018016.2	C|T	280	NP_001018016.1	P|S	70	**0.000**
	NM_001018017.2		253	NP_001018017.1		61	**0.000**
	NM_001204285.1		913	NP_001191214.1		281	**0.000**
	NM_001204286.1		940	NP_001191215.1		290	**0.000**
	NM_001204287.1		334	NP_001191216.1		88	**0.000**
	NM_001204288.1		280	NP_001191217.1		70	**0.000**
	NM_001204293.1		307	NP_001191222.1		79	**0.000**
	NM_002456.5		307	NP_002447.4		79	**0.000**
rs148332231	NM_001018016.2	A|G	785	NP_001018016.1	Y|C	238	**0.000**
	NM_001044390.2		602	NP_001037855.1		177	**0.000**
	NM_001044391.2		443	NP_001037856.1		124	**0.000**
	NM_001044392.2		470	NP_001037857.1		133	**0.000**
	NM_001204285.1		1418	NP_001191214.1		449	**0.000**
	NM_001204286.1		458	NP_001191215.1		458	**0.000**
	NM_001204287.1		839	NP_001191216.1		256	**0.000**
	NM_001204289.1		707	NP_001191218.1		212	**0.000**
	NM_001204290.1		644	NP_001191219.1		191	**0.000**
	NM_001204291.1		716	NP_001191220.1		215	**0.000**
	NM_001204292.1		710	NP_001191221.1		213	**0.000**
	NM_001204294.1		683	NP_001191223.1		204	**0.000**
	NM_001204295.1		560	NP_001191224.1		163	**0.000**
	NM_001204296.1		629	NP_001191225.1		186	**0.000**
	NM_001204297.1		524	NP_001191226.1		151	**0.000**
rs149173724	NM_001204286.1	G|T	298	NP_001191215.1	G|C	76	0.014
rs183700327	NM_001204286.1	A|G	1289	NP_001191215.1	Y|C	406	0.046
rs191544901	NM_001018016.2	G|A	776	NP_001018016.1	R|H	235	0.016
	NM_001018017.2		749	NP_001018017.1		226	0.017
	NM_001044390.2		593	NP_001031855.1		174	0.014
	NM_001044391.2		434	NP_001037856.1		121	0.013
	NM_001044392.2		461	NP_001037857.1		130	0.017

	NM_001204285.1		1049	NP_001191214.1		446	0.029
	NM_001204286.1		1436	NP_001191215.1		455	0.018
	NM_001204287.1		830	NP_001191216.1		253	0.008
	NM_001204289.1		698	NP_001191218.1		209	0.008
	NM_001204290.1		635	NP_001191219.1		188	0.009
	NM_001204291.1		707	NP_001191220.1		212	0.011
	NM_001204292.1		701	NP_001191221.1		701	0.010
	NM_001204294.1		674	NP_001191223.1		201	0.015
	NM_001204295.1		551	NP_001191224.1		160	0.012
	NM_001204296.1		620	NP_001191225.1		183	0.010
	NM_001204297.1		515	NP_001191226.1		148	0.013
	NM_002456.5		803	NP_002447.4		244	0.022
	NM_001018016.2	G|T	776	NP_001018016.1	R|L	235	0.001
	NM_001018017.2		749	NP_001018017.1		226	0.001
	NM_001044390.2		593	NP_001031855.1		174	0.001
	NM_001044391.2		434	NP_001037856.1		121	0.001
	NM_001044392.2		461	NP_001037857.1		130	0.001
	NM_001204285.1		1049	NP_001191214.1		446	0.001
	NM_001204286.1		1436	NP_001191215.1		455	0.001
	NM_001204287.1		830	NP_001191216.1		253	0.001
	NM_001204289.1		698	NP_001191218.1		209	0.001
	NM_001204290.1		635	NP_001191219.1		188	0.001
	NM_001204291.1		707	NP_001191220.1		212	0.001
	NM_001204292.1		701	NP_001191221.1		701	0.001
	NM_001204294.1		674	NP_001191223.1		201	0.001
	NM_001204295.1		551	NP_001191224.1		160	0.001
	NM_001204296.1		620	NP_001191225.1		183	0.001
	NM_001204297.1		515	NP_001191226.1		148	0.001
	NM_002456.5		803	NP_002447.4		244	0.001

Appendix B : Summary of deleterious nsSNPs predicted by PROVEAN tool

SNP ID	Variation in Nucleotide			Variation in Amino Acid			Tolerance Value Cutoff
	Accession Number	Allele variation	Location of Allele Variation	Accession Number	Residue variation	Location of Residue Variation	
rs139437006	NM_001018016.2	C|A	134	NP_001018016.1	T|K	31	**-2.92**
	NM_001018017.2		137	NP_001018017.2		22	-2.78
	NM_001044391.2		137	NP_001037856.1		22	**-2.61**
	NM_001044392.2		164	NP_001037857.1		31	**-3.15**
	NM_001044393.2		137	NP_001037858.1		22	**-5.39**
	NM_001204287.1		164	NP_001191216.1		31	-2.93
	NM_001204288.1		164	NP_001191217.1		31	**-3.55**
	NM_001204289.1		164	NP_001191218.1		31	**-2.88**
	NM_001204291.1		164	NP_001191220.1		31	**-3.02**
	NM_001204292.1		164	NP_001191221.1		31	**-3.29**
	NM_001204294.1		137	NP_001191223.1		31	-2.92
	NM_001204296.1		164	NP_001191225.1		31	**-3.38**
	NM_001204294.1		164	NP_001191226.1		31	**-2.86**
	NM_001018016.2	C|T	134	NP_001018016.1	T|M	31	**-2.99**
	NM_001018017.2		137	NP_001018017.2		22	**-2.97**
	NM_001044391.2		137	NP_001037856.1		22	**-2.72**
	NM_001044392.2		164	NP_001037857.1		31	**-3.22**
	NM_001044393.2		137	NP_001037858.1		22	**-5.39**

	NM_001204286.1		164	NP_001191215.1		31	-2.66
	NM_001204287.1		164	NP_001191216.1		31	**-3.04**
	NM_001204288.1		164	NP_001191217.1		31	**-3.57**
	NM_001204289.1		164	NP_001191218.1		31	**-3.01**
	NM_001204291.1		164	NP_001191220.1		31	**-3.02**
	NM_001204292.1		164	NP_001191221.1		31	**-3.30**
	NM_001204294.1		137	NP_001191223.1		31	-2.91
	NM_001204296.1		164	NP_001191225.1		31	**-3.48**
	NM_001204294.1		164	NP_001191226.1		31	**-3.04**
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	NM_001018017.2		418	NP_001018017.1		116	-2.54
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	NM_001204286.1		1105	NP_001191215.1		345	-2.94
	NM_001204287.1		499	NP_001191216.1		143	-2.67
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	NM_001204289.1		367	NP_001191218.1		99	-2.70
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	NM_001204292.1		710	NP_001191221.1		213	**-8.01**
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	NM_001204291.1		707	NP_001191220.1		212	-4.45
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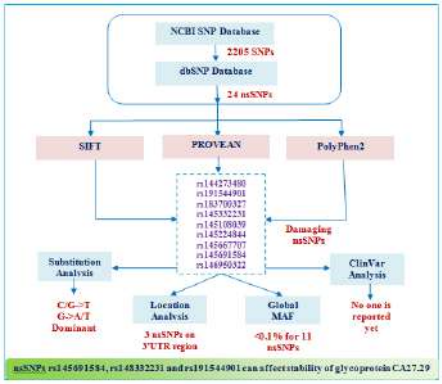

Fig.1.Graphical Abstract

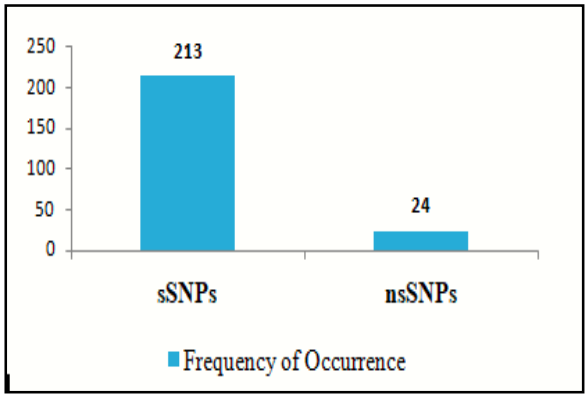

Fig.2. The frequency of occurrence of various sSNPs and nsSNPs associated with CA27.29 gene

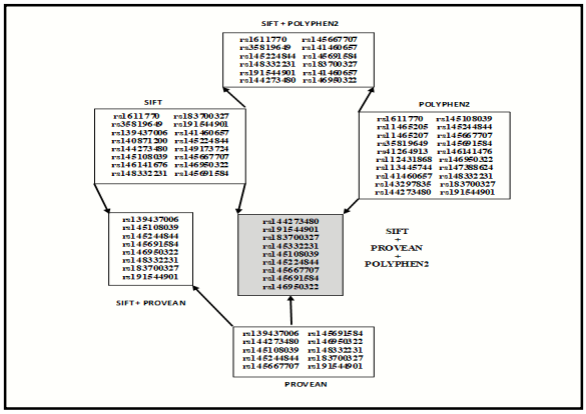

Fig.3. Details of overall nsSNPs predicted by SIFT, PROVEAN and PolyPhen-2 tools

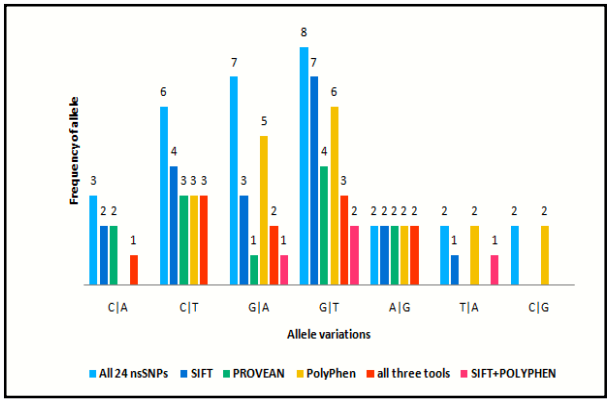

Fig.4. Frequency distribution of allelic variations in deleterious nsSNPs

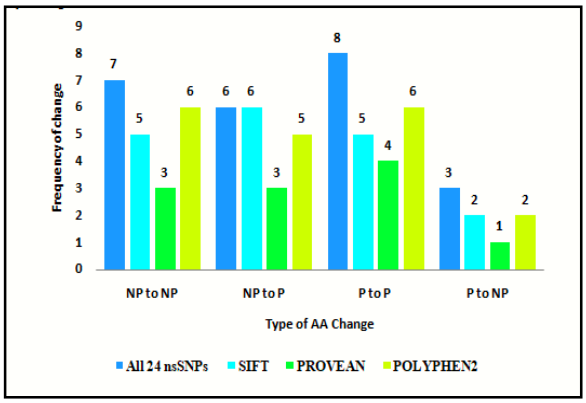

Fig.5. Frequency distribution of amino acid to amino acid (NP= Non-Polar; P= Polar) variations in deleterious nsSNPs

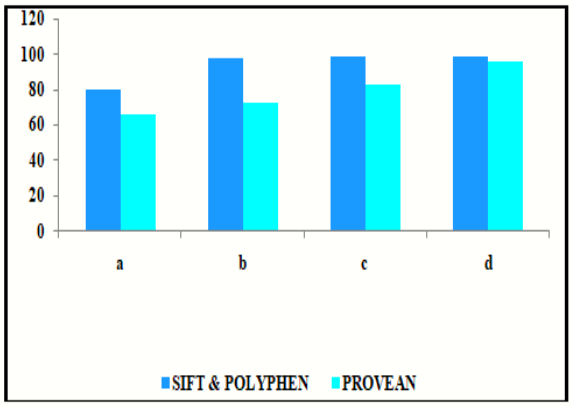

Fig.5 Benchmarking of predictions made by SIFT, PolyPhen2 and PROVEAN tools using clustering methods a. K_Means b. Make_Density_Based_Clustrer c. Farthest_First d. ACERAM

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Shelf-Life Study of Fish Silage Prepared from Freshwater Fish Waste

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Received: 30 Dec 2021 Revised: 14 Jan 2022 Accepted: 24 Feb 2022

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ABSTRACT

The present study was undertaken to investigate the suitability for the transformation of fish market waste into silage by using two different solvents; Inorganic (98% sulphuric acid) organic (98% formic acid). Indian aquaculture, which is dominated by carps, is extremely promising and has increased by more than six and a half times in the last two decades, with freshwater aquaculture accounting for more than 95% of total aquaculture production. Only 25-50% of the raw material is used for human consumption, according to estimates. The remaining 50-75% of raw material is processing waste and can be used to make low-valued goods. Fish silage was prepared using 3.5% sulphuric acid and formic acid with added Butylated Hydroxy Tolune (BHT). The storage is always a problem in silage production. The study was conducted for 60days at room temperature. The biochemical parameters (pH, TVB-N, TBARS, AAN) gradually increase during the course of storage but does not exceed the acceptable level. The present study shows that the quality of the fish silage is safe and does not deteriorate during the 60days and utilization of these fish wastes provides more revenue to fish and related sectors.

Keywords: Fish market waste, Fish silage, Biochemical changes

INTRODUCTION

Aquaculture is a fast growing agribusiness in India. Fishes are good sources of protein; the meat is rich in essential vitamins, minerals and omega-3-fatty acids. Besides its nutritious value it also plays an important part in raising the economy and standards of living (Huisman *et al*., 1989). Aquaculture accounts for about 50% of the world's total fish

production (Jayasankar, 2018). India is the 2nd largest producer of fish and the trend is increasing. It accounts for about 5.6% of world's fish production. Fishery accounts for 5% of the total agricultural GDP of India. Majority of fishery in India is from Inland Fisheries predominantly from aquaculture of which freshwater aquaculture accounts for about 95%. Indian aquaculture sector shows a growth rate of 7% (Jayasankar, 2018). Carp is the main culture in Indian freshwater aquaculture, some catfishes and freshwater prawns.

Feed is the key input and fish meal is the main ingredient as a source of valuable animal protein in fish diets (Rangacharyulu *et al*., 2003). Feed cost amounts to about 60% of the production cost. Fish meal is expensive and there is scarcity in supply due to overexploitation. The replacement of fish meal with alternative proteins or alternative processing methods has been developed but the adverse effect has been related to deficiencies of certain essential amino acids, particularly methionine and lysine. To combat this, fish nutritionists have supplemented the diet with crystalline amino acids to improve fish growth and health. On the contrary, animal proteins had an adequate concentration of these amino acids which are essential for normal growth. The advantage of animal protein is the low concentrations of anti-nutritional factors that might reduce the digestibility and assimilation of nutrients, as is the case when fish are fed plant proteins (Abdel-Fattah and El-Sayed, 1999). Thus, studies on the use of other efficient and cheaper sources of protein as substitutes for fish meal are necessary for aquaculture development.

Alternative resources such as meat and bone meal, hydrolyzed feather meal, fleshing-meal and blood meal, dried fish and chicken viscera (Paul *et al*., 1997; Millamena, 2002; Giri*et al.,* 2000), poultry silage, crayfish meal and shrimp meal (Middleton *et al*., 2001; Agouz and Tonsy, 2003; Al-Azab, 2005) have been used to replace fish meal either partially or fully. But these alternative sources are not sufficient to meet the growing demands of fish raising industry. Fish wastes can be processed into fish feed by fermentation with lactic acid bacteria. Fish waste account for about 30% after the processing which comprises of gills, fins, scales, visceral bones, etc.

Fish silage is defined as a liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic acid-producing bacteria are added (FAO, 2007). Fish silage is the liquefied product rich in protein and free amino acids (Martin, 1996). The liquefaction of fish mass carried out by enzymes already present in fish (Tatterson and Windsor, 1974). This is obtain by action of the naturally occurring enzymes presence in the whole fish, fish minced or fish offal. The enzymes, mainly from the digestive organ, break down protein into smaller soluble unit and the acid helps to speed up their enzyme activity while preventing bacterial spoilage (Al-Abri *et al.*, 2014). This procedure is safe, economically advantageous and environment friendly. The pH value of the fish pastes decreases below 4.5 during ensilage and this pH decrease in partly responsible for preservation (Maria *et al*., 2000). Fish silage is generally a product of high biological value presenting practically the same composition as the original raw material (Wassef, 1990, Fagbenro and Jauncey, 1994 and Vidotti and Carneiro, 2002). In developing countries like India, fish silage is cheaper to produce, involves simple artisanal technology and possesses good storage properties. It represents an alternative to fish meal in utilizing waste/trash fish (accounted for about 5% of annual farm production). The present study is aim to explore and produce high quality fish silage.

MATERIALS AND METHODS

Freshwater fish processing wastes (viscera, heads, scales, fins, skin and bones) were collected from local fish market. The fish wastes are mainly fromtilapia, catla, rohu, mirgal, amur carp, pangasius. The collected fish wastes are washed and stored at -20°C. For preparation of raw material for fish silage, the frozen wastes were thawed and grinded into paste using a mixer grinder.

Production of fish silage using organic acids and inorganic acids

Minced fish waste of 500g each was poured in a six glass container. 3.5% of 98% formic acids and sulphuric acids (Palkar et al., 2018; Mousavi *et al*., 2013) were added into three containers each as a triplicate and65mg of Butylated

Hydroxyl toluene (BHT). The mixture was kept in room temperature and stirred regularly using sterile glass rod and kept 60 days for fermentation. The change in pH was recorded regularly during this period.

Biochemical analysis

The proximate composition and pH were measured according to AOAC 2005 official methods. (TVB-N) Total volatile base nitrogen was determined by the Conway microdiffusion method (Conway, 1950). AAN measured according to(Pope and Stevens, 1939), Oxidation stability of the sample was assessed by measuring Thiobarbituricacid (TBA) value (Tarladgi*et al*., 1960).

Statistical analysis

The data was analysed using MS-Excel 2010's analysis of variances (ANOVA) tools to see if there was a significant difference. Duncan's multiple range tests (for Post hoc analysis) were used to compare the averages of the parameters evaluated for quality evaluation (p0.05) using statistical analyses (SPSS, version 16.0 for windows).

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of the fish waste is presented in Table 1. The fish waste contained74.82±0.09 % moisture, 16.02±0.14% protein, 4.37±0.22% of lipid and 3.86±0.07% of ash. Similar findings are recorded by (Palkar*etal*., 2017) from fish waste, where contained moisture 77.09 ± 0.14 %, crude protein 15.20 ± 0.15 %, fat 4.03 ± 0.07 % and ash 3.30 ± 0.11 %. (Hossain and Alam, 2015) found protein of 14.01±0.68%, lipid of 20.00±1.04%, moisture of 60.62±2.15% and 4.75±0.64% ash from fish viscera. The protein value was in consistent as obtained by (Bechtel, 2003) in fish viscera of 13.0-15.3% protein. Another study conducted by (Tanuja *et al*., 2014), a protein % of 37.7±0.42% on dry weight basis.

pH of the silage during storage period

The changes in pH during the storage period for sulphuric acid and formic acid treated samples are presented in Figure 1. Maintaining of the acidic pH of the fish silage during the storage period is important to prevent the growth of pathogenic organism and maintain the hygiene of the samples. In the present study, the sulphuric acid treated sample there is a decrease in pH (1.8) at the 3rd day of storage and slowly increases and a constant reading of pH (2.5) was observed from the 36th day till the 60th day of storage. This result is in consistence with (Mousavi *et al*., 2013), observed a stable pH of the sample at 2.58. A stable pH of 2.66 after the 30th day of storage was observed by (Palkar*et al.,* 2017). The time taken for obtaining a stable pH in the present study have taken more time as compare to studies reported by (Palkar*etal*., 2017: Mousavi *et al*., 2013). This difference could be due to the use of low-fat fish waste mostly from carps, the difference in acids use and concentration and the storage temperature. The formic treated sample, the pH increases slightly during the storage but stable below 4.2 till the 60th day of storage. This is due to the acid impact being neutralised by chemical compounds and reactions with fish waste. Due to rapid chemical changes and accumulation of pH-lowering impact, pH reduction or stability was occasionally seen in short time intervals. (Mousavi *et al*., 2013) reported that on day 56, the pH of the sample fixed at 3.88 when 90% formic acid with a weight percentage of 3.5 was employed. This can be ascribed to differences in chemical composition of raw materials, bacterial load, ambient conditions, and the kind and concentration of utilised acid.

TVB-N of the silage during storage period

The changed in the TBV-N value during the storage period of fish silage treated with sulphuric acid and formic acid is plotted in Figure 2. The acceptable limit of TVB-N for fresh fish is 35-40 mgN100g^{-1}. It is used as a criterion to measure the freshness of raw materials. In the present study, the TVB-N in both the treatment is below the acceptable level during the 60 days of study, though an increasing trend was observed during the 60 days study. The highest TVB-N content recorded till the end of the 60days study was 47.45 7 mgN100g^{-1} for formic acid and 26.21mg N100g^{-1} for sulphuric acid treated sample compared to 17mgN100g^{-1} and 16mgN100g^{-1} respectively. These findings were in consistent with that reported by (Tanuja *et al*., 2014) of below TVB-N value less than 20 mg N100g^{-1} using freshwater

fish waste. But the amount were much lesser as compared to those findings reported by (Kuhlmann *et al*,, 2011)level of more than 150mg% in fishmeal, (Haaland and Njaa, 1989)112 mg/100g in ensilage using 1.4% formic acid. Ali and Sahu (2002) has reported 79.8mg % for acid silage using marine fishes. (Ahmed and Mahendrarkar, 1996) using a carp visceral for silage production had reported 9mg % TBV-N. TVB-N consists of ammonia and trimethylamine, where the majority is contributed by trimethyl amine which is absent or found in very limited quantity in freshwater fish. This must be the reason for the low quantity of TVB-N in silage prepared from freshwater fish species waste.9% of total nitrogen as TVBN was reported by (Ahmed and Mahendrakar, 1996)in fish viscera ensilage. A similar trend was reported by (Nilsson and Rydin, 1963; Zuberi *et al*., 1993; Xavier *et al*., 2016).

TBARS of the silage during storage period

The change in TBARS value during the storage period plotted in Figure 3.TBA is use to measures malanaldehyde formed by the sample during oxidative rancidity. The final TBARS for sulphuric and formic acid treated sample for 60 days are 2.02 mg malonaldehyde/kg and 2.5 mg malonaldehyde/kg respectively. TBARS value increased steadily during the storage period. These reading are in consistent with the findings made by Tanuja *et al*., 2014. The addition of antioxidants in the silage helps in the slowing down of lipids oxidation(Ahmed and Mahendrarkar, 1996).Sajib*et al*., (2020) reported continuous opening of the storage can for stirring increased the TBARS value, therefore, that limiting the supply of oxygen is required if targeting a high-quality silage production. A four weeks study o carp visceral silage, the TBA value reached 1mg malonaldehyde/ kg oil as reported by (Bhaskar and Mahendrarkar, 2007).

Alpha Amino Nitrogen

Alpha Amino Nitrogen is used to measure the protein digestion which is determined by the production and liquefaction of NPN and NH_3 compounds. The AANcompounds of both the treatment are plotted in Figure 4. There was a steady increase in the AAN content of the fish silage in both the treatment during the 60 days storage. The AAN had increased from 10.04 mg-N100^{-1}g to 44.2 mg-N100 $^{-1}$g in sulphuric acid treated sample and 14.2 mg-N100 $^{-1}$g to 51mg-N100 $^{-1}$g for formic acid treated sample respectively. The rate of liquefaction is different when mineral and organic acids are used for silage production. Proteolysis is inhibited when highacids is used which lowers the pH. The rate of autolysis and yield of soluble materials were lower at pH 3 (Raa and Gildberg, 1982).Stone and Hardy (1986) reported there is no increase in the level of amino nitrogen after 42 days storage of pacific whiting silage using sulphuric acids at 2.45%, which indicates the absence of autolysis. Similar trends were also observed by (Palkar*etal*., 2017) that results in 47.71 mg-N100 $^{-1}$g in sulphuric acid treated sample and 52.15 mg-N100 $^{-1}$g in formic acid treated sample after 30days of storage.Endogenous enzymes in the fish viscera operate on the peptide bonds of protein structure during ensilaging. Proteinases catalyse the hydrolysis of peptide bonds, which is a frequent process in nature. Proteinases are multifunctional enzymes that catalyse the hydrolytic breakdown of proteins in aquatic animals. They are mostly produced by the digestive glands (Garcia-Carreno and Hernandez-Cortes, 2000). This will aid in activating the action of the acid or aspartyl proteinases group of endo peptidases (Whitaker, 1994). In the digestive organs of fish, pepsin, gastricsin, trypsin, chymotrypsin, collagenase, elastase, carboxy-peptidase, and carboxyl esterase were discovered (Haard, 1994; Simpson, 2000).

CONCLUSION

Freshwater fish processing generates a large amount of processing waste, the most common of which is visceral waste. Acid ensilation might be a potential option for converting these wastes into valuable byproducts. Only little changes in the dry matter, protein, lipid, and mineral fractions occurred throughout the acid ensiling process, demonstrating the methodology' applicability. In the production of high-quality, nutrient-rich powder fish silage, fish viscera might be a good replacement for expensive fishmeal. In the preparation of fish and animal feed, it will be feasible to partially substitute expensive fish meal. Fish and other animals' growth performance in farm culture conditions should be tested with fish silage. It was necessary to conduct research on the most acceptable packaging for such a product for local marketing. The addition of BHT to acid silages made from carp fish viscera delayed the process of auto oxidation, and the low pH inhibited microbe multiplication. The present study shows that the silage

quality is good and does not deteriorate during the 60days study period and could also be done on a small scale. More income and job possibilities may be produced from the fisheries and allied industries by properly using these wastes.

ACKNOWLEDGEMENT

The authors are grateful to Centurion University of Technology and Management, Odisha for granting permission to carry out the work and providing the necessary facilities.

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Table 1. Proximate composition of fish waste. Values expressed as mean ± SD (n = 3)

	Moisture %	Protein %	Lipid %	Ash %
Fish waste	74.82±0.09	16.02±0.14	4.37±0.22	3.86±0.07

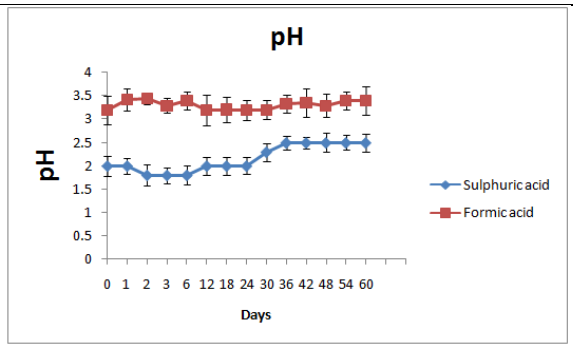

Figure1. Changes in pH of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3)

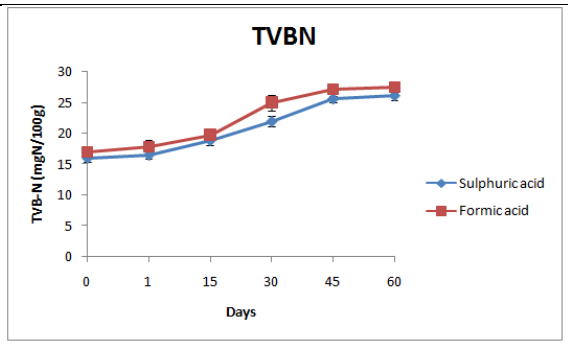

Figure 2. Changes in TVBN-N content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3)

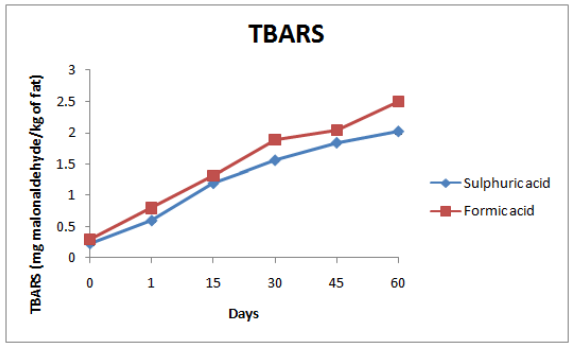

Figure 3. Changes in TBARS content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3)

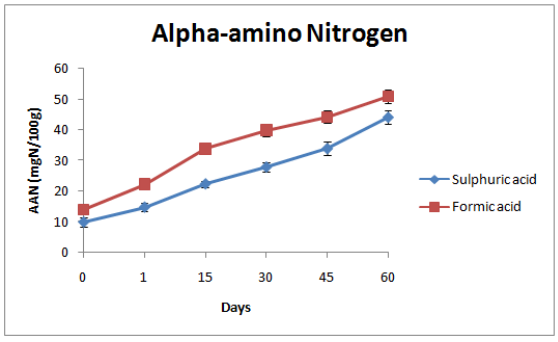

Figure 4. Changes in AAN content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Regional Variant Analysis of Spike Glycoprotein Mutations of SARS-CoV-2 and Its Implications in COVID-19 Pandemic Control

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Received: 05 Jan 2022 Revised: 06 Feb 2022 Accepted: 07 Mar 2022

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ABSTRACT

Mutations in the spike glycoprotein have various impacts on the receptor binding, antibody interaction, and host range of SARS-CoV-2. As the interaction of spike glycoprotein with the human ACE2 receptor is the entry point of SARS-CoV-2 in human cells, mutations in the spike protein itself contain numerous impacts on the pandemic. Here, we analysed all the mutations in the spike glycoprotein from123 strains isolated from Kerala, India. We also predicted the possible structural relevance of the unique mutations based on topological analysis of the residue interaction network of the spike glycoprotein structure.

Keywords: SARS-CoV-2; COVID-19; Next generation sequence analysis; Virus-Host interaction; Spike glycoprotein; Mutation; Network analysis

INTRODUCTION

The COVID-19 or coronavirus disease-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an ongoing worldwide pandemic [1]. Although the original source of this virus to humans is still unknown, it first appeared in Wuhan, Hubei, China, in late 2019 [2]. In India, the first case of COVID-19 was reported in Thrissur, Kasargode, and Alappuzha, in Kerala, from where three medical students returned from

Wuhan, China [3]. On 23 March 2020, the 1st lockdown was announced in Kerala. On 12 May 2021, Kerala was announced as the largest single-day state with 43529 new cases. On 24 June 2021, 2854325 are confirmed cases, a positive test case rate of 10.37%, and 2741436 recoveries and 12581 deaths in Kerala [4]. As of May 2021, Kerala has the 2nd highest number of confirmed cases of covid 19 after Maharashtra in India. In May 2021, more than 90% of known cases were reported due to community spread. The most affected districts are Ernakulam (12.2%), Malappuram (11.5%), and Kozhikode (10.6%) [5,6]. The RNA genome of coronavirus is covered by a helical capsid made up of N (nucleocapsid) proteins [7]. Due to its enveloped nature, the nucleocapsid is surrounded by M (membrane), E (envelope), and S (spike) protein [8]. In this viral structure, spike protein is especially important and draws research attention as the viral entry inside the host cell is mediated by this protein. Spike protein, which is a class 1 membrane fusion protein, has a transmembrane domain which helps to anchor the envelope [9]. The ectodomain radiates out from the viral structure which is solely responsible for receptor binding (via the S1 subunit) and membrane fusion (via the S2 subunit). S protein helps to fuse the viral and host membrane by changing its conformation. SARS-CoV interacts with the host through a hinge-like movement of the S1 subunit in the prefusion state which helps the S2 subunit into a dumbbell-shaped stable post-fusion conformation. This S2 subunit with its 6 helices brings the host and viral membrane together. S1 subunit has the receptor-binding domain for human ACE2 receptors as reported by various studies. Various mutations along the spike protein affect not only the receptor affinity but also the host range [10].

In the present study, we identified a total of 298 mutations in the spike protein from 123 SARS-CoV-2 isolates of Kerala, India. For the known mutations, we have reviewed their consequences in the SARS-CoV-2 structure, host range, and antibody binding capacity. But, for unknown mutations, which are unique to Kerala, we have tried to predict the mutational consequences using the residue interaction network. Here, the residue interaction network represents a complex network representation of protein structure where amino acids are nodes. We calculated network matrices and based on topological significance we predicted the possible effect of the mutations on the protein structure.

MATERIALS AND METHODS

Identification of spike mutations

The study was based on a total number of 123 SARS-CoV-2 full genomes of Kerala origin fetched from the GISAID database [11]. Multiple sequence alignment was performed with the reference sequence of hCoV-19/Wuhan/WIV04/2019, which has been isolated from Wuhan of China and has been considered the reference strain worldwide [1]. With the help of the CoV surver mutation analysis tool of SARS-CoV-2, we have identified all the mutations on the spike protein of SARS-CoV-2 isolates from Kerala, India [12].

Residue interaction network (RIN) construction

For the construction of a residue interaction network (Brinda et al. 2005) the respective PDB file of the protein is required [13]. Residue interaction network depends on the three-dimensional coordinates of each atom of amino acid. Here, we have mainly focused on the C-alpha network which is based on the C-alpha atom of amino acid. In this network each of the C-alpha atoms of each amino acid is considered as the node representing the amino acid in the network. An edge will be considered between two amino acids if the cutoff distance between the two respective C-alpha atoms is >= 7Å. For the construction of the network, we have used NAPS web-server [14].

Network metric analysis

We calculated the basic network metric for each mutated node of the network. Each of the network metrics has its biological significance as reported in various literature [18]. In a graph, a node's degree represents the number of connections the node has with other nodes in the network. Closeness centrality is defined by the reciprocal of the shortest path distance of that node to every other node in the graph. So, the closeness centrality of a node i in a network is represented by the following formula,

$C_i=N-1 / \sum(Dij)$

Where N is the total number of nodes in the network. Dij represents the shortest path distance between i and j in the network. Now the closeness centrality of a node in a biological network could reveal various importance.
Similarly, the betweenness centrality of a node represents the number of shortest paths passing through that node. Betweenness centrality of node v is represented as,

$Bv= \sum(x(ij)v) / x(ij)$

Where, x(ij) is the number of the shortest paths from node i to j in the network and x(ij)v is the number of the shortest paths from node i to j passing through v. Also, to compare a node's topological metric in respect to the same of other nodes, we have calculated the Z score values. The Z score of a measure of a node is defined as,

$Z= (x-\mu) / \sigma$

where x is the value of the measure of the node. μ and σ represent the mean and standard deviation of the same measure of the whole population. We have considered only Z value >=1.

RESULTS

We identified a total number of 298 spike mutations in various SARS-CoV-2 variants isolated from Kerala, India. In figure 1 we have included a pie chart of the various spike mutations with their abundance frequency.

RIN network analysis

To predict the effect of unique mutations on the protein structure, we have used topological analysis of the residue interaction network analysis based on node metrics. We have used spike glycoprotein of SARS-CoV (PDB id: 6ACC) for our protein residue interaction network construction [15]. We measured the change in node betweenness centrality metrics of every amino acid as the effect of the deletion of the mutated amino acids from the RIN. In figure 2, we represent the network view (a) of the spike glycoprotein with comparison to its three-dimensional helical structure (b).

It was observed that the mutation D138Y could be very crucial as the position has a very high betweenness value. It indicates that the amino acid of position 138 is very important as many shortest paths pass through this point. Mutation at this point changes the interaction which alters the overall structure of the protein. Residues with high betweenness indicate their importance in ligand binding [16]. This position also has a very high closeness value which indicates that mutation in this position also could affect various ligand or receptor binding as indicated in the previous literature [17]. Other than that mutation Y144del and D614G are also important based on their closeness values. Mutation in S982A and H69del has a very high degree which indicates that the amino acids in these two positions interact with many other amino acids in the protein structure. So, the mutation in these two positions also should have biological significance.

DISCUSSION

In this paper, we have summarised all the 298-point mutations in the spike glycoprotein which are isolated from Kerala, India [7,8]. Among them, we identified the top 14 mutations based on their abundance in the SARS-CoV-2 variants of Kerala. Further, we summarised the abundance frequency of these mutations in Kerala. To explore their biological significance, we used residue interaction network analysis. Based on some well-known topological measures we predicted the significant mutations among these 14 unique mutations. Our network analysis result is based on node degree, closeness, and betweenness centrality. In a previous study, a residue interaction network

study found that more than 1/3 of biologically essential residues have been identified to possess high closeness values [15]. Not only that, residues in the active sites and that are associated with ligand binding activity, also possess high closeness values. Betweenness centrality also indicates the ligand-binding sites as reported previously [5]. So, our prediction of the potential biological significance of these unique spike mutations needs to be studied further with experimental verifications. We believe this research will help to understand the SARS-CoV-2 spike mutations and would help researchers in the development of alternative vaccines.

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Table 1 The node centrality values for all the mutated amino acids which are unique to Kerala

Node	Abundance	Degree	Z_D	Closeness	Z_C	Betweenness	Z_B
D614G	69	8	0.25	0.062434	1.11	0.058986	0.21

N501Y	27	9	0.7	0.050503	-0.24	0.001544	-0.33
T716I	25	6	-0.65	0.050681	-0.22	0.003744	-0.31
S982A	25	11	1.59	0.047371	-0.59	0.008894	-0.26
P681H	25	6	-0.65	0.045263	-0.83	0.001228	-0.33
D1118H	25	7	-0.2	0.045317	-0.82	0.000634	-0.33
A570D	24	6	-0.65	0.055296	0.3	0.017623	-0.18
Y144del	7	4	-1.54	0.064724	1.36	0.005417	-0.29
D138Y	7	4	-1.54	0.066475	1.56	0.99097	8.89
V70del	6	6	-0.65	0.049148	-0.39	0.001561	-0.33
H69del	6	10	1.15	0.054494	0.21	0.063394	0.25
T20I	4	9	0.7	0.055047	0.28	0.001147	-0.33
E484K	4	9	0.7	0.058918	0.71	0.019155	-0.16
Y145H		7	-0.2	0.054106	0.17	0.002706	-0.32

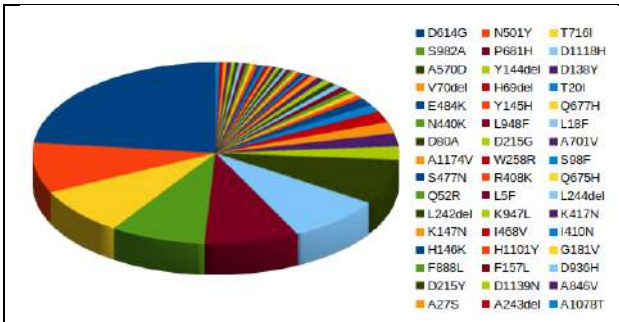

Figure 1. Frequency of 298 spike mutations in various SARS-CoV-2 variants isolated from Kerala, India

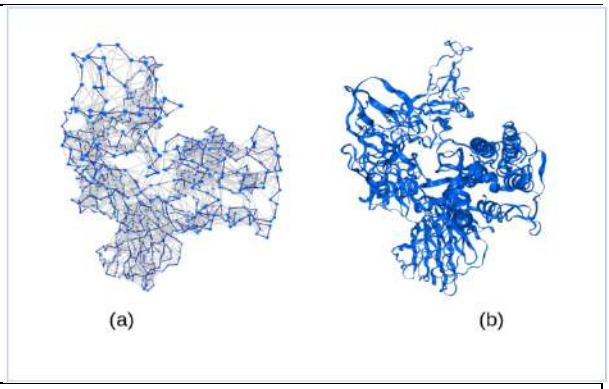

Fig 2. Network representation of spike glycoprotein in comparison to its three-dimensional helical structure

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Anti Product of (λ, μ) – Multi Anti Fuzzy Subgroup of A Group

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Received: 14 Jan 2022 Revised: 22 Feb 2022 Accepted: 31 Mar 2022

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ABSTRACT

In this paper, the product of (λ, μ)-multi anti fuzzy set, (λ, μ)-multi anti fuzzy subgroup of a group are defined and discussed some of their properties.

Keywords: Multi anti fuzzy set (MAFS), Multi anti fuzzy subgroup (MAFSG) , (λ, μ)- multi anti fuzzy subgroup ((λ, μ)- MAFSG).

INTRODUCTION

In 1965, L.A. Zadeh[16] introduced the notion of fuzzy set of any non empty set. Yuan et al. [14] introduced the concept of generalized fuzzy groups with thresholds. A fuzzy subgroup with thresholds λ and μ is also called a (λ, μ)-fuzzy subgroup. However, there is much more research on (λ, μ)-fuzzy subgroups if we consider rich results both in the classic group theory and the fuzzy group theory in the sense of Rosenfeld[10]. Shen researched anti-fuzzy subgroups in by a fuzzy subset of a non-empty set X we mean a mapping from X to the unit interval [0,1]. Throughout this article, we will always assume that $0 \leq \lambda < \mu \leq 1$. S. Sabu and T.V. Ramakrishnan[11] proposed the theory of multi fuzzy sets in terms of multi dimensional membership functions. The notion of (λ, μ)-multi fuzzy subgroup was introduced by the author[3]. KR. Balasubramanian ed al[4]. Muthuraj. R and S. Balamurugan [8] introduced the concept of Multi Anti fuzzy group and its Lower level subgroup. In this paper, we define product of (λ, μ)-multi anti fuzzy subgroups of a group and study some of their related properties.

PRELIMINARIES

Definition 2.1

Let X be a non-empty set. A fuzzy subset A of X is defined by a function A:X→[0,1].

Definition 2.2

Let X be a non-empty set. A multi fuzzy set A in X is defined by the set
$A = \{(x, (A_1(x), A_2(x), \dots, A_k(x), \dots)), : x \in X\}$.Where $A_i : X \longrightarrow [0,1]$ for all i.

Definition 2.3

Let X be a non-empty set. A k-dimensional multi fuzzy set A in X is defined by the set

$$A = \{(x, (A_1(x), A_2(x), \dots, A_k(x))), : x \in X\}.$$

Where $A_i : X \longrightarrow [0,1]$ for i = 1,2,3,. . , k.

Definition 2.4

Let X be a non-empty set. A k-dimensional multi fuzzy set A in X is defined by the set $A = \{(x, (A_1(x), A_2(x), \dots A_k(x))), : x \in X\}$, Where $A_i : X \longrightarrow [0,1]$ for i = 1,2,3, … , k.

Definition 2.5

For any three MFS's A, B and C , then we have

1. Commutative Law : $A \cap B = B \cap A$ and $A \cup B = B \cup A$
2. Idempotent Law : $A \cap A = A$ and $A \cup A = A$
3. De Morgan's Law :$\neg(A \cup B) = (\neg A \cap \neg B)$and $(\neg A \cap B) = (\neg A \cup \neg B)$
4. Associative Law : $A \cup (B \cup C) = (A \cup B) \cup C$ and $A \cap (B \cap C) = (A \cap B) \cap C$
5. Distributive Law : $A \cup (B \cap C) = (A \cup B) \cap (A \cup C)$ and
   $A \cap (B \cap C) = (A \cap B) \cup (A \cap C)$

Definition 2.6

Let A be a fuzzy subset of G. Then a (λ, μ)- fuzzy subset $A^{(\lambda,\mu)}$ of a fuzzy set A of G is defined as $A^{(\lambda,\mu)} = (x, A \vee \lambda \wedge \mu : x \in G)$.

Definition 2.7

Let A be a multi fuzzy subset of G. Then a (λ, μ)- multi fuzzy subset $A^{(\lambda,\mu)}$ of a fuzzy set A of G is defined as $A^{(\lambda,\mu)} = (x, A \vee \lambda \wedge \mu : x \in G)$.That is, $A_i^{(\lambda_i,\mu_i)} = (x, A_i \vee \lambda_i \wedge \mu_i : x \in G)$

Clearly, a (0, 1)-multi fuzzy subset is just a multi fuzzy subset of G, and thus a (λ, μ)- multi fuzzy subgroup is a generalization of fuzzy subgroup. Where (0, 1)-multi fuzzy subset A is defined as $A^{(0,1)} = \left(A_i^{(0_i,1_i)}\right)$

Definition: 2.8

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be any two (λ, μ)- multi fuzzy sets having the same dimension k of X. Then

(i) $A^{(\lambda,\mu)} \subseteq B^{(\lambda,\mu)}$, iff $A^{(\lambda,\mu)}(x) \leq B^{(\lambda,\mu)}(x)$ forall $x \in X$

(ii) $A^{(\lambda,\mu)} = B^{(\lambda,\mu)}$,iff $A^{(\lambda,\mu)}(x) = B^{(\lambda,\mu)}(x)$ forall $x \in X$

(iii) $^A^{(\lambda,\mu)} = \{(x, 1 - A^{(\lambda,\mu)}) : x \in X\}$

(iv) $A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)} = \{\left(x, \left(A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)}\right)(x) : x \epsilon X\right\}$,

where, $\left(A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)}\right)(x) = \min\{A^{(\lambda,\mu)}(x), B^{(\lambda,\mu)}(x)\} = \min\{A_i^{(\lambda_i,\mu_i)}(x), B_i^{(\lambda_i,\mu_i)}(x)\}$ for i = 1,2, … , k

(v) $A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)} = \{\left(x, A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)}(x)\right) : x \in X\}$,

where $\left(A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)}\right)(x) = \max\{A^{(\lambda,\mu)}(x), B^{(\lambda,\mu)}(x)\} = \max\{A_i^{(\lambda_i,\mu_i)}(x), B_i^{(\lambda_i,\mu_i)}(x)\}$ for i = 1,2, … , k

Here, $\{A_i^{(\lambda_i,\mu_i)}(x)\}$ and $\{B_i^{(\lambda_i,\mu_i)}(x)\}$ represents the corresponding i^{th} position membership values of $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$.

Definition 2.9

For any three MFS's $A^{(\lambda,\mu)}, B^{(\lambda,\mu)}$ and $C^{(\lambda,\mu)}$. We have,

1. Commutative Law : $A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)} = B^{(\lambda,\mu)} \cap A^{(\lambda,\mu)}$ and

$A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)} = B^{(\lambda,\mu)} \cup A^{(\lambda,\mu)}$

2. Idempotent Law: $A^{(\lambda,\mu)} \cap A^{(\lambda,\mu)} = A^{(\lambda,\mu)}$ and $A^{(\lambda,\mu)} \cup A^{(\lambda,\mu)} = A^{(\lambda,\mu)}$

3. De Morgan's Law: $\neg(A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)}) = \neg(A^{(\lambda,\mu)} \cap \neg B^{(\lambda,\mu)})$ and $\neg(A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)}) = \neg(A^{(\lambda,\mu)} \cup \neg B^{(\lambda,\mu)})$

4. Associative Law: $A^{(\lambda,\mu)} \cup (B^{(\lambda,\mu)} \cup C^{(\lambda,\mu)}) = (A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)}) \cup C^{(\lambda,\mu)}$ and $A^{(\lambda,\mu)} \cap (B^{(\lambda,\mu)} \cap C^{(\lambda,\mu)}) = (A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)}) \cap A^{(\lambda,\mu)}$

5. Distributive Law: $A^{(\lambda,\mu)} \cup (B^{(\lambda,\mu)} \cap C^{(\lambda,\mu)}) = (A^{(\lambda,\mu)} \cup B^{(\lambda,\mu)}) \cap (A^{(\lambda,\mu)} \cap C^{(\lambda,\mu)})$ and $A^{(\lambda,\mu)} \cap (B^{(\lambda,\mu)} \cup C^{(\lambda,\mu)}) = (A^{(\lambda,\mu)} \cap B^{(\lambda,\mu)}) \cup (A^{(\lambda,\mu)} \cap C^{(\lambda,\mu)})$

Definition 2.10

Let $A^{(\lambda,\mu)} = \{(x, A^{(\lambda,\mu)}(x)) : x \epsilon X\}$ be a (λ,μ) –MFS of dimension k and let $\alpha = (\alpha_1, \alpha_2, \dots, \alpha_k) \in [0,1]^k$, where each $\alpha_i \in [0,1]$ for all i. Then the α – cut of $A^{(\lambda,\mu)}$ is the set of all x such that $A_i^{(\lambda_i,\mu_i)}(x) \geq \alpha_i$, $\forall i$ and is denoted by $[A^{(\lambda,\mu)}]_{(\alpha)}$. Clearly it is a crisp set.

Definition 2.11

Let $A^{(\lambda,\mu)} = \{(x, A^{(\lambda,\mu)}(x)) : x \epsilon X\}$ be a (λ,μ) –MFS of dimension k and let $\alpha = (\alpha_1, \alpha_2, \dots, \alpha_k) \in [0,1]^k$, where each $\alpha_i \in [0,1]$ for all i. Then the strong α – cut of $A^{(\lambda,\mu)}$ is the set of all x such that $A_i^{(\lambda,\mu)}(x) > \alpha_i, \forall i$ and is denoted by $[A^{(\lambda,\mu)}]_{\alpha^*}$. Clearly it is also a crisp set.

Theorem 2.12

Let A and B are any two (λ,μ) –MFSs of dimension k taken from a non –empty set X. Then $A \subseteq B$ if and only if $[A^{(\lambda,\mu)}]_{(\alpha)} \subseteq [B^{(\lambda,\mu)}]_{(\alpha)}$ for every $\lambda,\mu \in [0,1]^k$.

Definition 2.13

A MFS A of a group G is said to be a (λ, μ)-multi fuzzy sub group of G ((λ, μ)-MFSG), if it satisfies the following: For $\lambda,\mu \in [0,1]^k, 0 \leq \lambda_i \leq \mu_i \leq 1, 0 \leq \lambda_i + \mu_i \leq 1$

(i) $A(xy) \vee \lambda \geq \min\{A(x), A(y)\} \wedge \mu$

(ii) $A(x^{-1}) \vee \lambda \geq A(x) \wedge \mu$ for all $x, y \in G$. That is,

(i) $A_i(xy) \vee \lambda_i \geq \min\{A_i(x), A_i(y)\} \wedge \mu_i$

(ii) $A_i(x^{-1}) \vee \lambda_i \geq A_i(x) \wedge \mu_i$ for all $x, y \in G$.

Clearly, a (0, 1)-multi fuzzy subgroup is just a multi fuzzy subgroup of G, and thus a (λ, μ)- multi fuzzy subgroup is a generalization of multi fuzzy subgroup.

An alternative definition for (λ, μ)-MFG is as follows:

Definition 2.14

A MFS A of a group G is said to be a (λ, μ)-multi-fuzzy sub group of G ((λ, μ)-MFSG), if it satisfies.

$A(xy^{-1}) \vee \lambda \geq \min\{A(x), A(y)\} \wedge \mu$ for all $x,y \in G$

Where, $A(xy^{-1}) \vee \lambda = (A_1(xy^{-1}) \vee \lambda_1, A_2(xy^{-1}) \vee \lambda_2, \dots, A_k(xy^{-1}) \vee \lambda_k)$ and $\min\{A(x), A(y)\} \wedge \mu = (\min\{A_1(x), A_1(y)\} \wedge \mu_1, \min\{A_2(x), A_2(y)\} \wedge \mu_2, \dots, \min\{A_k(x), A_k(y)\} \wedge \mu_k)$ for all x, y and xy^{-1} in G.

Definition 2.15

A (λ,μ) – MFSG $A^{(\lambda,\mu)}$ of a group G is said to be an (λ,μ) –multi fuzzy normal subgroup ((λ,μ) – MFNSG) of G, it satisfies $A^{(\lambda,\mu)}(xy) = A^{(\lambda,\mu)}(yx)$ for all $x, y \in G$.

MAIN RESULTS

Definition 3.1

Let $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \dots, A_n^{(\lambda_n,\mu_n)}$ be n (λ,μ) −multi anti fuzzy subgroups of n multi fuzzy subset $A_1, A_2, \dots, A_n$ of fuzzy order k of $G_1, G_2, \dots, G_n$ respectively. Then the anti product of $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \dots, A_n^{(\lambda_n,\mu_n)}$ is defined as, $(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \dots \times A_n^{(\lambda_n,\mu_n)})(x_1, x_2, \dots, x_n) = (A_1^{(\lambda_1,\mu_1)}(x_1) \vee A_2^{(\lambda_2,\mu_2)}(x_2) \vee \dots \vee A_n^{(\lambda_n,\mu_n)}(x_n)$

$= \begin{pmatrix} A_{11}^{(\lambda_1,\mu_1)}(x_1) \vee A_{21}^{(\lambda_1,\mu_1)}(x_2) \vee \dots \vee A_{n1}^{(\lambda_1,\mu_1)}(x_n), \\ \dots, (A_{1m}^{(\lambda_m,\mu_m)}(x_1) \vee A_{2m}^{(\lambda_m,\mu_m)}(x_2) \vee \dots \vee A_{nm}^{(\lambda_m,\mu_m)}(x_n) \end{pmatrix}$ for all $(x_1, x_2, x_3, \dots, x_n) \in G_1 \times G_2 \times \dots \times G_n$.

Definition 3.2

An anti product $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \dots \times A_n^{(\lambda_n,\mu_n)}$ of n (λ,μ) −multi anti fuzzy subgroups of the multi fuzzy subsets $A_1, A_2, \dots, A_n$ of the groups $G_1, G_2, \dots, G_n$ is said to be conjugate to an anti product $B_1^{(\lambda_1,\mu_1)} \times B_2^{(\lambda_2,\mu_2)} \times \dots \times B_n^{(\lambda_n,\mu_n)}$ of n (λ,μ) −multi anti fuzzy subgroups $B_1^{(\lambda_1,\mu_1)}, B_2^{(\lambda_2,\mu_2)}, \dots, B_n^{(\lambda_n,\mu_n)}$ of the multi anti fuzzy subsets $B_1, B_2, \dots, B_n$ of the groups $G_1, G_2, \dots, G_n$ if there exits x in $G_1 \times G_2 \times \dots \times G_n$ such that for all g in $G_1 \times G_2 \times \dots \times G_n$, $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \dots \times A_n^{(\lambda_n,\mu_n)}(g) = A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \dots \times A_n^{(\lambda_n,\mu_n)}(x^{-1}gx)$.

Theorem 3.3

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be two (λ,μ) −multi anti fuzzy subsets order m of the groups G and H respectively. Suppose that e and e′ are the unit elements of G and H respectively and if $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of $G \times H$, then at least one of the following statements must holds:

(i) $B^{(\lambda,\mu)}(e') \leq A^{(\lambda,\mu)}(x)$, ∀ x in G

(ii) $A^{(\lambda,\mu)}(e) \leq B^{(\lambda,\mu)}(y)$, ∀ y in H

Proof: Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be two (λ,μ) −multi anti fuzzy subsets of two multi fuzzy subsets A and B order m of the groups G and H respectively. By Contraposition, suppose that none of the above two statements (i) and (ii) holds. Then we can find the elements a in G and b in H such that,

$A^{(\lambda,\mu)}(a) < B^{(\lambda,\mu)}(e')$ and $B^{(\lambda,\mu)}(b) < A^{(\lambda,\mu)}(e)$

We have, $(A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(a,b) = A^{(\lambda,\mu)}(a) \vee B^{(\lambda,\mu)}(b)$

$\leq B^{(\lambda,\mu)}(e') \vee A^{(\lambda,\mu)}(e) = A^{(\lambda,\mu)}(e) \vee B^{(\lambda,\mu)}(e') = (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(e,e')$

Thus $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is not a (λ,μ) −multi anti fuzzy subgroup of $G \times H$. Hence we should have, either $A^{(\lambda,\mu)}(a) < A^{(\lambda,\mu)}(e')$ or $B^{(\lambda,\mu)}(b) < A^{(\lambda,\mu)}(e)$.

Theorem 3.4

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be (λ,μ) −multi anti fuzzy subset of multi fuzzy subsets A and B of the groups G and H respectively such that $A^{(\lambda,\mu)}(x) \geq A^{(\lambda,\mu)}(e')$, ∀ x in G, e′ being unit element of H. If $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of $G \times H$, then $A^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G.

Proof: If $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) − multi anti fuzzy subgroup of $G \times H$ and $x, y \in G$ then $(x,e'), (y,e') \in G \times H$.

Now, using the given condition $A^{(\lambda,\mu)}(x) \geq B^{(\lambda,\mu)}(e')$, ∀ x in G we get, $A^{(\lambda,\mu)}(xy) = A^{(\lambda,\mu)}(xy) \vee B^{(\lambda,\mu)}(e'e') = (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(x,e')(y,e')$

$\leq (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(x,e') \vee (y,e') = A^{(\lambda,\mu)}(x) \vee B^{(\lambda,\mu)}(e') \vee A^{(\lambda,\mu)}(y) \vee B^{(\lambda,\mu)}(e')$

$= A^{(\lambda,\mu)}(x) \vee A^{(\lambda,\mu)}(y)$.

Also, $A^{(\lambda,\mu)}(x^{-1}) = A^{(\lambda,\mu)}(x^{-1}) \vee B^{(\lambda,\mu)}(e'^{-1}) = (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(x^{-1}, e'^{-1})$

$(A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(x,e')^{-1} = (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(x,e') = A^{(\lambda,\mu)}(x) \vee B^{(\lambda,\mu)}(e') = A^{(\lambda,\mu)}(x)$. Hence, $A^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G.

Theorem 3.5

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be (λ,μ) −multi anti fuzzy subsets with respect to the multi fuzzy subsets A and B of the groups G and H respectively and $B^{(\lambda,\mu)}(x) \geq A^{(\lambda,\mu)}(e)$, $\forall\, x \in H, e$ being unit elements of G. Suppose $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G × H, then $B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of H.

Proof: Suppose that $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G × H, and $x, y \in G$. Then $(x,e),(y,e) \in G \times H$. Now, using the property $A^{(\lambda,\mu)}(e) \leq B^{(\lambda,\mu)}(y)$, $\forall$ y in H. We get,

$B^{(\lambda,\mu)}(xy) = B^{(\lambda,\mu)}(xy) \vee A^{(\lambda,\mu)}(ee) = \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(x,e)(y,e)$

$\leq \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(x,e) \vee \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(y,e)$

$= B^{(\lambda,\mu)}(x) \vee A^{(\lambda,\mu)}(e) \vee B^{(\lambda,\mu)}(y) \vee A^{(\lambda,\mu)}(e) = A^{(\lambda,\mu)}(x) \vee B^{(\lambda,\mu)}(y)$

Also,

$B^{(\lambda,\mu)}(y^{-1}) = B^{(\lambda,\mu)}(y^{-1}) \vee A^{(\lambda,\mu)}(e^{-1})$

$= \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(y^{-1}, e^{-1}) = \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(y,e)^{-1} = \left(B^{(\lambda,\mu)} \times A^{(\lambda,\mu)}\right)(y,e)$

$= B^{(\lambda,\mu)}(y) \vee A^{(\lambda,\mu)}(e) = B^{(\lambda,\mu)}(y)$. Hence $B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of H.

Corollary 3.6

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be (λ,μ) −multi anti fuzzy subsets of the multi fuzzy subsets A and B of the groups G and H respectively. If $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G × H, then either $A^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G or $B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of H.

Remark 3.7

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be (λ,μ) - multi anti fuzzy subsets of the groups G and H respectively. If $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) −multi anti fuzzy subgroup of G × H, then it is not necessary that both $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ should be (λ,μ) −multi anti fuzzy subgroups of G and H respectively.

Example 3.8

Let G = {e, a}, where $a^2 = e$, let H be the Klein four – group and

H = $\{e', x, y, xy\}$, Where $x^2 = e' = y^2$ and xy = yx.

Then G × H = $\{(e,e'),(e,x),(e,y),(e,xy),(a,e'),(a,x),(a,y),(a,xy)\}$

Let A = $\{< e,(0.4,0.3,0.6) >< a,(0.5,0.6,0.3) >\}$

B = $\{< e',(0.2,0.1,0.1) >, < x,(0.9,0.8,0.6) >, < y,(0.4,0.5,0.6) >, < xy,(0.5,0.4,0.6) >\}$.

Then, A × B = $\begin{Bmatrix} (e,e'), & (0.4,0.3,0.6) \\ (e,x), & (0.9,0.8,0.6) \\ (e,y), & (0.4,0.5,0.6) \\ (e,xy), & (0.5,0.4,0.6) \\ (a,e'), & (0.5,0.6,0.3) \\ (a,x), & (0.9,0.8,0.6) \\ (a,y), & (0.5,0.6,0.6) \\ (a,xy), & (0.5,0.6,0.6) \end{Bmatrix}$

Let λ = (0.2,0.7,0.6) and μ = (0.8,0.7,0.4). Then,

$A^{(\lambda,\mu)}$ = $\{< e,(0.4,0.3,0.4) >< a,(0.5,0.6,0.3) >\}$

$B^{(\lambda,\mu)}$ = $\{< e',(0.2,0.1,0.1) >, < x,(0.8,0.7,0.4) >, < y,(0.4,0.5,0.4) >, < xy,(0.5,0.4,0.4) >\}$

$$A^{(\lambda,\mu)} \times B^{(\lambda,\mu)} = \left\{ \begin{matrix} ((e,e'), & (0.4,0.3,0.4)) \\ (e,x), & (0.8,0.7,0.4) \\ (e,y), & (0.4,0.5,0.4) \\ (e,xy), & (0.5,0.4,0.4) \\ (a,e'), & (0.5,0.6,0.3) \\ (a,x), & (0.8,0.7,0.4) \\ (a,y), & (0.4,0.5,0.4) \\ (a,xy), & (0.5,0.5,0.4) \end{matrix} \right\}$$

Here, $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) –multi anti fuzzy subgroup of G × H, Even though $A_3{}^{(\lambda,\mu)}$ and $B_2{}^{(\lambda,\mu)}$ are not (λ,μ) –anti fuzzy subgroups of the group G. Because of $0.7 = B_2{}^{(\lambda,\mu)}(x) = B_2{}^{(\lambda,\mu)}(x.yy) \not\leq B_2{}^{(\lambda,\mu)}(xy) \vee B_2{}^{(\lambda,\mu)}(y) = 0.5$ and $0.4 = A_3{}^{(\lambda,\mu)}(a.a) \not\leq A_3{}^{(\lambda,\mu)}(a) \vee A_3{}^{(\lambda,\mu)}(a) = 0.3 \vee 0.3 = 0.3$

Theorem 3.9

Let $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ be two (λ,μ) –multi anti fuzzy subgroups of G and H respectively, then $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ is a (λ,μ) –multi anti fuzzy subgroup of G × H

Proof: Let $(a,b),(c,d),(a^{-1},b^{-1}) \in G \times H$.

$$\begin{aligned} \text{Then } (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})((a,b),(c,d)) &= (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(ac,bd) \\ &= A^{(\lambda,\mu)}(ac) \vee B^{(\lambda,\mu)}(bd) \\ &\leq A^{(\lambda,\mu)}(a) \vee A^{(\lambda,\mu)}(c) \vee B^{(\lambda,\mu)}(b) \vee B^{(\lambda,\mu)}(d) \\ &= A^{(\lambda,\mu)}(a) \vee B^{(\lambda,\mu)}(b) \vee A^{(\lambda,\mu)}(c) \vee B^{(\lambda,\mu)}(d) \\ &= (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(a,b) \vee (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(c,d) \end{aligned}$$

$$\begin{aligned} \text{Also, } (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})((a,b)) &= A^{(\lambda,\mu)}(a) \vee B^{(\lambda,\mu)}(b) = A^{(\lambda,\mu)}(a^{-1}) \vee B^{(\lambda,\mu)}(b^{-1}) \\ &= A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}(a^{-1},b^{-1}) = (A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(a,b)^{-1}. \end{aligned}$$

Theorem 3.10

Let $A^{(\lambda,\mu)}$ and α be two (λ,μ) –multi anti fuzzy subgroups of a group G and $A^{(\lambda,\mu)}$ be conjugate to α. And let $B^{(\lambda,\mu)}$ and β be another two (λ,μ) –multi anti fuzzy subgroups of a group H and $B^{(\lambda,\mu)}$ be conjugate to β. Then the (λ,μ) –multi anti fuzzy subgroup $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ of a product group G × H is conjugate to the (λ,μ) –multi anti fuzzy subgroup $\alpha \times \beta$ of G × H.

Proof: Let the two (λ,μ) –multi anti fuzzy subgroups $A^{(\lambda,\mu)}$ and $B^{(\lambda,\mu)}$ of a groups of G and H respectively are conjugate to a (λ,μ) – multi anti fuzzy subgroups α and β of a groups G and H respectively. Then there exists x in G and g in H such that, for all g in G and h in H,

$A^{(\lambda,\mu)}(g) = \alpha(x^{-1}gx)$, $B^{(\lambda,\mu)}(h) = \beta(y^{-1}hy)$ and

Now, $(A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(g,h) = A^{(\lambda,\mu)}(g) \vee B^{(\lambda,\mu)}(h) = \alpha(x^{-1}gx) \vee \beta(y^{-1}hy)$

$(A^{(\lambda,\mu)} \times B^{(\lambda,\mu)})(g,h) = (\alpha \times \beta)(x^{-1}gx),(y^{-1}hy)$

Hence, the (λ,μ) –multi anti fuzzy subgroup $A^{(\lambda,\mu)} \times B^{(\lambda,\mu)}$ of G × H is conjugate to the (λ,μ) –multi anti fuzzy subgroup of $\alpha \times \beta$ of G × H.

Theorem 3.11

Let $A_1{}^{(\lambda_1,\mu_1)}, A_2{}^{(\lambda_2,\mu_2)}, \ldots, A_n{}^{(\lambda_n,\mu_n)}$ be (λ,μ) –multi anti fuzzy subgroup of $G_1, G_2, \ldots, G_n$ respectively. Then $A_1{}^{(\lambda_1,\mu_1)} \times A_2{}^{(\lambda_2,\mu_2)} \times \ldots \times A_n{}^{(\lambda_n,\mu_n)}$ is a (λ,μ) –multi anti fuzzy subgroup of $G_1 \times G_2 \times \ldots \times G_n$.

Proof: Let $A_1{}^{(\lambda_1,\mu_1)}, A_2{}^{(\lambda_2,\mu_2)}, \ldots, A_n{}^{(\lambda_n,\mu_n)}$ be a (λ,μ) –multi anti fuzzy subgroup of same order of $G_1, G_2, \ldots, G_n$ respectively. Then

$$\begin{aligned} &\left(A_1{}^{(\lambda_1,\mu_1)} \times A_2{}^{(\lambda_2,\mu_2)} \times \ldots \times A_n{}^{(\lambda_n,\mu_n)}\right)((x_1,x_2,x_3,\ldots,x_n)(y_1,y_2,\ldots,y_n)) \\ &= \left(A_1{}^{(\lambda_1,\mu_1)} \times A_2{}^{(\lambda_2,\mu_2)} \times \ldots \times A_n{}^{(\lambda_n,\mu_n)}\right)(x_1y_1,x_2y_2,\ldots,x_ny_n) \\ &= A_1{}^{(\lambda_1,\mu_1)}(x_1y_1) \vee A_2{}^{(\lambda_2,\mu_2)}(x_2y_2) \vee \ldots \vee A_n{}^{(\lambda_n,\mu_n)}(x_ny_n) \end{aligned}$$

$$\leq A_1^{(\lambda_1,\mu_1)}(x_1) \vee A_1^{(\lambda_1,\mu_1)}(y_1) \vee A_2^{(\lambda_2,\mu_2)}(x_2) \vee A_2^{(\lambda_2,\mu_2)}(y_2) \vee \ldots \vee A_n^{(\lambda_n,\mu_n)}(x_n) \vee A_n^{(\lambda_n,\mu_n)}(y_n)$$
$$\leq \left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(x_1, x_2, \ldots, x_n) \vee \left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(y_1, y_2, \ldots, y_n)$$

Also, $\left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)((x_1, x_2, x_3 \ldots, x_n)^{-1})$

$$= \left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(x_1^{-1}, x_2^{-1}, \ldots, x_n^{-1})$$
$$= (A_1^{(\lambda_1,\mu_1)}(x_1^{-1}) \vee A_2^{(\lambda_2,\mu_2)}(x_2^{-1}) \vee \ldots \vee A_n^{(\lambda_n,\mu_n)}(x_n^{-1}))$$
$$= (A_1^{(\lambda_1,\mu_1)}(x_1) \vee A_2^{(\lambda_2,\mu_2)}(x_2) \vee \ldots \vee A_n^{(\lambda_n,\mu_n)}(x_3))$$
$$= \left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(x_1, x_2, \ldots, x_n).$$

Thus, $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}$ is a (λ, μ) −multi anti fuzzy subgroup of $G_1 \times G_2 \times \ldots \times G_n$.

Theorem 3.12

Let $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \ldots, A_n^{(\lambda_n,\mu_n)}$ is a (λ, μ) −multi anti fuzzy subgroup of order m of $G_1, G_2, \ldots, G_n$ conjugate to (λ, μ) −multi anti fuzzy subgroups $B_1^{(\lambda_1,\mu_1)}, B_2^{(\lambda_2,\mu_2)}, \ldots, B_n^{(\lambda_n,\mu_n)}$ of order m of $G_1, G_2, \ldots, G_n$. Then $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}$ and $B_1^{(\lambda_1,\mu_1)} \times B_2^{(\lambda_2,\mu_2)} \times \ldots \times B_n^{(\lambda_n,\mu_n)}$ are conjugate (λ, μ) −multi anti fuzzy subgroups of the groups $G_1 \times G_2 \times \ldots \times G_n$.

Proof: Let (λ, μ) −multi anti fuzzy subgroups $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \ldots, A_n^{(\lambda_n,\mu_n)}$ of $G_1, G_2, \ldots, G_n$ is conjugate to (λ, μ) −multi anti fuzzy subgroups $B_1^{(\lambda_1,\mu_1)}, B_2^{(\lambda_2,\mu_2)}, \ldots, B_n^{(\lambda_n,\mu_n)}$ of $G_1, G_2, \ldots, G_n$. Then there exits $x_i \in G_i$ such that for all $g_i \in G_i$.

$A_i^{(\lambda_i,\mu_i)}(g_i) = B_i^{(\lambda_i,\mu_i)}(x_i^{-1} g_i x_i)$, for i = 1,2, … , n.

Then $\left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(g_1, g_2, \ldots, g_n) = A_1^{(\lambda_1,\mu_1)}(g_1) \vee A_2^{(\lambda_2,\mu_2)}(g_2) \vee \ldots \vee A_n^{(\lambda_n,\mu_n)}(g_n)$

$$= B_1^{(\lambda_1,\mu_1)}(x_1^{-1} g_1 x_1) \vee B_2^{(\lambda_2,\mu_2)}(x_2^{-1} g_2 x_2) \vee \ldots \vee B_n^{(\lambda_n,\mu_n)}(x_n^{-1} g_n x_n)$$
$$= \left(B_1^{(\lambda_1,\mu_1)} \times B_2^{(\lambda_2,\mu_2)} \times \ldots \times B_n^{(\lambda_n,\mu_n)}\right)(x_1^{-1} g_1 x_1, x_2^{-1} g_2 x_2, \ldots, x_n^{-1} g_n x_n)$$

Hence, the (λ, μ) −multi anti fuzzy subgroup $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}$ of the groups $G_1 \times G_2 \times \ldots \times G_n$ is conjugate to the (λ, μ) −multi anti fuzzy subgroup $B_1^{(\lambda_1,\mu_1)} \times B_2^{(\lambda_2,\mu_2)} \times \ldots \times B_n^{(\lambda_n,\mu_n)}$ of $G_1 \times G_2 \times \ldots \times G_n$.

Theorem 3.13

Let $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \ldots, A_n^{(\lambda_n,\mu_n)}$ be (λ, μ) −multi anti fuzzy subsets of the groups $G_1, G_2, \ldots, G_n$ respectively, such that for all x_i in G_i,

$$A_i^{(\lambda_i,\mu_i)}(x_i) \geq \left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_{i-1}^{(\lambda_{i-1},\mu_{i-1})} \times A_{i+1}^{(\lambda_{i+1},\mu_{i+1})} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(e_1, e_2, .., e_{i-1}, e_{i+1}, \ldots, e_n)$$

Where, e_i being the identity element of G_i. If $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}$ is a (λ, μ) −multi anti fuzzy subgroups of $G_1 \times G_2 \times \ldots \times G_n$, then $A_i^{(\lambda_i,\mu_i)}$ is a (λ, μ) −multi anti fuzzy subgroups of G_i.

Proof: It is similar to Theorem: 3.12

Theorem 3.14

Let $A_1^{(\lambda_1,\mu_1)}, A_2^{(\lambda_2,\mu_2)}, \ldots, A_n^{(\lambda_n,\mu_n)}$ be a (λ, μ) −multi anti fuzzy subsets of the groups $G_1, G_2, \ldots, G_n$ respectively, such that

$$\left(A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_{i-1}^{(\lambda_{i-1},\mu_{i-1})} \times A_{i+1}^{(\lambda_{i+1},\mu_{i+1})} \times \ldots \times A_n^{(\lambda_n,\mu_n)}\right)(x_1, x_2, .., x_{i-1}, x_{i+1}, \ldots, x_n)$$
$$\geq A_i^{(\lambda_i,\mu_i)}(e_1, e_2, .., e_{i-1}, e_{i+1}, \ldots, e_n) \text{ for all, } (x_1, x_1, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n)$$

$\in G_1 \times G_2 \times \ldots . \times G_{i-1} \times G_{i+1} \times \ldots \times G_n$, e_i being the identity element of G_i.

If $A_1^{(\lambda_1,\mu_1)} \times A_2^{(\lambda_2,\mu_2)} \times \ldots \times A_n^{(\lambda_n,\mu_n)}$ is a (λ,μ) –multi anti fuzzy subgroups of $G_1 \times G_2 \times \ldots \times G_n$, then $A_i^{(\lambda_i,\mu_i)}$ is a (λ,μ) – mutli anti fuzzy subgroups of G.

Proof: It is similar to Theorem: 3.13.

CONCLUSION

In the theory of fuzzy sets, the level subsets are vital role for its development. Similarly, the (λ,μ) – mutli fuzzy subgroups are very important role for the development of the theory of multi fuzzy subgroup of a group. In this paper an attempt has been made to study some algebraic natures of (λ,μ) – mutli anti fuzzy subgroups and their product of (λ,μ) – mutli anti fuzzy subgroups.

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Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Dynamic Timer Based QoS in Mobile Adhoc Networks

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Received: 03 Jan 2022 Revised: 25 Jan 2022 Accepted: 18 Feb 2022

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ABSTRACT

Dynamic Routing in MANET is a significant problem that must be addressed in order to maintain reliable data transport. Increased mobility of mobile nodes in a MANET, on the other hand, would result in frequent route path failure. It offers a flexible environment for malicious nodes to insert attacks while there is no centralised node to monitor them. We addressed previous research effort by providing the Mobility Factor aware Routing Scheme (MFRS), which selects nodes for route path creation based on mobility factors. However, the previous work has a higher time complexity and resource consumption since it calculates trust and mobility behaviour. By introducing the Dynamic Timer-based QoS, we concentrated and addressed the suggested research approach (DTQoS). Our suggested work, optimum route path selection, is carried out utilising the Dynamic Timer Based Path Selection method while taking different QoS aspects such as mobility and bandwidth into account. High mobility is identified in our suggested study based on high traffic and malicious nodes by examining the packet-forwarding behaviours of surrounding nodes. If high mobility is generated by high network traffic received from numerous nodes, multipath transmission is allowed. If malicious node activity causes excessive mobility, information about the malicious nodes is conveyed to adjacent nodes and added to the prohibited node list. Otherwise, regular data transfer will resume. The suggested research technique is implemented in its entirety in the NS2 simulation environment. It has been shown that the suggested research technique may provide the best possible result of the present research approach.

Keywords: QoS, DTQoS, MFRS, MANET, Timer, High Mobility, DTPS

INTRODUCTION

Wireless mobile Ad hoc networks (MANET) are important components of modern communication networks. The growing demand for a corporate environment to offer cost-effective and mobile Internet connectivity has resulted in widespread use of wireless technologies. Because it has a very basic infrastructure configuration and no or minimum central management, a MANET network may be set up or deployed anywhere and at any time. These networks are mostly utilised by members of the community, including the military, researchers, companies, students, and emergency services [1]. Wireless MANET networks are often made up of wireless nodes that have Omni-directional antennas [2]. To avoid collisions, omni-directional antennas disseminate radio signals in all directions, and neighbours surrounding a transmitter or receiver inhibit broadcasting. The downside of Omni-directional antenna wireless networks is their restricted capacity due to severe interference and poor spatial reuse [3].

The responsive directing protocols are dynamic source steering and on-request remove vector directing [4] [8]. Numerous associations coordinate anycast direction [9]. Anycast is a crucial method communication when hub portability and connection breakage are visited. Anycast allows the sender to direct a packet to a single objective from a list of possible destinations. Customers submit a package to one of the targets that is nearest to them. As a goal, Anycast system management chooses a standout amongst the greatest administration from a gathering of administration. Overseeing the adaptability of hubs and the dynamically changing system architecture is a critical concern in MANET [12] [13]. In our work, DTQoS presented, optimal route path selection is completed by applying timer-based computation while taking into account various QoS criteria like as flexibility and data transfer. The goal of our effort is to sort high versatility based on high traffic and toxic hubs by examining the packet sending methods of nearby hubs [14] [15]. If high versatility is created by heavy system traffic from many hubs, multipath transmission is enabled at that moment. Otherwise, if high portability is achieved as a result of vengeful hub behaviour, info about malignant hubs is conveyed to neighbour hubs and put to the prohibited hub list [16]. Otherwise, normal information transfer will go place. The rest of this paper is structured as follows. Section II provides context for QoS definition, kinds, models, and QoS needs in MANETs. Section III describes our DTQoS setup; section IV discusses simulation data and analyses. Section V concludes with conclusions and future study.

BACKGROUND STUDY

T. Cakan and V. Wieser [3] Two wireless MANET network configurations were simulated in this article. In the first simulation, omni-directional antennas were used for all nodes, while directional antennas were used in the second simulation. As a simpler radiation pattern of a directional antenna, the flat-top model was utilised. Because it is simple, and its implementation is likewise simple, this model is often used in simulation environments. Jieying Zhou, et al. [5] Timer Forecasting Zone, a novel on-demand multicast routing system for MANET based on demand multicast routing protocol (TFZMP), is suggested in this study. TFZMP combines three MANET-appropriate mechanics: mesh-based, on-demand, and zone-based. On-demand approaches are often adaptable to changes in network architecture. Mesh-based multicast techniques have been shown to be mobile-resistant. Zone-based approaches, such as ZRP, have been shown to offer minimal overhead and high scalability. The TFZMP strategy delivers acceptable multicast service to MANET when bandwidth is restricted, topology changes often, and power is controlled by the cohesive integration of the aforementioned three approaches. A. Junnarkar, et al. [6] This work presents a QoS efficient ACO-based routing algorithm that uses node position information to address MANET mobility difficulties. DoS, blackhole, grayhole, selfish node, malevolent node, and other types of attacks are common. In order to address such issues, we submitted a secure low weight secure data transfer method to this study. In this study, we demonstrated both algorithms and their work. Malicious attackers compare the proposed SQMAA routing protocol to the ARA and QMAA protocols.

M. Kumar, et al. [7] In this study, we tend to impart safe tunnels between subnet gateways that are unit integrated with the flow and network management schemes—as MANETs expand, it will be necessary to guide and absorb the numerous protocols. These methods are being developed into a unified framework that facilitates trustworthy safe

communication in this very dynamic environment. However, the dynamic in MANET makes intrusion detection difficult, since it should take into account not only the input and output of nodes inside the network, but also their quality among them. Prathviraj N. and S. L. Deshpande [10] A rough set theory has shown to be the best-suited strategy to handling difficulties linked to a multi-criteria decision. The multi-criteria decision issue is connected to the multi-constrained QoS routing challenges in MANETs. As a result, rough set theory is relevant to MANET QoS routing difficulties. A feasibility study using rough set theory in multi-constraint QoS routing was completed, and it is already operational. G. Qian [11] QoS routing is an important part of QoS architecture. Because of the dynamic and physical nature of a wireless ad hoc network, building a QoS routing protocol presents several obstacles. Based on the AODV protocol, this study proposes an enhanced QoS routing system for wireless ad hoc networks. It expands the route table, enhances the request reply packet, and applies chosen flooding and local recovery mechanisms to effectively raise the packet delivery ratio while reducing control overload and route searching time. The results of simulations show that the design of this protocol fits our design aims.

T. Sanguankotchakorn and P. Maharjan [12] In this paper, we offer a technique for finding multiconstrained viable paths in MANETs using a novel approach to route selection. Because the original OLSR routing does not support QoS, we extend it to support QoS and then find a feasible path. When numerous routes to the same destination are available, we employed an unique composite function (a mix of both additive and non-additive QoS indicators) to determine which one is best. Zhu, X., and J. Lian [17] The study describes an MAODV-based QoS multicast routing system with mobile prediction (MPMRPQ). The MAODV is subjected to the mobile prediction process. MPMRPQ's routes are more stable.

PROBLEM DEFINITION

This initiative aims to tackle communication issues when communication infrastructure becomes restricted or unreliable, or when it reaches a tipping point. The network infrastructure might be static or movable. In this scenario, entities are networked using static infrastructure, which is represented by the Static Infrastructure Network (SIN), and entities are interconnected using mobile infrastructure, which is represented by the Mobile Infrastructure Network (MIN) (MIN). The SIN might be outfitted with communication towers or earth-based electronics. The MIN is made up of mobile phones, walkie-talkies, and other wireless media capable of MANET routing.

DYNAMIC TIMER BASED QOS

In our work, we choose the best route of action by applying timer based route searching calculations while keeping several QoS aspects in mind, for example, portability and data transfer. In our approach, the goal of high portability is determined based on heavy traffic and toxic hubs by dissecting nearby hubs' packet sending methods. If high portability is caused by high system traffic received from several hubs, then multipath transmission is enabled with a timer. Otherwise, if high portability is achieved as a result of malicious hub behaviour, data about malicious hubs are communicated to neighbour hubs and added to the prohibited hub list. Otherwise, standard information transfer will be preceded.

NETWORK MODEL

The system under examination has n nodes, some of which are movable and others of which are essentially fixed. Each node in the network is given a unique ID. At time t, each node occupies a location (xt, yt) within a defined geographic region (ll m2) and is initially equally distributed. Every node travels autonomously and follows the aforementioned mobility concept. The mobility model preserves the uniform geographical distribution of nodes across time. If the Euclidean distance d (ni, nj) between ni and nj is smaller than rtx, two nodes ni and nj are within transmission range rtx. Each node keeps a neighbour table N. (.). The topology graph G(t) = (V,E(t)) is made up of a collection of vertices V that represent network nodes and a set E(t) of undirected edges that indicate communication linkages between nodes at time t.

OPTIMAL ROUTE PATH SELECTION

The best route of action is chosen by applying dynamic timing computation while keeping several QoS

characteristics in mind, for example, adaptability and transfer speed. Our study presents a better route path determination method for competent MANET conditions. LEACH now includes the new Dynamic Timer Based Path Selection (DTPS) algorithm. DTPS is a bio-enlivened calculation (DTPS) ventures for guiding the progression process. While looking for supplements, the basic technique of timer advancements receives basic advancements (timer). DTPS is a timer strategy construction of virtual nodes (multicast steering search space), and each timer imparts to the others by transmitting signals (hub correspondence). The route seek systems used by DTPS include timer, generation, disposal, and dispersion. DTPS on multicast steering process completed to limit directing shakiness, restrict way tragedy rate, and increase bundle transmission rate.

FITNESS EVALUATION

The fitness factors studied here are mobility factor and bandwidth. Mobility factor: The versatility of hubs relies upon the speed, route, and delay time of hubs. Versatility factor estimates processed point for speed, route, and interruption time of hubs and is estimated co-operatively regarding neighbor hubs. The portability factor is also considered directing measurements; an appropriate hub will either be chosen to build up away or rejected. A hub having a high portability factor implies it has low speed, appropriate bearing, and high delay time. We characterize an edge esteem th ($0 \leq th \leq 1$), wherein the portability factor can run from 0 (zero) to 1 (one). If the portability factor is more noteworthy than or equivalent to the limit esteem, it will be considered a middle-of-the-road hub of dynamic route. If the portability factor is not exactly the edge esteem, it won't be considered in the dynamic route. Let TM_{ij} be the mobility factor of node j concerning the node i. We introduce two more terms in our work. M_{ij}^{S} is the mobility factor of a node j that is computed by any other node i which is in the radio range of node j. M_{ij}^{O} is the mobility factor of node j that is computed by the common neighbors of both nodes i and j. We also introduce two constants' a' and' b' which are weight value parameters for determining the weights of M_{ij}^{S} and M_{ij}^{O}. We therefore define TM_{ij} as follows:

$$TM_{ij} = aM_{ij}^{S} + bM_{ij}^{O}$$

where $[TM_{ij}, M_{ij}^{S}, M_{ij}^{O} \in \{0, 1\}; a, b \in \{0, 1\}; a + b = 1]$. The mobility factor between node i and j is independent and non-transitive, i.e., TM_{ij} is not equal to TM_{ji}.

Bandwidth: Bandwidth estimation is the main function needed to provide QoS in MANETs. Since each host has inaccurate network status and dynamic links, it is difficult to estimate the available bandwidth between nodes. Evaluate the capacity of a node and estimate the available bandwidth.
Available bandwidth = Channel Capacity - Utilized Bandwidth

Here Utilized bandwidth = N*S*8/T

where N- No. of packets, S- Size of the packet, and T- Time duration

ANALYSING PACKET FORWARDING BEHAVIOUR

Following the selection of the best route path, hub behaviour is analysed to determine the reason for the high bundle misfortune rate due to noxious hub proximity and the increased traffic rate. Our solution is completed by offering the eyewitness hub, which will monitor every ongoing trade taking place on the system. It will determine the variety present on each hub based on the number of packets delivered and the amount of receiving. Given those test results, it is possible that the dark opening assailant hub nearness was successfully identified. In our study, we differentiate high versatility caused by high traffic and malignant hubs by evaluating nearby hubs' bundle sending habits. If heavy system traffic from several hubs causes high portability, multipath transmission is enabled by tiny portioning the packets and sending them concurrently over multipath. If high versatility is caused by malicious hub behaviour, transmit information about spiteful hubs to adjacent hubs and add them

The middle of the road hubs are not authorised to respond to RREQs in order to acquire as much data as possible about the routes (including data about nearby hubs) and safeguard their unwavering quality. The information will be used to register zone-disjoint routes. To limit the number of to the prohibited hub list. Otherwise, continue with the information transfer.

MULTIPATH DATA TRANSMISSION

The route discovery is the first step of the multipath transmission protocol. It comprises two main processes: route request and route reply. Route Request: When s has a packet to deliver to d but does not have a route to do so, it sends an RREQ. Interestingly, each RREQ is distinguished by two identifiers: the RREQ's grouping number and the hub's location at the commencement of the RREQ. Figure 2 depicts the header of an RREQ (a). The way field provides a list of the middle of the road hubs between s and d. When s issues the RREQ, the rundown is empty. The way field will be updated during the propagation of the RREQ at that location. Each transfer hub's location will be included in the list of transitional hubs. The list allows for opposing directing circles and builds an arrival path for transmitting the response to RREQ 1. Because it is a source directing, the source hub also uses the list of middle of the road hubs to identify the whole path for information packets (the way will be typified in information packets). repeating RREQs and avoid the communication storm problem, the middle of the road hubs are only authorised to transmit the principal duplicate of each RREQ. When a middle-of-the-road hub obtains the primary duplicate of an RREQ, it saves the request in an RREQ store.

To determine zone-disjoint routes, the neighbours of the source hub (which starts the RREQ) and those of the target hub (which starts the RREP) are not taken into account. d sends an RREP to all of its neighbours who have sent the RREQ. Each moderate hub that receives the RREP ensures that there is no directing circle in the path (if there is a circle, the RREP is dropped). If there is no circle, the transitional hub refreshes the message by adding (I) its location to the way field and (II) its list of neighbours to the neighbours' field. The midway hub then delivers the updated RREP to its neighbours, from whom it obtained the route demand (the rundown is accessible in the RREQ store). The RREP generates till it is obtained by s.

Algorithm 3.1: Dynamic Timer Based Path Selection Algorithm

Step 1: The source node has a data packet for the destination and checks its route cache for the destination.

Step 2: If entry is not present in route cache, go to step 3; otherwise, go to step 11.

Step 3: Source broadcasts RREQ packet towards the destination with Trust_calculate() on every possible path.

Step 4: Template Generation() on Each intermediate node compares the required and available bandwidth at the node; if the required bandwidth is greater than the available bandwidth, drop the RREQ packet immediately.

Step 5: Template Matching() on each intermediate node calculate hop count with the help of node trace. If the count increases to MAX_H_COUNT, then drop the RREQ packet immediately; otherwise, add its identity in the node trace field of the RREQ packet and broadcast it further.

Step 6 For the amount of time stored in TIMER_DES, Destination sends Request-Reply packets against all RREQ packets of the same sequence number.

Algorithm 3.2: Trust Calculation

Function: *trust_calculate (a, c)*

Input: nodes a and c whose trust to be calculated

Output: Trust value between nodes a and c (T_{ac})

Begin

if a is friend with c then

Use Trust value tv

else

while ($T_{ac} = 0$ && $p_i \leq 60$)

$Tab = max\_trust\ (a,b)$

if $c \varepsilon b.f$ then

$T_{ac} = T_{ab} * T_{bc} + (1-T_{ab}) * (1-T_{bc})$

```
                    else
                        Tbx = max_trust(b,x)
                        if (Tax = 0) then
                            Tax = Tab*Tbx + (1-Tab)*(1-Tbx)
                        end if
                        b=x
                    end if
                end while
        end if
End
```

Algorithm 3.3: Template generation

```
Function: template_generate (messages)
Input: set of input messages Sp
Output: Optimal route templates
Begin
        SS = M_merge(Sp)  // Super sequences formation
                for i in Sp  // matrix formation
                    for node, key of i in SS
                        if key == word then
                            matrix[SS].append(node)
                        end if
                    end for
                end for
                for key in SS   //column-concatenation
                    for key1 in SS
                        for val1,val2 in key1,key2
                            if(!(val1 == ε and val2) || !(val1 and val2 == ε)) then
                                Break
                            end if
                        end for
                          list[key1].append(key2)
                    end for
                end for
                Optimal route templates list by concatenating the keys
End
```

Template matching is done using the created templates. In the testing phase, incoming messages are matched to a template. Algorithm 3.4 explains the template matching process. Template matching count Tm is zero at first. It is done for each incoming message (M) to see whether it is a subset of the templates (Te). The template's matching count grows by the same amount as the comparison is made. If the similarity value is less than 75%, the DTQoS algorithm sends the message. The output messages based on template matching is the next step.

Algorithm 3.4: Template matching

```
Function: template_match(M)
Input: message from user
Output: Optimal route / Not
Begin
        Tm = 0 //number of templates matched
        for each incoming message
                if M c Te then
                    Tm ++
```

```
            end if
        end for
        if Tm > 0.75 * length (Tδ) then
            report optimal route
        else
            report result (SF, BF)
        end if
End
```

Trust value and template matching (P) are calculated using the proposed method on optimal route path selection during the data transmission.

SIMULATION RESULTS

In the NS-2 network simulator, a simulation model with an extra timer function to enable QoS has been constructed. Omni-directional antennas were utilised at each node. Our simulation model had 50 wireless nodes spread over a 600 × 600 m region (Fig. 4). Each node's broadcast range was set to 200 metres. For traffic creation, 5 CBR (Constant Bit Rate) flows with packet sizes of 512 bytes were employed. Except for the channel data rate, which was adjusted to 11 Mb, the simulation model utilised default settings. Table 1 summarises the simulation parameters.

PACKET DELIVERY RATE

Every node sends packets using the routing protocols. The packet delivery rate is a measure of the packets received to those sent and is calculated as follows:

$$\text{PDR} = \frac{\sum_0^n \text{Packets Received}}{\sum_0^n \text{Packet Sent}}$$

The PDR of the methodology, namely MFRS and DTQoS, are compared together. Based on the result, DTQoS shows an improved performance than the other two methodologies, according to figure 3. DTQoS shows a 17% increased PDR than MFRS.

PACKET LOSS RATE

The Packet Loss Rate (PLR) is another imperative metric to measure in a protocol. to estimate the PLR, the expression is given as follows:

$$\text{PLR} = \frac{\sum_0^n \text{Packets Dropped}}{\sum_0^n \text{Packet Sent}}$$

The PLR of the methodology, namely MFRS and DTQoS are compared together. Based on the result, DTQoS shows an improved performance than the other two methodologies, according to figure 4. MFRS shows an 11% reduced packet loss rate than the DTQoS.

AVERAGE DELAY

The time difference between the current packets and the previous packet inward bound is defined as the average delay caused at a node. The following equation measures it.

$$\text{Average Delay} = \frac{\sum_0^n \text{Pkt Received Time} - \text{Pkt Sent Time}}{\text{n}}$$

The average delay of the methodology, namely MFRS and DTQoS are compared together. Based on the result, DTQoS shows an improved performance than the other two methodologies, according to figure 5.

THROUGHPUT

It is one of the dimensional parameters of the network which gives the fraction of the stability used for useful transmission selects a destination at the beginning of the simulation. i.e., information on whether or not data packets are correctly delivered to the destination. The result is shown in Figure 6. The throughput of various methodologies in the simulation environment from the source to each destination node is shown in Figure 6. As a result, it concludes that the DTQoS will produce better results compared to the standard MFRS.

END-TO-END DELAY

The end-to-end delay of data packets is the interval between the data packet generation time and when the last bit arrives at the destination. The end-to-end delay of various methodologies in the simulation environment from the source to each destination node is shown in Figure 7. As a result, it concludes that the DTQoS will produce better results compared to the standard MFRS.

SPEED VARYING CONDITION

The hub development speed is progressively changed dependent on the nature of the connection. Throughput and PDR of all the versatile hubs are shown in Figures 8 and 9 individually. Both throughput and PDR of all the portable hubs are diminished when the hubs move with higher speed. When the hubs move with speed lower than 10m/s, the exhibition of all conventions as far as throughput and PDR is very little unique. The throughput and Packet Delivery Ratio are compared together in various methodologies in the simulation environment from the source to each destination node is shown in Figure 8 & Figure 9. As a result, it concludes that the DTQoS will produce better results compared to the standard MFRS. The comparison of delay and movement speed of all the mobile nodes is done, illustrated in Figure 10. As a result, it concludes that the DTQoS will produce better results compared to the standard MFRS.

CONCLUSIONS

QoS routing is an important part of QoS architecture. Because of the dynamic and physical nature of MANET, building a QoS routing protocol presents several issues. The DTQoS routing mechanism in MANET is designed in this work using the AODV protocol. It increases the packet delivery ratio while reducing control overload and route searching time by extending the route table, improving request-reply packets, and using chosen flooding and local recovery mechanisms. The results of simulations show that the design of this protocol fits our design objectives. The suggested research approach is carried out in its entirety under the NS2 reproduction state. It is proven that the suggested research approach outperforms the present research technique in terms of achieving the best results. In the future, we will employ the 802.11 priority mechanism to create a classified QoS routing protocol for greater scalability.

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Table 1: Simulation Parameters

Parameter	Value
Area size	600 x 600
Propagation model	Two-way ground
Routing protocol	AODV
Type of antenna	Omni-directional
Data flow	5
Packet size	512 bytes
Simulation time	130 seconds

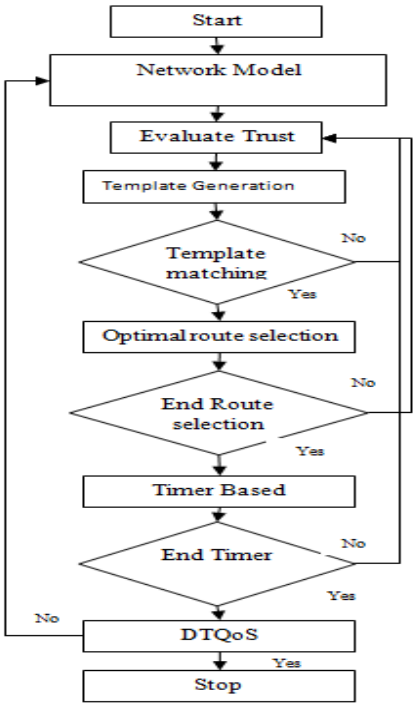

Figure 1: DTQoS Architecture Diagram

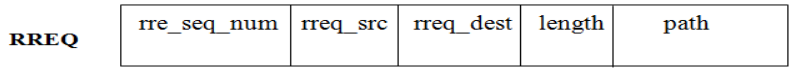

Figure 2 (a): RREQ header

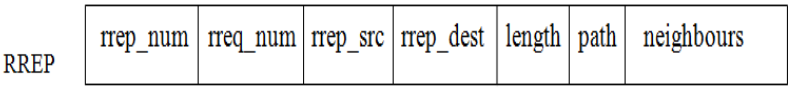

Figure 2(b): RREP header

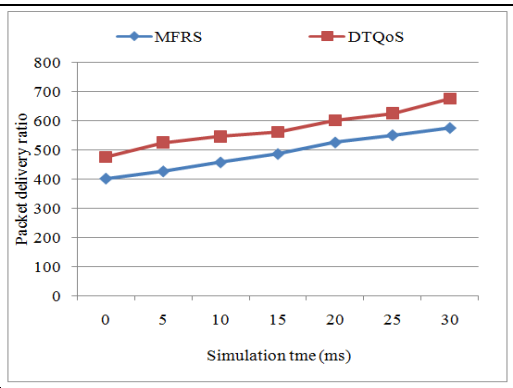

Figure 3: Packet Delivery rate

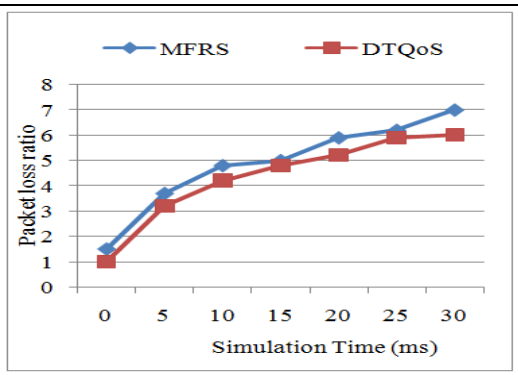

Figure 4. Packet Loss Rate

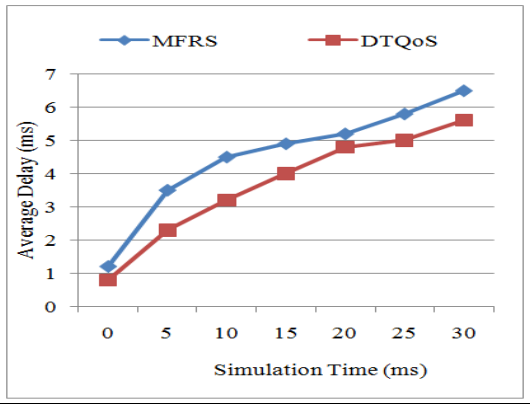

Figure 5. Average Delay

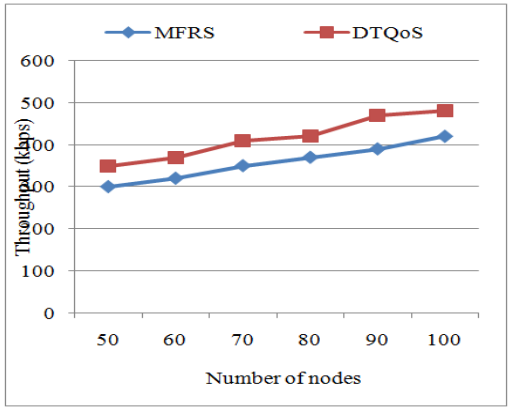

Figure 6: Throughput

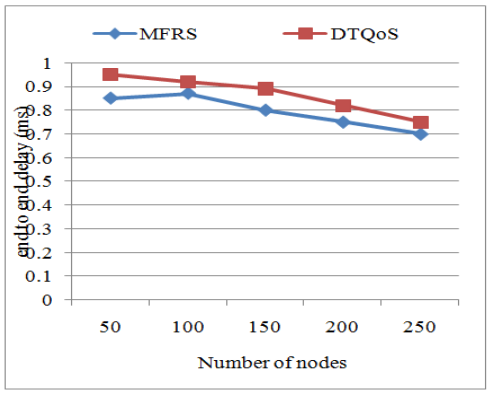

Figure 7: End to End delay

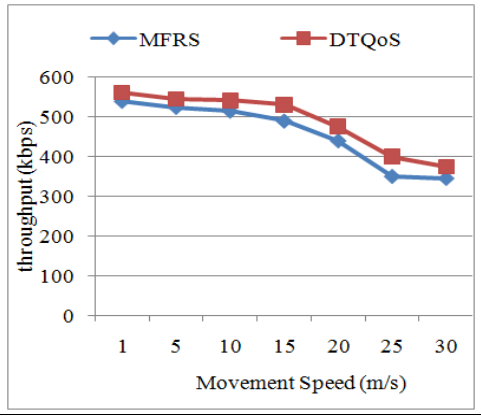

Figure 8: Throughput vs. Movement Speed

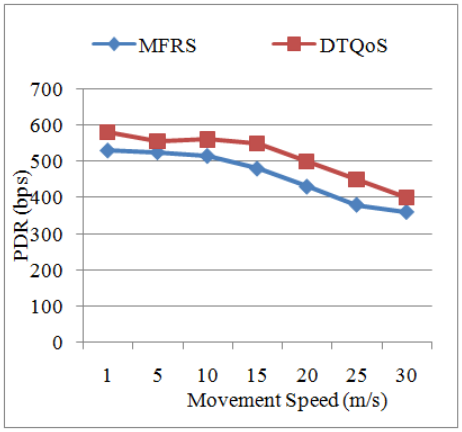

Figure 9: PDR vs. Movement Speed

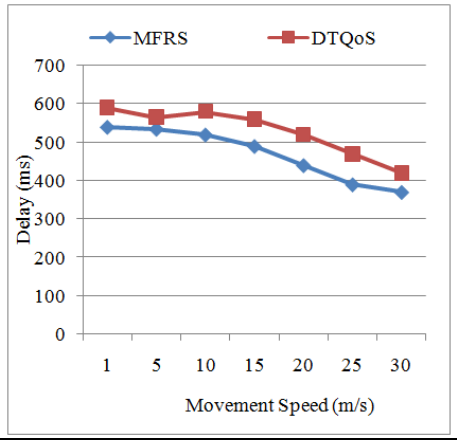

Figure 10: Delay vs. Movement Speed

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Implementation of Agile Scrum based MVP Architecture Model for Android Mobile Application Development

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Received: 20 Jan 2022 Revised: 17 Feb 2022 Accepted: 28 Mar 2022

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ABSTRACT

In the recent days, the mobile application (app) development industry has growing in rapid manner. The mobile users and the usage of mobile phones were increased rapidly. To satisfying the massive demand of mobile Apps and mobile users, the software development companies start taking up new projects based on the mobile app development to remain competitive. Traditional software development models and methods are not fit for mobile app development and lack of architecture and lack of models is still remain in the field of mobile app development. This kind of issues identified in various literatures. Very petite research work has done in the field of mobile app development. The need of effective Mobile App Development Life Cycle (MADLC) for mobile application development is mandatory. In this research, we propose a new architecture pattern ASM-MVP (Agile Scrum based – Model View Presenter) for android app development and it has compared with the existing MVC (Model View Controller) framework. Our findings show that the ASM-MVP model gives better results comparing the MVC.

Keywords: Mobile App, AB-MVP model, MADLC, MVP and MVC Framework

INTRODUCTION

The mobile application development is a process of making mobile applications. The mobile apps are designed specifically to execute on handheld devices like smart phones, tablets and PDA's (Personal Digital Assistant). In the present scenario, the mobile application development is very challenge and the app developers facing different challenges [1] due to the frequent requirement changes, dominance of new technologies, technical constraints of mobile systems, user's much expectations and needs. As now there are no appropriate models and methodologies available for developing mobile applications [2]. Accordingly the developers are pushed to develop an application with various issues. To overcome this kind of issues and challenges we introduced a new model called AB-MVP. In

this model we follow an Agile Based app development with Model View Presenter pattern. In this approach the agile and MVP plays an essential role in the context of mobile app development.

The Agile in Mobile Application Development

Agile is not software, not a programming language, is a philosophy or mindset which is used in software development methodologies based on incremental and iterative approach. The agile methodology is introduced by "The Agile Alliance", is a group of independent thinkers about software development process. They are introduced a Manifesto [4] with 12 major principles for Agile Software Development. Mobile application development can be categorized as either plan driven process or agile processes. According to [3], plan driven process where all the process activities are planned in advance and the progress is measured through this plan. In comparison to agile, plan driven are static and not an iterative method. It is not suitable for mobile App development. In the present scenario the agile based mobile application development is necessity because the competition in market place, quick delivery of the product, need to reduce the development cost and increase the speed in development requires a different approach than the traditional model [5]. Now a days there are various agile methodologies [7] followed by the industries, among that the scrum [6] methodology is a developers best choice and mostly used in IT industries. In this research Agile Scrum Model is incorporate with MVP (ASM-MVP) and the result was compared with MVC and MVP.

MVC Architectural Pattern

MVC & MVP are most popular software design patterns; it is mainly used to separate the applications in the context of visualizing, processing, and data management for User Interface (UI) applications. The main goal of this architecture is to increase the Modularity, Flexibility, Testability and Maintainability of the application. MVC design pattern shown in the Figure 1 is divides an application into three major aspects: Model, View, and Controller. Model having the capability of storing and managing the data and display the results to the view based on the users request via the controller. Based on the business rules and logic the data can be changed and manipulated. Figure 1 shows the MVC Architecture Pattern. View represents the UI components like HTML, DHTML and XML. View is responsible for displaying the data to the user through the screen which is received from the controller. Model and View interact with each other through the controller. Controller is responsible to take over the incoming requests from the view and processes the user's request through the Model than passing the results back to the View. Usually it acts as a mediator between the View and the Model. Controller is tightly coupled with view because of multiple user input at the same time.

MVP Architectural Pattern

The MVP architecture shown in the Figure 2 shows the MVP Architecture pattern which is similar to the MVC. It is derived from MVC; here the controller is replaced by the presenter. This architecture divides an application into three major aspects: Model, View and Presenter. Model and View are the same as in MVP and the presenter plays an impartment role. Presenter receives the input or request from the users via View, then process the user's data or request with the help of the Model and passing the results back to the View. Presenter communicates with View through an Interface. Interface is defined in presenter class, to which it pass the required data. The Presenter is also decoupled directly from the View and talks to it through an Interface. The major advantage of MVP over MVC is, The unit testing is much easier because of maximum testability surface; clean separation of the View and Model, The amount of code is reduced because of leveraged data binding, View and Presenter are loosely coupled from each other's and communicate to each other's by an interface.

Incorporate ASM (Agile Scrum model) in MVP

The Agile scrum model shown in the Figure 3 depicts the ASM-MVP Architecture Pattern. It is the combination of the agile philosophy and the scrum framework. It is a lightweight and sprint based project management system with the goal of delivering the highest value to stakeholders. Agile means "incremental", allowing software teams to develop projects in small increments. Scrum model is used for breaking projects down into size of software chunks called "sprints." Agile scrum methodology is good for businesses that need to finish specific projects quickly. This

methodology is a project management system that relies on incremental development. Each iteration consists of two to four week sprints. The goal of each sprint is to build the most important features first and come out with a potentially deliverable product. More features are built into the product in subsequent sprints and are adjusted based on stakeholder and customer feedback between sprints. In this proposed model we are incorporating ASM in MVP. During the development of each sprint we can follow the MVP architecture pattern for getting a well equipped, error free software product.

Literature Review

The study [9] emphasized that MVC has been the most used pattern over the past years and it continues to gain popularity. This is most traditional used pattern in mobile application development. Comparing to MVC, MVP is less popular method used in app development. A research [10] has compared the importance and effect of using MVP and MVVM (Model View and View Model) architectures on the performance of android applications by measuring memory usage, CPU usage, and execution time. Based on the experimental results it can be concluded that the MVP is better in various aspects comparing the other models. In this research [11] a method was introduced, the MVP design pattern is derived into Modern Driven Architecture (MDA). Using MDA and UML, the MVP was designed. In this study [12] Scrum, Kanban and JIRA was analyzed. The study says kanban is not suitable for android app development and JIRA plays a crucial role to track the project development activities during the each sprint in the Scrum model. [13] This paper reviewed the quality of the existing architectural MVVM pattern specifically in modifiability, testability, and performance in order to investigate the code and testing activities.MVC architectural pattern [14] separates the functionality of application from each other. Though, it shows how the user interactions are changed into functional behavior. MVP architecture allows the developers to address the functional behaviors and the user interface independently. The result shows the unit testing is very easy in MVP comparing to MVC. In this paper [15], the author compared the modularity and maintainability between two applications build with MVP design pattern and anti-pattern. This study proved empirically, use of MVP Design pattern significantly increase modularity by two times comparing the 'anti-pattern'.

METHODOLOGY

The proposed methodology shown in the Figure 4 is classified into two major stages. (1) Implementation phase and (2) Evaluation and Testing phase. In implementation phase, we build an Android application for Student Information System on the MVC and ASM-MVP architectures. In the evaluation and testing phase, the android application that has been built will be analyzed and measured through tools; the results of the testing phase will be discussed based on predetermined metrics.

Our Contribution

This research work aims to identify the effective project management and its outcome using Student Information System android app which is used MVC and ASM-MVP Model. The first part of this work is to build the android application (Student Information System) on the MVC and ASM-MVP architectures use the Java with Android Studio IDE. The second part of the work is to measures the performance of MVC and ASM-MVP through the JIRA Project management tool. The test results are then compared and analyzed, which performance is better between MVC and ASM-MVP. The results can be a consideration for android developers in using which architecture is better in terms of performance.

Evaluation Phase

The MVC and ASM-MVP based android applications that have been built will be tested for performance with the following experimental stages.

The MVC Architecture Pattern

1. Start to develop the app with MVC Architecture.
2. Track the project management activities in manual.
3. Test the written test cases for unit testing which is listed in the Table 1.

4. Test the application.
5. Find the result.

The ASM-MVP Architecture Pattern

1. Start to develop the same app with MVC Architecture.
2. Track the project management activities with JIRA Tool.
3. Test the written test cases for unit testing which is listed in the Table 2.
4. Compare the result with MVC.

Implementation Phase

In this phase, we created an android application for student information system as an experimental product. This application is developed using android studio with java language and incorporated agile scrum model in MVP architecture. Based on the methodology the overall system has been designed. During this phase we analyze the needs of the product, design the overall system, carry out the implementation, and perform unit, integration and performance testing to ensure that the application is running according to the requirement and needs.

Use-case Diagram

The Student Information Systems functional requirements are modeled in the form of use cases diagram as shown in Figure 5. This use-case diagram illustrates the interaction between the actors and systems. Students and admin can act as actors in this system and plays a crucial role. This android application has developed based on this use case. It contains add, edit, update, delete and report modules.

Class Diagram

The class diagram is a type of UML diagram; it is a graphical representation of all classes which is used in the android application Student Information System. It is drawn using rectangular box with three components like class name, attributes and operations. Attributes refers the data members and the operations refers that the member functions which is used in the class. The class diagram for Students Information System as shows in the Figure 6. It contains different classes, data members and member functions. Based on this diagram we complete the coding phase for this system with MVC & ASM-MVP architecture.

Testing with User Interface

This is a user interface of the android application. It was developed using the java with android studio. Using this we can login with MVC and ASM-MVP architecture in the same application. For unit testing we go through all the modules of this application. While developing ASM-MVP architecture we can track all the project management activities through JIRA Tool in each sprint.

Evaluation of MVC with Unit Testing

The MVP based student information system was developed without using any model. The planning, designing, implementation and testing were done in manual. The unit testing was done in MVP architecture with five test cases. In each test case we find the SLOC, actual development duration, testing and execution time. The expected development time has increased in all test cases because of no proper tool used to plan and track the project management. Development time is calculated based on the software development time duration which is calculated by the developer manually. The application was tested with unit testing and the testing time was calculated based on the test duration which is calculated by the tester. Source Line of Code (SLOC) was calculated by the developer based on the classes, data members and member functions used in this project. The build time is calculated in android studio while running the application. All the test cases were executed and the result is given in the Table 3.

FINDINGS AND RESULTS

Comparing to MVC, the SLOC in ASM-MVP is slightly increased in M2, M3 and M4 because of code separation. Even though the source code is increased at the same time the development and testing time was almost decreased

and maintained the same level except one or two module. The build time is moreover less comparing to MVC. Comparison of the results between the MVC, ASM-MVP is shown in the Table 5. The Figure 9 shows that the difference of SLOC in MVC and ASM-MVP. In ASM-MVP the source line of code is slightly increased in M2, M3 and M4 because of some additional number of classes, data members and member functions used in the application. It leads to the developer and tester to debug and test the application very quickly. In MVC the development time is measured by the developer in manual. The Figure 10 shows that the MVC architecture pattern of this application is to take little bit extra development time comparing to ASM-MVP. The reason behind ASM-MVP project management activities are planned and tracked through Agile Scrum model with JIRA Tool. It will help the developer to complete the task within the planned schedule. In MVC we follow the traditional waterfall model for this application development. So it takes some extra time for the development process. The comparison of testing time is shown in the Figure 11. The android application was developed using ASM-MVP architecture pattern is almost reduced the testing time in all the modules comparing to MVC because of bundled packages and the code separation in all the modules. It is calculated by the developer when doing the unit test of this application. It will not execute the whole package, it executes based on the events which is induced by the end users. Almost in all the modules the build time is also decreased in ASM-MVP because of code separation.

CONCLUSION AND FUTURE WORK

The Software development architecture pattern becomes very important in developing an application for both small and large scale projects. Specifically in the development of android applications, the architecture plays an important role. Due to the technological growth the emergence of MVC and MVP architecture can be a consideration for android developers. Based on the findings of this research the ASM-MVP model provides the better results. Besides productivity factors, this architecture can also affect performance factors. In this research we compared the efficiency of using MVC, MVP and ASM-MVP architectures. Based on the unit testing the performance of student information system an android application was measured by the metrics like SLOC, development, testing and build time. Based on the experimental results it can be concluded that the ASM-MVP architecture has better performance than MVC and MVP although not in all aspects. Even though the SLOC is slightly increased in ASM-MVP but the development and testing times is reduced and it remains in the expected level. It is measured in the JIRA Tool. The ASM-MVP architecture model build time is also measured with android studio and it's also less comparing with MVC and MVP. So the proposed ASM-MVP architecture model is a best choice of android developers. In addition that, the further research is going to be done with this model on real time environment with different developers and testers. Based on the suggestions this model will be improved much better in future.

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Table 1: Unit Testing Test Case for MVC

Test Case-ID	Module	Description	Findings
UTC-01	Login	Login with username and password	Find the SLOC, development, testing and execution time
UTC-02	Add Student Details	Add student details in add page	
UTC-03	Update Student Details	Update student details in update page	
UTC-04	Display Details	Display the record in display page	
UTC-05	Report	Report Generation	

Table 2: Unit Testing Test Case for ASM-MVP

Test Case-ID	Module	Description	Findings
UTC-06	Login	Login with username and password	Find (Sprint wise) the SLOC, development and testing time
UTC-07	Add Student Details	Add student details in add page	
UTC-08	Update Student Details	Update student details in update page	
UTC-09	Display Details	Display the record in display page	
UTC-10	Report	Report Generation	

Table 3: Evaluation Results of MVC

Test Case-ID	Module	Description	SLOC	Expected Development Time in Hours	Actual Development Time in Hours	Testing Time in Minutes	Build time in Seconds	Test Result
UTC-01	Login	Login with username and password	167	5	5	5	0.1	Pass
UTC-02	Add Student Details	Add student details in add page	324	10	13	10	0.2	Pass
UTC-03	Update Student Details	Update student details in update page	351	15	17	15	0.3	Pass
UTC-04	Display Details	Display the record in display page	289	9	12	5	0.2	Pass
UTC-05	Report	Report Generation	217	7	10	5	0.3	Pass

Table 4: Evaluation results of ASM-MVP

Test Case-ID	Product Backlog	Module	Description	SLOC (Source Line of Code)	Expected Development Time in Hours	Actual Development Time in Hours	Testing Time in Minutes	Build time in Seconds	Test Result
UTC-06	Sprint-1	Login	Login with username and password	167	5	5	5	0.1	Pass
UTC-07	Sprint-2	Add Student Details	Add student details in add page	**354**	10	10	8	0.1	Pass
UTC-08	Sprint-3	Update Student Details	Update student details in update page	**375**	15	**17**	12	0.2	Pass
UTC-09	Sprint-4	Display Details	Display the record in display page	**302**	9	9	4	0.1	Pass
UTC-10	Sprint-5	Report	Report Generation	217	7	7	5	0.2	Pass

Table 5: Result Comparison of MVC and ASM-MVP

Module	SLOC (Source Line of Code)		Actual Development Time (Hours)		Testing Time (Minutes)		Build time in (Seconds)	
	MVC	ASM-MVP	MVC	ASM-MVP	MVC	ASM-MVP	MVC	ASM-MVP
Login (M1)	167	167	5	5	5	5	0.1	0.1
Add Student (M2)	324	**354**	13	**10**	10	**8**	0.2	**0.1**
Update Student (M3)	351	**375**	17	**17**	15	**12**	0.3	**0.2**
Display (M4)	289	**302**	12	**9**	5	**4**	0.2	**0.1**
Report (M5)	217	217	10	**7**	5	5	0.3	**0.2**

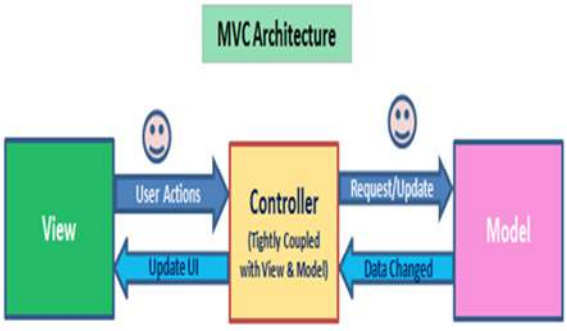

Figure 1: MVC Architecture Pattern

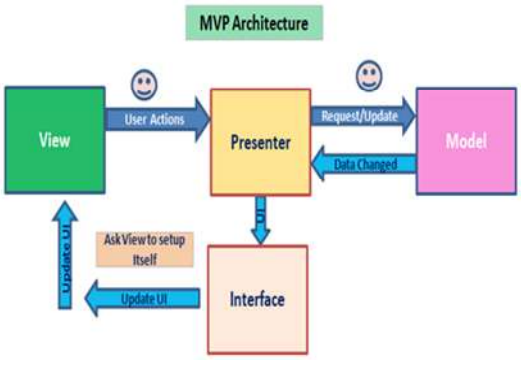

Figure 2: MVP Architecture Pattern

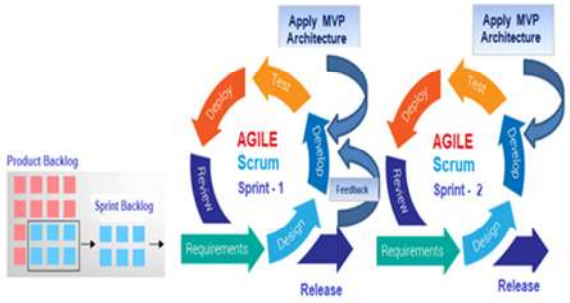

Figure 3: ASM-MVP Architecture Pattern

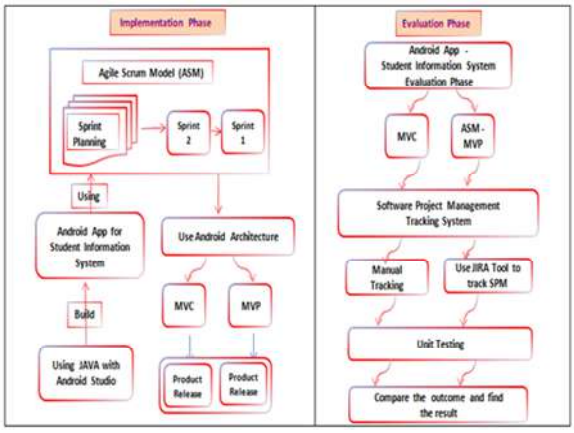

Figure 4: Proposed Methodology

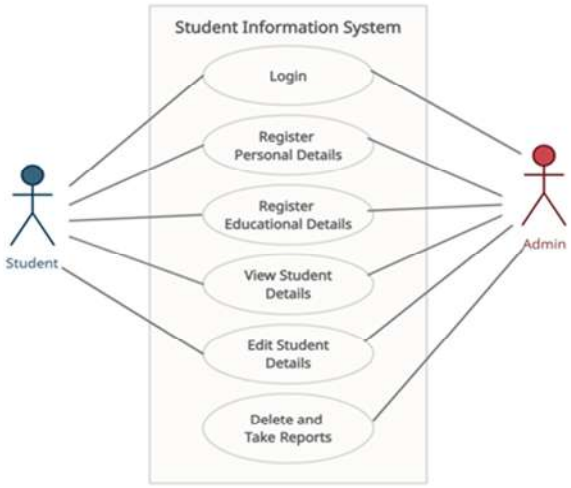

Figure 5: Use case Diagram for SIS

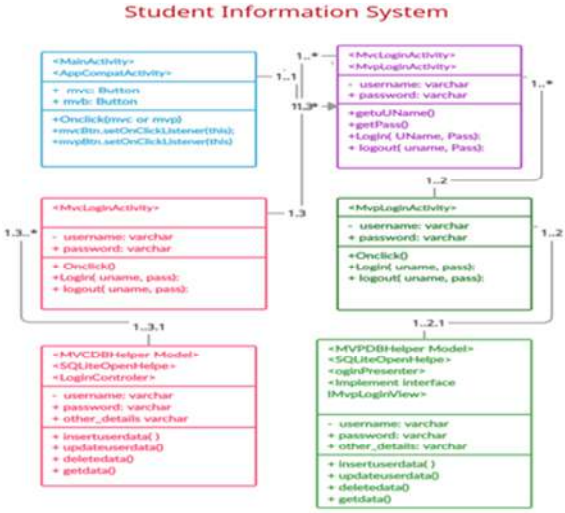

Figure 6: Class diagram for SIS

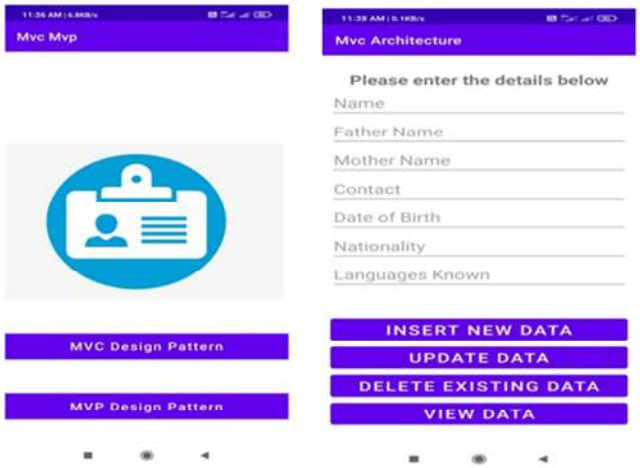

Figure 7: User interface of Student Information System

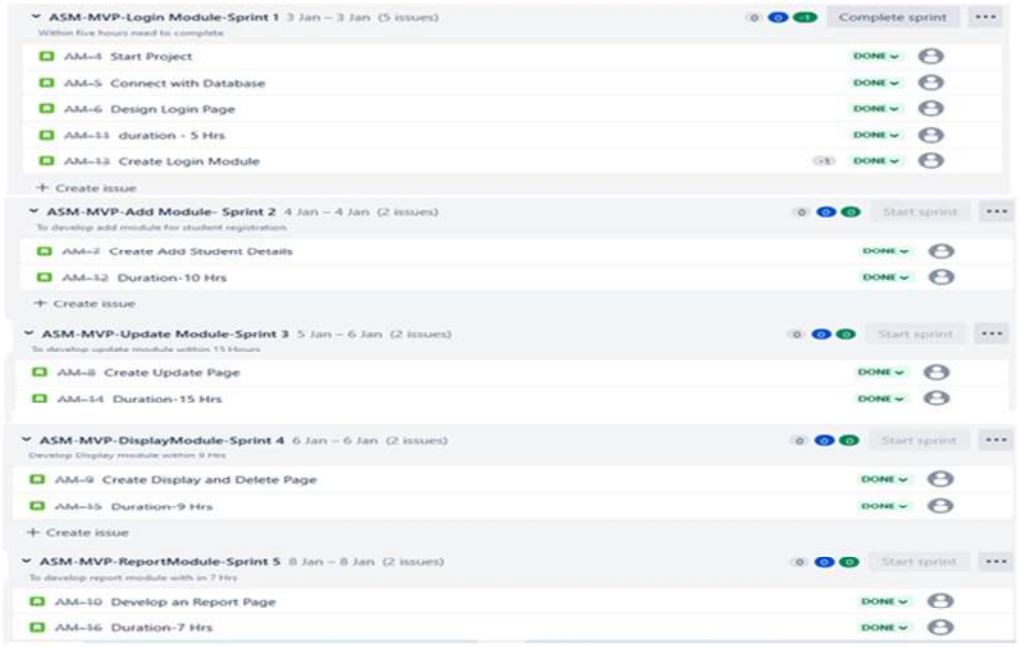

Figure 8: Implementation of ASM in MVP with JIRA

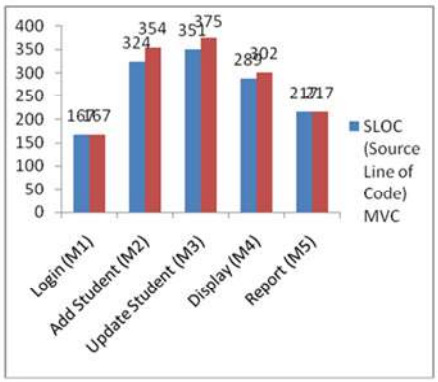

Figure 9: SLOC comparison between MVC and ASM-MVP

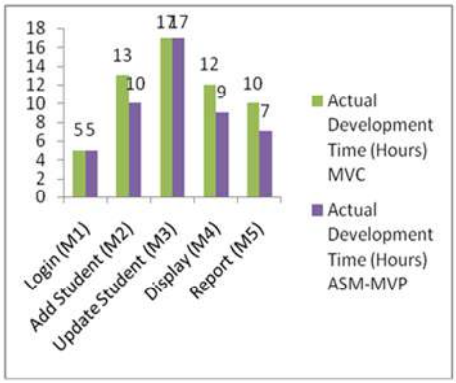

Figure 10: Development time comparison between MVC and ASM-MVP

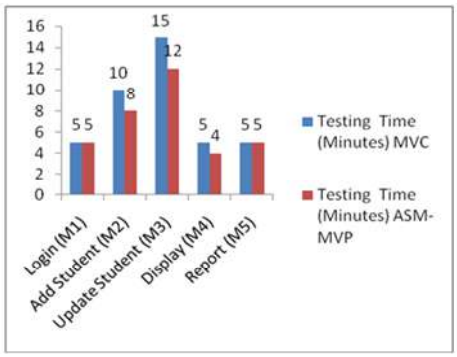

Figure 11: Testing time comparison between MVC and ASM-MVP

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Phytochemical Analysis and Antibacterial Potential of *Thuja occidentalis* Linn.

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Received: 19 Feb 2022 Revised: 04 Mar 2022 Accepted: 31 Mar 2022

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ABSTRACT

Thuja occidentalis Linn. is possess a potent medicinal value in the folklore system of medicine. *T.occidentalis* belongs to family Cupressaceae. It is a monoecious coniferous plant, it has hepatoprotective, antioxidant, antibacterial, antifungal, anticancerous and antidiabetic activity.*T.occidentalis*leaf and stem were selected for the experiment and extraction was done by soxhlet apparatus by using solvents methanol, hexane and petroleum ether. The extracts were subjected to preliminary antimicrobial screening against six pathogenic bacteria *Klebsiella pheumoniae, Vibrio cholera, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas gerugihosa, Salmonella typhi* by agar well diffusion method. The leaf extract showed presence alkaloids, glycosoids, flavonoids, saponins, tannins, and triterpenoids in methanol and petroleum ether extract and negative for all the metabolites in hexane extract. Whereas, stem extract showed similar metabolite presence in extracts. Results of antibacterial activity among these extracts methanol was found to be potent by exhibiting maximum of zone of inhibition at 75mg concentration for *K. pneumoniae*(10 mm), *V. cholera*(18 mm), *S. pneumonia*(15 mm), *S.aureus*(9 mm), *P.gerugihosa*(6 mm), *S.typhi*(10 mm) in leaf extract and *K. pneumoniae*(8 mm), *V. cholera* (17 mm), *S. pneumonia* (13 mm), *S. aureus*(7 mm), *P. gerugihosa*(8 mm), *S. typhi* (9 mm) in stem extract compared to other extracts.

Keywords: *Thuja occidentalis* Linn.,Soxhlet extraction, Phytochemical, Antibacterial activity, Cupressaceae

INTRODUCTION

Thuja occidentalis is a plant which belongs to Cupressaceae family, and commonly known as ''*Thuja''* is an evergreen coniferous tree which is used traditionally for treating many diseases, mainly used as antimicrobial agent to treat skin infection; it has been used to treat conditions such as bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [1].The 'Northern white cedar' is a monoeciousconifer with a height of 15-38 meter, stunted or prostrate in harsh, frigid environment. Occasionally the trunk is divided into two or three secondary stems, often reproduces from fallen trunks. The bark is reddish or greyish brown, 6-9 mm thick, fibrous, and fissured. The leaves of the branchlets are 1.5-3.5mm in length. Acute, dull yellowish green on both surfaces. The pollen cones are 1-2mm in length and reddish, the seed cones are ellipsoid, 9-14mm in length and brown in color. The leaf arrangement of *Thuja* is alternate with simple leaf and entire leaf margin. It has less prominent leaf venation with scaly leaves. The leaf as persistence fragrance and is evergreen. The length of the leaf blade is less than 2 inches. The color of leaf is green and when it falls, it does not change its colour.

Plant derived bioactive substances are good source of medicines that play a significant role for human health and also used against different types of microbial diseases[2, 3]. Plants have great medicinal relevance; infections have increased to a great extent and resistant against antibiotics become an ever increasing therapeutic problem. Moreover, in recent years, plant extract and their phytochemicals are getting more importance as they have the great potential sources for microbial and viral inhibitors. A number of researchers have focused their interest to investigate phytochemical constituents of plant for human health [4-6]. The bioactive constituents of plants such as tannins, flavonoids [7], saponins[8], terpenoids[9] and alkaloids [10] have great antimicrobial and other biological activities.

Medicinal Plants

In recent years, there has been a rising attention in drugs from medicinal plant origin is compared to the synthetics which are considered as unsafe to humans [Lu Y. Critical Care Nursing Clinics of North America. 2003]. Medicinal plants have been in existence for thousands of years [11]. They are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, tannins and saponins. The therapeutic value of the medicinal plants lies in the secondary metabolites present in it. It has been found to have antiviral, antibacterial, anti-inflammatory, antiulcer, and antioxidant properties for therapeutic applications [12]. Thus, the medicinal properties play an important role in developing newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs [13].

Many diseases are caused by oxidative stress that results from imbalance between the formation and neutralization of free radicals[14]. Oxidative stress initiated by reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide, and peroxynitrite damages. Cellular macromolecules are present such as DNA, protein, and lipids [15]. Among the effects, lipid peroxidation initiates inflammation processes. Therefore, inflammation is intertwined to oxidative stress[16]. Medicinal plants play important roles as source of antioxidant, inflammatory, and antibacterial agents. These bioactivities are mainly due to the presence of phenolic compounds [7]. Phytochemicals are known to possess antioxidant [17] antibacterial [18], antifungal [19], antidiabetic [20, 21], anti-inflammatory [22] and radio-protective activity [23], and due to these properties they are largely used for medicinal purpose. The development of the drug resistance and the undesirable side effects of certain antibiotics have led to the findings for new antimicrobial agents, mainly among plant kingdom, in order to find leads with unique chemical structures which may exert a unexploited mode of action. Deriving potential benefits from plants has always been a field of speculation for researchers and has formed the need for the development of drugs for treating various diseases. Hence forth, phytochemical screening of plants for the presence of natural products and beneficial properties is of a major avenue. The resistance acquired by microbes to the existing antibiotics seems to be a increased efforts in the development of new antibiotics. Although a number of plants with antimicrobial potential have been identified, a huge numbers still remains unidentified. Great range of bioclimatic variation from tropical to alpine brings richness in biological diversity. Many kinds of plants are prevalent in India and a large number of them

have been used for antimicrobial assay [24]. There is a urgent need of extensive studies of medicinal plants found with a special reference to their properties to fight against microbial diseases. The natural drugs are always a better substitute of synthetic drugs. The medicinal value of a plant lies on bioactive phytochemical constituents that produce a definite physiological action on the human body. These phytoconstituents work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions. The most important of these bioactive constituents of plants are tannins, flavonoids, carbohydrates, glycosides, steroids, terpenoids, lignin's, and fats[25].

MATERIAL AND METHODS

Collection of Plant Material and Extraction

The plant material was collected from Amanikere Park (Tumkur district). Stem and Leaf parts of plant were separated, washed carefully with tap water, rinsed with distilled water, air dried for 1 hour, and shade dried. They were ground into powder and stored in room temperature. The extract of the samples were prepared by soxhlet extraction method by using different selected solvents like methanol, n-hexane and petroleum ether. The extract was collected and test was carried out.

Preliminary Phytochemical Screening of Leaf and Stem Extract

The freshly prepared leaf and stem extracts of methanol, hexane and Petroleum ether were qualitatively analysed for the phytochemical screening using standard methodology as described by Sharangouda and Patil[26], Harborne[27]and Farnsworth[28] to determine alkaloids, glycosides, flavonoids, phenols, saponin, tannins, triterpenoids.

Antimicrobial Activity

Determination of Antimicrobial Activity

Antimicrobial activity of *Thuja occidentalis* methanolic, hexane and Petroleum ether extract were tested against the following bacterial strains *Klebsiella pneumoniae*, *Vibrio cholera*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* were obtained (procured) from Government I.D.S.G. College, Chikmagalur.

Agar Diffusion Assay

The modified agar well diffusion method was employed [29]. The bacterial suspensions were inoculated onto nutrient agar media by spread plate technique. Once it is dried, 4mm diameter wells were punched onto the media. Tetracycline was used as a standard antibiotic drug (2mg/mL), the leaves extracts of *Thuja occidentalis* were dissolved in DMSO (Dimethyl Sulphoxide) from which 25, 50 and 75 microlitres concentration of each extract were added into agar wells. The plates were sealed and incubated at 37^{o} C for 24 hr. The inhibition zones of diameter were recorded after the incubation period along the two cardinal diameters and were carried in triplicate to determine concurrent value. Same procedure was repeated with the stem extract and inhibition zone of diameter were recorded [30].

Statistical Analysis

All the experiments were carried out in triplicates and were expressed as mean ± standard error of the mean. The data were statistically analysed using Microsoft Office Excel 2007.

RESULTS

Phytochemical Analysis

The preliminary phytochemical screening of total methanolic extract, hexane extract and petroleum ether extract of leaf and stem were subjected to phytochemical test for identification of various plant constituents and they showed positive results for alkaloids, glycosides, flavonoids, saponins, tannins and triterpenoids in methanolic extract and petroleum ether extract, and negative results in hexane extract. The phenols were absent in this plant extracts.

Determination of Antibacterial Activity

The antimicrobial activity of *Thuja occidentalis* leaf extracts against the multidrug resistant pathogenic bacterial strains viz.,*Klebsiella pneumonia, Vibrio cholera, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* were tested. It has resulted in concentration dependent antibacterial activity in all the assays in minimum inhibitory concentration (MIC). The methanol extract was found to be potent by exhibiting maximum of zone of inhibition at 75mg concentration for *K. pneumoniae*(10 mm), *V. cholera* (18 mm), *S. pneumonia* (15 mm), *S. aureus*(9 mm), *P. gerugihosa*(6 mm), *S. typhi* (10 mm) in leaf extract and *K. pneumoniae*(8 mm), *V. cholera* (17 mm), *S. pneumonia* (13 mm), *S. aureus*(7 mm), *P. gerugihosa*(8 mm), *S. typhi* (9 mm) in stem extract compared to standard Tetracycline and control DMSO.

The petroleumether extract was found to be potent by exhibiting maximum of zone of inhibition at 75mg concentration for *K. pneumoniae*(10 mm), *V. cholera* (9 mm), *S. pneumonia* (13 mm), *S. aureus*(5 mm), *P. gerugihosa*(6 mm), *S. typhi*(1 mm) in leaf extract and *K. pneumoniae*(7 mm), *V. cholera* (7 mm), *S. pneumonia* (10 mm), *S. aureus*(4 mm), *P. gerugihosa*(5 mm), *S. typhi* (10 mm) in stem extract compared to standard Tetracycline and control DMSO. *T.occidentalis* of leaf and stem extract of methanol have active constituents to act as antibacterial potential. Methanolic and petroleum ether extract exhibited maximum inhibition against studied bacterial strains comparison with standard drug Tetracycline. The extracts showed prominent antibacterial activity with their inhibitory zones.

DISCUSSION

Over use of drugs and high incidence of their resistance is very common among the antimicrobial agents, the antibacterial effect evaluation on medicinal plant principles to treat the internal and external infections has a paramount importance in addressing animal as well as human health problems. The present study discusses the significance of phytochemical and antibacterial potential of *Thuja occidentalis* plant i.e., leaf and stem as a valuable source of secondary metabolites like alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids in methanolic and petroleum etherextracts. Among these, leaves exhibited potent extract of *Thuja occidentalis* revealed the remarkable antibacterial activity against *K. pneumoniae*(10 mm), *V. cholera* (18 mm), *S. pneumonia* (15 mm) maximum in methanol extract and *K. pneumoniae*(10 mm) and *S. pneumonia* (13 mm) maximum in petroleum ether extract compared to that of standard antibiotic drug. Similar studies observed by Sreedharan *et al* [10] reported the extracts from the seeds of *Rhussemialata* potent bioactive molecules and antimicrobial activity against five organisms, three bacterial strains (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*) and two fungal (*Aspergillusniger and Penicillium sp.*) using four different concentrations (10, 30, 60, 100 µl) of the dose dependent concentration of the extracts. Mummed *et al.,* [31] studied seven plants, namely *Cissus quadrangularis*, *Commelina benghalensis*, *Euphorbia heterophylla*, *Euphorbia prostrate*, *Momordicas chimperiana*, *Trianthema* spp., and *Solanum incanum* and were found antibacterial activity against any one bacteria in considerable results, in that the MIC value of *Euphorbia prostrata* ranged from 12.18 mg/mL to 390.00 mg/mL against *S. aureus* and *E, coli*, respectively to act as significant medicinal plant.

The antibacterial activities of the medicinal plants may be due to the presence of various phytochemical agents in the plant extracts, which are familiar to exhibit mechanism to use an antibacterial potential. In the present study, *T.occidentalis* possess active metabolite tannins and showed a better antibacterial activity in methanol and petroleum ether extract. Mecahnism and action of tannins may attribute to their ability to inactivate several enzymes, microbial adhesion, and membrane proteins during transport [32]. Flavonoids and saponins also present in both the extract to exhibit antibacterial activity, which could be emphasized their ability to form complex cellular proteins, soluble proteins and bacterial cell wall reactions [33–34].

CONCLUSION

The medicinal plants value lies in some phytochemical constituents that produce a definite physiological action on the human body. In the present investigation, phytochemical screening and antibacterial studies of *Thuja occidentalis* with methanol, petroleum ether and hexane solvents were carried out on leaf and stem extracts. The presence of active constituents was observed in methanol and petroleum ether extract and found maximum antibacterial activity against pathogenic organisms. Further studies on in vivo models may reveal better results and it will be highly applied to use it as antibacterial agent on infectious diseases.

ACKNOWLEDGEMENT

The authors are thankful to Department of Biotechnology, Government I.D.S.G. College, Chikmagalur, Karnataka, India for providing the laboratory facilities for the successful completion of the project.

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Table 1: Preliminary phytochemical screening of methanolic, hexane and petroleum ether extract of leaf of *Thujaoccidentalis*

Phytochemical tests	Methanolic extract	Hexane extract	Petroleum ether extract
Alkaloids	+	_	+
Glycosides	+	_	+
Flavonoids	+	_	+
Phenols	_	_	_
Saponin	+	_	+

Tannins	+	_	+
Triterpenoids	+	_	+

Table 2: Preliminary phytochemical screening of methanolic, hexane and petroleum ether extract of stem of *Thujaoccidentalis*

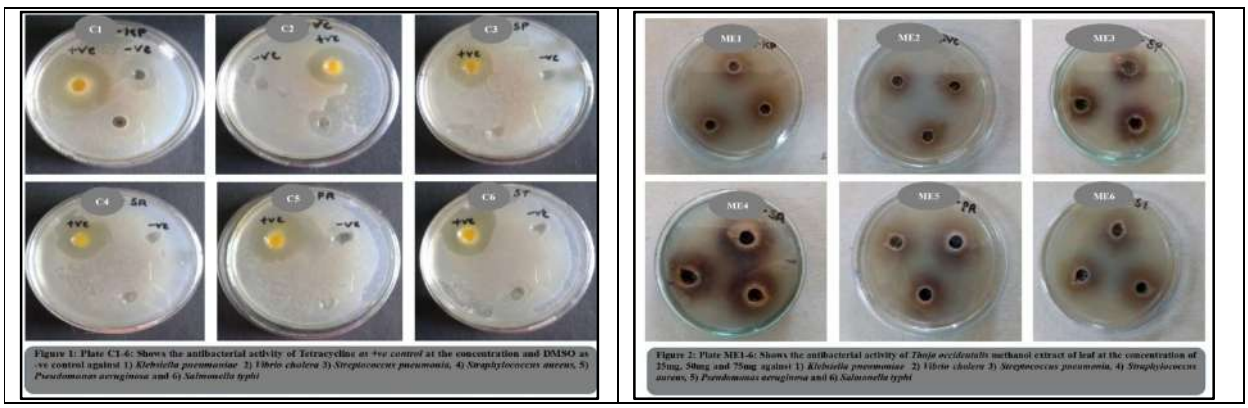
Phytochemical tests	Methanolic extract	Hexane extract	Petroleum ether extract
Alkaloids	+	_	+
Glycosides	+	_	+
Flavonoids	+	_	+
Phenols	_	_	_
Saponin	+	_	+
Tannins	+	_	+
Triterpenoids	+	_	+

Table 3: Antibacterial assay of methanol and petroleum ether extract of leaf of *Thujaoccidentalis*

Bacterial Strains	Methanol Extract			Petroleum Ether Extract		
	25mg	50mg	75mg	25mg	50mg	75mg
Klebsiella pneumonia	5mm	7mm	10mm	6mm	8mm	10mm
Vibrio cholera	13mm	15mm	18mm	4mm	6mm	9mm
Streptococcus pneumonia	9mm	12mm	15mm	8mm	10mm	13mm
Staphylococcus aureus	2mm	5mm	9mm	2mm	4mm	5mm
Pseudomonas aeruginosa	1mm	3mm	6mm	2mm	4mm	6mm
Salmonella typhi	6mm	9mm	10mm	8mm	9mm	1mm

Table 4: Antibacterial assay of methanol and petroleum ether extract of stem of *Thujaoccidentalis*

Bacterial Strains	Methanol Extract			Petroleum Ether Extract		
	25mm	50mm	75mm	25mm	50mm	75mm
Klebsiella pneumonia	4mm	6mm	8mm	4mm	6mm	7mm
Vibrio cholera	11mm	13mm	17mm	3mm	5mm	7mm
Streptococcus pneumonia	7mm	10mm	13mm	6mm	8mm	10mm
Staphylococcus aureus	2mm	4mm	7mm	1mm	3mm	4mm
Pseudomonas aeruginosa	1mm	2mm	8mm	2mm	4mm	5mm
Salmonella typhi	5mm	8mm	9mm	4mm	8mm	10mm



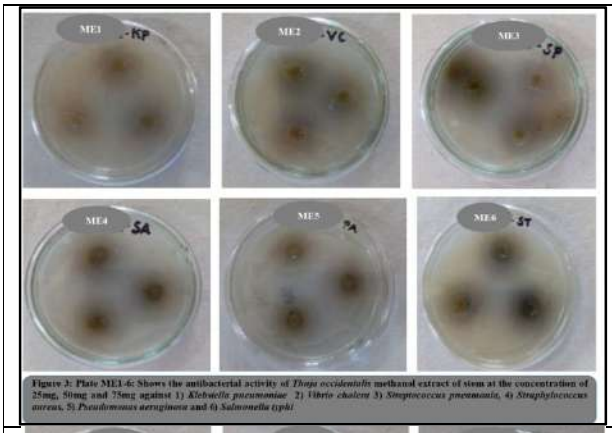


Figure 3: Plate ME1-6: Shows the antibacterial activity of *Thuja occidentalis* methanol extract of stem at the concentration of 25mg, 50mg and 75mg against 1) *Klebsiella pneumoniae* 2) *Vibrio cholera* 3) *Streptococcus pneumonia,* 4) *Straphylococcus aureus,* 5) *Pseudomonas aeruginosa* and 6) *Salmonella typhi*

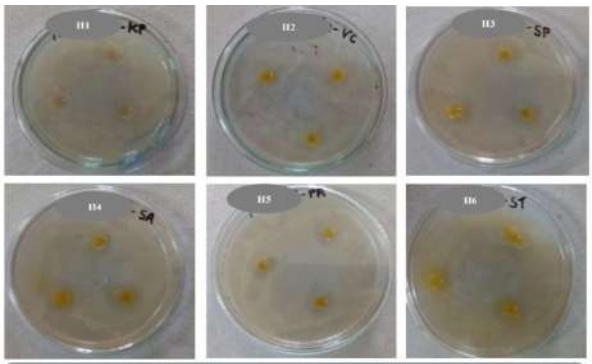


Figure 4: Plate H1-6: Shows the antibacterial activity of *Thuja occidentalis* n-hexane extract of leaf at the concentration of 25mg, 50mg and 75mg against 1) *Klebsiella pneumoniae* 2) *Vibrio cholera* 3) *Streptococcus pneumonia,* 4) *Straphylococcus aureus,* 5) *Pseudomonas aeruginosa* and 6) *Salmonella typhi*

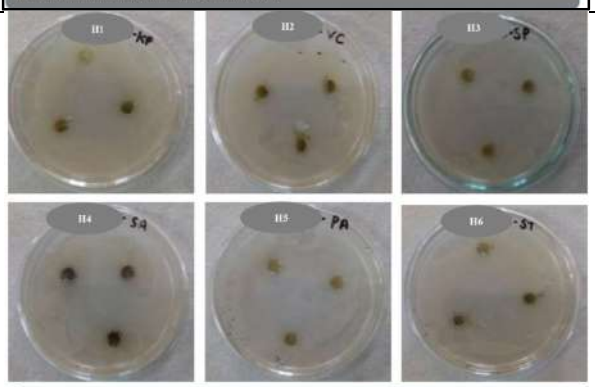


Figure 5: Plate H1-6: Shows the antibacterial activity of *Thuja occidentalis* n-hexane extract of stem at the concentration of 25mg, 50mg and 75mg against 1) *Klebsiella pneumoniae* 2) *Vibrio cholera* 3) *Streptococcus pneumonia,* 4) *Straphylococcus aureus,* 5) *Pseudomonas aeruginosa* and 6) *Salmonella typhi*

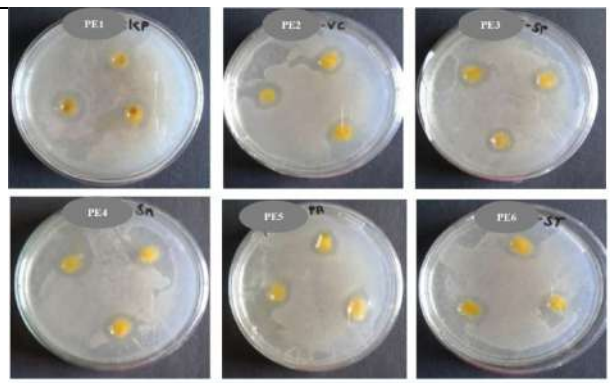


Figure 6: Plate PE1-6: Shows the antibacterial activity of *Thuja occidentalis* petroleum ether extract of leaf at the concentration of 25mg, 50mg and 75mg against 1) *Klebsiella pneumoniae* 2) *Vibrio cholera* 3) *Streptococcus pneumonia,* 4) *Straphylococcus aureus,* 5) *Pseudomonas aeruginosa* and 6) *Salmonella typhi*

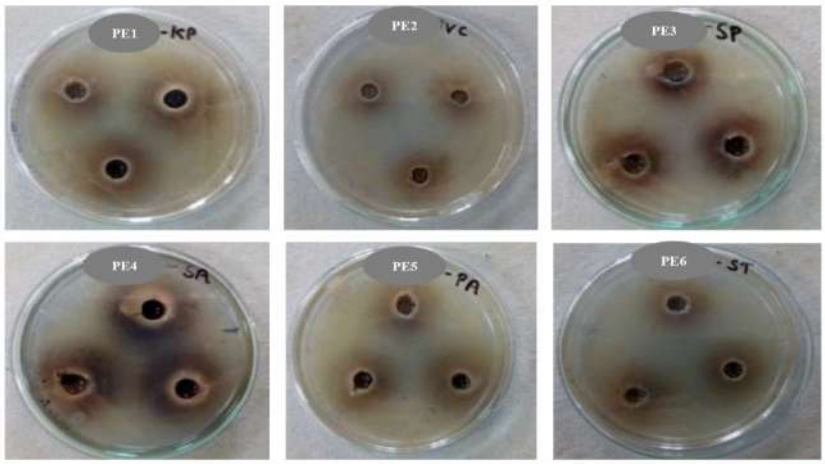


Figure 7: Plate PE1-6: Shows the antibacterial activity of *Thuja occidentalis* petroleum ether extract of stem at the concentration of 25mg, 50mg and 75 mg against 1) *Klebsiella pneumoniae* 2) *Vibrio cholera* 3) *Streptococcus pneumonia,* 4) *Straphylococcus aureus,* 5) *Pseudomonas aeruginosa* and 6) *Salmonella typhi*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Remote Factor Focused Intervention with the Knee Factor Focused Intervention Is More Effective Than Knee Intervention in Patellofemoral Pain Syndrome

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Received: 26 Jan 2022 Revised: 16 Feb 2022 Accepted: 15 Mar 2022

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ABSTRACT

A common orthopaedic problem seen in children, adolescents, adults and older age individuals is Patellofemoral pain (PFP). Pain around the patella is the primary symptom during activities that load the Patellofemoral Joint e.g. Squatting, Running and Stair Climbing. Patellofemoral pain (PFP) has an incidence rate as high as 40% among the general population. To examine the effect of remote factor intervention on pain and function capacity in patient with Patellofemoral pain syndrome 30Subject with patellof emoral pain syndrome were taken based on inclusion and exclusion criteria. There are two group A and B. Group A taken only Knee focused intervention and Group B taken remote factor focused intervention and knee focused intervention for 4 weeks, 3 days/week. Pre and post NPRS and KOOS-PF was checked. Pre and Post Analysis were done by Wilcoxon rank test through SPSS version 20.And the inter group analysis done by Mann-Whitney U Test. Improvement was seen in pain and function in both groups ($p < 0.05$). But greater improvement seen in remote factor focused intervention with knee focused intervention for 4 week. Hence from this study, it can be concluded that: four week (12 sessions) of either knee focused intervention or remote factor focused intervention were effective in improving function and reducing pain in patients with the patellofemoral pain syndrome. Improvements of pain and function were greater for the group who performed the remote factor focused intervention.

Keywords: Patellofemoral pain syndrome, Patellar maltracking, Q-angle, Remote factor focused intervention, Knee focused intervention, Patellar taping,

INTRODUCTION

A common orthopaedic difficulty seen in children, adolescents, adults and elderly age individuals is Patellofemoral pain (PFP) [1,2]. This pain limits their physical activities of choice and also altered the sports-related activities along with the activities of daily living[1, 2]. Pain around the patella is the primary symptomduring activities that load the Patellofemoral Joint e.g. Squatting, Running and Stair Climbing [3, 4].Swelling of the knee is not representative of Patellofemoral pain syndrome, although patients may report a sensation of stiffness, especially when the knee is flexed[5]. A "popping" or "catching" sensation may be described. Locking of the joint is not a symptom of PFPS5, however it can affect participation in daily work and exercise, with important implications for the prevention of conditions such as cardiovascular disease, diabetes and osteoporosis [4].

Patellofemoral pain (PFP) has an incidence rate as high as 40% among the general population6. PFPS is a common overuse injury in runners, accounting for nearly 20% of all running related injuries [7]. The 4th International Patellofemoral Research Retreat which was held in Manchester, UK, over 3 days (September 2–4th, 2015) has undergone peer review for scientific merit and relevance to the retreat. During this retreat, total 67 abstracts were accepted out of which 50 were podium presentations and 17 were short presentations. The podium and short presentations were grouped into five categories as mentioned below:
PFP;
Factors that influence PFP;
The trunk and lower extremity;
Interventions and

Systematic analyses
There were three keynote speakers who were chosen for their scientific contribution in the area of PFP. Among the three, Professor Andrew Amis gave highlights on the biomechanics of the patellofemoral joint [8]. Current literature suggests that the etiology of PFP is multifactorial in the absence of direct trauma. The most attention Factors related directly to the patellofemoral joint are vastus medialis obliquus insufficiency, decreased hamstring, quadriceps, or iliotibial band flexibility, patella alta, and femoral anteversion [9,10,11,12,13,14,15,16]. Suchfactors may also contribute to PFP through their role in causing patellofemoral malalignment, which is a universally accepted contribution to PFP [17, 14]. It is frequently found that a laterally positioned patella is a contributing factor to this pathology. This is because of its potential to overload the lateral articular surfaces of the patellofemoral joint during repetitive movement [18]. In patellofemoral pain, Radiological (MRI scan)and clinical measures19 have shown that there can be a significant laterally positioned patella. Maintaining the patellar alignment in the trochlear groove of the femur is necessary to restore functional efficacy of the patellofemoral joint [19]. Excessive training volume,rise in speed, and extended training on stairs or hills are some of the external variable that might cause overload of the PFJ [20]. Ground surface, footwear and anthropometry are some other factors contributing to Patellofemoral pain syndrome [20].

Internal variable are further classified into remote or local [20]. The distribution of load is interpreted as movement of the patella occurs within the femoral trochlear space otherwise known as Patellar Tracking [21]. It is also believed that some remote factors do effect patellar tracking. These remote factors are as mentioned below:
An elevated in femoral rotation
Elevated valgus stress at the knee
Raised tibial rotation
Excessive subtalar rotation, and
Inadequate flexibility [20].
Both local factors and remote factors have been identified through research such as a large navicular drop which suggests that both local and remote factors may increase an individual risk of developing patellofemoral pain [22, 23].Other important contributing factors of this syndrome are muscle imbalance between the medial and lateral

vastus and shortness of the iliotibial band [24, 25]. Factors which influence the load on the PFJ can be intrinsic or extrinsic [20]. There are also certain local factors which are assumed to be the contribution for abnormal tracking of patella. These local factors are patellar position, soft tissue contributions, and neuromuscular control of the vastus medialis, vastus lateralis and vastus intermedius [20]. The rehabilitation program should focus on correction of the maltracking of the patella by addressing the findings identified on the physical examination. Significant strengthening of the quadriceps may be required by some patients. Others may have excellent quadriceps strength but excessively tight lateral structures or poor quadriceps flexibility. There are techniques such as Soft tissue techniques and flexibility exercises which can prove to be helpful for these patients [5].

Distal and proximal strengthening has been suggested to be specifically important for some individuals with PFPS but has so far received little attention in research. Thus, the purpose of the study is to compare the effect of standard knee targeted exercise therapy versus adding a remote factor targeted exercises on pain and functional outcome among a subgroup of individuals.

Need of the Study

Distal and proximal strengthening has been suggested to be specifically important for some individuals with PFPS but has so far received little attention in research. Thus, the purpose of the study is to compare the effect of standard knee targeted exercise therapy versus adding a remote factor targeted exercises on pain and functional outcome among a subgroup of individuals.

Aims and Objectives

Aim

To examine the effect of remote factor intervention on pain and function capacity in patient with Patellofemoral pain syndrome.

Objectives

To find out and compare the effect of knee factor intervention on pain and functional capacity in individual with the patellofemoral pain syndrome

To find out and compare the effect of remote factor intervention on pain and functional capacity in individual with the patellofemoral pain syndrome.

To compare the effect of knee factor intervention and the remote factor focused intervention

METHODOLOGY

Method

Study Design: Experimental Study
Study Setting: Various Clinics in Rajkot City
Sampling Technique: Convenient Sampling
Study Population: Youngsters Age between 18-40 Years
Study Sample: 30 Subjects
Study Duration: 6 Months

Criteria for Selection

Inclusion Criteria: [26].

Anterior or retro patellar pain, insidious in nature, which was aggravated by at least two of the following common functional activities of daily life:-

Prolonged Sitting
Stair Climbing
Squatting
Running
Kneeling
Hopping/Jumping
Pain during an objective single leg squatting of 10-seconds
Aged less than 40 years (both genders)

Exclusion Criteria [26].

Any trauma, inflammatory or infectious pathology in the lower extremity
Any dislocation or subluxation in the Patellofemoral pain syndrome
Any history of surgical treatment in the knee joint
Any signs of mimic secondary osteoarthritis in the knee joint

Procedure

The ethical clearance for the study was taken from the Ethics Committee (ECR/259/Indt/GJ/2016) School of Physiotherapy, R K University, Rajkot and the trial was registered with CTRI/2018/09/10/015988. Several Orthopaedics in Rajkot city were approached for the subjects with Patellofemoral pain syndrome. The subjects were included in the study through convenient sampling according to the inclusion and exclusion criteria. Their consent was taken for the participation in the study. The baseline assessment of the subjects were taken, were asked for their pain intensity using the NPRS and their quality of life was measured using KOOS. The subjects were allocated the groups according to the sequence of their arrival. Group A had subjects with odd number arrival and were given Knee factor focused intervention while Group B had subjects with even number arrival and were given remote factor focused intervention for 4 weeks, 3 days per week on alternate days. The pain intensity and quality of life was measured using NPRS and KOOS, respectively after 4 weeks.

Interventions

Local Factor (Knee) Targeted Intervention [27].
Rigid Patellar Tapping(24 hour, 3 days/week, for 4 week)
Specific Strengthening Of VMO (3*10 repetition)
Sitting position 90^{o} hip and knee flexion, isometric knee extension
Small range of flexion & extension movement in walk standing position
Step up and step down
Patellar Mobilization
Remote Factor Targeted Exercise [28, 29].
Strengthening:
Hip abduction against elastic band (standing), 3 *10 repetitions
Hip external rotation against elastic band (sitting), 3*10 repetitions
Side stepping against elastic band ,3 *10 repetitions
Tib. Posterior (theraband)
Heel raise, 3*10 repetition
Short foot 3*10 repetition

Stretching of: (30 sec)
Hamstring muscle
Iliotibial band
Gastrocnemius muscle

RESULT

The present study was carried out to find out the effect of remote factor focused intervention in patellofemoral pain syndrome. To all subject given either the knee factor focused intervention or the remote factor focused intervention. Data was analysed using SPSS software version [21] and Microsoft excel. Before applying statistical tests data was screened for normal distribution. Changes in outcome measures were analysed intra group and inter group.

DISCUSSION

The purpose of the present study was to find out and compare the effectiveness of the remote factor focused intervention with knee focused intervention in patient with patellofemoral pain syndrome. The result of the present study indicated significant differences in pain intensity and quality of life in both the groups. Also significant improvement was seen in the remote factor focused intervention than knee factor focused intervention. There for, the null hypothesis is rejected and alternative hypothesis accepted. One of the reasons why we gave McConnell tapping to our subject that It was proposed that taping over the skin; stimulates cutaneous mechanoreceptors and leads to increased afferent feedback to the central nervous system which decreased the pain and also corrects the patellar tracking & centralizing it within the trochlear groove [33]. Patellar mobilization helps with correction of the patellar position disturbed due to alteration in the biomechanics and muscle imbalance.

Strengthening exercises for hip external rotators and abductor muscles helps in improving and normalizing their roles in knee biomechanics [34]. In patellofemoral pain syndrome, an increase in the Q-angle is observed, which leads to decrease in strength of quadriceps and hip external rotators[35]. Strengthening of these hip and knee muscles provides better stability and posture at both joints[36]. Poor foot arches and altered foot biomechanics is also an important factor for increase in the Q angle[37]. Therefore, short foot exercises helps in strengthening these foot muscles and correcting the biomechanics. This is one of the reason for significant improvement in pain intensity and quality of life of group B than Group A. Hence, a significant reduction in pain intensity and better quality of life is seen in remote factor focused intervention with knee focused intervention than only knee focused intervention. Flavio Fernandes Bryk At Al, 201029 studied on short term effect of hip abductors and lateral rotators strengthening in females with Patellofemoral pain syndrome by measuring pain intensity with NPRS as outcome measure.

Mitchell Selhorst et al, 201538 evaluated the treatment algorithm for patients with patellofemoral pain syndrome with NPRS as their outcome measure. KOOS-PF is a patient reported outcome measure (PROM). And it is specially developed for patellofemoral pain. Kay M Crossley At Al, 2018 developed the patellofemoral pain and osteoarthritis subscale of the KOOS (KOOS-PF)[32]. Individuals having increased calcaneal eversion had larger improvements in pain with specific foot exercises which was supported with distally oriented treatment program as observed by Moolgaard CM28. Ghoubanpour A. observed the patellar realignment with repeated stimulation of the vastus medialis obliques muscle with the help of its strengthening[39]. Fukuda TT observed increased adduction and internal rotation of femur is caused due to weakness of hip muscles during dynamic weight bearing activities causing overload of patellar facet which is corrected by its strengthening[29]. Augustsson J suggested the closed kinetic chain exercises in patellofemoral pain syndrome management which enhanced the proprioception, replicated the role of lower limb muscles and lowered the stress on patellofemoral joint40. Tulder MW concluded proposed the strengthening of the quadriceps to overcome its imbalance and correct the biomechanics improving the patellofemoral pain syndrome41.

Gringstaff TL found an immediate increase in quadriceps force output and activation after lumbopelvic joint manipulation which might address specific impairments associated with PFPS like asymmetries in hip rotations and decreased quadriceps activation [4]

CONCLUSION

Four week (12 sessions) of either knee focused intervention or remote factor focused intervention were effective in improving function and reducing pain in patients with the patellofemoral pain syndrome. Improvements of pain and function were greater for the group who performed the remote factor focused intervention.

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Table 1 Test used for Intra group Analysis

Outcome Measure	Test used to compare within group
NPRS	Wilcoxon test
KOOS-PF	Wilcoxon test

Table 2 Baseline Characteristics

Total no. of participants	30
Gender	M,F
Mean age	26.73

Table 3 Mean age of both group

	GROUP A	GROUP B
Mean	27.6000	25.8667
SD	4.76295	4.3893

GROUP A

Table 4 Group A NPRS Intra group Analysis

NPRS	Mean	SD	P- VALUE
PRE	7.5333	.63994	0.00
POST	4.4000	.50709	

Table 5 Group A KOOS-PF Intra group Analysis

KOOS-PF	Mean	SD	P- VALUE
PRE	56.51	3.19	0.00
POST	70.75	3.73	

GROUP B

Table 6 Group B NPRS Intra group Analysis

NPRS	Mean	SD	P-VALUE
PRE	7.4667	.7432	0.00
POST	2.6667	.6172	

Table 7 Group B KOOS-PF intra group Analysis

KOOS-PF	Mean	SD	P-VALUE
PRE	55.00	5.83	0.000
POST	82.57	3.17	

Table 8 Test used for Inter group Analysis

Outcome Measure	Test used to compare between group
NPRS	Mann-Whitney Test
KOOS-PF	Mann-Whitney Test

Table 9 NPRS Inter group Analysis

NPRS	Mean	SD	P- VALUE
Group A	3.1333	.74322	0.00
Group B	4.8000	.67612	

Table 10 KOOS-PF Inter group Analysis

KOOS-PF	Mean	SD	P-VALUE
Group A	14.2424	4.16	0.00
Group B	27.57	6.42	

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of *Coleus aromaticus* and *Ocimum basilicum* on Immune Response of *Oreochromis mossambicus* Infected with *Aeromonas hydrophila*

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Received: 06 Jan 2022 Revised: 31 Jan 2022 Accepted: 24 Feb 2022

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ABSTRACT

An investigation was carried out to evaluate the effect of Coleusaromaticus and *Ocimum basilicum* leaf powder as supplemented feed on the relative percent survival and antigen antibody titer. Four experimental feeds were prepared by adding 2.5 grams and 5 grams of leaf powders to the basal diet and one control feed without leaf powder. The *Oreochromis mossambicus* fishes were supplemented with these feeds for 45 days. After 30 days of feeding trial the fishes were infected with *Aeromonas hydrophila* and survival rate of fishes were noted and antibody titer were done on 38th and 45th day after treatment. The data obtained were analyzed using one way analysis of variance (ANOVA). The fishes fed with 5gm coleus aromaticusand *Ocimum basilicum* leaf powder supplemented feed showed greater effect in terms of survival rate and antigen antibody titer. This study indicated that inclusion of leaf powder in fish feed resulted in better survival and antigen production. The formulation of plant based diet for fish will provide new opportunities.

Keywords: Leaf powder, supplementation, *Aeromonas hydrophila*, relative percent survival rate and the antigen antibody titer.

INTRODUCTION

Aquaculture ascertained by FAO (1990) [1]. as the meadows of aquatic organisms containing crustaceans, fishes and aquatic plants with some species of invasion in the culturing process to reinforce productions such as furnishing,

feeding and conservation from predators. From ancient times, the aquaculture has been conducted and it is spread all over the world constantly it is revolutionized from a tradition into science (FAO, 1990)[1]. It increases its growing rate of 6 % annually and it has been gaining importance over capture fisheries since 1990s2. Disease outbreaks are increasingly being recognized as a potential constraint on aquaculture production and trade and cause massive financial loss either through mortality or reduced profit margins[3&4]. *Aeromonas hydrophila*is a ubiquitous organism present in the aquatic environment causing diseases in fish under stress[5]. *A. hydrophila*is a gram negative, motile rod recorded as an opportunistic pathogen in freshwater fish species and it is considered to have widespread geographical distribution[6]. *A. hydrophila*has been recognized as the causative agent of hemorrhagic septicemia/ motile *aeromonas* septicemia, skin ulceration, fin/tail rot and sore disease[7]. Often, healthy fish suddenly develop swimming abnormalities, pale gills, bloat (abdominal distension) and dermal/ ocular ulcerations. Many plant powders and extracts with antimicrobial, antibacterial and immunostimulant properties have been used as therapeutic agents against fish pathogens. Against bacteria, they disrupt the bacterial cell wall, block the synthesis of proteins and DNA, inhibit enzyme secretion and hinder the bacterial signalling mechanism via quorum sensing [8].
Coleus aromaticus Benth syn. *Coleus ambionicus*(Lour) Spreng or *Plectranthusambonicus* L our, one of the plants of genus *Coleus* which is native to India and Mediterranean. The plant is also known by different folk names such as Country-borage, Indian-borage (English), Ajwainpatta, Karpuravalli (Tamil). It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, Chronic asthma, hiccough, bronchitis, colic convulsions and epilepsy [9].

Sweet basil *(Ocimum bascilicum*L.) is native plant of Indo-Malayan region. It is called the "King of herbs" which contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits. Sweet Basil has shown unique health protecting effects due to its important flavonoids and volatile oils. The unique array of active constituents called flavonoids found in basil provides protection at cellular level[10]. So the investigation aimed at enhancing the immune system of *O.mossambicus*challenged with *A.hydrophila*andby supplementing the fisheswith *C.aromaticus* and *O.basilicum*leaf extract as supplemented feed.

MATERIALS AND METHODS

Experimental Fish Collection And Maintenance

The experimental animal selected for present study was Tilapia fish (*O.mossambicus*). The fingerlings of *O.mossambicus* was collected from Aliyar dam, near Pollachi, Coimbatore district, Tamil Nadu. They were acclimatized for 2-3 weeks in the laboratory and was then transferred in to the experimental tanks. They were fed with commercial feed during this period. The tanks were maintained with care and were kept clean. The excess food and fecal matter were removed on a daily basis. The water was changed once in a week keeping 50 % of the habitat water.

Collection Of Plant Samples

The plant selected for the present study are *C.aromaticus* and *O.basilicum*. The leaves from these plants were collected from in and around Coimbatore, Tamil Nadu.

Processing And Preparation Of Leaf Powder

Fresh leaves of *C.aromaticus* and *O.basilicum* were collected, washed and shade dried. The dried flowers were finely powdered and stored in air tight containers at room temperature for further use.

Preparation Of Feed

Fish feed was prepared by adding equal proportions of wheat flour and coconut oil cake in the ratio of 1:1:1 and corn flour as a binder. These substances were mixed thoroughly with hot water and it was steamed for 25-30 minutes and then cooled at room temperature for 30 minutes. Pellets were prepared by using domestic appliances with 0.5 mm

diameter. It was dried by keeping in the sun. Four experimental diets were prepared by adding 5 grams and 10 grams of flower powders separately and the feed without plant powder was kept as control.

Selection of Pathogen

The fish pathogen *A.hydrophila* was provided from the department microbiology, PSG Institute of research and management, Coimbatore. The pathogen was maintained on agar slopes at 4°C and was used for infecting the healthy fish. *A. hydrophila* was cultured in a nutrient agar broth for 24 hrs at 37°C in an incubator. The cultured broth was then centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the pelleted bacteria were washed thrice with phosphate buffer saline (PBS) and prepared to 108 cfu/ml as determined using Neubaur haemocytometer slide[11]. This bacterial suspension was used for further experiments.

Experimental Design

The fishes were randomly distributed into 5 experimental tubs. Each tub consisted of 8 fishes. The fish were fed with experimental feed at the rate of 5 % of body weight once in a day before 9:00 for 45 days. Group C was fed with basal diet and it acts as the control. The remaining groups were fed with 2.5g of *C.aromaticus*(T_1), 5g of *C.aromaticus*(T_2), 2.5g of *O.basilicum*(T_3), 5g of *O.basilicum*(T_4).

Pathogen Challenge Test

After 30 days of feeding trail, fishes in the control and treatments were injected intraperitoneally with 0.1 ml (or) 100 µl of 10-4 cfu/ml *A.hydrophila* suspension. Mortality was recorded until 15 days after post challenge. Behavioural alterations, feeding response and mortality were observed daily and dead fish were removed. The experiment was conducted in triplicate. The behavioural changes and survival were observed.

Relative Percent Survival

$$\text{Relative percent survival (RPS)} = \frac{\text{1- (\% of Mortality in treated group)}}{\text{(\% of Mortality in control group)}} \times 100$$

Antigen-Antibody Titer (Agglutination Test)

Serum antibody titer was measured using the agglutination protocol described by [12] after 7 days of infection. The agglutination test was assayed in well microtiter plates. Serum (15 µl) was diluted at 1:1 ratio in saline PBS in the first well and was serially diluted in the wells of the first row till the 11th well of the microtitre plate leaving the 12th well as a negative control. Similarly, other serum samples were also diluted serially in each row of the microtitre plate. 50 ml of the antigen was added to all the wells. Gently shake the microtitre plate for efficiently mixing of the reagents. Incubate the titer plates for one hour at room temperature. The highest dilution of the sample which shows detectable (macroscopic) agglutination was recorded and expressed as log2 antibody titre of the serum.

Statistical Analysis

The results of the present study were subjected to statistical analysis. The data obtained on immunological parameters were analyzed using one way ANOVA and the level of significance was defined at $p<0.05$.

RESULTS

Relative Percent Survival

The relative percent survival was 100% in $T_2$and T_4 fishes on 38th day after treatment when compared to control (86.6%)(Table -2). On 45th day of treatment, 100% survival was observed in T_2 and T_4 the treatments whereas control showed 73.3%relative percents urvival (Table -2).

Antigen-Antibody Titer

The antibody response to *A.hydrophila* by various experimental groups fed with different concentrations of leaf powders of *C.aromaticus* and *O.basilicum* were found out by using antibody titer plate. The antibody response produced by experimental fishes was higher than the control group. The antibody response T_2 and T_4 fishes were 1.041whereas control showed a minimum antibody titer value 0.698 on 38th and 45thday after infection.

DISCUSSION

The leaf powders used in this study would have enhanced the immune response in all experimental groups. The present study revealed that fishes fed with leaf powders supplemented feed showed higher survival rate when compared to control. This might be due to the action of the phytochemical components present in leaf powders. The secondary metabolites found in the leaf powders might be responsible for significant increase in the survival rate of the fishes in experimental groups. The results of present study correlate with the findings13who reported that *Mystus montanus* fingerlings fed with medicinal plants showed increased resistance and survival against *A.hydrophila. L. rohita*fed with garlic and mango kernel showed increased survival rate against *A.hydrophila* when compared to control [14]. A positive effect on the survival rate of common carp observed when treated with *Avena sativa* extract against *A.hydrophila [*15]. Fishes fed with 5%*Ocimum basilicum* showed greater effect in terms of survival and at 10% it showed better antigen antibody titer[16]. The antibody titer was significantly higher in experimental groups when compared to control and this increase in the production of antibody might be due to the immuno stimulatory effect of flower extracts in the experimental feed. The phytochemicals found in the leaf powders were able to resist against bacteria which resulted in enhanced antibody production.

The results of present study were similar [17]. who reported that the *Euphorbia hirta*plant leaf extract enhance antibody response but the extract which are in higher concentration (25 to 50g) were only able to stimulate higher antibody production. Leaf extracts of *Phyllanthusniruri* and *Acalyphaindica* stimulated the antibody response in Tilapia (*O.mossambicus*) [18]. *Ocimum sanctum* positively determined the immunomodulatory effect stimulate the antibody response and disease resistance in *O.mossambicus* against the infection of *A.hydrophila[*19].

CONCLUSION

The results on present study indicated the beneficial role of selected leaf powders of *C.aromaticus* and *O.basilicum*as immunistimulants against the pathogen *A.hydrophila* in *O.mossambicus.* The study revealed that leaf powders at all concentration enhanced the survival rate of the fish against the pathogen. In general, the immunostimulant was found to produce antibodies in greater amount against the pathogen. The study thus states that *C.aromaticus* and *O.basilicum*are suitable as supplemented feeds for fishes at farm level as they have been identified as effective immune stimulants against certain pathogens.

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Table 1: Ingredients Used In Feed Preparation

	Diet				
Composition	**C**	**T_1**	**T_2**	**T_3**	**T_4**
Wheat flour	50	50	50	50	50
Coconut oil cake	50	50	50	50	50
Corn flour	1	1	1	1	1
C.aromaticus	-	2.5g	5g	-	-
O.basilicum	-	-	-	2.5g	5g

Table -2: Relative Survival Rate in *O.mossambicus* fed with *C.aromaticus* and *O.Basilicum* Leaf Extracts As Supplemented Feed (5 And 10 Gm/Kg Of Feed) On 38thday.

Treatments	No. of fishes introduced	No. of fishes dead	% mortality	No.of fishes survived	% survivial
Control	15	2	13.4±0.08	13	86.6±11.57
T_1	15	1	6.67±0.38	15	93.3±5.57
T_2	15	0	0±0.00	15	100±0.00
T_3	15	1	6.67±0.38	15	93.3±5.57
T_4	15	0	0±0.00	15	100±0.00

Table -3: Relative survival rate in *O.mossambicus*fed with *C.aromaticus*and *O.basilicum*leaf powders as supplemented feed (5 and 10 gm/kg of feed) on 45th day.

Treatments	No. of fishes introduced	No. of fishes dead	% mortality	No.of fishes survived	% survivial
Control	15	4	26.67±7.64	11	73.33±22.7
T_1	15	1	6.67±0.38	15	93.3±5.57
T_2	15	0	0±0.00	15	100±0.00
T_3	15	2	13.4±0.08	15	86.66±11.57
T_4	15	0	0±0.00	15	100±0.00

Table -4: Antibody titer in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* leaf powders as supplemented feed (5 and 10 gm/kg of feed) on 38thand 45thday.

Treatments	Antibody titer (38th day)	Antibody titer (45th day)
Control	0.698	0.698
T_1	0.954	1.000
T_2	1.041	1.041
T_3	0.845	1.000
T_4	1.041	1.041

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Different Organic Manures on the Nutrient Release Pattern in Sandy Loam Soil

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Received: 10 Jan 2022 Revised: 25 Jan 2022 Accepted: 28 Feb 2022

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ABSTRACT

The study of nutrient release patterns from various organic manures is critical for ensuring enough nutrient availability to crop plants at the right time and in the right quantity. To assess the nutrient release pattern from several organic manures, including poultry manure, goat manure, pressmud, vermicompost, neem cake, and composted coir pith, a laboratory incubation experiment was done in a sandy loam soil. They were applied based on the organic manure's nitrogen content. The release pattern of available nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur in soil incubated with various organic manures at various days (0,15,30,45,60,75,90) under controlled aerobic conditions was determined using a Completely Randomized Design. The study revealedthat application of various organic manures substantial increase in primary (N,P and K) and secondary nutrients (Ca, Mg and S) release throughout the incubation period. Based on the release of nutrients, it was found that there was consistent and stable release of N, P, K, Ca, Mg and S from poultry manure, goat manure and press mud throughout the incubation period upto 90 DAI, compared to other three manures viz., vermicompost, neem cake and composted coir pith.

Keywords: Poultry manure, Goat manure, Press mud, Vermicompost, Neem cake, Composted coir pith, N, P, K, Ca, Mg, S release in soil, Incubation periods.

INTRODUCTION

It is critical to maintain proper nutrients in the soil in order to ensure long-term crop output. Organic manure breakdown in soils is vital for soil fertility maintenance and laboratory incubation studies are useful and widely used tools for researching it. By alternating leguminous and non-leguminous crops and adding organic manures, it might be accomplished and achieved in agricultural systems with low chemical fertiliser and pesticide inputs. Non-composted organic wastes from agriculture, industry, municipal activities, seaweed, or blood and bone meal can be used in crop plants instead of composted organic manures (Quilty and Cattle, 2011). The release of nutrients fromvarious organic manures viz., poultry manure, goat manure, pressmud, vermicompost, neemcake, composted coirpith at various period of incubation, depends on its chemical and biochemical characteristics referred to as its quality. Residue quality and environmental conditions regulate the rate and the extent of decomposition of organic matter by soil organisms (Moorhead *et al.,* 1996).The proportion of mineral N released from the material added may become part of the mineralized N pool; it may be immobilized by microbes and hence become part of a microbial biomass pool, or may be denitrified and lost as either nitrous oxide or dinitrogen (Calderon *et al.,* 2005).Organic manure application improves plant development and may reduce the requirement for mineral fertilisers (Mohanty *et al.,* 2011), lowering farmer expenditures. Organic manures replenish soil nutrients by preserving organic matter and maintaining soil fertility for agricultural output, especially in long-term crops, by slowly releasing nutrients (Tejada *et al.,* 2009). As a result, organic manures recycle nutrients and organic matter to boost crop output while also preserving soil quality (Whalen *et al.,* 2001).

Soil organic matter serves as a repository for nutrients like nitrogen, phosphorus, and sulphur, as well as improving the physical, chemical, and biological aspects of soils (Diacono and Mon-temurro, 2010). Soil organic matter stimulates the growth and activity of soil microorganisms, resulting in efficient crop nutrient mineralization (Tejada *et al.,* 2009). Plants, animals, and bacteria all contribute to soil organic matter. These organic materials decompose via mineralization to release the nutrients essential for crop growth and development, whether they are introduced to the field or existing on-site (Diacono and Montemurro, 2010).Organic manures have recently gained appeal in agriculture as an alternate technique for managing waste and increasing soil organic matter content in low-fertile soils (Flavel and Murphy, 2006).

Mineralization of nitrogen is a biological process. The chemical composition of organic matter (e.g., nitrogen content, C:N ratio, and contents of cellulose and hemicelluloses, lignin, and polyphenols) (Mohanty *et al.,* 2011) and the physical, chemical, and biological properties of soil microbes (Manojlovi'c *et al.,* 2010) influence the amount of nitrogen released to crops. Organic manures with a high nitrogen content and a low C:N ratio mineralize enough nitrogen to support plant growth (Cordovil *et al.,* 2005). Organic manures with lower N content and higher C:N ratios, on the other hand, can immobilise N. To avoid damaging the ecosystem, it is critical to apply organic manures properly (Manojlovi'c *et al.,* 2010). N and P availability in the soil increases as soil organic matter increases (Ewulo *et al.,* 2008). The application of organic manures to soils greatly enhanced the available P content, according to Ahmed and Ali (2005). Sarwar *et al.* (2010) also found that applying organic manures to the soil improved the amount of N, P, and K in the soil. According to Youssef (2011), applying organic manures alone or in combination with efficient microorganisms greatly enhanced the available soil phosphorus content.Furthermore, Adeleye*et al.* (2010) found that applying poultry manure increased total N, accessible P, and K levels. The use of organic and biofertilizers could result in an increase in the soil's accessible and exchangeable potassium (Dadhich *et al.,* 2011). Organic materials and effective microorganisms had a considerable favourable impact on both accessible and exchangeable K. (Youssef, 2011). However, nothing is known regarding the release of other plant nutrients from various organic manures. For optimum exploitation of organic nutrient sources in crop production, the availability of P, K, and S, as well as micronutrients, from organic manures must be examined (Villegas-Pangga *et al.,* 2000).

Various organic manures *viz.,* cattle manure, compost, and green dung have been researched to see how they affect N mineralization and the microbial population as major decomposers. In this study, we focused on the effect of various organic manures on the release of available nutrients for ensuring enough nutrient availability to crop plants at the right time. Organic matter aids in the optimization of nutrient efficiency in agricultural crop production systems. Understanding the nutrients released by organic residues will allow management techniques to be designed that make better use of these organic residues (Villegas-Pangga *et al.,*2000).

MATERIALS AND METHODS

An incubation study was conducted at Department of Soil Science & Agricultural Chemistry, Annamalai University, Tamil Nadu, India to study the release pattern of available Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sulphur from various organic manures *viz.* poultry manure (O_1), goat manure (O_2), press mud (O_3), vermicompost (O_4), neem cake (O_5) and composted coir pith (O_6). Prior to seeding the crop, bulk soil samples for the incubation study were taken from the field experimental location. It was used for an incubation study after being air dried, finely powdered, and sieved with a 2 mm sieve.This soil had the initial available nutrient content of 214.6 kg of N ha^{-1}, 13.2 kg of P ha^{-1}, 275.4 kg of K $ha^{-1}$and 7.4 mg of S kg^{-1}. The available Ca and Mg content of the soil was 8.21 and 5.85C mol (p+) kg^{-1}.

For the conduct of incubation experiment, triplicate of 200 g of soil samples were taken in plastic cups and were mixed with nitrogen based doses of poultry manure, goat manure, press mud, vermicompost, neemcake and composted coir pith. The experiment was laidoutin Completely Randomized Design. Distilled water was added to maintainthe moisture content at field capacity. Periodic soil samples were drawn at 15 days interval upto 90 days. The sampling for analysis of available N, P,K, Ca, Mg and S of soil was done at 0,15,30,45,60,75 and 90 days after incubation. The nutrient content of organic manures (Table 1)were determined as per standard methods viz., nitrogen content was determined by micro-kjeldahl method (Humphries,1956), phosphorus, potassium, calcium and magnesium content were determined by vanadomolybdate yellow colour method, flame photometer and versenate titration method as outlined by Jackson (1973) and sulphur content was determined by turbidimetric method by Chesnin and Yien (1951). The available nitrogen was determined using the alkaline potassium permanganate method (Subbiah and Asija, 1956), available phosphorus was determined using the ammonium–molybdate–ascorbic acid method using a Spectrophotometer as outlined by Olsen *et al.,* 1954, and available potassium was determined using the flame photometer method after extracting the soil with neutral normal ammonium acetate as outlined by Olsen *et al.,* 1954 (Hanway and Heidel,1952) The available Ca and Mg were determined using the versenate titration method with neutral normal ammonium acetate (Jackson, 1973), while the available Sulphur was evaluated using the turbidimetric method with 0.15 $CaCl_2$ extract (Jackson, 1973). (Chesnin and Yien, 1951). For correct interpretation, the data obtained for all of the traits under study were statistically analysed as proposed by Gomez and Gomez (1976). The critical difference was calculated at a 5% probability level for significant results

RESULTS AND DISCUSSION

Available Nitrogen (Table2)

The release of available nitrogen in incubated soil was significantly and positively influenced by the application of variousorganic manures and period of incubation. The available nitrogen content ranges from 107.20 mg kg^{-1} to 119.83 mg kg^{-1}in incubated soil at various period of incubation. This is observable since the incubation experiment was a controlled experiment and changes in the pattern of N release were solely due to the soil environment and manure characteristics. Application of poultry manure @10 t ha^{-1}(O_1) had the maximum available N content of 115.14 mg kg^{-1}, and it raised the available N content gradually with various periods of incubation, making it available to the plants for a longer period of time (Amanullah, 2007). The increased amount of nitrogen released from poultry

manure could be owing to the manure's greater capability for N-mineralization (Cordovil *et al.,* 2005). This treatment was followed by the treatments with the application of goat manure @ 10 t ha^{-1}(O_2), pressmud@10 t ha^{-1}(O_3), vermicompost @ 5 t ha^{-1}(O_4) and application of neem cake @ 2 t ha^{-1} (O_5). These treatments recorded the mean available N content of 112.97, 111.78, 110.39 and 110.14 mg kg^{-1} in soil, respectively. The lowest available N content (108.74 mg kg^{-1}) was noticed in the treatment with the application of composted coir pith @5 t ha^{-1}(O_6). The release of available N was significantly influenced with progressive increase in incubation period upto90DAI. VelMurugan and Swarnam (2013) reported that organic manures significantly increased the concentration of mineral nitrogen (NH_4^+ + NO_3^- nitrogen) in the soil during the incubation period. This could be attributed to the fact that a proportion of the organic N content has been converted to the inorganic form gradually through the incineration of manures, and hence the mineral N content of the manures were higher during various days of incubation. Among the various intervals of incubation periods evaluated, the treatment with 90DAI recorded the highest available N content (113.72 mg kg^{-1}). The treatments next in order were 75,60,45,30 and 15 DAI registered the available N content of 113.57, 113.19, 112.28, 111.22 and 109.26 mg kg^{-1}, respectively.

With regard to the interaction effect, the application of various organics and period of incubation was significant. Application of poultry manure @ 10 t ha^{-1}at90 DAI registered the highest available N content of 119.83 mg kg^{-1} and was comparable with45, 60 and 75DAI.The rapid rise of available N content is attributable to the decomposition of the easily decomposable nitrogenous substances present in the organic materials. This is in corroboration with the findings of GonzalexPriesto, Carballas, Villar and Carballas (1995). This process is also brought about by the activity of microorganisms present in soil which aid in release of nutrient by enhancing decomposition (Godlin and Olinevinch, 1966). In the organic manures *viz.,* goat manure, vermicompost and composted coirpith the release was gradual increase upto 90 DAI. But the application of neem cake @ 2 t ha^{-1} tremendously increases N release over upto 30 DAI and thereafter there is linear decrease in N upto 90 DAI, which might me due to high concentration and speedy release of N from neem cake, the large number of organic compounds released during decomposition are known to inhibit nitrification Debona and Audus (1970)

Available phosphorus

Various organic manure applications and incubation periods have a substantial impact on the available P content of soil. Fig 1 a, b, and c show the release of available P from various organic manures during the incubation period.

The application of poultry manure (O_1) produced the greatest available P of all the organic manures tested (9.92 mg kg^{-1}). With the addition of organic matter to the soil, the availability of P improves (Ewulo *et al.,* 2008). This could be owing to the organic forms covering sesquioxide, which lowered soil P fixing capability and increased phosphorus availability (Kumar et al. 2015). The treatments O_2 (7.83 mg kg^{-1}), O_3 (7.46 mg kg^{-1}), O_4 (7.40 mg kg^{-1}) and O_5 (7.01 mg kg^{-1}) considerably increased the available P content. The least available P content (6.89 mg kg^{-1}) was noticed in treatment O_6(composted coir pith). With regards to period of incubation, the available P content showed a progressive increase upto90 DAI. Among the period of incubation evaluated, the treatment with 90 DAI registered the highest available P content of 8.53 mg kg^{-1}as against 6.71 mg kg^{-1} at the initial stage of incubation and was comparable with 75 DAI (8.43 mg kg^{-1}).

This was followed by the treatments 60 DAI(8.18 mg kg^{-1}), 45 DAI(7.95 mg kg^{-1}), 30 DAI(7.54 mg kg^{-1}) and 15DAI(6.91 mg kg^{-1}), respectively. The reason might be due to the fact that decomposition of organic materials increasingly continued accompanied with increase in the release of P upto the 90 days. The increase in the release of Phosphorus depends upon the type of the organic manures and the incubation period (Singh and Patel, 2016).The interplay between organic manures and period of incubation was significant. The highest available phosphorus content of 12.21 mg $kg^{-1}$was registered in the treatment O_1 at 90 DAI, which received poultry manure @ 10t ha^{-1}. However, this was comparable with the values of 75 DAI. In case of goat manure, pressmud and vermicompost the same trend was followed with reference to interactions. But in case of neem cake and Composted coirpith the increase of available P was gradual upto 75 DAI and thereafter it declines

Available Potassium

A distinctive effect on available K was observed from variousorganic manures during incubation at variousperiods (Table 3). With regard to organic manures available K ranged from 142.49 mg $kg^{-1}$to 147.63 mg kg^{-1}.Of the various organics evaluated, application of goat manure @ 5 t ha^{-1}(O_2) was significantly superior in increasing the available K contentin soil. This might be due to the fact that application of sheep manurein bulk quantity and subsequently their slow mineralization resulted in gradual build up of available K (Chetri *et al.,* 2012). A similar trend of result was recorded by Gokul, 2020. The increase in available K was attributed not only to the enrichment of K by organic manures, but also to the action of organic acids generated during the decomposition of organic matter, which made local K more available (Dhanorkar *et al.,*1994). The treatment was followed by the treatments O_1, O_3, O_5, and O_6.These treatments recorded the available K content of 146.11mg kg^{-1}, 144.44mg kg^{-1}, 142.95 mg $kg^{-1}$and 142.66mg kg^{-1}, respectively. The vermicompost has the lowest available K content (142.49 mg kg^{-1}) (O4). Brar *et al.,* (2008) similarly found that manured soil had higher K availability than unmanured soil. The results of the incubation investigation on K availability were comparable to those of Kaur and Benipal (2006). In terms of incubation time, the treatment with the greatest available K (147.72 mg kg^{-1}) was recorded at 90 DAI. This could be owing to the longer incubation time required for K mineralization, which could have resulted in a considerable increase in accessible K and boosted the available K pool in soil due to the release of more organically bound potassium during the decomposition of organic waste. However, the treatment was found to be on par with 75 DAI and 60 DAI. This was followed by the treatments O_4, O_5 and O_6.The available K was progressively increased upto90 DAI.

The interaction effect between addition of organic manures and period of incubation on available K was significant. Application of goat manure @ 10 t ha^{-1} registered the highest available K content of 153.60 mg kg^{-1}at 90 DAI, which was statistically on par at 75 DAI and 60 DAI. In case of pressmud and composted coirpith the release of available K was progressive increaseupto90 DAI. But, in case of vermicompost the release was gradually increase upto 75 DAI and thereafter it declines.

Available Sulphur

The results of incubation experiment revealed that application of variousorganic manures and period of incubationsignificantly influenced the available sulphur contentin soil (Fig. 2 a, b and c) The available S content ranged from 7.40 to 8.23 mg kg^{-1} in incubated soil at variousperiods of incubation. Among the various organics evaluated, the application of pressmud@ 10 t ha^{-1}(O_3)registered the highest available S content of 7.93 mg kg^{-1}, which was significantly superior to the rest of treatments. This might be due to mineralization of sulphur from native as well as from added organic source. Pressmud acts as an energy source for sulphur oxidizing microorganisms which in turn make in their activities and increased the sulphur mineralization rate in soil. With regard to the period of incubation, available S content gradually increased upto 90 DAI. Among the period of incubation evaluated, the treatment with 90 DAI registered the highest available S content of 7.81 mg kg^{-1} as against 7.42 mg kg^{-1} at the initial stage of incubation. The reason might be due to the release of S release from pressmud during the process of mineralization maintaining optimal soil nutrient levels over a prolonged period of time (Bationo *et al.,* 2004). A similar trend of results were given by Poonkodi *et al.* (2018) and Aravindh Ramnathan *et al.* (2019).This treatment was followed by the treatments 75 DAI(7.79 mg kg^{-1}), 60 DAI (7.76 mg kg^{-1}), 45 DAI (7.73 mg kg^{-1}), 30 DAI (7.64 mg kg^{-1}) and 15 DAI (7.55 mg kg^{-1}) in registering the available S content over a period of incubation. The interaction effect between addition of organic manures with varioustime interval was not significant.

Available Calcium

In the present study, it was observed from table 4 that the available calcium content in soil was significantly enhanced with the application of variousorganic manures and incubation time. While, the interaction of organic manures and period of incubation was not significant. With regard to the treatments imposed, available calcium was maximum (9.13 C mol (p+) kg^{-1}) with poultry manure @ 10 t ha^{-1} compared to other organic manures. It was significantly superior to the rest of the treatments. This might be due to the release and availability of calcium from the soil also along with release from of poultry manure. A part of free available calcium in soil might have adsorbed

into colloidal sites of humic substances present in poultry manure and thereby released into available pool. These results are in conformity with findings of Premamali *et al.* (2019).The available calcium was increased up to 90 DAI 9.30 C mol (p+) kg^{-1}. This increase might be due to the supply of Ca through the release from poultry manure (Chaurasia*et al.,* 2005) This treatment was followed by the treatments at 75, 60, 45, 30 and 15DAIrecording the available calcium content of 9.29, 9.16, 9.01, 8.85 and 8.51 C mol (p+) kg^{-1}, respectively.

Available magnesium

The application of various organic manures increased the availability of magnesium during incubation period was depicted in Table 5. while, their interaction effect was not significant. With respect to organic manures application, the release of available magnesium content ranged from 6.33 C mol (p+) kg^{-1} to 6.72 C mol (p+) kg^{-1}at various period of incubation. The application of goat manure @ 10 t ha^{-1} recorded the highest (6.72 C mol (p+) kg^{-1}) amount of available magnesium at all the stages of incubation. This might be due to the release of organic acids from the decomposition of goat manure which in turn might have released magnesium from exchange sites in the soil (Muthuraja *et al.,* 2005). The treatment was on par with O_1 and this was followed by treatment application of composted coirpith @ 5 t ha^{-1} and it increased the availability of magnesium at all stages of incubation, Elayaraja *et al.* (2020).With regard to period of incubation, the available magnesium showed a progressive increase upto 75DAI (6.94 C mol (p+) kg^{-1}), then turn to decline in 90 DAI (6.93 C mol (p+) kg^{-1}). This was comparable with 60 and 90 DAI. A similar result was recorded by Ha *etal.*(2007).This might be due to the initial fast release of nutrients from water soluble compounds such as sugars, amino acids and organic acids, whereas at the later stage of slow release might be attributed to resistant compounds such as cellulose, lignin, phenol or waxes.

CONCLUSION

According to the findings of an incubation study, various organic manures significantly increased the release of primary (N, P, and K) and secondary nutrients (Ca, Mg, and S). In compared to the other three manures, vermicompost, neem cake, and composted coir pith, poultry manure, goat dung, and pressmud released N, P, K, Ca, Mg, and S continuously and steadily all through the incubation period up to 90 DAI

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Table 1. Properties of organic manures

Parameters	Poultry manure	Goat manure	Pressmud	Vermi compost	Neem cake	Composted coir pith
pH	6.45	7.90	7.62	6.40	5.23	5.53
EC (dSm^{-1})	0.7	1.23	1.34	0.63	0.28	0.32
Total carbon (%)	8.09	6.16	5.24	6.84	2.28	7.80
Nitrogen (%)	3.20	2.97	1.44	1.64	4.30	1.06
Phosphorus (%)	2.03	1.13	0.82	0.87	0.91	0.06
Potassium (%)	1.57	2.01	1.20	0.60	3.00	0.74
Calcium (%)	2.99	0.96	0.63	0.78	0.54	0.50
Magnesium(%)	0.67	0.98	0.42	0.46	0.31	0.48
Sulphur (%)	0.59	0.46	1.02	0.003	0.32	0.002

Table 2: Influence of various organic manures on available nitrogen content (mg kg^{-1}) of soil during incubation at various days

Organic Sources	Days After Incubation (DAI)							Mean
	0	15	30	45	60	75	90	
O_1	107.46	109.86	113.00	117.03	119.06	119.73	119.83	**115.14**
O_2	107.60	109.87	111.66	113.63	114.56	116.16	117.33	**112.97**
O_3	107.20	109.40	110.50	112.23	113.63	113.93	115.60	**111.78**
O_4	107.23	107.96	108.90	110.90	113.03	112.76	111.96	**110.39**
O_5	107.66	110.56	114.93	111.23	109.80	108.83	107.96	**110.14**

O₆	107.63	107.93	108.30	108.63	109.03	110.03	109.66	**108.74**
Mean	**107.46**	**109.26**	**111.22**	**112.28**	**113.19**	**113.57**	**113.72**	
	Organic Sources			**DAI**			**O x DAI**	
F Test	**			**			**	
S Ed	0.59			0.64			1.58	
CD (P = 0.05)	1.19			1.28			3.14	

Table 3: Influence of various organic manures on available potassium content (mg kg^{-1}) of soil during incubation at various days

Organic Sources	Days After Incubation (DAI)							Mean
	0	**15**	**30**	**45**	**60**	**75**	**90**	
O₁	137.60	143.10	145.10	146.50	149.20	150.30	151.00	**146.11**
O₂	137.50	142.20	146.40	149.80	151.60	152.30	153.60	**147.63**
O₃	137.20	141.30	142.50	144.20	146.60	148.80	150.50	**144.44**
O₄	137.80	139.50	140.70	142.40	144.70	146.60	145.70	**142.49**
O₅	137.90	144.40	147.80	145.30	143.70	141.60	139.60	**142.90**
O₆	137.40	140.80	141.90	142.20	144.90	145.50	145.90	**142.66**
Mean	**137.57**	**141.88**	**144.07**	**145.07**	**146.78**	**147.52**	**147.72**	
	Organic Sources			**DAI**			**O x DAI**	
F Test	**			**			**	
S Ed	0.58			0.62			1.53	
CD (P =0.05)	1.15			1.24			3.05	

Table 4: Influence of various organic manures on available calcium content (C mol (p+) kg^{-1}) of soil during incubation at various days

Organic Sources	Days After Incubation (DAI)							Mean
	0	**15**	**30**	**45**	**60**	**75**	**90**	
O₁	8.22	8.69	9.04	9.15	9.35	9.71	9.73	**9.13**
O₂	8.22	8.54	8.95	9.16	9.29	9.33	9.35	**8.98**
O₃	8.21	8.42	8.89	9.00	9.18	9.29	9.31	**8.90**
O₄	8.22	8.56	8.95	9.09	9.16	9.25	9.25	**8.93**
O₅	8.21	8.49	8.74	8.92	9.02	9.16	9.12	**8.81**
O₆	8.22	8.38	8.51	8.74	8.97	9.00	9.04	**8.69**
Mean	**8.22**	**8.51**	**8.85**	**9.01**	**9.16**	**9.29**	**9.30**	
	Organic Sources			**DAI**			**O x DAI**	
F Test	**			**			**NS**	
S Ed	0.07			0.07				
CD (P =0.05)	0.14			0.15				

Table 5: Influence of various organic manures on available magnesium content (C mol (p+) kg^{-1}) of soil during incubation at various days

Organic Sources	Days After Incubation (DAI)							Mean
	0	**15**	**30**	**45**	**60**	**75**	**90**	
O₁	5.75	6.24	6.34	6.74	7.11	7.14	7.17	**6.64**

O_2	5.76	6.25	6.54	6.83	7.19	7.22	7.25	**6.72**
O_3	5.75	6.02	6.24	6.53	6.72	6.77	6.81	**6.41**
O_4	5.77	6.15	6.3	6.65	6.73	6.86	6.77	**6.46**
O_5	5.76	5.95	6.13	6.46	6.64	6.72	6.65	**6.33**
O_6	5.78	6.15	6.24	6.65	6.88	6.94	6.92	**6.51**
Mean	**5.76**	**6.13**	**6.30**	**6.64**	**6.88**	**6.94**	**6.93**	
	Organic Sources			**DAI**			**O x DAI**	
F Test	**			**			**NS**	
S Ed	0.04			0.04				
CD (P =0.05)	0.08			0.08				

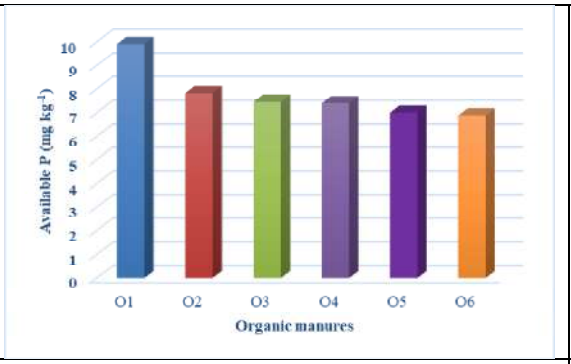

Fig. 1 (a) :Influence of various organic manures on the incubation soil's available phosphorus content (mg kg^{-1})

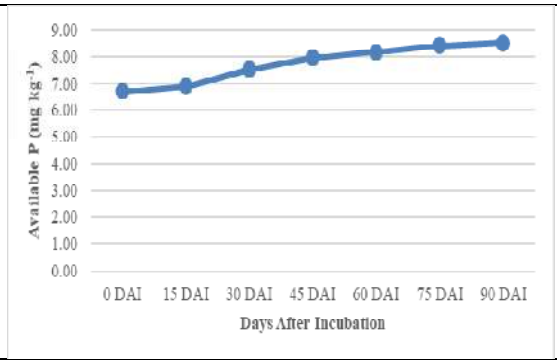

Fig. 1 (b) :Influence of various organic manures on available phosphorus content (mg kg^{-1}) at various incubation periods

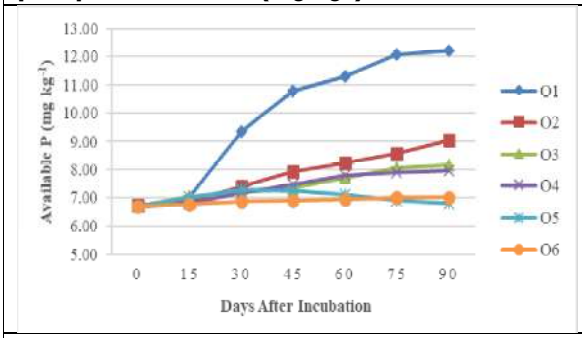

Fig. 1 (c) :Influence of various organic manures on soil available phosphorus concentration (mg kg^{-1}) during incubation at various days

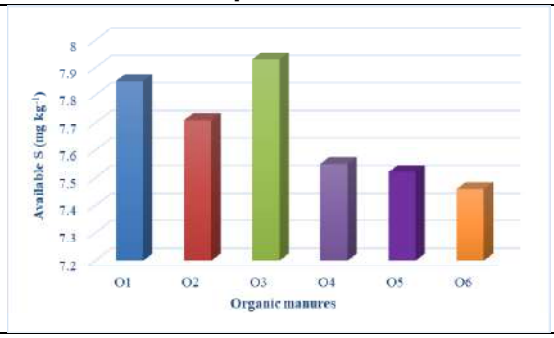

Fig. 2 (a) :Influence of various organic manures on the incubation soil's available sulphur content (mg kg^{-1})

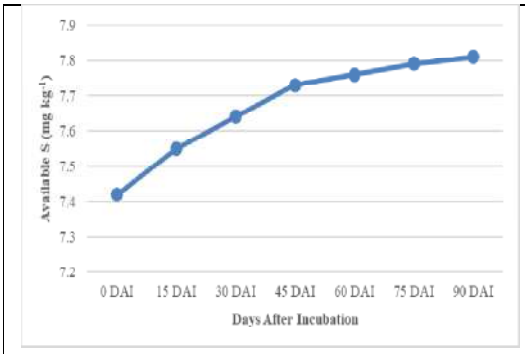

Fig. 2 (b) :Influence of various organic manures on available sulphur content (mg kg-1) at various incubation periods

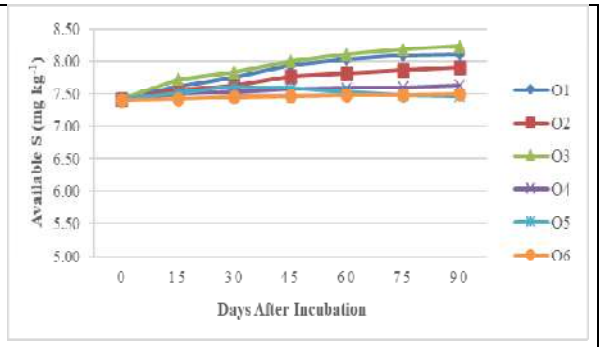

Fig. 2 (c) :Influence of various organic manures on soil available sulphur concentration (mg kg-1) during incubation at various days

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Impact of Covid-19 on Micro, Small and Medium Enterprises in India

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Received: 23 Jan 2022 Revised: 20 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

MSMEs, which make up about 8% of India's GDP, are responsible for nearly half of its total modern output and about a third of its full fares. In the first two weeks of June, almost 500 Indian Micro, Small and Medium Enterprises(MSMEs) were infected by the Coronavirus. Thirty-three percent of those polled said they had temporarily shut down their business to resume regular operations. MSMEs are pleading with the Government for assistance in dealing with the current crisis. Many small businesses (more than 50 percent) believe that the administration should offer exemptions or duty limits, followed by 36 percent of small businesses who want loans at zero interest or lower rates. In addition, 30% of MSMEs have started a business website or increased their internet business capabilities since the COVID-19 pandemic began. Ultimately, this study hopes to learn more about how different Micro, Small, and Medium Enterprises can affect the economy. For the investigation, secondary data has been gathered. Small and medium-sized enterprises (MSMEs), which make up more than a third of India's economy and employ more than 114 million people, have had the most difficult three years. The twin problems of unemployment and poverty pose a significant improvement challenge for our economy, and MSMEs will play a substantial and crucial role in that improvement. MSMEs face several challenges. India's economy will continue to grow at a rate of 8 to 10 percent for many years to come, thanks to the efforts of the Government, banks, and financial institutions.

Keywords: Coronavirus, Covid-19, Lockdown, Micro, Small and Medium Enterprises, Pandemic.

INTRODUCTION

Micro, Small, and Medium Enterprises (MSMEs) are also known as SSI (Small Scale Industries). They have been widely accepted as the driving force behind economic growth and more equitable distribution of resources worldwide. MSMEs are categorized into various industries based on the amount of investment. For example, if they are starting as a manufacturing company, they may purchase plant and machinery, while in the service sector, they may purchase equipment. MSMEs, or micro, small, and medium-sized enterprises, are easily frustrated by the importance of MSMEs for the growth and development of the country. This division employs 69 million people in India, 40% of the country's workforce, through 44 million businesses. Although they are an essential source of vocation, they have enriched the lives of many families over the years. Around 45% of India's total modern yield and 40% of the country's full fares are attributable to the country's MSMEs, which account for about 8% of GDP. Ninety percent of India's total mechanical units are found in the MSMEs' assembling division, more significant than the administration sector. Only 55% of the total MSMEs are located in urban areas. In states like Uttar Pradesh, Maharashtra, Tamil Nadu, West Bengal, Andhra Pradesh, and Karnataka, 45 percent of the units have rural areas. They promote fair distribution of public salaries, a reduction in poverty, and a rise in overall financial resources. This division employs 69 million people in India, 40% of the country's workforce, through 44 million businesses.

STATEMENT OF THE PROBLEM

Micro, small, and medium-sized enterprises (MSMEs) are a rapidly expanding segment of the modern global economy. For social and economic reasons, small and medium enterprises are necessary because of their ability to speak to the level of all undertakings and provide independent work, which helps alleviate unemployment. Many micro, small, and medium-sized businesses have temporarily shut down because of the COVID-19 emergency. On 1st June 2019, the first two weeks of June, nearly 500 Indian micro, small, and medium-sized enterprises (MSMEs) were affected by the Coronavirus. Of those polled, 33% admitted to closing their business for some time before things returned to normal. To stay afloat during this crisis, MSMEs are looking to the Government for assistance. Most small businesses expect the administration to offer exemptions or duty limits, followed by 36 percent of small companies requesting low-interest or zero-interest loans from the Government. Since the COVID-19 pandemic began, 30 percent of MSMEs have either started or facilitated the use of their websites or other internet-based business tools.

Many small- and medium-sized enterprises (SMEs) in the educational sector use advanced media. According to a Small Business Administration (SBA) report, more than half of the MSMEs' total income came from web-based businesses with lockdown measures in place. The increase in income commitment from the online business was 53% for retail MSMEs and 65% for instructional or educational services. More than half of the MSMEs in the study used video conferencing equipment and WhatsApp to keep their businesses running smoothly during these trying times. "The Coronavirus has forced everyone to re-evaluate their day-to-day activities. As a result of the lockdown, MSMEs with a grasp of computerized presence maintained some semblance of regularity and continued to serve or draw in customers or clients.

NEED AND RELEVANCE OF THE STUDY

The MSMEs segment is the engine of development for the Indian economy. The investigation uncovers an expanding design in the number of units, work, and market estimation of advantages. It is essential to note the severe connection between the all-out production line unit and labor. The investigation examination has discovered that MSMEs have shown a decent exhibition as of late. Simultaneously, its difficulties are likewise not overlooked. The MSMEs division is developing quickly, and they have become a pushed region for future development for the provincial and metropolitan turn of events. Its financial uplift men's have a great deal of strategy suggestion. With uncertainty and over the length of the COVID-19 emergency, it is the need of great importance for MSMEs and the Government to meet up to pursue purposing business restarting tasks and secure the employees and laborers.

Recently, the task estimated that around 47 percent of Indian new businesses and SMEs have short of one month of money left, and 27 percent are out of assets. Many others are searching for strategic and transformational changes in their business to deal with remaining significant and enduring the droop. The Micro, Small, and Medium Enterprises (MSMEs) structure or know that it's the backbone of India's economy. This sector adds to and contributes 30% of India's GDP and utilizes an expected 28 percent of the work power, supporting jobs of more than 100 million individuals. Appraisals recommend that the part made around 13.5 million to14.9 million new openings for every annum somewhere within the range of 2015 and 2019.

OBJECTIVE OF THE STUDY

- To study the impact of the sectors of Micro, Small, and Medium Enterprises.

METHODOLOGY

This investigation significantly centered around miniature, little and medium endeavors situated in modern regions and conspicuous business sectors of MSME. Different makers, specialist co-ops, new businesses from the food, IT, and material assembling industries met as an investigation aspect. The exploration started with get-together foundation data, for example, history, current condition and centrality of miniature, little and medium endeavors, and fundamental investigation of the division. Two kinds of examination techniques most generally utilized for information assortment are as per the following: quantitative and subjective strategies. The quantitative test uses information that is then changed over into numbers, charts, tables, and measurements to arrive at a resolution. However, emotional strategies frequently allude to contextual analyses where data have been gathered from a couple of contemplating objects. Subjective techniques underscore getting, translation, perceptions in traditional settings, and closeness to information with such an insider see. The secondary data have been gathered for the investigation. Additional information was gathered from different reports, articles, and other web sources. Important information was collected by expressly talking with individuals or sending them surveys. Various MSMEs were met to catch a wide range of issues, including producing area ventures, Government's part endeavors, food industry, new businesses, and undertakings enrolled as Small-Scale Industry (SSI). The example isn't illustrative of the Indian populace; it comprises endeavors picked haphazardly from Delhi Markets and accessible contacts.

DATA COLLECTION

- Secondary data that has been collected for an other purpose
- It involves the fewer efforts
- Secondary data is the data that is being used usually in a different context, and other sites were used to collect more information about that
- Collected the data even through newspapers and friends and collected the data from the websites
- Collected data from magazines and books

IMPACT OF COVID-19 ON MSME SECTOR

The global economy has been shaken and degraded due to this pandemic. As a health crisis, the Coronavirus has evolved into an economic crisis. This pandemic wiped out the entire global economy. The public fringe has been closed in more than 100 countries. The chain had been gracefully broken for the previous month throughout the world. Because of the Covid19 pandemic, the global economy could shrink by nearly 1% in 2020. India is a developing country. India's Micro, Small, and medium undertakings (MSME) are the biggest in the world after China. MSME assumes a critical function to quicken the development of the Indian economy. However, the situation of MSME will be truly inconceivable and unpredictable after this epidemic. MSMEs have risen as a profoundly lively and dynamic part of the Indian economy throughout the most recent fifty years. It contributes essentially to the nation's financial and social advancement by encouraging business enterprise and creating the biggest business openings at a relatively lower capital expense, following as it were horticulture. MSMEs are correlative to enormous ventures as auxiliary units, and this are a contributes fundamentally to the country's comprehensive modern

improvement. The MSMEs are enlarging their area across divisions of the economy, delivering the different scope of items and Government to fulfill the needs of homegrown just as worldwide business sectors. The small-scale manufacturing business started at home, any area, or small rental premises. Small business does not include huge investments. The existences of MSMEs are inevitable for the countries of (Developed, Developing and Underdeveloped). Miniature, Small, and Medium Enterprises (MSME) assume a crucial part during events. It creates work with ease and encourages the public to thrive and develop. As more than 65% of Indians live in towns and as there is a little extension for the foundation of Large-scale ventures in these regions, MSME is a significant perspective on the advancement of provincial areas.

STRENGTHS, WEAKNESSES, OPPORTUNITIES, AND CHALLENGES (SWOC) OF THE RESEARCH

Strength

The results discovered by the owners' the management have scored the highest mean worth, trailed by ideal capital yield proportion, modest work, co-activity from employees, and closeness to advertise. It shows that owners' management gives adaptability and comfort. It encourages the simplicity of cost control and overseeing by significant operational issues. Specifically, the owners must close with the market feel and versatility to increase quick reactions to changing business sector needs, developing business strength.

- MSMEs have be gun at an exceptionally minimal effort.
- Adaptability is critical in these divisions, and the absence of regulatory dormancy makes it easier to respond quickly to the commercial center.
- Increased responsibility and advancement are two of the benefits of a solid client-provider relationship. When a business treats its customers as part of the family, they are almost sure they will return.
- Furthermore, these parts have the advantage of working for themselves.
- In addition, a small business can adapt its products and services to meet the unique needs of its customers. The typical business visionary or director of an independent venture better understands their clientele than in a large organization.
- Don't just meet, but exceed, the expectations of your customers. Item or service adjustments, or even the length of time a company is in business, could benefit customers; this is something an organization can do.
- Individuals– the business visionary, any accomplices, counsels, representatives, or even relatives – have an enthusiastic, practically enthusiastic desire to succeed. It makes them work more enthusiastically and better

Weaknesses

- It can be challenging to find enough potential customers to build a solid client base. If you have many clients, they may not want to work with you.
- Provides little in products, administrations, and services widely recognized.
- Raising accounts is probably the most common weak point for small business owners. Institutional moneylenders like banks and government budgetary organizations are generally reluctant to send money to small businesses, so many entrepreneurs stash their cash when starting a business or when the company encounters difficulties.
- Entrepreneurs who start their businesses have to deal with the high costs of starting up, which is one of the weak spots for independent companies.
- The majority of smaller organizations lack a well-trained workforce. Because of this, these organizations require evidence of mechanical activities for improvement consulting and guiding administrations, as well as providing mechanical training and development for their employees.
- Independent companies don't utilize Information innovation and its applications, for example, model machines for items distinguished by nation assets and requirements.
- It often turns out to be extremely hard to get the authorization of and permit, or to take the permission from Industrial Development of the state, nearby bodies, and so forth.
- Marketing is one of the shortcomings for independent ventures or small businesses.

Opportunities

The opportunities enjoyed in the current situation by the MSMEs with the help of government support, reservation of item things, extract the resource, and auxiliary for colossal business. Keeping the weaknesses aside, MSMEs have admittance to opportunities like various reciprocal and multilateral economic alliances, which opens the door to an extraordinary wellspring of income for business entrepreneurs/businesses. In like manner improved uphold for increasingly more government activities for their advancement are offered. Subsequently, business visionaries/entrepreneurs can anticipate some alleviation regarding admittance to credit offices. Also, with the appearance of advanced time (web), the admittance to fresher openings has gotten simpler and expanded essentially. A portion of the business people has also persevered against syndications and imprinted in the industry. Like this, the development of commercial web centers and elective financing has made it simpler for business visionaries to make safe business subsidizing. Again, NSIC (National Small Industries Corporation) has dispatched a few showcasing projects to advance.

MSMEs. Along these lines, entrepreneurs can use satisfactory showcasing support benefits from Government offices to develop MSMEs.

- Less capital concentrated
- Most crucial work creating sectors. It gives half of the private-sector business to the employees
- Compelling apparatus for the advancement of adjusted local turn of events
- It has been widely promoted and supported by the Government Fund and government subsidies
- The government purchases produced goods.
- MSMEs account for 40% of all fares in India.
- Acquiring raw materials and hardware
- Micro, Small, and Medium Enterprises (MSMEs) have been given new opportunities by globalization.
- For countries to grow economically, trade admissions and presentations were essential

Challenges

By contributing 45 percent of current yield, 40 percent of exports, and employing 60 million people, Small and Medium Enterprises (SMEs) play an essential role in the Indian economy's development. Produce more than 8000 high-quality items for the Indian and global markets each year, supporting 1.3 million jobs. Seventeen percent of GDP was contributed by small and medium-sized enterprises in 2011, which is expected to rise to 22 percent by 2012. Approximately 30 million MSME units and 12 million people in India will be required to enter the workforce in the next three years. However important MSMEs are to Indian economic development, the area or sectors face difficulties. They do not receive the necessary help from the relevant government departments, banks, financial institutions, and corporations, which is a barrier to MSMEs' growth. Existing and new Micro, Small and Medium Enterprises have to deal with the following issues:

Absence of a financially sound and satisfying account
lack of funds and knowledge
Lack of availability of appropriate technologies
Production capacity is limited.
Advertising methods that aren't working
Recommendations for upgrading and expanding
Talented work that isn't available at a reasonable cost

Get in touch with various government agencies to figure out problems caused by a lack of labor and information. Get involved. It is critical for Micro, Small and Medium Enterprises (SMEs) to maximize their limited resources (human and financial) in the most efficient manner possible. SMEs should be taught and educated on the most recent international advancements and assisted in acquiring the skills necessary to keep up with global events. There are more open doors than ever before for small businesses to grow and expand into new markets. Manufacturing,

precision engineering, food processing, pharmaceuticals, textiles and apparel, retail, information technology and telecommunications equipment and services (IT and ITES), agro-processing, and the service sector are industries where Indian entrepreneurs make significant gains. The Chamber has developed critical strategies to advance and support the SME sectors to meet the challenges faced by this sector and gain access to the market. SMBs are encouraged to think outside the box and develop new ways to grow their business by the Chamber. A primary goal of the Chamber of Commerce is to organize educational and awareness-raising activities for Micro, Small, and Medium Enterprises (SMEs). As part of MSME's efforts to empower and educate the SME sector, the organization has conducted various exchange-specific training and exercises.

OUTCOMES OF THE STUDY

India at present has more than 75 million MSMEs (Micro, Small, and Medium Enterprises), and near a fourth of these organizations will confront conclusion if the lockdown, actuated by Covid-19, goes past about a month while a stunning 43% will close shop if the frenzy stretches out. After more vocations, it lost than lives because of COVID-19. In light of ground sway data accumulated from different sources, we accept that many MSMEs in the nation will disappear if the panic continues beyond the month. MSMEs, which are the backbone or the strength of the Indian economy and give work to more than 114 million employees and add to over 30% of the GDP, has experienced the most troublesome occasions over the most recent three years. "It is a well-known, established truth that they confronted one mishap after another, first being demonetization at that point followed by glitch GST execution. At that point came monetary lull of land and auto-area to which the more significant part of the MSMEs were fundamental wellsprings of providers. At that point came the financial and banking emergency. Presently, COVID-19 is the last hit to the MSMEs by all accounts. Indications or the signs of decay are as of now visible. MSMEs have just started facing the problems financial of unpaid pay rates (will, in the end, bring about loss of work), unpaid EMIs (will affect the accounting reports of budgetary establishments) lastly, the conclusions of such activities. Misfortune making monetary records doesn't permit them to get any money from banks or NBFCs according to RBI standards, bringing about expanded unsold stock heap up and getting into dead speculations and resulting in gigantic misfortunes.

MC KINSEY 7S MODEL

It is a strategy tool that analyzes the company's hierarchical plan by seeing seven key inner components: strategy, structure, systems, shared values, style, staff, and skills, to recognize if they are effectively adjusted and permit the association to achieve it star gets.

The model can be applied to numerous circumstances and is essential for questioning the hierarchical plan. The most well-known employments of the structure are:

- To encourage organizational change.
- To help make or implement a new methodology.
- To recognize how every area may change in the future.
- To promote the merger of associations.

Strategy

A company's strategy is to gain an advantage in the marketplace and compete effectively. An effective strategy should be clear, long-term, and assist in achieving the upper hand. It should also be based on a solid vision, mission, and values. Whatever the case, it's hard to tell if such a methodology is aligned with the various components when broken down alone; instead of examining your organization to see if it has a fantastic process, structure, frameworks, the key in the 7s model is to see if different components are aligned. An organization's decision to use the momentary technique is usually futile; however, it could yield solid results if the other six team members support it.

Structure

A company's divisions and units are laid out to show who is responsible for each. Thus, the design serves as an authoritative depiction of the company. It is also one of the system's most frequently and quickly replaced parts.

System
A company's divisions and units are laid out to show who is responsible for each. Thus, the design serves as an authoritative depiction of the company. It is also one of the system's most frequently and quickly replaced parts.

Skills
Company representatives consistently excel in these abilities. In addition, they include skills and skills. When a company is undergoing significant change, people often wonder what skills to strengthen its new method or structure.

Staff
One of the most pressing concerns of a company's staff is finding and training, motivating, and rewarding its future leaders for taking over the company at a higher level in the market. The author believes that this is an essential part of the framework.

Style
The company's staff is concerned about the types and numbers of employees it will require and the methods used to recruit, train, motivate, and reward them for taking over the company at a higher level in the market. The author thinks that this is the essential part of the framework in terms of technology.

Shared Value
The McKinsey 7s model is based on shared values. The standards and norms that guide the behavior of employees and friends and the formation of each group are derived from these standards and norms. It explains to workers how to develop the new product's design.

Experience
The MSME sectors in India is the strength of our Indian economy, and it helped me come up with new ideas about the sectors. The MSME sectors are such some help for the rural area people and the urban regions. And after we learned about that, it helped me develop a new awareness about why it is essential in the Indian economy. Almost as if we've been taught, the MSME sector is the backbone of our economy. 'The importance of Micro, Small and Medium Enterprises (SMEs) cannot be overstated. Because of its expanding economy, it employs people out of work. In addition to creating jobs, this process also generates new concepts for the product and other items. This pandemic situation has the potential to shut down as many as 25 percent of India's 6,9 core small and medium businesses. When the sudden lockdown began, they went under the losses.

Learning
Destroying Covid-19's flare-up will have an impact on nearly every country in the world. The economies of the world's most powerful countries have become defenseless and uncontrollable. Nevertheless, India's quick and ideal choice to go back is calculable. The devastating impact of the Coronavirus cannot be ignored. What if we compare India and some developed nations, such as the United States or Italy, whose examination is not supported by analysis? As a result, India is in a far better position. It results from the country's rapid lockdown, which emphasizes social removal. Until now, India has had a tremendous need to keep the virus under control. Unless India makes a quick decision, the pandemic's effect will be more dangerous, and its impact will be truly stunning in the future. The pandemic affects every part of the world. The Covid-19 pandemic may or may not affect India shortly, but that doesn't mean it won't impact the country. The question of how much money is needed to get back on track remains unanswered. India has just gone through a period of unemployment. We learn something new every time we experience something new in our daily lives. It could be both positive and negative. The good news is that this pandemic can help India realize its full potential. India's dependence on other countries to bring in goods is excessive. Instead of relying on imports, India has implemented a strategy based on domestic sourcing. The eradicating flare-up of Covid-19 has an impact on every country in the world. It's become impossible to change the course of events, even in countries with significant economies. However, the ricochet back from India's quick and

ideal decision is truly calculable. There is no way to ignore the devastating impact of the Coronavirus; on the other hand, if we compare India to some developed nations, such as the United States or Italy, whose correlation has not yet been defended, India is in a much better position. There has been an increase in social removal due to the country's rapid lockdown. India is under extreme pressure to keep the virus under control for the time being. With each passing day, the pandemic's impact on India grows more dangerous, and its future impact will be truly stunning if India doesn't act quickly. Because of the pandemic, everything is affected. We learn something new that we can use throughout our lives here and now. Positive or negative, it could be both. In a positive light, this pandemic provides an opportunity for India to examine its potential. India has developed a home-sourcing strategy given this dependence on other countries instead of importing foreign products.

CONCLUSION

Every country on the planet is affected by the devastation caused by the Covid-19 outbreak. Countries with the most robust economies have become more vulnerable as the world has become more chaotic. India has made a significant leap back by taking advantage of the right moment. When compared to countries like the United States and Italy, whose correlation isn't yet proven, India is in a far better position than the United States or Italy, which have been created. Lockdown has led to increased social removal due to the nation's sudden shutdown. For India, containing the spread of infection is of utmost importance. If India didn't act quickly at that point, the pandemic's impact would be increasingly dangerous, and its perception would be truly stunning in the coming future. The pandemic affects every aspect of society. However, shortly after, India may not be spared a pandemic caused by the Covid-19 virus. How much money will invest if the development track can be re-established? Indians are currently unemployed. We learn something new every time we encounter a unique situation. Positive or negative, it's up to the individual. In a positive light, this pandemic provides an opportunity for India to discover its potential. Rather than importing products from other countries, India has opted to source its goods. As a result, the country's BOP situation is expected to improve. An entirely new mindset will emerge in India thanks to the work-from-at-home concept! It means that the use of computerized practice will rise. Digital and Make-in-India initiatives aim to empower more Indians. New businesses and start-ups may be forced to shut down due to this. As a result, a few start-ups will go out of business. As the twin problems of unemployment and poverty pose a significant improvement challenge, MSMEs will play an essential role in our economic future MSMEs face several challenges. If the Government, Bank, and Financial Institutions take appropriate actions in MSME, India's economic growth will continue at a rate of 8-10 percent for the foreseeable future.

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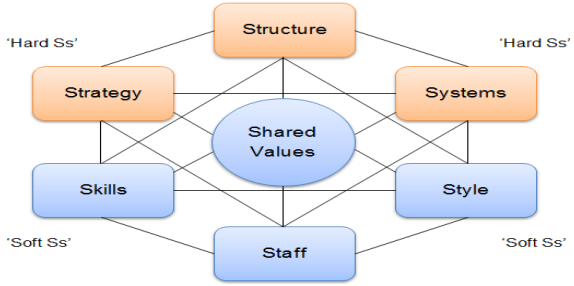

Fig.1. MC KINSEY 7S MODEL

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Urbanization and its Effects on Periya Mathagondapalli Village Water Tank, Krishnagiri District, Tamil Nadu, India

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Received: 09 Nov 2021 Revised: 04 Jan 2022 Accepted: 23 Feb 2022

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ABSTRACT

Today freshwater is a scarce resource, and it is being felt the world over. Urbanization affects the natural hydrological cycle and causes the degradation of water resources. The study is conducted to assess the impact of urbanization on surface water resources in the Periya Mathagondapalli tank, Krishnagiri District, Tamil Nadu. The data for this study is collected from Mathagondapalli village. Water sample from the tank is collected and analyzed to check the quality of water. The findings indicate that the storage capacity of the tank is reduced, the supply channels are blocked, the tank bed is infested with unwanted thorny trees and weeds and the tank is used to dispose of the solid waste and the grey water from the town contaminates the water body. The tank bed is also contaminated by residents through open defecation. Dissolved Oxygen and Phosphate are exceeded than the permissible level. Strategies for the solution of problems are identified that indicates that there is an urgent need to take up de-weeding, desilting works, cleaning of the supply channels, stabilization of the tank earthen bund, sewage management, and community awareness activities.

Keywords: Urbanization, water tank, water quality, pollution, weeds

INTRODUCTION

"The global water use has increased over the last 100 years at a constant rate of roughly 1% each year. In India, the average annual rainfall is 1160 mm. India's average yearly water availability has been estimated to be 1869 billion cubic metres (BCM). The utilizable water has been projected to be around 1123 BCM due to topographical, hydrological, and other restrictions, with 690 BCM surface water and 433 BCM replenish able ground water"[10]. This shows the importance of freshwater bodies. "Urbanization is the process by which cities expand and a growing percentage of the population moves to the city"*[9].* It's important to remember that the urbanization process entails more than just population growth; it also entails changes in a region's economic, social, and political structures. *(Bhatta, 2010).* According to census data of 2011, India's total population has risen from 1.02 billion in 2001 to 1.21 billion in 2011 [4].Around 70% of the world's population is anticipated to reside in cities by 2050. Over 60% of the land that is expected to become urban by 2030 has yet to be developed [12].

"Several consequences of urbanization are observed and recorded. The number of people on the planet grows every year, but the number of natural resources available to support this population, improve human life quality, and eradicate widespread poverty remains finite"[3]. This means that the management and distribution of accessible natural resources are also affected by the increasing urbanization process. for the urban population, and it cannot grow as fast as the human population. Because fresh water is one of life's needs, the human race has worked to find and develop it throughout history. Water, an essential source of life, is pollution-free in its natural state, but when man tampers with it, it loses its natural status*[1].* The major reason for contamination of water is wastewater drainage into water bodies, the release of chemicals and effluents into rivers, streams, and other surface water bodies [6]. Contaminants are produced in both nonpoint and point sources in urban environments. Industrial and municipal waste discharges are examples of point sources that have an impact on surface water*[8].*Rainfall over and through the earth causes pollution from non-point sources. As the runoff progresses, it takes up and transports contaminants, both natural and man-made, finally depositing them in lakes, rivers, wetlands, coastal waters, and groundwater. In surface waters, potential pathogen sources include point sources such as municipal sewerage, as well as non-point sources such as contaminated runoff from agricultural areas and areas with sanitation through on-site septic systems and latrines [11]. India to store only 2.4 % of rainwater, compared to developed nations'' [7]. So, the Conservation and management of freshwater bodies are necessary: Considering all these aspects, it is important to study the trend of urban growth and its various implications of it on natural resources, especially surface water bodies and there is a need to identify the measures to address the emerging challenges.

MATERIALS AND METHODS

Study Site

The locale of the study was Periya Mathagondapalli situated in Mathagondapalli Pancayat, Thally Block in Krishnagiri District of Tamil Nadu State.The village is 20 km from the industrial town of Hosur. As per the census data 2011, the total population of this Panchayat was 4979 and the total number of houses was 1148. The working population was about 44.2 % of total population. The geological formations of the Krishnagiri district belong mainly to Archaean age along with rock of Proterozoic age.The former is represented byKhondalite Group of rocks, Charnockite Group of rocks, Migmatites Complex, Sathyamangalam Group of rocks, while the latter is represented by Alkaline rocks. The area has predominantly the Quartzo feldspathic rock. The Study area map is presented in Figure 1. The total land area in Mathagondapalli Pancyat is 1012.48 ha.The dry land is 771.10 ha and irrigated land is 89.84 ha. The net cropped area is586.08 ha. The farmers mainly grow millet, horse gram, sorghum, red gram, and mustard in dry land. In garden land, the vegetables such as tomato, cabbage, bean flowers like rose, chrysanthemum, and marigold are grown. Coconut, mango, eucalyptus plantations are also there in the area. Most of the farmers grow three crops in a year.

The average annual rainfall and the 5 years rainfall collected from Indian Meteorological Department (IMD) is 850. 58 mm. To meet domestic and irrigation requirements in the past the groundwater was exploited through shallow dug wells. The deep bore wells with depth ranging from 100-360 mis exploiting the Presently groundwater status mainly. Now the top weathered zone is practically dry and most of the dug wells are dry.

Source of water in Mathagondapalli Panchayat

- Check dams in Mathagondapalli Panchayat – 4
- Tanks in the Panchayat- 12
- Dug wells in Periya Mathagondapalli - 46
- Bore wells in Periya Mathagondapalli – 378

One of the surface level fresh water bodies called Gow ramma tank that spreads over in 5.72 ha (14.13 ac) in this Panchayat. (Geo coordinates 12.629886 N, 77.726930 E). The elevation of this location is 908 MSL(Figure 2 Catchment area) .The tank is more than 100 years old. There are three feeder channels to this tank. Thorny bushes are covered in two channels. In one of the channels, gabion structures are constructed. The height of these gabions is equal to the ground level that restrict the flow of water during the monsoon to the tank. The third feeder channel brings grey water from the urban households to the tank. The tank bed is also used by the public as the open defecation ground. The tank bed is covered with thorny trees and bushes and a lot of silt accumulation is also observed. As the result, the tank looks like a small pond with polluted water The domestic animals drink water from the tank. The bore wells in the command area depends on the water in this tank. Most importantly the bore well, which is used to supply the water to the water ATM which is dried now; the functionality of this water ATM depends on the availability of water in this tank. In command area, farmers grow varieties of crops in 10.71 ha of land. They also grow trees like Eucalyptus adjacent to the tank which also absorb a big quantity of water.

Research Design

The details that are collected from the villagers, VAO office of Mathagondapalli and secondary data collected from Government records are used for investigation. Data is collected from 66 bore well owners about the ground water scenario. Lab test is conducted to assess the quality of water that is collected from the tank

METHODOLOGY

RESULT AND DISCUSSION

The Rapid Growth of Urban Life in Mathagondapalli is observed in the last few decades. As per 2011 census, the population was 4979. The Panchayat is closer to Hosur where the Industrialization process is taking place in a rapid manner. Similarly, there are new industries emerging in Mathagondapalli area. The area has the advantage of providing employment opportunities as well as have the availability of some basic infrastructure. Transportation facilities which connect theHosur via public transport and affordable accommodation prices are also some of the deciding factors for urbanization. At the same time urban development has been observed to be spreading in an unplanned and unstructured manner *(Figure 3 map shows the Gowramma tank and urbanization).*

Shrinking of water storage capacity of the tankdue to weeds growth, siltation

The local community uses the tank as the dumping ground to dispose the solid wastes. Due to reduced dissolved oxygen, the weed growth has been increased in the water body. Due to lack of soil conservation measures, the top soil from the catchment area has been eroded and deposited in the tank. There are many thorny, bushy types are trees occupied the tank area and the trees also absorb the water and lead to quick depletion of water during the summer months (vide *Figure 4 Maps showing the infestation of tank bed with development of weeds, new feeder road across the tank)*

Water Contamination due to disposal of solid wastes and liquid waste

The urban household waste water leads to contamination of water. In addition to this, the open defecation is also observed. These practices have deteriorated the water quality of the tank.To analyze the effect of urbanization on the freshwater quality in Mathagondapalli, the water sample was collected and analyzed during Jan 2021. The HDPE Jerry Cans used for the water sample collection had a volume of five liters for physical and chemical analysis. Before sampling, the can was rinsed one time with the same water. Samples were taken from different locations in the tank and the water was given for test, transported to the laboratory of the Annamalai University, Chidambaram. The canwas labelled with the sampling location and date. The water sample was analyzed for physical and chemical parameters. The results are compared with the limits provided by Indian Standards IS10500:2012 (Bureau of Indian Standards, 2012).

pH: The permissible limit of pH (Potential of Hydrogen ions) in drinking water is within 6.5 – 8.5 according to the Bureau of Indian Standard (BIS). The value of pH in tank water samples of the study area is 6.95 which is within the accepted limits.

Chloride: The chloride concentration can be utilized as a useful metric for detecting sewage contamination. The chloride anion is found in most natural fluids. A high chloride concentration in natural fresh water is considered a contamination sign. Excreta from humans and animals, as well as industrial effluents, contain large amounts of chlorides and nitrogenous chemicals. Organic wastes have higher chloride concentrations, and higher chloride concentrations in natural water indicate pollution from home sewage. Metal pipelines and structures, as well as growing plants, may be harmed by high chloride levels. Chlorides in excess give water a saline flavor. The research region has a chloride concentration of 24 mg/l. Chloride has a permitted limit of 250 mg/l.

Sulphate is abundant in nature and may be found in natural water.In present study, the sulphate concentration is found 1.2 which is very low compared to the maximum permissible level of 250 mg/lit

Phosphate: During the study period, the concentration of phosphate is 4.5 mg/l. The BIS standard does not specify a permitted maximum for phosphate in drinking water, but WHO (1993) set it at 0.1 mg/l.The high concentration can be attributed to decay and subsequent mineralization of dead organic matter and surface runoff.

Nitrate: During the investigation period the values of nitrate recorded was 5.9 mg/l. The maximum permissible limit is 45 mg/lit.

Dissolved oxygen(DO): It is one of the most important parameters of water quality analysis. The presence of oxygen is critical to the chemical and biological processes that take place in a water body. The measurement of dissolved oxygen is an important test in the regulation of water pollution and waste treatment processes. According to Indian standards, the maximum allowable value for DO is 5 mg/L. The dissolved oxygen concentration in this study is 6.3 mg/l. The elevated DO, which could be related to enhanced oxygen solubility at lower temperatures.As per WQI, the Dissolved oxygen must be< 4.0 mg/l, it is observed that the DO is 6.3 which is higher than the satisfactory level.

BOD Biological Oxygen Demand is the amount of oxygen required to degrade organic matter present in the water body. BOD determination is used for assessing the organic pollution and also used for the measurement of the number of organic materials in an aquatic system supporting the growth of microorganisms Biological Oxygen

Demand > 3.0 mg/l is considered as satisfactory level and in the tank it is observed that the level is 5.8 mg/ lit

COD: The COD value in this study is 30 mg/l. According to IS 10500, there is no guideline for BOD or COD in drinking water, however it should ideally be zero or minimal.BOD up to 3 ppm is generally considered clean.

Temperature: In the aquatic environment, temperature is one of the most important factor. It affects the physical and chemical properties of water and affects the aquatic vegetation, organisms, and their biological activities. Temperature value in the tank in the study area is 28^{o}C.

Total Hardness: The capacity of water to create soap precipitates and scales with particular anions present in the water is measured by its hardness. The total hardness of water reveals the calcium and magnesium content of the water. The study tank has a hardness content of 44 mg/lit. The desired overall hardness limit is 300 mg/l. The sample values are found to be within the desirable limit when compared to the desired limit.

Total Suspended Solids: TSS are particles in water that filter and capture the Silt, rotting plant and animal stuff, industrial wastes, and sewage are just some of the elements that can be found in TSS. Suspended particles in high concentrations can wreak havoc on tank health and aquatic life. In the case of the tank, the TSS is 89 mg/lit.

Total Dissolved Solids: The term "total dissolved solids" (TDS) refers to the numerous minerals that can be found in water. The allowed value for TDS is 500 mg/l, as defined by IS 10500 and BIS. The current investigation found low TDS in tank water samples, indicating that the water is less mineralized and contains fewer contaminants. (91 mg/lit) TDS of Less than 300 mg/lit is coming under excellent category

Total Solids: The total solid found in the tank is 180 mg/lit (Suspended solid 89 mg/lit + Dissolved Solid 91 mg/lit)
Turbidity: The maximum of 10 NTU is accepted. The turbidity of the water sample analyzed is 3.9 NTU which is within the acceptable level

Poor Community participation in freshwater tank maintenance
Community awareness about the protection of water bodies is not adequate as a result the main water source of this village is losing its significance and becoming like a waste disposal tank

CONCLUSION

The objective of this study was to analyze the urban growth and its impact on the fresh surface water storage structure in Mathagondapalli. Community survey, tank visit, water test analysis, the use of google maps along with demographic information from census data are used in the detection of the changes. The study shows that tank ecosystem and water storage capacity have been severely affected. Dissolved Oxygen and Phosphate are higher in the water than the permissible level. The quality of water is also deteriorated due to rapid urbanization process. The study was limited to understand the effect of urbanization on surface water storage structure. There is also a need to study on its effect on ground water availability and quality. The surface water body available in this village which needs to be protected from urban encroachment and pollution. The following suggestions are provided based on the study for future researchers / development professionals who are working in this field

De-weeding, Desilting, cleaning of supply channels, stabilization of earthen bund
"The Hon˝ble National Green Tribunal Principal Bench, New Delhi in its order M.A. No. 26/2019 in Original Application No. 325/2015 dated: 10.05.2019" has directed that "Identification, Protection and Restoration of water bodies like Ponds, Tanks, Lakes or any other inland water bodies etc., including water bodies having an area less than 0.5 acre or more as recorded in the revenue record have to be done by the State/Union Territories".

Sewage Management

Survey to identify the source of pollution and prepare long term preventive measures on Sewage Management should be given immediate attention. A- Filter bed system should be constructed just before the entry of drain water into the tank. The plants that absorb the harmful substances and bad odor from household sewage such as Kalvazhai (*Canna indica)* should be planted before the filter bed. (In sewage inlet of the tank) These plants are simple to grow and grow quickly in practically any weather or soil type. They don't require any human attention once they've been established. Flowers will create a lovely backdrop for the water bodies

Community awareness on protection of water tank

Creating awareness and training programme for tank conservation, promoting public participation are necessary as the sustained measures to prevent pollution of the water tank

Regulating measures

Strict regulation should be followed to stop the dumping of waste along the water tank or inside the tank bed. The small-scale industries which are located inside the residential areas are needed to be provided with the proper disposal network to dispose there effluents.

Public amenities and biodiversity development

The basic amenities such as public toilets should be constructed for the people who do not have the space or resources to construct individual toilets. In addition to this, the de silted soil shall be used to create an island inside the elevated part of the tank where the useful trees shall be planted to attract the birds and to improve the biodiversity of the tank. The species such as *bamboo*, *ziziphus jujuba syzygium cumuni, vitex negundo, mimusops elengi Cherry* trees, *ficus* species, *pithecellobium dulce* shall be planted in this island and *Pongamia* species shall be planted on the tank bund.

During the process of urbanization, some degree of degradation will be unavoidable. Examining the effects each urban activity have on the water resources can help develop the integrated approach so necessary to avoid serious long-term degradation and to encourage sustainable use of the resources.

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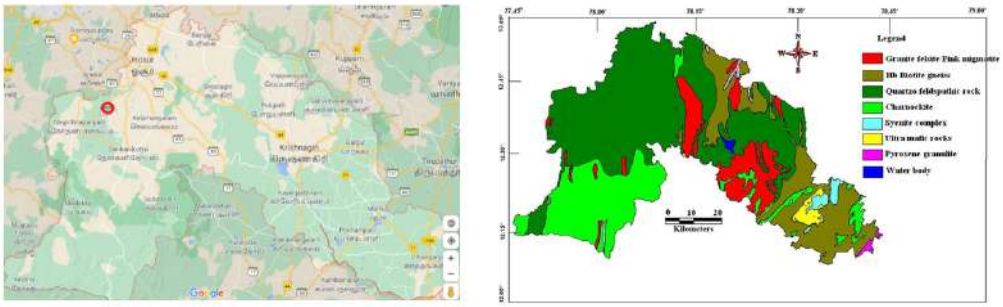


Figure 1 Study area map

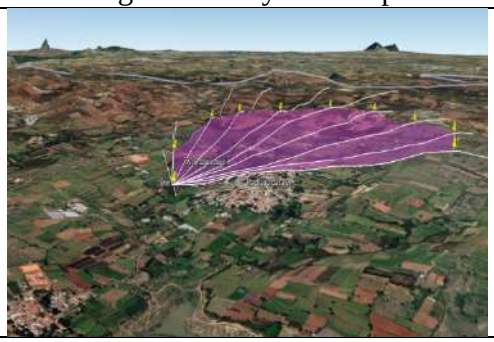
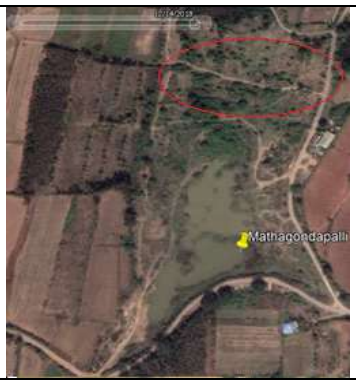


Figure 2 Catchment area of the Gowramma tank

08.12.2002

14.12.2018

Figure 3 map shows the Gowramma tank and urbanization



08.12.2002 | 14.12.2018

Figure 4 Maps showing the infestation of tank bed with development of weeds, new feeder road across the tank

Source: Google earth pro

Figure 5 De-weeding, Desilting, and stabilization of earthen bund (Marked portion- strengthening of tank bunds)

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Correlation and Path Coefficient Analysis in Groundnut (*Arachis hypogaea* L.) Genotypes

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Received: 02 Feb 2022 Revised: 26 Feb 2022 Accepted: 21 Mar 2022

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ABSTRACT

The present investigation was carried out on 30 groundnut genotypes during late Rabi 2019-2020 to access the genetic variability for quantitative parameters following complete Randomized block design with three replications at Bagusala Farm, M.S. Swaminathan School of Agriculture, CUTM, Paralakhemundi, Odisha. Correlation studies revealed significantly positive association of dry pod yield per plant with number of pegs per plant, number pods per plant and fresh pod yield per plant. Therefore these characters could be used as criteria for selection of genotypes with high pod yield. Path coefficient analysis revealed a high positive direct contribution of number of pods/plant and fresh pod yield /plant to the dry pod yield/plant and their correlations with dry pod yield/plant were also significantly positive. Therefore, direct selection based on these morphological traits (number of pods/plant and fresh pod yield/plant) would be more effective in simultaneous improvement of dry pod yield in groundnut breeding programs.

Keywords: Correlation, path coefficient, residual effect**,** direct effect, indirect effect.

INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is a member of the sub-family, Papilionaceae of the family Leguminosae. Groundnut is the sixth most important oilseed crop in the world [1]. Nowadays groundnut is considered to be a high energy food crop due to rich in rich nutrients and vitamins. Groundnuts contain 48-50 per cent of fats consisting mostly of

mono- and polyunsaturated fatty acids. A large percentage of the crop is used for edible oil extraction. Groundnut is an important source of plant-based proteins constitutes around 25-28 per cent of total calories whereas it contains low carbohydrates around 13-16 per cent. Due to low carbohydrate and a rich source of fat, proteins, dietary fibre groundnut has a low glycaemic index (GI), the 564 kcal of energy is produced from 100 g of kernels [2]. It also contains vitamins and mineral such as biotin, copper, niacin, folate, manganese, vitamin E, thiamine, phosphorus and magnesium.

Despite its importance, there are some constraints for the low production due to some environmental changes. Genetic diversity serves as an indispensable aid to study how these genotypes possess variations and adoption to changing environments. This can be achieved by estimating various parameters that affect growth and yield of the crop in a particular environment. Thus the overall study is to investigate genetic variability and identify the superior groundnut genotypes for further exploitation in breeding programs. The study was generally conducted by recording yield contributing parameters in an isolated environment. Therefore the overall study is to investigate genetic variability and identify the superior groundnut genotypes for further exploitation in breeding programmes.

MATERIALS AND METHODS

The experimental material for the genetic divergence studies comprised of 30 diverse genotypes of Groundnut derived from various breeding programmes. The material was made available for study by Agricultural Research Station, Kadiri (Andhra Pradesh). Field study was conducted at Centurion University Agricultural College Farm, Bhagusala, Paralakhemundi, Odisha. The experiment was laid out in a randomized block design replicated thrice. Each genotype was sown in three rows of three meter length with a spacing of 22.5cm between the rows and 10cm within the row. Observations were recorded on ten randomly selected plants in each treatment and in each replication. The plants were selected from the middle of the row excluding the border plants. The crop was harvested at maturity stage on plot basis. Data was collected for characters like days to 50% flowering, days to maturity, plant height (cm), Number of primary branches per plant, number of secondary branches per plant, number of pegs per plant, number of pods per plant, number of filled pods per plant, number of unfilled pods per plant, fresh pod yield per plant (g), dry pod yield per plant (g), 100 pods weight (g), 100 seeds weight (g), oil content (%) and protein content (%). Phenotypic and genotypic correlation was worked out by adopting the formulae suggested by [3]. Path coefficient analysis suggested by [4] and elaborated by [5], was used to calculate the direct and indirect contribution of various traits towards seed yield.

RESULTS AND DISCUSSION

Correlation analysis

The phenotypic and genotypic correlation coefficients estimated for morphological and maturity parameters of 30 groundnut genotypes presented in table 1. and table 2. [6] has suggested the minimum values of correlation coefficient necessary for indirect selection to be more efficient than direct selection for the yield. The dry pod yield per (DPY/P) plant exhibited positively significant correlation with number of pegs per plant (0.392P, 0.642G), number of pods per plant (0.814P, 0.839G), number of filled pods per plant (0.965P, 0.9912G) and fresh pod yield per plant (0.999P, 0.999G) at both genotypic and phenotypic level while, recorded positive significant association with plant height (0.261G) and number of unfilled pods per plant (0.258G) at genotypic level only. Further dry pod yield per plant showed non-significant positive correlation with days to maturity (0.076P, 0.094G) and 100 kernels weight (0.030P, 0.037G) at both genotypic and phenotypic level. Dry pod yield per plant displayed significantly negative correlation with number of primary branches per plant (-0.355P, -0.483G), oil content (-0.246P, -0.299G) and protein content (-0.406P, -0.430G) at both genotypic and phenotypic level while, recorded significant negative association with days to 50% flowering (-0.120G) at genotypic level only. The significant correlation indicates that there is strong association between various traits and DPY/P.

A positive correlation between desirable characters is favorable to the plant breeder because it helps in simultaneous improvement of both characters [7]. Similar findings were reported by [8] for number of pegs per plant, number of pods per plant, number of filled pods per plant and fresh pod yield per plant that showed positive significant correlation with DPY/P. These findings are in agreement with the observations of [9] wherein DPY/P had positive significant correlation with number of pods per plant. Likewise similar results were reported earlier by [10] in that the DPY/P had significant positive correlation with plant height while, recording significant negative correlation with number of primary branches per plant. The results are in consonance with [7, 11] wherein the DPY/P showed positive significant correlation with number of filled pods per plant whereas, positive non-significant correlation with days to maturity. Oil content had negative correlation with DPY/P and similar results were reported by [12].

Path coefficient analysis

In general genotypic path effects were higher than phenotypic. Correlation between different characters is vital in planning selection programmes. However the correlation coefficients only denote the mutual association existing between a pair of characters which themselves are the results of the interaction between various features of the plant. Nevertheless a dependent character is an interaction production of many mutually associated component characters and alteration in any component will disturb the entire network of cause-and-effect system. Path coefficient analysis was carried at both the phenotypic and genotypic levels considering the dry pod yield as dependent character and the attributes as independent character. Each component has two path actions, direct and indirect effect on DPY/P per plant. Both the phenotypic and genotypic correlations were partitioned into direct and indirect effects on DPY/P per plant and the data presented in table 3. (genotypic) and table 4. (phenotypic). The path coefficient analysis is a statistical device developed by [4]. The analysis takes into consideration the cause-and-effect relationship between the variable and is exclusive in partitioning the association into direct and indirect effects through other independent variables. The path coefficient analysis also calculates the effect of causal factors involved. In order to get a transparent picture of the inter-relationship between different characters, the direct and indirect effects of the important quantitative characters on the grain yield had worked separately using path coefficient analysis at both genotypic and phenotypic level. Direct or indirect effects were divided as, negligible when values are between 0.00 and 0.09, low when values range from 0.10 to 0.19, moderate for values between 0.20 to 0.29, high for values 0.30 to 0.99 and the values more than 1.00 are categorized as very high [13].

Path coefficient analysis at genotypic level revealed that number of pods/plant exerted the highest positive direct effect on dry pod yield/plant followed by fresh pod yield/plant and number of secondary branches/plant. [8] reported fresh pod yield per plant had high positive direct effect, 100 seed weight had positively negligible direct effect on dry pod yield whereas, oil content had negatively negligible direct effect on dry pod yield per plant. [14] reported that number of pods per plant had highest positive direct effect whereas, number of filled pods per plant and number of unfilled pods per plant had high negative direct effect on dry pod yield per plant. Days to maturity, number of primary branches/plant and 100 seed weight recorded negligible, positive direct effect on dry pod yield/plant. Conversely plant height, days to 50% flowering, number of pegs/plants, number of filled pods/plant, number of unfilled pods/plant, oil content and protein content recorded negative and negligible direct effect on dry pod yield per plant. Consistent with the above findings [9] reported that the traits oil content and protein content had negative direct effect on dry pod yield per plant. Number of primary branches per plant resulted negligible direct effect on dry pod yield per plant. These results are in concurrence with the findings of [11]. Plant height, days to maturity, days to 50% flowering, number of filled pods per plant and number of unfilled pods per plant resulted with negative indirect effect on dry pod yield through 100 seed weight and the similar findings were reported by [15].

CONCLUSION

The results on character associations revealed that dry pod yield per plant showed a positively significant correlation with the number of pegs per plant, number of filled pods per plant, number of pods per plant and fresh pod yield

per plant. These characters resulted in high positive associations that can be rewarded and should form the selection criteria in breeding programmes. Results on path coefficient analysis for dry pod yield per plant revealed a high positive direct effect with respect to the number of pods per plant and fresh pod yield per plant. Number of secondary branches per plant recorded lower positive direct effect on dry pod yield. The traits day to maturity, day to 50% flowering, plant height, number of primary branches per plant, hundred kernel weight, oil content and protein content resulted in a negligible direct effect on dry pod yield per plant. Path coefficient analysis revealed a high positive direct contribution of number of pods/plant and fresh pod yield /plant to the dry pod yield/plant and their correlations with dry pod yield/plant were also significantly positive. Therefore direct selection based number of pods/plant and fresh pod yield /plant would be more effective in simultaneous improvement of dry pod yield in groundnut breeding programs.

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Table 1. Phenotypic correlation analysis of the morphological and maturity to the dry pod yield/plant (yield)

	DM	DFF	PH	NPB	NSB	NPEP	NPOP	NFP	NUFP	FPY	HSW	OC	PC	DPY
DM	1	-0.074	-0.248 *	-0.148	-0.225 *	-0.112	-0.193	0.005	-0.390 ***	0.074	-0.029	-0.183	-0.052	**0.076**
DFF		1	-0.064	0.270 **	0.160	-0.135	-0.139	-0.097	-0.127	-0.120	-0.162	-0.268 *	0.201	**-0.12**
PH			1	-0.135	0.137	0.109	0.231 *	0.143	0.240 *	0.115	-0.143	0.126	-0.089	**0.114**
NPB				1	0.419 ***	0.030	-0.311**	-0.400 ***	-0.010	-0.356 ***	0.122	0.015	-0.009	**-0.355** ***
NSB					1	0.342 ***	0.052	-0.113	0.275 **	-0.134	-0.291 **	0.011	-0.130	**-0.131**
NPEP						1	0.563 ***	0.426 ***	0.470 ***	0.390 ***	-0.151	-0.155	-0.238 *	**0.392** ***
NPOP							1	0.868 ***	0.664 ***	0.812 ***	-0.038	-0.096	-0.297 **	**0.814** ***
NFP								1	0.206	0.965 ***	-0.030	-0.262 *	-0.356 ***	**0.965** ***
NUFP									1	0.148	-0.029	0.204	-0.050	**0.15**
FPY										1	0.030	-0.246 *	-0.404 ***	**0.999** ***
HSW											1	0.225 *	-0.163	**0.03**
OC												1	-0.089	**-0.246** *
PC													1	**-0.406** ***

Table 2. Genotypic correlation analysis of the morphological and maturity to the dry pod yield/plant (yield)

	DM	DFF	PH	NPB	NSB	NPEP	NPOP	NFP	NUFP	FPY	HSW	OC	PC	DPY
DM	1	-0.093	-0.455 ***	-0.167	-0.247 *	-0.176	-0.202 *	0.020	-0.439 ***	0.092	-0.034	-0.188	-0.067	**0.094**
DFF		1	-0.217 *	0.349 ***	0.194	-0.214 *	-0.189	-0.180	-0.121	-0.224 *	-0.178	-0.303 ***	0.254 *	**-0.221** *
PH			1	-0.410 ***	0.330 ***	0.253 *	0.489 ***	0.262 *	0.610 ***	0.261 *	-0.310** **	0.218 *	0.120	**0.261** *
NPB				1	0.485 ***	-0.020	-0.381 ***	-0.509 ***	-0.031	-0.485 ***	0.187	0.023	0.012	**-0.483** ***
NSB					1	0.470 ***	0.054	-0.131	0.301** *	-0.158	-0.319** **	0.008	-0.146	**-0.155**
NPEP						1	0.830 ***	0.683 ***	0.687 ***	0.637 ***	-0.171	-0.235 *	-0.348 ***	**0.642** ***
NPOP							1	0.885 ***	0.737 ***	0.838** *	-0.043	-0.109	-0.305 ***	**0.839** ***
NFP								1	0.339 ***	0.991 ***	-0.039	-0.320 ***	-0.364 ***	**0.991** ***
NUFP									1	0.256 *	-0.031	0.243 *	-0.089	**0.258** *
FPY										1	0.035	-0.300 ***	-0.428 ***	**0.992** ***
HSW											1	0.234 *	-0.188	**0.0371**
OC												1	-0.095	**-0.299** **
PC													1	**-0.430** ***

Table 3. Direct and indirect path coefficients (genotypic) of yield components on dry pod yield per plant in 30 groundnut (*Arachis hypogaea* L.) genotypes.

		DM	DFF	PH	NPB	NSB	NPEP	NPOP	NFP	NUFP	FPY	HSW	OC	PC
DM		**0.0316**	-0.003	-0.0144	-0.0053	-0.0078	-0.0056	-0.0064	0.0007	-0.0139	0.0029	-0.0011	-0.006	-0.0021
DFF		0.0088	**-0.0939**	0.0204	-0.0328	-0.0182	0.0201	0.0178	0.017	0.0114	0.021	0.0167	0.0285	-0.0239
PH		0.0183	0.0087	**-0.0403**	0.0165	-0.0133	-0.0102	-0.0197	-0.0106	-0.0246	-0.0106	0.0125	-0.0088	-0.0049
NPB		-0.0103	0.0214	-0.0251	**0.0612**	0.0297	-0.0012	-0.0233	-0.0312	-0.0019	-0.0297	0.0115	0.0014	0.0008
NSB		-0.031	0.0243	0.0414	0.0608	**0.1253**	0.0589	0.0068	-0.0164	0.0377	-0.0198	-0.04	0.001	-0.0183
NPEP		0.0478	0.0581	-0.0688	0.0054	-0.1275	**-0.2711**	-0.2251	-0.1854	-0.1863	-0.1728	0.0466	0.0639	0.0946
NPOP		-0.2412	-0.2262	0.5843	-0.4555	0.0651	0.9911	**1.1937**	1.0572	0.8804	1.0005	-0.0521	-0.1307	-0.3648
NFP		-0.0035	0.0309	-0.0448	0.087	0.0224	-0.1167	-0.1512	**-0.1707**	-0.058	-0.1692	0.0067	0.0547	0.0621
NUFP		0.2212	0.0609	-0.3074	0.0159	-0.1516	-0.3463	-0.3716	-0.1711	**-0.5037**	-0.1291	0.016	-0.1228	0.0452
FPY		0.0456	-0.1099	0.1284	-0.2379	-0.0777	0.3126	0.411	0.4861	0.1256	**0.4904**	0.0176	-0.1472	-0.21
HSW		-0.0003	-0.0017	-0.0029	0.0017	-0.003	-0.0016	-0.0004	-0.0004	-0.0003	0.0003	**0.0093**	0.0022	-0.0018
OC		0.007	0.0113	-0.0081	-0.0009	-0.0003	0.0088	0.0041	0.0119	-0.0091	0.0111	-0.0087	**-0.0371**	0.0036
PC		0.0008	-0.0029	-0.0014	-0.0001	0.0017	0.004	0.0035	0.0041	0.001	0.0048	0.0021	0.0011	**-0.0113**
DPY	G	0.0949	-0.2219	0.2613	-0.4839**	-0.1554	0.6427**	0.8391**	0.9912**	0.2583	0.9992**	0.0371	-0.299**	-0.4309**
	R^2	0.003	0.0208	-0.0105	-0.0296	-0.0195	-0.1742	1.0016	-0.1692	-0.1301	0.4904	0.0003	0.0111	0.0049

Residual effect= 0.0310

Table 4. Direct and indirect path coefficients (phenotypic) of yield components on dry pod yield per plant in 30 groundnut (*Arachis hypogaea* L.) genotypes.

		DM	DFF	PH	NPB	NSB	NPEP	NPOP	NFP	NUFP	FPY	HSW	OC	PC
DM		**0.0041**	-0.0003	-0.001	-0.0006	-0.0009	-0.0005	-0.0008	0	-0.0016	0.0003	-0.0001	-0.0008	-0.0002
DFF		-0.0001	**0.0008**	-0.0001	0.0002	0.0001	-0.0001	-0.0001	-0.0001	-0.0001	-0.0001	-0.0001	-0.0002	0.0002
PH		0.0002	0.0001	**-0.0009**	0.0001	-0.0001	-0.0001	-0.0002	-0.0001	-0.0002	-0.0001	0.0001	-0.0001	0.0001
NPB		-0.0001	0.0002	-0.0001	**0.0006**	0.0003	0	-0.0002	-0.0002	0	-0.0002	0.0001	0	0
NSB		-0.0006	0.0004	0.0004	0.0011	**0.0027**	0.0009	0.0001	-0.0003	0.0007	-0.0004	-0.0008	0	-0.0004
NPEP		0	0	0	0	0.0001	**0.0002**	0.0001	0.0001	0.0001	0.0001	0	0	-0.0001
NPOP		-0.4203	-0.3022	0.5023	-0.6758	0.1138	1.2232	**2.1694**	1.8841	1.4406	1.763	-0.0829	-0.2102	-0.6463
NFP		-0.0082	0.1609	-0.237	0.6606	0.1877	-0.7024	-1.431	**-1.6477**	-0.3396	-1.5908	0.0503	0.4323	0.5876
NUFP		0.4272	0.1399	-0.263	0.0112	-0.3017	-0.515	-0.7275	-0.2258	**-1.0956**	-0.1621	0.0323	-0.224	0.055
FPY		0.074	-0.1191	0.1138	-0.3527	-0.1334	0.3861	0.8038	0.9549	0.1464	**0.9891**	0.0297	-0.2438	-0.3999
HSW		0	-0.0003	-0.0002	0.0002	-0.0005	-0.0003	-0.0001	-0.0001	0	0.0001	**0.0017**	0.0004	-0.0003
OC		0	0	0	0	0	0	0	0	0	0	0	**0.0001**	0
PC		0.0001	-0.0005	0.0002	0	0.0003	0.0006	0.0007	0.0009	0.0001	0.001	0.0004	0.0002	**-0.0024**
DPY	P	0.0763	-0.1202	0.1144	-0.3551**	-0.1317	0.3928**	0.8143**	0.9658**	0.1508	0.9998**	0.0305	-0.2461	-0.4067**
	R^2	0.0003	-0.0001	-0.0001	-0.0002	-0.0004	0.0001	1.7666	-1.5913	-0.1652	0.9889	0.0001	0	0.001

Residual effect= 0.0165

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Overview of Diabetes and Recent Advances in Its Treatment Strategy

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Received: 19 Jan 2022 Revised: 24 Feb 2022 Accepted: 25 Mar 2022

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ABSTRACT

Diabetes is one of the most common metabolic disorders characterized by insulin insufficiency or insulin resistance or hyperglycemia that assists as the major biomarker for the diagnosis of diabetes as well. It has a significant impact on health and has evolved into a major non-communicable illness with a rising mortality and morbidity rate over the world. Insulin is a hormone produced by the beta cells of the pancreas' Langerhans and is a key regulator in the body's glucose levels Patients with mild hyperglycemia may go unnoticed at first, but as time Diabetes mellitus has (WHO) divided diabetes into four types: type 1 diabetic mellitus (T1DM), type 2 diabetic mellitus (T2DM), gestational diabetes, and gestational diabetes, and some specific types of diabetes. Patients with diabetes have co-existence of multiple complications that could lower the quality of life resulting in diabetic nephropathy, diabetic neuropathy, retinopathy, etc. people with diabetes are also on the verge of increasing risk of heart diseases, nerve degeneration etc. At the moment, modern medical care focuses on healthy lifestyle changes, prevention of complications, increased self-care and recently discovered pharmaceutical interventions aimed at preventing and treating hyperglycemia. Insulin, insulin analogs, non-insulin oral hypoglycemic medicines, and genetic treatments techniques are the most commonly used pharmaceuticals in clinical treatment, depending on the type of diabetes. This review provides a brief about the causes, pathophysiology, complications, risks, diagnosis, and recent developments in the treatment of diabetes mellitus, as well as the evolving trend of medication therapy and the use of delivery systems.

Keywords: diabetes mellitus, insulin, diagnosis, complications, gene therapy.

INTRODUCTION

Diabetes mellitus is a collection of metabolic diseases characterized by chronic hyperglycemia brought on by insulin synthesis, insulin action, or both. Diabetes causes anomalies in glucose, lipid, and protein metabolism due to insulin's ineffective action on target tissues. [13]. According to the World Health Organization (WHO), that affects a large part of the population. The underlying cause is the defective production or action of insulin hormone.[2]. Diabetes is a dangerous, chronic condition that happens when the pancreas produces insufficient insulin (a hormone that regulates blood glucose) or when the body's insulin is ineffectively used. Uncontrolled diabetes causes elevated blood glucose, which can cause catastrophic damage to the heart, blood vessels, eyes, kidneys, and nerves over time. Diabetes affects over 400 million people worldwide. Patients with diabetes are growing more frequently all across the world, and the disease is approaching epidemic proportions. Diabetes and related illnesses account for a sizable portion of annual health spending. There are several concepts engaged in the etiopathogenesis of the disease and the pandemic dissemination of the disease. Although there is no cure for diabetes, it appears that it can be managed by regular exercise, eating healthy, etc.[3]. complications affecting the eyes, kidneys, and nervous system. Although diabetes also substantially increases the risk for cardiovascular disease, cardiovascular disease[4]. Furthermore, serious acute metabolic disorders will be extremes in the range of dysglycemia. diabetic ketoacidosis and hyperglycemia are examples of dysglycemia. Therefore, Diabetes mellitus has become a major public health concern in recent years. Diabetes mellitus is classified by the World Health Organization (WHO). Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes, as well as other kinds of diabetes.

An Overview of Insulin

Insulin is a polypeptide hormone produced mostly by beta cells in the pancreatic islets of Langerhans. In conjunction with glucagon, the hormone may influence blood glucose levels, hence playing a function in carbohydrate metabolism regulation. Insulin binds to the insulin receptor (INSR) on the plasma membrane of target cells to exert all of its recognized physiological effects.[5]. Insulin controls blood glucose levels and causes glucose storage in the liver, muscles, and adipose tissue, resulting in weight gain. Glucose-induced insulin stimulation is the process through which insulin is released after eating glucose. This mechanism necessitates both intracellular glucose uptake and metabolic breakdown. Insulin insufficiency prevents cells from using glucose as an energy source. As a result, elevated glucose levels in the bloodstream cause a condition called hyperglycemia. Long-term hyperglycemia can lead to diabetes mellitus and other health problems, including damage to the brain system and eye and kidney malfunction [6]. Impaired insulin secretion is common in diabetes mellitus and is responsible for many of the metabolic abnormalities associated with this disease. [7].

Pathology of Diabetes Mellitus

Diabetes is caused by several different pathogenic mechanisms. These range from autoimmune death of pancreatic cells, resulting in insulin shortage, to anomalies that result in insulin resistance. Diabetes causes abnormalities in glucose, lipid, and protein metabolism due to insulin's insufficient action on target tissues. Polyuria, polydipsia, weight loss, occasionally polyphagia, and blurred vision are all symptoms of severe hyperglycemia. Retinopathy, which can lead to vision loss; nephropathy, which can lead to renal failure; peripheral neuropathy, which can lead to foot ulcers, amputations, and Charcot joint; and autonomic neuropathy, which can cause gastrointestinal, genitourinary, and cardiovascular symptoms as well as sexual dysfunction. [8].Atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease are more common in diabetic patients. People with diabetes are more likely to have hypertension and impaired lipoprotein metabolism. Insulin stimulatory actions.

Classification of Diabetes Mellitus and Other Glucose Regulation Categories

The circumstances existing at the time of diagnosis play a large role in assigning a type of diabetes to an individual, and many diabetics do not neatly fall into a single category. A person with gestational diabetes mellitus (GDM) may, for example, remain hyperglycemic after birth and be diagnosed with type 2 diabetes[8]. The present classification system is based on both the etiology and pathophysiology of a disease, and it is effective in clinical disease

assessment and therapeutic selection. Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and diabetes-induced or related with particular specific illnesses, conditions, and disorders are the four basic forms or categories of diabetes, according to this classification[9].

Type 1 Diabetes (Absolute Insulin Deficiency Due To Destruction Of Beta-Cells)

A shortage of insulin synthesis in the body causes diabetes mellitus (also known as insulin-dependent diabetes, juvenile diabetes, or childhood diabetes) or immune-mediated diabetes. This kind of diabetes, which affects only 5–10% of diabetics and was previously known as insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes, is caused by cellular-mediated autoimmune destruction of the pancreatic beta-cells. T1DM that progresses rapidly is most frequent in youngsters, although it can also affect adults. Ketoacidosis may be the first symptom of the disease in certain people, particularly children and adolescents[10]. Markers of the immune destruction of the beta-cell include islet autoantibodies against cells, insulin autoantibodies, glutamic acid decarboxylase (GAD65) autoantibodies, and tyrosine phosphatases IA-2 and IA-2 autoantibodies. The rate of beta-cell breakdown in this kind of diabetes is extremely diverse, being fast in some people (mostly infants and children) and slow in others (mostly adults). In this form of diabetes, the rate of beta-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Others have mild fasting hyperglycemia, which can quickly progress to severe hyperglycemia and/or ketoacidosis if they are exposed to an infection or other infections. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. Autoimmune destruction of beta-cells is linked to a variety of genetic predispositions as well as yet-to-be-identified environmental factors. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia. [8]. To control the quantity of glucose in their blood, people with type 1 diabetes need to take insulin every day. They will perish if they do not have access to insulin. Type 1 diabetes has no known etiology and is not yet preventive. Excessive urine and thirst, persistent hunger, weight loss, eyesight abnormalities, and weariness are all symptoms[11]. Insulin is the first line of treatment for type 1 diabetes, and injections or an insulin pump can be used to deliver insulin. Insulins can be classified into three categories: rapid-acting, long-acting, and intermediate-acting. Regular insulin (HUMULIN 70/30, NOVOLIN 70/30), insulin isophane (HUMULIN N, NOVOLIN N), insulin glulisine (APIDRA), insulin lispro (HUMALOG), and insulin aspart (HUMALOG) are some of the insulins available (NOVOLOG). Glargine (LANTUS), detemir, and other long-acting insulins are available (LEVEMIR) [12].

Type 2 Diabetes (Insulin Resistance with Relative Insulin Insufficiency to Insulin Resistance with an Insulin Secretory Defect)-

This form of diabetes, which accounts for 90–95% of those with diabetes, is a common and serious global health problem that has evolved in response to rapid cultural, economic, and social changes, such as population aging, increasing and unplanned urbanization, dietary changes such as increased consumption of highly processed foods and sugar-sweetened beverages, obesity, reduced physical activity, unhealthy lifestyle and behavioral patterns, fetal malnutrition, and increased fetal exposure to hyperglycemia during pregnancy. T2DM is most common in adults, however, it is now affecting an increasing number of children and adolescents[10].Type II diabetes, often known as adult-onset diabetes or non-insulin dependent diabetes, refers to people who have insulin resistance and have a relative (rather than absolute) insulin deficit. These people do not require insulin medication to survive, at least at first and often throughout their lives. This type of diabetes is likely caused by a variety of factors. Despite the absence of particular etiologies, autoimmune destruction of beta-cells does not occur. Because hyperglycemia develops gradually and is often not severe enough for the patient to detect any of the usual signs of diabetes in its early stages, this kind of diabetes frequently goes untreated for many years. Despite this, such patients are more likely to suffer macrovascular and microvascular problems. While patients with this kind of diabetes may have normal or raised insulin levels, the higher blood glucose levels in these diabetic patients are predicted to cause complications. If their beta-cell function was normal, they would have even greater insulin levels. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may

improve with weight loss and/or pharmaceutical hyperglycemia therapy, although it is seldom recovered to normal. The chance of having this type of diabetes rises as you become older. obesity, and lack of physical activity. It is more common in women who have had previous GDM and in people who have hypertension or dyslipidemia, and the occurrence varies by racial/ ethnic minority. A combination of genetic and metabolic variables determines type 2 diabetes.

Gestational Diabetes Mellitus

Gestational Diabetes Mellitus or GDM is a clinical problem where a woman especially and commonly without previously diagnosed diabetes mellitus presents with high blood glucose level during pregnancy particularly in the third trimester. It is caused when insulin receptors malfunction or is insensitive or resistant destroyed. Pregnancy-related variables such as the presence of HPL (human placental lactogen) interacting with receptors may cause GDM. As a result, blood glucose levels are abnormally high. GDM commonly does have a few minor symptoms and thus it needs to be diagnosed very often by screening of the pregnant women. As with GDM, babies born to mothers with untreated diabetes are generally and typically at increased risk of problems such as being large babies for pregnancy duration with its attendant puerperal complications, hypoglycemia, icterus, etc. If left untreated or neglected and overlooked, it can also cause hyperglycemic convulsions and stillbirths. GDM plus its attendant complications are preventable[13]. The hallmark of GDM has increased. Gestational hormones and other contributors are incriminated to interfere with insulin as it links its receptors. Gestational hormones and other contributors are incriminated to interfere with insulin as it links its receptors. The interference is most likely occurring at the level of the insulin receptor's cell signaling pathway Since insulin causes glucose entry into cells, insulin resistance prevents glucose entry, resulting in hyperglycemia. Thus, additional insulin is required to counteract this resistance; about 2 times more insulin is produced in GDM than otherwise normal gestation.

Drug- Or Chemical-Induced Diabetes

Insulin secretion and action can both be hampered by a variety of medications. These medications have the potential to cause diabetes in those who have insulin resistance or moderate-cell dysfunction. Toxins like pyrinuron (a rat poison) and pentamidine can kill pancreatic cells irreversibly. Fortunately, adverse medication reactions are uncommon[10]. Because the sequence or relative importance of cell dysfunction and insulin resistance is uncertain in these situations, the classification is ambiguous. Toxins like Vacor (a rat poison) and intravenous pentamidine can kill pancreatic beta-cells irreversibly. Such drug reactions fortunately are rare. There are several drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Interferon-treated patients have been documented to develop diabetes with islet cell antibodies and, in some cases, severe insulin insufficiency. Vacor, Pentamidine, Nicotinic acid, Glucocorticoids, Thyroid hormone, Diazoxide, adrenergic agonists, Thiazides, Dilantin are some drug or chemical-induced instances. [8].

Uncommon Forms of Immune-Mediated Diabetes

For many years, a slowly evolving form of immune-mediated diabetes has been described, most commonly in adults who have evidence 16 of pancreatic autoantibodies that can react with non-specific cytoplasmic antigens in islet cells, glutamic acid decarboxylase (GAD), protein tyrosine phosphatase IA-2, insulin, or ZnT8 [10]. There are two known conditions in this category, with more on the way. The stiff-man syndrome is a central nervous system autoimmune illness marked by axial muscular rigidity and severe spasms. Patients frequently have high GAD autoantibody titers, and about one-third of them will develop diabetes. By attaching to the insulin receptor, anti-insulin receptor antibodies can cause diabetes by preventing insulin from binding to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Patients with systemic lupus erythematosus and other autoimmune disorders may have anti–insulin receptor antibodies Ethnicity, family history of diabetes, and previous gestational diabetes combined with older age Obesity and overweight, as well as physical inactivity, are thought to account for a large portion of the global diabetes burden. As in other states of extreme insulin resistance, insulin activity can also be hampered by a variety of medications and hormones.

Risk Factors for Diabetes

Type 1. Although no specific environmental risk factors have been established to produce a considerable number of cases, it is widely accepted that type 1 diabetes is the result of a complex interaction between genes and environmental variables. The majority of type 1 diabetes occurs in children and adolescents, environmental risk factors have been shown to cause a significant number of cases. The majority of type 1 diabetes occurs in children and adolescents[11].

Type 2. A combination of genetic and metabolic variables influences the risk of type 2 diabetes. Ethnicity, family history of diabetes, and past gestational diabetes all raise risk, as do older age, overweight and obesity, a poor diet, lack of physical activity, and smoking. Overweight and obesity, as well as physical inactivity, are thought to be responsible for a significant percentage of the global diabetes burden. Excessive intake of saturated fatty acids, high total fat intake, and inadequate dietary fiber consumption have all been linked to unhealthy body weight and/or type 2 diabetes risk. High intake of sugar-sweetened beverages, which contain considerable amounts of free sugars, increases the likelihood of being overweight or obese, particularly among children[11].

Gestational diabetes- The two principal risks GDM entailing the baby are growth anomalies and chemical imbalances following birth, which may need hospitalization a neonatal ICU. Infants born to women with If GDM is mismanaged, it can result in both large for gestational age (macrosomic) and small for gestational age, as well as intrauterine growth retardation or mortality. [11]. Untreated GDM also affects maturation, resulting in dysmature babies who are more prone to have respiratory distress due to a lack of lung growth and maturation, as well as decreased surfactant production. [11].

Diagnostic Criteria for Diabetes Mellitus

The presence of chronic hyperglycemia must be confirmed to diagnose diabetes. Diabetes is diagnosed when two or more tests, performed on different days, reveal the presence of the disease,[14] End organ damage, dysfunction, and failure in organs and tissues such as the retina, kidney, neurons, heart, and blood vessels are all linked to the chronic hyperglycemia of diabetes mellitus (DM). Because untreated diabetes can lead to major complications, getting a diagnosis early can help you avoid serious complications. High blood glucose levels over an extended length of time, frequent urination, increased thirst, and increased hunger are all common diabetes symptoms [3**].** To diagnose prediabetes or diabetes, some biochemical tests are performed regularly basis. Oral glucose tolerance tests (OGTT) and glycosylated hemoglobin (HbA1c) are routinely used to check for diabetes. The OGTT test determines how well body cells absorb glucose after a specified amount of sugar is consumed. The suspected person is usually given 75 grams of glucose orally, and the plasma glucose level is tested two hours later. If the blood glucose level is less than 11.1 mmol/L, the person is diagnosed as diabetic [15]. Another reliable routine method for diagnosing diabetes is a fasting plasma glucose test. Fasting plasma glucose test: This test should be taken after an eight-hour fast. A blood glucose level of more than 126 mg/dl on two or more separate tests are considered a diabetes diagnosis [16]. HbA1c is also commonly used as a diabetes diagnostic test. The glycosylated hemoglobin level in T2DM patients is 48 mmol/mol (6.5 DCCT percent) [15]. Another prognostic sign for detecting diabetes is random blood sugar monitoring [3].

Complications of Diabetes

When diabetes isn't properly treated, it can lead to complications that are dangerous to one's health and life. Acute complications are a major cause of death, high expenditures, and poor quality of life. If abnormally high blood glucose causes illnesses like diabetic ketoacidosis (DKA) in types 1 and 2, and hyperosmolar coma in type 2, it can be fatal. All types of diabetes can cause abnormally low blood glucose, which can lead to seizures or loss of consciousness. It can happen if you skip a meal or exercise more than normal, or if you take too much anti-diabetic medicine.[11].The high blood glucose level in the delicate vessels of the retina increases osmotic pressure, causing the capillaries to leak or burst in some cases, resulting in a reduced blood supply to the retina in uncontrolled diabetes. Collateral blood vessels sprout out of the retina to compensate for the ruptured retinoid vessels, causing scar tissue to form and impaired vision. Diabetic nephropathy is one of the major complication that occurs when long-term diabetes damages the basement of glomerular capillaries, disrupts protein crosslinking and allows proteins in the

urine to flow through. Diabetes patients frequently develop ketoacidosis as a result of the constant synthesis of ketone bodies. Diabetic ketoacidosis (DKA) is a symptom of insulin insufficiency rather than T2DM's characteristic insulin resistance [3].Diabetic retinopathy is the most prevalent diabetic microvascular problem. Every year, it is responsible for 10,000 new cases of blindness. Growth factors such as vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor have also been shown to have a role in diabetic retinopathy development [17].

Treatment Strategy

1- Drug therapies

Anti-diabetic medications include insulin, insulin analogs, and non-insulin hypoglycemic treatments that include insulin sensitizers, insulin secretagogues, and glucose regulators, as well as gene therapy [18]. The various drug groups function in different ways, but they all help to maintain normal blood glucose levels.

Insulin and its Analogs

Insulin is one of the most important and necessary exogenous medications in the treatment of T1DM and severe T2DM because of the absolute or relative insufficiency of insulin secretion. Insulin and its analogs principal physiological roles are to regulate sugar, fat, and protein metabolism in vivo and to keep blood glucose levels in a normal range. They stimulate the target cell membrane carriers in muscle and adipose tissue to transport glucose into cells, accelerate glycogen synthesis in liver and muscle cells, and inhibit glycogen breakdown and PEP carboxykinase synthesis. The rapid-acting analogues were created to speed up the absorption of insulin, and at the same time, minimize postprandial glucose increases more effectively and lower the risk of hypoglycemia due to the high exogenous insulin concentrations for a longer period time. Insulin lispro (Humalog), insulin glulisine (Apidra), and insulin aspart are three rapid-acting analogs now available (NovoLog). These insulin analogs are particularly for individuals with postprandial hyperglycemia and can be injected 0–15 minutes before or after a meal, with excellent compliance [18]. Supplement therapy, replacement therapy, and intensification therapy are the three basic therapeutic techniques for insulin and its analogs. Injecting intermediate-acting insulin or long-acting insulin analogs before bedtime is commonly recommended for patients on supplemental medication to decrease hepatic sugar output and manage to fast blood glucose levels. The hypoglycemic impact peaks 6–8 hours after injection, which could successfully prevent the "dawn phenomenon." Some individuals with poor blood sugar management after dinner can choose to increase their injection before breakfast to ensure enough insulin concentration after dinner. At the same time, this treatment should pay special attention to the risk of hypoglycemia at night [18]. T1DM patients with severely damaged pancreatic function and absolute loss of insulin secretion, as well as T2DM patients with the long-term disease who are intolerant to oral hypoglycemic drugs and have liver and kidney hypofunction these patients, are suitable for insulin replacement therapy. Insulin replacement treatment is currently being used routinely in clinics. For patients, there are always two options: injecting premixed insulin twice a day before breakfast and dinner or three times a day before or soon after a meal, or injecting short-acting insulin before meals in combination with basic insulin injection before bedtime [18].

Non-insulin hypoglycemic drugs- are first-line treatments for patients who are unable to maintain normal blood glucose levels just by adjusting their diet and exercising moderately. These medications are currently available in a variety of forms, including biguanide, sulfonylurea, thiazolidinedione, and glinide. Dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide1 receptor agonist, and sodium-glucose cotransport protein 2 inhibitors are among the many novel medications in development. In addition, considerable focus was placed on free fatty acid receptor 1 agonists, glucokinase agonists, and protein tyrosine phosphatase-1B inhibitors, which are still being studied. Drugs can be classified as insulin sensitizers, insulin secretagogues, or glucose regulators based on their mode of action [18].

Glucose Regulators

A novel family of hypoglycemic medicines known as sodium-glucose cotransporter 2 (SGLT2) inhibitors has been proposed that is not dependent on the insulin secretion route. Sodium-glucose cotransporters 1 (SGLT1) and 2

(SGLT2) are critical mediators of glucose transport across epithelial membranes. While SGLT1 is responsible for the bulk of dietary glucose uptake in the gut, SGLT2 is in charge of the majority of glucose reuptake in the kidney tubular system. Approximately 180 g of glucose is filtered from the original urine every day under normoglycemic conditions, and almost all of it is reabsorbed by the proximal tubules, with SGLT2 mediating 97 percent and SGLT 1 mediating 3% (Bonner et al., 2015). But for diabetic patients who have already experienced hyperglycemia, increasing glucose reabsorption in the renal tubules would result in a significant increase in blood glucose concentration. As a result, SGLT2 inhibitors bind glucose to transporters competitively, restrict renal tubular reabsorption of glucose, and aid excess glucose excretion with urine to regain euglycemia. At the same time, the inhibitors do not operate on pancreatic or intestinal cells to increase the burden of insulin secretion, which is protective to pancreatic-cell function. SGLT-2 inhibitors not only provide a good hypoglycemic impact, but also protect the cardiovascular and kidney systems by decreasing blood pressure, lipids, and uric acid. However, clinical investigations have revealed that SGLT-2 inhibitors have several side effects, the most common of which are ketoacidosis, hypoglycemia, and hypoglycemia. h and urogenital system infection. Daglitazone, englenet, ruglietin, caglione, eglitoglione, and other SGLT-2 inhibitors are listed on the market [18].

Gene Therapy

Alternative ways for treating diabetes are being studied due to the inherent limitations of exogenous insulin and transplant-based therapy. Future treatments will aim to restore dynamic blood glucose control without the need for time-consuming daily injections, major surgical procedures, or lifetime immunosuppressive regimes. Future medicines must also be affordable to all patients. Gene therapy has emerged as a promising treatment option for T1DM that meets all of the criteria [19]. To restore or replace lost biological capabilities, therapeutic genes are given to target cells using a vector, such as a virus. In the case of T1DM, therapeutic gene transfer can enhance diabetic patients' clinical outcomes by preventing autoimmune beta-cell destruction prior to the beginning of disease, non-beta-cells can be reprogrammed to act as surrogate cells, or lost cells can simply be replaced. replacing the function of beta-cells using insulin gene therapy [19]. Gene therapy is a potential technique for the treatment of diabetes mellitus because it targets the disease's fundamental cause and allows us to stop or reverse the disease's progression. DNA, small interfering RNA (siRNA), mRNA, micro RNA, and antisense oligonucleotides are the most common genetic medicines utilized in gene therapy. Replacement gene therapy, immunological gene therapy, and regulatory gene therapy are three types of gene therapy for diabetes mellitus [18].

Replacement Gene Therapy:

Non-beta-cells are capable of secreting insulin and can be used to replace damaged pancreatic beta-cell cure and rectify insulin synthesis and secretion deficiencies. A successful replacement gene therapy must meet several requirements. An effective insulin gene transfer mechanism; a glucose-responsive regulatory system that controls insulin expression and release; transfected cells that can transform proinsulin into mature and active insulin; target cells that have biochemical features comparable to -cells but are immune-resistant [18]. To transfer genes into target tissues or cells, such as the pancreas, liver, intestinal endocrine K cells, and muscle cells, viral and non-viral vectors such as lentivirus and adeno-associated virus, as well as liposomes and plasmids, have been used. Intestinal endocrine K cells, which resemble pancreatic -cells in many ways, have been shown to produce glucose-dependent insulinotropic polypeptide (GIP) and contain prohormone converting enzymes required for proinsulin processing [20]. After introducing the GIP promoter into K cells of the gastrointestinal tract area, transgenic mice generated by streptozotocin (STZ) displayed long-term euglycemia. These findings suggested that K cells could produce enough insulin to keep glucose level in check [21]. Attempt to repair the impaired beta-cell function by injecting adeno-associated viral vectors containing insulin and glucokinase genes into the skeletal muscle of STZ-induced diabetes mice and dogs [21]. A substantial fraction of beta-cells is found in human islets. The co-expression of these two genes increased GLUT4 and glucokinase translocation, as well as glucose transport into muscle cells. Furthermore, glucokinase may operate as a "glucose sensor," regulating insulin release in response to variations in blood glucose levels. Infused adeno-associated viruses as vectors to contain Pdx1 and MafA expression cassettes to reprogram alpha-cells into functional beta-cells and repair damaged beta-cell function. Alpha cells provide the following

advantages as a potential source of beta-cells replacement. The growth mechanism of alpha cells is similar to that of beta-cells since they are endocrine cells, which may be advantageous for reprogramming. Human islets, in particular, contain a high number of alpha cells, making them a potentially rich source for reprogramming. Appropriately reducing the number of alpha cells does not effect on normal glucose metabolism and is beneficial to blood glucose [15].

Immune Gene Therapy

Immune gene therapy is generally applied to patients with early T1DM. Because of the intricate autoimmune mechanism of T1DM, which involves a wide range of cells and many signaling pathways, current research focuses on transducing target genes to stop or reverse the autoimmune response. Immunological intervention may be able to safeguard islet cell function and lessen the patient's reliance on insulin therapy. IL-10, an anti-inflammatory cytokine with multiple biological functions, can alter the organism's immune response as well as the expression of MHC class II antigens. It may also have a role in mediating the mutual control of Th1 and Th2 cells, as well as having a suppressive function in the prevention of autoimmune illness [18]. IL-10, an anti-inflammatory cytokine with multiple biological functions, has the ability to alter the organism's immune response as well as the expression of MHC class II antigens. It may also have a role in mediating the mutual control of Th1 and Th2 cells, as well as having a suppressive function in the prevention of autoimmune illness [20].

Regulatory Gene Therapy

Dozens of cytokines that regulate the development and maturation of pancreatic -cells, as well as the synthesis and production of insulin. A collection of precise mechanisms regulates the expression of numerous genes as well as the activation and inactivation of certain proteins. As a result, the researchers attempted to introduce genes for linked cytokines into the organism to improve insulin secretion and blood glucose balance [18].Insulin-like growth factor 1 (IGF1) is a beta-cell mitogen and pro-survival factor that has been shown to boost glucose and amino acid uptake, glycogen synthesis, and organ insulin sensitivity. In addition, IGF1 modulates immunological processes and is one of the primary players in the immune-endocrine system crosstalk. In transgenic mice, it was discovered that over expressing IGF1 in beta-cells stopped the over expression of human interferon-beta (IFN-beta) in beta-cells, preventing islet infiltration and immune cell-mediated beta-cell death. In a study, AAV serotype 8 (AAV8-IGF1-dmiRT) expressing IGF-1 was created and injected into the pancreatic alveolar cells of adult mice, with tissue-specific gene expression achieved using micro RNA target sequences [18].The findings demonstrated that the expression of IGF1 in the pancreas could prevent the occurrence of diabetes in non-obese mice by blocking the β-cell directed autoimmune attack. As a result, microRNA-mediated AAV-mediated IGF-1 gene transfer has a lot of therapeutic potential for T1DM therapy and prevention [22].

Drug Delivery Systems in Diabetes Mellitus Treatment

Due to the difficulties of pharmacological therapy and the superiority of nanoparticles (NPs) in drug transport and imaging [23]. Researchers are increasingly interested in nanoparticles (NPs) in nanocarriers for diabetes therapy and management of diabetes. The majority of drug delivery systems are made up- Liposomes, polymer-based NPs, and inorganic NPs are all examples of nanoparticles. Various polymer-based NPs, such as nanospheres, are among them. As a result, nano-capsules, micelles, and dendrimers have been produced carriers of drugs showing the various types of nanocarriers that have been used for loading insulin and other anti-diabetic medications, as well as in vivo effects, have been reported. These nanocarriers have been developed to be potentially advantageous in a variety of ways, including shielding medicines from enzymatic breakdown and enhancing their effectiveness in vivo stability, as well as overcoming various biological hurdles and Bioavailability is being improved. They could also serve as an intelligent assistant a fully automated system that mimics endogenous insulin administration has a non-linear reaction to environmental stimuli, this lowers the risk of hypoglycemia and improves patient compliance. Furthermore, they show excellent performance in more precisely delivering pharmaceuticals to targeted areas and sustaining and controlling drug release inside targeted sites over a long periods time, reducing unwanted side effects and maximizing therapeutic efficacy [18]. Because of their distinctive photoluminescent features, quantum dots and

metal-oxide NPs are frequently used in the detection of pH and chemical analytes, as well as imaging in drug delivery. At the same time, polymer material features such as mean particle size and polydispersity, surface electrical charge, and hydrophilicity of nanoparticles are critical for antidiabetic medication delivery [23]. As a result, developing optimal NP delivery systems for efficient diabetic therapy is both required and important.

Nanoliposome- Liposomes are spherical vesicles that are made up of one or more lipid bilayers generated by the self-assembly of phospholipids. Drugs having poor permeability, both hydrophilic and hydrophobic, could be enclosed in hydrophilic interior watery cores or hydrophobic lipid bilayers, or even bonded to the vesicle's surface [18]. Liposomes are appealing vehicles in the field of drug administration because of their high biocompatibility, biodegradability, protective action against enzymatic degradation, and cell-specific targeting. Cationze Cas9 proteins with a single guide RNA (sgRNA) form a highly unionized RNP (ribonucleoprotein) complex in the CRISPR/Cas9 system. The complex might be encapsulated with cationic liposomes and then supplied to cells via endocytosis and macropinocytosis for improved cell membrane permeability and protein stability. Cationic liposomes interact with negatively charged gene therapy medications that are successfully compressed from extended structures to smaller particles through electrostatic contact to form a transfection complex as positively charged lipid vesicles. The lecithin liposome was created as a nanocarrier to contain Cas9-RNP complexes for target delivery to the liver via polymer fusion self-assembly. The sgRNA targeting the dipeptidyl peptidase-4 gene (DPP-4) was particularly designed for this delivery method to block the breakdown of glucagon-like peptide 1 and increase insulin secretion [24]. T2DM animals injected with nano carrier Cas9-RNP complexes demonstrated a significant reduction in DPP-4 gene expression, as well as euglycemia, insulin responsiveness, and reduced liver and kidney damage. These findings imply that the nano-liposomal carrier system containing therapeutic Cas9-RNP has a lot of promise for treating T2DM.

Polymer Nanogel- Smart nanohydrogels loaded with antidiabetic medicines could rapidly shift their structure-swell or shrink-in response to pH and temperature changes in the surrounding media, which are common triggers changing hydrogels from "off" to "on" state. Nanogels could shield protein pharmaceuticals from enzymatic degradation, carry them unmolested to the intestine, and effectively control the release rate of preloaded drugs by acting as innovative polymeric devices. [24]

CONCLUSION

The recent advances in the treatment of diabetes mellitus, epidemiology of diabetes, and the proper management of the condition were the emphasis of this review. Diabetes has evolved into a serious chronic illness, and the number of diabetic people is rising at an alarming rate, making it a major public health concern a crucial research subject. The failure of the insulin hormone is the primary mechanism of diabetes. The failure to utilize glucose results in diabetes, which is characterized by chronic hyperglycemia. Based on the available evidence, it can be determined that changing one's lifestyle, getting enough exercise, maintaining a healthy weight, and exercising, maintaining a healthy lipid profile and lowering blood glucose levels are both very beneficial. Up to now, owing to the development and advances of drugs therapy, the clinical treatment of diabetes has made significant progress and the condition of patients with diabetes has been well controlled. The clinical treatment of diabetes has made tremendous progress, and the condition of diabetic patients has improved, thanks to the creation and advancements of medication therapy. Diabetes has been well-controlled. With a thorough examination of the etiology and treatment options, anti-diabetic medications have been gradually expanded, the new non-insulin hypoglycemic objectives are receiving a lot of attention. pharmaceuticals, as well as the development and use of nanocarriers but, also actively seeking treatments that are predicted to be effective. diabetes mellitus is totally cured, incorporating gene therapy and stem cell therapy. For non-insulin hypoglycemic drugs, new targets such as enzymes and receptors directly related to carbohydrate metabolism and upstream regulatory factors related to energy metabolism have received extensive attention and research. At present, there are many methods of drug administration for diabetes treatment, such as transdermal delivery, oral delivery, nasal insulin delivery, and pulmonary delivery. Therefore, the

development of many drugs and treatments has provided tremendous promise for the clinical treatment of diabetes, but researchers still face a significant task in elucidating the pathogenic mechanism of diabetes and totally curing diabetes.

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Isolation, Characterization and Production of Menaquinone-7 (MK-7) From *Bacillus* Sp.

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Received: 30 Jan 2022 Revised: 18 Feb 2022 Accepted: 10 Mar 2022

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ABSTRACT

Soil samples were subjected to serial dilution and plating on nutrient agar by which 14 isolates were studied for morphological characteristics. Nine of the isolates were identified as *Bacillus* sp. and was further analyzed for production of Vitamin K2-7. All the nine *Bacillus* isolates were found to grow proficiently on specified medium for MK-7 production ranging from 10 mg – 38 mg/L, and out of these isolate PY-01 was found to consistently produce MK-7 and on analysis was found to produce a maximum of 38 µg/ L. The crude experimentation with minimum requirement resulted in higher yield. The PY-01 Bacillus strain seems to have potential to grow rapidly in batch experiments. This preliminary work can be further subjected to optimization studies, which could help in increasing the yield by either submerged or solid state fermentation.

Keywords: *Bacillus* sp., Menaquinone 7, fermentation, glycerol, isoprenoids

INTRODUCTION

Quinones are a broad category of membrane based electron transfer molecules known to play an indispensable role in metabolism and has been found to be an integral part of prokaryotes and eukaryotes respiration process [1]. Major quinones of relevance are Ubiquinone(U), phylloquinone(K1) and menaquinone(MK) and among them the menaquinone of prokaryotes are further diversified into MK-1 to MK-17 in terms of their isoprenyl side chain length[2]. MK and phylloquinones (produced exclusively by plants and cyanobacteria) are often referred to as vitamin K indicating their indispensable role in blood coagulation in humans (herein the isoprenyl tail of K1 are mostly saturated in comparison to MK with unsaturated tail) [2], [3]. MK (vitamin K2) is efficiently transported within human system and plays an additional role in cartilage deposition thus strengthening heart muscles and bone development [4], [5]. Their red-ox capabilities have been further efficiently used by eukaryotic system for roles

pertaining to blood coagulation and bone development thus finds diverse applications in the field of therapeutics [2]. Menaquinones are specific to each species of aerobic and anaerobically respiring bacteria, phylogenetic classification has been attempted to broadly classify bacteria on the basis of MK isoprenyl side chain length [6]. MK is found to be produced via two different pathways shared among prokaryotes with chorismate being the common precursor for the process. MKs are also naturally found in human intestine produced by natural microbial flora and among the MKs, Menaquinone-7 (MK-7) has been found to have maximum reach, wider benefits and most effectively absorbed by human system with the longest retention time in body (even in comparison to K1) [7]. Fermented foods like natto have been reported to be rich in MK-7 (chiefly attributed to role of *Bacillus subtilis* sp.) but their presence in other natural foods is found to be minimal. Their indispensible role as multifunctional vitamin and its role in varied biological functions from reducing inflammation to several age related diseases [8]. Though MK-7 is found in fermented foods and produced by microbial flora in human intestine but its contribution to daily requirement is debated [5], [9]. MK-7 is exclusively produced by *Bacillus* strains and in the present study an attempt has been made to isolate *Bacillus* strains from soil and identify their ability to produce MK-7.

MATERIALS AND METHODS

Isolation of Bacterial Strains From Soil Samples

Well aerated surface soil samples were collected in sterile polythene bags from farm lands around Bangalore and brought to the laboratory. The samples represented different farm lands with various soil textures. They were powdered and stored at 4^0C for further studies. Six soil samples thus collected were serially diluted and inoculated on to nutrient agar medium incorporated with Nystatin to prevent fungal growth. The inoculated plates were incubated at 37^0C for 24-48 h.

Morphological Identification of Bacteria

The bacterial colonies grown on the nutrient agar medium were observed and selected based on the standard bacterial characteristics colony features and further 9 Bacillus colonies (PY-01 to 09) were subjected for morphological characterization such as gram staining, motility and sporulation as per Bergey's manual of systematic bacteriology. The selected prominent colonies of bacteria were sub-cultured and maintained on nutrient agar at 4°C for further studies.

Screening of the Bacterial Isolates for Menaquinone Production

For the detection the MK-7 production by the bacterial isolates, each isolate was inoculated in Erlenmeyer flask containing 100 ml cultivation media composed of (g/L) soya peptone (5g), yeast extract (5g), glycerol (5g) and K_2HPO_4 (0.06g) of pH 7. Thus, inoculated flasks were incubated at 37°C for 5 days in shaker incubator at 120 rpm and, biomass was harvested for MK-7 extraction. From the harvested biomass, lipid content was extracted by the modified Bligh and Dyer method described [10], [11]. After 5 days of fermentation process, the broth was centrifuged at 10,000 rpm for 10 mins at 22°C and the cell pellets were collected as described [12]. Thus, obtained pellet was mixed with 3 ml of phosphate buffer, 7.5 ml of methanol and 3.75 ml of chloroform, shaken for few minutes and transferred to a separating funnel and allowed to stand for 2 h. Then 3.75 ml of chloroform and 3.75 ml of water were added, mixture was shaken well and allowed to separate the two layers for 1 h. The lower phase was collected and dried under the stream of nitrogen gas. The extraction and purification was carried out rapidly in dim light to prevent photo-oxidation in presence of strong light and oxygen, further extremes of pH was avoided as MKs are known to be susceptible to strong acid an alkali conditions.

Identification of MK 7 by Thin Layer Chromatography and HPLC

The dried extract was subjected to TLC wherein the extract was dissolved in few microlitres of chloroform as described [12]. TLC silica gel 60 F254 pre coated plates (Merck make) was used for the purpose. Working standard used for the experiment was Menaquinone 7 (Vitamin K2-7) assay (on as such basis) 98%. The silica gel sheets were spotted with sample and standard (MK7). The TLC was performed with Petroleum ether and Diethyl ether (85:15) as

an solvent system as described [13],[12]. The simple and rapid method of separating and detection menaquinone was also mentioned by other workers[6] with an Rf value of 0.7 and can be eluted using chloroform[6].

RESULTS AND DISCUSSION

The serial dilution and plating of six different soil samples yielded several colonies of nutrient agar plates supplemented with nystatin. After 24 hours of incubation the nutrient agar plates were observed for their colony characteristics. Well isolated colonies were considered for further sub-culturing, colonies were selected based on pigmentation and colony morphology, and sub-cultured on nutrient agar medium for further studies. By pure culture techniques a total of 14 strains of bacteria were isolated, out of which 09 were found to be Bacillus strains. Further work was carried with Bacillus isolates as they were known to be potent strains for MK7 production from literature survey [14, 15, 16]. All the nine bacillus strains were found to produce MK7 but in varying amount, one isolate (PY-01) was considered as potential strain for favourable physiological properties, better yield and culture stability (Figure 1a; 1b; 1c). All the nine Bacillus isolates grew luxuriently in media enriched with soya peptone and yeast extract when subjected to aerated shake flask experiments in liquid medium for 5 days at 120 rpm. The growth efficiency of each isolate was tested by centrifugation and checked for bacterial biomass. The bacillus isolates showed different growth properties on these 5 days of incubation. Few of the isolates (PY-09) were sticky with gummy properties and was not free flowing, gelly and mucillagenous growth of few strains were noticed. Two of the bacillus isolates (PY-03 and PY-04) exhibited matted growth even at 120 rpm, and it was difficult to subculture or transfer them from one flask to another. Some of the isolates could not be centrifuged for pellet formation, as it was not forthcoming even at 10,000 rpm. Further studies are required to identify the properties of these isolates which could lead to the production of biosurfactants and PHB, which is outside the preview of the present research on menaquinone. The isolates were any how subjected to centrifugation for further studies (Figure 1d and 1e).

The flasks were inoculated and maintained in duplicates, menaquinone production was tested in duplicates and the experiments were repeated, to check the efficiency of the process followed. Though the shake flask supported growth, the menaquinone could be identified only on 5th day of incubation. Any observation after 6th day yielded less amount of menaquinone, the reason could be probably attributed to death of active cells. The aerobic environment was also found to be essential for maximum menaquinone production, as reduction in rpm resulted in lesser or no yield of menaquinone. During the work, it was observed that glycerol supplementation to the medium, enhanced the menaquinone production in almost all the bacillus strains. For the determination of the menaquinone production the broth was centrifuged and pelleted cells were subjected to extraction of menaquinone (Figure 1f). The centrifuged pellets of bacterial isolate PY-09 on further extraction with methanol and chloroform failed to form layers and could not be processed further for MK-7 identification. presence of MK-7 and purity level of MK-7 thus assessed could prove the production of MK-7 by all the Bacillus isolates. (Figure 2) Thus the potential isolate PY-01 identified as Bacillus strain is capable of producing MK7 which had been confirmed by TLC and HPLC. The MK-7 concentration was evaluated and it was found to vary from 10 – 38 mg/ L. Various studies, on the isolation and optimization of Bacillus strains have reported higher yield of MK-7 upto 73mg/L [12, 17]. Further studies on the optimization of physical parameters and process economization have to carried out to obtain enhanced MK7 production.

And among all the 9 isolates PY-01 was found to be consistently producing MK-7 after repeated testing by thin layer chromatography and then by HPLC (Figure 1g, 1h and 1i). As per earlier reports the menaquinone was found to be photo-oxidizing rapidly in the presence of oxygen and strong light and care was taken to do the entire extraction under dim light 6. The menaquinone spot was identified by exposure to ultravoilet light of different wavelengths and from the spots using chloroform, the MK-7 was eluted from TLC plates. Thus eluted sample was subjected to analysis by HPLC,

CONCLUSION

The MK-7 is solely a microbial product and specifically produced by Bacillus strains. In the present study, the best isolate PY-01 has been tentatively identified as *Bacillus mesentericus*, possessing stable growth properties. Further molecular identification of the PY-01 strain has to be carried out and followed by optimization of vitamin K2-7 production. Strain improvement studies have to be carried out, to obtain higher yield and improved menaquinone production. Menaquinone-7 has been receiving an increasing global wide review for its possible multifunctional and beneficial role in cancer treatment, diabetes, bone health and cardiovascular [18] **,** hypothesized that MK-7 supplementation will slow down the calcification process in Aortic stenosis [19]. Vitamin K2 reported to have a positive impact on osteoporosis, cardiovascular disease, parathyroid disorders, cerebral palsy and sperm motility [20].

ACKNOWLEDGMENT

Financial support provided by Department of Science & Technology, Government of India under FIST program (SR/FST/College-332/2016 dates 15.2.2018) is gratefully acknowledged.

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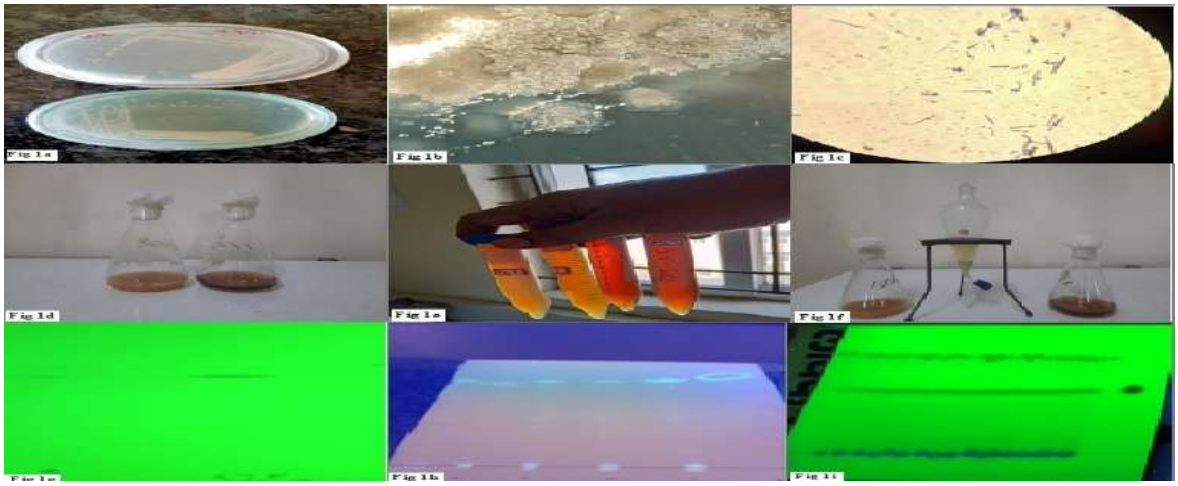

Fig 1a: Potential bacillus strain PY-01; **Fig 1b**: bacillus strain on nutrient agar;
Fig 1c: gram staining of the bacterial isolate; **Fig 1d**: Shake flask culture of PY-01;
Fig 1e: microbial cultures subjected to centrifugation; **Fig 1f:** Solvent extraction of MK7
Fig 1g: TLC @ UV - 354nm (spot 1 from extreme right – standard MK7; spot 2 to 6 – samples);
Fig 1h: TLC @ UV – 265 nm ((spot (right) – standard MK7; spot (Left) – sample; **Fig 1i**: MK7 spot for extraction and assessment by HPLC

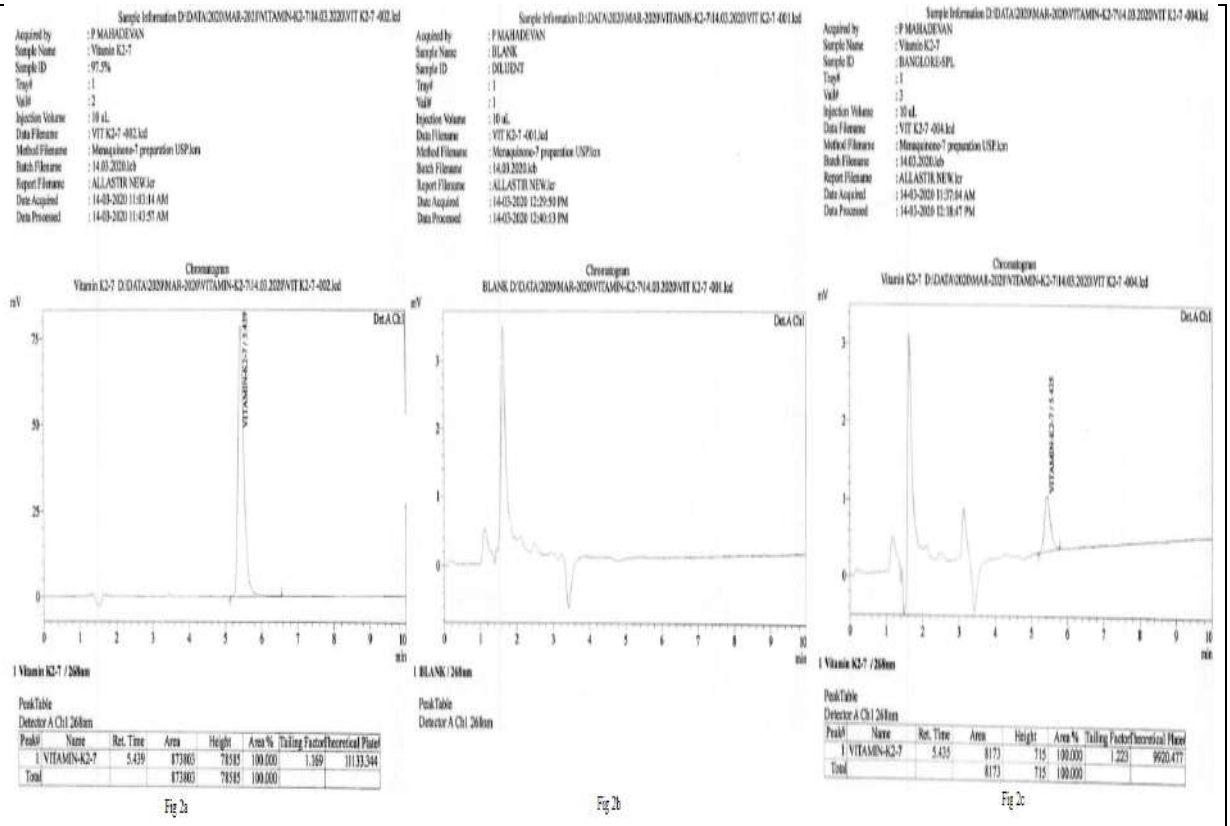

Fig 2a: Standard MK7 detection under HPLC (extreme right); Fig 2b: Blank (Middle image);
Fig 2c: sample MK7 detection under HPLC (extreme left);

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Drug Review on Siddha Polyherbal Formulation Thiratchai Chooranam with Special Reference to Vatha Paandu (Megaloblastic Anemia)

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Received: 09 Dec 2021 Revised: 05 Jan 2022 Accepted: 23 Feb 2022

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ABSTRACT

Anemia is one of the extensive blood disorders that affect people of all age group across the globe. The treatment strategy of anemia involves administration of the iron-rich diet, folic acids, amino acids and vitamins. Megaloblastic anemia, a type of anemia in which the bone marrow produces unusually large, morphologically abnormal, immature red blood cells. Nowadays, immense research has been focussing on exploring the benefits of phytochemicals in the management of anemia. The clinical features of megaloblastic anemia can be correlatedand referred with Vatha paandu in Siddha medicine based on the ancient text Yugi vaithiyachinthamani.Numerous studies reported the beneficial effect of phytochemical on anemia but there was no report on megaloblastic anemia. Siddha medicine is one of the most ancient traditional medical systems of India and it recommends the usage of herbs in anemia treatment. Thiratchai chooranam (TIC) is a polyherbal formulation consisting of twenty-one herbs and prescribed for anemia in siddha text Agathiyar Paripooranam 400. This study will discuss the phytochemicals and pharmacological activities of Thiratchai chooranam constituents with special reference to megaloblastic anemia.This review revealed the presence of potential phytochemicals including resveratrol, viniferin, β-carotenein Thiratchai chooranam and disclosed the pharmacological activities such as anti-diarrheal, anti-anemic and antioxidant capacity.

Key words: Anemia, Phytochemicals, Thiratchai chooranam, Antioxidant, Hepatoprotective

INTRODUCTION

Anemia is a condition in which the number of red blood cells (and consequently their oxygen carrying capacity) is inadequate to confront the body's physiological requirements[1]. Macrocytosis is a blood condition in which the morphology of red blood cells (RBC) are larger than normal condition [2]. Megaloblastic anemia (MA) is a condition in which the bone marrow produces unusually large, structurally abnormal, immature red blood cells (megaloblasts). The deficiencies of Cobalamin (vitamin B_{12}) or Folate (vitamin B_9) are the two most common causes MA. These vitamins are vital in the production of red blood cells. Vitamin B_{12} and folic acid are coenzymes required for the synthesis of thymidine, one of the four bases found in DNA [3]. A deficiency of these vitamins or impairment in their metabolism results in large erythroblast, defective nuclear maturation and ineffective erythropoiesis. Symptoms of megaloblastic anemia includes pallor with slight icteric skin, light headedness, vertigo, glossitis, anorexia with moderate weight loss, numbness in extremities, retinal haemorrhage, dermatitis, constipation or diarrhoea and paraesthesia in extremities (Glove and stocking paraesthesia) [4]. Current treatment in modern science includes intramuscular or subcutaneous administration of cyanocobalamin injection. Also, hydroxocobalamin is suggested every1–3 months as an effective therapy. The above therapies should be followed till the deficiency is corrected [2].

Siddha medicine is one of the most ancient traditional medical systems of India. According to siddha system of medicine, diseases are classified into 4448 types. Paandu is described in siddha text as anemic condition based on its clinical description. Paandu is characterized by a decreased amount of red blood cells which leads to immediate weakness of the body [5]. Etiology of Paandu are increased intake of salty and sour foods, worm infestation, liver disorders, menorrhagia and tobacco chewing as mentioned in Yugi vaithiyachinthamani. The signs and symptoms of vathapaandu are lower abdominal pain, thirst, anorexia, roughness of skin, hyper pigmentation of skin, tremors, redness of eyes, constipation, headache, an a sarca and pallor [6]. Hence the clinical features of Vatha paandu can be correlated with megaloblastic anemia. Thiratchai chooranam is used in the treatment of paandu as described in the siddha text Agathiyar Paripooranam 400 [7]. TIC is a polyherbal formulation consisting of twenty-one herbal drugs described below. There are no reports stating the efficacy and scientific validation of TIC. Therefore, this review discusses the therapeutic properties of constituents of the Thiratchai chooranam from previous studies, thereby unravel its effect on megaloblastic anemia.

Vitis vinifera

Grape seed extract at a concentration of 100mg/kg plays a protective role in cardiac cells and pancreas from oxidative damage. Isolated tannins from grape seed at various concentration prevents red blood cells from oxidative damage caused by excessive reactive oxygen species production. Similarly, phenolic extract from grape seed increased the glutathione level in human platelets treated in platelets [8]. Raisin increased the iron status of the subjects who consumed raisin (8g) for 20 days. It moderately increased the hemoglobin, ferritin, serum iron levels and also decreased the Total Iron binding capacity and transferring [9]. The beneficial value of grape molasses in the management of anemia was demonstrated in this study conducted in rat model. Grape molasses was administered daily for 20 days at different concentrations increased the blood cell parameters [10].

Phoenix dactylifera

Hematopoietic activity of aqueous and methanolic extract of *P.dactylifera* was studied in the rat model. The extract of crude fruit was orally administered once daily for 112 days has shown dose dependent increase in blood parameters such as RBC, Hb, PCV, reticulocytes and platelet count and it may contribute to an increase in the erythropoietin synthesis [11].

Illicium verum

The ethanol extract of *I.verum* fruit showed anti anemic and anxiolytic activity in the rat model. Illicium verum fruit extract of 80 mg/kg for 6 weeks in rats significantly increased the hematological parameters like RBC, PCV, mean

corpuscular hemoglobin concentration and platelets also exhibited the same effect as common medicine ferrous sulphate on rat model [12].

Glycirrhiza glabra

The therapeutical effect of aqueous extract of *G.glabra* was studied on hemolytic anemia induced in rats. Different concentrations of the extract were given orally for 15 days significantly enhanced the levels of white blood cell (WBC), platelet, RBC, Hb, PCV and high-density lipoprotein [13].

Cyperus rotundus

Methanol extract of *C.rotundus* was studied for cardio protective potential in rabbits. Myocardial infarction is induced with isoproterenol in rabbits and the extract were given at doses of 100, 150 and 200 mg per kg daily for 21 days significantly reduced the elevated level of cardiac enzymes and reinstated the level of antioxidant enzymes level in heart tissues [14].

Coriandrum sativum

The antifibrotic effect and antioxidant effect of *C.sativum* extract were studied against liver fibrosis in the male rat model. the combined dose of 200mg/kg coriander extract and 25mg/kg silymarin was given orally once daily for 15 days. There was a significant increase in RBC count, Hb, platelet count and total WBC count and reduced the elevated plasma aspartate aminotransferase and alanine aminotransferase. The extract may protect against microcytic hypochromic anaemia, thrombocytopenia and reduce hepatic damage by preventing oxidative stress [15]**.**

Nymphaea alba

In *N.alba* rhizomes, 64 phenolic compounds were identified using LC-MS. Five phenolic compounds were isolated for the first time and analysed its Radical scavenging activity using 2.2Di (4-tert-octylphenyl)-1- picryl-hydrazyl (DPPH) assay. The result unravels the reliable antioxidant activity of phenolics compounds in the rhizome of *N.alba* [16].

Myristica fragrans

Oral administration of Myristicin in albino mice showed protective effects against ulcerative colitis induced by acetic acid. There was a significant decrease in the levels of inflammatory mediators in mice pre-treated with myristicin at a dose of 200 mg per kg when compared to the acetic acid treated group [17]. Aqueous extract of nutmeg has cardioprotective and hepatoprotective effects against isoproterenol induced hepatotoxicity and oxidative stress in male rats [18].

Syzygiumaromaticum

A study by Shekar *et al*., 2018 explored the potential antioxidant role of ethanolic extract of *S.aromaticum* in neuronal cells. The result elucidated that *S.aromaticum* has significantly scavenged the neurotoxicity induced ROS production and simultaneously increased the activity of antioxidant enzymes [19].

Zingiber officinale

The efficacy of Ginger and its bioactive component 10-gingerol in hematologic disorder was studied in zebra fish by elevating the levels of hematopoietic progenitor markers and also rescue from acute hemolytic anemia by increasing the circulating erythroid cells [20]. In addition to antioxidant, anti-inflammatory properties, ginger was found to combat the immune suppressive drug Azathioprine in modulating erythropoiesis. Treatment with ginger extract in guinea pig found to increase the erythrocyte count, Hb and PCV in the drug treated group, thereby the extract nullifies Azathioprine drug and further confirmed the beneficiary effect in erythropoiesis [21].

Hemidesmus indicus

Methanolic extract of *H.indicus* root exhibits antihepatotoxic activity against paracetamol and carbon tetrachloride induced hepatic damage in rats. Pre-treated rats with root extract elevated the levels of serum liver enzymes, total

and direct bilirubin but it was significantly less as compared to those treated with paracetamol or carbon tetrachloride [22].

Cinnamomum tamala

A study by Eswaran *et al*., 2010 demonstrated the free radical scavenging activity of *C.tamala* leaves in ethanol induced ulcer model in rats. Potential gastroprotective activity was evaluated as extract significantly decreased the lesion index, whereas increased the level of gastric wall mucus, pH and antioxidant enzyme catalase [23].

Taxus baccata

The alcoholic leaf extract of *T.baccata* at concentrations of 100 mg/kg and 200 mg/kg reduced the intestinal secretions and also lowered the intestinal propulsive movement, thereby *T.baccata* leaf extract could be a potential anti-diarrheal agent in herbal medicine [24].

Piper longum

The potential therapeutic role of neuroprotective effect in alkaloids from *P.longum* was demonstrated in mouse model of Parkinson's disease. The extract has shown neuroprotective effects by increasing the levels of dopaminergic neurons, DOPAC (3,4-Dihydroxyphenylacetic acid), glutathione level and superoxide dismutase activity and decreased the lipid peroxidation of malondialdehyde, thereby extract has ameliorative properties in dopaminergic neurons[25].A molecular docking study revealed that piperine when conjugated with iron, reduced the activity of cytochrome P450 redox system[26].

Plectranthus vettiveroides

The hydro alcoholic extract of *P.vettiveroides* shows high cytotoxicity in cancer cell line (breast cancer cell line, hepatocellular carcinoma cell line) and also exhibit potent antioxidant activity even at low concentration in oxidant scavenging assays [27].

Mesua ferrea

The antiulcer activity in albino rats was evaluated using the xanthones of *M. ferrea* by pyloric ligation method. The xanthone pre-treated animals shown only scattered areas of hyperaemia and occasional haemorrhagic spots whereas the control animals showed extensive ulceration, haemorrhage and perforation [28]. The extract treatment results in a significant increase in biochemical parameters such as Liver superoxide dismutase level and posed a hepatoprotective effect by decreasing the levels ALT and AST and also reduced CAT activity [29].

Santalum album

The methanol extract of Sandalwood was studied for anti-diarrheal activity in mice. The extract was administered intragastrically at different concentrations inhibited gastric emptying and reduced the small intestinal motility in castor oil induced diarrhoea in mice [30]. The hydro alcoholic extract of *S.album* stem possess significant anti-ulcer potential in rat model [31].

Cinnamomum zeylanicum

Cinnamon was found to have a diverse range of pharmacological activity and also possess a potent immune modulator effect [32]. Anti-diarrheal activity of *C.zeylanicum* bark aqueous extract was studied in albino mice. There was a significant decline in the number of feces and gastrointestinal motility at doses of 100 and 200 mg/kg in mice [33].

Bambusa arundinacea

Silica deposits from the bamboois a potential drug extracted from the substance accumulated at the hollow internodes of bamboo and is reported to possesses stimulant, febrifuge, cooling tonic, antispasmodic agent and aphrodisiac [34].

Phyllanthus emblica

A study by Sriwatcharakul demonstrates the seed extract has a high content of phenolic, tannin and flavonoid and exhibited high antioxidant capacity [35].

Oryza sativa

Puffed rice balls with jaggery given as an iron supplement to 25 female subjects for 60 days at a dose of 100g/day significantly increased the hemoglobin level and decreased serum total iron binding capacity [36].

CONCLUSION

In South east Asian countries, the higher cost of medicine, side effects of an overdose of biomolecules and not adequate screening of the disease may surge the prevalence of anemia. Moreover, national wide prevalence of anemia and its types were required for effective anemia treatment. So, there is an imperative need for exploring alternative strategy such as nutraceuticals and phytochemical based methods to alleviate anemia. In Siddha system of medicine, TIC has used for years for treating anemia, but there is no scientific validation against anemia. This review first time extensively revealed the potential of the polyherbal formulation Thiratchai chooranam based on its phytochemical constituents and their effective pharmacological activity. This review may pave for further preclinical and clinical studies for the effective treatment against megaloblastic anemia.

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Table 1. Bioactive Constituents and Pharmacological Activity of TIC

S. No	Botanical name	Bioactive constituents	Pharmacological activity
1	*Vitis vinifera* Linn.	• **Stilbenes** : Resveratrol, viniferin, astringin • **Flavonoids** : Catechins, anthocyanins, proanthocyanidins • **Tannins** : Procyanidins, prodelphinidins[37,38]	Antioxidant Hepatoprotective Neuroprotective Cardioprotective [37]
2	*Phoenix dactylifera* Linn.	• **Carotenoids** : Lutein, β-carotene • **Phytosterols** : β-Sitosterol, stigmasterol, isofucosterol • **Phenolic acids** : p-Coumaric acid, ferulic acid [39]	Haemopoietic Antioxidant Gastroprotective Hepatoprotective [40]
3	*Illicium verum*Hook.f.	• **Phenylpropene derivative** : Anethole, estragol • **Flavonoids** : Quercetin, kaempherol • **Terpenes** : Limonene, linalool, Caryophyllene [41]	Anti anemic Hepatoprotective Antioxidant [42]
4	*Glycyrrhiza glabra* Linn.	• **Triterpenoid saponin** : Glycyrrhizin • **Flavonoids** :Glucoliquiritinapioside, shinflavanone, shinpterocarpin, glabridin • **Monoterpenes** : α-Terpineol, geraniol [43]	Anti anemic Neuroprotective Gastroprotective
5	*Cyperus rotundus* Linn.	• **Sesquiterpenoid** : α-Cyperone, cyperene, α-selinene, mustakone • **Iridoid glycoside** :Rotunduside G, rotunduside H, negundoside, nishindaside[44]	Anti-diarrheal Neuroprotective Gastroprotective [45]

6	*Coriandrum sativum* Linn.	• **Terpenes** : Linalool, α-pinene, γ-terpinene, p-cymene [46]	Anti anemic Antioxidant Hepatoprotective [47]
7	*Nymphaea alba*Burm.f.	• **Flavonoids** : Kaempferol, apigenin • **Polyphenols** : Catechin, corilagin, rutin, naringenin, castalin[48,49]	Anti-diarrheal [48] Antioxidant Anti-inflammatory
8	*Myristica fragrans*Houtt.	• **Lignans** :Myrisfragransin, macelignan, myrislignan • **Phenyl propene** : Myristicin, elemicin [50] • **Terpenes** : α-Thujene, sabinene, β-pinene [51]	Hepatoprotective Anti-inflammatory
9	*Syzygiumaromaticum* (Linn.) Merr. &L.M.Perry.	• **Flavonoids** : Quercetin, kaempferol • **Phenolic acids** : Ferulic acid, caffeic acid, ellagic acid, salicylic acid [52]	Gastroprotective Anti-inflammatory Antioxidant [52]
10	*Zingiber officinale*Rosc.	• **Phenolic compounds** : Gingerols, shogaols, paradols, quercetin, zingerone, gingerenone-A • **Terpenes** : Zingiberene, α-curcumene, β-sesquiphellandrene[53]	Anti anemic Hematopoietic Antioxidant Neuroprotective
11	*Hemidesmus indicus* (Linn.) R.Br.	• **Pregnaneglycosides** :Desinine, hemidine, hemidescine, emidine, indicine, hemisine medidesmine • **Coumarino-lignoid** :Hemidesminine • **Terpenoids** :α-amyrin, ursane, oleanane, lupine, lupeol [54]	Anti-diarrheal [55] Hepatoprotective Anti-inflammatory
12	*Cinnamomum tamala* (Buch.-Ham.) T.Nees&Eberm.	• **Terpenes** : Camphene, myrcene, limonene, linalool, α-pinene • **Phenolic compound** : Eugenol [56]	Anti-diarrheal Hepatoprotective Antioxidant [57]
13	*Taxus baccata* Linn.	• **Flavonoids** • **Taxoids** :Taxusin, baccatin • **Steroids [**58]	Antioxidant Immunomodulator
14	*Piper longum* Linn.	• **Alkaloids** :Piperine, piperlongumine, pipernonaline, pellitorine, piperettine[59]	Immunomodulator Anti-inflammatory Hepatoprotective Antioxidant Anticancer
15	*Plectranthusvettiveroides* (Jacob) N.P.Singh&B.D.Sharma.	• **Diterpenoids** :Abietanoids, royleanone • **Sesquiterpene** :1(10)-aristolen-13-al [60]	Anti cancer Antioxidant
16	*Mesua ferrea* (Burm.f.) Kosterm.	• **Phenylcoumarins**:Mesuol, mesuagin, mammeisin, mammeigin, mesuone[61]	Antioxidant Hepatoprotective [62] Immunomodulator
17	*Santalum album* Linn.	• **Oxygenated terpenes** : α-Santalol, β-santalol, teresantalol, nuciferol, α-bergamotol • **Monoterpenes** :Camphene, α-terpinene • **Sesquiterpenes** : α-Santalene, α-farnesene, α-cedrene [63]	Hematopoietic Antioxidant Anti-diarrheal [64]
18	*Cinnamomum verum*	• **Phenyl propanoid** : Cinnamaldehyde	Immunomodulator

	J.S. Presl.	• **Phenolic compound** : Eugenol • **Aldehyde** :Methoxycinnamaldehyde • **Carboxylic acid** : Cinnamic acid, methyl Cinnamate [65]	Anti-diarrheal
19	*Bambusaarundinacea* (Retz.) Willd.	Silica deposits from the nodes of bamboo	-
20	*Phyllanthus emblica* Linn.	Essential oil [66]	Antioxidant [67]
21	*Oryza sativa* Linn.	Puffed rice	-

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Hepatoprotective Activity of Novel Pyridazinone Derivative against D-Galactosamine Induced Hepatotoxicity in Rats

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Received: 08 Jan 2022 Revised: 10 Feb 2022 Accepted: 11 Mar 2022

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ABSTRACT

To evaluate the hepatoprotective activity of novel pyridazinone derivative against d-galactosamine induced hepatotoxicity in rats. A series of novel pyridazinone derivative were synthesized in good yield. Modern spectroscopic techniques and elemental analysis were used for the identification of the synthesized compounds. The hepatoprotective properties of compound 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione was evaluated against D-galactosamine (GalN) -induced hepatotoxicity rat model. Administration of D-galactosamine induced oxidative stress by elevating the liver enzymes and antioxidant enzymes and MDA levels. Pretreatment with the novel pyridazinone derivative, 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione at 30 and 60mg/kg decreased these elevated MDA and liver enzymes levels and improved the antioxidant enzymes in a dose dependent manner. Histopathological examination of liver tissues also confirmed the hepatoprotective activity of compound. These results demonstrated that the novel Pyridazinone Derivative at a dose 30mg and 60mg /kg possesses hepatoprotective and antioxidant activityagainst d-galactosamine induced hepatotoxicity in rats.

Keywords: Hepatoprotective activity, Pyridazinone, 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione, D-galactosamine.

INTRODUCTION

Throughout the world, liver disease ranks first among leading causes of death. Excessive alcohol consumption, malnutrition, and exposure to pollutants, medicines, chemicals, viruses, and infections are all factors that contribute to the disease's development (1). It is inevitable that the liver would be exposed to various exogenous substances, which will result in various liver diseases. Except for a few naturally occurring medicinal plants, there are no effective treatments for liver illnesses available at this time (2). Silymarin has long been recognised as a potent antihepatotoxic substance. It is derived from the seeds of the plant *Silybum marianum* (milk thistle) (3). Pyridazinone having six-member heterocyclic compounds, two nitrogen atoms are present at adjacent positions. Pyridazin-3-one, a saturated or unsaturated arrangement of pyridazine with carbonyl group on third carbon, bygone expressed as a magic moiety which acquire all kinds of biological activities. The pyridazine nucleus represents a functional scaffold to establish new pharmacologically active compounds. Diazines and their derivatives have become very important to the field of chemistry and to the general population in terms of their priceless biological activities. Diazines contain two azomethine nitrogen atoms. There are three types of diazines - pyridazine, pyrimidine and pyrazine are stable, colorless compounds, which are soluble in water [4,5]. Many pyridazinones have attracted considerable attention as they are endowed with a variety of pharmacological activities. These derivatives serves as One of the most active class of compounds possessing broad spectrum of biological activity varying from cardiovascular, anti-asthmatic, anti-inflammatory, anti-HIV-1, anti-diabetic, antidepressant, anticonvulsant, antiproliferative, analgesic, anti-AIDS, analgesic, anticancer, antimicrobial, cardiotonic, antihypertensive, antimicrobial and insecticidal activities etc. Some of the pyridazinone derivatives have reached till the clinical trial as biologically active agents [6-13]. .Benzoxazole belongs to one of the most important class of heterocyclic compounds and has got importance as a medicine. It has been incorporated in many medicinal compounds that made it versatile heterocyclic compound possessing wide spectrum of biological activities viz: antimicrobial [14, 15] and got anti-diabetic activity [16] Addition benzoxazole to the pyridazinone moiety could help explore potential antidiabetic actions of the substituted compounds.The model system of liver damage produced by D-galactosamine (GalN) in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view [17]. As a result, this model is now accepted as one of the most authentic test systems of liver damage in experimental animals. GalN has great liver specificity because the hepatocytes have high levels of galactokinase and galactose-1-P-uridylyltransferase. Meanwhile, other organs are not affected [18,19]. GalN causes liver cell injury, with spotty hepatocyte necrosis and prominent portal and parenchymal inflammation [20].GalN also causes depletion of uridine diphosphate (UDP)by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis,leading to deterioration of the cell membranes [21, 22].

We designed this study to assess the hepatoprotective activity of the novel pyridazinone derivative1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione for a specific period of time.

METHODS

Synthesis of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydrop yridazine-3,6-dione.

The research work was focused on the synthesis of biologically potent molecules. benzoxazoles are known for diverse pharmacological activities. In this study, Addition benzoxazole to the pyridazinone moiety reacts and forms 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione. The structure of the compound was confirmed by the spectral studies such as Infrared spectroscopy, NMR spectroscopy, and MASS spectroscopy. The percentage yield of the compound was found to be 0.3271 (89 %) and the melting point was found to be 136-138^0 C; The IR spectrum showed the bands in the following regions.IR (KBr vmax cm-1): 1660.23, (NH-C=O str), 1695.13 (N-C=O str), 3454.86 (-NH str). 1H NMR of the synthesized compound was as follows. (DMSO-d6, 400MHz) δ. 6.86-8.97 (m, 4H, Ar-H), 11.42 (s, 1H, -NH), 2.53 (s, 3H, -CH3). 13C NMR (DMSO-d6, 100 MHz) δ: 173.64, 172.50, 155.78, 152.05, 150.73, 149.46 149.22, 141.67, 136.08, 124.73, 121.16, 22.49 was shown in fig 1&2. The elemental analysis was done and

calculated (%) for C12H8ClN3O3; C,51.91; H,2.90; N,15.13; observed C,51.88; H,2.86; N,15.09. M+1, 277.66, M+2, 279.66.

Animals

Healthy adult male wistar rats were used for the hepatoprotective study. The animals were procured from CPCSEA listed suppliers of Srivenkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12 h dark schedule at 25°C and 55–65% humidity levels. The rats had been given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of animal

Healthy animals were randomly selected for the study and kept in their cages for at least 1week prior to dosing to allow for acclimatization to the laboratory conditions. Before test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (IAEC/REETESH KUMAR RAI/VU/PhD/PRMOC17B02/KMCP/106/2020-21) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration
in experimental animals.

Experimental design

Animals were randomly distributed into five groups each containing six animals and treated as follows:

Group I: Normal Control: The animals received distilled water 5 ml/kg b.w. p.o. for 21 days.

Group II: Toxicant N-Galactosamine Group: Also received distilled water 5ml/kg b.w. p.o. for 21 days. A dose of D-Gal N 400 mg/kg b.w was given i.p. after one hour of vehicle [23].

Group III: Standard Silymarin Group: The animals received silymarin 75 mg/kg b.w. p.o. was given for 21days. The animals received a single dose of D-Gal N 400 mg/kg b.wi.p after 1 hour of std drug on the 21th day.

Group IV Toxicant + novel pyridazinone derivative at a dose of (30mg/Kg through intraperitoneally) for 21 days. A dose of D-Gal N 400 mg/kg b.w. i.p. after 1 hour of test drug on the 21th day.
Group V Toxicant +novel pyridazinone derivative at a dose of (60mg/Kg through intraperitoneally) for 21 days. The animals received dose of D-GalN 400 mg /kg b.w i.p. after 1 hour of test drug on the 21th day.

Biochemical Parameters: On the 22nd day, after overnight fasting, the blood was collected from retro- orbital plexus. The blood was allowed to clot and centrifuged (Remi-R 8C Centrifuge) at 2500 rpm for 10 minutes. The serum was separated and used for the assay of alanine transaminase [ALT] [24], aspartate transaminase [AST] [25], alkalinephosphatase [ALP] [26], γ –glutamyl transferase [γGT] [27] and bilurubin by using standard methods using enzyme assay kits. Transasia Bio-medicals Ltd Kit for ALT, AST, LDH and Accurex Biomedicals Ltd Kit for γ GGT & ALP. The enzyme assays were performed on a semi-autoanalyser ERBA Chem.

Evaluation of Biochemical Parameters: The livers were dissected out immediately, washed with ice-cold saline and 10% homogenates in phosphate buffer solution (pH 7.4) were prepared. The following biochemical measurements were carried out in the liver tissues. Liver homogenate was used for the assay of MOA while some fraction of homogenates was centrifuged at 2500 rpm for 10 min at 4 ºC using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) [28,29,30] standard methods using enzyme assay kits. The enzyme assays were performed on a semi-auto analyser ERBA Chem.

Histopathological Observation: The liver from each group was aseptically excused stored separately for analysis of oxidative stress-related biomarkers and in phosphate - buffered formalin (10%) for histopathological evaluation.
Statistical Analysis: The Statistical analysis was carried out by oneway analysis of variance (ANOVA) followed by NewmannKeul's multiple range tests. The values are represented as Mean ± SEM. Probability value at $p < 0.01$ was considered as statistically significant.

RESULTS

BIOCHEMICAL OBSERVATIONS

Significant increase in (P< 0 .01) AST, ALT, ALP, Total bilirubin and Gamma-glutamyl transpeptidase and significant decrease in (P< 0.01) Total protein, and Total albumin levels were observed in animals treated with galactosamine 400mg/kg (Group II) as compared to normal control group (Group I). Pretreatment with Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg for 21days decreased the levels of above indices like AST ,ALT , ALP, TB, GGTP and increased levels of TP and TA significantly(P <0.01) in group IV and V. Silymarin pretreatment produced significant decrease in (P< 0.01) serum AST, ALT, ALP, TB,GGTP and significant increase in TP and TA at (P< 0.01) in group III.

BIOCHEMICAL OBSERVATION IN LIVER HOMOGENATE TISSUE

In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine 400mg/kg as compared to normal control group. Pretreatment with Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg for 21 days increase the levels of above indices like SOD, CAT and GPx levels and decrease levels of LPO significantly (P<0.01) in group IV and V. Silymarin pretreatment produced significant increase in (P< 0.01) Liver homogenate enzyme such as SOD, CAT, GPx levels and decrease the levels of LPO significantly (P<0.01) in group III. Table no 3 shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the tissues (liver) of D-galactosamine hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine hepatotoxic rats significantly decreased. Novel Pyridazinone Derivative at a dose 30mg and 60mg/kg showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats.

HISTO PATHOLOGICAL OBSERVATIONS

Histology of liver sections of normal control animals (Group I) showed normal liver architecture with were brought out central vein, were preserved cytoplasm and prominent nucleus and nucleolus (Fig no:1). The liver sections of galactosamine treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells. Silymarin (Group-III) exhibited protection from galactosamine induced changes in the liver. Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg, pretreatment also caused marked decrease in inflammatory cells.

DISCUSSION

D-galactosamine is a well-established hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis. Liver damage induced by D-galactosamine, reflects disturbances of liver cell metabolism, which lead to characteristic changes in the serum enzyme activities. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte (31). When the liver cell plasma membrane is damaged, a variety of enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilurubin and gamma-glutamyl transpeptidase are released into the blood stream. Their estimation in the serum is useful as a quantitative marker of the extent and type of hepatocellular damage.

In D-galactosamine induced toxicity, increased activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total biluribin and gamma-glutamyl transpeptidase and decrease activities of total protein and total albumin were observed in serum. Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg, seems to preserve the structural integrity of the hepatocyte membrane as evidenced from the significant reduction in the activities of these enzymes. The 60mg/kg dose had a better effect than the low dose of Novel Pyridazinone Derivative(60mg/kg). The higher concentration might have resulted in the production of more by products that would have interfered with the activity. Treatment with Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg, significantly decreased these enzyme activities, indicating that Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg , has a hepatoprotective effect against a D-galactosamine-induced liver injury.

D-galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane (32). D-galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney (33). The previous studies show that D-galactosamine-induced rats significantly increased thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in liver and kidney. In the present study, we observed an increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipid peroxidation in various tissues has long been known to cause functional degradation; thus, the degradation of vital tissue leading to complications may be indirectly due to increased oxidative stress.

Treatment with Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg, and silymarin showed a significant reduction which might be due to the antioxidant ability of these compounds and the consequent reduction in lipid peroxidation. Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg, possesses antioxidative and free-radical scavenging effects. Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes (34). The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase. Reduced activities of these enzymes in the tissue of D-galactosamine- hepatotoxic rats were observed in our study. Superoxide dismutase protects against the superoxide radical (O2−), which damages the membrane and its biological structure. Catalase primarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues (35). Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. Significant increases in the activities of these enzymes were observed on Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg administration.

The second line of defense consists of the non-enzymic scavenger's glutathione, ascorbic acid, and α-tocopherol, which scavenge residual free radicals escaping from decomposition by the antioxidant enzymes. Moreover, enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals (36). Glutathione a major non-protein thiol in living organism's plays a central role in coordinating the antioxidant defense process. Glutathione reacts directly with reactive oxygen species and electrophilic metabolites, protects the essential thiol group from oxidation, and serves as a substrate for several enzymes including glutathione peroxidase. The lowered glutathione in D-galactosamine induced rats represents the increased utilization of glutathione as a result of oxidative stress. Perturbation in the redox status of glutathione not only impairs cellular defense against toxic compounds but also results in enhanced oxidative stress and oxidative injury (37). Apart from glutathione, α-tocopherol and ascorbic acids are important free-radical scavengers which protect cell membrane against toxic agents. Both vitamins C and E have a synergistic action in scavenging oxygen-derived free radicals (38). Vitamin C functions as a free-radical scavenger of oxygen radicals and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid appears to trap the peroxyl radical in the aqueous phase with a rate large enough to lipids and

dehydroascorbate is produced in this reaction. A thiol cycle converts the dehydroascorbate into ascorbate. The thiol cycle consists of a GSSG/GSH couple. Thus glutathione in blood keeps up the cellular levels of the active form of vitamin C. When there is a reduction in glutathione, the cellular level of ascorbic acid is also lowered. The observed decrease in the levels of α-tocopherol and ascorbic acid in the D-galactosamine rats might be due to an antioxidant defense against increased ROS or due to a decrease in glutathione levels in D-galactosamine-hepatotoxic rats. In this respect,reported that ascorbic acid and α-tocopherol decreased in liver diseases, particularly in D-galactosamine-hepatotoxic rats. Our study observed increase the levels of these antioxidants in Novel Pyridazinone Derivative at a dose 30mg and 60mg/kg and silymarin administered rats. The ability of Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg to enhance the levels of antioxidants along with its anti-lipid peroxidative activity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. Studies on the anti-oxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the anti-oxidative properties of polyphenols depend on hydroxylation of ring B. The present results corroborate the protective action of Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg in D-galactosamine intoxication of rats, particularly noticeable with the high dose used by us (60 mg/kg body weight). Supplementation with this flavonoid ameliorated the hepatoprotective and antioxidant activity in D-galactosamine-induced hepatitis in rats.

In conclusion, our findings demonstrated that Novel Pyridazinone Derivative at a dose 30mg and 60mg /kg possesses hepatoprotective and antioxidant activity, which is evidenced by lowered serum hepatic marker enzyme activities. Among the two dosages tested, 60 mg/kg/body weight showed more promising hepatoprotective and antioxidant activity, and is comparable to the standard drug Silymarin.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Vinayaka Mission's Research Foundation (Deemed to be University), Salem for providing the facilities for carrying out this research.

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Table.1. Effects of novel derivative of pyridazinone on biochemical parameters in D-galactosamine induced hepatotoxicity on rats

Groups	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	Total Protein (gm/dl)	Total Billurubin (mg/dl)	GGTP (mg/dl)	Total Albumin (mg/dl)
I Control	50.40±2.55	31.30±1.60	30.65±1.43	5.20±0.60	1.85±0.12	102.85±3.75	3.85±0.20
II D-galactosamine (400mg/kg, i.p)	112.70±4.55[a]	90.15±3.45[a]	38.15±5.15[a]	3.45±0.22[a]	4.46± 0.48[a]	170.25± 5.60[a]	2.20±0.10[a]
III DG + Silymarin(75mg/kg, p.o)	58.20±2.60[b]	39.25±1.85[b]	50.40± 2.20[b]	4.75± 0.55[b]	2.58± 0.55[b]	120.30± 3.05[b]	2.90±0.18[b]
IV DG+ NPD (30mg/kg, p.o)	64.75±2.85[b]	48.55± 2.20[b]	60.50± 2.45[b]	4.10± 0.36[b]	3.15± 0.70[b]	132.15± 3.58[b]	2.45±0.15[b]
V DG+ NPD (60mg/kg, p.o)	60.90± 2.74[b]	45.60± 2.05[b]	55.60± 2.34[b]	4.43± 0.45[b]	2.80±0.60[b]	125.60±3.34[b]	2.58±0.18[b]

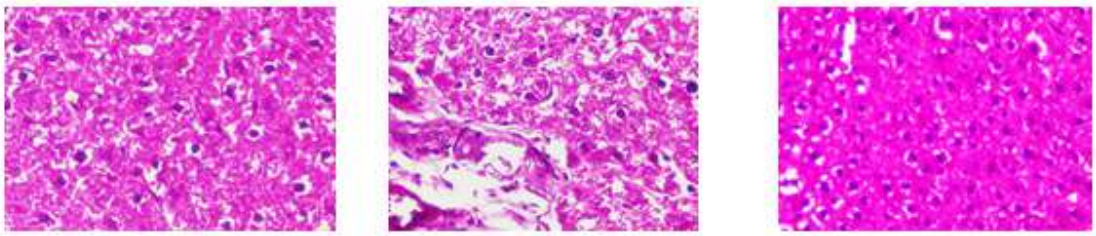
Values are expressed as Mean ± SEM. Values were analyzed by using oneway ANOVA followed by Newmannkeul's multiple range tests. *a – values are significantly different from Normal control at P< 0.01, *b – values are significantlydifferent from Toxic control (G2) at p< 0.01.

Table.2. Effects of novel derivative of pyridazinone on antioxidant enzymes of liver in D-galactosamine induced hepatotoxicity on rats

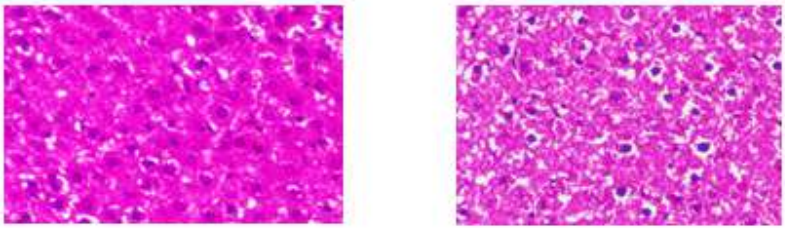
Groups	SOD (U/mg) Protein	CAT (U/mg) Protein	GPx (U/mg) Protein	MDA (U/mg) Protein	GST (mg/100 gm Tissue)	Vitamin C (mg/100 gm Tissue)	Vitamin E (mg/100 gm Tissue)
I Control	126.30± 3.45	285.38± 4.12	1.25± 0.13	3.90± 0.25	0.49±0.02	5.49±0.24	4.58 ±0.25
II D-galactosamine (400mg/kg, i.p)	68.20± 1.22[a]	190.90± 3.60[a]	0.38±0.02[a]	7.40±0.42[a]	0.28±0.01[a]	2.98±0.28[a]	2.45±0.06[a]
III DG+ Silymarin (75mg/kg, p.o)	101.85± 3.24[b]	258.40± 3.78[b]	0.90±0.08[b]	4.50±0.30[b]	0.42±0.01[b]	5.18±0.06[b]	4.15±0.09[b]
IV DG+ NPD (30mg/kg, p.o)	91.22±2.40[b]	232.60±3.40[b]	0.60± 0.06[b]	5.08±0.43[b]	0.37±0.02[b]	3.98±0.14[b]	3.25±0.18[b]
V DG+ NPD (60mg/kg, p.o)	96.90±2.75[b]	245.80± 3.64[b]	0.75± 0.09[b]	4.76± 0.33[b]	0.41±0.02[b]	4.92±0.02[b]	3.96±0.11[b]

Values are expressed as Mean ± SEM. Values were analyzed by using oneway ANOVA followed by Newmannkeul's multiple range tests. *a – values are significantly different from Normal control at P< 0.01, *b – values are significantlydifferent from Toxic control (G2) at p< 0.01.

Fig.no.1. Effects of novel derivative of pyridazinone on liver in D-galactosamine induced hepatotoxicity.



Control D-galactosamine 400mg/kg DG+ Silymarin 75mg/kg



DG+ NPD 30mg/kg DG+ NPD 60mg/kg

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Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effects of Work-Life Balance on the Job Satisfaction of Doctors at Bengaluru City's Multi-Specialty Hospitals

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Received: 31 Jan 2022 Revised: 14 Feb 2022 Accepted: 28 Mar 2022

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ABSTRACT

The study explored the effects of work-life balance on doctors' job satisfaction at Bengaluru city's multi-specialty hospitals. The data in this study was meticulously organized and analyzed scientifically. Proportionate Quota sampling was employed to gather data for this study. There were 1,040 people included in the study's sample size calculation. This project uses a well-crafted questionnaire to collect primary data using the survey approach. Secondary data refers to data that wasn't gathered from the ground up but rather from published or unpublished sources. The effect of shared value on performance was studied using multiple regression analysis. The study concluded that creating a mutually beneficial relationship between the doctor and employer is the goal of effective work-life programs. A happier and more contented doctor can manage the demands of their time effectively. As a result, their performance will improve. As a result, they're becoming more and more critical to corporate branding and employee retention.

Keywords: Job Satisfaction, Employee Retention, Modern Life, Personal Life, Work-Life Balance.

INTRODUCTION

The term "work-life balance" refers to workplace practices that recognize and support doctors' need to strike a balance between the demands of their personal (life) and professional (work) lives. According to the Work Foundation, formerly known as the Industrial Society, the key to achieving work-life harmony is for individuals to have autonomy over their schedules, locations, and methods of employment. If an individual's right to a fulfilled existence both inside and outside of paid labor is understood and respected as the norm, it has attained. The recognition that a person's work-life and personal/family life might impose competing demands on each other gave rise to the notion of work-family (life) balance. Government, researchers, managers, and doctors emphasize the need

for work-life balance in Human Resource Management (HRM). Global rivalry revived interest in personal lives and family values, and an aging workforce contributes to the growing concern over work-life balance. Changes in technology, business transactions, and demographic trends are all to blame for the increasing importance of work-life balance in industrialized cultures. There is also an increase in homes with both a working and a homemaker member and a growing proportion of women in the workforce.

Review of Literature

Malik and his colleagues (2010) investigated the association between job satisfaction and emerging concepts like Work-Life Balance (WLB), doctors' turnover intentions, and their level of exhaustion. The study showed how WLB practices in underdeveloped nations like Pakistan affect doctors' job satisfaction and burnout. According to the findings, doctors who are better able to balance their professional and personal lives report lower rates of burnout and higher levels of job satisfaction, both of which are associated with lower staff turnover.Australian GPs' WLB has been studied by Shrestha Durga et al. (2011). Baseline on the Medicine in Australia cohort: Balancing Employment and Life (MABEL) lengthways research provided the data for this investigation. According to the findings, women and GPs from Generation X had superior WLBs than men and GPs from the Baby Boomer generations. They worked much fewer hours than individuals with good WLB, which is a paradox. Better WLB was seen among doctors who reported having access to various leisure activities and a positive outlook on their health. Those who had trouble taking time off when they wished and those who worked irregular and long hours had a lower WLB. Significantly, GPs who had a bad WLB were more likely to plan to work fewer hours in the future. Several families and social conditions characteristics did not influence the WLB of GPs in this study. One in four primary care physicians (PCPs) reported high WLB. The WLB can be improved, and more GPs in the workforce can be encouraged to participate if they have better freedom in their work schedules, access to leisure activities, and good health.

A study by Goyal Bindiya (2014) examined the work-life balance of nurses and female doctors working in private clinics and hospitals. Nurses and doctors benefited from the hospitals' policies and practices regarding work-life balance. In addition, nurses and female doctors will be more devoted and productive in clinics and private hospitals with a successful work-life balance, leading to complete job satisfaction.Work-life balance experiences of female medical professionals in Nigeria were examined by Akanji et al. (2020), who analyzed factors such as perceived stress and coping mechanisms used by female doctors in an African environment. Medical professionals are aware of the detrimental effects that work-home interference has on their personal lives. In research on work-life balance, patriarchal tendencies and task-pay discrepancy have appeared sources of stress and work-family conflicts, leading female doctors to develop coping skills as mitigation strategies.

Need for the Study

Individuals, families, and communities are under tremendous stress due to their various responsibilities, including employment, children, household, volunteering, spouse, and elderly parent care. Workers, employers, and communities are all affected by the severe problem of work-life conflict.The multi-specialty hospital employer is quite concerned about how much money the doctors bring in. One of the main factors is the efficiency with which doctors complete their work. Work-life balance has a significant impact on doctor output. The multi-specialty hospital may employ doctors from many specialties. A different company could hire somebody else. Because of this, they should investigate more information on multi-specialty hospitals.

Doctors' quality of life depends on their job happiness because they spend one-third of their waking hours at work. It's safe to assume that doctors' job satisfaction will rise if they have a positive work experience or a sense of purpose. The doctors' ability to strike a healthy work-life balance significantly impacts their employees' overall well-being. Because of this, this research is vital for improving doctor-related information from a patient's perspective.
The quality of people's lives has traditionally been viewed as a measure of their contribution to society. The community standard would rise if people could adapt to modern life and work hard. Multi-Specialty Hospital Doctors are becoming more common in today's culture.

Objects of the Study

To explore the effects of work-life balance on doctors' job satisfaction at Bengaluru city's multi-specialty hospitals.

Research Methodology

The data in this study was meticulously organized and analyzed scientifically. Proportionate Quota sampling was employed to gather data for this study. There were 1,040 people included in the study's sample size calculation. This project uses a well-crafted questionnaire to collect primary data using the survey approach. Secondary data refers to data that wasn't gathered from the ground up but rather from published or unpublished sources. The effect of shared value on performance was studied using multiple regression analysis.

Data Analysis and Interpretation

Multi-Specialty Hospital Doctors (the dependent variable) had their work-life balance and job satisfaction explained using multiple regression analysis, which considered (the independent variable) the quality of their personal lives, their relationships and friendships, their work lives, their financial positions, and their experiences with family and work-related challenges.Many variables, such as the quality of personal life, relationships and friendships, the quality of work, financial security, family responsibilities, and work-related challenges, were considered when constructing a multiple regression model to explain the variation in doctors' work-life balance and job satisfaction (the dependent variable).

The Regression row contains information about the model's variation. Using the Residual row, you can see the interpretation that wasn't included in the model. For Multi-Specialty Hospital doctors, roughly eighty-seven percent of their work-life balance and job satisfaction score can be explained by this model's regression sum, which is more significant than residual squares. According to the F statistic, the observed change cannot be explained by chance because the significance level is less than 0.01.Table 3 demonstrates that the predictor Work-related problems have non-significant coefficients, despite the model's apparent positive fit. It suggests that the variables in this section have little effect on the model's final fit.Dependent Variable: Multi-Specialty Hospital Doctors' work-life balance and job satisfaction score. The coefficients of the regression line are shown in this table. Looking at the significance values, we can see that the quality of personal life is significant (P = 0.01) in predicting the work-life balance and job satisfaction of Multi-Specialty Hospital Doctors and the quality of personal relationships and friendships.

Suggestions

The hierarchical level of work-life balance programs, techniques, and activities are notable but insufficient. People's ability to benefit from work-life programs must be linked to legislative, business, and division-level regulations. The Doctors must be well-versed in and interested in the association's strategies, and they must continue to preserve their interests. The hierarchical atmosphere and environment may, in the interim, indicate that legislative arrangement changes are frequently shortened by their lack of support, overall, for representatives who go above their legally established powers. Consequently, achieving a healthy work-life balance is a complex issue requiring the involvement of social performers at all levels, including national, administrative, authoritative, and worker-level.

CONCLUSION

This study examines the effect of work-life balance on doctor job satisfaction in Bengaluru's multi-specialty hospitals. Multi-specialty hospital doctors' quality of work-life balance is reviewed. We can give more weight to the doctor's feelings and the shifting quality of work-life balance because it has a good and vital relationship with personal life, relationships, friendships, work-life and financial status. It's beneficial to both the person and the entire hospital to establish initiatives to promote work-life balance. Creating a mutually beneficial relationship between the doctor and employer is the goal of effective work-life programs. A happier and more contented doctor can manage the demands of their time effectively. As a result, their performance will improve. As a result, they're becoming more and more critical to corporate branding and employee retention.

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TABLE 1: DESCRIPTIVE STATISTICS

	Mean	SD	R^2
Multi-Specialty Hospital Doctors' work-life balance and job satisfaction	41.46	6.93	0.413
Quality of personal life	23.54	5.18	
Quality of relationship and friendship	21.43	3.90	
Quality of work-life	33.87	5.07	
Quality of financial position	11.32	2.82	
Family-related challenges	27.13	4.61	
Work-related challenges	32.49	4.70	

TABLE 2: ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	20640.12	6	3440.02	121.37	< 0.001
Residual	29278.49	1033	28.34		
Total	49918.61	1039			

TABLE 3: REGRESSION COEFFICIENTS

	B	Std. Error	t	p
(Constant)	7.518	1.481	5.08	< 0.001
Quality of personal life	-0.201	0.037	5.47	< 0.001
Quality of relationship and friendship	0.402	0.051	7.91	< 0.001
Quality of work-life	0.331	0.044	7.51	< 0.001
Quality of financial position	0.780	0.061	12.76	< 0.001
Family-related challenges	0.463	0.039	11.93	< 0.001
Work-related challenges	-0.078	0.047	1.66	0.097

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

REVIEW ARTICLE

An Overview on Sublingual Spray

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Received: 21 Jan 2022 Revised: 22 Feb 2022 Accepted: 26 Mar 2022

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ABSTRACT

Oral mucous membrane drug delivery is an alternative to intravenous drug delivery. A sublingual dosage form must be placed under the tongue, which has a large vascular supply beneath the mucosal lining, to get the drug into the bloodstream. For medications that take effect quickly, sublingual sprays are commonly used instead of oral tablets because patients are more likely to take them. In terms of permeability, the sublingual and palatal areas are more permeable than the vocal and palatal areas, respectively. Misting sublingual sprays under the tongue is the most effective way to use them. Because it is three to ten times more effective than the oral route, the sublingual route of drug absorption can be used in emergency situations. When it comes to ensuring consistent dosing, sublingual film formulations present a significant challenge. Spraying saves time and money because no special equipment is required. Sublingual spray has a number of advantages, including quick absorption, a large surface area, and high efficacy. This method of administration involves three stages: sublingual, jugular/superior vena cava, and sublingual capillaries.

Keywords: Oral mucous membrane, Sublingual, Palatal.

INTRODUCTION

Drugs bypass first-pass liver metabolism and the digestive tract, entering the bloodstream directly through the oral mucosa. For some drugs, intravenous injection is not the best route of administration. When using sublingual sprays, drugs can be absorbed more quickly and easily through the mucosal layer of the tongue [1-2].

Advantages [2,4,10]

1. You'll see results faster and they'll last longer because it's a sublingual spray. The device's simplicity will benefit people who are unable to swallow pills.

2. It is not necessary to break down the drug.
3. Because the medication does not dissolve in saliva, patients with dry mouths may benefit from using the spray.
4. Because the dosage form does not require water, first-pass metabolism and a pleasant mouth feel are not an issue.
5. High efficacy and fewer side effects can be achieved by avoiding hepatic first-pass metabolism.
6. Improved drug absorption and bioavailability, saliva moves from the mouth to the pharynx and esophageal passages.
7. Because of the large surface area of the teeth, drugs are absorbed quickly and completely in the mouth.
8. Sublingual Spray have an advantage over injections and oral medication for reducing pain, administering drugs to patients who are unconscious or incapacitated, and making the process easier.

Disadvantages

1. Taste masking is a common issue in sublingual spray formulations.
2. High blood concentrations or large doses are not required for all medications.
3. Because it interferes with eating, drinking, and talking, sublingual delivery is ineffective for sustained-delivery systems.

Mechanism of Sublingual Absorption

Ionisation and molecular weight affect the oral mucosa's ability to absorb the solution in addition to lipid solubility and permeability. When the carrier's pH is lower, absorption through the oral mucosa is increased (less alkaline). Oral mucosal absorption decreases as the carrier's alkalinity rises (more alkaline). Endocytosis, or the process of surrounding an object with your own body, allows these cells to take up particles. They have engulfed particles that are too large to diffuse through their walls on numerous occasions. This mechanism will not benefit the stratified epithelium [3]. Also unlikely are activated transport processes in the oral mucosa. Increased blood flow and stimulation of salivary glands can improve vitamin and mineral absorption. Squamous epithelium, which contains numerous glands, covers the mucous membrane in the mouth. The buccal mucosa and the sublingual mucosa have a lot in common. They are divided into two categories: those that produce saliva and those that do not. The three pairs of salivary glands on the floor of the mouth are the parotid, sublingual, and submandibular salivary glands. Those who have a higher acid sensitivity level produce more saliva [2,5].“As you travel forward, the sublingual artery supplies blood to the sublingual gland and its surrounding muscles and tissues (tongue and gums). Two symmetrical branches meet beneath the tongue to form the tongue's rounded tip. When the submental branches of the facial artery meet in the neck, they form an anastomosis. The lingual artery transports blood from the external carotid artery to the tongue and the floor of the mouth. Because it is close to the majority of the cerebral hemisphere, the internal carotid artery is easily accessible [3].”

Sublingual Glands

The salivary glands get their name from their location on the mouth's floor, just below the tongue. The sublingual route, which has a large blood supply and high permeability, can be used to deliver drugs to patients. Sublingual administration is capable of providing a quick response time. Lubrication and binding are also performed by sublingual glands, making eating easier. The pH and volume of saliva, for example, influence the mouth's primary physiological environment [6]. Mineralization and demineralization occur in a cycle when teeth are exposed to oral fluids. Saliva is produced by salivary glands in the sublingual, parotid, and submaxillary regions. Saliva keeps the mouth's acidity and enzyme activity in check. Salivary glands in the sublingual and supramaxillary regions produce viscous and watery saliva. Saliva helps keep the mouth moist and prevents tooth decay, in addition to preventing tooth demineralization and making swallowing easier. The salivary glands produce between 0.5 and 2.0 litres of saliva per day. In comparison to the gastrointestinal tract, the constant availability of 1.1ml of saliva provides a relatively small volume of fluid for drug release from delivery systems. Saliva flow is affected by the stimulus, the

time of day, and the intensity of the stimulation. Figure 1 depicts the absorption of a sublingual dosage form through the mucosa [7].

Factors Affecting the Sublingual Absorption[8]

Lipophilicityof Drug: The lipid solubility requirements for sublingual drug absorption differ from those for gastrointestinal permeation.

Solubility in Salivary Secretion: The fact that salivary secretions must undergo a lipid-to-water transition before the drug can be absorbed into the bloodstream is referred to as biphasic solubility.

pH and pKa of the Saliva: The pH of saliva must be at least 6.0 in order for the human body to absorb drugs that are still unionised. The oral mucosa acts as a conduit for drug absorption are given based on 2 acid and base less than 10 is base and greater than 2 is acid [14].

Binding to Oral Mucosa: Oral mucosa-bound drugs have a very low bioavailability in the systemic circulation as a result of this.

Thickness of Oral Epithelium: The buccal epithelium is much thicker than the sublingual epithelium when it comes to oral epithelium thickness. When there is less saliva and the epithelium is thinner, drug absorption is accelerated.

Oil to Water Partition Coefficient: The oral mucosa quickly absorbs compounds with a favourable oil-to-water partition coefficient. The Coefficient of Partition between Oil and Water. The optimal oil-water partition coefficient for sublingual medication absorption is between 40 and 2000 [15].

IDEAL PROPERTIES OF DRUG IN SUBLINGUAL DRUG DELIVERY SYSTEM [12]

1. The medication should not have an unpleasant taste.
2. For example, you should not take Nifedipine for more than 20 minutes at a time.
3. Furthermore, the size of the molecules can be changed at any time.
4. Water and saliva are also unable to pass through the material.
5. Ketotifen fumarate, for example, has a first-pass effect.
6. The mouth's pH should not be ionised as a result of the drug's use.
7. Sublingual tablets' efficacy is influenced by their solubility, crystal structure, particle size, hygroscopicity, and bulk density.
8. Sublingual dosage forms are better suited because some drugs undergo a significant amount of first-pass metabolism.
9. The most appropriate dosage form for parenterally unstable drug formulations is sublingual.

Aerosol

Colloidal aerosol is one of the most common types of aerosols and can be used in a variety of applications. The aerosol is classified as colloidal because of its finely dispersed liquid or solid particles. Aerosols necessitate precision metering valves. They can use this valve to dispense aerosol in amounts ranging from 25 to 100 microliters [13].

Advantages of Aerosol System [16]

1. The effect of the first pass can be turned off.
2. In a matter of seconds, you can make a decision.
3. It is critical to take this step in order to protect the digestive system.
4. The dosage should be reduced to avoid side effects.
5. The product's safety is ensured by seals on both the container and the valve closure. Seals that are impenetrable to tampering
6. When two or more drugs interact in a harmful or harmful way, this is known as an adverse reaction.

The various Aerosol systems that can be used are shown in Table 1 [17,20].

Aerosol systems use a variety of hydrocarbons as propellant to power the spray. Some of them are listed in Table 2 below [18-19].

Composition of Sublingual Spray [21]: In order to create a spray formulation, you need two things: Product concentrate and Propellant.

Product Concentrate: Sweeteners, hydrophilic polymers, and antioxidants are all important ingredients in concentrated products [22].

Penetration Enhancers:A drug's bioavailability can be increased in a variety of ways. Penetration enhancers like bile salts, oleic acid, sodium lauryl sulphate, and 1dodecylazacycloheptan-2-one can improve the fluidity of intracellular lipids (expansion of intracellular spaces) [17].

Propellants: During the extraction process from its container, a propellant is required to keep the concentrate moving in the right direction (i.e., spray, foam, and semisolid). A gaseous gas mixture can be used to transport the product concentrate in a variety of ways [23].

Paragon properties of propellants [25]:

1. It should not be harmful in any way.
2. There should be no impurities in the mixture.
3. Relax, there's nothing to be concerned about.
4. Wide availability of therapeutic active ingredients with good dissolving properties is essential.
5. A chemically inert substance must be used to accomplish this.
6. It is necessary to use a non-reactive product.

Evaluation Parameters

Qualitative Tests

Spray Patterns: "Using Whatman filter paper, determine the diameter and ovality ratio of the spray pattern (available at hardware stores). For better vision, Sudan red or brilliant blue are the colours of choice.) The ratio of Ovality is give using following formula.

Ovality ratio= Dmax/ Dmin

The maximum and minimum diameters of the spray pattern, Dmax and Dmin, are investigated [5,8].

pH: Following the manufacturer's instructions and using two buffers, pH metres can be calibrated. The tip of the probe is cleaned with water before inserting it into the samples. Allow time for the metre to adjust. The pH level is recorded when it reaches equilibrium [26].

Leak Test: The valve's crimping had been thoroughly tested for leaks; it was discovered. To ensure that the dimensions of the crimps met specifications, measurements were taken and verified. The leak tests were conducted with water, so the containers were completely filled [27].

Quantitative Tests [26,28]

Vapor Pressure: To measure vapour pressure, all you need is a pressure gauge. It can be accessed using a water bath, gauges, and other simple tools.

Density: The density of a substance can be measured with a Pycnometer by first filling it with 25ml of the substance and then reweighing it. The volume of a filled Pycnometer is divided by the volume of an empty Pycnometer to determine the density of a product.

Prime Test: You must squeeze the container repeatedly for the first test. Only one event is recorded once the formulation has been dispensed from a container.

Flame Projection: This test can be used to determine the effect of an aerosol formulation on flame projection. The project is ready to be inserted into the flame after four seconds. Depending on the formulation, the length of the flame can be precisely measured with a ruler.

Aerosol Valve Discharge Rate: The discharge rate of an aerosol valve can be evaluated by discharging a known weight aerosol product for a predetermined amount of time. Once the time limit has elapsed, the discharge rate (in grammes per second) can be computed.

Net Content: There is a significant difference in net weight between the two. The after and before filling sealing are empty containers

Drug Content: After dilution with one millilitre of spray solution, the concentration of the spray solution is measured using an ultraviolet (UV) spectrophotometer. The percentage of drug content are calculated below.
"Drug Content = Actual Drug content / Theoretical Drug Content X 100"

Drug Content per Spray: The contents of two sprays are determined using diffusion media in a beaker. After shaking the solution for a few minutes, analytical methods are used to determine the drug concentration.

Spray Angle: In this project, a spray impingement on a piece of paper is used. Sudan red (10 mg) dissolved in water helps with formulation visualisation. Spraying large areas of paper while keeping a precise distance from the spray tip is possible. The radius of a paper circle is calculated using three different angles.

Spray Profiling (Delivered Dose Uniformity): Spray profiling is recommended by the USP to determine whether a dosage can be repeated (Delivered Dose Uniformity). The amount of active ingredient in a spray determines the size of the spray. A test is given at the beginning, middle, and end of the course to ensure that the content is consistent.

Flash Point: The Tag Open Cup apparatus is used to determine it. The aerosol product is immediately transferred to the test apparatus for further analysis at a temperature of approximately -250 degrees Fahrenheit after cooling.

Ex-vivo Drug Diffusion Studies: Drug diffusion in a living organism is examined in the laboratory. The Franz diffusion method is used to conduct an ex-vivo drug diffusion study on the optimised formulation. The experiment on sublingual goat mucosa is carried out in an acidic buffer with a pH of 6–7. The aliquot is analysed using a UV spectrophotometer at predetermined intervals. Researchers conduct a drug diffusion study for a set period of time.

"Flux and Apparent Permeability Determination: Flux and apparent permeability are used by this formula are given below

$$Jss\ (Flux) = \Delta Qt/\Delta t * S$$

"Where, ΔQt / S is the cumulative drug permeation per unit of mucosal surface area (µg/ cm2) t is time expressed in hour.

Papp (Apparent permeability) = Jss / Cd Where, Jss is the flux and Cd is the concentrate of drug in donor compartment".

Stability Studies [24]: Electrochemical and long-term static stability testing are two types of stability testing. Despite the fact that it only provides a limited amount of information, electrochemical testing is a useful screening method. A vapour pressure metre can be used to measure concentration/propeller saturation changes, as well as weight loss, corrosion, and the stability of a concentrate (separation, coagulation, chemical change, gloss, and odour change). The most common method for long-term static testing is to run it at 120°F for three months to a year.

CONCLUSION

Sublingual sprays have a number of advantages over traditional sublingual formulations such as tablets or films. For drugs with a low molecular weight, low pH, high clearance rate, and high lipid solubility, sublingual spray formulations can be effective. Researchers should conduct field studies in the field to better understand patient compliance, the need for SL spray in next-generation personalised medicine, and regulatory feasibility.

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Table 1: Different types of Aerosol Systems

Different Types of Aerosol Systems	
Solution System	Two-phase system: Vapour and liquid phase. When the active ingredients are soluble in the propellant, no other solvent is required.
Water-Based System	Three-phase system: Propellant, water and vapour. Large amount of water can be used to replace all or a part of the non-aqueous solvents.
Suspension or Dispersion System	Dispersion of active ingredients in the propellant or a mixture of propellants. Decrease the rate of settling of the dispersed particles, various surfactants or suspending agents have been added.
Foam System	Consists of active ingredients, aqueous or non-aqueous vehicles, surfactant, and propellant and are dispensed as stable or quick breaking foam. The liquefied propellant is emulsified and is generally found in the Internal phase.

Table 2

		Composition (%)		
Designation	Pressure (psig at 70 °F)	N-Butane	Propane	Isobutane
A-108	108 ± 4	Traces	99	1
A-31	31 ± 2	3	1	96
A-17	17 ± 2	98	Traces	2
A-24	24 ± 2	49.2	0.6	50
A-40	40 ± 2	2	12	86
A-46	46 ± 2	2	20	78
A-52	52 ± 2	2	28	70
A-70	70 ± 2	1	51	48

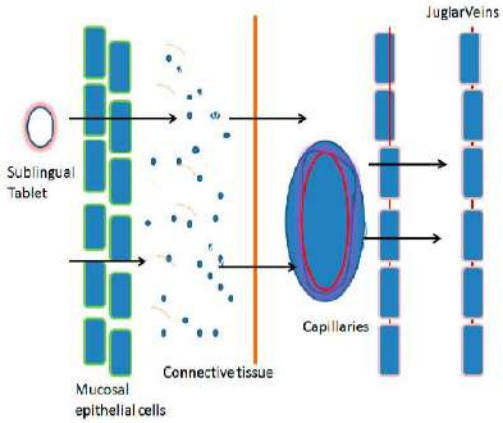

FIG. 1: MECHANISM OF ABSORPTION THROUGH SUBLINGUAL MUCOSA

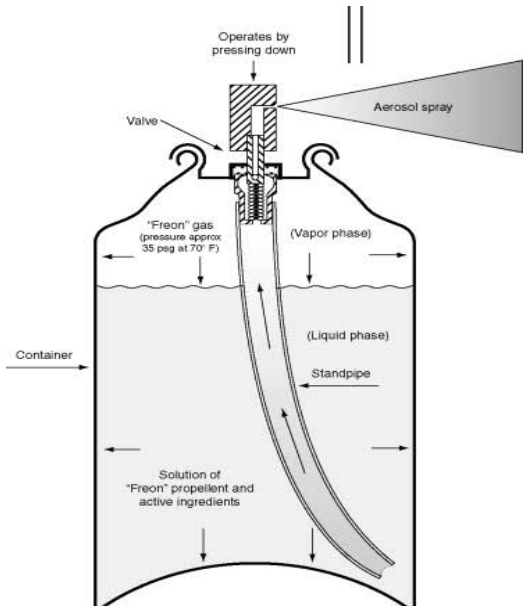

Figure 2: shows a cross-section of an aerosol container

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.13 / Issue 71 / April / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Acute Toxicity Study of Novel Synthetic Pyridazinone Derivatives

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Received: 08 Jan 2022 Revised: 10 Feb 2022 Accepted: 11 Mar 2022

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ABSTRACT

Pyridazinone are six-membered heterocyclic compounds, which acquire all kinds of biological activities. The pyridazine nucleus represents a functional scaffold to establish new pharmacologically active compounds. modifications of the Pyridazinone nucleus have resulted in a large number of compounds having diverse pharmacological activities. Testing the safety of the synthetic drug is must before it could be used as medicines.This study was carried out to analyze the novel synthetic pyridazinonederivative for their acute toxicity profile. The present study was carried out to evaluate the safety of the novel synthetic Pyridazinone derivative as per OECD guidelines 423. In this study, single oral administration of 5, 50, 300 & 2000 mg/kg doses of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dionedid not showed any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence, it was concluded from the results that the possible oral toxic doses of1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione are more than 2000mg/kg and found to be safer and non -toxic to rats and further chronic studies are required to confirm its therapeutic efficacy in animals and humans.

Keywords: Acute toxicity study, Pyridazinone, Benzoxazole,1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, occurs when the pancreas is not producing insulin or produced insulin cannot be used by the body, these may lead to raise blood glucose levels. Hyperglycemia for the long-term are associated with damage to the various organs and tissues. The number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030. IDF also estimates that as many as 183 million people are unaware that they have diabetes [1]. It can be predicted that by 2030, India, China and United States will have the largest number of diabetic patients [2]. There are two types diabetes: type 1 diabetes mellitus and type 2 diabetes mellitus. Despite the great interest in the development of new drugs to reduce the burden of this disease, the scientific community has raised interest to evaluate either raw or isolated natural products in experimental studies; few were tested clinically in humans [3]. Experimental studies of diabetes in animal models and advanced *in vitro* techniques are essential for the improvement of knowledge and clear understanding of the pathology and pathogenesis, and to find new therapy. Animal models of diabetes are therefore, greatly useful in biomedical studies because they offer the promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, shorter generation intervals and economic considerations. Experimental diabetes mellitus studied by several methods that include: chemical, surgical and genetic manipulations [4]. It is also very important to select appropriate animal model for the screening of new chemical entities and other therapeutic modalities for the treatment of diabetes [5].

Pyridazinone having six-member heterocyclic compounds, two nitrogen atoms are present at adjacent positions. Pyridazin-3-one, a saturated or unsaturated arrangement of pyridazine with carbonyl group on third carbon, bygone expressed as a magic moiety which acquire all kinds of biological activities. The pyridazine nucleus represents a functional scaffold to establish new pharmacologically active compounds. Diazines and their derivatives have become very important to the field of chemistry and to the general population in terms of their priceless biological activities. Diazines contain two azomethine nitrogen atoms. There are three types of diazines - pyridazine, pyrimidine and pyrazine are stable, colorless compounds, which are soluble in water [6, 7]. Many pyridazinones have attracted considerable attention as they are endowed with a variety of pharmacological activities. These derivatives serves as one of the most active class of compounds possessing broad spectrum of biological activity varing from cardiovascular, antiasthmatic, antiinflammatory, anti-HIV-1, antidiabetic, antidepressant, anticonvulsant, antiproli-ferative, analgesic, anti-AIDS, analgesic, anticancer, antimicrobial, cardiotonic, antihypertensive, antimicrobial and insecticidal activities etc. Some of the pyridazinone derivatives have reached till the clinical trial as biologically active agents [8-15].

Benzoxazole belongs to one of the most important class of heterocyclic compounds and has got importance as a medicine. It has been incorporated in many medicinal compounds that made it versatile heterocyclic compound possessing wide spectrum of biological activities viz: antimicrobial [16, 17] and got antidiabetic activity [18]. Addition benzoxazole to the pyridazinone moiety could help explore potential antidiabetic actions of the substituted compounds. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used. The main aim of our study was to evaluate the novel synthetic benzoxazole derivative for their toxic effects before it can be used for applications that are of importance to the public. Hence, this study aims to evaluate its acute toxicity study as per OECD guidelines.

METHODS

Synthesis of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione.

The research work was focused on the synthesis of biologically potent molecules. benzoxazoles are known for diverse pharmacological activities. In this study, Addition benzoxazole to the pyridazinone moiety reacts and forms

1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione. The structure of the compound was confirmed by the spectral studies such as Infrared spectroscopy, NMR spectroscopy, and MASS spectroscopy.

Animals

Healthy adult female Swiss albino mice (25-30gm) were used for the acute toxicity studies. The animals were procured from CPCSEA listed suppliers of Sri venkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12 h dark schedule at 25°C and 55–65% humidity levels. The animals had been given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of Animal

Healthy animals were randomly selected for the study and kept in their cages for at least one week prior to dosing to allow for acclimatization to the laboratory conditions. Before test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals

Toxicity Studies

Acute toxicity study was performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 423 (19).

Acute Oral Toxicity Studies

The acute toxicity studies were performed as per OECD guidelines 423. A total of 12 mice weighing between 25-30g were randomly divided into four groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld overnight). Following the period of fasting, the bodyweight of the animals was measured and the 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione was administered orally to each group at single doses of 5, 50,300, and 2000 mg/kg, respectively. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 14 days. All the animals were then allowed free access to food and water and observed for signs of acute toxicity. It includes changes in body weight, food and water intake, skin and fur, eyes and mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity, and behavior patterns. The number of deaths within this period was recorded. The urine analysis was performed to investigate any abnormalities in the excretion pattern after exposure to the test drug for 14 days

RESULTS

Synthesis of1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione

The novel synthetic pyridazinone derivative 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione was synthesized by standard procedure. The percentage yield of the compound was found to be 0.3271 (89 %) and the melting point was found to be 136-138^0 C; The IR spectrum showed the bands in the following regions.IR (KBr vmax cm-1): 1660.23, (NH-C=O str), 1695.13 (N-C=O str), 3454.86 (-NH str). 1H NMR of the synthesized compound was as follows. (DMSO-d6, 400MHz) δ. 6.86-8.97 (m, 4H, Ar-H), 11.42 (s, 1H, -NH), 2.53 (s, 3H, -CH3). 13C NMR (DMSO-d6, 100 MHz) δ: 173.64, 172.50, 155.78, 152.05, 150.73, 149.46 149.22, 141.67, 136.08, 124.73, 121.16, 22.49 was shown in fig 1&2. The elemental analysis was done and calculated (%) for C12H8ClN3O3; C,51.91; H,2.90; N,15.13; observed C,51.88; H,2.86; N,15.09. M+1, 277.66, M+2, 279.66.

Acute Toxicity Studies

Acute toxicity studies are performed to determine the short-term adverse effects of the drug when administered in a single dose orally. It also indicates the safety of the drug in-vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals. The LD50 determination was performed in mice as per OECD guidelines 423 and LD50 of the 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione was found to be non-toxic upto 2000 mg/kg and the ED50 values were 200 mg/kg, respectively was shown in table.no.1.

DISCUSSION

Any therapeutic compounds derived from synthetic chemistry must be scientifically validated for their safety. The aim of performing acute toxicity studies is for establishing the therapeut'ic index of a particular drug and to ensure the safety in-vivo. In the present study, the animals treated with -(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any of the four groups. Hence, it was safe up to 2000mg/kg.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Vinayaka Mission's Research Foundation (Deemed to be University), Salem for providing the facilities for carrying out this research.

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Table 1. LD_{50} value of the 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione.

S.No	Groups	No. of Animals/group	Dose mg/kg	No. of death animals
1	I	3	5	0
2	II	3	50	0
3	III	3	300	0
4	IV	3	2000	0
	LD_{50}		2000	
	ED_{50}		200	

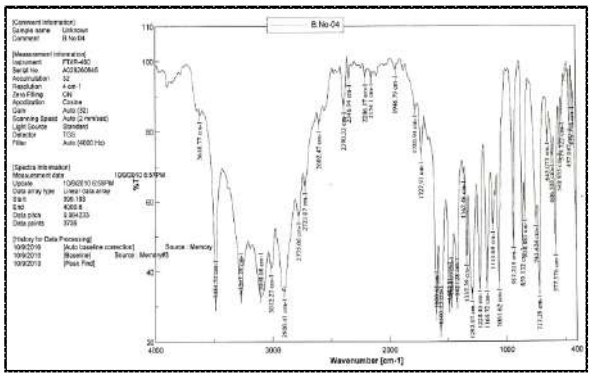

Figure 1. IR spectrum of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione

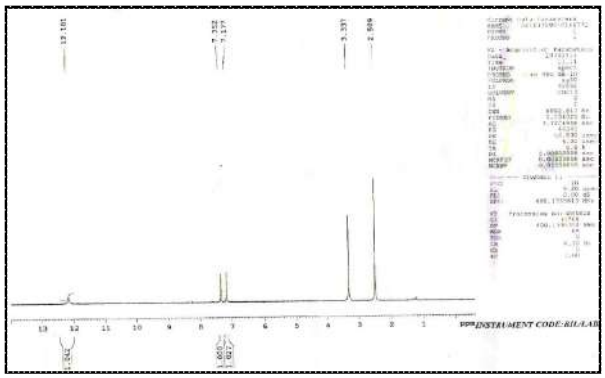

Figure 2. H-NMR spectrum of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione