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RESEARCH ARTICLE

Fuzzy Ranking Game Problem solving by using Dominance Principle with Fuzzy Triangular Numbers

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ABSTRACT

In many real world application problems are involves the elements with only partial membership of a set. But, fuzzy set theory accepts all possible memberships.In this paper, the focus is on solving Fuzzy ranking game problem with imprecise entries are assumed to be triangular fuzzy number and also illustrated by using some numerical examples with dominance method .

Keywords: Fuzzy game, Fuzzy Numbers, Triangular Fuzzy number, Dominance Principle.

INTRODUCTION

Game theory provides a mathematical process for selecting an optimal strategy. It is required to selecting an optimal strategy. It is required to take decision in a situation where there are two (or more) opposite parties with conflicting interests and the action of one depends upon the action of the opponents. The outcome of the situation is controlled by the decisions of all the parties involved. This problem arises frequently in social, military, political advertising and marketing. The mathematical approach of this game theory was made by John Von Newmann in 1944 and his approach to solve the game problem based on best out of the worst. That is the selection of strategies by A and B was based upon maximin and minimax principle which guarantees the best. When Maximin value of the game is equal to the minimax value of the game then the corresponding pure strategies are called optimum strategies. When, Maximin is not equal to the minimax, then pure strategy fails, therefore each player with certain probabilistic fixation is called mixed strategy.

Fuzzy concepts are applied in Managerial decision making problems. Logic is refers to the study of methods and principles of human reasoning because fuzzy logic is an approach which deals with an unclear situation and

it'sbased on degrees of truth rather than the usual "true (or) false". Generally it is not connected with fixed or exact value but it deals with approximate value. In this, the situation possibly lies between o and 1. This is called as membership function. Fuzzy logic is the extension of Boolean logic in which the situation is either 1and 0 respectively. Fuzzy sets are introduced by Zadeh.L.A (1965) provides natural way of dealing with problems.Fuzzy logic contributes in the association of uncertainty on parameters, properties, etc., It also depicts the physical world in a realistic manner than the original number. Aubin and Butnariu are developed fuzzy game research, Campes has explored Fuzzy linear programming models to solve fuzzy matrix games.

In the early 1970, fuzzy logic systems added a new dimension in all fields and hard approaches of fuzzy logic based theory helped in many fields and produced many exciting results in real world systems.

PRELIMINARIES

The aim of this section is to present, some important notations and results which were useful for the further discussion.

Fuzzy set

Let X is a nonempty set. A fuzzy set A in X is characterized by its membership function A→ [0, 1] and A(x) is interpreted as the degree of membership of element x in fuzzy A for each $x \in X$. The mapping A is called as the membership function of fuzzy set A. The value '0' is representing complete non-membership and the value '1' is represents complete membership. The values in between are represents intermediate degrees of membership. That is, the integration of the elements having a changing degree of membership in the set is called as fuzzy set.

Fuzzy Numbers

A Fuzzy set A defined on the set of real numbers R is said to fuzzy number if its membership function μ_A: R → [0, 1] has the following characteristics.

- A is normal that is there exist an x ∈ R such that $\mu_A(x)=1$
- A is convex that is for every $x_1, x_2 \in R$, $\mu_A(x)=1$
- μ_A is upper semi-continuous
- Sup (A) is bounded in R

Triangular Fuzzy number

A fuzzy number A in R is said to be triangular if its membership function has the following characteristics

$$\mu_A = \begin{cases} \dfrac{x-a_1}{a_2-a_1}, & a_1 \le x \le a_2 \\ 1 \quad , & x = a_2 \\ \dfrac{a_3-x}{a_3-a_2}, & a_2 \le x \le a_3 \end{cases}$$

Ranking of Triangular Fuzzy number

There are so many ideas regarding ranking of fuzzy numbers in the literature. An effective result for comparing the fuzzy numbers is by using ranking function based on their grade means. That is, for every A =(a_1 , a_2, a_3) ∈F(R), the ranking function $\mathbb{R}: F(R) \to R$ by graded mean is defined as $\mathbb{R}(A) = \frac{a_1+4a_2+a_3}{6}$

PROPERTIES OF FUZZY RANKING

Let $A = (a_1, a_2, a_3)$ and $B = (b_1, b_2, b_3)$ be any two triangular numbers in $_F(R)$ and $x \in \mathbb{R}$, then

i) $xA = \{(xa_1, xa_2, xa_3), x \geq 0 \; and \; (-xa_1, -xa_2, -xa_3), x < 0\}$
ii) $A + B = (a_1 + b_1, a_2 + b_2, a_3 + b_3)$
iii) $A - B = (a_1 - b_1, a_2 - b_2, a_3 - b_3)$
iv) $A \leq B \; iff \; a_1 \leq b_1, a_1 - a_2 \leq b_1 - b_2, a_1 + a_3 \leq b_1 + b_3$
v) $A < B \; iff \; \mathbb{R}(A) < R(B)$
vi) $A > B \; iff \; \mathbb{R}(A) > R(B)$
vii) $A \approx B \; iff \; \mathbb{R}(A) \approx \mathbb{R}(B)$
viii) If $\mathbb{R}(A) > 0 \; then \; A > 0$
ix) If $\mathbb{R}(A) = 0 \; then \; A \approx 0$
x) If $\mathbb{R}(A) = \mathbb{R}(B) \; then \; A \approx B$

METHODOLOGY

Fuzzy Payoff Matrix

In the game problem, let $A_1, A_2, \ldots, A_m$ be the strategies of player A and $B_1, B_2, \ldots, B_n$ be the strategies of player B. It is assumed that player A is always the gainer and Player B is always loser. Let a_{ij} be the payoff which player 'A' gains from player 'B'. Then the payoff matrix is of the form

PLAYERB				
PLAYERA		B_1	…	B_n
	A_1	a_{11}	…	a_{1_n}
	…	…	…	…
	A_m	a_{m1}	…	a_{mn}

Solving Game Problem using Dominance property

It is observed that the one of the pure strategies of either player is inferior to at least one of the remaining ones. The superior strategies are said to dominate the inferior ones. Clearly we can reduce the size of the payoff matrix by deleting those strategies which are dominated by others. Procedure for solving fuzzy ranking game by using triangular fuzzy number is as follows:

Step-1: Represent the triangular fuzzy numbers in the fuzzy game problem.
Step-2: Identify any two rows. If all the elements of a row say k^{th}, are less than or equal to the corresponding elements of any other row say r^{th}, then k^{th} row is dominated by r^{th} row.
Step-3: If all the elements of a column, Say k^{th} are greater than or equal to the corresponding elements of any other column, say r^{th} then k^{th} column is dominated by r^{th} column.
Step-4: Dominated rows and columns are deleted to reduce the size of payoff matrix.

NUMERICAL ILLUSTRATION

Consider the following fuzzy ranking game problem:

	B_1	B_2	B_3
A_1	(7,5,6)	(2,4,6)	(3,5,6)
A_2	(2,6,8)	(4,1,5)	(4,3,5)
A_3	(8,7,9)	(1,6,7)	(5,7,4)

Now convert the above fuzzy problem into a crisp value problem by using the measures with the help of Maximin and Minimax principle with saddle point:

	B_1	B_2	B_3	Row Minima	Minimax
A_1	5.5	4	4.83	4	5.33
A_2	5.67	2.17	3.5	2.17	
A_3	7.5	5.33	6.17	5.33	
Column Maxima	7.5	5.33	6.17		
Maximin	5.33				

MaximinValue=5.33
Minimax Value = 5.33
HencetheValue ofthe game =5.33

Consider the following fuzzy ranking game problem:

	B_1	B_2	B_3
A_1	(3,4,6)	(2,3,6)	(4,5,8)
A_2	(5,7,8)	(1,2,5)	(8,7,9)
A_3	(4,6,9)	(4,1,5)	(5,3,4)

Now convert the above fuzzy problem into a crisp value problem by using the measures using Dominance Principle:

	B_1	B_2	B_3
A_1	4.17	3.33	5.33
A_2	6.83	2.33	7.5
A_3	6.17	2.17	3.5

Now all the elements of rowA_3 are less than the corresponding elements of A_2. Therefore A_3 is dominates the A_2 and it is deleted. All the elements of B_3 are greater than the corresponding elements of B_2 and it is deleted. Now the payoff matrix is reduced to 2x2.

	A_1	A_2	Row Minima	Minimax
B_1	4.17	3.33	3.33	3.33
B_2	6.83	2.33	2.33	
Column Maxima	6.83	3.33		
Maximin	3.33			

Maximin Value = 3.33
Minimax Value = 3.33
The value of the game = 4.33

CONCLUSION

In this paper, we have obtained the optimum solution of the fuzzy ranking game problem using Triangular Fuzzy numbers using (i) Maximin and Minimax (ii) Dominance principle. We may get different game value for different triangular fuzzy numbers. A Numerical example has been considered and solved to illustrate the proposed method.

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RESEARCH ARTICLE

A Study to Assess the Knowledge Regarding Prevention of Domestic Violence among Adolescent Girls at Selected Higher Secondary School, Salem

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ABSTRACT

A descriptive study was conducted to assess the knowledge of adolescent girls on prevention of domestic violence at government higher secondary school, Attayampatti, Salem. Data was collected from 50 adolescent girls. The simple random sampling technique was adopted and information was gathered. Highest percentage (40%) of the adolescent girls was in the age group of 16 years. Highest percentages (40%) of the adolescent girls were studying 11 th standard. Majority (52%) of adolescent girl's father educational status were primary and secondary education. Highest percentages (50%) of adolescent girl's mother educational status were primary and secondary education. Majority (80%) of adolescent girls was nuclear family. Highest percentage (52%) of adolescent girls had two siblings. Highest percentage (52%) of adolescent girls was living in rural. Most of the (36%) adolescent girl's hobbies were watching television. Highest percentage (40%) of adolescent girl's mode of transport was two wheeler. Majority (32%) of adolescent girls was received from teacher. Further area wise distribution of mean, SD and mean percentage of knowledge adolescent girls regarding prevention of domestic violence reveals that mean percentage (13.94 ± 2.52) which is 3.04% was obtained by the adolescent girls. The overall level of knowledge shows that highest percentage (52%) of adolescent girls had moderate knowledge. Lowest percentage (6%) of adolescent girls had adequate knowledge and (42%) of adolescent girls had inadequate knowledge. The adolescent girls knowledge level was found to be moderate further awareness regarding prevention of domestic violence need to be created to improve adolescent girl's level of knowledge.

Keywords: Domestic Violence, Adolescent girls, Knowledge.

INTRODUCTION

The term adolescence derived from Latin: adolescence means "to grow up" is a transitional stage of physical and mental human development generally occurring between puberty and legal adulthood. According to Erick Erikson's stages of human development, adolescent girls are a person between the ages of 13 and 19, KP Neeraja (2017). In the world, India has the largest number of children (375 million), covering 40% of its population, out of which 69% of Indian children are victims of physical, emotional and sexual abuse. New Delhi, The nation's capital, has an abuse rate over 83%.More than 70% of cases are unreported or unshared even with family members. Global prevalence of child sexual abuse study conducted by the Centers for Disease control. The U.S 19.7% of women globally experienced sexual abuse prior to the age of 18. The highest prevalence rate of child sexual abuse geographically was found in Africa (34.4%). Europe showed the lowest prevalence rate (9.2%). America and Asia had prevalence rates between 10.1% and 23.9 %. In Indian society adolescence place an additional burden on females with biological development. The adolescent girl is often pressurized towards social role conformity, requiring major changes in the psychological sphere. The young lady is often confronted with difficulties and problems related to family society and their own physical aspects and emotional needs (www.ncbi.nlm.govt). Domestic violence also referred to as molestation, is the forcing of undesired sexual behavior by one person upon another. When that force is immediate, short duration, or frequent, it is called sexual assault. The offender is referred to as sexual abusers or molester. When the victim is younger than the age of consent, it is referred to as sexual abuse. Sexual abuse or violence against adolescent girls is defined as a situation in which children or adolescent girls are used for the sexual pleasure of an adult or older adolescent girl which ranges from petting, fondling of genitalia, breasts or anus, voyeurism, pornography, exhibitionism, pressuring a child to engage in sexual activities, indecent exposure of the genitals nipple etc, Marlow (2009).

Statement of the problem

A Study to assess the knowledge regarding prevention of domestic violence among adolescent girls at Selected Higher Secondary School, Salem

Objectives

- To assess the level of knowledge regarding domestic violence among adolescent girls
- To compare the level of knowledge regarding domestic violence among adolescent girls with their selected demographic variables.

METHODOLOGY

Research approach

A cross sectional survey approach was used to assess the knowledge regarding prevention of domestic violence among adolescent girls

Research design

A descriptive research design was adopted to assess the knowledge regarding prevention of domestic violence among adolescent girls

Setting of the study

The study was conducted in Vidhya Mandir higher secondary school, Attayampatti, Salem. Located 8 km away from Vinayaka Mission's Annapoorana College of Nursing, Salem.

Population and Sample

The population under study was adolescent girls in the age group of 14-16 years, who are all studying Vidhya Mandir higher secondary school, Attayampatti, Salem and who were presented during the period of data collection was the sample of the study.

Sample size

The sample comprised of 50 adolescent studying in Vidhya Mandir higher secondary school, Attayampatti, Salem.

Sampling technique

Simple random sampling was adopted to select the samples for this study.

Description of the tool

Closed ended questionnaire was used to assess the knowledge regarding prevention of domestic violence among adolescent girls

The tool consists of two sections.

Part A – Demographic data

Part B – Knowledge questionnaire

Part A: Demographic data

It consist of demographic characteristics such as age, education, father education, mother education, type of family, number of siblings, residence, hobbies, mode of transport and source of information.

Part B: Knowledge questionnaire

Structured questionnaire consist of 30 multiple choice questions, related to information domestic violence / sexual exploitation, consequences, prevention. Each right answers carrying one mark. Each wrong answers carrying zero mark.

RESULT AND DISCUSSIONS

A descriptive cross sectional study was conducted to assess the knowledge regarding prevention of domestic violence among adolescent girls in higher secondary school, Attayampatti, Salem. The data collected from 50 adolescent girls by using simple random sampling technique.

Assessment of level of knowledge of the adolescent girls regarding prevention of domestic violence

Percentage wise distribution of adolescent girls according to their overall level of knowledge shows that highest percentage (52%) of adolescent girls had moderate knowledge. Lowest percentage (6%) of adolescent girls had adequate knowledge and (42%) of adolescent girls had inadequate knowledge. (Fig. No. 1.1)

Area wise distribution of mean, SD and mean percentage of knowledge adolescent girls regarding prevention of domestic violence

Area wise distribution of mean, SD and mean percentage of knowledge adolescent girls regarding prevention of domestic violence reveals that mean percentage (13.94 ± 2.52) which is 3.04% was obtained by the adolescent girls. (Table No. 4.2.1)

Association between the knowledge score of adolescent girls with their selected demographic variables.

The variables such as age in year, educational status, father education, mother education, type of family, number of siblings, residency, mode of transport, showed non- significant association except hobbies and sources of information with the overall level of knowledge of the study subjects. The study finding reveals that there is no association of level of knowledge regarding prevention of domestic violence.

CONCLUSION

The present study assessed the knowledge of adolescent girls regarding prevention of domestic violence. The adolescent girls knowledge level was found to be moderate further awareness regarding prevention of domestic violence need to be created to improve adolescent girls level of knowledge.

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Area wise distribution of mean, SD and mean percentage of knowledge adolescent girls regarding prevention of domestic violence

n=50

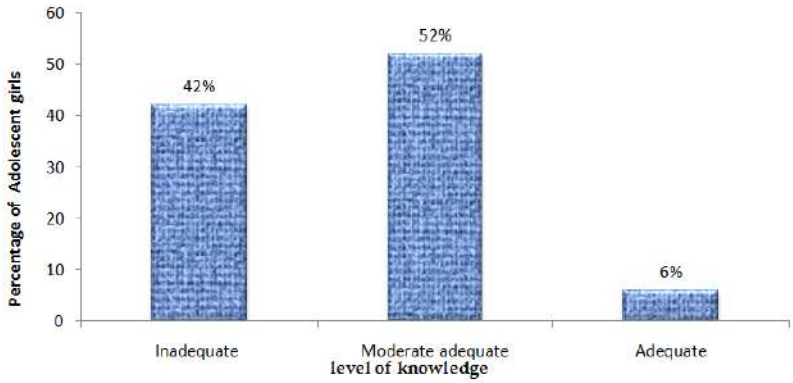
Variables	Total score	Mean	Standard deviation	Mean percentage
Knowledge	30	13.94	2.52	3.04

Association between the knowledge score of adolescent girls with their selected demographic variables.

n=50

S. No	Demographic variables	x^2	Significance
1	Age in year	1.99	Non-significant
2	Educational status	1.99	Non-significant
3	Father education	2.33	Non-significant
4	Mother education	0.76	Non-significant
5	Type of family	2.85	Non-significant
6	Number of siblings	0.66	Non-significant
7	Residency	0.66	Non-significant
8	Hobbies	27.19*	Highly Significant
9	Mode of transport	2.02	Non-significant
10	Sources of information	11.27*	Highly Significant

***significant at P<0.05 level**



Bar diagram showing overall percentage of adolescent girls according to their level of knowledge about prevention of domestic violence

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REVIEW ARTICLE

Regulatory Aspects in New Drug Application (NDA)-A Review

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ABSTRACT

Current compel of Regulatory Affairs uncovers assorted nations had the opportunity to follow distinctive administrative necessities for Marketing Authorization Application (MAA) endorsement of most recent medications. Each nation has its own administrative organization which is dependable to authorize the standards and guidelines and issue the principles to deal with the promoting of the medications. When a lead drug particle has been found, nonclinical investigations of a medication ought to be led to ensure adequacy and security. Then, at that point, clinical preliminaries are regularly performed, after an application is submitted to skilled authority of the concerned country. The three periods of clinical preliminaries are led according to the conventions. The capable position audits an application submitted to encourage endorsement for promoting the medication and supports it whenever fulfilled that the medication upholds quality, security and viability concerns. Even after the endorsement of most recent medication, government should screen its security by post promoting observation which is considered as stage IV clinical preliminaries. However certain parts of medication endorsement measure are comparative on various nations, a few contrasts do happen. In this current effort concentrate on communicates the medication endorsement measure and administrative prerequisites predictable with US Food and Drug Administration (UDFDA), European Medical Agency (EMA) and Central Drug Standard Control Organization (CDSCO). This audit diagrams progresses in treatment and the principle spot light for an improvement and advance of cell treatments that are being faced today.

Keywords: Drug endorsement; Regulatory necessities; USFDA; CDSCO; EMA

INTRODUCTION [1-6]

As of now various nations need to follow diverse administrative necessities for endorsement of most recent medication. For Marketing Authorization Application (MAA) one administrative methodology is material to shifted nations is very nearly a troublesome undertaking. In this manner, it is important to have information about administrative necessity for MAA of every country. Every nation has its own administrative necessities which should be fulfilled to endorse a substitution drug in that specific country. It is hard to go for one administrative methodology for endorsement of a substitution drug in a few nations. Thus there's a necessity for acquiring mindfulness on administrative issues with different nations. It is reported that the US of America (USA) and in this way the European Union (EU) are the chief likely business sectors for drug items inside the world; various organizations center around their drug enactments. Thus, this text features the administrative techniques people, EU and India. Initially, when a lead particle is distinguished for an objective illness, it ought to be enhanced. After the creation of a medication, pre-clinical preliminaries are directed on creatures to ensure security and viability. An application ought to be submitted to equipped authority of a concerned nation to encourage authorization for directing clinical investigations. Clinical preliminaries are acted in four stages to guarantee security, adequacy then the medication portion is advanced in people. A Marketing Authorization Application (MAA) is then submitted, which is supported by the capable power, if the medication fulfills the needs of security and adequacy and demonstrates that its advantages offset its dangers (Figure 1).

New Drug Application (NDA) is an application submitted to the individual administrative organization for approval to plug a substitution drug for example creative item. To acquire this consent a support submits preclinical and clinical preliminary information for examining the medication data, portrayal of creating preliminaries.

VARIOUS PHASES OF CLINICAL TRIALS [7-9]

- Pre-clinical review
- Phase I - Clinical preliminary
- Phase II - Exploratory preliminary
- Phase III-Confirmatory preliminary
- Phase IV-Post Marketing preliminary.

After NDA get by the organization, it goes through a specialized screening. This assessment guarantees that adequate information and information are submitted in every space to legitimize "recording" the apparatus. At the finish of the audit of a NDA, there are 3 potential activities which will ship off support:

- Not approvable - in this letter rundown of insufficiencies and clarify the explanation.
- Approvable - changes and conceivable solicitation obligation to do post-endorsement examines.
- Approval-it express that the medication is supported.

On the off chance that the activity taken is either an approvable or a not approvable, the administrative body furnishes candidate with an opportunity to fulfill with office and examine the inadequacies.

MEDICATION APPROVAL IN INDIA [10]

The Drug and Cosmetic Act 1940 and Rules 1945 was broadcasted by the India's parliament to deal with the import, production, dispersion and offer of medication and beauty care products. The Central Drugs Standard Control Organization (CDSCO) and consequently the workplace of its chief, the Drugs Controller General (DCGI) was set up. In 1988, the Indian government adds Schedule Y to the Drug and Cosmetics Rules 1945. Timetable Y gives the principles and necessities to clinical preliminaries, which was additionally amended in 2005 to bring it at standard with universally acknowledged strategy. At the point when a partnership in India needs to make/import a substitution drug it's to use to chase authorization from the permitting authority (DCGI) by documenting in Form 44 likewise presenting the information as given in Schedule Y of medication and Cosmetics Act 1940 and Rules 1945. To

demonstrate its adequacy and security in Indian populace it's to lead clinical preliminaries as per the standards spread out in Schedule Y and present the report for some clinical preliminaries in indicated design.

RULE [11-22]

122A of the Drug and Cosmetics Act says that the clinical preliminaries could likewise be postponed inside the situation of most recent medications which are endorsed and getting utilized for quite a while in different nations. Segment 2.4 (a) of Schedule Y of medication and Cosmetics Act 1940 and Rules 1945 says for a those medication substances which are found in India all periods of clinical preliminaries are required. Segment 2.4 (b) of Schedule Y of medication and Cosmetics Act 1940 and Rules 1945 says that those medication substances which are found in nations beside India; the candidate ought to be present the information accessible from different nations and the permitting authority might expect him to rehash every one of the investigations or license him to continue from Phase III clinical preliminaries.

Show of security and adequacy of the medication item to be utilized in people is significant before the medication item are regularly endorsed for import or assembling of most recent medication by the candidate by Central Drugs Standard Control Organization (CDSCO). The guidelines under Drugs and Cosmetics Act 1940 and its standards 1945, 122A, 122B and 122D portray the information needed for endorsement of an application to import or make of new medication for showcasing .For an investigational new medication, the support should give definite data to the DCGI about:

- Generic name
- Patent status
- Brief depiction of physico-substance/organic
- Technical data
  a) Stability
  b) Specifications
  c) Manufacturing measure
  d) Worldwide administrative status
  e) Animal pharmacology and poisonousness contemplates
- Published clinical preliminary reports
- Proposed convention and professional forma
- Trial span
- During ace record
- Undertaking to Report Serious or Life-compromising Adverse Drug Reactions.

The requirement for neighborhood clinical preliminaries in India relies upon the situation with drug in different nations. On the off chance that the medication is as of now supported in different nations, for the most part stage III clinical preliminary preliminaries are required. Stage I preliminaries aren't permitted in India except if the data is out there from different nations. Authorization is allowed by DCGI to direct Phase 1 preliminaries in India, if the medication has exceptional significance to a weakness in India, similar to jungle fever or tuberculosis.

Bioavailability and bioequivalence (BABE) studies ought to be led according to BABE rules. The far reaching data on the advertising status of the medication in different nations is furthermore required beside the information on security and viability. The data in regards to the remedy, tests and testing conventions, item monographs, names should even be submitted. It generally requires 3 months for clinical test endorsement in India. The clinical preliminaries can be enlisted in the Clinical Trials Registry of India (CTRI) giving subtleties of the clinical preliminaries and the subjects engaged with the preliminaries. The standards to be followed

- Rule 122 - A: Application for consent to import new medication
- Rule 122-B: Application for endorsement to make new medication other than the medications determined under Schedule C and C (1).

- Rule 122 - D: Permission to import or fabricate fixed portion mix.
- Rule 122 - DA: Application for consent to direct clinical preliminaries for New Drug/Investigational New Drug.
- Rule 122 - DAB: Compensation on account of injury or demise during the clinical preliminaries.

The progressions in the Drugs and Cosmetics Act incorporates, building up definitions for Phase I-IV preliminaries and clear responsibilities regarding agents and supporters. The clinical preliminaries were additionally separated into two classes in 2006. In one class (classification A) clinical preliminaries are frequently led in different business sectors with able and mature administrative frameworks while the leftover ones fall in to an alternate class (classification B) Other than A. Clinical preliminaries of classification A (endorsed inside the U.S., Britain, Switzerland, Australia, Canada, Germany, South Africa, Japan and European Union) are qualified for optimizing in India, and are probably going to be supported inside about two months.

The clinical preliminaries of classification B are under more investigation, and endorse inside 16 to 18 weeks. An application to direct clinical preliminaries in India ought to be submitted close by the data of science, assembling, control and creature studies to DCGI. The date with respect to the preliminary protcol, specialist's pamphlets, and educated assent reports ought to likewise be joined. A duplicate of the application should be submitted to the moral panel and the clinical preliminaries are directed solely after endorsement of DCGI and moral board of trustees.

PHASES OF ENDORSEMENT [23-24]

- Submission of Clinical Trial application for assessing wellbeing and adequacy.
- Requirements for consent of new medications endorsement.
- Post endorsement changes in organic items: quality, wellbeing and viability archives.
- Preparation of the quality data for drug accommodation for new medication endorsement.

Most nations have embraced the CTD design. Subsequently, CDSCO has likewise chosen to embrace CTD design for specialized prerequisites for enrollment of drug tems for human use (Figure 2).

MEDICATION APPROVAL IN EUROPE [25-28]

The European Medicines Evaluation Agency (EMEA) was set up in London, inside the year 1995, to organize the ecu Union (EU) part states for assessing and managing the restorative items for both human and veterinary use. It presented a straightforward method for the turn of events, meeting, conclusion and execution of drug rules. The medication endorsement measure in European nations is cultivated in two stages:

- Clinical preliminary.
- Marketing approval.

A clinical preliminary application (CTA) is documented to the capable authority of the state to lead the clinical preliminary inside European Union (EU). The able authority of that part state assesses the apparatus. The clinical preliminaries are directed solely after the endorsement. Showcasing approval application is recorded solely after every one of the three periods of clinical preliminaries are finished. The European Legislation containing the drug orders has been distributed inside the accompanying volumes entitled the standards Governing Medicinal Products inside the European Union.

Volume 1: Pharmaceutical Legislation for Medicinal Products for human use
Volume 2: Notice to Applicants for Medicinal Products for human use
Volume 3: Scientific Guidelines for Medicinal Products for human use
Volume 4: Good Manufacturing Practices Guidelines for Medicinal Products for human and veterinary use
Volume 5: Pharmaceutical Legislation for Medicinal Products for veterinary use
Volume 6: Notice to Applicants for Medicinal Products for veterinary use.
Volume 7: Scientific Guidelines for Medicinal Products for veterinary use
Volume 8: Maximum Residue Limits

Volume 9: Pharmacovigilance Guidelines for Medicinal Products for human and veterinary use
Volume 10: Clinical Trials Guidelines.

Europe has numerous designs and authoritative strategies for acquiring market approval of drugs. There are four distinct courses in the European Union to get promoting endorsement of drugs (Figure 3).

CENTRALIZEDPROCEDURE [29, 30]

The incorporated method is one which permits candidates to get an advertising approval that is legitimate all through the EU. In this strategy, applications are acknowledged concerning results of bio-innovative sciences and New Chemical Entities (NCEs). All the Biotechnological items are gathered as "Rundown A" and NCEs are assembled as "Rundown B". As indicated by this method, just one advertising approval is legitimate for whole European Union. The EMEA staff, on getting the Marketing Authorization Approval (MAA), actually takes a look at the culmination and consistence of the machine with EU rules. This evaluation should be finished inside ten days from the date of documenting the application. The support pays the fitting charges. Then, at that point, EMEA has 210 days to ponder the machine. It can delegate rapporteurs, who evaluate the machine and report Committee for Medicinal Products for Human Use (CHMP). CHMP offers a viewpoint whether to acknowledge or dismiss the application; it is sent to the European Commission, which can require 90 days to arriveata choice. The absolute an ideal opportunity for endorsement is around 300 days (210+90) [5].

Under this item is perceived by all part nations through one application to EMA. Model: CEP entries, eDMF, CTD accommodation on exceptional item like allMeds, Specified Antiviral Medicines, Specified ZS Medicines for Neurode generative Disorder including diabetes and Specified Medicines for Auto insusceptible Diseases/dysfunctions.The dismissal of use under these plans bansentry to EU.

- Results in a solitary approval substantial in EU, Norway, Iceland and Liechtenstein
- Application assessed by relegated Reporters.
- Timeline: EMA assessment gave inside 210 days, and submitted to European Commission f or last endorsement.

Concentrated interaction is obligatory for:

- Those meds which are gotten from any biotechnology measures, like hereditary designing
- Those meds which are expected for the therapy of Cancer, HIV/Aids, diabetes, neurodegenerative issues or immune system sicknesses and other safe dysfunctions.

Medicines authoritatively assigned 'vagrant prescriptions' (medications utilized for uncommon sicknesses) (Figure4).

DECENTRALIZED PROCEDURE [31]

Under this, an item is perceived by a gathering of part's nations all the while. It is considered as exceptionally productive method. Decentralized technique is followed to acquire promoting approvals in a few part states. The support submits to a public administrative power, the application and a rundown of all Concerned Member States (CMSs), determining Reference Member State (RMS). The RMS must legitimate ate the application and Summary of Product Characteristics (SPCs); set up a draft appraisal report inside 210 days and send a duplicate to the CMSs; this report can be endorsed inside 90 days. In the event that a restorative item should make potential genuine danger a general wellbeing. CMSs can mention any criticisms and afterward the CHMP intercedes and takes an ultimate conclusion inside 30 days.

In any case, an adverse choice can influence the enlistment in numerous nations under this plan likewise following item can't be enrolled: Orphans Medicinal Product, All biotechnology based product, Specified Aids and Cancer Medicines, Specified Antiviral Medicines ,Specified Medicines for Neuro degenerative Disorder including diabetes and Specified Medicines for Auto resistant Diseases/dysfunctions (Figure5).

NATIONAL PROCEDURE[32-34]

In Europe every country has its own administrative body. Public system is technique taken on by every country autonomously of different countries. The charges are reasonable in any event, for little firms. It saves money on interpretation cost to English or territorial dialects. It makes a base for Mutual acknowledgment Procedure Biotechnical systems can't be enlisted through public methodology. The Centralized recording through EMA is necessary for the equivalent. The application, presented by the support under the public guidelines to the public capable power, is surveyed and a showcasing approval is conceded. Under this plan likewise following item can't be enrolled: Orphans Medicinal Product, All Biotechnology Based Product, Specified Aids and Cancer Medicines, Specified Antiviral Medicines, Specified Medicines for Neurodegenerative Disorder including diabetes and Specified Medicines for Auto resistant Diseases/dysfunctions (Figure6).

MUTUAL RECOGNITION PROCEDURE (MRP)(35-37)

Under this an item enrolled in one nation is commonly perceived by the other country. The application is needed to make application just a single time for beginning enrollment. Similar applications for certain provincial changes is acknowledged by another part country. Appraisal Report of therapeutic Product by Member Countries in EU: During Mutual acknowledgment measure the evaluation report of Reference part state is looked into previously allowing endorsement. The accommodation can be made to quite a few the other part states and the RMS sends a duplicate of the appraisal report to the CMSs, who can mention any criticisms inside 90 days. Every CMS gives a public advertising approval with an indistinguishable SPC. Under this plan following item can't be enrolled: Orphans Medicinal Product, All biotechnology based item, Specified Aids and malignant growth Medicines, Specified Antiviral Medicines, Specified Medicines for Neurodegenerative Disorder including diabetes and Specified Medicines for Autoimmune Diseases/dysfunctions (Figure7).

DRUG APPROVAL PROCESS IN UNITED STATES [38-41]:

They has maybe the world's most severe guidelines for endorsing new medications. Medication endorsement guidelines inside the us are considered by numerous individuals to be the first requesting inside the world. In 1820, the new period of USA drug guideline was begun with the foundation of U.S. Pharmacopeia. In 1906, Congress passed the main Food and medications Act, which necessitate that medications should satisfy official guidelines of solidarity and virtue. In any case, in 1937, on account of sulphanilamide misfortune, the Federal Food, Drug and Cosmetic Act (of 1938) was sanctioned and added new arrangements that new medications should be shown protected prior to promoting.

Further, in 1962, the Kefauver-Harris Amendment Act was passed which necessitate that producers should demonstrate that medication is protected and successful (for the cases made in naming). The Food and Drug Administration (FDA) is responsible for ensuring and advancing general wellbeing. Like general medication endorsement measure, FDA's new medication endorsement measure is moreover refined in two stages: Clinical Trials (CT) and New Drug Application (NDA) endorsement. The new medication item are controlled through a new medication application (NDA). At present such applications are acknowledged for survey in eCTD design. The significant worry about NDA is that the item will be security and successful .FDA endorsement measure starts solely after accommodation of investigational new medication (IND) application. The US Drug Law and Regulations United States Pharmacopeia (USP) were begun in1820to set norms for strength and immaculateness of medications. Significant achievements inside the advancement people drug law are:

- Food and Drugs Act (1906): It necessitates that the medications should fulfill official guidelines of solidarity and virtue.
- Federal Food, Drug and Cosmetic Act (1938): It was established after sulphanilamide misfortune, to demonstrate the security of a medication prior to being advertised.
- Kefauver-Harris Amendment (1962): It was passed after the thalidomide debacle. It requires the makers to

demonstrate that medication is protected and compelling ll the organizations ought to send antagonistic impact reports to FDA.

- Orphan Drug Act (1973): This permits charge derivations for drug organizations to foster vagrant medications.
- Generic drug implementation Act (1992): It manages feelings identified with ANDA endorsements.
- FDA Modernization Act (1997): It contains a few changes in Federal Food, Drug and Cosmetic Act with respect to assortment and appraisal of client charges and sped up endorsement measures.

INVESTIGESTIONAL NEW DRUG APPLICATION [42-44]

It is an application documented to FDA before human testing. It gives a full portrayal of science, assembling and controls, pharmacology and toxicology data, any past human experience.

Types of IND

- **An Investigator IND:** It is put together by a doctor who the two starts and leads an examination and under whose prompt bearing the investigational drug is directed or administered. A doctor may present an exploration IND to propose examining an unapproved drug, or a supported item for another sign or in another patient populace.
- **Emergency Use IND:** This permits the FDA to approve utilization of a trial drug in a crisis circumstance that doesn't permit time for accommodation of an IND.
- **Treatment IND:** It is submitted for trial drugs showing guarantee in clinical testing for genuine or promptly hazardous conditions while the last clinical work is directed and the FDA audit happens.

The two IND classes are business and exploration (non-business) types. The IND application should contain data in three wide regions:

- Animal Pharmacology and Toxicology Studies
- Manufacturing Information and
- Clinical Protocols and Investigator Information.

When the IND is presented, the support should stand by 30 schedule days prior to starting any clinical preliminaries. During this time, FDA has a chance to audit the IND for security to guarantee that exploration subjects won't be exposed to absurd danger.

IND CONTENT AND FORMAT

The necessities for the substance and arrangement of IND application are given in the 21 Code of Federal Regulations (CFR), Section312.A support (business association) or an agent who means to direct a clinical examination ought to present an "Investigational New Drug Application" in the accompanying request (Figure8):

- Form FDA1571
- Table of substance
- Introductory proclamation and investigational plan
- Investigator's leaflet
- Protocols
- Chemistry, assembling and control (CMC)information
- Pharmacology and toxicology data
- Previous human experience
- Additional data.

NEW DRUG APPLICATION (NDA) [45-47]

A New Drug Application is recorded to get endorsement for promoting another medication in the USA. A NDA contains data remembered for the IND, just as the consequences of clinical investigations demonstrating security and efficacy. The FDA shall start the review process within 60 days from the accommodation of a NDA. Substance and

Format of NDA Two duplicates of the application are: (a) Archival duplicate and (b) Review duplicate. Archival Copy: It fills in as a kind of perspective hotspot for FDA commentators to find data not contained in the survey duplicate; and it contains duplicates of arrangements and clinical review case report structures. It contains the accompanying components:

- Application structure FDA356
- Index
- Summary
- Technical areas: further composed to-
- Chemistry, assembling and controls area
- Non-clinical pharmacology and toxicology area
- Human pharmacokinetics and bioavailability area
- Microbiology area
- Clinical information area
- Statistical area
- Pediatric use area
- Samples and Labeling
- Case report structures

Review Copy: Each specialized area is independently establishing every organizer. Every specialized order particle ought to contain:

- Index
- Copy of FDA Form 356h
- Copy of introductory letter
- Letters of approval
- Copy of utilization outline.

The FDA can direct gatherings with the support no less than multiple times; once toward the finish of Phase 2 clinical preliminaries and one more before a NDA is submitted i.e., a pre-NDA meeting. The audit group will examine the review results and settle on a choice whether to endorse the application (Figure 9).

ABBERIVATED NEW DRUG APPLICATION (ANDA) [48-49]:

ANDA is applied for items with same or firmly related dynamic fixings, measurement structure, and strength, course of organization, use and naming as item previously demonstrated to be protected and viable. It is utilized when the patent has terminated for an item, and a company needs to plug its duplicate. Such medications are called conventional medications, which should meet bio and drug comparable guidelines. An ANDA is submitted to Center for Drug Evaluation and Research, Office of Generic Drugs, where it's surveyed and supported.

Content and Format of ANDA.

- Application structure
- Table of substance
- Basis for ANDA accommodation
- Conditions of utilization
- Active fixings
- Route of organization, measurement from, strength Bioequivalence
- Labeling
- Chemistry, assembling and control
- Human pharmacokinetics and bioavailability
- Samples

- Analytical strategies
- Case report structures and classifications.

The Division of Bio equality's Office of Generic Drugs of CDER gave "Direction on measurable Procedures for Bioequivalence Studies utilizing a Standard Two Treatment Crossover Design" distributed in July 1992, which gives guidelines on legitimate factual investigation for bioequivalence appraisal. This guarantees the legitimacy of bioequivalence evaluation. The FDA has later given a draft direction named "in vivo bioequivalence concentrates on upheld populace and bioequivalence" that offers proposals to backers of INDs, NDAs, ANDAs, who will perform studies dependent on an examination of pharmacokinetic measurements. All endorsed drug items, including marked and nonexclusive medications are recorded in FDA's "Supported Drug Products with Therapeutic Equivalence Evaluations", called Orange Book. It incorporates items that are evaluated by FDA for both wellbeing and adequacy and for which NDAs or ANDAs have been endorsed. It additionally gives helpful proportionality assessments to multisource physician recommended drug items that contain similar dynamic fixings (Tab 1).

SUPPLEMENTAL NEW DRUG APPLICATION (SNDA) [50]

After endorsement of NDA or ANDA, all critical changes inside the conditions depicted inside the applications should be supported, by recording a supplemental NDA or ANDA. Such changes like those in pressing or fixings ought to be supported by the CDER. New-utilizes endorsements of currently supported medications going under this classification are a superior advancement as they need lesser assets to survey than that required for unique use approvals (Tables1and 2).

CONCLUSION

The Drug endorsements in the India, Europe and US are the most idea due on the planet. The basic role of the standards administering therapeutic items in India, Europe and US is to safe gatekeeper general wellbeing. It is the job of public administrative specialists to ensure that drug organizations suits guidelines. There are enactments that need medications to be created, tried, followed, and made in understanding to the guidelines all together that they're protected and patient's prosperity is secured.

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Table 1: Difference between India, EU and US.

Requirements	India	EU	US
Agency	Single agency DCG I (CDSCO)	Multiple agencies • EMEA • CHMP • National health agencies	Single agency USFDA
Registration process	Single registration process	Multiple registration process • Centralised (European community) • Decentralised (at least 2 member states) • Mutual recognition (at least 2 member states) National member state)	Single registration process
TSE/BSE study data	Required	Required	Required
Braille code	Braille code is not required on labelling	Braille code is required on labelling	Braille code is not required on labeling
Post approval changes	Post approval changes: • Major • Moderate	Post variation in the approved drug: • Type IA • Type IB • Type II	Post approval changes in the approved drug: • Minor • Moderate • Major

Table 2: Manufacturing and control requirements.

Requirements	India	EU	US
Number of batches	1	3	1
Packaging	Not addressed	Not required	A minimum of 1,00,0000
Process validation	Required	Required	Not required at the time of submission
Batch size	Pilot scale batch	2 pilot scale plus 1 lab batch or minimum of one lakh units whichever is higher	1 pilot scale or minimum of one lakh units whichever is higher

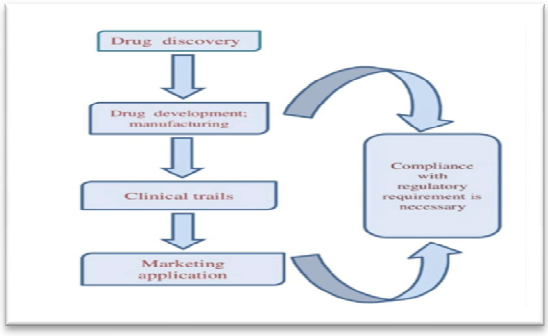

Figure 1: Regulation of Drug Approval Process

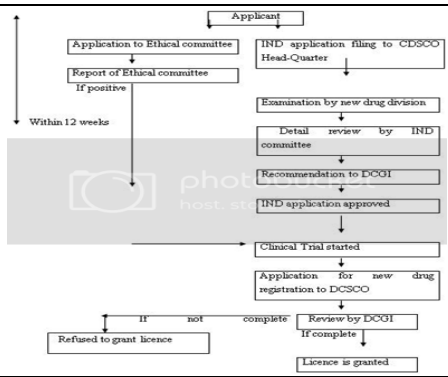

Figure 2: Drug approval process in India.

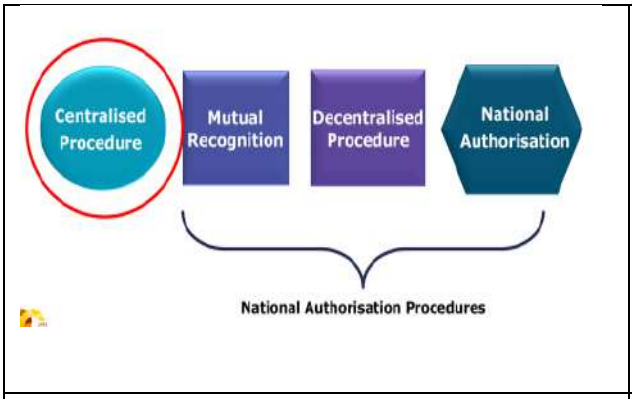

Figure 3: Shows different routes in the European Union to obtain marketing approval of pharmaceuticals.

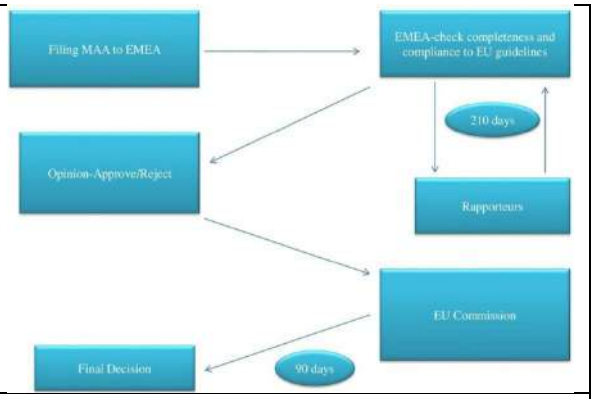

Figure 4: Shows Centralized Procedure.

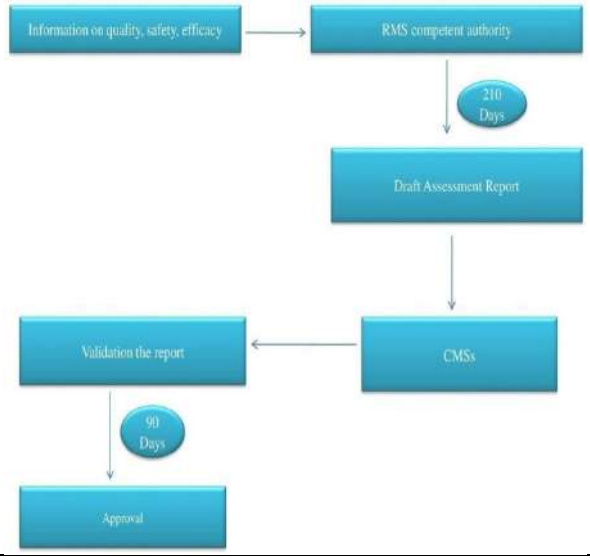

Figure 5: Decentralized Procedure.

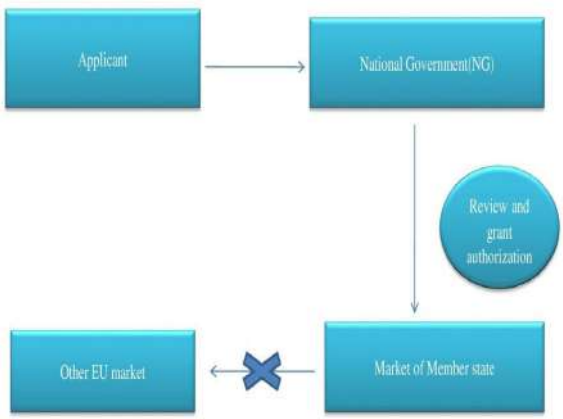

Figure 6: National Procedure.

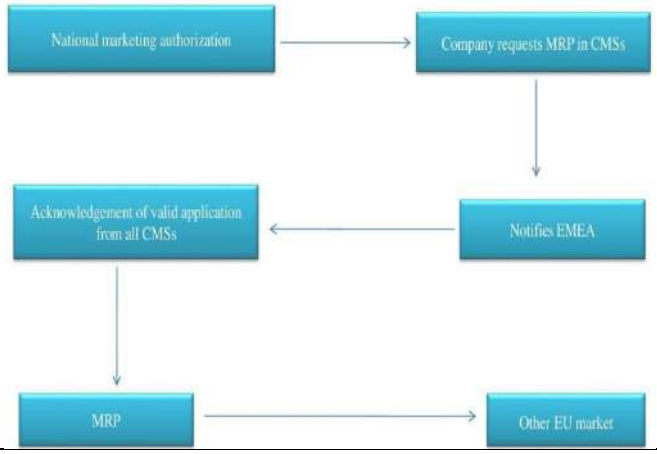

Figure 7: Mutual Recognition Procedure (MRP).

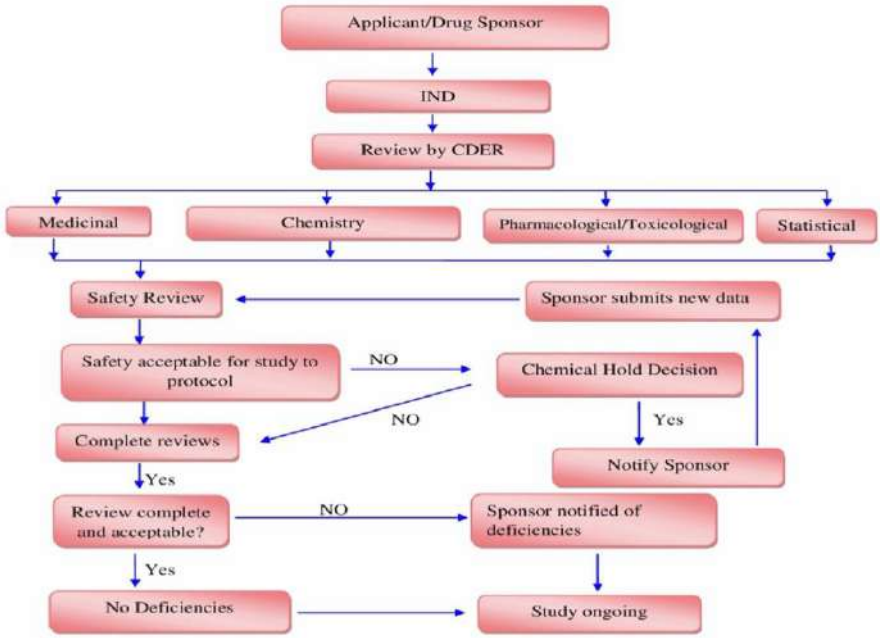

Figure 8: Investigational New Drug Application.

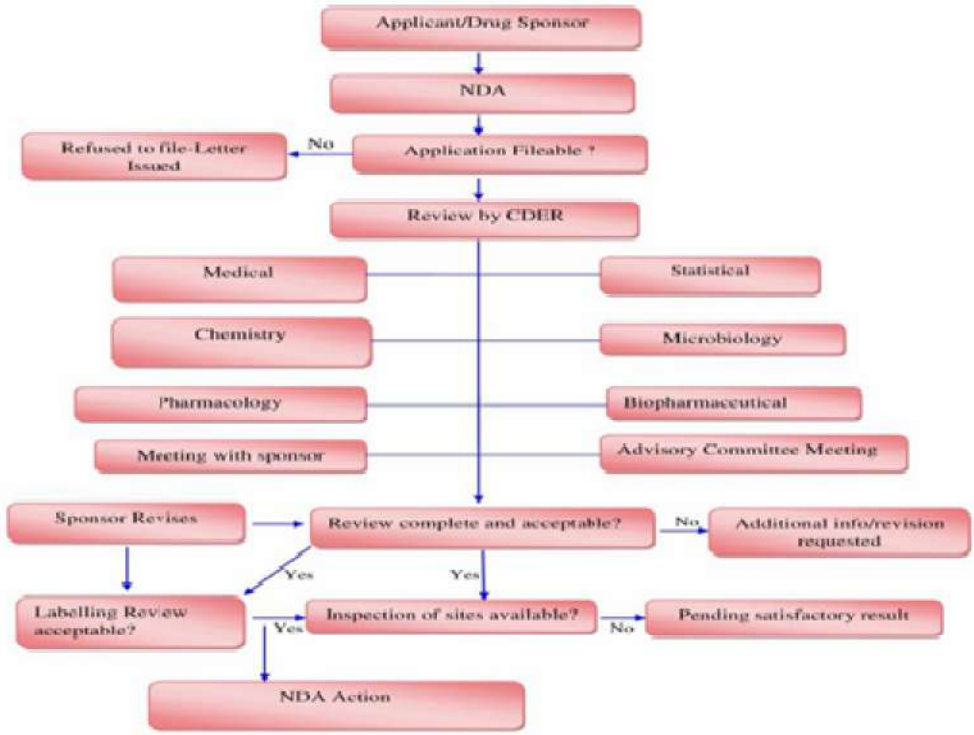

Figure 9: New Drug Application.

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RESEARCH ARTICLE

Modified Spatial Attention Generative Adversarial Networks for Cloud Removal in Optical Remote Sensing Images

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ABSTRACT

Optical remote sensing images are used in wide range of applications like in natural resource management, hazard assessment, disaster management, ocean and atmospheric related applications, etc. However, clouds pose a major challenge by blocking sunlight to reach ground and the reflected energy to be recaptured by sensors leading to complete loss of useful information. Hence, cloud removal is an important pre-processing task in remote sensing images. In this work, we modified Spatial Attention Generative Adversarial Network (SpA GAN) by adding pre and post processing layers for removing thin clouds/haze and thick clouds/cloud shadows in remote sensing images. The accuracy of our model is calculated using standard performance parameters like PSNR, SSIM and MSE. We observe that the best working model, when applied on 50% cloud covered satellite image, removes cloud and regenerates image with PSNR value 32.440 dB, SSIM as 0.921 dB and MSE of 0.0019. Our work is useful in many aspects especially to policy makers to efficiently plan their efforts in reaching to the masses.

Keywords: Remote-sensing, Cloud-removal, Neural-networks, Deep-learning, Satellite-images.

INTRODUCTION

Remote sensing is the science and art of acquiring information about an object, area, or phenomenon by detecting and recording its reflected or emitted energy using remote sensors that are not in physical contact with the object under investigation. It is being used for a wide range of applications, such as natural resource management and planning, agriculture, forest monitoring, weather forecasting, disaster management support

etc. Due to its high-resolution, stable geometric properties, multispectral capabilities ease and expertise in data analysis, passive or commonly known as optical remote sensing is the most commonly used technology for satellite imagery. However, there are several challenges faced in optical remote sensing, mainly owing to the earth's atmosphere which often lead to partial or even complete loss of information. One such major challenge is presence of cloud. Clouds block certain portions of the sunlight in reaching different features present on ground. They also block the reflected energy from the ground surface in reaching to the satellite sensors, hence drastically affecting the quality of imagery produced. This is most common and inevitable occurring phenomenon. As images generated from active remote sensing, commonly known as microwave (cloud-penetrable) are quite difficult to interpret. Thus optical remote sensing still remains the most used and preferred choice and cloud removal becomes an indispensable pre-processing step for further analysis. Generally, clouds can be categorised as: a) Thin Clouds / Haze, where ground features are captured and only a part of information is lost. b) Thick Clouds, where ground information is completely lost under the clouds c) Cloud Shadows where sunlight is blocked by thick clouds forming shadows. These are depicted in figure 1. Removing thin cloud is a single-image problem as hidden ground truth information can be recovered using a single image. However, removing thick clouds is a condition-limited problem, solving which often requires multi-temporal images. While it often becomes infeasible to wait for better quality images as it may take an uncertain amount of time.

Many traditional image processing techniques such as Fourier transform, wavelet analysis and interpolation techniques are used for image reconstruction. However, they require fair amount of computation and manual work, along with many prerequisites like temporal data, terrain and sensor information etc. This leads to explore new technologies, like artificial intelligence, machine learning and deep learning. With the availability of data to train the models, deep learning algorithms are explored to perform the task of cloud removal from remote sensing images [1]. SpA GAN (Spatial Attention Generative Adversarial Network), proposed by Heng Pan [2] and applied for thin cloud removal is relatively new in this field and further explored in this proposed work. The organization of the paper is as follows; Section 2 briefs about the related works in this area. We present our methodology and modified architecture in Section 3. Section 4 contains about the datasets used while Section 5 presents the experimental work. Conclusion and future scope are presented in Section 6 followed by References.

LITERATURE REVIEW

Presence of cloud is a very prominent and common problem in optical remote sensing. It adversely affects the quality of images resulting in information loss and restricts their applications for earth observation. The detection and removal of various types of clouds from satellite images have always been preprocessing steps for further analysis. A variety of methods and techniques were evolved and applied to address this problem. Many traditional approaches like time series reconstruction methods [3], usage of Filters [4], Fourier analysis [5], Wavelet transform [6] etc. were applied however either they require many prerequisites or have limited applications with dependence on topology, resolutions and terrains. At times, it requires lot of manual intervention and expertise. With the advent of new technologies, Artificial neural networks and its successors, an era of data-centric approach evolved [7], giving a new dimension to tackle this issue. The major breakthrough in this field gave rise to many algorithms and approaches for solving the task of image classification and reconstruction [8, 9]. Conditional adversarial networks were investigated in [10] as a general-purpose solution to image-to-image translation problems. These networks not only learn the mapping from input image to output image, but also learn a loss function to train this mapping. This approach is effective at synthesizing photos from label maps, reconstructing objects from edge maps, and colorizing images.

Generative Adversarial Networks (GANs) are one of the most innovative and useful, proposed recently. They are among the most interesting and popular applications of Deep Learning. GANs works using two different models; the Generator and the Discriminator. It is an unsupervised model for generating new elements from a set of similar

elements [11, 12]. Generative Multi-column Convolutional Neural Network (CNN) [13] uses Spatial Attentive Networks based on the Generative Adversarial Networks (GAN). This model when applied on clouded images, trained to identify and remove the small cloud portions in the satellite images without an explicit cloud mask. It reconstructs the images by replacing the cloud patches with the underlying ground truths in a local-to-global manner. The original GAN approach used unsupervised concept of working. Later, many researchers [10, 14] devised supervised formulations, the "conditional" approaches, in which some prior knowledge was given to the network. CycleGAN [14] architecture is the most worked upon approach. It deals with Image-to-image translation cases in the absence of paired training samples. They have applications in super-resolution, style transfer etc.

Another novel approach is using the concept of image-to-image translation technique that uses Spatial attention Generative Adversarial Network (SpA-GAN) for thin (haze) cloud removal [2]. It comprises a Spatial attentive network as a generator model and a fully connected CNN as a discriminator model. This approach avoids the need of any additional, expensive, spectral source of information such as Synthetic Aperture Radar imagery which is cloud penetrable. The model provides the state-of-the-art results that can be evaluated using SSIM, PSNR and MSE values [15, 16]. An altogether different approach in the field of deep learning was recently explored which does not require any pre-training dataset and the handcrafter prior or the distorted image and randomized neural network trains the model. The author applied basic SpA-GAN model and concluded that it could reconstruct to a fairly good limit in case of haze and thin clouds but did not applied the model for removing thick clouds or cloud shadows. Also, the paper does not say anything about cloud cover percentage which the model removes satisfactorily or the number of iterations or time taken for cloud removal. Thus, it was felt to further explore the SpA-GAN approach and improve the model for its better applicability for image reconstruction.

In this work we propose an modified model and apply it on a large date set to remove different types of clouds; thin clouds/haze and thick clouds/cloud shadows, and analyzed its performance. The output of our work was validated using visual observations and by PSNR, SSIM and MSE evaluation metrics. We also added pre and post processing layers to the model, thus proposing a model which works as an end-to-end solution for cloud removal in Remote Sensing Images. There is always a trade-off between performance and time taken or resources required. Thus, number of iterations for best optimum performance is also analyzed.

METHODOLOGY

We studied many deep learning algorithms like Image Inpainting using Partial Convolutions and Generative Multi-column Convolutional neural network, Conditional GAN, Cyclic GAN, SpAGAN and for cloud removal in remote sensing images. It is observed that SpAGAN approach is more efficient and provide better results and therefore we explored it in our work. Before we discuss our methodology we present the following evaluation parameters in our experiments. Mean squared error (MSE): It measures the quality of an estimator and is always non-negative, values closer to zero are consider as good. Peak Signal-to-noise ratio (PSNR): Higher values of PSNR denotes better quality of the compressed or reconstructed image. Structural similarity (SSIM) : It signifies the symmetry between the output and the original image. The higher the values, better is the reconstructed output image.

General Structure of a GAN

Given generator G and discriminator D, GAN is a minimax problem as shown below:

$$\min_G \max_D V(D,G) = E_{x\sim pdata}(x)[\log(D(x))] + E_{z\sim pz}(z)[\log(1-D(G(z)))]$$

where, x denotes real samples, pdata denotes distribution of x, z is random noise, pz is the data distribution of z. D(x) is the discriminator output that represents the probability of x (for real sample) and G(z) is the output generated by the generator. The basic model of SpAGAN does not provide good results if the cloud density exceeds 30%. It also fails to regenerate ground information beneath thick clouds. Thus, keeping these factors into account we are proposing a new model by making changes in the architecture and adding pre and post processing layers. Changes

in various hyper parameters like optimizers, activation functions, weight initialization functions etc., are also done in the basic model and experimented. We generated a new dataset, containing multi-temporal thick clouded and cloudfree paired images and hosted on the web, for experimental work. We now present the modified and upgraded architecture of our proposed model. The improved model is used to remove thin clouds/haze and thick clouds/cloud shadows and reconstruct satellite image. The performance is judged in terms of more accurate output images generated in less time with minimal manual intervention and resources. Also, additional parameters like cloud cover percentage present in the scene is also calculated. The model consists of additional layers at both the input and output ends of the original model, as shown in the figure 2. The additional input layer performs several preprocessing tasks, namely image cropping, partitioning, format conversion from Tiff to PNG and pixel resolution conversion to 512 x 512. This makes the original model more convenient to use with remote satellite images.

The additional output layer performs tasks as generating the cloudless and attention heat maps separately instead of a stacked image as in original mode, computing the cloud cover percentages in cloudy and ground truth images (if cloud masks are explicitly provided) and computation of PSNR and SSIM values of the cloudless images produced to estimate accuracy of the model. The new model uses Xavier initialization for all Conv2d layers and normal initialization for batchnorm2d layers instead of normal initialization for both the types of layers as used in original model. While the actual no. of epochs to attain the optimal model depends on the chosen PSNR and SSIM thresholds to stop the training process, on an emergency call where these thresholds can be slightly compromised to yield a model with less training time, the improved model has been observed to perform much faster than the original model. with an average PSNR of 30 dB and SSIM of 0.90 dB in only around 73 epochs as compared to around 200 epochs of the original model. Also, with equal number of epochs (200), improved model provides much better output in terms of PSNR and SSIM as compared to the original model. For thick cloud/cloud shadow removal, the improved model was trained with a self-prepared multi-temporal dataset of the area of interest and was observed more efficient and accurate.

DATASET

Datasets from Landsat satellite covering Northeast India forest regions, acquired during the Monsoon season to have maximum cloud cover, and are obtain from “Remote sensing Image Cloud rEmoving (RICE)”, an open source dataset for cloud removal, for experiments [17]. The RICE dataset contains two sub sets containing images of thin and thick clouds respectively, called RICE1 and RICE2. The forests of Northeast India are considered as regions of interest with following details:

1. Molai Forest, Kokilamukh, Assam (26°51′0″N 94°9′8″E)
2. Mawphlang Sacred Grove, Nongrum, Meghalaya (25°28′N 91°46′E)
3. Dibang Forest, Roing, Arunachal Pradesh (29.047°N 95.79°E)

The various resolutions of Landsat 8 OLI/TIRS Collection 2 Level-1 dataset as follows: Spatial - 30mRadiometric - 8 bits Temporal - 16 days. For all images (in PNG) constituting training and testing datasets following formats are considered: Bands: RGB (Bands B2- Blue, B3- Green, B4- Red). Bit Depth: 24 bits (8 bits/channel) Spatial resolution- 30m (full original resolution). RICE1 contains 500 sample pairs of thin cloud and their corresponding cloudless images under 512 x 512 resolutions. It is composed from Google Earth. The cloudy/cloudless paired images are obtained by setting the layer visibility to yes/no respectively with varied values of layer transparency percentage for different images. However, for thick cloud removal is a condition-limited problem and therefore it requires multi-temporal images of the testing region. Not many images, which are required to train the SpA GAN model for thick cloud/cloud shadow removal, are present as an open source data set. To solve this issue for and for good experimental work, prepared a multi-temporal data set, consisting of 150 sample pairs of thick cloud/cloud shadow and their corresponding cloudless images fewer than 512 x 512 resolutions.

Testing dataset is obtained from USGS Landsat 8 OLI/TIRS Collection 2 Level-1 dataset on Earth Explorer. Ground truth dataset for evaluating test performance is obtained from multitemporal Landsat 8 OLI/TIRS Collection 2 Level-1 dataset for corresponding testing dataset within 30 days with cloud cover between 0 to 5%.

EXPERIMENTAL WORK AND RESULTS

The training and testing of various models and architecture of SpAGAN was written in Python (Version 3.6.9) and PyTorch deep learning library on Google Colaboratory using GPU during runtime.

Training

We consider 500 thin cloud images to train the model for thin cloud/haze removal and 150 thick cloud images to train model for thick cloud/cloud shadow removal separately. For validation, 20%of the images from each set were used. Hence, the model was trained on 400 images and validated on 100 images for thin cloud removal. Similarly, it was trained on 120 images and validated on 30 images for thick cloud removal. As the model requires long training time of around 7 minutes per epoch for 736 training images, all modifications are first tested on a smaller training dataset of 56 images for 11 epochs (as it is considered optimal epoch value for most neural networks) and then tested on the overall training dataset if any improvements are found with respect to the PSNR and SSIM values. The training is terminated earlier than the pre-specified number of epochs to restrain the model from over fitting using 'Early Stopping' function in Py Torch with 'PSNR' and 'SSIM' as monitoring parameters. The batch size for both training and validation sets was set to 1 and all models were trained for 100 epochs to determine the best set of optimizer and activation function. During the training phase, the SpA GAN model generates an attention map which guides the cloud removal process in the subsequent residual blocks. The map is also used to calculate the loss which contributes in the training process of the model. The bit depth of all images used in this work is 8 bits/channel results in the maximum pixel value to 255. As we know, for 8-bit images, the PSNR value lies in the range of 20-40, hence, a PSNR value ~ 30 dB and SSIM value ~ 0.95 is taken as the threshold for the training process. The best model is chosen and further trained upto the epoch where PSNR value of ~ 30 dB and SSIM value of ~ 0.95 is obtained for both thin and thick cloud removal.

Testing

The cloudy and ground truth test images of the Northeast India forest regions were first transformed to the same extent and partitioned to several 512 x 512 pixel resolution images. Then images with thin and thick clouds were fed to the trained models for thin and thick cloud removal respectively. The generated cloudfree images were then compared with corresponding ground truth images, visually as well as using evaluation parameters PSNR, SSIM and MSE [15, 16], to evaluate the model's performance.

Modifications in the hyper-parameters

Following parameters are modified in the basic model [2] and performance is evaluated.

- Numbers of Convolutional layers are changed. Initially from 4 layers, these layers are respectively increased to 5, 6, 7 and 8 and performance is analyzed. Then it was to 3 and analyzed. In both the cases, it was observed that by using 4 layers, best results are obtained.
- *Relu, Leaky ReLU & Sigmoid* Activation functions in the discriminator layer are used and evaluated for best performance, as shown in Table 1.
- *Adam, SGD & RMSprop* Loss optimizers are used.
- *Normal, He, Xavier & Constant* Weight initialization techniques are evaluated for both *Conv2d* and *Batchnorm2d* layers.
- Learning rate for *Adam* optimizer is 0.0004 and 0.001 for SGD and RMSprop optimizers.

We have trained, implemented and evaluated nine SpA GAN models by modifying different parameters in the discriminator layer and applied on thin and thick clouds and analysed them.

We observe that Model 2 with leaky *ReLU* activation function in discriminator and *Adam* optimizer gave the best performance metrics for the validation data sets at the end of 100 epochs, when applied on thin and thick cloud removal. Now, this Model 2 is used for testing purpose. It is further trained till the PSNR value of ~ 30 dB and SSIM value of ~ 0.95 is obtained for both thin and thick cloud removal. The epoch value for thin cloud is 202 and for thick cloud is 203, approximate training time taken is 17 hours and 5 hours respectively.

For epoch value = 202 (thin cloud),
===> Avg. MSE: **0.0015**
===> Avg. PSNR: **31.267 dB**
===> Avg. SSIM: **0.973 dB**

For epoch value = 203 (thick cloud), outputs are:
===> Avg. MSE: **0.0019**
===> Avg. PSNR: **30.813 dB**
===> Avg. SSIM: **0.945 dB**

Output Cloudless Image Visualization

The selected model (Model 2) was first tested on the entire Landsat scenes re-sampled to 512 x 512 resolution and spatial resolution- 450 x 460 m. In the below figures (from figure 3 to 12), the output stack contains three images; Cloudy Image, generated Cloudfree image and the Attention Heatmap, from left to right respectively. The fourth image is the ground truth image for evaluating the generated cloud free image. Thick Clouds are in figure 3, 4 and 5.

Observations

SpA GAN is able to remove clouds from the images, but unable to restore the ground information at places which are completely lost under the thick clouds. This may have also resulted due to less training of the model owing to less availability of thick clouded satellite images. Thus, we have made our own dataset, containing multi-temporal thick clouded and cloudfree paired satellite images, and trained the model with this dataset also. This has resulted in improving the PSNR and SSIM values of the output cloudfree images. Also, to address boundary distortion problem in output images, the model was then tested on clipped Landsat images (partitioned using 'BuildVRT' function of GDAL library in Python, with spatial resolution- 30 x 30 m and pixel resolution- 512 x 512) extracted from the above scenes. It is observed that the problem is solved using this method. The model was also tested on different cloud cover percentage. Thin Clouds are presented in figure 6, 7 and 8.

CONCLUSIONS AND FUTURE SCOPE

We studied nine different SpA GAN models with different set of activation function and optimizer in the discriminator layer were applied to remove thin cloud/Haze, thick clouds/shadow clouds in remote sensing images. The activation functions used were ReLU, Leaky ReLU, Sigmoid and optimizers used were Adam, SGD and rmsprop. The model with 'Leaky ReLU' activation and 'Adam' optimizer was observed with the better results for both thin and thick cloud removal. Several weight initialization techniques, such as He, Xavier, Normal and Constant were applied on the model's Conv2d and batchnorm2d functions. The model with 'Xavier initialisation' for Conv2d weights and 'normal initialisation' for Batchnorm2d weights was observed with the better results. A threshold value of 30 dB for PSNR and 95 dB for SSIM was used as terminating condition for the training process. Finally, the model was tested on the Landsat Northeast India forest datasets and the results were analyzed both visually and using the performance metrics to determine the image quality. Post-processing layer produces the cloudfree image output and automates the computation of PSNR and SSIM performance metrics. While we used the RICE dataset to train the model for thin cloud/haze removal, a self-prepared dataset with multi-temporal images of the interested area was used to train the model for thick clouds/cloud shadows removal. It was observed that self prepared dataset produces much better results than RICE dataset for thick cloud removal.

For images with thin clouds/haze, it is observed that the modified SpAGAN model is able to remove the clouds to a very good extent by visual interpretation and comparison with corresponding ground truth images. The best observed values are: PSNR of 32.440 dB, SSIM of 92.10 dB with MSE of 0.0019, for cloud cover range of 5% to 59.93%. The model's performance degrades for cloud cover range from 60% to 100%. Below 5% cloud percentage, the model changes other aspects of image, such as brightness and contrast.

For images with thick clouds/cloud shadows, it is observed that the model is able to restore the lost ground information upto a manageable extent by visual interpretation and comparison with corresponding ground truth images. The observed PSNR values are with an average PSNR of 29.585 dB and SSIM of 77.662 dB, for cloud cover range from 10% to 34.89%. The model's performance degrades for cloud cover range from 35% to 100%. Below 10% cloud percentage, the model changes other aspects of image, such as brightness and contrast.

The future scope of this work may include experimenting with the number of layers in the model architecture for further improvement. For insufficient data to train model on thin cloud removal, the open-source RICE dataset was used in our work. However, the future scope may include training model on self-prepared multi-temporal image datasets for thin cloud removal as well, which is expected to give even better results.

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Table 1: Models for thin clouds/haze and thick clouds/cloud shadows removal

Model	Activation	Optimizer	Avg. PSNR	Avg. SSIM	Avg. MSE	Avg. PSNR	Avg. SSIM	Avg. MSE
			Thin Cloud			Thick Cloud		
Model_1	ReLU	adam	24.8160	0.9123	0.0058	23.2544	0.8102	0.0061
Model_2	leaky ReLU		25.9711	0.9438	0.0054	24.2679	0.8317	0.0042
Model_3	sigmoid		18.3201	0.7612	0.0113	19.5672	0.8304	0.0079
Model_4	ReLU	sgd	20.7610	0.8203	0.0071	19.4361	0.7971	0.0090
Model_5	leaky ReLU		22.5471	0.8145	0.0061	19.0217	0.7745	0.0102
Model_6	sigmoid		20.4308	0.8123	0.0089	18.3201	0.7612	0.0111
Model_7	ReLU	rmsprop	18.2301	0.7634	0.0121	20.7610	0.8203	0.0077
Model_8	leaky ReLU		20.7832	0.8217	0.0078	22.5471	0.8145	0.0067
Model_9	sigmoid		22.7156	0.8177	0.0068	20.4308	0.8123	0.0088

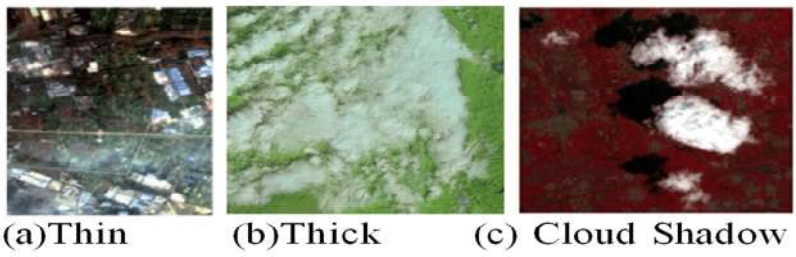

Figure 1: Different Cloud categories

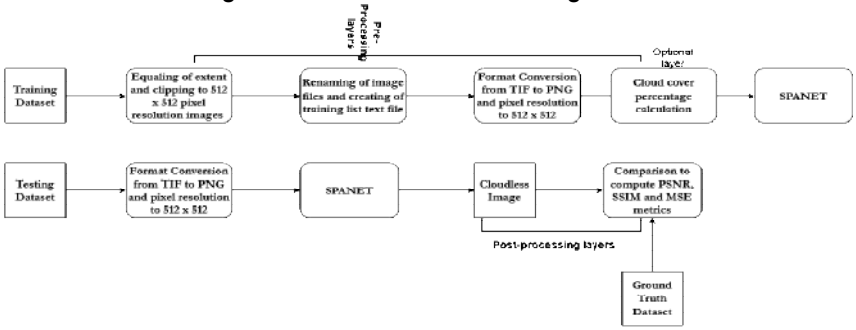

Figure 2: Proposed SpAGAN model

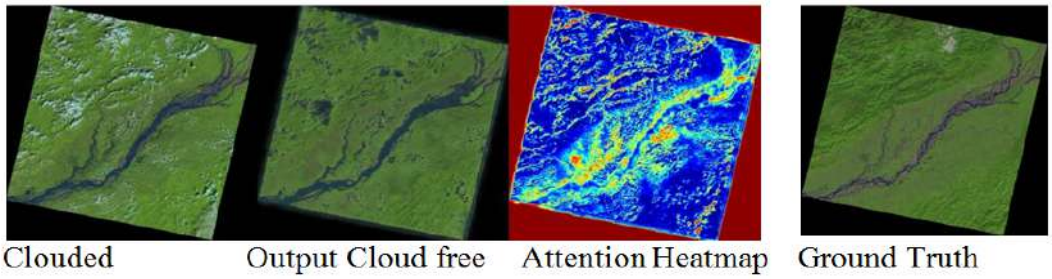

Figure 3: Molai Forest, Kokilamukh, Assam (Dt: 10 Oct, 2020; Cloud Cover: 14.62%) with PSNR: 29.377, SSIM: 0.758

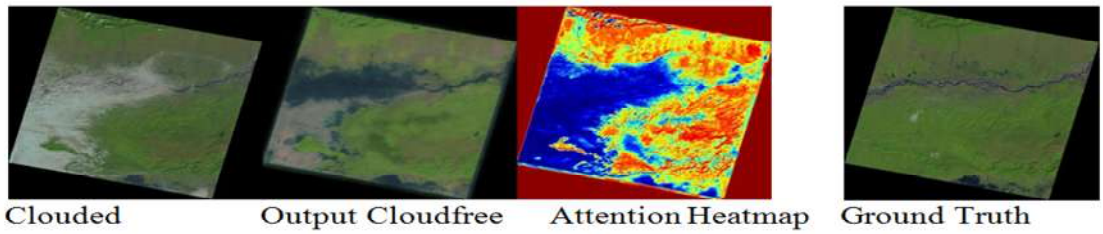

Figure 4: Mawphlang Sacred Grove, Nongrum, Meghalaya (Date: 11 Dec, 2020; Cloud Cover: 26.16%) with PSNR: 28.350, SSIM: 0.660

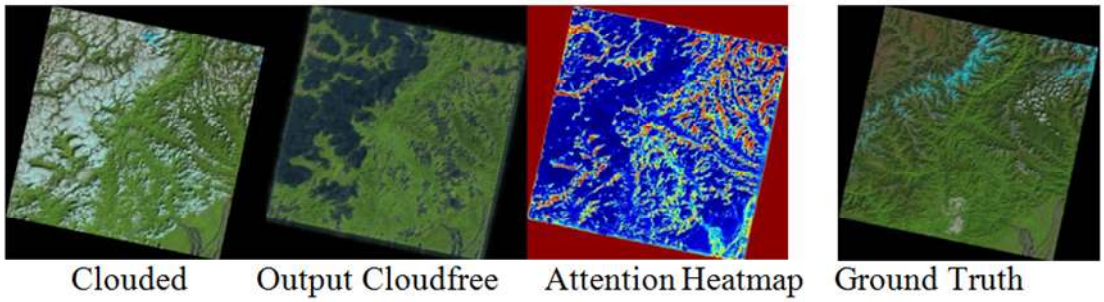

Figure 5: Dibang Forest, Dibang Valley, Roing, Arunachal Pradesh (Date: 10 October, 2020; Scene Cloud Cover: 42.03%) with PSNR: 27.301, SSIM: 0.637

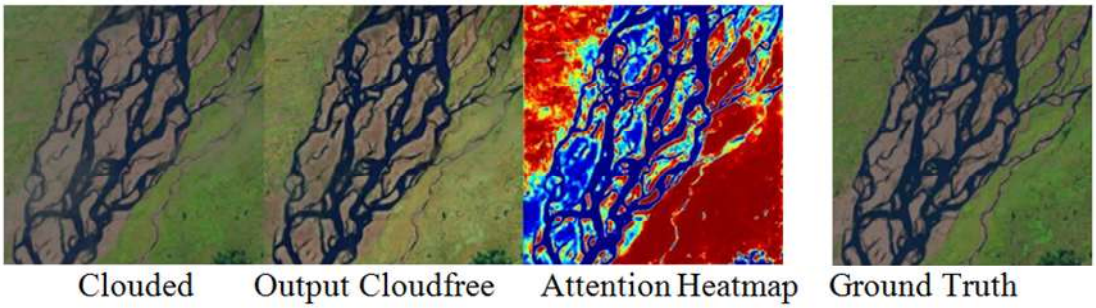

Figure 6: Molai Forest, Kokilamukh, Assam (Date: 27 Nov, 2020); Cloud Cover: 14.62%) with PSNR: 31.574 dB, SSIM: 0.884 dB

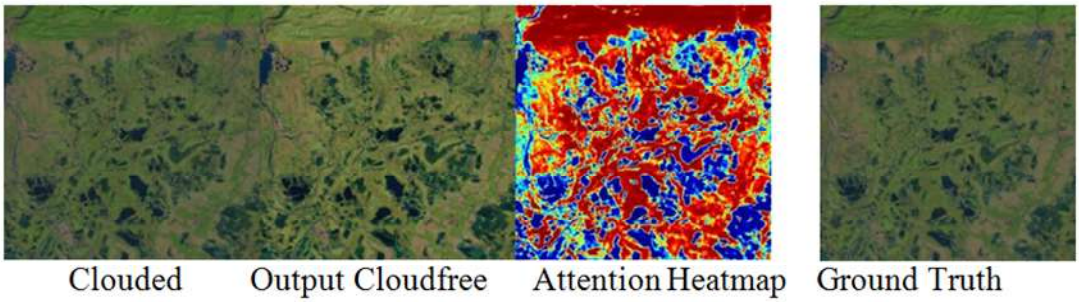

Figure 7: Mawphlang Sacred Grove, Meghalaya (Date: 4 Dec, 2020; Cloud Cover: 10.12%) PSNR: 29.438 dB, SSIM: 0.806 dB

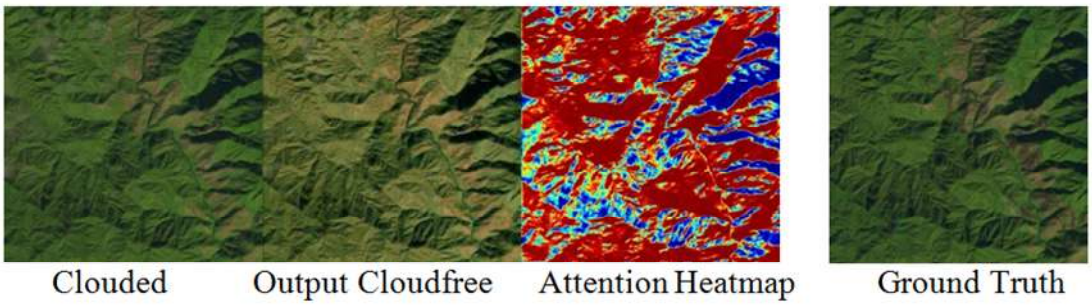

Figure 8: Dibang Forest, Roing, Arunachal Pradesh (Dt: 1 Dec, 2018; Cloud Cover: 8.29%) with PSNR: 30.347 dB, SSIM: 0.856 dB

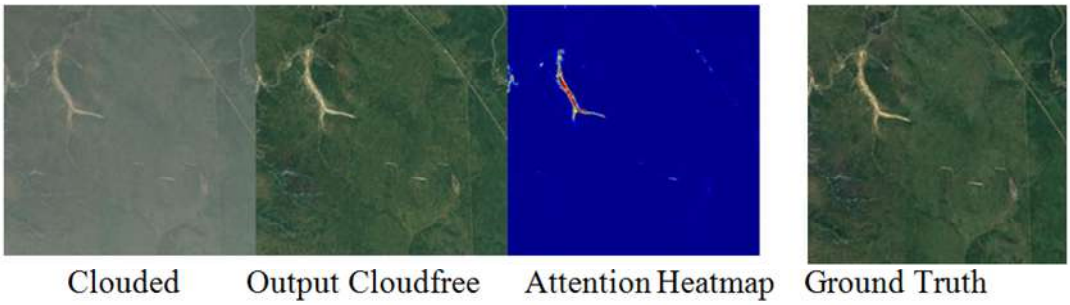

Figure 9: Dibang Forest, Roing, Arunachal Pradesh (Image from Google Earth for Dec, 2018; Cloud Cover: 50%) with PSNR: 32.440 dB, SSIM: 0.921 dB.

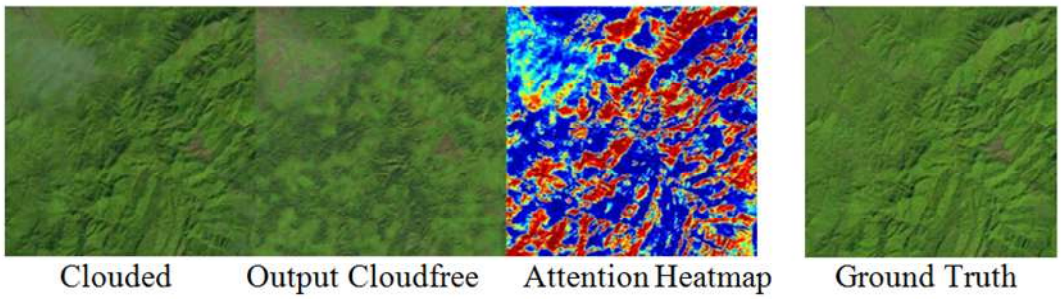

Figure 10: Molai Forest, Kokilamukh, Assam; Cloud Cover: 15%) with PSNR: 28.739dB, SSIM: 0.518dB

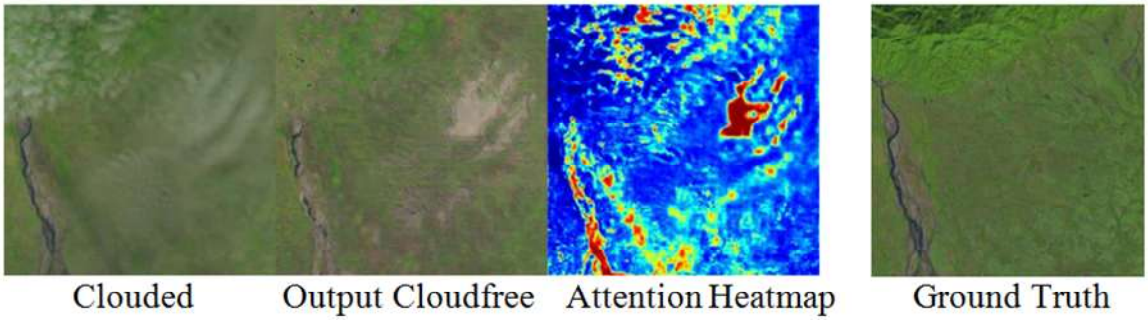

Figure 11: Mawphlang Sacred Grove, Meghalaya (Cloud Cover: 10%) PSNR: 28.680dB, SSIM: 0.546dB

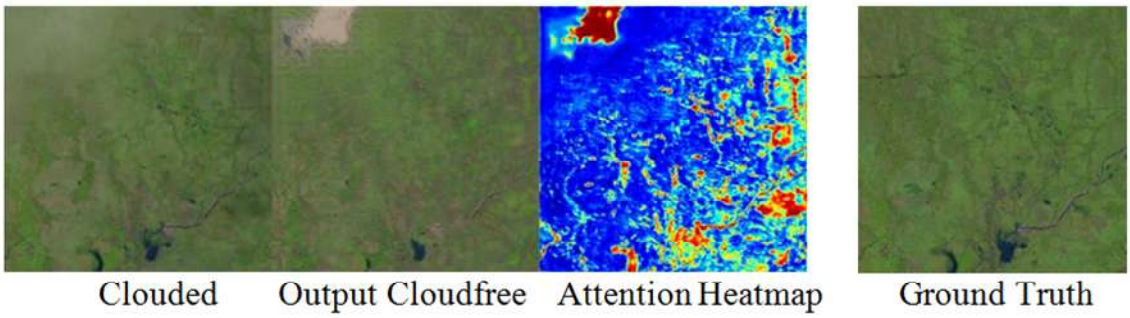

Figure 12: Dibang Forest, Roing, Arunachal Pradesh (Cloud Cover: 8%) with PSNR: 29.600dB, SSIM: 0.663dB

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RESEARCH ARTICLE

Classification of Covid-19 using Deep Neural Network

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ABSTRACT

Covid-19 pandemic is cause by corona virus. Detection of covid-19 from patient infected with pneumonia is crucial as steps must be taken for treatment. In this paper Covid-19 chest X-ray images has been collected from Kaggle, and Convolution Neural Network (CNN) is used for the classification of the dataset as Covid-19 or not Covid-19. The CNN models used are Convolution Neural Network sequential (CNN1) and Convolution Neural Network parallel (CNN2). The dataset is pre-processed, and CNN model is applied on the original and pre-processed dataset. The experimental result of the models on two datasets, shows that CNN1 model have better classification accuracy on original dataset with sigmoid activation function on the classification layer.

Keywords: Convolutional neural network, classification, covid-19, sigmoid, softmax

INTRODUCTION

COVID-19 is a transmissible disease caused by corona virus [1]. It is an illness that causes mild to severe breathing problems and the symptoms of the disease are dry cough, fever, muscle aches, and fatigue [2]. If the virus enters the respiratory track, it causes inflammation that leads to the difficulty to take oxygen and release carbon dioxide. Almost 9 million cases of COVID-19 have now been reported in India, and almost 133,773deaths [18]. People who are at risk include aged people and people having medical problems like heart disease, diabetics, chronic respiratory disease and cancer. Early diagnosis is the only way to save life before the lungs are affected and damaged. Lot of research is going on to find the best possible way to diagnose and treat the disease. The infection rate of a patient can be determine by taking the CT (Computed Tomography) scan image of the lungs and radiologist observe that its sensitivity reaches up to 97% [3]. For middle age patient radiologists suggest that lungs X-ray can be used to identify high risk patient which is cheaper and easy to disinfect as compared to CT scan [3].

Recently many scientific publications have emerged on classification of Covid-19 from X-ray image using different classifier. The most used classification techniques include deep learning and SVM. In [6] the author proposed a model COVIDX-Net for the diagnosis of COVID-19 from X-ray images applying seven CNN models. The chest X-ray taken from the COVID-19 patient has low sensitivity. Overlapping of the lungs with other diseases makes identification of the disease by the radiologist very complicated and challenging. To overcome this challenges computer aided detection of the disease is a need for automated detection to help physician to diagnose the disease faster and determine high risk patient to give proper treatment in less time to fight against the disease.

In this paper CNN models, CNN sequential (CNN1) and parallel (CNN2) architecture are used on Covid-19 chest image dataset for classifying into Covid-19 and no Covid-19. The dataset is collected from [19], and it consists of total 346 dataset of chest X-ray and CT images of Covid-19 infected and suspected with pneumonias patients. The image dataset is pre-processed by rotating horizontal and vertical, and rotating an angle of 90 degree, 180 degree and 270 degree. The dataset with the process image has dataset in total 2146. CNN1 and CNN2 models will be applied on the 346 original dataset and 2146 pre-processed dataset and the performance of the classifier will be evaluated using accuracy and ROC (receiver operator characteristic curve) and AUC (area under the curve).

Review of the Related Work

In [5] the author introduced a model using deep convolution neural network COVID-Net, for COVID-19 detection from chest X-ray image dataset. It is believed that COVID-Net is the first automated detection developed from CXR, when CXR is released the first time. The neural network architecture, COVID-Net network architecture is used for predicting a) no infection (normal), b) non-COVID19 infection (e.g., viral, bacterial, etc.), and c) COVID-19 viral infection. The performance of COVID-Net achieved higher test accuracy than the VGG-19 and ResNet-50 network architectures. In [7] the authors collected COVID 19 X-ray datasets from websites and publications for better understanding of the dynamics and treatment. This data has been created for reducing the death rate of COVID 19, and the dataset can be used for prediction using deep neural network. In [8] the authors develop a model COVID-CAPS by using Capsule Networks. The model is applied on small Chest X-ray image dataset of Covid-19 for the diagnosis of the disease. It is found that COVID-CAPS performance is better as compared to CNN models. The performance is evaluated in terms of accuracy, sensitivity, specificity and AUC.

In [9] the authors proposed a deep domain adaption model called COVID-DA for COVID-19 diagnosis. This new model diagnoses COVID-19 from few annotated data. Experimental results show that effectiveness of COVID-DA and its great potential for real world applications.In [11] the authors proposed a deep learning model DeTraC which help detect the irregularities present in the Chest X-ray image dataset for COVID-19. The performance of the model on classifying Covid-19 image dataset shows an accuracy of 95.12% with sensitivity of 97.91%, and a specificity of 91.87% from normal, and severe acute respiratory syndrome cases. In [12] the authors proposed a deep convolutional neural network (DCNN) based on inception V3 and the model is tested and train on dataset containing 864 COVID-19, 1345 viral pneumonia and 1341 normal. The model accuracy percentage is 96%.

In [13] the authors proposed a deep learning mode DarkCovidNet and is experimented on patients having pneumonia and found that the diagnosis is true whereas the response is relatively low for multiclass prediction. The model was train and tested on a dataset containing 3550 X-ray images of non-covid, covid-19 and viral pneumonia. In [16] the author used SVM and deep learning methodology resnet50 model and observe that the accuracy of the model is better as compared to four different models. In [15] the authors used CNN models: ResNet50, InceptionV3 and InceptionResNetV2 for COVID-19 pneumonia detection from Chest X-ray radiology. The experimental results of the three models on the X-ray image datasets shows, the accuracy of 98% for ResNet50 which is higher as compared to the other two models.

System Approach

ANN is a model created that work in a similar manner as per the minimal perception of the learning system of human brain. Similarly, to the human brain ANN learns from the datasets and makes decision. A simple architecture of ANN has three layers: an input, a hidden and an output layer. Each node in each layer is interconnected and associated with a weight as shown in Figure. Different neural network architecture of ANN is created by adding more than one hidden layer. This neural network architecture is called Deep neural network which begins deep learning. A deep neural network model with two hidden layers is shown in figure 2. ANN has perform amazing benefit in disease diagnosis especially breast cancer [17,20,21,22,23,24]. The network architecture of CNN is like the standard neural network. The network has an input layer, a fully connected output layer and in between has a stack of convolution layer and pooling layer. For the inner layer ReLU (Rectifier Linear Activation) activation function and for the output layer sigmoid or softmax activation functions are used. The input in the input layers is a matrix of pixel values; the output to the input layers is features which serve as an input to the convolution layers. The last convolution layer output is flatten and is given as an input to the output layer for classification. Figure 3 shows a CNN with two Convolution layer two pooling layer and two fully connected layers.

In our approach CNN sequential and parallel model are used for the classification of COVID 19 X-ray datasets as Covid-19 or no Covid-19. The X-ray image datasets is pre-processed by rotating horizontal and vertical, and rotating an angle of 90 degree, 180 degree and 270 degree. Two models each of sequential and parallel are created and tested on two datasets: the first dataset is the original datasets, and the second datasets is the pre-processed datasets. The network model is train using learning rate 0f 0.0001. The complete flow chart of the model is shown in figure 4.

Analysis of COVID-19 X-ray Datasets

Covid-19 chest X-ray images dataset is collected from [19]. The image dataset consists of 361 X-ray images of which 285 images are Covid-19 and 76 images are no Covid-19. Pre-processing of the dataset is performed by first transforming the images into grey scale, in the second step the image is rotated at an angle of 90-degree, 180 degree and 270 degree. The image is also rotated horizontal and vertical. The total images after pre-processing consists of 2146 dataset of which 1690 are Covid-19 and 456 are no Covid-19. The pre-processed dataset is named dataset A, and the original dataset is named dataset B. From the dataset A, 1431 dataset are taken for training and715 datasets are kept for testing. From the dataset B, 240 datasets are used for training and 121 datasets are kept for testing.

Two classifier models CNN1 and CNN2 are created on the dataset A and B. CNN1 is a sequential CNN model and CNN2 is a parallel CNN model. CNN1 and CNN2 models consist of an input layer, four convolution layers, ReLU activation function for the inner layer. The output layer is a fully connected feed forward neural network. The learning rate used is 0.0001 and the models performances are observed by taking sigmoid activation function and softmax activation function in the output layer. The two models are experimented on the two datasets A and B and the classifiers performance is evaluated using accuracy and roc curve. The average classification accuracy of the two classifiers CNN1 and CNN2 on the two datasets A and B, for different input shape 128X128 and 250X250 with activation function sigmoid and softmax for the classification layer is shown in the table 1. Figure 5,6,7,8 shows the accuracy graph for the two models for different activation function on the outer layer for datasets A and B.

CONCLUSION

In this paper we perform an analysis of Covid-19 chest X-ray image datasets using CNN models CNN1 and CNN2. The dataset is pre-processed and the models are train and tested on the processed datasets. The experimental results shows that the classification performance of CNN1 on dataset A for sigmoid activation is 90% with ROC and AUC 0.95 which is better as compared to CNN1 on dataset B, CNN2 on dataset A and CNN2 on dataset B. It can be also be

seen from figure 1,2,3,4 that on an average the classifier performance of CNN1 and CNN2 is better on the original dataset B as compared to the pre-processed dataset A.

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Table I: Classification accuracy of Cnn1 and cnn2 on datasets a and b

CNN Model	Input Image size	Accuracy using Sigmoid Activation function	ROC	Accuracy using Softmax Activation function	ROC
CNN1 on Dataset A	128X128	74.46%	67.23%	74.46%	77.51%
CNN1 on Dataset B	128X128	90.00%	95.44%	81.43%	86.32%
CNN1 on Dataset A	250X250	77.59%	80.00%	76.39%	79.44%
CNN1 on Dataset B	250X250	82.86%	89.51%	82.86%	87.00%
CNN2 on Dataset A	128X128	77.83%	82.23%	77.83%	82.23%
CNN2 on Dataset B	128X128	82.86%	89.51%	84.86%	89.55%
CNN2 on Dataset A	250X250	78.31%	81.67%	78.31%	83.42%
CNN2 on Dataset B	250X250	84.29%	90.00%	84.29%	90.00%

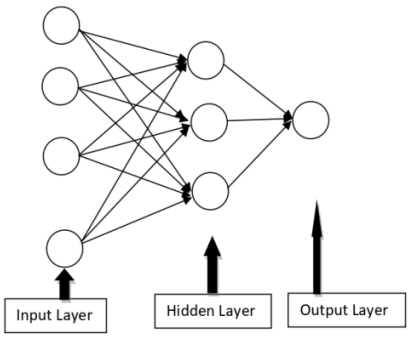

Fig. 1 Neural Network

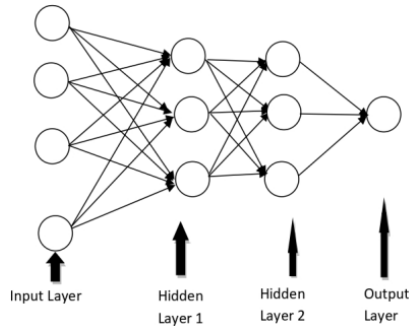

Fig. 2 Neural Network

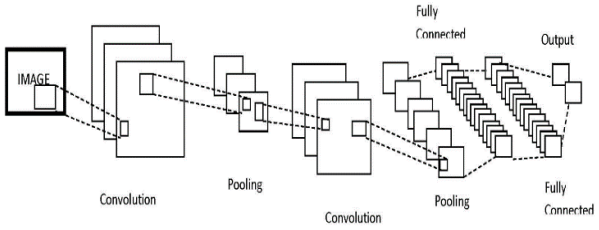

Fig 3. CNN

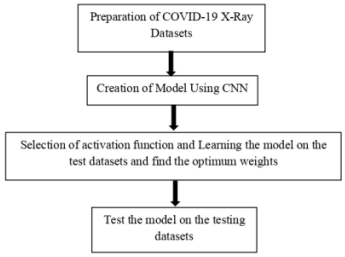

Fig 4. Prototype model of CNN

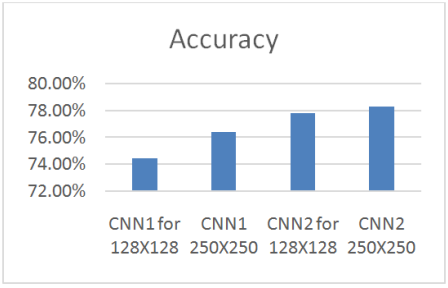

Fig 5. Accuracy of the two models on dataset A for sigmoid activation function.

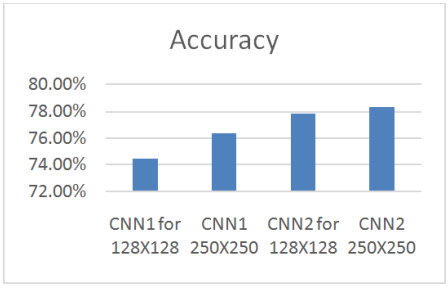

Fig 6. Accuracy of the two models on dataset A for softmax activation function.

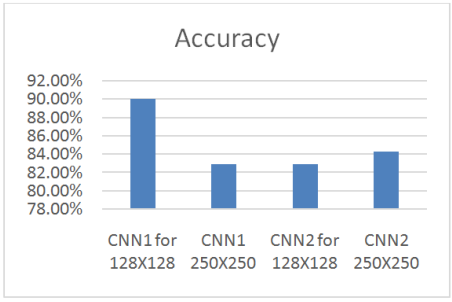

Fig 7. Accuracy of the two models on dataset B for sigmoid activation function.

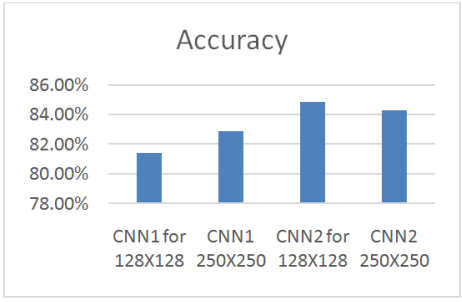

Fig 8. Accuracy of the two model on dataset B for softmax activation function.

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RESEARCH ARTICLE

Design, Development and Evaluation of a Model System for Teaching and Learning Mechatronics

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ABSTRACT

The application of a model system in instructional design through instructional technology training enhances learning. The purpose of this paper was to design, developed, and evaluate a trainer that resembles real-world experience that creates a correlational or contextual groupings of attributes through a model technology. The designed model showed that it is possible to reduce cost while maintaining the balance between quality and usefulness of technology. Experts and practitioners rated the developed technology as highly valid and did not vary much from the real-world models. Although, the model produced a good evaluation results, it requires in-deep analysis and further testing in order to strengthen both implied and actual performance.

Keywords: low cost trainer, instructional technology, instructional design, model system, conceptual learning .

INTRODUCTION

With the rise of technology and the prevalent increase of its benefits to society, educational systems are investing large amounts of money into instructional technology. Global innovation index 2021 report showed that Philippines ranks 51st overall, which indicates that the country is caching up. However, several indicators where the country need to further improve in order to move to upper middle-income group. Lower middle-income group of economies where Philippines is a part of, still struggle to compete in the race of developing cutting edge technology (WIPO, 2021). For instance, secondary education classrooms in the Philippines have limited number of computer laboratories, let alone technologies to enhance instructional methods (Barrot, Llenares, & Del Rosario, 2021; Orleans, 2007). Technology designed for instructional setting are limited due to infrastructure due to inadequate financial

support and national budget constraints (Dotong, et al., 2016).However, in the case of Higher Education Institutions (HEIs) both public and private presentsa different scenario (Birdsall, 1996). Government allocates funds for public HEIs based on the four-fold functions: research, instruction, extension and production. These funds are given under the general appropriation for public HEIs including those HEIs with mandates of providing higher vocational, professional and technological instruction and training in the fields of agriculture, fisheries, engineering and sciences, as well as short-term technical courses. One of the HEIs with the same mandate to deliver the four-fold function of research, instruction, extension and production is Surigao State College of Technology (SSCT) created under Republic Act number 8650.

Although SSCT can use general appropriation funds as leverage of either purchasing or developing their own technology but cost and functionalities must be put into consideration. One framework to consider in developing instructional technology is the concept of appropriate technology (Basu & Weil, 1998). Moreover, there is another roadblock towards developing such technology, the extravagant price of the equipment and the limited to almost zero availability of equipment as a training mechanism (Coffman, 2002). While considering the complexity of operations and the fundamentals of a physical systems, synergistic integration adds purpose of improving technology for instructional design (Harashima, Tomizuka, & Fukada, 1996). Thus, a need to balance all the factors involved in developing instructional technology.

Short term courses in technology, vocational training and even fundamentals of engineering design benefits most from a hands-on instructional technology training (Regan, & Sheppard, 1996), opportunities for social interaction (Ekmekci, & Gulacar, 2015), effective means for illustrating (Clarkson & Shipton, 2015), take greater advantage of the visual simulation power, while simultaneously observing the external functionality (Regan & Sheppard, 1996). Thus allows students to better understand and explain the design process embedded in the technology. Development of instructional technology for teaching in the area of mechatronics and automation highlights the importance of balancing cost to functionalities. Galita and Asuncion (2012) initiated a strategy in order to address the recurrent problems encountered by state colleges and universities (SUCs) in the Philippines on the inadequacy of laboratory equipment needed in the training of students. They developed a low cost industrial automation trainer. The evaluation of the instruments showed that the developed device can function according to expectations both novice and experts. Meanwhile, the developed device may improve its functionality if embedded with a simulation software that can manage the operations while maintaining automations that are based on freely available software application. Similarly, Carreon (2010) introduced an *Industrial Motor Control Trainer* for instructional purposes in Don Mariano Marcos Memorial State University Mid La Union Campus, City of San Fernando, La Union. The study was to design, construct and develop an Industrial Motor Control Trainer (IMCT). The study applies the method of designing, constructing, testing and revising the model. The goal of the overall design is to provide adequate instructional equipment for laboratory activities and improve technical skills of students in electrical technology department in school.

One of the courses offered in SSCT where students benefits most when exposed to instructional technology is the course on Fundamentals of Pneumatic Electro-pneumatic Controller. This course deals on the use of compressed air to do mechanical work – that is to produce motion and to generate forces. It also covers design, function and applications of various electro-pneumatic elements commonly used in industry. In this course, the students will use relays, electric or electronics switches and sensors to control pneumatic circuits instead of the conventional mechanical controlling methods. Sufficient time is allocated for hands-on exercises to achieve hook-up and troubleshooting skills. The course also include pneumatic training software as a teaching tool for simulating basic pneumatics and runs using freely available but reliable software. This will support learning through visualizing pneumatic components and diagrams which are explained with textual description, figures and animation that illustrate underlying working principle.

Certain roadblocks are present in delivering the course in order to attain its outcomes, for example, the extravagant price of the equipment needed in the lesson delivery and the limited to almost zero availability of equipment as a training mechanism. Hence, developing a trainer that is low cost and parts-replaceable but without sacrificing its instructional benefits can help the students not be afraid to practice, since the parts are accessible and can easily be replaced. These can result to the desired quality of education with a competitive edge. This can also help boost student's morale, knowing that they can be as good as any other students in the country through the help of this low cost competitive trainer. The developed trainer can perform various functions of basic motor starting, star-delta starting, forward-reverse starting, part-winding starting and sequence control. The trainer can perform exactly as the commercial one. Electrical connections, diagrams and symbols were made simple and based on international standards. Students could easily comprehend with the simplicity of the design of the trainer. The present study and the reviewed study are related in methods applied, from designing to revising. They are both electrical–based trainers with similar objectives and purpose. It differs in the application in the sense that the present study applies automation and mechatronics.

Specifically, it sought to accomplish the following objectives: design and construct a Low Cost Mechatronics Trainer; compare the Low Cost Mechatronics Trainer to existing designs and commercially available mechatronics trainer in terms of cost and technical features. To evaluate and validate the Low Cost Mechatronics Trainer in conformity to the Technology Assessment Protocol (TAP) of the DOST using TEEPS: technical feasibility, economic and financial viability, environmental soundness, political acceptability, and social acceptability. Moreover, Competency Based Learning Materials (CBLM) was validated in terms of: face validity, and content validity.

Conceptual Framework

The conceptualized, designed and constructed a Low Cost Mechatronics trainer responded to the need of developing training equipment while balancing the cost without sacrificing the quality and enhancing the knowledge and skills of students in automation and mechatronics.It aimed to address the problem of inadequate training facilities in SUC's because of extravagant price of training equipment. The design could compete against existing design and expensive commercial mechatronics trainer using low cost materials, thus most of the materials used were recycled and second hand items.

The developed instructional trainer is based on the concept of instructional design theory for concept teaching and learning (Reigeluth, 1999) where learning is viewed in terms of the two processes of knowledge: formation and development. The process of developing conceptual knowledge starts when senses observe information i.e., concept acquisition, from the environment, these information are carried it into the working memory- short term memory. These information in the working memory are then divided into two dimensions: meaningful and not meaningful. Only those meaningful information are then processed, i.e., concept development and transferred into the storage system. When the senses encounters same entity in the new environment, the meaningful dimensions of the memory select information from the memory storage and matches this information from the known examples and connect this entity in a given domain of information.

These process of learning are demonstrated by the model system provided by the trainer. The real-world concepts are simulated automatically using the system designs feature of the low cost mechatronics trainer which will allow students todevelop meaningful information. Which in turn be stored into the memory storage and hence becomes transferable to future situations. The LCMT consist of six main parts: the AC power source, pneumatic power source, signal input, signal processing, signal output and command execution. Under these six main parts includes various components that perform specific function for the proper operation of the trainer. In order to provide *AC power source*, a cord with male plug is used and a circuit breaker is connected to this line. The power supply is part of the AC power source. *The pneumatic power source* includes: a mobile portable compressor, air service unit with filter and regulator, and a series regulator attached in the main panel. To deliver the compressed air to various pneumatic and

electro-pneumatic devices, a pneumatic hose of 6mm and 4mm is used. *The signal input* includes various types of input elements such as: push button switch, sensors (limit switch, reed switch, inductive proximity sensors and optical proximity sensors. Their functions are part of the electrical signal control section, which is logically associated with those components in the signal processing. *The signal processing* includes various types of processing elements such as: relay, contactors, timer, counter and programmable logic controller. These are also part of the electrical signal control section associated with signal input. *The signal output* consists of final control element which is the electro-pneumatically controlled solenoid valve. This element forms a link between the electrical signal control section and the pneumatic power section. *The command execution* includes power components such as cylinders, motors lamps and buzzer. Command execution takes place at a high power level such as for fast ejection of a work piece from a machine or to exert a high force for pressing and cutting a work piece. Command execution belongs to the power section of a control system.

The study used the Input-Process-Output scheme as shown in Figure 1. The Input includes: 1) conceptual design of low cost mechatronics trainer; 2) sample mechatronics utility models; 3) supplies and materials; and 4) syllabus, training modules /methodologies and manual. The Process includes 1) design and construction of Low Cost Mechatronics Trainer (LCMT); 2) comparison of the LCMT to existing designs and commercial mechatronics trainers in terms of cost, and. technical feature; 3) validation using TAP of DOST on TEEPS: technical feasibility, economic and financial viability environmental soundness, political and social acceptability; and 4) validation of Competency Based Learning Materials. The target output is the viable low cost mechatronics trainer and the competency based learning materials which serves as a tool in performing laboratory activities such as projects and exercises.

MATERIALS AND METHODS

The design, development and construction, and evaluation of Low Cost mechatronics trainer (LCMT) followed the DOST-TAPS TEEPS method (de Guzman, 2005). In evaluating the developed traineir, a 5-point Likert scale was used to quantify responses of the evaluators, with 5 being the highest and 1 being the lowest. The descriptive evaluative design describes the device based on its evaluation made by the experts in engineering designs and also practitioners of the filed. In the developmental phase, the technical feasibility of the design was considered. While in the descriptive phase, perceptual assessment on performance, economic feasibility, environmental soundness, political acceptability and social acceptability (TEEPS) were evaluated.

Respondents

The respondents who made an evaluation of the performance was composed of eleven (11) engineering design experts and thirty (30) end users. The experts were TESDA National Certificate holder in Mechatronics Servicing and Electrical Installation and Maintenance (EIM) which was composed of Eight (8) faculty members from the state university. One (1) faculty from the Department of Industrial and Controls Engineering Technology, one (1) faculty from the College of Trades and Industries, and one (1) faculty/trainor from Technical Educational Skills Development Authority (TESDA), Regional Training Center and thirty (30) end users.

Instrumentation

Upon completion of the design and construction of the developed LCMT, the validation process was conducted. Eleven (11) identified expert evaluators demonstrated the actual operation of the LCMT. Two samples of exercises were used to demonstrate the operation. The 30 end users observed the demonstration process. A questionnaire or was used to evaluate the LCMT in terms of Level of Technical Performance (safety of operation, precision of the trainer, simplicity of the mechanism, and portability) and Economic viability, Environmental soundness, Political acceptability and Social acceptability of the Developed Low Cost Mechatronics Trainer (Ventura, & Guillermo, 2020)

RESULTS

The Low Cost Mechatronics Trainer is powered by a 220VAC source. A short-circuit-proof power supply unit (input: 220VAC; 60 Hz; output: 24VDC, maximum 5A) is used for DC supply. All electrical and electro-pneumatic components are 24VDC, except for the PLC that is 220VAC. For compressed air supply, a mobile portable compressor 220VAC, maximum 6 bar = 600 kPa is used as the pneumatic power source. From thence, all exercises, (PLC) programmed in the computer and hardwired electro-pneumatic control can now be performed in the trainer.

Similar to commercially available mechatronics trainer, the designed and developed mechatronics trainer is divided into four functions as ca be seen in Figure 2. *Signal input, signal processing, signal output and command execution.* Signals from the signal input are logically associated (signal processing). Signals for signal input and signal process are low power signals. Both functions are part of the electrical signal control section. At the signal output stage, signals are amplified from low power to high power. Signal output forms the link between the electrical signal control section and the pneumatic power section. Command execution takes place at a high power level – that is, in order to reach a high speed (such as for fast ejection of a work piece from a machine) or to exert a high force (such as for pressing and cutting). Command execution belongs to the power section of a control system.

Both pneumatic and electro pneumatic controllers have a pneumatic power section. The signal control section varies according to type. In a pneumatic control, pneumatic components used are: various types of valves, sequencers, and air barriers. In an electro-pneumatic control, the signal control section is made up of electrical components, for example with electrical input push buttons, proximity switches, relays, or a programmable logic controller. In contrast to a purely pneumatic control system, electro pneumatic controllers are not shown in any single overall circuit diagram, but in two separate circuit diagrams - one for the electrical part and one for the pneumatic part. For this reason, signal flow is not immediately clear from the arrangement of the components in the overall circuit diagram.

Validation of the Low Cost Mechatronics Trainer Using the DOST TEEPS

The trainer was evaluated by both expert and end users as basis for its technical performance. The operation of the trainer was demonstrated by eleven (11) expert and thirty (30) end users as respondents. Two exercises were performed by the trainer, after which they accomplished the evaluation form which covers five indicators. Table 3 presents the summary of table for the TEEPS assessment of the Low Cost Mechatronics Trainer to determine its level of validity. The trainer was evaluated by eleven (11) experts and thirty (30) end users. For Technical Feasibility, the overall mean that the experts and end users gave was 4.67 with a descriptive equivalent of very higly validity. Safety operation of the trainer got the highest mean in this area. The evaluators agreed that the LCMT is safe to use. They also agreed that portability is not a problem because it does not require much effort to store and transfer from one place to another. They believed on the accurateness of the trainer in terms of components quality and standards. They believed that parts and component could be readily available when needed because of some accessible second hand parts. In terms of Econnomic viability, the evaluator agreed that the trainer was the lowest cost among other three samples of mechatronics trainer based on the data they gathered in the comparison of LCMT to other mechatronics trainer.

But they are in doubt on the gap between the prices, and the availability of parts. The evaluators gave the lowest mean on this area of 4.53 with a descriptive equivalent of very high validity.The Environmental Soundness was the highest rating among all areas under the TEEPS. The evaluators rated 4.86 or very high validity. They believed that when the trainer was used, it did not bring harm to the environment. Political Acceptability has a weighted mean of 4.78 also describe as very high acceptable, second to the highest. Evaluators believed that the trainer follows with the regulatory requirements and standard.Social Acceptability gained 4.76 weighted mean, still a very high acceptability. Evaluators saw that the developed LCMT does not violate to their culture and traditions. It implies also that it can be

operated by both sexes. The LCMT with the used of TEEPS criteria for evaluation got an overall grand mean of 4.72, a very high validity/acceptability trainer.The effectiveness of the developed LCMT was apparently evident when the researcher took the assessment for Mechatronics Servicing NC II and was able to pass the assessment rated as "competent". The researcher uses the trainer as part of his tool to pass the assessment.

Validation of the Competency Based Learning Materials (CBLM)

Face Validity

Table 3 shows the level of face validity of the CBLM. The total mean given by the evaluators was 4.45 with descriptive equivalent of very high validity. It implies that in general, the CBLM was appropriate. The different creteria as to 1) general apperance, 2) grammar and 3) layout have all a descriptive rating of very high; thus there is no need for an improvement of the format.

Content Validity

As gleaned from the table, the total mean of the level of content validity of the CBLM for mechatronics is 4.72 which means that the CBLM is with very high validity in terms of its content. As reflected from the table, the first and third criteria have the highest rating of 4.91 (very high validity). It implies that the concept learned from the topics are provided with laboratory exercises with corresponding evaluation such as self check and that every activity and exercise in electro pneumatics and PLC are appropriate and comprehensible to the users of the CBLM. As to the second criterion, one expert evaluator commented that the CBLM in their program was not only with in the 3rd year level of students but it was also comprehensible and appropriate to 2nd year level of students based on their course syllabus. He further suggested that performance evaluation sheet in the learning materials has to be included. The comments and suggestions were carried and noted by the researcher and he was further given an explanation that in other universities the topic or subject in electro pneumatics and PLC are given only for 3rd year student. On criterion number 5, one of the evaluator suggested that the learning sequence of the topics must be observed. It implies that this evaluator notice a particular laboratory activities not in sequence with the topic, he might have used different sequence of activities on his syllabus. However, the suggestion was noted.

The evaluator in TESDA had the most comments and suggestions and that gave the reseracher more insights. He commented and suggested that pictures used in the CBLM shall be labelled and if necessary, provide it with wiring diagram. He further write comments and suggestion on some pages of the learning materials such as: self check must be separated in a one page and no other topics or exercises be included in this page; labelling of the name of diagram as to electrical diagram or pneumatic diagram. He suggested that "remarks" on "table input/output assignment" is to be replaced with "function" in order to identify what function should the input device will do when circuit is ON. The grand mean of the CBLM is 4.56 which means that it is with a very high validity manual.

DISCUSSION

The design, construction, development and evaluation of Low Cost Mechatronics Trainer were the main purpose of this study in response to the need of developing training equipment in Mechatronics. The designed and constructed a mechatronics trainer that could compete against the expensive existing and commecially available mechatronics trainer using low cost materials, devices and components, thus most of the materials used were recycled and surplus (second hand items). The DOST TAP TEEPS protocol criterion was used to evaluate the developed LCMT. The developmental phase covered the technical feasibility of the design while in the descriptive phase, it covered perceptual assessment on the performance, economic feasibility, environmental soundness, political acceptability and social acceptability (TEEPS). Experts from three Universities along with their specialization and end users evaluated the trainer using the said assessment protocol. The developed LCMT was also compared to the existing design and commercial mechatronics trainer in terms of cost and technical feautures. The same group of expert

evaluators except for the end users, had evaluated the level of validity of the Competency Based Learning Materials in terms of face and content validity.The developed LCMT was conceptualized and underwent methodical procedures in designing and construction and came out with the most functional designed trainer. This characteristic of the model gives a sound evidence that it can help improve the instructional practice (Pianta, Hamre, &Nguyen 2020). The trainer is significant to the students since the system provides low-cost, and flexible trainer platform in the application of mechatronics.

Based on the given three samples of mechatronics trainers, the developed LCMT has the lowest cost of all three samples. This is due to the market value of some of the components used in the designed trainer which is low in cost. Most of the components were recylced materials and second hand items (surplus). The LCMT has advantages as to portability and precision as compared to the existing design and commercial mechatronics trainer. All criteria under technical feasibility were rated with a very high validity. The developed LCMT was found to have a very high acceptability in accordance with the regulatory requirements and standard as set by the implementing body. The validators of the trainer agrees that the LCMT has a very high rating as to social acceptability and it is user-friendly. Due to its users adaptability, it can be easily used by any gender.

CONCLUSION

The technical performance, economic viability, environmental soundness, political acceptability and social acceptability of the design resembles the actual process and requirement for a system. Experts and practitioners in the field of engineering designs perceived the quality and the reliability of the developed trainer as highly valid. These features of model system where experts confirm the criteria sets out helps advance the appropriateness of a model used for teaching (Lavigne& Good2020; Pianta, Hamre, &Nguyen, 2020). The evaluation showed that the developed trainer although low in cost did not compromise the quality. The developed trainer serves as a prototypical model for learning conception and formation.Learning as demonstrated by the designed trainer resembles the concrete future entities of the real-word where students can experience, i.e., hands-on, therefore creates a correlational or contextual groupings of attributes that forms an independent stimulus dimensions (Reigeluth, 1999). This hands-on experience increases confidence of storing and forming information when a given instance is similar to a prototypical example of the category.

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Table 1*. Production Cost*

Sources	Cost
1. Supplies and materials	P 25,963.00
2. Labor Cost (25%)	6,490.75
3. Overhead Cost (10%)	2,596.30
Total Production Cost	P 35,050.05

Table 2.*TEEPS Assessment*

Indicator	Expert	users	Mean	DR
a. Technical Feasibility	4.72	4.62	4.67	VHV
b. Economic Viability	4.63	4.44	4.53	VHV
c. Environmental Soundness	5	4.73	4.86	VHV
d. Political Acceptability	4.81	4.75	4.78	VHA
e. Social Acceptability	4.84	4.68	4.76	VHA
Grand Mean	4.80	4.64	4.72	VHV

Legend: DR – Descriptive Rating VHV – Very High Validity VHA - Very High Acceptability

Table 3. *Level of Face Validity*

Indicator	Mean	DR
1. General Appearance	4.55	VH
1.1 Neatness and attractiveness		
1.2 Typing and alignment		
2. Grammar	4.36	VH
2.1 Correct tense		
2.2 Parallelism		
3. Layout	4.46	VH
3.1 Spacing and indention		
3.2 Balance on sheet		
Mean	4.45	VH

Legend: DR – Descriptive Rating VH – Very High

Table 4.*Level of Content Validity*

Indicator	Mean	DR
1. The laboratory activities are representative of the concept learned from the topics in electro pneumatics and PLC.	4.91	VH
2. The content of each laboratory activity is comprehensive and appropriately suitable for 3rd year college, electrical, electronics and mechatronics courses.	4.67	VH
3. Laboratory activities are appropriate to the topics in electropneumatics and PLC.	4.91	VH
4. The language used is appropriate to the vocabulary level ofstudents taking up electrical, electronics, and mechatronics.	4.55	VH
5. The laboratory activities are arranged according to the sequence of topics presented in mechatronics	4.55	VH
Mean	4.72	VH
Grand Mean	4.56	VH

Legend: DR – Descriptive Rating VH – Very High

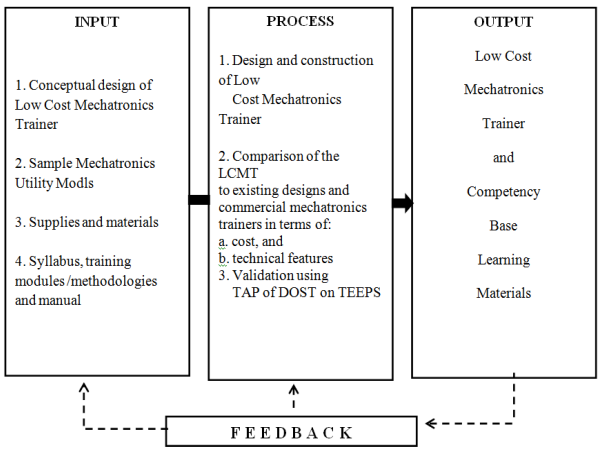

Figure 1. Input-Process-Output scheme

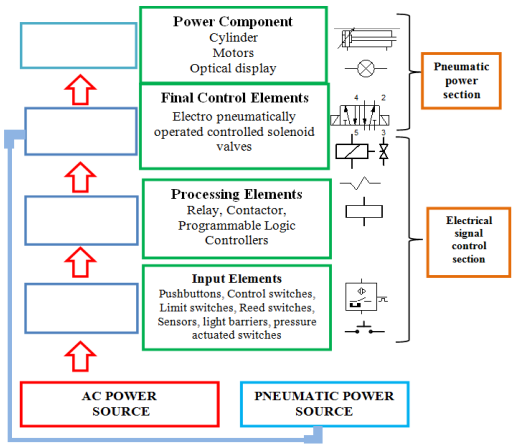

Figure 2. Signal/Process Flow of a Mechatronics System

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RESEARCH ARTICLE

Novel Technology of Plant Extraction in Pharmaceutical Sector

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ABSTRACT

Extraction, as the term is utilized pharmaceutically, includes the separation of therapeutically dynamic bits of plant from the inactive or inert components by utilizing specific solvents in standard extraction strategies. Along these lines, normalization of extraction techniques contributes essentially to the last nature of the home grown medication. Methods of extraction of medicinal plants are Maceration, Hot continuous extraction (Soxhlet), Decoction, Percolation**,** Counter current extraction (CCE) these are method it will be help to extract the medicinal plant. Novel technologies for aqueous extraction of plant are Bioactives pulsed electric fields, Supercritical fluid utilization and Ultrasound Assisted Extraction (UAE), if this innovative technique based on using ultrasonic waves for the extraction of numerous compounds from a diversity of matrices (microbial, plant, etc). The microwave radiations are electromagnetic waves that propagate in the vacuum at the speed of light. They are characterized by a frequency ranging between 300 MHz and 300 GHz. Ultrasound-assisted extraction is occasionally faster than microwave-assisted leaching.

Keywords: Extraction, Percolation, Fermentation, Biological, Menstruum, Microbial.

INTRODUCTION [1, 2]

Extraction, as the term is utilized pharmaceutically, includes the separation of therapeutically dynamic bits of plant from the inactive or inert components by utilizing specific solvents in standard extraction strategies. The products so acquired from plants are somewhat tainted fluids, semisolids or powders expected distinctly for oral or outside use. These incorporate classes of arrangements known as decoctions, imbuements, liquid concentrates, colors, semisolid concentrates and powdered concentrates. The reasons for normalized extraction techniques for unrefined medications are to achieve the restoratively wanted part and to wipe out the inactive material by treatment with a

specific dissolvable known as menstruum. The concentrate consequently acquired might be prepared for use as a therapeutic specialist as colors and liquid concentrates, it could be additionally handled to be consolidated in any measurements structure like tablets or cases, or it very well might be fractionated to segregate individual substance elements, for example, ajmalicine, hyoscine and vincristine, which are present day drugs. Along these lines, normalization of extraction techniques contributes essentially to the last nature of the home grown medication.

METHODS OF EXTRACTION OF MEDICINAL PLANTS

MACERATION [3]

In this process, the entire or coarsely powdered unrefined medication is set in a stoppered holder with the dissolvable and permitted to remain at room temperature for a time of no less than 3 days with continuous unsettling until the solvent matter has broken up. The blend then, at that point, is stressed, the marc (the damp solid material) is squeezed, and the joined fluids are explained by filtration or decantation subsequent to standing.

Application [4]

1. Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time.
2. This technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction.
3. This technique was used for the extraction of essential oils and active compounds from plant materials.
4. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent.

Advantages

1. Maceration is a simple method using non-complicated utensil and equipment [5].
2. Generally, maceration is conducted at cool temperature [6].
3. Extraction method is rapid and low expensive [7].

HOT CONTINUOUS EXTRACTION (SOXHLET) [8]

In this method, the finely ground crude drug is set in a permeable pack or "thimble" made of solid channel paper, which is set in chamber E of the Soxhlet mechanical assembly. The separating dissolvable in jar An is warmed, and its fumes gather in condenser D. The dense extractant trickles into the thimble containing the rough medication, and concentrates it by contact. At the point when the degree of fluid in chamber E ascends to the highest point of siphon tube C, the fluid substance of chamber E direct into flask A. This cycle is persistent and is done until a drop of dissolvable from the siphon tube doesn't leave buildup when vanished. The benefit of this strategy, contrasted with recently depicted techniques, is that a lot of medication can be separated with a lot more modest amount of dissolvable. This influences gigantic economy as far as time, energy and thus monetary data sources. At limited scope, it is utilized as a bunch cycle just, however it turns out to be substantially more practical and reasonable when changed over into a ceaseless extraction strategy on medium or huge scope.

Application [9]

1. Soxhlet extraction has been used widely for extracting valuable bioactive compounds from various natural sources.
2. In this extraction, a small amount of dry sample is placed in a thimble, which is placed in a distillation flask containing the solvent of particular interest.

Advantages

1. It is able to extract solute from insoluble impurities [10].
2. It is mechanically gentle on the samples but still efficient in separation [11].
3. It is often used as a benchmark when developing new extraction methods [12].
4. Soxhlet extraction is that it can be extracted multiple times. Compared with the general soaking method [13].
5. It requires small solvent dosage, high efficiency and complete extraction [14].

DECOCTION [15]

In this interaction, the unrefined medication is bubbled in a predefined volume of water for a characterized time frame; it is then cooled and stressed or separated. This technique is reasonable for separating water-dissolvable, heatstable constituents. This cycle is ordinarily utilized in planning of Ayurvedic extricates called "quath" or "kawath". The beginning proportion of unrefined medication to water is fixed, for example 1:4 or 1:16; the volume is then brought down to one-fourth its unique volume by bubbling during the extraction technique. Then, at that point, the concentrated concentrate is separated and utilized all things considered or handled further.

Application [16]

1. Decoction involves first mashing the plant material to allow for maximum dissolution, and then boiling in water to extract oils, volatile organic compounds and other various chemical substances.
2. Decoction can be used to make tisanes, tinctures and similar solutions**.**
3. Decoction is an extraction procedure that has been used especially for water-soluble and thermostable constituents**.**
4. Typically used in preparation of ayurvedic extracts.

PERCOLATION [17, 18]

This is the system utilized most regularly to extract active ingredients in the arrangement of colors and liquid extracts. A percolator (a narrow, cone-molded vessel open at the two closures) is for the most part utilized. The strong fixings are dampened with a proper measure of the predefined menstruum and permitted to represent around 4 h in a very much shut compartment, after which the mass is pressed and the highest point of the percolator is shut. Additional menstruum is added to shape a shallow layer over the mass, and the blend is permitted to macerate in the shut percolator for 24 h. The power source of the percolator then, at that point, is opened and the fluid contained in that is permitted to trickle gradually. Extra menstruum is added as needed, until the permeate measures around 3/4 of the necessary volume of the completed item. The marc is then squeezed and the communicated fluid is added to the permeate. Adequate menstruum is added to deliver the necessary volume, and the blended fluid is explained by filtration or by standing followed by tapping.

Application [19]

1. Percolation theory can be used as a simple idealized model for predicting the distribution of the oil or gas inside porous rocks or oil reservoirs.
2. To the probability with which a site is occupied in the percolation problem it corresponds the porosity or the average concentration of oil in the rock.

AQUEOUS ALCOHOLIC EXTRACTION BY FERMENTATION [20]

Some medicinal preparations of Ayurveda (like asava and arista) adopt the technique of fermentation for extricating the dynamic standards. The extraction methodology includes dousing the unrefined medication, as either a powder or a decoction (kasaya), for a predetermined timeframe, during which it goes through aging and creates liquor in situ; this works with the extraction of the dynamic constituents contained in the plant material. The alcoholsubsequently created additionally fills in as an additive. On the off chance that the aging is to be completed in an earthen vessel, it ought not be new: water should initially be bubbled in the vessel. In large-scale manufacture, wooden tanks, porcelain containers or metal vessels are utilized instead of earthen vessels. A few instances of such arrangements are karpurasava, kanakasava, dasmularista. In Ayurveda, this strategy isn't yet normalized at the same time, with the exceptionally serious level of progression in maturation innovation, it ought not be hard to normalize this method of extraction for the creation of home grown medication extricates.

COUNTER CURRENT EXTRACTION [21]

In counter current extraction (CCE), wet crude material is crushed utilizing toothed circle disintegrators to deliver fine slurry. In this process, the material to be removed is moved one way (generally in the form of fine slurry) inside

a barrel shaped extractor where it interacts with extraction dissolvable. The further the beginning material moves, the more focused the concentrate becomes. Complete extraction is hence conceivable when the amounts of dissolvable and material and their stream rates are streamlined. The interaction is exceptionally proficient, requiring brief period and representing no danger from high temperature. At long last, adequately focused concentrate comes out toward one side of the extractor while the marc (for all intents and purposes liberated from apparent dissolvable) drops out from the opposite end.

Application [22]

1. The separation of substances with different distribution coefficients (ratios). ... The liquid-liquid extractions are taking place simultaneously in all tubes of the apparatus which is usually driven electromechanically**.**
2. The CCD technique was employed in many notable separations such as polycyclic aromatic hydrocarbons, bile acids, ribonucleic acids, penicillin insulin, Taxol, Streptomyces antibiotics. and many other antibiotics.
3. Aromatic compounds are recovered from paraffin fraction of the petroleum oil, Isolation of chemical compounds from the aqueous systems using small quantities of organic solvents in the production of synthetic drugs and intermediates

This extraction process has significant advantages

1. A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction and percolation [23].
2. CCE is commonly done at room temperature, which spares the thermo labile constituents from exposure to heat which is employed in most other techniques [24].
3. As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermo labile constituents from exposure to heat [25].
4. The extraction procedure has been rated to be more efficient and effective than continuous hot extraction [26].

NOVEL TECHNOLOGIES FOR AQUEOUS EXTRACTION OF PLANT BIOACTIVES

PULSED ELECTRIC FIELDS [27]

Because of high temperature and long time used in conventional methods and consequently, degradation possibility of thermo sensitive compounds, PEF, as a nonthermal extraction technique, is considered a promising alternative. During PEF, high-voltage pulses ranging from 20 to 80 kV/cm are applied between electrodes containing the food product which result in the electro oration of the cell membranes and improvement of the intracellular compounds extraction. To enhance PEF efficiency, three major parameters should be taken into account, including

1. Generation of high electric field intensity,
2. Uniform treatment to all parts of intended food, which highly depends on treatment chamber,
3. Assurance of the absence of electrolysis in the food material.

Applications of PEF [28]

1. Mild preservation of beverages and semi-liquid food products
2. Treatment of potatoes to replace thermal preheating
3. Extraction processes such as extraction of antioxidants, extraction of oil and protein from algae, extraction of sugar from sugar beets and extraction of nutrients or fibers from peels and stems.
4. Furthermore PEF processing can be applied for the removal of acrylamide, concentration of protein from potatoes and enhancement of production processes for cooked ham and dry sausage.

SUPERCRITICAL FLUID EXTRACTION [29]

Supercritical fluid utilization for extraction purposes began with its discovery by Hannay and Hogarth in 1879 and since then, an ever-increasing interest led to successful application of this technique in different fields, including environmental, pharmaceutical, cosmetics, polymer, and food industries. SFE offers several advantages compared to conventional extraction such as faster extraction rate, easier diffusion, high selectivity, and being environmentally

friendly. Among different possible solvents that could be used as supercritical fluids, carbon dioxide is the most commonly used one mainly due to three reasons, including its harmlessness for humans and environment, suitability for temperature-sensitive components [moderate critical temperature (z31C)], and ability to preserve the extract from contact with air and lowering the oxidation possibility.

Applications of Supercritical Fluid Extraction [30]

1. SFE has been used in industries for the extraction of various commercial natural products.
2. In case of natural product extraction. Extraction via SCF is predominantly limited to plant natural products and just handfuls of studies are carried out for extraction of microbial natural products via SFE.
3. Sources from where the main bioactivities are extracted by SFE are antioxidant (41%), antitumor (18%) and antibacterial activity (10%), followed by antiviral, antimicrobial, anti-inflammatory and anti-cholinesterase (up to 5%)

Advantages

1. That slight changes in temperature and pressure within the critical region give extremely large changes in solvent density and solubility [31].
2. There is greater flexibility in the process operating parameters of pressure and temperature as compared with conventional liquid–liquid extraction processes [32].
3. Elimination of organic solvents and reduce the risk of storage [33].
4. Susceptible to thermal degradation [34].

NEGATIVE PRESSURE CAVITATION [35, 36]

To extract bioactive substances from plant tissues, NPCE is considered as a novel technique, categorized as a type of hydrodynamic cavitations. While using this method, an intense cavitation effect and a rigorous stirring effect are generated by a continuous air flow introduced into the liquid solid system. These phenomena results in easier mixing of substrate and solvent and better mass transfer. There are several advantages in NPCE application, including environmentally friendly, low cost, energy efficient, scalable technology, time-saving, and mild operating conditions. NPCE equipment consists of five sections including an extraction pot, an assortment pot, a warming framework, a vacuum siphon, and a condenser.

In this framework, vacuum siphon makes negative tension, which thus shapes serious cavitations to erode the outer layer of strong particles. Ceaseless expansion of air into the framework by means of the valet brings about more disturbance and impact; subsequently mass exchange upgrades between the extraction dissolvable and the strong network and extraction measure speeds up. The extraction productivity is influenced by certain boundaries like tension, temperature, time, type, and centralization of dissolvable, presence of disintegrated gas (air or nitrogen). To accomplish the most elevated extraction productivity, control and advancement of these boundaries ought to be considered.

Application [37]

1. Negative pressure cavitation (NPC) as potential tool to recover valuable compounds. NPC can be combined with other technologies to improve extraction process.
2. It is a useful tool to prevent degradation of thermolabile compounds.
3. NPC is a simple eco-friendly process, requiring low cost, and with high efficiency.

ULTRASOUND ASSISTED EXTRACTION (UAE) [38, 39]

Ultrasound Assisted Extraction (UAE) is an innovative technique based on using ultrasonic waves for the extraction of numerous compounds from a diversity of matrices (microbial, plant, etc). The propagation of ultrasonic waves causes the implosion of bubbles, usually called cavitation phenomenon that induces macroturbulence, high-velocity interparticle collisions, and perturbation in the microporous particles of the sample. As a result, the solute quickly

diffuses from the solid phase to the solvent. UAE offers environment friendly, clean extraction with several advantages. This technique is simple, effective, and inexpensive. Its most significant advantages are related to the increase of the extraction yield and an acceleration of the kinetics compared to conventional extraction. However, the instrument possesses a significant shortcoming in the aging of the instrument, as power progressively declines with attenuation of the intensity, thus decreasing the reproducibility of the experiments. Generally, the extraction temperature during UAE should be relatively low to avoid the degradation of the thermo sensitive compounds. UAE could be performed with numerous solvents such as water, methanol, acetone, and ethyl acetate. Moreover, the effect of ultrasonic extraction on yield and extraction kinetics is related to the nature of the plant matrix. This technology has been successfully applied to extract different bioactive compounds such as carotenoids, polysaccharides, proteins, phenolic compounds, aromatic compounds, and sterols.

Applications [40, 41]

Protein extraction

1. The continuous high-intensity application extracted 54% and 23% more protein for aqueous and alkali extraction respectively, compared with the batch extraction using comparable processing times and volumes.
2. Ultrasound-assisted extraction has been successfully used in the extraction of various medicinal compounds including alkaloids, flavonoids, glycosides, phenolic compounds and polysaccharides from plants in laboratory investigations
3. This technique has been used in the development of methods for the analysis of numerous contaminants, including organic compounds (pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons, polyhalogenated flame retardants, etc.) and heavy metals.

Advantages

1. Ultrasound-assisted extraction is occasionally faster than microwave-assisted leaching [42].
2. The ultrasonic procedure is safer as it requires no high pressure [43].

MICROWAVE-ASSISTED EXTRACTION [44]

The microwave radiations are electromagnetic waves that propagate in the vacuum at the speed of light. They are characterized by a frequency ranging between 300 MHz and 300 GHz. On the electromagnetic spectrum, microwaves are located between the radio frequency and infrared. The effectiveness of electromagnetic fields for heating dielectric materials has been stipulated by the American physicist Percy L. Spencer in 1949. This discovery was later adopted by some firms such as General Electric and Raytheon, producing heating systems for industrial and domestic use. A better understanding of this new generation of heating systems was required. Thus, the nature and mechanisms of interaction between the electromagnetic waves and the matter were studied by Von Hippel. According to his macroscopic studies, microwave heating is induced by two major phenomena:

1. ionic conduction
2. dipolar polarization

PHYTONICS PROCESS [45, 46]

A new solvent based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant materials offer significant environmental advantages and health and safety benefits over traditional processes for the production of high quality natural fragrant oils, flavors and biological extracts. Advanced Phytonics Limited (Manchester, UK) has developed this patented technology termed “phytonics process”. The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment. The process is advantageous in that the solvents can be customized: by using modified solvents with HFC-134a, the process can be made highly selective in extracting a specific class of phytoconstituents. Similarly, other modified solvents can be used to extract a broader spectrum of components. The biological products made by this process have extremely low residual solvent. The residuals are invariably less than 20 parts per billion and are frequently

below levels of detection. These solvents are neither acidic nor alkaline and, therefore, have only minimal potential reaction effects on the botanical materials. The processing plant is totally sealed so that the solvents are continually recycled and fully recovered at the end of each production cycle. The only utility needed to operate these systems is electricity and, even then, they do no consume much energy. There is no scope for the escape of the solvents. Even if some solvents do escape, they contain no chlorine and therefore pose no threat to the ozone layer. The waste biomass from these plants is dry and "ecofriendly" to handle.

Advantages of the Process

1. Unlike other processes that employ high temperatures, the phytonics process is cool and gentle and its products are never damaged by exposure to temperatures in excess of ambient [47].
2. It is less threatening to the environment and it requires a minimum amount of electrical energy [48].
3. The solvents used in the technique are not flammable, toxic or ozone depleting [49].
4. The solvents are completely recycled within the system [50].

Applications [51-53]

1. The phytonics process can be used for extraction in biotechnology (e.g for the production of antibiotics), in the herbal drug industry, in the food, essential oil and flavor industries, and in the production of other pharmacologically active products.
2. In particular, it is used in the production of top quality pharmaceutical-grade extracts, pharmacologically active intermediates, antibiotic extracts and phytopharmaceuticals.
3. The technique is being used in the extraction of high-quality essential oils, oleoresins, natural food colors, flavors and aromatic oils from all manner of plant materials.
4. The technique is also used in refining crude products obtained from other extraction processes. It provides extraction without waxes or other contaminants.
5. It helps remove many biocides from contaminated biomass.

CONCLUSION

This study was dealing with the use of novel extraction techniques for extraction of bioactive molecules from plant matrices. The technologies described in this work were Maceration, Soxhlet Extraction, Decoction, Percolation, PEFs, SFE, NPCE, MAE, and UAE. However, some limitations are associated with their use. Although the application of some emerging systems in aqueous extraction of plant bioactives has already been demonstrated at the laboratory scale, the advantageous and disadvantageous of using these systems in the industrial scale need to be further investigated. The development of cost-effective and more sustainable extraction and separation processes is the critical step toward the recovery and commercialization of new and low-cost bioactive products for the nutraceutical, cosmetic, and pharmaceutical sectors, while envisaging their widespread use in the near future to boost the quality of modern society.

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RESEARCH ARTICLE

An Empirical Study of Data Mining Techniques in Agriculture Influencing Soil and Weather Factors

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ABSTRACT

Agriculture plays a vital role in a day-to-day life and globally it is used to accomplish a food demand. Nowadays due to the some calamities like bad weather condition, drop of irrigation, soil fertilization and so on farmers facing a difficulties in yielding a production and as well as profit. Data mining and machine learning techniques are sought for solving various problems such as crop prediction, increasing yield production, fertilizer recommendation etc. and also it is very helpful to the farmers to take a right decision at right time. The success of crop yield prediction is made by considering the two important input factors such as soil and weather information. The main focus of this study is to summarize the findings and recommendations which are described by different researchers by applying various algorithms in the agriculture field. Through the huge size of data set and the features selection in input parameters how accurately the algorithm produces the results are evaluated. In data mining and machine learning, clustering and classification techniques are frequently implemented and gave ingenious information in research to solve the problem of yield production and crop prediction for smart farming.

Keywords: Crop prediction, feature selection, soil and weather information, yield productivity, recommendation

INTRODUCTION

Analyzing a large amount of data is a necessity in agriculture. Analyzing such data is an important need so we can use a data mining techniques as a resource to evaluate this process. Agriculture is the main source of Indian economy to solve an issue in agriculture which was faced by the farmers and also an analysis is made in order to help the farmers to increase the crop prediction, yielding more production, fertilizer recommendation, improving nutrients in soil, weather forecasting and controlling the pest disease. The issue which was said above can be predicted and suggest or recommend a result using various data mining techniques. To improve more efficiency a machine learning algorithms also plays a vital role in analyzing and predicting an agricultural data. Agriculture depends on mining to produce a healthy product to the generations. Figure1 shows District wise agricultural productivity in tamil nadu.

In data mining, unknown properties are discovered in the data by using predictive and descriptive method. Predictive method such as classification and regression uses existing variables in the database to predict unknown or future value. Descriptive method like clustering and association rule focuses on finding patterns to describe the data and user interpretation are presented. In machine learning, from the training data prediction is made based on the known properties. In machine learning and data mining, some of the algorithms and methods will have same features and significance. Main difference is based on properties of these two algorithms.
Data mining procedure is isolated into seven methods:

- *Data cleaning* - Finding a missing/null values in an agricultural dataset
- *Data integration* – Merging a agricultural data from multiple heterogeneous data source into a coherent data
- *Data selection* – Retrieving the data which are relevant for analyzing a crop prediction or yield productivity
- *Data transformation* – Dataset values are transferred from unstructured to structured data
- *Mining techniques* – Applying predictive or descriptive algorithms in agricultural dataset for prediction
- *Pattern estimation* - To find interesting and previously-unknown patterns within said set of data.
- *Knowledge discovery* – Presenting the prediction result in a graphical representation

LITERATURE REVIEW

Yogesh Gandge, Sandhya[10]. had made a study about various data mining techniques to predict the crop yield. They take soil quality as input variable and compare different data mining algorithm and produce the accuracy of the algorithm. Algorithm also used to predict which type of crop can be yield according to the recommendation soil parameter values. The study makes clear that the drawbacks of the algorithm and also to boost the working of the algorithm. Kiran M P. Deepak N[2]. they set a main goal of their research work is to know about the ways to improve the crop prediction by considering some of the parameters are soil, water, atmosphere. pesticides and crop informational collection by using data mining techniques. The Various Classification algorithm utilized in study and experimental results show predicted crop, suitable algorithm and algorithm accuracy in that particular state of India respectively.

Vaishnavi S, Shobana M, Sabitha R, Karthik S[3]. in their research they implement chine Learning techniques for crops recommendation based on its climatic factors and quantity. Agricultural crop recommendations are based on productivity and season. The various machine learning algorithm gives a different accuracy value to predict a crop.
Deone jyoti Bhanudas, Khan rahat Afreen[4]. in this paper they overlook on farmer's choice of crops, irrigation facilities and type of soil to predict the crop. By considering two type of soil Red and Black depending on this type of soil examination is made to find the accuracy of the JRip and Navie Bayes algorithm. In the result, JRip classification technique gives more accuracy as compared to Naïve Bayes. Chandraprabha M, Rajesh Kumar Dhanraj[5]. in their research the evaluation on diverse prediction algorithms like support vector machines (SVM), recurrent neural networks (RNN), K nearest neighbour regression (KNN-R), Naive Bayes, BayesNet, support vector regression (SVR)

are used to predict the crop yield. Finally in the result depend on the error rates and accuracy levels the performance is evaluated. It shows BayesNet has the higher accuracy of about 97.53% and RNN has less percentage error rates that dominate other algorithms in harvest prediction. Prof. Amol Pande, Sarvan Purhit, Shruti Jadhav, Krishba Shah[6]. in their study they develop a web application system for farmers in selecting the suitable crop for their land. More than one parameters like soil color, moisture, ph values, season, rainfall and temperature are taken as input to the system. Applying Decision Tree(ID3 Algorithm), and based on the rule base after getting input from the user the cop will be predicted efficiently. Jharna Majumdar, Sneha Naraseeyappa and Shilpa Ankalaki[11]. they mainly focuses to maximize the crop production and finding the optimum parameter using PAM, CLARA and DBSCAN and Multiple Linear Regression. Comparing these three algorithms, clustering quality can be found better in DBSAN than PAM and CLARA.

METHODOLOGY

In data mining and machine learning, clustering and classification techniques make ingenious information in research and knowledge acquisition from integrated farming. And that produces better solution for the farmers about their cultivation (yield) [14]. A detailed overview of the methodology followed in this paper is explained in Figure 2

Identifying the Issue in Agricultural Field

There are numerous issues was faced by the researchers when analyzing the agricultural field. Most of the research papers that were studied have considered some of the problems they taken to solve in agricultural field.

- Prediction of Crop
- Increasing the yield
- Fertilizer recommendation
- Weather forecasting
- Detection of Crop infection disease

Study of Input Parameters

Input parameters are referred as important factors that is influencing in agriculture for crop production. In this study, data set values are collected from various factors. Table 1 shows the detail about the attributes and their values in the dataset for crop prediction. Table 1 shows the Dataset Input types and its parameters

Preprocessing and Splitting the Data Set

The huge amount of dataset is required for analyzing any application to be made successful. Preprocessing is states that removing a noise values and finding any missing or null values in the dataset. A given dataset is split into training and testing data. Training data is used as train the model which algorithm is selected for prediction. Test data is send to the model and the approximately a result is produced from a model. For evaluation purpose and also to check the accuracy of the algorithm we can send a whole dataset in the percentage ratio of train and test data(80%:20%).

Attribute Selection

The issues stated above can be resolved by choosing the attribute efficiently from the input parameter. According to the extraction of best feature from the dataset we can manipulate the prediction or recommendation in feasible manner. Attribute selection can be differ in multi dimensional view.

For example: Crop prediction can be done based on the two factors such as soil and weather information. By considering these factors, we need to choose a particular attribute for crop prediction in the following manner:

- Combination of soil type and rainfall
- Merging seed and soil nutrients

- Appending weather and irrigation level
- Recommending pest disease and fertilizer

Analyzing the Algorithm or Models

In data mining and machine learning techniques are mainly divided in two groups:

- Classification
- Clustering

Classification techniques are intended to categorize unknown samples using information provided by a set of classified samples. This set is usually referred to as the training set it is used to train the classification technique to perform its classification. In case a training set is not there, then there is no knowledge about the data to categorize. In such cases clustering technique can be used to split a set of unknown samples into clusters [15].. Listing some of the frequent algorithms used in most of the papers for crop predictions is showed in Table. 2

Tools Used for Analysis

According to the issue the researcher were choose either classification or clustering algorithms for predicting the crop. To train the model, algorithms should be implemented in any one of the tool to produce the result such as accuracy, error rate, MAE etc., In this study, the authors were implemented the algorithm in the following tools/languages to find the output:

- WEKA
- Python
- Java
- R Programming

RESULT

An output received from the model is considered as result to predict the crop and production rate. From the confusion matrix we can obtained a True Positive, True Negative, False Positive and False Negative based on the features extracted in the dataset such as soil type Soil Nutrients level, Weather, temperature and rainfall, extract which crop can increase yield production can be forecasted. Comparison is made between the various algorithm by considering the Precision, Recall and F-measures values among it. The result can be represented in graphical manner which algorithm is used to predict the crop, production rate or any other recommendations effectively.

Review of Soil and Weather Factors

Most of the authors in their research papers they used soil and weather factors as input parameters for crop prediction, yield productivity, fertilizer recommendations in efficient way. In this study, Table 3 shows the research papers where the authors taken soil as their input dataset for predicting a crop effectively and Table 4 depicts the algorithms frequencies and chart is displayed in Figure 3.Table 5 listed the papers that the authors had chosen weather as their one of the factor for evaluating the yield production and crop prediction for the farmers. Table 6 shows the algorithm frequencies and Figure 4 displays the chart of algorithm frequencies.

CONCLUSION

Selection of a crop and predicting the yield production is more important for the farmer in their life. Data Mining Techniques achieve this goal very efficiently by implementing the various algorithms. For predicting the crop and yielding we want consider the two important factors that influence in agriculture that is Soil and Weather. In this study, more concentrates take place both on soil and weather factors for crop prediction. According to the soil type and soil parameters, researchers applied a lot of data mining techniques in that J48 algorithm is frequently used in lot of papers is showed in Table 3 and Figure 3 because it achieved a higher accuracy than other algorithms.

Crop prediction can also be done efficiently by considering the Weather, it also helps farmer for more yield in production. Data mining and machine learning techniques are applied to the weather data and finds that K Nearest Neighbor (KNN) algorithm is used most frequently by more researchers to predict the crop productivity is depict in Table 5 and Figure 4 and also it was used to estimate the payout insurance area in the agriculture. In future work, when combining the two important factors such as soil and weather, it is able to predict more suitable crop and increase the yield production efficiently.

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Table 1: Dataset Input types and its parameters

Attributes	Parameters
Soil	Soil Type – Alluvial, Red, Black, Laterite, Desert, Forest Soil Nutrients – Nitroen(N), Phosphorous(pPh), Potassium(K), magnesium(Mg), Calcium(ca), Zinc(Zn), Sulfur(S), Chloride(Cl)
Weather	Rainfall, Humidity, Temperature, Climate
Land	Area of Production
Crop	Crop type, Crop price
Seed	Seed type, length, width, area of perimeter
Water	Irrigation level, Season
Pest	Fertilizers, Pesticides

Table 2: Data Mining Techniques and its Algorithm

Techniques	Algorithms	Illustration
Classification	K-Nearest Neighbor Navie Bayes Decision Tree Random Forest Support Vector Machine(SVM)	Segmenting which crops will grow under the soil type or climatic condition
Clustering	BIRCH DBSCAN PAM Hierarchical Clustering CLARA	Grouping the similar crop details in a chosen attribute
Regression	Liner Multiple Lasso Logistic	To predict the crop, determine the strength of the relationship between soil nutrient with the soil type
Association Rule	Apriori Hashing&Pruning	Discovering important relations between the variables such as soil, temperature, rainfall, crop type in large databases.

Table 3: Data Mining Techniques Applied for Soil Factor in Agriculture

S. No.	Name of the Author	Title of the Paper	Data Mining Techniques Applied	Findings Obtained	Accuracy Value
1	Jay Gholap	Performance tuning of j48 Algorithm for prediction of Soil Fertility	J48	To increase the accuracy level Selection and Boosting techniques are implied	91.90%
2	Dr. K. Arunesh V. Rajeswari	Agricultural Soil Lime Status Analysis Using Data Mining Classification Techniques	J48 Random Tree JRip, OneR Naïve Bayes	Naïve Bayes classification algorithm gives better results	93.81%
3	B.Jayalakshmi, M.Savitha Devi	Soil Fertility Prediction for Yield Productivity and	J48 Navie	J48 classifier achieve higher performance for predicting	97.6%

		Identifying the Hidden Factors through Machine Learning Algorithms	Bayes REPTree	the corn cropwith the soil attributes	
4	S.S.Baskar, L.Arockiam, S.Charles	Applying data mining techniques on Soil fertility Prediction	Regression J48 JRip Navie Bayes	J48 Algorithm produces a high accuracy level than other algorithms	93.86%
5	K.Samundeeswari Dr.K.Srinivasan	Data Mining Techniques In Agriculture Prediction Of Soil Fertility	J48 JRip Navie Bayes	Based on soil classification, J48 produces high accuracy than other algorithms to predict the crop	93.86%
6	Vrushali Bhuyar	Comparative Analysis of classification techniques on soil data to predict fertility rate for Aurangabad District	Random Forest J48 Navie Bayes	J48 classifier perform better to predict fertility index and also to recommened fertilizer	98.17%
7	N. Saranya, A. Mythili	Classification of Soil and Crop Suggestion using Machine Learning Techniques	K-Nearest Neighbour (K-NN) Support vector machine(SVM) Logistic regression	SVM has obtained the maximum accuracy based on soil and crop database	96%
8	Navneet Nasib Singh Gill	Algorithm for Producing compact decision trees for enhancing classification accuracy in fertilizer recommendation of soil	J48 J48 & K-Means Schwarz Criterion (SC)-Decision tree	Incorporating both classification and clustering, produce a result more accurate for fertilizer recommendation	97.17%
9	Keerthan Kumar T G Shubha C Sushma S A	Random Forest Algorithm for Soil Fertility Prediction and Grading using Machine Learning	Random Forest SVM Navie Bayes	By analyzing the important soil properties, Random Forest Classifier which gives the best and accurate output to predict the crop	72.74%
10	Anguraj.K Thiyaneswaran. B Megashree.G Preetha Shri.J.G Navya.S Jayanthi.J	Crop Recommendation on Analyzing Soil Using Machine Learning	Random Forest Navie Bayes Gradient Descent	Soil data are collected from sensors using IOT and ML Technique such as Gradient Descent have produced high accuracy to chose the precise crop	96.89

Table 4 Comparison of Algorithm Types and its Frequency of used

Algorithm Type	Frequency
Random Forest	1
Navie Bayes	1
Support Vector MAchine	1
J48	5
Gradient Descent	1
Schwarz Criterion (SC)- Decision tree	1

Table 5: Data Mining Techniques Applied in the Agriculture by Considering Weather

S.No.	Name of the Author	Title of the Paper	Data Mining Techniques Applied	Findings Obtained
1	SanjayD.Sawaitul, Prof.K.P.Wagh& Dr.P.N.Chatur	Classification and Prediction of Future Weather by using Back Propagation Algorithm An Approach	Neural Networks	Focuses on weather forecasts to increase the yield
2	V.K.Somvanshi, *et al.,*	Modeling and prediction of rainfall using artificial neural network and ARIMA techniques	Neural Networks	Prediction of rainfall for yielding
3	S.Veenadhari, Dr. Bharat Misra, Dr. CD Singh	Data mining Techniques for Predicting Crop Productivity	Decision Trees	kharif and rabi crops production effected by climatic factors
4	Tripathi, S., Srinivas, V. V., & Nanjundiah, R. S.	Downscaling of precipitation for climate change scenarios: a support vector machine approach	Bayesian network	Using Bayesian network learning method developing the model for agriculture
5	Shalvi D & De Claris N,	Unsupervised neural network approach to medical data mining techniques	KNN	Daily precipitations simulation of weather and other conditions
6	Altannar Chinchulunn, Petros Xanthopoulos, Vera Tomaino, P.M.Pardalos	Data Mining Techniques in Agricultural and Environmental Sciences	Support Vector Machine	Classifying the weather sample information into linearly severable
7	B. Rajagopalan & U. Lal	A K-nearest neighbor simulator for daily precipitation and other weather variable	Support Vector Machine	Conducting climate impact studies
8	K.P.Mangani, R.Kousalya	Designing Weather Based crop insurance payout estimation based on Agro-Meteorological data using Machine Learning Techniques	KNN (CBKNN-PAYRULE)	Estimate and Predict the weather insurance payout
9	M Ramzon Talib, etl.,	Application of Data mining techniques in weather data	KNN & Decision Tree	Examining the changing patterns of

		analysis	Algorithm	weather parameters
10	M.Mayilvaganan, P.Anitha	Correlation Analysis of meterological data in region of tamilnadu districts based on K-Mean Clustering Algorithm	KNN (K-Mean Clustering Algorithm)	Analyze the reliability factors of weather information

Table 6: Frequency of the Algorithm usage

Algorithm Type	Frequency of Used
Neural Networks	2
Decision Trees	1
Bayesian network	1
Support Vector Machine	2
KNN	4

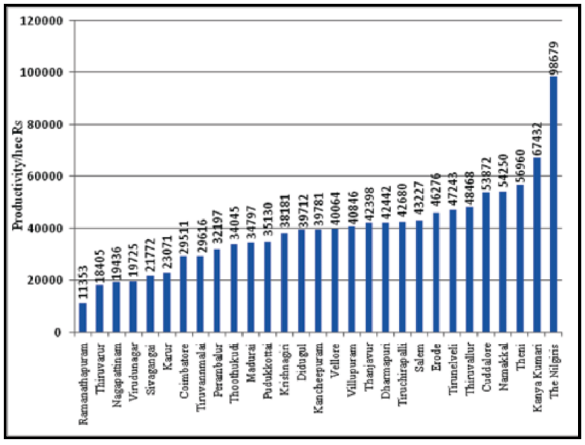

Figure 1: Productivity Details

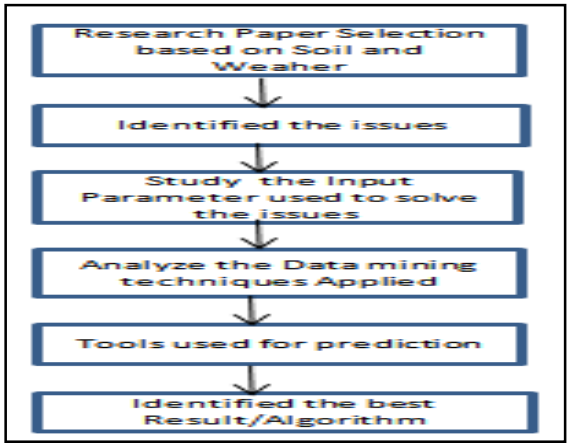

Figure 2: Process of Systematic Literature Study (SLR)

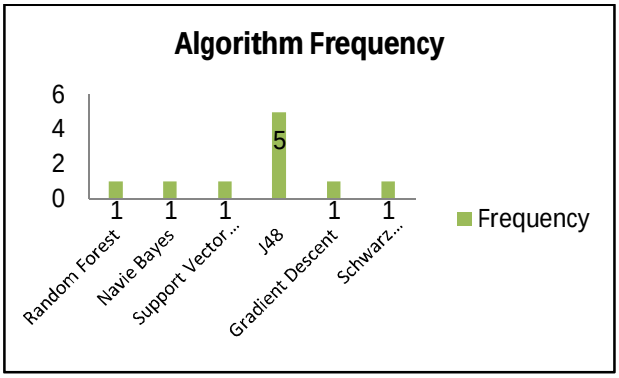

Figure 3: Comparison of Algorithm and its Frequency

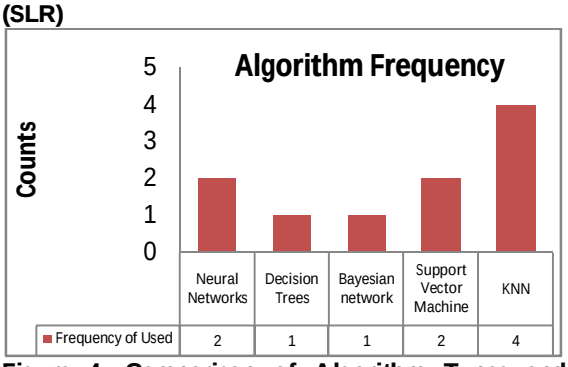

Figure 4: Comparison of Algorithm Types and Frequency of used

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RESEARCH ARTICLE

Simultaneous Estimation and Validation of Paracetamol, Cetirizine Hydrochloride and Phenylephrine Hydrochloride Inbulk and Tablet Dosage Form by using Different Spectrophotometric Method

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ABSTRACT

A simple, precise, accurate and economic simultaneous UV spectrophotometric method has been developed for the estimation ofParacetamol, Cetirizinehydrochloride and Phenylephrine Hydrochloride in combination in bulk mixture and tablet. The estimation was based upon measurement of absorbance at absorbance maxima of 248nm, 230 nm and 215 nm for Paracetamol, Cetirizinehydrochloride and Phenylephrine Hydrochloride in methanol, respectively in bulk mixture and tablet. The Beer Lambert's law obeyed in the concentration range 4-20µg/ml, for Paracetamol, Cetirizinehydrochloride and Phenylephrine Hydrochloride respectively. The estimation of bulk mixture and tablet was carried out by simultaneous equation, Q-analysis and area under curve method for estimation of Paracetamol, Cetirizine hydrochloride and Phenylephrine Hydrochloride. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICHguidelines.

Keywords: Paracetamol, Cetirizinehydrochloride, Phenylephrine Hydrochloride, Ultraviolet spectroscopy, Simultaneous equation method, Absorption Ratio Analysis Method, Area Under Curve Method.

INTRODUCTION

Paracetamol (PARA) is chemically N-(4-hydroxyphenyl) acetamide,It has analgesic and antipyretic activity [1]. Various analytical methods, such as, spectrophotometry [2,3], HPLC [4,5], HPTLC[6] have been reported for the estimation of paracetamol from its formulations. Phenylephrine Hydrochloride is [(R)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride], and used as alpha-adrenergic, sympathomimetic agent as well as

vasoconstrictor with little effect on the myocardium or the central nervous system [1]. From literature survey Phenylephrine Hydrochloride has been determined alone or in combination by using UV spectrophotometry [2,3], HPLC[5], RP-HPLC[7]methods. Cetirizine Hydrochloride used as an antihistaminic, antiallergics. A mixture of this combination is widely used as ananalgesic, antipyretic, decongestant and antihistamine. A combination of paracetamol, phenylephrine hydrochloride, Cetirizine Hydrochlorideis commercially available in tablet dosage form. Literature reveals that no analytical method is available for simultaneous determination of these three drugs in combination. So we communicate here rapid and cost- effective quality-control tool for their routine quantitative analysis in pure and combined dosage forms by spectrophotometry.

MATERIAL AND METHOD

Materials

UV-visibledoublebeamspectrophotometer,Shimadzumodel1900i with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. The commercially available tablet, Oncet - CF (Label claim: Paracetamol I.P.-500 mg, Cetirizine hydrochloride 5mg and Phenylepinephrine hydrochloride 10mg) was procured from local market, Methanol, API of Paracetamol, Cetirizine hydrochloride and Phenylepinephrine Hydrochloride.

Selection of common solvent

After assessing the solubility of drugs in different solvents Methanol was used as common solvent for developing spectral characteristics.

Preparation of standard stock and calibration curves

The standard stock solutions (1000 µg/ml) of each of Paracetamol, Cetirizine hydrochloride and Phenylephrine HCl were prepared separately by dissolving accurately about 50 mg of API in 50 ml of Methanol and volume was madeup to 100 ml with methanol. Working standard solutions of 20 µg/mlwere scanned in the entire UV range of 400-200 nm to obtain the absorbance. Solutions of 20 µg/ml of Paracetamol, Cetirizine hydrochloride and Phenylephrine HCl were prepared separately. All these solutions were scanned in the spectrum mode from 200 - 400 nm. The maximum absorbance of Paracetamol, Cetirizine hydrochloride and Phenylephrine HCl were at 248 nm, 230 nm, 215 nm respectively. Paracetamol, Cetirizine hydrochloride and Phenylephrine HCl showed linearity in the concentration range of 4-20µg/ml at their respective maxima. Accurately measured standard stock solution of Paracetamol, Cetirizine hydrochloride and Phenylephrine HCl (2ml, 4ml, 6ml, 8ml, 10ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with Methanol. The absorbance of resulting solutions were measured at their respective max and plotted a calibration curve against concentration to get the linearity and regression equation.

Method 1: Simultaneous Equation Method

Simultaneous equation method of analysis is based on the absorption of Paracetamol, Cetirizinehydrochloride and Phenylephrine HCl at the wavelength (λ- max) of each other.λ-max for Paracetamol, Cetirizine hydrochloride and Phenylephrine hydrochloride are 248 nm, 230 nm and 215 nm respectively. The absorptive values determined at 248 nm, 230 nm and 215 nm for Paracetamol 0.037855 (az1), 0.049965 (az2), 0.090490 (az3), for Cetirizinehydrochloride 0.029583(ay1), 0.030408 (ay2), 0.00294(ay3) and for Phenylephrine hydrochloride 0.030205 (ax1), 0.011730 (ax2), 0.001073 (ax3). These values are means of five estimations. The absorptive coefficients were substituted in equation 1,2 and 3 to obtain the concentration of drugs [2,3,10-12].

A1 = 0.090490 x Cpa + 0.0002940 x Cct + 0.001073 x CPh (1)

A2 = 0.049965 x Cpa + 0.030408 x Cct + 0.011730 x CPh (2)

A3 = 0.037855 x Cpa + 0.029583 x Cct + 0.030205 x CPh (3)

Where, CPA, CCT and CPH are concentrations of Paracetamol,Cetirizine hydrochloride and Phenylephrine Hydrochloride respectively inμg/mL, and A1, A2, and A3 are the absorbance of the sample at 248 nm, 230 nmand 215 nm respectively.

Concentration	Absorbance		
	Phenylephrine HCl	Cetirizine HCl	Paracetamol
Lambda max	215	230	248
0	0	0	0
4	0.129	0.109	0.417
8	0.245	0.259	0.662
12	0.372	0.378	1.146
16	0.468	0.493	1.332
20	0.558	0.602	1.734

Method 2: Absorption Ratio Method

For Q method, 242 nm (isobestic point) and 248 nm (λ-max of PARA) were selected for PARA and PHEN as wavelengths of measurements 250 nm (isobestic point) and 215 nm (λ-max of PHEN) were selected for PHEN and CTHZ as wavelengths of measurements. 234 nm (isobestic point) and 230 nm (λ-max of CTHZ) were selected for CTHZ and PARA as wavelengths of measurements. Concentrations of PARA, CTHZ and PHEN were determined using following equations[2,3,10-13].

$$CPARA = \frac{(Qm1 - QPHEN)}{(QPARA - QPHEN)} X \frac{A1}{aPARA1} \quad \text{.....(4)}$$

$$CPHEN = \frac{(Qm2 - QCTHZ)}{(Q\,PHEN - QCTHZ)} X \frac{A1}{aPHEN1} \quad \text{.....(5)}$$

$$CCTHZ = \frac{(Qm3 - QPARA)}{(QCTHZ - QPARA)} X \frac{A1}{aCTHZ1} \quad \text{.....(6)}$$

Where; Qm1 = A2 / A1, Qm2=A3 / A2, Qm3=A4 /A3, QPARA = aPARA2/ aPARA1, QPHEN = aPHEN2 / aPHEN1, QCTHZ=aCTHZ2 /aCTHZ1,

A2= Absorbance of Mixture at 248nm, A1= Absorbance of Mixture at 233 nm, A3= Absorbance of Mixture at 250 nm, A4= Absorbance of Mixture at 234 nm

aPARA1= absorptivity of PARA at 233 nm, aPHEN1= absorptivity of PHEN at 233 nm, aPARA2= absorptivity of PARA at 248 nm, aPHEN2= absorptivity of PHEN at 248 nm, aPHEN1= absorptivity of PHEN at 250nm, aCTHZ1= absorptivity of CTHZ at 250 nm, aPHEN2= absorptivity of PHEN at 215nm,aCTHZ2= absorptivity of CTHZ at 215nm,

aCTHZ1= absorptivity of CTHZ at 234 nm, aPARA1= absorptivity of PARA at 234 nm, aCTHZ2= absorptivity of CTHZ at 230nm, aPARA2= absorptivity of PARA at 230nm

Method 3: Area under Curve Method

Area under curve method for PARA and PHEN in presence of CTHZ

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (250 μg/ml) of PARA, PHEN and CTHZ were prepared separately in Methanol. The solutions of drugs were scanned in the range of 200-400 nm. For Area Under Curve method, calibration curve was plotted and the sampling wavelength ranges selected for estimation of PARA, PHEN and CTHZ are 244 nm - 255 nm (λ1-λ2) and 208 nm - 220 nm (λ3-λ4) and 225 nm – 237 nm (λ5-λ6) respectively and area were integrated between these selected wavelength ranges for three drugs, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations. By using integrated areas three simultaneous equations were constructed and solved to determine concentrations of analytes [14,15].

$$C\ PARA = \frac{XPHEN\ at\ (254-265)\ x\ AUC\ PHEN\ at\ (232-244) - XPHEN\ at\ (232-244)\ x\ AUC\ PARA\ at\ (254-265)}{XPHEN\ at\ (232-244)\ x\ XPARA\ at\ (232-244) - XPHEN\ at\ (232-244)\ x\ XPARA\ at\ (254-265)} \ldots(7)$$

$$C\ PHEN = \frac{XPARA\ at\ (232-244)\ x\ AUC\ PARA\ at\ (254-265) - XPARA\ at\ (254-265)\ x\ AUC\ PHEN\ at\ (232-244)}{XPHEN\ at\ (254-265)\ x\ XPARA\ at\ (232-244) - XPHEN\ at\ (232-244)\ x\ XPARA\ at\ (254-265)} \ldots(8)$$

Where; $C_{PARA}$and C_{PHEN}-Concentration of PARA and PHEN, respectively, $AUC_{PARA}$and AUC_{PHEN}- Area under curve of PARA and PHEN in bulk mixture.

Similar procedure was applied for determination PARA and PHEN in tablet solution.

Estimation of CHTZ in presence of PARA and PHEN by standard curve method

The absorbance of standard CHTZ solutions at different concentration ranging from 4-20μg/ml at 230 nm was measured. The regression equation was established by plottingthecalibrationcurveofabsorbanceVsconcentration. The absorbance of bulk mixture and tablet solution was measured at 230 nm for CHTZ. The concentration of CHTZ was estimated by regression equation,

$$y = 0.0306x + 0.001$$

Where, y-absorbance of CHTZ in bulk mixture, x- concentration of CHTZ in tablet solution

Analysis of the tablet formulations

Ten tablets of marketed formulation were accurately weighed and powdered. Standard addition method was used for analysis of drugs. A quantity of powder equivalent to 50 mg of Paracetamol was weighed and dissolved in 100 ml of Methanol. Then the solution was filtered through Whatman filter paper no 41. From the above 10 ml of solution was diluted to 50 ml with Methanol to get 100 μg/ml of Paracetamol and corresponding Phenylephrinehydrochloride and Cetirizine hydrochloride. From above 2.5 ml of solution was transferred in 10 ml volumetric flask. To this add 0.2 ml of stock solution (250 μg/ml)of pure Phenylephrine Hydrochloride and Cetirizine hydrochloride and make-up volume up to the mark with Methanol. The purpose of this addition is to bring the concentration of Phenylephrine hydrochloride and Cetirizine hydrochloride in linearity range. With this addition, concentration of Paracetamol, Phenylephrine Hydrochloride and Cetirizine hydrochloride inthesampleswasbroughtto2.5, 5.5 and 5.1 μg/ml respectively. Analysis procedure was repeated five times with tablet formulation and result reported in Table 1.

Validation

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range is 4-20μg/mL for all drugs. The linearity data for all methods are presented in Table 3 [1,16].

Accuracy

Accuracy of the developed method was confirmed by recovery study as per ICH norms at three different concentration levels of 80 %, 100 %, 120 % by replicate analysis (n = 3). Here to a pre analyzed sample solution, standard drug solutions were added and then percentage drug content was calculated. The result of accuracy study was reported in Table 2. The recovery study indicates that the method is accurate for quantitative estimation of Paracetamol, Phenylephrine Hydrochloride and Cetirizine hydrochloride in tablet dosage form as the statistical results are with in the acceptance range (S.D.<2.0).

Precision

Precision was determined by studying the repeatability and intermediate precision.

Repeatability

Repeatability result indicates the precision under the same operating conditions over a short interval of time and inters- assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for five times with tablets formulation. The results of statistical evaluation are given in Table 1.

Intermediate Precision (Inter-day and Intra-day precision)

An intermediate precision was carried out by intra and inter day precision study. In intra-day study concentration of drugs were calculated on the same day at an interval of one hour. In inter day study the drug contents were calculated on three different days. Study expresses within laboratory variation in different days. In both intra and inter-day precision study for the methods % COV were not more than 1.0 indicates good intermediate precision (Table3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Paracetamol, Phenylephrine Hydrochloride and Cetirizine hydrochloride by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 /S and 10 /S respectively, where S is the slope of the calibration curve and is the standard deviation of response. The results of the same are shown in Table 3.

RESULT

The proposed methods for simultaneous estimation of PARA, PHEN and CTHZ in combined dosage form were found to be accurate, simple and rapid which can be well understood from va1lidation data as given in Table 3 and 4. The % R.S.D. Linearity was observed by linear regression equation method for PARA, PHEN and CTHZ in different concentration range. The Correlation coefficient of these drugswasfoundtobecloseto1.00, indicating good linearity. The assay results obtained by proposed methods as shown in Table 2, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. It can be easily and conveniently adopted for routine quality control analysis. These methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

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Table 1: Analysis data of tablet Formulation

Method	Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.	% C.O.V.	S.E.
I	PARA	500	495.411	99.08	0.7608	0.7563	0.3106
	PHEN	10	9.957	99.57	0.1625	0.1611	0.0665
	CTHZ	5	4.950	99.01	0.5137	0.4987	0.2105
II	PARA	500	495.0	99.10	0.5674	0.7811	0.3086
	PHEN	10	10.11	100.05	1.0077	1.2015	0.3537
	CTHZ	5	4.901	98.02	0.5480	0.4557	0.12227
III	PARA	500	486.98	94.55	0.2640	0.2640	0.1080
	PHEN	10	9.8732	97.32	1.0775	1.0865	0.4424
	CTHZ	5	4.900	98.01	0.9230	0.9289	0.3562

Table 2: Result of recovery studies

Method	Recovery level (added amount)	Drugs		
		PARA	PHEN	CTHZ
I	80 %	99.99 ± 0.1456	99.20 ± 0.1445	100.20 ± 0.3538
	100 %	98.90 ± 0.2052	99.70 ± 0.4567	98.89 ± 0.1406
	120 %	98.50 ± 0.6384	98.30 ± 0.1423	100.30 ± 0.3578
II	80 %	99.40 ± 0.2395	98.95 ± 0.5569	99.5 ± 0.0856
	100 %	100.30 ± 0.6568	99.90 ± 0.7560	99.32 ± 0.0856
	120 %	99.80 ± 0.3401	100.1 ± 0.3382	99.90 ± 0.0704

III	80 %	99.60 ± 0.1423	99.47 ± 0.3985	98.95 ± 0.9345
	100 %	99.75 ± 0.2376	98.92 ± 0.6258	99.47 ± 0.5641
	120 %	98.89 ± 0.2576	99.87 ± 0.5832	98.83 ± 0.3281

Table 3: Optical Characteristics data and validation parameters

Parameter	**Values**		
Drugs	PARA	PHEN	CTHZ
Working (nm)	248	215	230
Beer's law limit µg/ml	4-20	4-20	4-20
Absorptive	0.0724	0.0430	0.0424
Correlation coefficient	0.9912	0.9954	0.9977
Intercept	0.0319	0.0143	0.001
Slop	0.085	0.0281	0.0306
LOD	0.4607	0.0785	0.3518
LOQ	1.6078	0.6851	0.5365
Intra-day (precision) (% C.O.V.)	0.7136	0.3213	0.3813
Inter-day (precision) (% C.O.V.)	0.9728	0.9588	0.5701

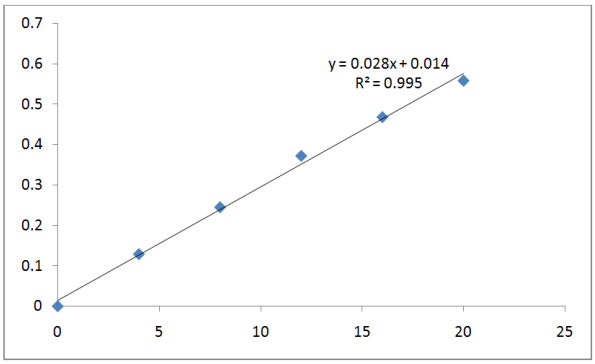

Fig 1 Calibration Curve of Phenylephrine HCl in Methanol

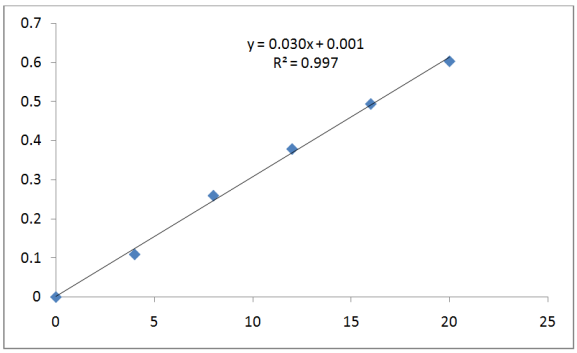

Fig 2 Calibration Curve of Cetirizine HCl in Methanol

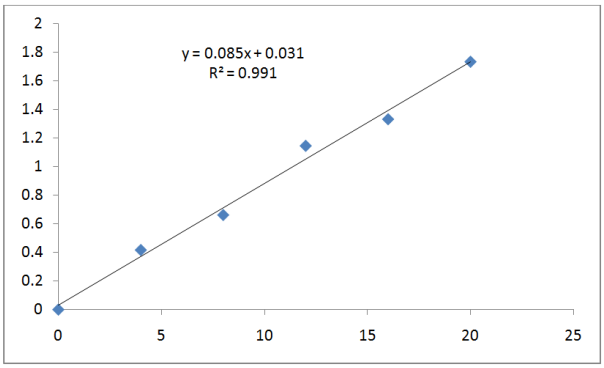

Fig 3 Calibration Curve of Paracetamolin Methanol

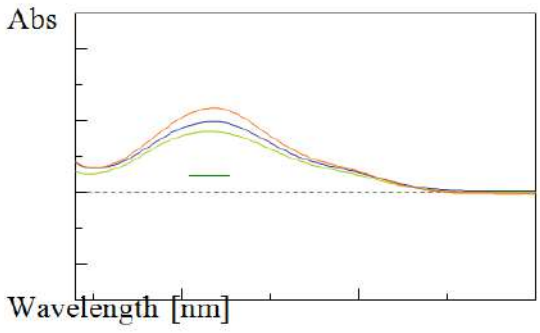

Fig 4: Overlay spectrum of Paracetamol

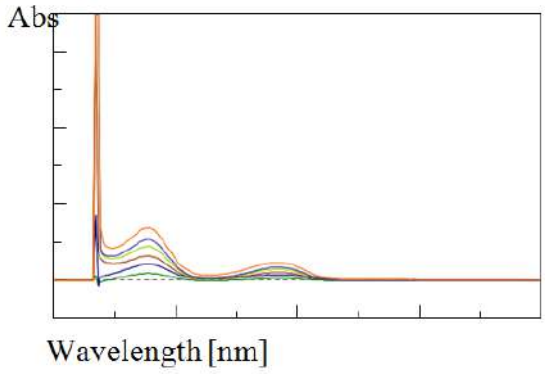

Fig 5: Overlay spectrum of Phenylephrine

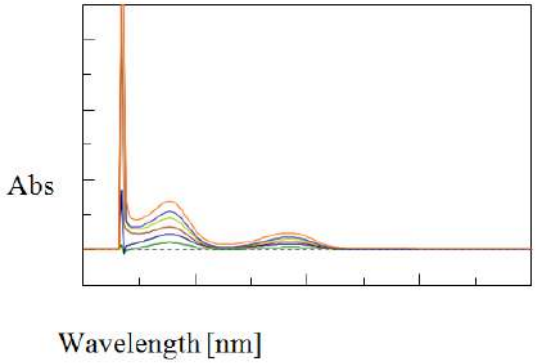

Fig 6: Overlay spectrum of CTHZ

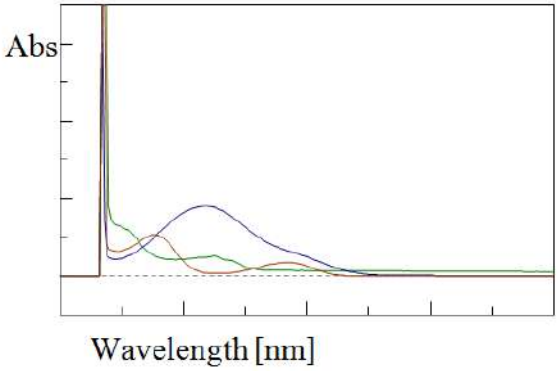

Fig 7: Overlay spectra of PARA, PHEN and CTHZ

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RESEARCH ARTICLE

Insolvency and Bankruptcy (Amendment) Act, 2021- Integration and Analysis of Recent Literature

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ABSTRACT

Whole world is going through health crisis amid COVID-19 since 2019 December. In India the Pandemic raged in havoc situation since March 2020. Due to this country been seeing many indirectly related crisis like unemployment, hunger, market uncertainties etc. MSME sector on which India's large population is dependent for daily earnings were also affected. Many MSMEs are indebted to lenders because of slowdown in businesses. Due to which many of them are at verge of closing down that highly impact the employment and GDP statistics of the Country. One survey has shown that disruptions caused by the Covid-19 pandemic have impacted MSMEs earnings by 20-50%, micro and small enterprises faced the maximum heat, mainly due to liquidity crunch. To overcome the situation President of India promulgated The Insolvency and Bankruptcy Code (Amendment) Act, 2021 on 4th April 2021. This review paper incorporates the latest literature available in 2021 and the review of experts on passing of the ordinance. It will help in giving brief assistance to the researcher on the present topic to develop future research points.

Keywords: Pre-Packs, MSME, Insolvency, COVID-19

INTRODUCTION

India has been facing financial, economic crisis since COVID-19 has entered the country, strict lockdowns has caused loss in earnings, reduced demands, cash-flow shortage, un-employment etc. Micro, small, medium enterprises are largest producer of GDP and employment after agriculture in India. New entrepreneurs are highly dependent on lending company and finance banks for their capital requirements, due to the pandemic businesses have been seeing steep decrease in earnings, which in turn caused a debtor stress to payback loan amounts on time. Insolvency and

Bankruptcy code 2016 has been dealing with the distressed company through corporate insolvency resolution process. But the process took more time than stipulated in the code, which cause slow recovery, decrease in value of assets of corporate. This caused the object of resolution at expected price got defeated. Pre-packs are blend of informal and formal process of insolvency that consumes less time and money, also the value of the business doesn't get depreciated due to pre-arranged agreement between the investors and corporate debtors. The process is prevalent in USA and U.K. India in respect of MSMEs has promulgated the amendment ordinance on 4th April 2021 to resolute the insolvent corporate through Pre-Packs. On 11th August 2021 the Act to this effect received the assent of President of India. It is expected to focus on the fast insolvency and resolution process of distressed MSMEs due to COVID-19 in efficient way.

Methodology for the Review

The Literature is present on the basis of researcher's view point on the pre-packs utility. As ordinance has not been implemented to its full form. It is interesting to read the viewpoints of experts in the particular field to examine the further aspects and reaction the ordinance. This paper will review 22 literature items.

S.No	Author	Title	Key Takeaways	Observation/ Conclusion
1.	Gupta, Rehan,(2021)	Pre-Pack- New Stress Resolution Tool in India.	The pre-packs are cost effective, consumes less time and also helps in preserving the value of business. But India should be cautious about the dependency of Players of Indian debt market on scrutiny from various regulatory and investigating agencies. "Safe Harbor' rule from US can be adopted.	Positive impact of Pre-Packs will be seen in recovering MSMEs from COVID-19 downfall.
2.	Jain, (2021)	Analysis of Pre-Packaged Insolvency Resolution Process Under IBC Code(Amendment) Ordinance, 2021	The debtor-in-possession strategy would grant substantial consent privileges to financial creditors. Proposal assessment mechanism will create competitive tension which would result in least impairment to the interest of creditors.	Positive about the Pre-Packs in MSME but also shows concerns regarding NCLT facilities that can affect the pre-pack scheme.
3.	Sinha, (2021)	Ordinance Passed in Insolvency and Bankruptcy Code, 2020: A Menace for Indian Financial Institutions.	Emphasizing on the need of Pre-Packs in all sectors, not restricting to sector. Will decrease dependency and streamline the concept of Atmanirbhar.	Negative about the conventional insolvency process.
4.	Sharma , Jain (2021)	Pre-Packaged Insolvency Resolution Scheme For MSMEs- An Insight Into.	For best implementation, there is need of regular monitoring on Insolvency professional's standards in terms of working.	Positive impact with few concerns like less loan amount involved in MSME , that may not be beneficial in Pre-Packs

5.	Sharma, Ajmera (2021)	The Pre-Pack Ordinance 2021 - The Challenges And Way Forward	Act doesn't covers all MSME, Benefit can be seek only by registered ones which substantially impede the 94% from availability of process. Ordinance doesn't provide any mechanism in case Pre-Pack Fails. Which would lead to CIRP.	Negative about the drafting.
6.	Prasad, (2021)	About 60% of all Active Cos are eligible for Pre-Pack Turnaround Scheme: MCA	With a minimum default of 10 lakh will exclude the MSMEs having less loan amount. Because of Lack of sufficient infrastructure and manpower will affect the implementation.	Does only tell positive and negative aspect without forming any opinion.
7.	Unnikrishnan, (2021)	Explained: Why pre-packaged insolvency resolution is great for MSME borrowers	It will be a win-win situation for both borrower and creditors. As borrower has chance to regain control over the company and creditors will be able to complete it in less time that is 120 days. But the concern is MSMEs are not resolved through IBC as the default amount is less. Will it be beneficial?	Positive about the draft but skeptical about the efficacy of Ordinance.
8.	Singh, (2020)	Pre-Packaged Insolvency in India: Lessons from USA and UK.	Pre-Packs in India are untested and hence the implementation should be experimental and concise first. In USA the cases are filed to district courts first, from there the matter is referred to specialized bankruptcy court which comprises of dedicated bankruptcy judges, similarly India can adopt it by renovating infrastructure in subset forms of NCLT. The powers of IRP to be defined in extensive manner. To ensure creditors cooperation in the process they should be incentivized.	Concerned regarding niche market in India and stresses on desirable changes in start.
9.	Singh, (2021)	Why Most MSMEs are not eligible for the Pre-Pack insolvency resolution Process.	There is restricted applicability of the resolution process. It only applies to company and limited liability partnerships and keeps sole proprietorship and HUFs out of purview. Because the date of incorporation is mandatory will restrict many MSMEs from the benefit of ordinance.	Negative about the effectiveness

10.	The Indian Express, (2021)	Pre-Pack Scheme for MSMEs will bring down Costs, Lead to faster resolution of cases.	Considering the present data of recovery rate through Conventional CIRP method around 86.3 percent of total registered cases are undergoing the process in the NCLT for more than 270 days. Anticipating that it will positively decrease the burden of the tribunals and hence accelerates the recovery rate.	Positive impact.
11.	Financial Express, (2021)	Pre-Pack insolvency for MSMEs: How it differs from corporate insolvency resolution process; Key features	Briefly discussed the salient features of Pre-Pack resolution process which were not present in the CIRP. Like restriction on running CIRP and Pre-pack in parallel, moratorium period that will save the debtor from numerous suits.	Neutral opinion.
12.	BFSI, (2021)	How the Pre-Packaged Insolvency scheme for MSMEs will operate	Highlighting the role of Resolution Professional, the role will be decreased to administration of property of debtor to suggesting and coordinating the whole process. The right RP can lead to the plan work in good direction.	Positive impact on the MSME sector.
13.	Financial Express, (2021)	Pre-Packaged Insolvency resolution under IBC for MSMEs: Challenges that may rise in implementation	As the Base resolution plan is to be submitted by debtor it may face some issues like hesitation in raising additional capital from investor in fear of non-recovery from the lenders. Another issue that may arise is the timeline for approving the resolution plan, according to the researcher it is less. Researcher shows concern about the corporate and personal guarantors as the ordinance is not clear whether they will be released on approval of base resolution plan or application against them can be filed in NCLT.	Negative as it lacks clarity on some points.

14.	Rathi, (2021)	Can IBC Pre-Packs be the "Six Packs" for MSMEs?	The most promising aspect of the PIRP is defaulters can still work during the settlement process. The creditors will be more attracted towards prepacks because of its benefits of moratorium and washout of all liabilities. In the process lot of homework need to ready beforehand which includes agreement of financial creditors, shortlisting of resolution applicant. Pre-packs in companies need to be implemented with more caution in respect to debtor-in-possession.	Positive impact.
15.	Chawla, (2021)	Can Pre-Packaged insolvency resolution under IBC be helpful for MSME?	The ordinance in silent discretionary power of NCLT on admitting Corporate insolvency resolution process during PPIP. There is no withdrawal clause of the process.	Warning to all MSMEs to look into the issues related to the ordinance.
16.	Mittal, (2021)	Pre-packs for MSME-A Vaccine that doesn't work?	The process seems costly as it requires three fold approval from different stakeholder. The DIP is infructuous as the power lies in committee of creditors.	Negative impact of pre-packs.
17.	Ajmera, Nigam (2021)	Pre-Pack Paradigm Vis-à-vis Indian Insolvency Laws	CIRP process took 415 days on average to complete the process of insolvency. That can be regularized by Pre-Packs. Features like confidentiality, control of business under existing management will increase the preservation of jobs of existing employees. Concern has been shown for unsecured creditors as their value can be captured by stakeholders	Positive impact if the implementation is done properly.
18.	Ravi, (2021)	The next phase of the IBC- Introducing the pre-packs for MSME	Relaxations from Section 29A need to be addressed in relation to pre-packs for other entities as it would demotivate corporate debtor to initiate the process.	Positive Impact.

19.	Singh, (2021)	IBC Amendment ordinance 2021- A pre-pack resolution?	Process seems complicated to the author. As it covers two fold approval, it will become time consuming. Option of commencing CIRP at any time may cause additional delay.	Negative
20.	EPA Law Offices	Insolvency and Bankruptcy Code (Amendment) Ordinance 2021	It will provide in time remedy and increase in number of resolution per year in the adjudicating authority.	Positive
21.	Karwa, Karwa (2021)	A Critique of 'Debtor-in-possession model' Under Pre-Pack Insolvency in India	Powers given to Resolution Professional are limited in nature as compared to the responsibilities he has to deliver. It contradicts the jurisprudence of insertion of section 29A of the IBC 2016.	Negative
22.	Rao, 2021	Insolvency Procedures- Investigating The Pre-Pack Paradigm in India	The interest of creditors may dissolve over the debtor's interest. Unsecured creditors will have no say in the transaction. But it will be helpful in curbing NPA.	Both positive and negative for specific areas

CONCLUSION

Majority of experts are in opinion that the said ordinance would be helpful for distressed MSME business in viable manner. Few of them are concerned as to how it will be implemented, the changes need to be done according to the coming market situation and MSME ease. COVID 19 distress has effected most of the MSME but pre-packs will benefit them is yet to be seen. There are also negative opinion towards the new move. Like it will be "create an impression that the corporate debtor shall conduct a meeting of all its unrelated OCs – in view of the fact that the number of OCs may be significantly higher *vis-à-vis* FCs, the process ought to become costly, both in terms of cost and time" expressed by *Megha Mittal*. Overall the review of the above literature concludes that it would be beneficial for MSMEs, the amendments can be brought after reviewing its utility in market.

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RESEARCH ARTICLE

Advanced Preservation Fresh Freeze Technology used for Medicinal Plants

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ABSTRACT

Freezing technique is one of the perseveration process and it is the old method of preservation compared with recent preservation technique, and most generally used methods for drug preservation, which preserve the taste, and nutritional value in pharmaceutical product which is best than the other methods. The process of freeze can be carried out under completely sterile conditions, thereby prevention microbial contamination. The fresh freeze technology is formed by Japan; it is one of the Japan's top inventions in freezing technology. The fresh freeze that focused to assist freeze drug without destroying its cell structure a "fresh freezing" system. This technique has been with success applied to numerous biological materials, like meats, coffee, juices, farm product, cells, and bacterium, and is customary observe for antibiotic drug, hormones, plasma, victuals preparations, etc. Freezing could be a very well-established drug preservation process that produces long storage life to the drug and will be maintains the standard of the pharmaceutical product.

Keywords: Freezing, Preservation, Cryopreservation, Pisum sativum, Biologicals, Plant based product.

INTRODUCTION [1-5]

Freezing is technique is one of the perseveration process and it is the old method of preservation compared with recent preservation technique, and most generally used methods for drug preservation, which preserve the taste, texture, and nutritional value in pharmaceutical product which is best than the other methods. The freezing process could even be a combination of the beneficial effects of low temperatures at which microorganisms cannot grow,

chemical reactions are reduced, and cellular metabolic reactions are delayed. Freezing well is an extremely well-established drug preservation process that produces long storage self-life to the drug and may be maintains the standard of the pharmaceutical product. However, freezing is not suitable for all drugs, and freezing can cause physical and chemical changes in some drugs that are consider as reducing the quality and standard of either the defrosted material or the end product. The physical state of drug material is modified when energy is removed by cooling below freezing temperature at 18°C. Freezing methods are employed like as cryogenic freezing, air blast freezing, indirect contact freezing, direct contact freezing, and immersion freezing. These traditional methods, it is very difficult to preserve the perishables because there occurs discoloration. Several emerging technologies are recently proposed for ice nucleation control during freezing.

HISTORY OF FREEZE TECHNOLOGY [6-8]

Freezing technique used as a way of preservation, and history reveals it had been mostly shaped by the technological developments within the process low quantity of ice produced without employing a "natural cold" in 1755 was considered the primary discovery within the freezing process. The process of freeze-drying was invented in 1906 by Arsened Arsonval and his assistant Frederic Bordas at the laboratory of biophysics of school de France in Paris. In 1911 Downey Harris and Shackle established the lyophilization process of preserving live rabies virus which eventually led to development of the first antirabies vaccine. Freeze drying was first actively established during World War-II for transport of serum. The fluidized bed freezer, can be a recent modified reasonably air-blast freezer for particular product types, consists of a bed with a perforated bottom through which cold air is blown vertically upwards Rahman in 1992. The fresh freeze technology is formed by Japan; it's one amongst the Japan's Top inventions in freezing technology. This time, an invention that uses attraction to assist freeze drug without destroying its cell structure: a "fresh freezing" system. It had been developed by a Japanese venture firm in 1995 as a technology which will help freeze drug without altering its therapeutic effect.

GOAL OF FREEZING [9-10]

- To prevent growth of microorganisms by
  - ✓ Killing some bacteria
  - ✓ Reduced water activity
  - ✓ Mechanical formation of ice crystals and Osmotic changes in cell fluids
  - ✓ Typing up some free water (reduced the amount of free water)
- Physical, biochemical and microbiologic degradation of drugs controlled by heat removing process.

NEED FOR FREEZING [11]

1. The process of freezing involves lower temperature which acts as a productive agent.
2. The process of freeze can be carried out under completely sterile conditions, thereby prevention microbial contamination.
3. Dehydration of the material takes place in a rapid manner.
4. The process of freeze for drugs and food materials is completely approved by food and drug safety regulations and is safe for consumption for users.

FACTORS AFFECTING DURING FREEZING

Freezer Burn [12]

Freezer burn is caused by the sublimation of ice on the surface region of the merchandise when the water pressure of ice is on top of the pressure within the environment. Freezer burn produces changes within the looks and texture on the merchandise surface and may be the reason for off-odors and flavors. Moisture migration causes weight losses during freezing and frozen storage, unless the merchandise is packed employing a cloth with tide vapor permeability.

Freezing Burn And Dehydration [13]

If the freezing burn and dehydration, the air when and where it has access to product, starts withdrawing water from the product and the sport or area from where water is withdrawing gets dehydrated and appears as white spot called freezer burns. Unpacked foods and drugs are more susceptible to freezer burns. Longer freezing time will result in larger freezing losses IQF type of freezing result in 2- 4%; slower methods may contribute to even 10% or more weight loss.

Biological and chemical changes [14]

The biological changes include reduction of micro flora on the surface and interior of drugs, freezing has inhibiting effect on the metabolism and reproduction of microbes. As water gets converted into ice and it is not freely available to microbes for their metabolic and physiological activity. Due to this effect reduction in the bacterial load during freezing is witnessed however freezing is not a sterilization process.

FRESH FREEZE TECHNOLOGY [16-21]

Fresh freeze technology is also known as Magnetic resonance assisted freezing in this system patented by the Japanese company of ABI Corporation Ltd, Chiba, Japan. It's one of the Japan's Top inventions in freezing technology. It had been developed by a Japanese venture firm in 1995 as a technology which will help freeze drug without altering its therapeutic effect. The concept of fresh freeze technology is provide a process where a product can be frozen 'in such a manner attraction to assist freeze drug without destroying its cell structure: a "fresh freezing" system. The thought came from the experiments conducted on freezing the water droplets with magnetic fields under 0–0.5 T, which resulted in significant degree of super cooling of water molecules and increased the steadiness in hydrogen bonding. Later, this study was granted patent when same results were found that magnetic resonance is assisted to helped in maintaining stability of the drugs. They proposed that application of magnetic assisted freeze in unidirectional motion created an imbalance within the electronic spin of drug molecules and thus ultimately prevented the freezing by providing force field. It also resulted in removing heat energy of freezing from the water molecules without undergoing into the frozen form.

PRINCIPLE INVOLVED IN FRESH FREEZE [22-26]

Fresh freezing with induced magnetic field has the potential to enhance the freezing rate and to improve the quality of frozen drugs. The process of magnetic resonance assisted freezing or fresh freezing includes the conversion of the material directly from the solid phase to gaseous phase, without going through the liquid phase. For perishables, freeze is the most appropriate method for preservation.

- ✓ Refrigeration not necessary
- ✓ Material can be stored at the room temperature
- ✓ Reconstituted with water within a short period of time
- ✓ Long-term stability(for about two years)

It is also a universal truth that magnetic field greatly affects the properties of water which is associated with the movement of tides in the sea called process of geomagnetism. It has been proposed by the various scientists that magnetic field act on water molecules by aligning the electronic and nuclear spins of the atoms in the direction of the magnetic field. The latter is more acceptable as it is possible to vary the strength of filed applied as per the need and freezing requirements of a particular food product. The electro-magnetic field can be further divided into two categories, i.e. (Static magnetic field, oscillating magnetic field).

WORKING OF FRESH FREEZE TECHNIQUE [27-32]

Fresh freeze technology is also known as Magnetic resonance assisted freezing. Freezing with induced magnetic field has the potential to enhance the freezing rate and to improve the quality of frozen drugs. An electrical current flowing during a wire creates a magnetic flux round the wire, consistent with the Ampere's law.

Consistent with Ampere's law, magnetic fields are associated with the electrical current produced in them. The law specifies the magnetic flux that's related to a given current or vice-versa, as long as the electrical field doesn't change with time

$$B = \mu o \times N/L \times I$$

Where;
B = Magnetic field, **μ0** = Permeability of free space ($4\pi \times 10–7$), **N** = Number of loops , **L** = Number of turns per unit length (m) , **I** = Current flowing through the wire (A)

So, as to generate the magnetic field in an electro-magnet, a coil of wire is wound around the magnetic core with many turns. So, when the electric current is passed through the wire, the magnetic field of all the turns of wire passes and penetrates the iron coil, causing the domains to turn and tiny magnetic fields of core are added to the magnetic field of wire, thus creating a large magnetic field. The intensity of magnetic field generated is influenced by the number of turns in the winding **N** and the current flowing through the wire **I**. The changes brought by the magnetic field depend on the strength of magnetic field, exposition time, and the temperature.

Magnetic field could be applied in the form of permanent magnets or by using electromagnetic coils, The latter is more acceptable as it is possible to vary the strength of filed applied as per the need and freezing requirements of a particular food product. The electro-magnetic field can be further divided into two categories, i.e. (static magnetic field and oscillating magnetic field). The magnetic field assisted freezing system with metallic coil generating magnetic field around the drugs product is prevented which would cause the cold air to transmit easily to the inner portion of the object and enhance the cooling rate and brings free water in a super cooled state. It also results in the cold air to be transmitted quickly inside the core of the product, thus resulting in even and quick cooling of product
This phase change leads to temperature shift which is proportional to face of the applied magnetic flux.. The decreasing cluster size of free water makes it possible to increase the amount of non-freezable bound water and finishes up in better freshness of products. .

QUALITY LOSSES IN FROZEN PRODUCT

Frozen product has expanded worldwide over the last 50 years to include a variety of pharmaceutical products. Even if a drug product is adequately frozen, physico-chemical and biochemical changes during storage can lead to degradation in its quality. Quality of frozen drugs is extremely captivated with storage temperature and there's a requirement for a continuing and systematic control on maintaining the specified temperature throughout the frozen product distribution within the cold chain, from production to final consumption.

Physical Changes During Frozen Storage [33-37]

The main physical changes during storage of frozen drugs are moisture migration and ice recrystallisation.

Moisture migration

A slow freezing process can allow sufficient time for water migration because of osmotic forces from the inner region of a product to the freeze-concentrated intercellular region. These phenomena affect not only the texture of the frozen product, but also a major drip loss during thawing and resulting in a loss of therapeutic effect. During frozen storage the existence of temperature gradients within a product creates water vapors pressure profiles leading to moisture migration and relocation of the water, both within and from the merchandise.

Recrystallisation of Ice

Slow freezing leads to a coffee rate of nucleation and therefore the production of a little number of huge ice crystals, whereas fast freezing causes a high rate of nucleation leading to the formation of an outsized number of small ice crystals. Recrystallisation reduces the advantages of fast freezing and includes any change within the number, size,

shape, orientation, or perfection of crystals following completion of initial solidification. In frozen aqueous solutions recrystallisation is that the method by which the standard ice crystal size increases with time.

Chemical Changes During Frozen Storage [38-42]

During the freezing of product, water is transferred into ice crystals and solutes concentrate in the unfrozen matrix. Slow freezing results in a maximum ice crystal purity and maximum concentration of solutes in the unfrozen phase, leading to equilibrium conditions.

Oxidation

Oxidation is a reaction that severely limits the shelf-life of a frozen product, leading to loss of quality flavor, appearance, nutritional value, and therapeutic effect. Oxidation is a complex process that proceeds upon a free radical process.

Flavor Deterioration

Flavor deterioration is produced in both plant and animal products. It's identified more with frozen muscle than with frozen vegetable products, because blanching is usually applied to vegetables before freezing.

FREEZING PLANT MATERIALS [43-45]

The freeze technique of making ready tissue for microscopic anatomy observation has been applied to an outsized type of stuff with sensible results. This technique involves state change the tissue quickly and dehydrating at a temperature below −30°C below state change with a chemical agent. The plant part, once freeze, is infiltrated with paraffin below vacuum. The equipment uses magnetic resonance assisted freeze and liquid nitrogen-cooled condenser because the chemical agent. Using root, stem, leaf, and procreative tissue of common experimental species like us,

- ✓ *Vicia faba,*
- ✓ *Zea mays,*
- ✓ *Allium cepa,*
- ✓ *Lillium longiflorium,*
- ✓ *Pisum sativum* and
- ✓ *Phaseolus vulgaris*,

Dried or preserved plant materials complement freezing in each formal and informal arrangements. They're going to last nearly indefinitely if rigorously done and need little or no care. Flower arrangements, wreaths, ironed footage, potpourri and wall hangings square measure some of the artistic prospects with preserved plant materials. The simplest time to collect materials for preserve depends on the individual species. As a general rule, collect doubly the maximum amount material as expect to use, since some are going to be broken within the freezing method. Materials like dry grasses, reeds, pine cones, and most seed heads ought to be harvested within the fall at the top of their season however before they become withered in look. Decide cattails after they initial flip brown and flowers square measure still visible at the highest of the spike.

Easy grooming is typically all that's necessary for protective these materials below the recent freeze technique. It'll forestall shattering of fragile seed heads in while. Removal of wetness whereas retentive the first form, color, and texture is that the goal of stuff in freezing techniques. Decide flowers before they reach full bloom as a result of they're going to open additional as they dry before freeze.

CHARACTERISTICS OF PLANT-BASED PRODUCTS [46, 47]

Plant-based products are derived from vegetables, grains, nuts, seeds, legumes, and fruits. Two forms of plant-based products are utilized in cooling method: solids and consistent solutions/suspensions like juices or purees. Solid plant products gift intrinsic characteristics in terms of structure, anatomy, and composition, which can cause challenges on

one hand, however otherwise sometimes facilitate to ease the cooling operation. To start, solid plant-based products are in the main cellular solids. Polysaccharide and nano plastic (hemi-celluloses and pectic) polysaccharides are the most polymers forming the cytomembrane of plant-based products. Polysaccharide is that the single most luxuriant saccharide part of vegetable cell walls, presenting areas of crystallinity conveyance a substantial durability.

Also, the mechanical response of cellular materials is increased by their arrangement and native pure mathematics. during this sense, if the cooling step is correctly done at adequate low temperatures (without ice crystals destroying/weakening the cell walls), cellular materials are higher ready to face throughout cooling. It will be aforementioned that once dehydration solid cellular products, mechanical properties and structural strength might play a additional necessary role keep product integrity than glass transition temperature so as to avoid collapse throughout primary/secondary drying and storage of freezing products.

EFFECTS OF FREEZING TEMPERATURES ON CROPS [48-52]

=To properly evaluate the benefit of freeze prevention methods it is necessary to understand the effect of below freezing temperatures on the crops concerned. Some effects square measure standard whereas others square measure less clear and need a lot of analysis. The minimum temperature referred to as the "critical" temperature that should be reached before harm happens is also influenced by several factors. These embrace plant species, variety, growth or development stage, plant vigor, soil conditions, surface cover; freeze intensity and duration; thawing conditions, cloud and wind conditions throughout the freeze; et al.. Many plants have less freeze-resistance once they become mature than throughout earlier stages of growth.

A healthy, growing plant will usually face up to a frost higher than a weak plant. The crucial temperatures required for harm to occur might vary reckoning on the period that temperatures stay below physical change. as an example, buds of fruit trees is also broken if exposed to -2°C for quite twenty four hours, however might survive if exposed to -6°C for fewer than a pair of hours. The result that physical change temperatures wear crops can vary. In some cases it leads to a complete loss of the plant elements affected. As an example, frozen apple blossoms won't manufacture fruit. In alternative instances it'll solely lead to a decline in yield or quality. If potato A-one square measure frozen untimely, the result are going to be solely a partial loss in yield or quality of tubers. A premature frost will have an effect on each yield and quality of feed and grain corn further as alternative cereal crops.

PHARMACEUTICAL APPLICATIONS [53-57]

The fresh freeze process is one of the important applications in the pharmaceutical and biotechnology industries, and pharmaceutical fresh freeze is latest process used to stabilize, store or increase the shelf life of drug products and other biologicals. Pharmaceutical companies often use freeze to increase the shelf life of products, such as vaccines and other injectable. Pharmaceutical companies use freeze as tool to extend the shelf-life of medication and vaccines. If a liquid drug is converted to its powdered from and stored in an exceedingly vial, it are often easily reconstituted as necessary. Pharmaceuticals is newly subjected to the fresh freeze process include vaccines, hormones, proteins, plasma, antibiotics, ect. In chemical synthesis, products are often lyophilized to form them more stable, or easier to dissolve in water for subsequent use. However, the freezing process is employed more commonly within the pharmaceutical industry.

ADVANTAGES 0F FRESH FREEZE TECHNIQUE

The most important advantages of fresh freeze are listed below:

- ✓ Minimum damage to the heat labile material [58].
- ✓ Speed and completeness of rehydration [59].
- ✓ The ability to sterile filter liquids just before dispensing [60].
- ✓ The substance may be stored at room temperature without refrigeration and be protected against spoilage for many years [61].

DISADVANTAGES 0F FRESH FREEZE TECHNIQUE

The most important advantages of fresh freeze are listed below:

- ✓ Maintaining frozen storage is costly and takes up a lot of space [62].
- ✓ High capital cost equipment, and high energy cost [63].
- ✓ Possible damage to products due to change in pH and tonicity [64].
- ✓ Transportation of frozen materials can be difficult and expensive [65].
- ✓ Failure of freezing equipment would risk the total loss of the product [66].

PHARMACEUTICALS AND BIOLOGICALS SUITABLE FOR FRESH FREEZE [67-69]

- ✓ Vaccines and antibodies
- ✓ Blood plasma
- ✓ Proteins
- ✓ Enzymes and hormones
- ✓ Viruses and bacteria.

CONCLUSION

Pharmaceutical companies use freeze as tool to extend the shelf-life of medication, vaccines and other injectable. In fresh freeze is latest process used to stabilize, and maintain the freshness of drugs. By removing the water from the product and water proofing the material in vial, the drugs can be easily stored. Freezing is wide wont to dehydrate the plant-based substance together with fruits, vegetables, spices. Despite the long interval and being a fashionable drying methodology, fresh freeze technology is also known as Magnetic resonance assisted freezing system. In this process increase the shelf-life of drugs and other biologicals compared with other methods of freezing technique. Though some losses in vitamins and different valuable biocompounds are often found when dehydration, this sort of dehydration methodology is that the best to preserve nutritionary qualities in comparison to different freezing ways. Fresh freeze technique is newly invented method it is very useful in know a days for storing the vaccines and plant based herbal product.

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RESEARCH ARTICLE

Combined Effect of Chest Physiotherapy along with Abdominal Exercise on Peak Expiratory Flow Rate and Oxygen Saturation in Upper Abdominal Surgery Patients- A Randomised Controlled Clinical Trial Study

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ABSTRACT

Purpose of the study is to find the combined effect of chest physiotherapy along with abdominal exercises on peak expiratory flow rate and oxygen saturation in upper abdominal surgery patients. Lung function which is declined following abdominal surgery due to anaesthetic effect and the incisional pain which alters the normal ventilatory function. Due to this there is a reduction in pulmonary function which causes the increased rate of post-operative pulmonary complications that significantly increases the post-operative morbidity and mortality. Therefore the study is aimed to analyse the outcomes of peak expiratory flow rate and oxygen saturation in participants subjected to upper abdominal surgery on performing chest physiotherapy along with abdominal exercises in the post-operative period. 30 subjects between the age of 30 to 60 years who have undergone urgent and elective abdominal surgery were included in the study.15subjects were given chest physiotherapy while another 15subjects were given chest physiotherapy along with abdominal exercises. The exercises were given for a period of 6 days, and the outcome measure of PEFR and SpO_2 are measured on the first day and 6th day for the comparison. Statistical analysis were done using students "t" test.

The subjects who had performed only chest physiotherapy for a period of 6 days shown significant improvement in both PEFR and SpO_2. Similarly the subjects who performed chest physiotherapy along with abdominal exercises also shown improvement on PEFR and SpO_2. The PEFR on the Day 6 in Group B (258.67±42.74) had significant difference than Group A where the PEFR was (204±26.94). The SpO_2 on the Day 6 in Group B (99.93±0.26) had no significant difference than Group A where the PEFR was (99.6 ±0.63). The above study had proved that abdominal exercises increases the peak expiratory flow rate and SpO_2, the lung function along with chest physiotherapy which helps to reduce the post-operative pulmonary complications in participants who have undergone upper abdominal surgery. This accepts the alternate hypothesis.

Keywords: Chest physiotherapy, Abdominal exercises, Peak expiratory flow rate, Oxygen saturation, Upper abdominal surgery.

INTRODUCTION

Surgical procedures in the abdomen affects respiratory muscles, coordinated motion of the chest wall, loss of muscle integrity and change in thoracoabdominal mechanics. Upper abdominal surgery is associated with a reduction in lung volumes, rapid shallow breathing that leads to the decrease in transdiaphragmatic, expiratory and maximum inspiratory pressures. An increase in the ratio of oesophageal pressure to gastric pressure shifts the rib cage breathing with change in abdominal to rib cage excursions that causes the more active function of intercostal muscles due to the reduced function of diaphragm in upper abdominal surgery [1]. The respiratory function is affected during and after abdominal surgery due to the factors including anaesthesia and thoracco abdominal mechanisms. This is the reason for respiratory muscle dysfunction to develop post-operative pulmonary complications such as atelectasis and pneumonia. It is shown that the incidence of respiratory muscle dysfunction following abdominal surgery is 22- 40 % compared to the lower abdominal surgery which is 2-5%. The diaphragmatic weakness is said to be the reason for the respiratory muscle dysfunction which changes the breathing pattern to rapid shallow and decrease in lung volumes [2]. The diaphragm is the main muscle that is affected during upper abdominal surgery. Anaesthesia and pain suppresses the action of diaphragm which reduces its strength that is mandatory to perform normal breathing and forceful expiratory maneuverer. The respiratory muscle dysfunction leads to the reduction in vital capacity, tidal volume and total lung capacity which produces coughing mechanism insufficient to clear the secretions this in turn affects the gas exchange properties of the lungs with a mismatch of ventilation and perfusion in spite of inefficient cough that develops the atelectasis,[3]which is considered to be the risk factor off pulmonary complications that causes significant increase in morbidity and mortality in participants following upper abdominal surgery. Also, the impairment in mucociliary clearance reduces the effectiveness of cough, that increases in the secretion collection which in turn drops the saturation of oxygen [4].

Pulse oximetry is a very important monitoring helps to measure the oxygenation of the patient to detect hypoxemia [5]. Hypoxemia, which is defined as the inadequate level of oxygen in the blood which are analysed by low PaO2 of 60mmHg in the arterial blood gas or SpO2 below 90% in the pulse oximetry [6]. Because of surgical pain, anaesthesia, hemodynamic impairment causes ventilation perfusion mismatch, atelectasis, alveolar hypoventilation that reduces lung function, diminished activity of diaphragm and chest wall becomes the reason to develop postoperative hypoxemia following abdominal surgery [7,8,9]. By measuring the oxygen saturation, it helps the physiotherapists to teach physiotherapy which helps to increase oxygen saturation by maintaining the ventilation perfusion ratio while performing incentive spirometry and breathing exercises. This further helps to reduce the post -operative pulmonary complications. Finally, physical manoeuvres such as intermittent positive pressure breathing, incentive spirometry, and deep breathing exercises have been shown to preventpulmonary complications after abdominal surgery [10,11]. As the abdominal muscles contributes 20% in the work of breathing in pulmonary function, therefore the study is

made to find the effect of chest physiotherapy and abdominal exercise on PEFR and oxygen saturation in upper abdominal surgery patients.

Statement of the problem

Abdominal muscle exercises and chest physiotherapy is very safe and beneficial in improving peak expiratory flow rate and oxygen saturation in subjects who have undergone upper abdominal surgery

Objective

To evaluate the importance of chest physiotherapy and abdominal exercises in improving the peak expiratory flow rate and oxygen saturation in upper abdominal surgery patients

Hypothesis

Null hypothesis

There is no significant improvements in peak expiratory flow rate and oxygen saturation following chest physiotherapy and abdominal exercises in subjects following upper abdominal surgery.

Alternate hypothesis

There is significant improvement in peak expiratory flow rate and oxygen saturation following chest physiotherapy and abdominal exercises in subjects following upper abdominal surgery.

Need of the study

Subjects who performes chest physiotherapy along with abdominal exercises following upper abdominal surgery has significant benefits in improving lung function, the quality of life, reduce the length of hospital stay and prevention of post-operative pulmonary complications.

MATERIALS AND METHODOLOGY

An experimental study was conducted at Civil Hospital, Dahod undertaken by Zydus Medical College from January2021 - August 2021. Ethical clearance was obtained from the Institutional ethical committee. Subjects of both genders were included for the study.30 subjects with the age group between 30 to 60 years who have undergone emergency and elective upper abdominal surgery were included in this study. Unstable and complicated abdominal surgical patients, laparoscopy patients, age more than 65 years, case of chronic respiratory diseases, cardiovascular diseases, neurological conditions and musculoskeletal problems were excluded in the study. Informed consent were taken. By random sampling they were divided into two groups with 15 in each group.

Group A were given chest physiotherapy only which includes breathing exercises, incentive spirometry, ankle, toe movement and thoracic expansion exercises , Breathing Exercises(diaphragmatic breathing exercises prior and after incentive spirometry) for 6 days post operatively, Incentive Spirometry (10 times with 3 secs hold every 4 waking hours)for 6 days post operatively, ankle, toe movement after breathing exercises, thoracic expansion exercises in sitting position with active deep inspiration followed by relaxed expiration. Like upper extremity flexion and extension(inspiration in shoulder extension, expiration in shoulder flexion), adduction and abduction, (inspiration in shoulder abduction, expiration in shoulder adduction) and hands on head, while Group B were given chest physiotherapy along with abdominal exercises which includes deep abdominals, pelvic tilting, pelvic bridging and knee rolling exercises. The outcome measure of PEFR and SpO_2 were recorded on the day 1 and at the end of sixth day. The collected data will be tabulated and analysed using students "t" test that will compare between the two groups by Unpaired "t" test and within the groups by Paired "t" test.

RESULTS

Demographic data: Table 1: Demographic data (n=30)

Characteristics	Chest physiotherapy group (n=15)	Chest physiotherapy and abdominal exercises (n=15)
Age (years)	43.33±10.22	45.07±8.82
Height (cm)	157±4.9	156.33±4.0
Weight (kg)	57.93±5.01	56.13±6.25
BMI	23.55±2.34	22.95±2.8

Gender distribution: Table 2: Gender distribution (n=30)

Sex	Chest physiotherapy group (n=15)	Chest physiotherapy and abdominal exercises (n=15)
Male	9 (60 %)	10 (67%)
Female	6 (40%)	5 (33%)

PEFR (L/Min): Table 3: Comparison of PEFR within the groups

	Chest physiotherapy group (n=15)				Chest physiotherapy and abdominal exercises (n=15)			
	Mean ± SD	t-value	p-value	Statistical significance	Mean ± SD	t-value	p-value	Statistical significance
Day 1	116±16.39	19.58	0.0001	Significant difference	111.33±13.56	14.496	0.0001	Significant difference
Day 6	204±26.94				258.67±42.74			

The above table shows that there is a significant difference of PEFR exists within both the group, chest physiotherapy group, "t" value 19.58 and in Chest physiotherapy and abdominal exercises group, the "t" value is 14.496 and "p" value (0.0001) < 0.05.

Table 4: Comparison of PEFR between the groups

	Groups	Mean ± SD	t-value	p-value	Statistical significance
Day 1	Chest physiotherapy group (n=15)	116±16.39	0.8498	0.4027	No Significant difference
	Chest physiotherapy and abdominal exercises (n=15)	111.33±13.56			
Day 6	Chest physiotherapy group (n=15)	204±26.94	4.1908	0.0003	Significant difference
	Chest physiotherapy and abdominal exercises (n=15)	258.67±42.74			

When comparing the PEFR values in Day 1 in both groups, there shows no significant difference between the group, as the "p" value (0.4027) > 0.05 and "t" value 0.8498, while PEFR values in Day 6 shows significant difference between the groups with "p" value (0.0003) < 0.05 and "t" value 4.1908.

SpO_2% : Table 5: Comparison of SpO_2% within the groups

	Chest physiotherapy group (n=15)				Chest physiotherapy and abdominal exercises (n=15)			
	Mean ± SD	t-value	p-value	Statistical significance	Mean ± SD	t-value	p-value	Statistical significance
Day 1	94.67 ±0.62	21.6209	0.0001	Significant difference	95.07±0.8	22.6053	0.0001	Significant difference
Day 6	99.6 ±0.63				99.93±0.26			

Comparison of SpO_2% within the groups shows the significant difference in both groups with "t" value 21.6209 in chest physiotherapy group and "t" value 22.6053 in chest physiotherapy and abdominal exercise group with "p" value 0.0001 < 0.05.

Table 6: Comparison of SpO_2% between the groups

	Groups	Mean ± SD	t-value	p-value	Statistical significance
Day 1	Chest physiotherapy group (n=15) [Group-A]	94.67 ±0.62	1.5346	0.1361	No Significant difference
	Chest physiotherapy and abdominal exercises (n=15) [Group-B]	95.07±0.8			
Day 6	Chest physiotherapy group (n=15) [Group-A]	99.6 ±0.63	1.8898	0.0692	No Significant difference
	Chest physiotherapy and abdominal exercises (n=15) [Group-B]	99.93±0.26			

On comparing the SpO_2 of Day 1 in both the groups there is no significant difference exists between the groups since the "p" value 0.1361 > 0.05 similarly in Day 6 also there is no significant difference exists between the groups since the "p" value 0.0692 > 0.05.

DISCUSSION

When considering the upper abdominal surgery patients in the post-operative period, the respiratory dysfunction is the important change to be considered. Post-operative pulmonary complications which are common following upper abdominal surgery increases the morbidity rate and length of hospital stay due to the reduction in lung volumes, ineffective expiratory maneuverer that increases the secretion in the lungs causing atelectasis, pneumonia, and reduced oxygen saturation. While comparing the mean values of PEFR day-1 and day-6 in chest physiotherapy group [Group-A], peak expiratory flow rate in day-1were 116±16.39, whereas in day-6 were 204±26.94. Since the p value < 0.0001 and "t" value is 19.58 with 28 degrees of freedom, there is a statistically significant difference exists within the group. (Table 3). Similarly in chest physiotherapy and abdominal exercises group [Group-B], peak expiratory flow rate in day-1were 111.33±13.56, whereas in day-6 were 258.67±42.74. Since the p value < 0.0001 and "t" value is 14.496 with 28 degrees of freedom, there is also statistically significant difference exists within the group. This has proven that the chest physiotherapy and the abdominal exercises are much beneficial in improving the lung function following upper abdominal surgery. (Table 3). When analyzing the mean values of PEFR in day-6 between both the groups, chest physiotherapy group [Group-A] were 204±26.94 and chest physiotherapy and abdominal exercises group [Group-B] were 258.67±42.74shows the significant difference between the groups, since the "p" value is 0.0003 < 0.05. This shows that abdominal exercises along with chest physiotherapy [Group-B] shows statistically significant difference than only chest physiotherapy [Group-A] with the "t" value 4.1908. This proves that performing abdominal exercises along with chest physiotherapy show significant improvement in PEFR than only chest physiotherapy. Shazly B. Ali et al., (2018) [12] on his study have recommended incentive spirometry and diaphragmatic breathing exercises for all patients undergoing upper abdominal surgery that helps to improve the postoperative pulmonary functions that in turn reduces the length of hospital stay with prevention of pulmonary complications. This was also supported by the Biplab et al., (2015) [13] who has shown the improvement in PEFR following incentive spirometry in post abdominal surgery. The influence of abdominal muscle strength on pulmonary function in post upper abdominal surgery by Abbina et al., (2013) [14] have suggested that good abdominal muscle strength maintains the pulmonary function which helps to perform abdominal exercises along with diaphragmatic breathing exercises to reduce the incidence of post-operative pulmonary complications following upper abdominal surgery.

While comparing the mean values of SpO_2 day-1 and day-6 in both the groups, chest physiotherapy group [Group-A], SpO_2 in day-1were 94.67 ±0.62, whereas in day-6 were 99.6 ±0.63. Since the p value < 0.0001 and "t" value is 21.6209 with 28 degrees of freedom, there is a statistically significant difference exists within the group. (Table 5). Similarly in Chest physiotherapy and abdominal exercises group [Group-B], SpO_2 in day-1were 95.07±0.8, whereas in day-6 were 99.93±0.26. Since the p value < 0.0001 and "t" value is 22.6053 with 28 degrees of freedom, there is also statistically significant difference exists within the group. This has proven that the chest physiotherapy and the

abdominal exercises are much beneficial in improving the oxygen saturation following upper abdominal surgery. (Table 5). When analyzing the mean values of SpO_2 in day-6 between both the groups, chest physiotherapy group [Group-A], were 99.6 ±0.63and chest physiotherapy and abdominal exercises group [Group-B], were 99.93±0.26shows no significant difference between the groups since the "p" value is 0.0692< 0.05 with "t" value 1.8898. Post-operative hypoxemia which is more common following surgery are caused due to the problem of gas exchange because of anaesthetic drugs.[15]By performing chest physiotherapy and abdominal exercises in post upper abdominal surgery patients, the oxygen saturation gets good improvement along with improved lung function. Though there is no significant difference exists between the groups, both shows the complete improved status of SpO2. Roberta Munhoz et al., (2008)[16]have concluded that chest physiotherapy during the immediate post-operative period have shown the improvement in the oxygen-haemoglobin saturation following upper abdominal surgery. According to Fagevik Olsen M et al., (1997)[17] in his randomized controlled trial of prophylactic chest physiotherapy in major abdominal surgery have stated that chest physiotherapy improves the oxygen saturation. As the incidence associated with post-operative pulmonary complications are about 21.7%[18]in surgical patients, the findings in the present study have suggested that chest physiotherapy along with abdominal exercises should be included in the post-operative period following upper abdominal surgery which gives significant increase in the PEFR and SpO2 that helps to prevent the post-operative pulmonary complications like atelectasis, pneumonia, lung function and chest wall improved motion. This also helps to reduce the length of hospital stay, increased strength of abdominal muscles with reduced morbidity and mortality rate. By early recovery of patients, the quality of life is increased.

CONCLUSION

The results of the above study, "Combined effect of chest physiotherapy along with abdominal exercise on peak expiratory flow rate and oxygen saturation in upper abdominal surgery patients" shows that there is more improvement of PEFR and SpO_2 on performing chest physiotherapy and abdominal exercises. Hence, it is mandatory to include chest physiotherapy as well as abdominal exercises in the post-operative period following upper abdominal surgery that improves lung function with reduction in the prevention of pulmonary complications.

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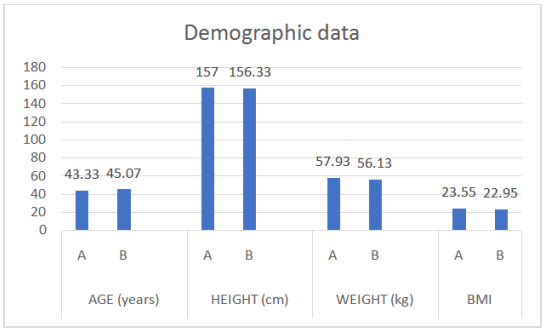

Figure 1: Demographic data

Figure 2: PEFR (L/min)

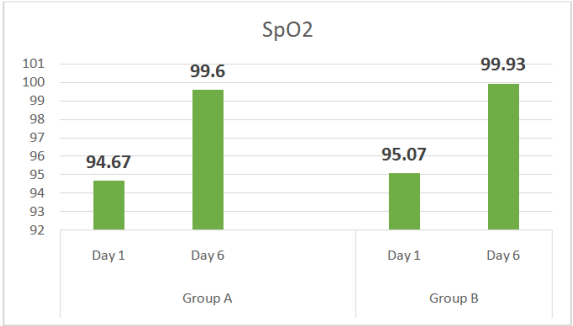

Figure 3: SpO_2

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RESEARCH ARTICLE

Study of Achievement Motivation on the Performance of Swimming Athletes

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ABSTRACT

Achievement motivation is the proclivity to take steps toward achieving a single goal that improves personal performance, even if it is necessary to overcome some challenges. *The current research investigates the relationships between the performance of well-trained swimmers and achievement motivation and compares* the swimming performance among different levels (High, Moderate and Low) of achievement motivation. A total of two hundred subjects were randomly selected from the school state-level swimming championship. The data were evaluated using descriptive statistics, Pearson product-moment correlation, and Analysis of Variance. The results of this study revealed a significant relationship between achievement motivation and swimming performance (r=-0.692, p0.00), as well as a significant difference in swimming performance among different levels of achievement motivation (High, Moderate, and Low) (F=325.167, p0.00).The findings aid both researchers and applied sport psychology practitioners in understanding how achievement motivation affects athletes' performance.

Keywords: Achievement Motivation, Sport, Swimming, Performance, athletes

INTRODUCTION

The desire to achieve success, persevere in the face of setbacks, and take pride in accomplishments is achievement motivation. The behavior of an individual who strives to achieve something, do his best, excel in performance is referred to as achievement motivation. This entails a competition with himself or others to attain a certain level of

performance excellence. This feature of achievement sets it apart from other motivations. Achievement motivation is a broad term that refers to various factors, including persuasive convictions, task esteems, objectives, and achievement goals(Wigfield *et al.,* 2016). Swimming games are both a competitive sport and a form of physical and physiological rehabilitation. Over the last five decades, competitive swimming has shown consistent and significant improvement. Progressive training, sophisticated selection approaches, superior stroke method, standardization, and variations in swimming regulations (complexity of pool, types of lane lines used, height and angle of starting blocks, water temperature), increased access to the sport, or new swimwear technologies are some of the reasons for this improvement(Costa *et al.,* 2010; O'Connor and Vozenilek, 2011). Several researchers (e.g., Marsh & Perry, 2005; Mooney *et al.,* 2016; Wilson *et al.,* 2014) have examined the factors influencing elite swimming performance. Swimming performances are influenced by boosting power and minimizing the resistance to advance in the liquid environment. Performance improvement is likely to attain by a suitably planned maintenance of strength and reduction of the training load (Mujika and Padilla, 2003). Swimming performance has been linked to various anthropometrical, physiological, and biomechanical parameters in studies(Hue *et al.,* 2006; Tsekouras *et al.,* 2005).
In theory, a successful training program should allow for consistency in training workloads and adequate rest periods. Athletes risk hormonal changes, mood disturbances, and performance declines if they combine high training volume and intensity with limited recovery periods in their training regimen. (Halson *et al.,* 2002; Kreider *et al.,* 1998)

Psychological and physiological factors are essential in determining game and sports performance(Schilling & Hyashi, 2001). Murray (1938) was the first to describe achievement motivation as a desire to complete a difficult task.Achievement motivation, participation motivation, intrinsic motivation, and extrinsic motivation have all been studied in the context of sport and physical activity participation(Biddle & Mutrie, 2007). The achievement motivation is one of the vital motives, as it guides and directs the individual to relieve his tension needs, expand consecutive plans to get consecutive goals and to execute these plans the technique that permits more than another calming urgency of the individual needs and his motives (El-Nabulsi, 1986). Nicholls' (1984, 1989) goal perspective theory has been the foundation for many studies in achievement motivation. Many sports psychologists have studied the features and theories of athletes' task and ego goals in a sporting environment(e.g., Duda, 1989; Lewthwaite, 1990; Lochbaum & Roberts, 1993). Individuals struggle to demonstrate high ability while avoiding demonstrating low ability, according to the theoretical perspective. In addition, the terms "success" and "failure" have two distinct goal orientations.A task goal orientation, which highlights exertion, task grasp, and performance upgrading, is defined by self-referenced capability insights. A norm-referenced insight of capability, a focus on winning, and favorable social comparisons by others define an ego orientation.

Swimming fosters an environment that encourages athletes to set and achieve realistic goals to learn, succeed, persevere, and enjoy their sport. The achievement goals that an athlete adopts are thought to be influenced by their performance as measured by cues and feedback. The term "need for achievement" refers to the desire to succeed and excel. It refers to the force or energy that propels a person toward a specific goal. Achievement motivation, in general, expects satisfaction from mastering challenging performances, whereas it stands for the pursuit of excellence in education. When a person's desire for achievement becomes their primary concern, it ignites restless driving energy in them to achieve excellence, get ahead, improve on previous recodes, beat competitors, do things better, and come up with unique solutions to complex problems. As a result, achievement motivation aims to provide students with positive human characteristics such as motivation, curiosity, and adaptability. As a result, we can conclude that achievement motivation is critical in setting higher goals and establishing higher standards in learning and performing activities from an academic standpoint and working toward their realization. Several recent studies have examined achievement motivation with sports such as Basketball, wrestling, baseball, etc. (Atta, Iqbal, & Mushtaq, 2020; Rutkowska, & Gierczuk, 2020; Gill, 2019). Only a few studies have investigated the performance of swimmers related to achievement motivation (Bono & Liyi, 2020; Halder & Phulkar, 2019). There are fewer published studies of the variability of swimming performance athletes related to achievement motivation. In the current study, the investigator has tried to investigate the relationships between swimming performance and achievement

motivation and compared the swimming performance among different levels of achievement motivation, which will enable sports executors to manage positively undesirable emotional situations and accomplish to their full capabilities.

METHODOLOGY

A total of two hundred swimmers were randomly selected from the state-level swimmers swimming championship held at Madhya Pradesh. The subjects belonged to different parts of Madhya Pradesh and had different socio-economic statuses. All the subjects had good health and fitness during the administration of the test. The research scholar met with the respected coaches and managers of the swimmers before administering the tests. The testing procedures' requirements were explained to them in detail so that there would be no ambiguity about the effort required of them. All of the subjects willingly decided to participate in the study and were aware of the study's terms and procedures. Even though no special techniques were used to motivate and encourage the subjects to give their all during the research, they appeared to be very enthusiastic and cooperative throughout the process. Achievement motivation was considered an independent variable assessed with the help of a questionnaire made by Kamlesh (1990), contains 20 unaccomplished statements, which can be accomplished by selecting either of the two proposed parts compared to each statement. The subjects were asked to tick (√) the second part in which their belief fits in best using the first part. Correct answers for each statement indicated above were awarded "2" marks, and incorrect answers for each statement were given "0" marks. The item, which was left unanswered, was not for consideration. The score was added, and it was pondered as an individual score. The score ranges from 0 to 40, which is further divided into three levels, i.e., High (30-40), Moderate (26-28), and Low (0-24).

Swimming performance was considered as a dependent variable which was measured with the help of 50 meters swimming. The time was recorded to the nearest 1/100 of the seconds. The data were analyzed using mean and standard deviation to determine the relationship between achievement motivation and swimming performance. Pearson product-moment correlation was used, and analysis of variance was employed to compare the swimming performance among different levels of motivation.

RESULTS

The mean and standard deviation values of achievement motivation of athletes for swimming performance among different levels were calculated and are given in Table 1.

Descriptive Statistics

It is clear from Table 1, among the swimming performance between different motivation levels, the achievement motivation belonging to Low level (39.57) have low and High-level achievement motivation (30.59) have high motivation level when compared to different levels of motivation.

Analysis of Variance of Swimming Performance among Various Motivation Groups

Above table 2 shows that there is a significant difference among the various motivation groups in context to swimming performance as the p-value is less than 0.05 level.

Post hoc comparison of Swimming Performance

Comparison of pair wise difference of means with a tabulated difference, it is apparent from Table 3 that there is a significant difference between high and moderate swimming performance (MD = -3.29); high and low (MD = -8.99); and moderate and low (MD = -5.69) swimming performance of athletes.

Relationship between Swimming Performance and Motivation

Support for the hypotheses was found in the correlation (Table 4), and there was a significant correlation between swimming performance and motivation. The findings of this study show that there is a significant difference in achievement motivation between high, moderate, and low performers. At the 0.05 level of significance, Table 1 revealed significant differences in sports achievement motivation between high, moderate, and low swimming athletes.

CONCLUSION AND DISCUSSION

The achievement motive, or the desire to succeed, is the foundation of achievement motivation. Those who engage in a task for the sake of achieving a goal are said to be working in the soul of achievement motivation. Need for Achievement (N-Ach) is defined by McClelland (1961) as an individual's desire for significant accomplishment, mastery of skills, control, or high standards. In his 1938 book "Explorations in Personality," Henry Murray coined the term to describe various behaviors. One of them is "penetrating, protracted, and frequent hard work to achieve to some degree problematic." The hierarchal model of achievement motivation was investigated by Elliot and McGregor (1999). They proposed that achievement goals directly influence achievement-relevant outcomes, whereas achievement motives have an indirect or distal influence. The term "achievement motivation" has been defined in several different ways. We now have an improved understanding of the effects of achievement on cognition and behavior. Many achievements motivation methods have been established independently, implying that furthermost achievement motivation theories agree rather than competition, despite their similarities in nature. The study determined to examine the relationships between the performance of well-trained swimmers and achievement motivation and compare the swimming performance among different levels (High, Moderate and Low) of achievement motivation. According to the findings of this study, there is a significant difference between achievement motivation and swimming performance. It was found that achievement motivation is rated lower by the low-level swimming athletes, probably because of the lower competition existing on those teams and higher by the higher-level swimming athletes probably because of their experience. These findings follow Rathee and Singh's (2011) findings, which found significant differences between national and international performance levels.

Moreover, it is interesting that the various motivation groups reported significantly to swimming performances. As such, Swimming Athletes with a higher level of motivation will effectively handle the demands experienced by them within their sports environment. The following research findings support the researcher's conclusions: Khan, Khan, and Ahmed (2010) and Ali (2010).As a result, one of the most important psychological factors influencing swimming athletes' performance is achievement motivation; without it, all tasks will be monotonous. Without sufficient motivation, no one can accomplish higher goals. This study has several limitations. This study consists of athletes from only the swimming context. Future studies should concentrate on different sports. In the future, researchers must focus on specific samples and experimental studies. More respondents and other psychological components should be used in future studies on mental toughness and sports performance.

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Table 1: Descriptive Statistics

	N	Mean	Std. Deviation
High	37	30.59	1.29
Moderate	40	33.88	1.29
Low	123	39.57	2.39
Total	200	36.77	4.22

Table 2 :Analysis of Variance of Swimming Performance among Various Motivation Groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2715.444	2	1357.722	325.167	0.000*
Within Groups	822.566	197	4.175		
Total	3538.010	199			

*significant as p<0.05

Table 3 Post hoc comparison of Swimming Performance

High	Moderate	Low	Mean Difference	Sig.
30.59	33.88		-3.29	0.000*
30.59		39.57	-8.99	0.000*
	33.88	39.57	-5.69	0.000*

*significant as p<0.05

Table 4 Relationship between Swimming Performance and Motivation

		Swimming Performance	Motivation
Swimming Performance	Pearson Correlation	1	-0.692*
	Sig. (2-tailed)		0.000
	N	200	200

*significant as p<0.05

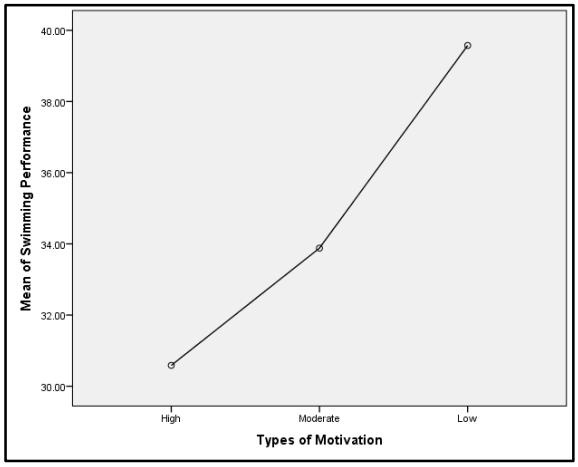

Fig.1 Types of Motivation and Swimming Performance

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RESEARCH ARTICLE

Automatic Generation Control Parameter Tuning Strategies for an Interconnected Reregulated Power System with Hydrogen Aqua Electrolyser Unit

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ABSTRACT

In this paper, an interconnected Automatic generation control (AGC) in a two-area restructured power system without / with HAE-FC Unit is proposed for load frequency control (LFC) in deregulated power environment. The PI[2] controller and proposed PI Controller interacting with Proportional – Derivative terms (PD interacted PI controller) are designed using JAYA algorithm and when implemented in the restructured power system it has been found that the proposed (PD interacted PI controller) shows a better performance. An extensive analysis is done for LFC and it is found that dynamic responses obtained satisfy the LFC requirements in deregulated power environment. The parameters of modified PD controllers are tuned to the optimum value using various available optimization methods. The whole system is design in MATLAB / SIMULINK model different load pattern is applied in place of modified PD controller.

Keywords: Automatic Generation control, Reregulated system, Deregulation, Restructuring, Jaya Algorithm, PID integrating controller, Hydrogen Aqua Electrolyser, Two- area interconnected thermal system.

INTRODUCTION

The fundamental function of automatic generation control (AGC) in a power system is to keep the system frequency and tie-line flow at their scheduled values during normal periods as well as when the system is subjected to modest step load perturbations. The problem of power and frequency oscillations owing to unforeseen load variations has become increasingly critical as the size and complexity of electrical power systems has grown, as has the number of interconnections. AGC regulator designs integrating parameter variations/uncertainties, load characteristics, excitation control, and parallel ac/dc transmission links have been added to the AGC problem from time to time as useful research contributions. Aside from the impact of energy storage units in power systems, the AGC control approach has a significant impact on system performance. Researchers have developed many conventional and intelligent techniques to address the AGC issue in the restructured system, such as the Artificial Cooperative Search Algorithm (ACSA) [6,10], to address the AGC issue in the restructured system. Sun

Flower Optimization (SFO) [1, Imperialist Competition algorithm (ICA) [2,3], Sine Cosine algorithm (SCA) [5, Particle Swarm Optimization (PSO) [4,14], Bacterial Foraging Optimization (BFO) [15,25], Fuzzy logic [8,18], Grey theory [16], and Beta wavelet Neural Network (BWNN) [11] are some of the algorithms used. However, optimal control strategies implemented on restructured systems show superior and robust dynamic performance compared to other methods. The efficiency and utilization of the BESS in the context of regulation and grid integration is examined [7].

Further, the different energy storage techniques such as Redox flow battery [9], Battery Energy storage [12, 19-20], Hydrogen Storage Tank [17] are implemented and designed in the two-area interconnected deregulated power system. The consolidate literature of the LFC of the interconnected power system is shown in Table 1.A robust control strategy for reducing system frequency deviation, caused by load fluctuations and power obtained from renewable energy resources, in a smart microgrid system with attached storage was detailed in [13]. Moreover, the objective of the current research work is to design and tune a PID interacting controller for LFC applications of an interconnected restructured power system under a deregulated environment which refers to reregulation.

Furthermore, this paper is organized as follows: section 2 illustrates AGC control in restructured power system for the 2-area interconnected thermal system. In section 3, the proposed the design and development of control strategy for AGC. In section 4 discussed the design of Hydrogen Aqua Electrolyser (HAE) unit. In Section 5, Jaya algorithm for solving the AGC problem. Section 6, pronounces the evaluation of restoration index. Finally, section 7concludes the paper.

AGC CONTROL IN RESTRUCTURED POWE SYSTEM

The goal of power system restructuring is to increase competition, allow open transmission access, and lower electricity costs for consumers. The former VIUs, which were used to regulate all electricity-related activities in the generation, transmission, and distribution subsystems, have been decomposed into separate companies called Generation Companies (GENCOs), Distribution Companies (DISCOs), and Transmission Companies (TRANSCOs). Each has committed to its own tasks, as has an Independent System Operator (ISO). The ISO oversees the operation of many ancillary services, one of which is frequency regulation, which is based on the concept of Automatic Generation Control (AGC). In DPM, the number of rows has to be equal to the number of GENCOs and the number of columns has to be equal to the number of DISCOs in the system. Any entry of this matrix is a fraction of total load power contracted by a Disco towards Genco. As a result, total of entries of column belong to Disco_i of DPM is $\sum_i cpf_{ij} = 1$. In this study two-area interconnected power system in which each area has two GENCOs and two DISCOs. Let Genco1, Genco2, Disco1, Disco2 be in area 1and Genco3, Genco4, Disco3, Disco4 be in area 2 as shown in Fig. 1. The corresponding DPM is given as follows

$$\boldsymbol{DPM} = \begin{vmatrix} cpf_{11} & cpf_{12} & cpf_{13} & cpf_{14} \\ cpf_{21} & cpf_{22} & cpf_{23} & cpf_{24} \\ cpf_{31} & cpf_{32} & cpf_{33} & cpf_{34} \\ cpf_{41} & cpf_{42} & cpf_{43} & cpf_{44} \end{vmatrix} \quad (1)$$

Where, *cpf* represents ''Contract Participation Factor" and is like signals that carry information as to which the GENCO has to follow the load demanded by the Disco. The actual and scheduled steady state power flow through the tie-line are given

$$\Delta P_{tie,1-2,schduled} = \sum_{i=1}^{2}\sum_{j=3}^{4} cpf_{ij} * \Delta P_{Lj} - \sum_{i=3}^{4}\sum_{j=1}^{2} cpf_{ij} * \Delta P_{Lj} \quad (2)$$

$$\Delta P_{tie,1-2,actual} = (2\pi T_{12}/s) * (\Delta F_1 - \Delta F_2) \quad (3)$$

And at any given time, the tie-line power error $\Delta P_{tie,1-2,error}$ is defined as

$$\Delta P_{tie,1-2,error} = \Delta P_{tie,1-2,actual} - \Delta P_{tie,1-2,schduled} \quad (4)$$

The error signal is used to generate the respective ACE signals as in the traditional scenario

$$ACE_1 = \beta_1 * \Delta F_1 + \Delta P_{tie,1-2,error} \quad (5)$$

$$ACE_2 = \beta_2 * \Delta F_2 + \Delta P_{tie,2-1,error} \quad (6)$$

For two area system as shown in Fig. 1, the contracted power supplied by ithGenco is

$$\Delta P_{gi} = \sum_{j=3}^{4} cpf_{ij} * \Delta P_{Lj} \quad (7)$$

Also note that $\Delta P_{L1,Loc} = \Delta P_{L1} + \Delta P_{L2}$and $\Delta P_{L2,Loc} = \Delta P_{L3} + \Delta P_{L4}$. In the proposed LFC implementation, the contracted load is fed forward through the DPM matrix to Genco set points. i.e., apf_{11}, apf_{12}, apf_{21} and apf_{22}

DEVELOPMENT OF CONTROL STRATEGY FOR AGC

Design and development of Proportional double Integral (PI²) controller

Belanger and Luyben was the first researcher to introduce the concept of the proportional plus double integral (PI²) controller [22] as a low frequency compensator. The main aim of the additional double integral compensation is to reject the effects of ramp-like disturbance.

Belanger and Luyben proposed thefollowing proportional plus double-integral (PI²) controller as a low-frequency compensator to deal with ramp-like disturbances:

$$u(t) = K_P\left(ACE + \frac{1}{T_{I_1}s}\int ACE\,dt + \frac{1}{T_{I_2}s^2}\int ACE\,dt\right) \quad (8)$$

The ideal continuous time domain PI controller for a single input single output process is expressed in the Laplace domain as follows:

$$U(s) = K_P\left(1 + \frac{1}{T_i s}\right) \quad (9)$$

The PI² controllers are expressed in Laplace form as follows [22]

$$U(s) = K_P\left(1 + \frac{1}{T_{I_1}s} + \frac{1}{T_{I2}s^2}\right) \quad (10)$$

Where $U(s)$ is the computed control input,K_P is the controller gain, and T_{I1} and $T_{I2}$are the integraland double-integral time constants, respectively. It canbe easily shown that a purely proportional plus integral (PI) controller (i.e.,T_{I2} = ∞) cannot reject efficiently ramp like disturbances. Even if the load-transfer function time constant is so large that it resembles an integrator over a wide range of frequencies, the closed loop performance of the system will be very poor. In this way, the objective of the double-integral action$\frac{1}{T_{I2}s^2}$is to introduce compensation at low frequencies toasymptotically reject the effects of frequently occurringramp-like disturbances. Belanger and Luyben developedtuning rules for the PI² compensator under the assumption that the process model can be approximated with a (first-order) integrator-dead time model. The proposed tuning rules for the controller parameters {K_P,T_{I1},T_{I2}}are given in terms of ultimate gain, K_u, and ultimate period, T_u, of the assumed integrator plus dead time process transfer function. Simple formulae were used to define the tuning parameters for PI² controller and the settings are given by K_P=0.33K_u, T_{I1}= 2.26T_u and T_{I2}= 20.5T_u^2 [22].

The purposes of this paper are (i) to interpret the double integral as an estimator of ramp-like disturbances and clarify its role into the feedback loop; (ii) to provide a parameterization of the PI² controller gain Kc, and integral times,T_{I1} and T_{I2}, in terms of a nominalclosedlooptime constant and a disturbance estimation time constant; (iii) to show that the PI² compensator is robust against unmodeled nonlinearities. Under the assumption that the dynamics of the plant can be approximated by a first-order model, a reduced order observer is constructed to estimate ramp-like disturbances, which is subsequently coupled with a feedback loop to counteract the effects of the disturbance. The Proportional–Double Integral (PI²) controllers are used for AGC loop of an interconnected power system is shown in Fig. 2.

Design and development of PD interacted PI controller

An integral term is almost always used for the feedbackcontroller to eliminate steady-state offset. The integral term is dominant at low frequencies compared with the proportional and derivative terms; hence, the term may be applied at a slower rate. With this concept, Lee and Edgar proposed adual-rate control system with improved stability robustness where the integral action is sampled more slowly. The sampleand hold in Lee and Edgarwere approximated witha time delay or with a low pass filter. A filtered integralterm will also have better stability robustness. A time delay or a low pass filter added to the integral term which can increase the gain and phase margins for some processes.

Ideal PID Controller

The transfer function form of ideal PID controller is as follows,

$$G_i(s) = \frac{U(s)}{E(s)} = K_C(1 + \frac{1}{T_i s} + T_d s) \quad (11)$$

Where, K_c, T_i, and T_d are the controller gain, the integral time, and the derivative time, respectively. A block diagram of a PID controller is shown in Fig 3. This controller structure is also termed as the standard, parallel, non-interacting or ISA (Instrumentation, Systems, and Automation Society) controller.

The Classical PID Controller

The transfer function of this controller is given as

$$G_s(s) = \frac{U(s)}{E(s)} = K_{C1}\left(1 + \frac{1}{T_{i1} s}\right)(1 + T_{d1} s) \quad (12)$$

Where, Kc_1, Ti_1, and Td_1 are the controller gain, the integral time, and thederivative time, respectively. The block diagram representation is shown inFig 4. This type of controller is also termed as the cascade, interacting, or series PID controller. The controller (12) can always be represented as a noninteracting controller (11), whose coefficients are given by

$$K_c = K_{c1}\frac{T_{i1}+T_{d1}}{T_{i1}} \quad (13)$$

$$T_i = T_{i1} + T_{d1} \quad (14)$$

$$T_d = \frac{T_{i1}T_{d1}}{T_{i1}+T_{d1}} \quad (15)$$

Equations (13) through (15) are obtained by comparing the coefficients of sin (11) and (12). Conversely, the noninteracting PID controller (11) can be written in the equivalent interacting controller from (12) with the following equivalence:

$$K_{c1} = \frac{K_c}{2}\left(1 + \sqrt{1 + 4\frac{T_d}{T_i}}\right) \quad (16)$$

$$T_{i1} = \frac{K_i}{2}\left(1 + \sqrt{1 - 4\frac{T_d}{T_i}}\right) \quad (17)$$

$$T_{d1} = \frac{K_d}{2}\left(1 - \sqrt{1 - 4\frac{T_d}{T_i}}\right) \quad (18)$$

In fact, this parallel structure of PID controller is the most general form. This is because the integral action (derivative action) can be switched off by letting $K_i = 0$ ($K_d = 0$). Note that in the ideal and classical structures of PID.

The Parallel PID Controller

In this structure, the three control actions are completely separated and the transfer function is given as

$$G_p(s) = \frac{U(s)}{E(s)} = K_p + \frac{K_i}{S} + K_d s) \quad (19)$$

Proposed PD interacted PI Controller

The basic PID controller structures given by (11), (12), and (19) are not suitable for practical applications. There are two main coupled reasons behind this fact. First, the controller transfer function is not proper and therefore it cannot be implemented in practice. This problem is due to Practically speaking, this term of the control signal with high amplitude and frequency may cause damage to the actuator. These problems can be rectified by including at least a first-order low-pass filter to filter out the derivative control action. In this regard, we may have two possibilities. One possibility is to replace the derivative action Tds of theideal structure by Tds/ (1 + (Td/N) s) or the term (1 + Td1s) of the classical structure by (1 + Td1s)/ (1 + (Td1/N1) s). Therefore, the modified versions of (11) and (12) take the following forms, respectively:

$$G_{cm}(s) = K_{c1}(1 + \frac{1}{T_{i1}s})(\frac{1+T_{d1}s}{1+(T_{d1}s/N_1 s)}) \quad (20)$$

The block diagrams of (20) are presented in Fig 5.

DESIGN OF HYDROGEN AQUA ELECTROLYSER UNIT

The hydrogen aqua electrolyser (HAE), hydrogen storage tank, and fuel cell (FC) combination is designed to provide long-term and large-scale load demand support to the power grid system. During normal time, the HAE is used to produce hydrogen (H_2) by electrolyzing water and compressing it before storing it in the tank. A proton exchange membrane FC absorbs stored H_2 and produces electricity directly, which can be used to satisfy immediate load demands.

In this, hydrogen and oxygen are obtained as by-products from the water. As compared to any single energy conversion method, this process is extremely effective. The HAE-FC unit performs well even in Hybrid Renewable Energy Systems (HRES) that include wind turbines, photovoltaic (PV) arrays, and/or battery stacks (BS).Thus, the transfer functions of HAE and FC are represented by first-order lag as follows [11]:

The transfer function of HAE is given by

$$G_{HAE}(s) = \frac{K_{HAE}}{1+sT_{HAE}} \quad (21)$$

The transfer function of HAE is given by

$$G_{FC}(s) = \frac{K_{FC}}{1+sT_{FC}} \quad (22)$$

Where, K_{HAE} and K_{FC} are the gains of HAE and FC, respectively. T_{HAE} and T_{FC} are the time constants of HAE and FC, respectively. The values of these parameters are taken from [11] as given in Appendix.

JAYA ALGORITHM FOR SOLVING THE AGC PROBLEM

A simple yet powerful optimization algorithm is proposed in this paper for solving the constrained and unconstrained optimization problems. This algorithm is founded on the idea that the best solution for a given problem should be pursued, while the worst option should be avoided. There are no algorithm-specific control

parameters required for this algorithm, simply the standard control parameters. Since its development, Jaya is recognized as a global optimization technique, but very little work is seen in the field of interconnected power system. Venkata Rao developed a newer form of simple roptimization technique [27] called Jaya which means victory. For the first time, Jaya AGC strategy is used to optimize PID structured regulator for interconnected diverse-source power system [28]. The advantage of this algorithm is that it involves very less control parameters, whereas the entire evolutionary and swarm intelligence are probabilistic algorithms and it considers a large number of algorithm-specific parameters along. Jaya algorithm is inspired by TLBO technique. Unlike two phases in TLBO, Jaya has one phase, which makes it a simple algorithm. It works on the simple concept of an approaching best solution and avoiding worst solution. The process of updating solution, during optimization of PID structured regulator, is according to Equation 23 and can be given as:

$$x_{j,k,i}^{new} = x_{j,k,i} + r_{1,j,i}\left(x_j, Best_{,i} - \left|x_{j,k,i}\right|\right) - r_{2,j,i}\left(x_j, Worst_{,i} - \left|x_{j,k,i}\right|\right), \quad (23)$$

Where $x_{j,k,i}$ is the value of j[th] variable for k[th] population during i[th]iteration; $x_j, Best_{,i}$ *and* $x_j, Worst_{,i}$are the values of j[th] variable for best and worst population. The optimization from which the performance index value of Equation. (23) is minimum in the last iteration predicts the optimized PID structured regulator parameters to be applied in simulation.

ASSESSMENT OF POWER SYSTEM RESTORATION

The Feasible Restoration Indices [25] are calculated as follows, The FRI_1 (ε_1) is defined as the ratio between the settling time of frequency deviation in area1 (ζ_{s1}) and power system time constant (T_{p1}) of area 1

$$\varepsilon_1 = \frac{\zeta_{s1}}{T_{p1}} \quad (24)$$

The FRI_2 (ε_2) obtained as the ratio between the settling time of frequency deviation in area 2 (ζ_{s2}) and power system time constant $(T_{p2})$of area 2

$$\varepsilon_2 = \frac{\zeta_{s1}}{T_{p1}} \quad (25)$$

The FRI_3 (ε_3) is obtained as theratio between the settling time of tie-line power deviation (ζ_{s3}) and synchronous power coefficient T_{12}

$$\varepsilon_3 = \frac{\zeta_{s3}}{T_{12}} \quad (26)$$

The FRI_4 (ε_4) is obtained as thepeak value frequency deviation$\Delta F_1(\zeta_p)$ response of area 1 exceeds the final value $\Delta F_1(\zeta_s)$

$$\varepsilon_4 = \Delta F_1(\zeta_p) - \Delta F_1(\zeta_s) \quad (27)$$

The FRI_5 (ε_5) is obtained as thepeak value frequency deviation$\Delta F_2(\zeta_p)$ response of area 2 exceeds the final value $\Delta F_2(\zeta_s)$

$$\varepsilon_5 = \Delta F_2(\zeta_p) - \Delta F_2(\zeta_s) \quad (28)$$

The FRI_6 (ε_6) is obtained as thepeak value tie-line power deviation $\Delta P_{tie}(\zeta_p)$ response exceedsthe final value $\Delta P_{tie}(\zeta_s)$

$$\varepsilon_6 = \Delta P_{tie}(\zeta_p) - \Delta P_{tie}(\zeta_s) \quad (29)$$

The FRI_7 (ε_7) is obtained from thepeak value of the control input deviation $\Delta P_{c1}(\zeta_p)$ response ofarea 1 with respect to the final value $\Delta P_{c1}(\zeta_s)$

$$\varepsilon_7 = \Delta P_{c1}(\zeta_p) - \Delta P_{c1}(\zeta_s) \quad (30)$$

The FRI_8 (ε_8) is obtained from thepeak value of the control input deviation $\Delta P_{c2}(\zeta_p)$ response ofarea 2 with respect to the final value $\Delta P_{c2}(\zeta_s)$

$$\varepsilon_8 = \Delta P_{c2}(\zeta_p) - \Delta P_{c2}(\zeta_s) \quad (31)$$

Apart from the normal operating condition of the test system few other cases studies such as one-unit outage in any area and outage of one distributed generation in both areas are considered individually. The optimal controller gains and their performance of the system various case studies the corresponding Comprehensive Restoration Indices (CRI)(ε_9; ε_{10}; ε_{11}; ε_{12}; ε_{13}; ε_{14}; ε_{15}; ε_{16}) is obtained from (24)– (31).

Feasible Restoration Indices

Poolco based transaction.

In this, the DISCOs should contract with the GENCOs in the similar area only. It is presumed that the two DISCOs in controlarea-1 demands 0.1 p.u.MW of power from the GENCOs of the same control area and the DISCOs in control area-2 have not any contract with the GENCOs in area-2. Hence, the load deviation inarea-1 is 0.2 p.u.MW and that in area-2 is zero. Based on DPM (1) the DISCOs and GENCOs is simulated in case of poolco based contractsis as follows

$$DPM = \begin{vmatrix} 0.5 & 0.5 & 0 & 0 \\ 0.5 & 0.5 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix} \quad (32)$$

Each GENCO participated in AGC as defined by following contract participation factors (cpfs) and in this case, it is presumed that all the DISCOs participated equally in their respective areas i.e., cpf11 = cpf12 = cpf21 = cpf22 = 0.5. In this case the scheduled tieline power is zero.Considerscenario-1 again with a modification that Disco demands as given in Tables 2 and 3which shows the tuning parameters of the existing and proposed controllers. From the simulation results Power System Restoration Indices such as Feasible Restoration Indices are evaluated using in (24)–(31) from dynamic output responses of the proposed test system T-TIPS using either PI^2or PID interacting controller is shown in Table 4 -6 Case (1-4). Moreover, the proposed approach is also compared with tuned PI^2controller. It is concluded from Table 7 and 8 that, system performance greatly increases in terms of settling times, peak overshoots and under shoots with proposed approach

Bilateral transaction

In bilateral based transaction, the DISCOs can contract with any GENCOs of any area. It is assumed that all the DISCOs having a demand of 0.1 p.u.MW and therefore, load deviations in each area becomes0.2 p.u.MW. Based on the following DPM the system is simulated

$$DPM = \begin{vmatrix} 0.5 & 0.25 & 0.5 & 0.4 \\ 0.2 & 0.25 & 0.2 & 0.2 \\ 0 & 0.3 & 0.2 & 0.25 \\ 0.3 & 0.2 & 0.1 & 0.15 \end{vmatrix} \quad (33)$$

In this case, the Disco1, Disco2, Disco3 and Disco 4, demand 0.15 pu.MW, 0.05 pu.MW, 0.15pu.MW and 0.05 pu.MW from Gencos as defined by cpf in the DPM matrix and each Gencos participates in LFC as defined by the following ACE participation factor apf11= apf12= 0.5 andapf21= apf22 = 0.5. The comparative transient performances of two-area Power System using PID interacting controller for given load perturbation with HAE unit is shown in Fig.7.

Thecorresponding Feasible Restoration Indices are evaluated from dynamic output responses of the proposed test system T-TIPS using either PI 2 or PID interacting controller is shown in Table 4-6.
(case 5-8).

Comprehensive Restoration Indices

Apart from the normal operating condition of the test systems few other cases such as outage generating unit in any area and uncontracted power demand in any area during outage the corresponding Power System Restoration Indices is called Comprehensive Restoration Indices (CRI). In this studyGenco-4 in area 2 is outage and uncontracted power demanding any area and Disco Participation Matrix (31) is considered. The comparative transient performances of two-area Power System using PI^2 or PID interacting controller for given load perturbation and corresponding Comprehensive Restoration Indices (CRI) are calculated and tabulated in Tables 7-9 for the restructured power system with HAE-FC unit.

SIMULATION AND DISCUSSION

The Proportional Derivative (PD) interacted with Proportional Integral (PI) are designed and implemented in the two-area interconnected power system. The proposed controller for the two-are structured power system without/with HAE in area 1 are designed and implemented. Power system restoration based on the modified PID controller is designed and implemented for the above-mentioned power system for a step load disturbance in area 1/area 2 and the corresponding frequency deviations, tie-line power deviations and control input deviations are plotted for easy comparison and are presented in Figs. 8-9. It is observed from the output responses that, FRI and CRI based modified controller when incorporated in the two-are a interconnected power system has not only improved the transient response of the system but also has reduced the settling time. Various control strategies have been proposed to optimize the AGC to enhance a better control over not only the inter-area tie- line power flow but also to optimize the control input requirements. Simulation results reveals that the output response with the proposed controller (PD interacted PI controller) with and without HAE provides a high-quality transient and steady state response when compared to that of the output response obtained using PI 2 controller of the system

CONCLUSION

This study has been carried out using JAYA algorithm which is used to tune the PID controller parameters and PD interacted PI controller parameters for various control strategies in enhancing a better AGC in a two-area restructured power system without / with HAE-FC Unit. The JAYA Algorithm is found to be easy to implement without additional computational complexity, with quite promising results and ability to jump out the local optima. The design procedure used for the assessment of the restructured power system provides information in adopting the remedial measures to minimize the frequency deviations, tie-line power deviation and control input deviations of the two-area restructured power system to ensure more reliable operation of the power system. The PI^2 controller and proposed PI Controller interacting with Proportional – Derivative terms (PD interacted PI controller) are designed using JAYA algorithm and when implemented in the restructured power system it has been found that the proposed (PD interacted PI controller) shows a better performance in improving the system reliability when compared with the output responses of the system with PI^2 controller. It may be concluded that the proposed design concept had the advantage of damping out the inertia mode and inter-area modes of the interconnected restructured power system thereby enhancing a good margin of stability.

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Table: 1 Tuned control parameters for the corresponding Load demand change of the Thermal- Thermal Interconnected Restructured Power System (T-TIPS) using PI² controller.

T-TIPS	PI² controller gain of area 1			PI²controller gain of area 2			Load demand in p.u.				Uncontracted load demand in p.u.	
	K_{P1}	K_{I1}	K_{I2}	K_{P1}	K_{I1}	K_{I2}	Disco 1	Disco 2	Disco 3	Disco 4	Area 1	Area 2
Case1	0.113	0.067	$1.12x10^{-2}$	0.063	0.012	5.51×10^{-4}	0.1	0.1	0	0	0	0
Case2	0.127	0.046	$4.16\ x10^{-3}$	0.039	0.007	3.47×10^{-4}	0.1	0.1	0	0	0.1	0
Case3	0.136	0.071	$9.35\ x10^{-3}$	0.094	0.016	7.12×10^{-4}	0.1	0.1	0	0	0	0.1
Case4	0.127	0.052	$5.17\ x10^{-3}$	0.085	0.018	1.02×10^{-3}	0.1	0.1	0	0	0.1	0.1
Case5	0.104	0.075	$1.33\ x10^{-2}$	0.041	0.043	1.11×10^{-2}	0.15	0.05	0.15	0.05	0	0
Case6	0.129	0.085	$1.35\ x10^{-2}$	0.043	0.044	1.14×10^{-2}	0.15	0.05	0.15	0.05	0.1	0
Case7	0.107	0.072	$1.21\ x10^{-2}$	0.071	0.053	9.86×10^{-3}	0.15	0.05	0.15	0.05	0	0.1
Case8	0.126	0.082	$1.34\ x10^{-2}$	0.082	0.054	8.76×10^{-3}	0.15	0.05	0.15	0.05	0.1	0.1
Case9	0.139	0.101	$1.79\ x10^{-2}$	0.082	0.027	2.18×10^{-3}	0.12	0.08	0.14	0.06	0	0
Case10	0.144	0.102	$1.78\ x10^{-2}$	0.084	0.026	2.01×10^{-3}	0.12	0.08	0.14	0.06	0.1	0
Case11	0.137	0.098	$1.75\ x10^{-2}$	0.083	0.025	1.89×10^{-3}	0.12	0.08	0.14	0.06	0	0.1
Case 12	0.143	0.086	$1.71\ x10^{-2}$	0.086	0.027	2.13×10^{-3}	0.12	0.08	0.14	0.06	0.1	0.1

Table. 2 Tuned control parameters for the corresponding Load demand change of the T-TIPS using PD interacted PI controller

T-TIPS	PI²controller gain of area 1			PI²controller gain of area 2			Load demand in p.u.				Uncontracted load demand in p.u.	
	K_P	K_I	K_d	K_P	K_I	K_d	Disco 1	Disco 2	Disco 3	Disco 4	Area 1	Area 2
Case1	0.394	0.461	0.401	0.311	0.529	0.499	0.1	0.1	0	0	0	0
Case2	0.419	0.389	0.218	0.356	0.371	0.667	0.1	0.1	0	0	0.1	0
Case3	0.505	0.469	0.897	0.228	0.406	0.168	0.1	0.1	0	0	0	0.1
Case4	0.423	0.432	0.828	0.342	0.302	0.221	0.1	0.1	0	0	0.1	0.1
Case5	0.369	0.586	0.251	0.380	0.221	0.243	0.15	0.05	0.15	0.05	0	0

Case6	0.482	0.641	0.311	0.311	0.508	0.127	0.15	0.05	0.15	0.05	0.1	0
Case7	0.393	0.559	0.458	0.294	0.483	0.328	0.15	0.05	0.15	0.05	0	0.1
Case8	0.396	0.618	0.624	0.238	0.403	0.795	0.15	0.05	0.15	0.05	0.1	0.1
Case9	0.529	0.819	0.618	0.366	0.387	0.467	0.12	0.08	0.14	0.06	0	0
Case10	0.529	0.856	0.822	0.447	0.361	0.203	0.12	0.08	0.14	0.06	0.1	0
Case11	0.519	0.647	0.764	0.491	0.340	0.488	0.12	0.08	0.14	0.06	0	0.1
Case 12	0.529	0.734	0.886	0.237	0.418	0.361	0.12	0.08	0.14	0.06	0.1	0.1

Table.3 FRI for T-TIPS using PI²controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_1	ε_2	ε_3	ε_4	ε_5	ε_6	ε_7	ε_8
Case1	0.853	0.837	40.12	0.336	0.294	0.033	0.126	0.096
Case2	0.915	0.851	41.88	0.541	0.382	0.046	0.213	0.106
Case3	0.881	0.927	44.91	0.413	0.438	0.051	0.126	0.231
Case4	1.192	1.332	51.42	0.596	0.702	0.073	0.208	0.219
Case5	0.901	0.871	38.24	0.309	0.396	0.058	0.113	0.053
Case6	0.923	0.892	39.73	0.524	0.453	0.061	0.223	0.105
Case7	0.914	0.961	42.22	0.367	0.618	0.067	0.124	0.119
Case8	1.224	1.294	50.74	0.608	0.949	0.069	0.213	0.136

Table. 4 FRI for T-TIPS using PD interacted PI controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_1	ε_2	ε_3	ε_4	ε_5	ε_6	ε_7	ε_8
Case1	0.822	0.814	39.12	0.316	0.278	0.031	0.114	0.094
Case2	0.884	0.842	40.54	0.524	0.342	0.041	0.209	0.104
Case3	0.855	0.923	42.56	0.409	0.398	0.048	0.123	0.223
Case4	1.074	1.117	49.85	0.577	0.658	0.067	0.199	0.208
Case5	0.894	0.868	37.23	0.285	0.278	0.049	0.103	0.052
Case6	0.914	0.872	38.54	0.514	0.443	0.058	0.222	0.103
Case7	0.895	0.945	41.87	0.347	0.608	0.065	0.117	0.112
Case8	1.190	1.192	49.72	0.526	0.924	0.066	0.207	0.121

Table. 5 FRI for T-TIPS for HAE unit using PD interacted PI controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_1	ε_2	ε_3	ε_4	ε_5	ε_6	ε_7	ε_8
Case1	0.741	0.941	27.912	0.432	0.640	0.026	0.129	0.111
Case2	0.765	1.021	31.451	0.419	0.433	0.046	0.126	0.219
Case3	0.805	0.996	28.662	0.354	0.431	0.037	0.083	0.273
Case4	0.709	1.004	29.472	0.473	0.512	0.059	0.064	0.378
Case5	0.791	0.902	27.947	0.327	0.764	0.024	0.097	0.348
Case6	0.849	0.757	33.113	0.384	0.801	0.051	0.081	0.188
Case7	0.836	0.941	28.634	0.338	0.755	0.039	0.184	0.113
Case8	0.895	0.719	24.262	0.347	0.750	0.040	0.186	0.319

Table 6 CRI for T-TIPS using PI²controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_9	ε_{10}	ε_{11}	ε_{12}	ε_{13}	ε_{14}	ε_{15}	ε_{16}
Case9	1.386	1.456	54.96	0.503	0.514	0.131	0.182	0.159
Case10	1.702	1.598	55.13	0.559	0.611	0.148	0.191	0.165
Case11	1.511	1.736	58.54	0.573	0.836	0.151	0.183	0.169
Case12	1.716	1.771	58.87	1.185	1.122	0.167	0.215	0.184

Table.7 CRI for T-TIPS using PD interacted PI controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_9	ε_{10}	ε_{11}	ε_{12}	ε_{13}	ε_{14}	ε_{15}	ε_{16}
Case9	1.367	1.432	54.26	0.487	0.508	0.129	0.180	0.149
Case10	1.622	1.580	54.13	0.524	0.599	0.146	0.188	0.161
Case11	1.435	1.610	57.79	0.569	0.789	0.147	0.171	0.158
Case12	1.671	1.626	58.54	1.108	1.114	0.159	0.197	0.174

Table.8 CRI for T-TIPS for HAE unit using PD interacted PI controller for different case studies

T-TIPS	Settling time(ζ_s)			Peak Over/ under shoot (m_p)			Control input deviation (ΔP_c)	
	ε_9	ε_{10}	ε_{11}	ε_{12}	ε_{13}	ε_{14}	ε_{15}	ε_{16}
Case9	1.089	1.314	53.706	0.969	0.875	0.147	0.181	0.181
Case10	1.144	1.313	54.998	0.462	1.096	0.149	0.182	0.182
Case11	1.215	1.306	54.261	0.339	0.735	0.145	0.187	0.187
Case12	1.268	1.396	54.547	1.064	0.925	0.140	0.181	0.181

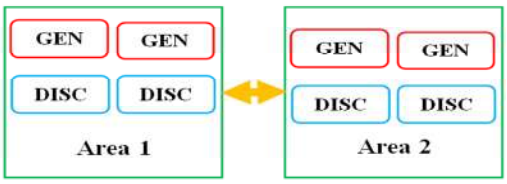

Figure.1 Schematic diagram of restructured interconnected system

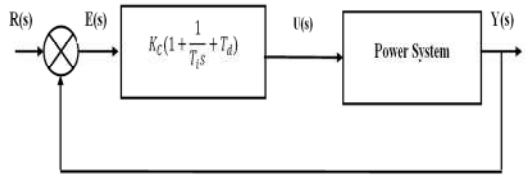

Figure.2 Transfer function diagram of conventional PI² control

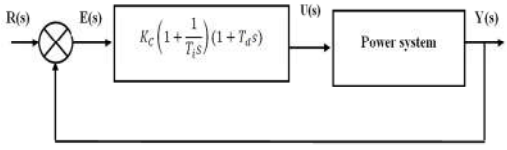

Figure.3 Transfer function diagram of Ideal PID controller in a unity feedback

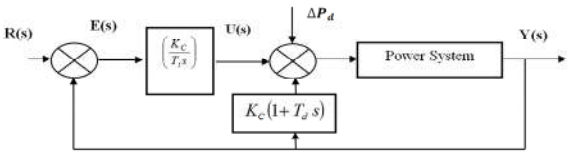

Figure.4 Transfer function diagram of classical PII controller in a unity feedback

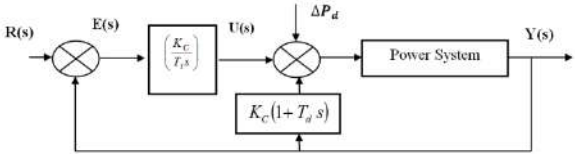

Figure.5 AGC loop using Interacting PI Controller with Proportional – Derivative terms acting on the output for closed loop control structure

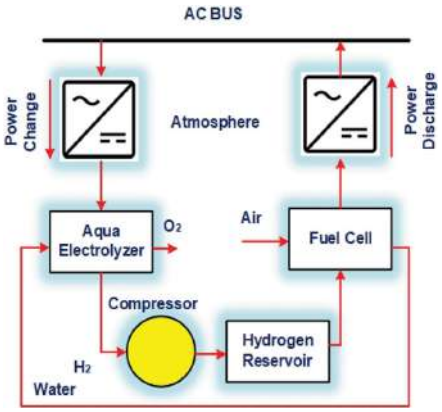

Figure. 6 Energy exchange processes of the Hydrogen Aqua Electrolyserunit with Fuel Cell

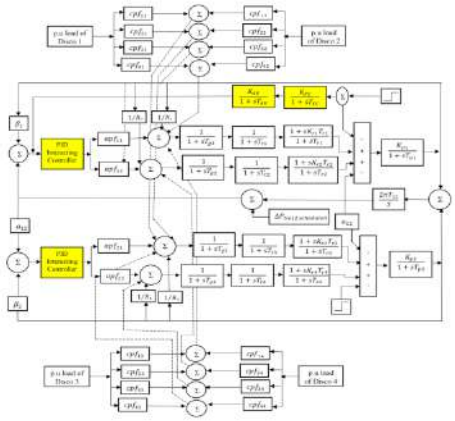

Figure.7 Block diagram of a two-area interconnected power system with Hydrogen

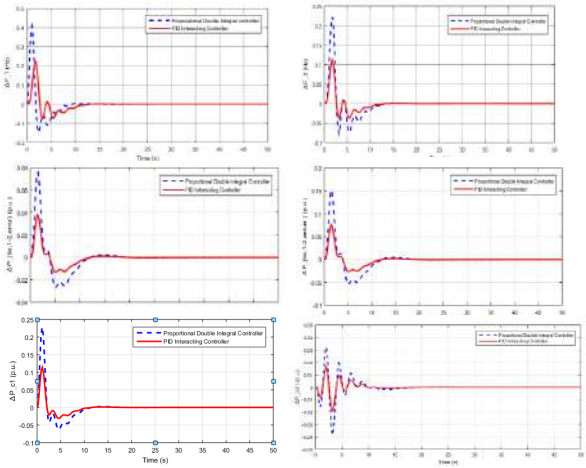

Figure 8. Dynamic responses of the frequency deviations, tie-line power deviations, and control input deviations in a two area LFC system using PI²controller and PD interacted PI controller in the restructured scenario(A) DF1 (Hz) Vs Time (s), (B) DF2 (Hz) Vs Time (s), (C) DPtie12; actual (p.u.MW) Vs Time (s), (D) DPtie12; error (p.u.MW) Vs Time (s), (E) DPc1 (p.u.MW) Vs Time (s), and (F) DPc2 (p.u.MW) Vs Time (s).

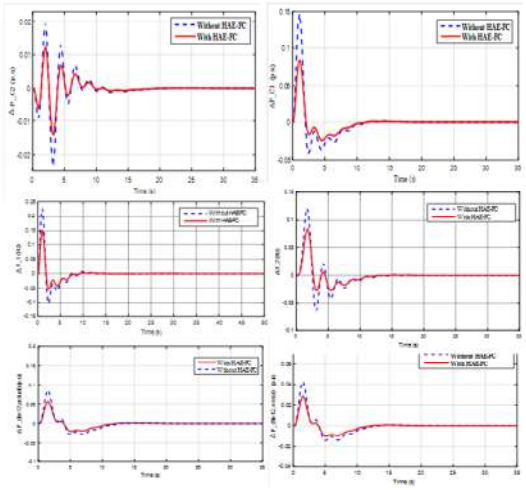

Figure 9. Dynamic responses of the frequency deviations, tie-line power deviations, and control input deviations in a two area LFC system with and without HAE unit using PD interacted PI controller in the restructured scenario(A) DF1 (Hz) Vs Time (s), (B) DF2 (Hz) Vs Time (s), (C) DPtie12; actual (p.u.MW) Vs Time (s), (D) DPtie12; error (p.u.MW) Vs Time (s), (E) DPc1 (p.u.MW) Vs Time (s), and (F) DPc2 (p.u.MW) Vs Time (s).

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RESEARCH ARTICLE

Microneedles: A Clever Techniques with Developed Drug Delivery System

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ABSTRACT

Hypodermic needles, topical creams, and transdermal implants are the most common approaches for transdermal drug administration. Since most therapeutic agents are restricted by the stratum corneum layer of the skin, which acts as a shield for molecules, only a few molecules are able to penetrate it. arrive at the action location. Microneedles are a modern type of delivery mechanism that helps to improve drug delivery via this path while also solving the issues that come with traditional formulations. The main idea is that the skin layer is disrupted, resulting in micron-sized channels that contribute to the drug. clear application to the epidermis or upper dermis zone where the drug can directly go into the systemic circulation without facing the barrier. This review describes the various potential and applications of the microneedles. The various types of microneedles can be fabricated like solid, dissolving, hydrogel, coated and hollow microneedles. Fabrication method selected depends on the type and material of the microneedle. This system has increased its application to many fields like oligonucleotide delivery, vaccine delivery, insulin delivery, and even in cosmetics. Many microneedle devices have entered the industry in recent years. But, before the microneedles can be successfully launched into the market, a lot of testing must be undertaken to solve the numerous challenges.

Keywords: Transdermal Drug delivery Microneedle Solid microneedle Hydrogel microneedle

INTRODUCTION

When it comes to drug distribution through the scalp, hypodermic needles and topical creams are the most widely used methods. Owing to the discomfort involved with needles and topical anaesthetics, patients are less accepting of them. Creams have a lower bio availability.The skin acts as a major barrier to drug delivery through the topical route. The stratum corneum, the middle epidermis, and the dermis are the three primary layers of skin. Dermis is the thickest of all. Only some molecules, such as lipophilic and low molecular weight products, can move through the stratum corneum membrane, which acts as a main barrier. The layer's lower permeability causes a slew of issues when it comes to formulating topical formulations Various topical or transdermal delivery devices, such as nanocarrier-loaded topical creams, transdermal patches, and microneedles, have been studied for enhancing opioid permeation across the skin. Various researchers have investigated microneedles (MNs) for drug delivery through the transdermal route and for overcoming the shortcomings of traditional approaches. Computer with microneedles consists of micron-sized needles that are arranged on a thin patch.The main issue with transdermal technologies is that certain medications do not have the ability to cross the skin at the desired pace for therapeutic action. Researchers have perfected a microneedle-based technology that allows hydrophilic high molecular weight compounds to join the stratum corneum. Using a microneedle instrument to administer medications allows drug molecules to cross the stratum corneum membrane, causing more drug molecules to penetrate the blood. The quicker onset of operation, greater patient behaviour, self-administration, increased permeability, and effectiveness are all characteristics of this technology. The hypodermic needle is inserted into the dermis, which contains pain receptors. As a result, it can produce 90–100% of the primed medication, but it is extremely unpleasant, resulting in low patient compliance. The stratum corneum barrier is bypassed, and the drug is delivered directly into the epidermis or upper dermis membrane, delivering 100% of the primed drug without discomfort.

MECHANISM OF DRUG DELIVERY

Topical route follows diffusion mechanism for the delivery of drug through the T route. The skin is temporarily disrupted in micro needle drug delivery system. In hundreds of micro needles arranged in the devices and arrays on the tiny patch. It delivers sufficient amount of drug in therapeutic response.By passing the barrier layer, it pierces the stratum corneum. In epidermis or upper epidermis drug is placed and it enters into the systemic circulation and it shows therapeutic response on reaching the targeted site.

DIMENSIONS OF MICRONEEDES

Depending on the sizes materials and micro needles are used. Since epidermis think is 1500 µm as same as the needle thick should have 1500 µm. it is sufficient to release the drug in epidermis. Larger needles can go deeper into the dermis and it damages the nerve and it causes pain. Mostly larger needles are 150-1500 microns long, 50- 250 micron wide and it have 1-25 micron thickness of tip. Micro needle device is to create micron size in transportable way, And the diameter of needles is kept between in few microns, micro needles tip can be cylindrical,triangle,pointed, pentagonal, octagonal and other many more shapes etc.

COATED MICRONEEDLES

Dipping or spraying the micro needles with an aqueous solution of increased viscosity to retain more formulation during drying and which contain a surfactant, the active agent and a stabilizing agent. Micro needles can be dipped one time or more than one time into a coating solution, each individual micro needles can be dipped into micro well containing drug solution or a film of drug solution previously formed on the roller can be applied, layer-by-Layer coating techniques.

DISSOLVING MICRONEEDLES

Encapsulated drugs are delivered by polymeric, microscopic needles in a minimal invasive manner. Now a days ,the superimposed patches of dissolving microneedle arrays that facilitate their insertion into the skin.

HOLLOW MICRONEEDLES

Micro-electromechanical system (MEMS) techniques –laser micro machining, deep reactive ion etching of silicon, an integrated lithographic molding techniques, deep X-ray photolithography, wet chemical etching and micro fabrication.

METHOD OF DELIVERING DRUG

Various methods to deliver the drug into the epidermal layer. First approach is to poke the skin and create the holes and drug patch over it and thus make direct pathway to the drug travel into the skin. Electric field makes better effect for the process. The second approach is to cover the surface of the needle with the drug and insert the drug needle into the surface the skin. The third approach is dipping the needle into the drug containing solution and scrape on the skin.

EVALUTION OF MICRONEEDLES

CHARACTERIZATION METHODS

The drug is loaded into the needle in suspension, encapsulation (or) dispersion form. The drug can be applied as a patch and it coated with the polymer solution. Loaded drugs are depending on the various physiochemical characteristic and the type of formulation used in the micro needles, adhesion, permeation, drug release tests are performed for a patch and it is applied after pre-treated. In different temperature, PH and simulated In-vivo physiological condition are studies of drug dispersion. Other test like drug content, solubility studies, bio-compatibility studies and In-vivo release test are performed on the designed micro needles.

DIMENSIONAL EVALUATION

To measure the tip radius, height and length of the micro needles and evaluate the needle geometry has various method. Optical or electrical microscopy is most common methods. Analysis of 3D image helps in quality control and it gives better picture of needle geometry. Confocal laser microscope and scanning electron microscope are used for the analysis of 3D image. Confocal laser microscope produce high resolution images and SEM produce an image of sample; it gives details about sample surface topography and composition.

MECHANICAL PROPERTIES (OR) INSERTION FORCES

The sharp and slender needles are easily penetrated into the skin.it does not break inside, while penetrate into the skin. Before using the needle mechanical tests are performed on the needle. The force at which the needles loses its structural integrity and insertion force are the two most important factors for the safe and efficient design of micro needles. The ratio of the two forces is called “safety factor”.

***In-vitro* skin permeation studies**

The drug's permeation through the skin is measured using a diffusion cell apparatus. The experiment mostly uses pig ear skin, which is placed between the receptor and donor compartments. The cumulative permeation profiles of skin that has been micro needled and skin that has not been micro needled are compared.

***In-vivo* animal model studies**

The research can be done with hairless rats. To anaesthetize the animal, a suitable technique must be used. Trans-epidermal water loss (TEWL), which is calculated before and after micro needling, is one of the parameters considered. This parameter is measured with a Delfin Vapometer.

PATIENT COMPLIANCE AND SAFETY

Skin recovery process

Micron-sized holes are left behind when a micro needle device is inserted into the skin and removed after the procedure. Re-sealing these pores can take some time. These holes must be resealed as soon as possible to avoid infection. The amount of time it takes for the skin to regain its barrier properties is critical. Electrical impedance measurements can be used to investigate pore resealing. Depending on whether the skin is occluded or not, as well as the geometry of the needle, recovery will take anywhere from 2 to 40 hours. Pore resealing can also be studied using TEWL and tissue staining.

Skin irritation

Transdermal injections, which are commonly used, cause a mild swelling around the injection site. This is due to the disruption of the skin layer when a foreign substance is injected into the skin.

Skin irritation and infection

Skin bears various protective mechanisms to defend itself when it is subjected to various environmental stresses. Micro needles can cause mild to moderate skin irritation or allergy in people with sensitive skin.There is redness, discomfort, and swelling. Itching can be a source of pain for patients. Unless the needles are sterile, holes created by inserting micro needles into the skin may become a source of infection. Despite the fact that the pores produced by micro needles are much smaller than those created by hypodermic needles, they allow for less microbial penetration.

Pain

Since micro needles do not penetrate far into the dermis to reach pain receptors, they cause less pain than a hypodermic needle. The number of micro needles on a patch, the length of the micro needle, and the tip angle or needle shape all influence pain intensity.Micro needles are less painful than a 26-gauge hypodermic needle, according to Gill et al. The pain associated with therapy is reduced by reducing the duration and amount of micro needles on the patch.

APPLICATION

Oligonucleotide delivery

Short DNA or RNA molecules are referred to as oligonucleotides. It's difficult to get oligonucleotides to their intracellular sites of action. As a result, numerous methods for improving distribution have been discovered. The micro needle method was used to deliver 20-merphosphorothioated oligodeoxynucleotide.The poke with patch method was used to deliver oligonucleotides using solid micro needles made of stainless steel or titanium. In comparison to intact skin, more drugs were found to enter the site of action. When iontophoresis was combined with a micro needle approach, the findings were stronger than when iontophoresis was used alone.

Vaccine therapy

A biological preparation is a vaccine. It offers disease-specific active acquired immunity. Vaccines are made up of a disease-causing microbe that has been destroyed or weakened, as well as its toxins or one of its surface proteins.Vaccine therapy strengthens the body's immune system and provides protection from future microbe encounters. The use of micro needles in vaccine therapy has been shown to be successful.A micro needle was used to administer the DNA vaccine. Immune responses were much higher than those seen with standard injections. There was also an effort to create a micro needle patch that could be used to administer influenza vaccine.When compared to intramuscular injection, a lower dosage is needed when the medication is administered using hollow micro needles. The use of hollow micro needles to administer anthrax and rabies vaccines was also investigated.To improve the intradermal vaccination quality, Ogai and colleagues created hollow micro needles out of poly-glycolic acid. Immunity is boosted thanks to the drug's specific distribution in the upper dermis.Antibody titers were substantially higher after intradermal vaccination with micro needles compared to subcutaneous injection on the 15th day after vaccination. Intradermal vaccination with dissolving micro needles was also examined.

Peptide delivery

When peptides are taken by mouth, they are enzymatically degraded. While transdermal delivery prevents this, it allows for a smaller amount of peptide to cross the skin. Peptide delivery through micro needles may aid in peptide penetration through the skin.Desmopressin is a potent peptide hormone that is synthesized from vasopressin. It's used to compensate for low vasopressin levels. Diabetes insipidus, bedwetting in young children, and haemophilia A are all treated with this drug. The use of micro needles to deliver desmopressin was investigated, and it was discovered that micro needle delivery was both safe and effective as compared to other methods.Cyclosporin A is a water-insoluble cyclic peptide with a high molecular weight that is used to treat a variety of skin diseases. Molding was used to create dissolving micro needles containing cyclosporine A with dimensions of 600 m in length and 250 m in width. Fabricated micro needles containing 10% cyclosporine A were pressed into the porcine skin for 60 minutes, resulting in the dissolution of approximately 65 percent of the micro needle and the delivery of 34 6.5 g of drug [62]. Liu et al fabricated GAP- 26 gap junction blocker loaded polyethylene glycol diacrylate dependent micro needles for delivering peptides through the swelling effect in one sample.The permeation of loaded peptide by the engineered micro needles was improved, which was validated by the inhibition of keloid fibroblast proliferation and collagen I expression.

Hormone delivery

Insulin is a hormone that is made up of peptides. The drug is used to help people with elevated blood sugar levels. The use of a micro needle to deliver insulin was found to be more effective in lowering blood glucose levels. Li et al created solid micro needles and investigated how they affected blood glucose levels and insulin delivery in diabetic mice. Li et al created solid micro needles and investigated how they affected blood glucose levels and insulin delivery in diabetic mice. The findings showed a reduction in blood glucose levels to 29% of the initial level after 5 hours, indicating that micro needle therapy increased insulin permeability to the skin. Micro needles integrated with pancreatic-cell capsules that sense blood glucose were studied by YE and colleagues. Insulin levels and secretion However, the patch was found to be ineffective.Thus, a micro needle matrix containing synthetic glucose signal amplifiers (GSAs) consisting of Nano vesicles containing glucose oxidase, - amylase, and glucoamylase an enzyme was developed. These amplifiers demonstrated insulin secretion from the -cell capsules. In comparison to traditional injection therapy, the findings of a clinical trial using parathyroid hormone (I-34) coated micro needles revealed a 3 times shorter Tmax and 2 times shorter apparent T1/2.These studies showed that micro needle therapy can be used effectively for hormone therapy. Furthermore, through the use of appropriate polymers, these can be adjusted for long-term action. In addition, iontophoresis in conjunction with micro needles may be used to deliver a variety of hormones.

Cosmetics

The use of micro needles in cosmetics is gaining popularity, especially for improving skin appearance and treating blemishes and scars. The micro needle method was used to deliver certain cosmetic active ingredients such as ascorbic acid, eflornithine, and retinyl retinoate.Melanin was added to phosphatidylcholine liposomes (nanoliposomes), which resulted in improved lipid solubility. When using an e-roller, the amount of pigment that penetrated deep near the hair structures was found to be higher. Micro needles were also used to investigate improved distribution of melanostatin, rigin, and pal-KTTKS.

Lidocaine delivery

Lidocaine is a local anaesthetic agent. When compared to a hypodermic injection, using a microneedle to administer lidocaine induces less discomfort and thereby improves patient compliance. Lidocaine was applied to the micro needle tips by Baek et al. In vitro, these micro needles demonstrated consistent skin penetration and improved drug delivery in 2 minutes. Micro needles can thus be used to provide painless and fast local anesthesia. When compared to a topical formulation, micro needles coated with PEG-lidocaine dispersions demonstrated improved drug delivery within 3 minutes in one trial.

Pain therapy

Polydimethylsiloxane moulds were used to make meloxicam-loaded polymeric micro needles. In-vitro permeation tests revealed that approximately 100 percent of the compound was released in 60 minutes. The drug deposition was found to be 63.37 percent, and the transdermal flux was increased to 1.60 g/cm2/hr. As compared to a free drug solution, permeation increased 2.58 times. Neuropathic pain is notorious for being difficult to manage. The available medications do not offer adequate pain relief and have certain side effects. The use of dissolved micro needles to relieve neuropathic pain was investigated. These delivered a selective calcitonin gene-related peptide (CGRP) antagonist peptide with high receptor specificity. There were no skin irritation or side effects from the analgesic micro needle pad. On the application, about 75% of the micro needle dissolved in 20 minutes. The successful delivery of therapeutics through micro needle has created enormous opportunities for the pain management industry.

Ocular delivery

Targeted drug delivery can be used to treat a variety of posterior segment indications. Nanoparticles were delivered via the suprachoroidal space using iontophoresis. The particles were found to localize at the injection site without iontophoresis. More than 30% of nanoparticles were administered to the posterior segment of the eye when paired with micro needles.

Cancer therapy

Every year, cancer strikes a large number of people around the world, and cancer care is fraught with difficulties. Micro needles have been studied for the delivery of anticancer drugs. Anti-PD-1 (aPD1) was delivered in a continuous manner using self-degradable micro needles for melanoma treatment. A micro needle was used to deliver anti-PD-1 and glucose oxidase-loaded pH-sensitive dextran nanoparticles. Basal cell carcinoma is treated with a topical cream containing 5-fluorouracil. When the cream was applied to skin that had been treated with strong micro needles, the permeability of 5-fluorouracil was increased by up to 4.5 times. Micro needle efficacy was further verified by significant inhibition of tumour growth. Bhatnagar et al studied the use of micro needles to deliver chemotherapeutic agents such as tamoxifen and gemcitabine for the treatment of breast cancer. The side effects of these medications could be reduced if they were delivered locally. Skin cancer and localized delivery of anticancer drugs were also studied using polymeric micro needles.

CLINICAL TRIALS AND SAFETY

Many pre-clinical experiments on micro needles have been conducted and found to be successful in many areas, but only a few have had success in human subjects. In the year 2001, Kaushik et al. performed the first human research on micro needles. As compared to a 26-gauge hypodermic needle, the aim was to see if the silicon micro needles were small enough to avoid pain. The micro needles were inserted into the forearms of the 12 healthy men and women who agreed to participate in the research. The discomfort caused by micro needles was found to be less than that caused by hypodermic needles in the study. Arya and her colleagues performed tests to see whether micro needles cause local skin reactions and are suitable to patients. A total of 15 people took part in the research. The micro needles did not cause any swelling, discomfort, or erythema at the patch application site, according to the report.The patches were self-administered by the patients without the use of an applicator. These were favored by the human subjects over traditional needles. The randomized clinical trial was performed on 21 men to see whether pretreatment with micro needles improved lidocaine delivery. After 60 minutes, a topical lidocaine cream containing 4% lidocaine provided anesthesia. Within 30 minutes, anesthesia was achieved thanks to the micro needle pre-treatment. A ten-patient open trial was performed to explore the therapeutic effects of a hyaluronic acid-based micro needle patch in the treatment of psoriasis.On the skin, calcipotriol-betamethasone ointment was added. Over this, a micro needle patch was applied once a day for a week. When compared to the traditional cream application, the one-week application significantly reduced the psoriatic plaques and was thus found to be effective.

CONCLUSION

Microneedles may be made in a range of sizes, forms, and textures using a number of different techniques. The right needle configuration will ensure a secure injection into the skin, reducing the risk of needle fracturing and patient discomfort. These painless systems are steadily gaining traction, and in the future, they may be one of the most critical applications for managed drug release.It was concluded that these devices represented effective and superior carriers for transdermal delivery as compared to other needle-based formulations.

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Table 1. MICRONEEDLE FABRICATION MATERIAL AND ITS PROPERTIES

Material type	MN type	Manufacturing method
α – aluminium (III) (α-al2o3),	ceramic (solid),	Lithography and ceramic sintering
Zirconia	hollow	micro molding, two-photon polymerization (2PP)
Glass	Hollow	pulling pipettes
Mesoporous silicon	coated	post-synthesis grafting method
Nickel\ iron	solid , Hollow ,coated	Laser-ablated ion, micro molding, Electroplating, wet etching
Nitinol	Hollow	multiple- pulse laser micro hole drilling
Silicon	solid,hollow,coated	etching, lithography
Stainless steel	solid,hollow,coated	Laser cutting, laser ablation, Etching, electro polishing, Lithography and microstereolithography.
Titanium	solid,hollow,coated	microelectromechanical system(MEMS)
Amylopectin	dissolving	photolithography
Chondroitin sulphate	hollow	2PP
CMC	hollow, dissolving	2PP, droplet-born air blowing DAB)Method
Dextran	hollow	2PP,atomized spraying process
Galactose,trehalose,maltose	solid, dissolving	Micromolding,atomized spraying process

Fructose,raffinose		
Thermoplastic	dissolving	Electro-discharge machining process
PLA	solid,dissolving	Fused deposition modeling (FDM)
PLGA	hollow, solid, dissolving	2PP, Micro molding
Polycarbonate	Solid	UV lithography, electroforming
PMVE\MA	Polymeric	Laser-based method for micro molding
Copolymer	hydrogel dissolving	
PVA	hydrogel	Atomized spraying process
PVP	Dissolving , hollow	2PP, atomized spraying process

Table 2. FABRICATED TECHNIQUES FOR DIFFERENT TYPES OF MICRONEEDLES

Types of micro needles	Fabrication technology
SOLID MICRONEEDLES	
Silicon micro needle	Silicon dry-etching process, isotropic etching, Anisotropic Wet etching, Dicing a silicon substrate and then acid Etching. And three - dimensional laser ablation.
Metal micro needles	Laser cutting, wet etching, metal electroplating methods.
Polymer micro needles	Photolithography.
Ceramic micro needles	Ceramic micro molding and sintering lithography.

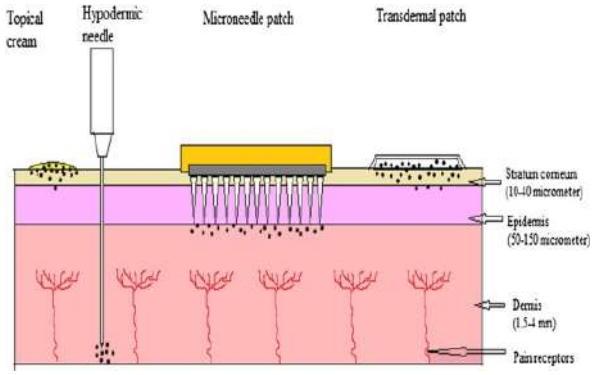

Fig.1.Transdermal Delivery Devices

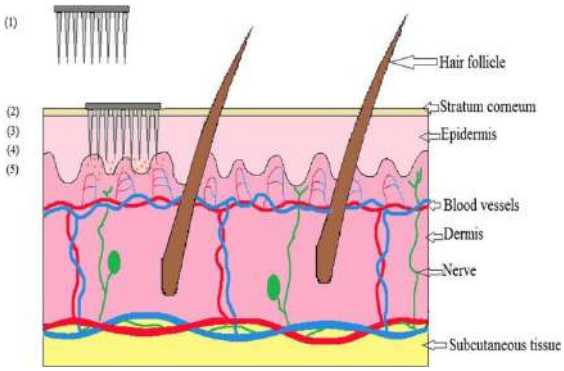

Fig.2.Fabricated Techniques

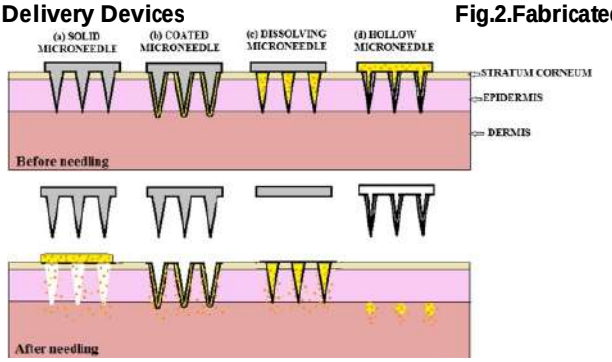

Fig.3.Method of Delivering Drug

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REVIEW ARTICLE

Review on Dental Caries

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ABSTRACT

Dental caries is a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the phase demineralization and remineralization of dental hard tissues. Caries can occur throughout life, both in primary and permanent dentitions, and can damage the tooth crown and, in later life, exposed root surfaces. The balance between pathological and protective factors influences the initiation and progression of caries. This interplay between factors underpins the classification of individuals and groups into caries risk categories, allowing an increasingly tailored approach to care. Dental caries is an unevenly distributed, preventable disease with considerable economic and quality-of-life burdens. The daily use of fluoride toothpaste is seen as the main reason for the overall decline of caries worldwide over recent decades. This aims to provide a global overview of caries, acknowledging the historical era dominated by restoration of tooth decay by progressive and more holistic long-term, patient-centred, tooth-preserving preventive care.

Keywords: Dental, biofilm-mediated, multifactorial, sugar-driven

INTRODUCTION

Dental caries consists in an exceedingly post-eruptive microorganism in a very characterized by a progressive demineralization method that affects the mineralized dental tissues. It's thought-about to be the foremost prevailing oral illness worldwide and also the main reason for tooth loss among the population [1]. Tooth decay area unit

chargeable for a high rate of morbidity among the population and area unit related to a reduced quality of life. It's familiar that the prevalence of tooth decay among the overall population has been coupled to socio-economic and demographic conditions, likewise as behavioral aspects [2]. Therefore, in most developed countries, the prevalence of tooth decay show a transparent tendency to say no within the last 3 decades of the 20th century and early twenty-first century [3]. Throughout this text we have a tendency to shall review some vital aspects concerning tooth decay and also the main etiological factors evolved, so health professionals will intervene within the treatment and interference of diseases.

Dental caries involves interactions between the tooth structure, the microbial bio-film shaped on the tooth surface and sugars, likewise as secretion and genetic influences. The dynamic dental caries method consists of apace alternating periods of tooth demineralization and remineralization, which, if web demineralization happens over decent time, leads to the initiation of specific dental caries lesions at bound anatomical predilection sites on the teeth. it's vital to balance the pathological and protecting factors that influence the initiation and progression of tooth decay. Protecting factors promote remineralization and lesion arrest, whereas pathological factors shift the balance within the direction of tooth decay and illness progression. Though the ravages of dental caries will build teeth seem to be extremely liable to destruction by illness, from associate degree biological process biology perspective, human teeth area unit a high-valued organ system concerned within the taking hold and process of food, and may conjointly perform in defense, sexual attraction and phonetic articulation . The outer surface of the tooth crown consists of enamel, the toughest substance within the body with spittle, a specialized fluid, being secreted throughout the day to preserve its integrity. The morphology of the trendy dentition has evolved principally supported our diet references, that have modified over the millennia[4]. Apparently, diets high in sugar tend to be soft and infrequently liquid; teeth don't seem to be needed for his or her bodily function, which could make a case for why teeth will be apace lost. This Primer aims to supply a balanced international summary of tooth decay, each as a fancy, complex illness and as a dynamically unsteady illness method. The article covers the total varies of views from medical specialty to quality of life via pathophysiology, diagnosis, risk assessment and interference. Public health aspects area unit a very important complement however don't seem to be lined comprehensive for reasons of area. Current analysis proof helps to chart the thanks to a lot of biologically based mostly manner of designing and delivering dental caries interference and care, at each the population and also the individual levels. These issues underpin the event of science, apply and policy to optimize patient care and health. Dental caries is wide recognized as Associate in nursing communicable disease evoked by diet. The most players within the a etiology of the sickness a cariogenic bacterium

- fermentable carbohydrates
- a prone tooth and host and
- time

However, in young youngsters microorganism flora and host defense systems a within the method of being developed, tooth surfaces a fresh erupted and will show hypo plastic defects, and their oldsters should hash out the dietary transition through breast/bottle feeding, 1st solids and childhood tastes. So it's thought that there could also be distinctive risk factors for dental caries in infants and young youngsters [5]. The pattern of decay is usually that several teeth a affected, with dental caries developing speedily, typically shortly once the teeth have erupted. Surfaces typically at low risk of developing dental caries a affected like the buccal surfaces of jaw incisors with the plain consequence of moving the child's facial look. It's this pattern of dental caries that has been labeled multifariously as 'baby bottle tooth decay', 'nursing caries' and 'night bottle mouth'. However, since these terms counsel that the prime reason for such dental caries is inappropriate bottle feeding and current proof suggests that though use of a sugar-containing liquid in an exceedingly bottle at night-time could also be a very important etiological issue, it should not be the sole or the foremost vital issue, it's currently suggested that the term 'early childhood dental caries' be used once describing any variety of caries in infants and pre-school youngsters (Reisine and Douglass), at inflated risk (frequent exposure to dietary carbohydrates, poor oral hygiene, and a cariogenic biofilm containing bacterium capable of zymosis carbohydrates and manufacturing a decrease in pH) [6]. As Fontana and Wolff (2011) note, it's vital to develop effective tools for bar and management that a risk primarily based

and patient focused. Investment in effective nonsurgical (medical) management of caries can pay off by reducing the necessity for dentistry to get rid of some or all of the tooth structure. Effective nonsurgical (medical) management of caries, as well as sickness bar and interventions in its earlier stages, needs early assessment to spot people in danger before visual indications of sickness prevalence (dental cavities) combined with person- and/or family– focused education concerning the importance of oral health and its link to overall health [7]. We have a tendency to believe a person's health acquisition is particularly vital to think about as a result of their understanding of the factors associated with the promotion of oral health and bar of sickness can impact their ability to require action and incorporate acceptable home care as a regular routine. Health acquisition as practiced may be a shared responsibility of the health care supplier, clinical setting, and patient/caregiver. The dental team will enhance a patient's self-efficacy by incorporating basic health acquisition principles, like the utilization of clear language and therefore the use of the "teach back" methodology in clinical apply. Ancient approaches to the treatment of caries have centered on repairing the implications of the sickness (cavities) instead of the sickness itself. From our perspective, person-centered approaches, like individual risk assessment, active police work, oral health acquisition, and preventive interventions/therapies, supplemented, once necessary, by surgical care (drilling, filling, extraction) ar the essential evidence-based approaches for the effective management of this sickness. we have a tendency to see that factors like worry, total and reimbursed prices, supplier convenience, transportation, and even parent or caregiver characteristics, as well as monetary distress, depressive symptoms, and restricted social networks, will be barriers to worry [8]. Management techniques that effectively arrest the {caries|cavity |dental dental caries | tooth decay |decay} method and permit remineralization of the caries lesion will facilitate conserve tooth structure and stop future surgical interventions. Moreover, in our opinion, inhume skilled collaboration with risk assessment and delivery of dental caries preventive interventions is important to cut back the two burdens of caries, because it can enhance access to the population teams that suffer disproportionately from this sickness method.

Caries Lesion Detection

To diagnose caries implies not solely finding a lesion (caries lesion detection) however, most significant, deciding if it's active, progressing speedily or slowly, or already in remission. While not this data, a logical call concerning treatment is tough [9] .Traditional dental caries detection tools embody visual and photography examination of teeth. New devices that concentrate on police investigation early lesions use technologies that distinction areas of tooth mineral loss to healthy tooth structure and, in our opinion, will facilitate monitor early stages of the sickness method and so monitor the result of non surgical interventions over time, instead of simply initiate early surgical care [10]. The Yankee Dental Association dental caries system proposes. Helpful nomenclature for clinicians to stage dental caries lesions by activity, severity, and that we believe this method facilitates deciding relating to management of the lesion. Activity assessment determines that lesions need treatment (surgical or nonsurgical). Lesion severity is vital choose once to surgically intervene with a restoration or filling as a result of true "cavity" formation permits bacterium to enter the deeper layers of the tooth. Location may be a key issue due to the distinctive challenges related to identification of lesions between teeth and on the occlusal (biting) surfaces of teeth[11]. Management techniques that effectively arrest the {caries |cavity| Dental caries |tooth decay| decay} method and permit remineralization or arrest of the caries lesion will facilitate conserve tooth structure and stop future surgical interventions.

Mechanisms / Pathophysiology

The mechanisms and pathophysiology underlying the event of decay |cavity| caries |tooth decay |decay} area unit currently progressively well understood and area unit best thought-about 1st from the arduous tissue-related aspects (as the sickness affects the calcified dental tissues) and so from the biology (biofilm)-related aspects (as these represent the driving force of the caries method if equilibrium imbalance is maintained) [12]. However, due to the many-sided nature of the sickness method, these factors don't seem to be independent. The dental arduous tissues that are exposed to the oral setting (crowns and, later, roots following animal tissue recession) area unit the targets of the tooth decay sickness method, and every one tooth surfaces area unit inclined throughout associate degree individual's period. However, tooth decay won't occur within the absence of a cariogenic (that is, pathogenic) dental

biofilm and frequent exposure to dietary carbohydrates, primarily free sugars, and thus tooth decay should be thought-about a dietary–microbial sickness. a contemporary idea of tooth decay conjointly includes thought of however behavioral , social and psychological factors, similarly as biological factors, area unit concerned. The importance of halide in modifying sickness expression can't be overemphasized (BOX 1)[13]. Maybe decay may be represented best as a fancy Biofilm mediate sickness that may be largely ascribed to Behaviour involving frequent activity of possible macromolecule (sugars like aldohexose, fructose, plant product and maltose) and poor oral hygiene together with inadequate halide exposure [14].

Demineralization and Remineralization.

Dental caries usually begin at and below the enamel surface (the initial demineralization is subsurface), and is that the results of a method during which the crystalline mineral structure of the tooth is dematerialized by organic acids created by biofilm bacterium from the metabolism of dietary possible carbohydrates, primarily sugars. Though a large vary of organic acids may be generated by dental biofilm microorganisms, carboxylic acid is that the predominant end-product from sugar metabolism and is taken into account to be the most acid concerned in tooth decay formation [15]. As acids build-up within the fluid section of the biofilm, the hydrogen ion concentration drops to the purpose at that conditions at the biofilm–enamel interface become under saturated, and acid part demineralizes the surface layer of the tooth. The loss of mineral results in augmented porousness, widening of the areas between the enamel crystals and softening of the surface, that permits the acids to diffuse deeper into the tooth leading to demineralization of the mineral below the surface (subsurface demineralization)[16]. The build-up of reaction merchandise, primarily atomic number 20 and phosphate, from dissolution of the surface and subsurface raise the degree of saturation and may part shield the surface layer from any demineralization. Additionally, the presence of halide will inhibit the demineralization of the surface layer. Once sugars area unit cleared from the mouth by swallowing and secretion dilution, the biofilm acids may be neutralized by the buffering action of spit. The hydrogen ion concentration of biofilm fluid returns towards neutrality and becomes sufficiently saturated with atomic number 20, phosphate and halide ions so demineralization stops and re-deposition of mineral (remineralization) is favored. as a result of the dynamic nature of the sickness method, the terribly early (subclinical) stages of tooth decay may be reversed or inactive particularly within the presence of halide [17].

As demineralization progresses into the subsurface of the enamel and dentine within the case of root tooth decay, with a unbroken acid challenge and hydrogen ion concentration drop, the speed of mineral loss becomes larger within the subsurface than at the surface, leading to the formation of a subsurface lesion. once comfortable mineral is lost, the lesion seems clinically as a white this can be a clinically vital stage of the tooth decay method, because the lesion may be inactive or reversed by modifying the motor faspot ctors or applying preventive measures; but, the repair method is usually largely restricted to the surface layer. At this stage in development, initial-stage tooth decay (ICDAS codes one and 2) area unit significantly demineralizes. With changes within the native ecology, dietary practices and halide convenience they may: arrest and stay as inactive lesions that don't progress however will still be detected as a scar due to the changes within the optical properties of the enamel; remineralize and effectively heal, whereby reprecipitation of mineral within the lesion associate degreed presumably some superficial surface wear leads to an apparently sound surface; or stay active and get to a a lot of in depth stage of destruction. If the tooth decay method progresses any, the surface porousness will increase with the formation of micro cavitations within the enamel (ICDAS code 3) or, in root tooth decay, a progressive softening of the surface dentine layer. In tooth decay of the tooth crown, the surface layer of the lesion might eventually collapse, leading to physical cavitations (a macroscopically hole — ICDAS code five or six). Even at this a lot of in depth stage of tooth decay severity, a lesion might in optimum circumstances still arrest, though the biofilm holding cavity can persist. Once associate degree irreversible stage of lesion extent is reached. (Typically, in most developed countries, at ICDAS codes five and six), combined with symptoms and/or concerns of the practical or aesthetic desires of the patient, operative intervention is indicated. If the tooth decay method continues, eventually the dental pulp are compromised and either a passageway treatment or tooth extraction are necessary. For optimum tooth health, the most goal is to keep up the mineral physiological condition of tooth surfaces. As teeth area unit oft exposed to acidic conditions either from

biofilm or dietary acids, the power to remineralize is important to maintaining tooth integrity. spit is important for the preservation of tooth health by providing the minerals necessary for remineralization. Low levels of halide greatly enhance this method, that for the most part explains the outstanding effectiveness of halide in many delivery forms in reducing decay (BOX 1). decay may be a dynamic sickness method that involves recurrent cycles of demineralization and remineralization throughout the day [18]. Teeth area unit most liable to tooth decay after they 1st erupt within the mouth and over time become a lot of proof against sequent acid challenge. The clinical implication is that there ought to be larger specialize in observance the tooth decay standing of teeth and delivering preventive care throughout the periods once teeth area unit erupting.

Microbiology and Dental Biofilms

Oral Microbiota in Health.

The mouth, like alternative surfaces of the body, is inhabited from birth by a various array of microorganisms (collectively referred to as the oral micro biota). The foremost common cluster of microorganisms is microorganism, but yeasts, viruses, mycoplasmas, protozoa and Achaea may be present. The oral micro biota contains a dependent or mutuality relationship with the host [19]. The resident oral microorganisms enjoy a heat and alimentary environs provided by the host and, in return, act to repel in cursive microorganisms, contribute to the host's defenses and interact in interference with the host to down regulate probably excessive pro inflammatory responses to communal microorganism. Secretion contains a crucial role in maintaining this helpful micro biota by buffering the oral atmosphere at a neutral hydrogen ion concentration (which is perfect for the expansion and metabolism of most of the oral micro biota), whereas providing proteins and glycoprotein's as nutrients [20].

Dental-Biofilms

The oral micro biota grows on surfaces as structurally and functionally organized communities of interacting species, termed plaque. Plaque is AN example of a biofilm, the formation of that involves many stages. Tooth surfaces a lined by a learning film of proteins and glycoprotein's that a derived primarily from secretion, however it additionally contains microorganism elements and their merchandise, animal tissue reticular fluid (that seeps from the junction between the gum and therefore the tooth), blood and food. The no heritable investment provides binding sites for adherence by early microorganism colonizers of the tooth surface, resulting in dental biofilm formation, and acts as a physical barrier preventing acid diffusion. Microorganism may be control infirm and reversibly close to the surface by long-range van der Waal forces (forces that don't involve valence or ionic bonds) between the external layers of the microorganism and this learning film. Attachment becomes stronger and additional permanent if interactions occur between molecules on the microorganism (adhesions) and complementary receptors within the learning film. Secondary colonizing species attach to the first colonizers (co-adhesion), and therefore the quality of the biofilm will increase. The biofilm undergoes maturation, and various synergistic and antagonistic microorganism interactions occur. A matrix is made, that consists of microorganism exopolymers (polymers secreted within the external environment), together with polysaccharides derived from sugar metabolism and DNA; the matrix helps to retain the biofilm on the surface and might influence the penetration and movement of molecules among the biofilm. The biofilm protects the microorganism against antimicrobial agents[21]. The composition of those biofilm varies on completely different surfaces of the tooth as a result of refined variations within the native environmental conditions.

Microbial Etiology of Dental Caries

The usually synergistic relationship between the resident micro biota and therefore the host is dynamic will |and may |and might be flustered by changes in fashion or alterations to the biology of the mouth; these changes can incline sites to sickness. Risk factors for dental caries embody the frequent consumption of possible dietary carbohydrates (especially sucrose) and/or a reduced secretion flow. Various cross-sectional and longitudinal medicine studies have rumored a shift within the balance of the micro biota at sites with dental caries compared with sites with sound surfaces. Early studies of dental caries lesions found higher proportions and incidence of eubacteria mutants and eubacteria serious than sound enamel; Lactobacillus were isolated from advanced lesions. These observations' junction rectifier to the proposal that dental caries is just caused by a restricted set of the various

species found in dental biofilm (the 'specific plaque hypothesis'). However, as additional medicine studies were performed, dental caries were determined within the apparent absence of those microorganisms, whereas these organisms may persist on alternative surfaces that remained sound resulting laboratory studies confirmed that alternative microorganism found among dental biofilm may additionally generate a coffee hydrogen ion concentration from sugars, whereas others may scale back the doubtless damaging result of carboxylic acid by victimization it as a nutrient supply and changing them to weaker acids, or by generating alkali from the metabolism of essential amino acid or organic compound in secretion [22]. These findings provided support for the 'nonspecific plaque hypothesis', during which dental caries may be a consequence of World Wide Web metabolic activity of the biofilm. Additional recently, studies classic culture or molecular approaches have found associations between dental caries and alternative teams of acid-producing and acid-tolerating microorganism, together with a variety of *Bifidobacterium* spp., *eubacterium* spp., *Propionibacterium* spp, *Scardovia wiggsiae*. Afterwards, different ideas are projected supported ecological principles that describe the events related to {caries| cavity| dental caries| tooth decay| decay} these ecological plaque hypotheses ar currently typically accepted because the most plausible explanations of the microorganism of caries. The initial 'ecological plaque hypothesis' recognized the consistency of microorganism operate (that is, speedy acid production and tolerance of the acidic conditions generated) within the absence of specificity in microorganism name, and stressed the essential demand of a caries-conducive atmosphere (that is, sugar-rich diet and/or low secretion flow). Microorganisms with traits that ar relevant to dental caries may be in biofilm on sound enamel, however at level or activity that's too low to be clinically relevant.

Dental caries may be a consequence of AN unfavorable shift within the balance of the resident micro biota driven by changes within the dental atmosphere. The regular exposure of plaque to possible. Dietary sugars lead to continual conditions of low hydrogen ion concentration within the biofilm that favor the expansion and metabolism of acid ¬tolerating microorganism whereas inhibiting helpful organisms that preferentially grow at neutral hydrogen ion concentration. Implicit this hypothesis is that the thought that sickness may be controlled, not solely by directly inhibiting the concerned microorganism however additionally by busy with the factors that drive the injurious shifts within the micro biota (that is, reducing the quantity and frequency of sugar intake to forestall acidic conditions or promoting the utilization of snacks containing different sweeteners that can't be metabolized to acid by oral bacteria). The ecological plaque hypothesis has recently been developed additional to replicate the flexibility of some oral microorganism to adapt to acid stress throughout regular and prolonged conditions of low hydrogen ion concentration, termed the 'extended dental caries ecological hypothesis. Again, natural action of the plaque acts because the main issue choosing AN acid-generating and acid-tolerating microorganism community, the event of that will increase the chance of dental caries. Thus, tooth decay isn't AN example of a classic communicable disease however may be a consequence of AN ecological shift within the balance of the usually helpful oral micro biota, driven by amendment in fashion and within the oral atmosphere. An appreciation of those principles opens new avenues for dental caries interference.

Environment and Dental Caries

Although biofilm formation could be an activity and is a necessary step for dental caries formation, the presence of a biofilm on a tooth surface isn't in and of itself a sign that sickness is present. It's solely once a posh interaction of host factors together with the tooth surface, no heritable investment and secretion, and free sugars within the diet that the presence of the dental biofilm will cause sickness expression over time. The distinctive environmental conditions that exist at every tooth website justify the extremely localized and complicated nature of the dental caries method, whereby dental caries will occur at a particular location on the tooth surface associate degree not on an adjacent tooth surface, even once each seem to be lined by biofilm [23]. These embody tooth-related factors that impact acid solubility (for example, tooth composition (such as amiss fashioned structure as in hypoplasias) and structure, pre-eruptive and post-eruptive halite exposure, and post-eruptive age of the tooth), and people that influence biofilm thickness and pathogenic by making areas of plaque stagnation (for example, tooth morphology, arch form, occlusion and tooth position). Development defects (for example, enamel dysplasia, a condition characterized by skinny enamel) might cause enlarged acid solubility and loss of surface structure, that creates sites of plaque

stagnation and will increase the danger of dental caries in primary teeth. Dental caries status also can be full of the proximity of teeth to exocrine gland orifices, and secretion film thickness and rate at specific tooth sites. Dental appliances (such as dental medicine appliances and dentures) and faulty restorations also can increase dental caries status at specific tooth sites by making areas of stagnation, encouraging biofilm formation.

Diagnosis, Screening and Prevention

Diagnosis, risk assessment, screening and hindrance an all vitally necessary issues for the winning understanding and management of caries at each the individual and therefore the population levels. In several countries, screening features a specific public health definition that's distinct from clinical apply, however this subject is on the far side the scope of this Primer. The main target here is on what happens at the individual patient level, whereby high numbers of patients daily act with oral health professionals round the world. It should be emphasized that, to forestall and management dental caries, each public health and individual level interventions got to be optimized and aligned. The International Dental Federation (FDI) and dedicated meetings46 have reviewed the dental caries risk and classification systems Though wonderful add developing a spread of assessment systems has been administrated in some countries (BOX 2), there's a shortage of comprehensive, internationally applicable, evidence-informed, holistic clinical systems, including ones developed by formal accord processes. Thus, though totally acknowledging that there ar a spread of alternative systems for endeavor some elements of the clinical tasks needed to tell fashionable dental caries management, we have a tendency to use the International dental caries Classification and Management System as a unifying framework as an example the key points. Within the ICCMSTM, the weather of dental caries risk assessment at each the patient and therefore the intra-oral levels, alongside the classification of dental caries by staging lesion severity and assessing lesion activity supported the ICDAS system, a brought alongside deciding [24]. This data is employed to provide a personalized care set up, which might then be undertaken with a stress on tooth-¬preserving dental caries hindrance and management, followed by a risk-based follow-up set up (dental recall). The four key parts of the system (simplified for general apply because the 'ICCMSTM 4D {caries |cavity |dental caries| tooth decay |decay} Management') permits a comprehensive assessment and formulation of a personalized caries care set up.

Prevention Public Health Management

The goal of caries hindrance is to preserve sound tooth structure, forestall demineralization of enamel and promote natural healing processes. Interventions may be enforced at the population level with health policy, legislation, regulation and public health approaches to push healthy behaviors and have an effect on broader social determinants of health. Hindrance approaches might target a complete population (for example, water addition and sugar taxes) to assure equity, or they'll target higher-risk teams to hunt to extend cost-effectiveness. The topic of halide and dental caries is taken into account in BOX one, and there's any thought of fluorides and dental caries hindrance in BOX three. Some dental caries risk factors at the population level embody low family financial gain, restricted aid access, low halide exposure, low oral health acquisition and high dental caries prevalence. Analysis is in progress to seek out the most effective ways that to focus on people with a high risk of developing dental caries. Hindrance programs may be targeted at teams with medical or special health care desires, like those with compromised immunity (for example, people with HIV infection or leukemia), psychological feature or biological process disabilities that may create oral hygiene tough, genetic disorders that a related to oral conditions (for example, birth defect and roof of the mouth, and ectodermic dysplasia), secretion pathology from Sjögren syndrome, diabetes, or frequent use of some medications that cause waterlessness (for example, antihistamines). As caries could be a complex sickness, complementary interventions could also be simpler than single interventions. The WHO's oral health action set up emphasizes the requirement for oral health hindrance programs to be combined with alternative chronic sickness hindrance and academic programs and policies that share common risk factors. Within the future, shared electronic health records, mobile sensible devices and social media might assist in these efforts[25]. The Alliance For a Cavity-Free Future (an international public health support charity) has chapters worldwide promoting a comprehensive agenda of activities and resources to forestall cavity initiation and progression. Support

and education efforts embody increasing public awareness and activity changes to boost oral hygiene and reduce sugar consumption, advancing analysis and clinical cavity management.

Diagnosis Risk Assessment

The first part in assessing a personal for {caries |cavity |dental {caries |cavity |dental {caries |cavity |dental cavity tooth decay| decay}|tooth decay |decay}|tooth decay |decay} in step with the ICCMSTM system is to work out patient-level caries risk by taking a comprehensive history asking a series of queries legendary to be related to increased caries risk or caries protecting factors. This includes assessment of the case history and therefore the relevant social history (for example, wherever the patient was born and raised, this residence, education level and occupation). Finally, the patient is asked concerning diet conditions in terms of daily sugar intake and frequency, the amount of between-meal snacks and therefore the form of dentifrice used: all data vital to assess the cavity risk at the individual level. A large vary of risk assessment tools are often used and a compatible with the ICCMSTM system. One such risk assessment is Cariogram, that there's a lot of proof than for several systems; studies have shown moderate accuracy on youngsters and young adults. Cavity Management by Risk Assessment (CAMBRA) and different university-compiled risk issue questionnaires a alternatives [26]. The Cariogram uses 9 predictors in its full form: the DMFT, connected diseases, diet content, diet frequency, quantity of plaque, levels of S. mutant, halide use, spit secretion and buffer capability. A coffee score (that is, zero or 1) indicates that a specific predictor contributes to low risk, whereas a high score (of two or 3) indicates high risk; so, Associate in Nursing overall risk are often calculable if the patient's profile remains stable. The danger assessment (independently of however it's derived) are often designed later into the ICCMSTM {caries |cavity |dental cavity| tooth decay| decay} risk likelihood matrix which mixes clinical caries activity with risk-level assessment. Eventually, it's doable to assess whether the patient is in low, moderate or high risk of developing a lot of new lesions or progression of the present cavity lesions inside consecutive few years. The danger of developing decay is often lowered by effective dietary recommendation, improved plaque management and increased use of halide, as an example, by using 1,450Ppm fluoridated dentifrice rather than one,050 Ppm toothpaste80, forward that the patient is compliant.

Clinical Assessments

In order to search out and assess any cavity lesions on individual tooth surfaces, a clinical examination is performed (the Discover and assess part of the ICCMSTM 4D methodology). The goal is to search out any cavity lesions gift and assess their severity, activity and therefore the risk factors at the tooth level. Assessments will embody spit secretion (and in some countries, buffer capability and therefore the presence of S. mutants a measured). Though these latter tests may increase patient motivation to Associate in Nursing assessment of cavity activity of the detected and staged lesions will then be created. Within the ICDAS organization many predictors a used: the situation of the lesion (whether it's a plaque stagnation area); the color of the lesion (whitish versus brownish); tactile feeling (rough or smooth) once a dull probe is run over the lesions, whether the lesion is matte or shiny, cavitated or non- cavitated; and, finally, whether the lesion is found on the animal tissue line and whether the gum bleeds once inquiring. A lesion is categorized as active if it's a lot of the subsequent characteristics: incorporates a plaque stagnation space, is whitish, incorporates a rough tactile feeling, is matte, cavitated, and if there's animal tissue bleeding; the ultimate identification of the lesion is assessed as initial, moderate or in depth active. If the lesion is categorized as inactive, the ultimate identification of the lesion is assessed as initial, moderate or in depth in remission.

Pulp Response to Dental Caries

Host pulp response to decay may be a key part in understanding the unhealthy method and its consequences. During this regard, enamel acts as a physical mineralized barrier preventing microorganism infiltration into the dentin and pulp. Also, the underlying dentin microscopic anatomy, composition, and performance give vital data on however bacterium invasion is hindered by the dentin itself and the way this dentin provides signaling molecules to induce dentin regeneration throughout the unhealthy method. Within the case of a deep unhealthy lesion reaching the odontoblasts, the pulp tissue it- self has additionally elaborate economical methods to hinder or perhaps arrest the unhealthy lesion progression and therefore the microorganism infiltration into the pulp.

Treatment of Caries

Restorative Materials.

The effects of bar on {caries |cavity| dental cavity| tooth decay| decay} prevalence and therefore the benefits of improved dental materials have shifted the main target in caries management from surgical strategies and restoring tooth structure to development and use of dental materials to forestall wellness, remineralization procedures, minimally invasive treatments for difficult-to-access regions and materials with that early lesions are often fertilized to forestall more progression. The table summarizes key historical events within the us involving restorative dental materials, instrumentality and techniques associated with the treatment of decay in single teeth. Whereas this table focuses on accomplishments in this country, we must always note that scientists in Japan (who developed dentin bonding systems and glass ionomers, for example) and Europe (who developed metal, salt cements, microfilm composites, hybrid composites and glass ionomers, among others) even have created several vital contributions. Continued decay wellness typically leads to tooth loss. Contributions associated with restoration for tooth replacement aren't enclosed here [27]. Throughout the long history of restorative odontology, U.S. dental corporations have developed several specialized hand instruments, dental burs, diamond cutting instruments and special finishing instruments. All of those technical developments in materials and treatment to revive unhealthy lesions have concerned a robust partnership of educational, corporate, association-based and governmental dental analysis entities and scientists. We tend to acknowledge the progress they need enabled odontology to attain in fighting oral wellness.

Treatment of Dental Caries with Antimicrobial

Dental caries may be a wellness caused by dysbiotic being that additionally produces biofilm and characterized by prolonged periods of acidic pH scale within the oral fissure that leads to web loss of mineral from the teeth. Associate in Nursing bactericide treatment ought to be thought-about as a part of the general management if the patient incorporates issue of microorganism load or metabolic dysfunction of the biofilm. The most bactericide agent used for decay has been antiseptic gluconate (0.12 %) rinse that acts as Associate in Nursing ant plaque agent. The antiseptic molecule acts on the cytomembrane of bacterium and disrupts its integrity. It's necessary to try and do the microorganism testing before the conduct of test to grasp the frequency of use of antiseptic rinse. Antimicrobic rinse also can be used as Associate in Nursing antiplaque agent, Associate in Nursing antimicrobial agent that acts on the cytomembrane and additionally agitates the biofilm. Within the terribly early stages it's doable to revive solid body substance and typically even will reverse cavity formation by mistreatment halide. A silver primarily based formulations (SBF and nano silver) has additionally shown antimicrobial activity against the predominant cariogenic flora significantly from dentin lesions[28]. The amount of cavity in childs are often reduced by native application of ten nothing povidone iodine resolution to the dentine of the infant each second month shown by a test placebo controlled test. The iodine is free slowly from povidone iodine that permits an extended amount bactericide effects. Xylitols may be a five-carbon sugar alcohol that has Associate in Nursing antimicrobial activity. Daily xylitol wipe application considerably reduced the cavity incidence in young youngsters as compared with wipe while not xylitol. The utilization of xylitol wipes is also a helpful adjunct for cavity management in infants. True bacteria mutants is vulnerable to 2 active ingredients glycyrrhizol A and B of the Chinese herb Glycyrrhiza uralensis. The extract of leaves of Bridelia scandens is extremely effective against true bacteria mutants and eubacteria. The antibiotics that act specifically on cariogenic microorganisms is a lot of ideal for treatment of decay than general antibiotic [29]. At a similar time indiscriminate use of antibiotics will result in development of resistance or persistence. To avoid the development of antibiotic resistance, the utilization of a mix of 2 or a lot of bactericide agents may be a sensible and quick suggests that of developing new medical aid for decay. The foremost ordinarily used antibiotics in practice was Polymox, penicillin V, antiprotozoal and the mix of Polymox and clavulanic acid.

Role of Saliva

Saliva maintains the traditional oral flora and tooth surface integrity through microorganism clearance, buffering capability, direct medication activity and remineralization. Once spittle flow is reduced, oral health issues like caries

and oral infections develop. Complications of caries embrace symptom, fistula, cellulites, odontogenic cysts, zoonotic disease, craniofacial phlebitis, jaw inflammation, septicemia and osteitis. The results of these infections will amendment in line with medicine standing of the patient further because the microbe resistance to the ordinarily used antibiotics. The 3 mechanisms connecting oral infections to secondary general effects-Transient bacteraemia leading to pathologic process unfold of infection from mouth. The current toxins created by oral organism which may cause pathologic process injury. The immunological injury caused by oral microorganisms. may result in pathologic process inflammation. Periodontitis as a significant oral infection could have an effect on the host's condition to general illness in 3 ways: by shared risk factors, sub gingival biofilms acting as reservoir of gram negative bacterium and also the periodontium acting as a reservoir of inflammatory mediators like cytokines. Cardiovascular diseases associated with dental Dental caries square measure coronary artery disease and infarction that happens as results of advanced set of genetic and environmental factors. The factors that make to vessel diseases related to caries will be classified into genetic factors like avoirdupois, lipoid metabolism, diabetes, high blood pressure and environmental factors like diet, smoking socioeconomic standing. The oral origin of strep mutants is obvious from its concurrent presence within the vessel region and within the bacterial plaque. strep mutans is thought to be related to bacteraemia and infective carditis. strep mutans strains square measure classified into four serotypes C, E, F and K supported their cell surface rhamnose-glucose polymers[30]. A High frequency of serotype K strain has been known in strep mutans positive extirpated heart valve specimen from infective carditis patients. The most vital factors in the pathologic process of infective carditis because of strep mutans is living substance aggregation. The issue| clotting factor} binding affinity of strep mutans is additionally thought of to be a virulence factor for infective carditis. These 2 properties square measure a lot of ordinarily found in serotype K strains. Infective carditis due to eubacterium is comparatively uncommon as compared to infective carditis due strep mutans. eubacteria even have been found to combination platelets, bind each fibronectin and factor I and cling to scleroprotein sorts one and five, that compose the animate thing matrix of epithelium cells. A recent study showed that the bacterium that cause caries square measure connected to AN response which can be protecting against cancer such as head and neck squamous cell cancer.

CONCLUSION

Dental caries is one of the most common and expensive diseases that can affect individual's health and quality of life considerably. This review highlights the significant role of Streptococcus and Lactobacillus in the development of dental caries. Important methods used to reduce the risk of dental caries usually involve decreasing the growth or activity of Streptococcus mutants. Several risk factors of dental caries are also documented in this review. It is predicted that diagnostic, preventive and treatment strategies directed towards specific bacterial species will not be universally effective because of polymicrobial nature of dental caries. The caries lesion is a result of the imbalance in the equilibrium between mineral loss of tooth and biofilm fluid usually results in caries lesion. Caries is an endemic and potentially both preventable and curable. Development of increased resistance by the antibiotics currently used in dental practice hinders the prevention of oral bacterial growth, adhesion and colonization. In order to be secured from these troublesome infections it is necessary to take necessary precautions like brushing twice a day, reduction in intake of sucrose rich food, regular mouth washing and flossing.

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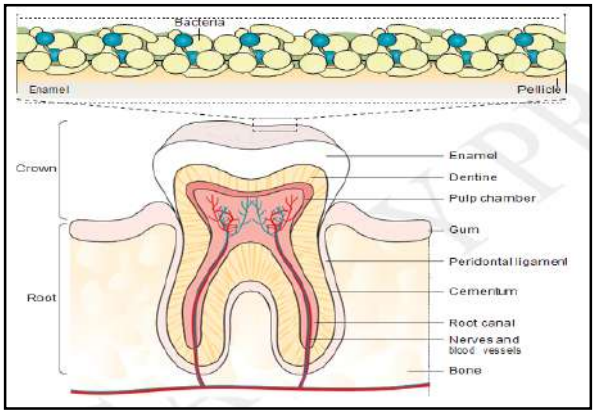

Fig.1 : Demineralization and Remineralization.

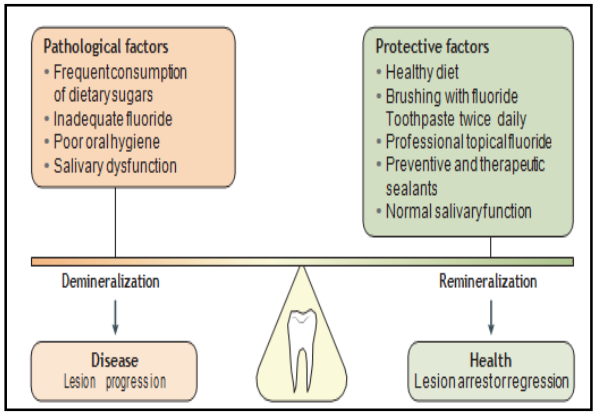

Fig.2 : Microbial Etiology of Dental Caries

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RESEARCH ARTICLE

Study and Investigation of a Jet Fighter Aircraft

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ABSTRACT

The manufacturing of airplane is much complicated and costlier rather than designing of airplane. The manufacturing company encounters a major loss if the plan of manufacturing of an airplane is dropped after starting the manufacturing process for any fault design etc..The main objective of this paper is aimed to design and investigate a supersonic fighter jet with a comparatively good performance in all aspects because of the importance of fighter jets in military purpose is increasing in now a days. This project mainly focused on performance improvement on airplane cruises at supersonic speed with quick and steep turn angles. So single pilot can carry a ammunition of large quantities. In this regard, the designing of aircraft gives way to both innovative design as well as a improvement of a older ones to the highly safe design of today's world requirement and scenario.

Keywords: Airplane, Design, Major loss, Supersonic, Steep turn angle.

INTRODUCTION

The design process is the first step towards the manufacturing of airplane. The airplane to be manufactured has to be designed first we need to ensure that their design is correct and it would fly if it is set to fly. Initially it will be the blue print of the airplane to be manufactured because we can edit the design as per the market requirement and also we can make any changes if needed any. In this project, it is planned to design supersonic jet fighter airplane in order to accommodate steep turn angle effectively for reducing the work load of the pilot. Initially each components need to be designed based on the theoretical calculation and final version of the proposed airplane is to be designed.

Landing gear design

The landing gear must absorb the shock of a bad launching and smooth out the ride when taxing. The tires itself provide some shock absorbing ability by deflecting when a bump is encountered. Today The most commonly used landing gear arrangement is 'tricycle' gear with two main wheels after the centre of gravity and an auxiliary wheel

forward of the centre of gravity. With a tricycle landing gear the centre of gravity is ahead of the main wheels so the aircraft is stable on the ground and can be landed at a fairly large "crab" angle. Also tricycle landing gear improves forward visibility on the ground surface and permits a flat cabin floor for passenger and cargo loading. To prevent the aircraft from overturning, the main wheels should b e laterally separated beyond a 25degree angle of the centre of gravity.

Diameter or width of the wheel
$D = A W_w^B$
For main wheel that value is 80% of the total aircraft weight and for auxiliary wheel it is 20% of total aircraft weight. So
W_w for main wheel = 0.8 * 4350 = 3480 kg.
W_w for auxiliary wheel = 0.2 * 4350 = 870 kg.
Diameter of the main wheel, from the Ramer book constant values for A and B will be taken as A= 1.51 and B= 0.349.
We have choosing a tricycle configuration, so two m ain wheels and one auxiliary wheel. So for one main wheel W_w is 5245.22.
Diameter of the main wheel $D = 1.51 * 5425.22^{0.349} = 30.35$ cm
Diameter of the auxiliary wheel $D = 1.51 * 2712.6^{0.349} = 23.83$ cm
To calculate width of the wheels take A= 0.1043 and B= 0.480
Width of the main wheel $W = 0.1043 * 4350^{0.480} = 5.8$ cm
Width of the auxiliary wheel $= 0.1043 * 2175.6^{0.480} = 4.17$ cm
Pavement or contact area for main wheel is $R_f = 31.46$ cm
Pavement or contact area for auxiliary wheel is $R_f = 21.56$ cm.

When used as a shock-strut, the oleo itself must provide the full required amount of wheel deflection, which can lengthen the total landing gear height. Also, the oleo strut must be strong enough to handle the lateral and breaking loads of the wheels. To repair or replace the oleo strut, the entire wheel assembly must be removed because it has attached to the bottom of the strut. The drag brace breaks at the middle for retraction and the drag brace may be behind the wheel with the gear retracting rearward or it may be in front of the wheel with the gear down in the event of a hydraulic failure. The diameter and width parameters for main as well as auxiliary wheel is calculated and this value is need to used for further process.

Wing design

Wing is a main component for aircraft and lift force which is essentially required to take off aircraft is generated by wing component. The first step involves a stability and control calculation to determine the required lift on the horizontal tail to balance the wing pitching moment at the critical condition because of supersonic jet fighter aircraft need to produce more lift and need more stability and control. Note that the required tail lift will increase or decrease the required wing lift to attain the same load factor. Complicated methods for estimating the lift on the trimmed tail and wing for a given load factor are calculated. This can be initially approximated by a simple summation of wing and tail moments the aircraft center of gravity, ignoring the effect of downwash, thrust axis etc… According to classical wing theory, the span wise lift distribution is proportional to the circulation at each span station. A vortex lifting-line calculation will yield the span wise lift distribution. For a elliptical plan-form wing, the lift and load distribution is of elliptical shape. For a non-elliptical wing, a good conventional semi-empirical method for span wise load estimation is known as Schrenk's Approximation. This method assumes that, the load distribution on an untwisted wing or tail has a shape that is the average of the actual platform shape and an elliptical shape of the same span and area. The total area under the lift load curve must sum to the required total lift.

As tip chord = 1.12 m
Root chord = 3.87 m
Span (b) = 12.7 m
Plan form area is $18.96 m^2$

Wing loading

Wing loading is the loaded weight of the aircraft divided by the area of the wing. The faster an aircraft flies, the more lift is produced by each unit area of wing. So a smaller wing can carry the same weight in level flight, operating at a higher wing loading. Correspondingly, the loading and take-off speeds will be more and high wing loading also decrease the manoeuvrability. Wing loading is a useful parameter of the maneuvering performance of an aircraft. Wings generate lift owing to the motion of air over the wing surface. Larger wings move more air, so an aircraft with a large wing area relative to its mass will have more lift at any given speed. Therefore an aircraft with lower wing loading will be able to take-off and land at a lower speed and also able to turn faster.

Thrust –to-weight ratio

It is a ratio of thrust to weight of a rocket, jet engine, propeller engine, or a vehicle propelled by such an engine. The thrust-to-weight ratio and wing loading are most important parameters to determine the performance of the aircraft for both steady and unsteady condition. The thrust-to-weight ratio varies continually during a motion. Thrust varies with throttle setting, airspeed, altitude and air temperature. Weight varies with fuel burn and changes of payload. For an aircraft, the quoted thrust-to-weight ratio is often the maximum static thrust at sea level divided by the maximum take-off weight. In cruising flight, the thrust-to-weight ratio of an aircraft is the inverse of the lift-to-drag ratio because thrust is equal to drag and weight is equal to lift.

Lift-to-drag ratio

The lift-to-drag ratio, or L/D ratio, is the amount of lift generated by a wing or vehicle, divided by the drag it creates by moving through the air. A higher or more favourable L/D ratio is typically one of the major goal in aircraft design. Since a particular aircraft's required lift is set by its weight, delivering that lift with lower drag leads directly to better fuel economy, climb performance and glide ratio. Coefficient of lift, coefficient of drag, drag due to lift and zero lift drag is calculated for different altitudes starting from sea level and values are tabulated. Velocity is taken as a input parameter to determine the above parameters with respect to different altitudes. All the parameters have been calculated with a difference of 4m altitude from sea level.

V-n diagram (velocity – load factor diagram)

In accelerated flight, the lift becomes much more compared to the weight of the aircraft. This implies a net force contributing to the acceleration. This force causes stresses on the aircraft structure. The ratio of the lift experienced to the weight at any instant is defined as the load factor(n). V-n diagram is also called as a velocity-load factor diagram and it is a important parameter because its limited by the structural design of the airplane. Now assume that, the angle of attack is increased to that for obtaining maximum lift co-efficient $C_{Lmax,}$ keeping the velocity constant at V_1, the lift increases to its maximum value for the given V_1, and hence the load factor reaches its maximum value for the given V_1. If the angle of attack is increased further, the wing stalls and load factor drops. However maximum load factor cannot be allowed to increase indefinitely. Beyond a certain value of a load factor, structural damage may occur to the aircraft. As velocity greater, the dynamic pressure becomes so large that again structural damage may occur to the airplane. In fact, the structural design of most airplanes are such that the maximum velocity allowed the V-n diagram is sufficiently greater than the maximum diving velocity for the airplane. At maneuver point, both lift coefficient and load factor are simultaneously at their highest possible values that can be obtained anywhere throughout the allowable flight envelope of the aircraft. Consequently this point corresponds to the smallest possible turn radius and the largest possible turn rate for the airplane. The corner velocity can be evaluated by using velocity yielding.

For our design calculation,
Load factor positive limit = 6
Load factor negative limit = -3
Starting point of structural failure positive load factor limit = 4
Starting point of structural failure negative load factor limit = -2

Cabin design

Cabin – 10 to 12 passengers fully enclosable toilet and refreshment centre
Flight deck – 2 pilots
Baggage Compartment Volume
Front (in cabin) – 700 1 / 25 Cu ft
Rear (in cabin) – 370 1 /13 Cu ft
Pressurization 0.59 bar /8.245 psi
Cabin height at 9,144 m / 30,000 ft

Primarily cabin safety focuses on aircraft occupant safety, and the safety responsibilities and roles of aircraft cabin crew members. This safety interests overlap into many areas of the aviation industry, including aircraft design, configuration, operations, in-flight service, maintenance, and flight crew training etc...

RESULT AND DISCUSSIONS

It has calculated many specifications and parameters which are essentially required to design a jet fighter aircraft has been summarized in the following tables

CONCLUSION

Maximum speed, altitude and some other important specifications and basic parameters which are essentially required for basic design have been taken from literature survey. The parameters discussed in above section was calculated and compared with existing data for verification. We have calculated the performance analysis parameters at the interval of 4m altitude from the sea level and plotted the calculation for sea level, 4m height and 8m height. So totally 3 iterations have been conducted to estimate the lift, drag, load factor and thrust-to weight ratio. With help of calculated parameters theoretical design have been verified and concluded.

ACKNOWLEDGEMENTS

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Table 1.Design Specifications

Type of the aircraft	Jet Fighter Aircraft
Crew Capacity	2 pilots
Mach No	0.9
Service Ceiling	18790 m
Range	1520 m
Payload	2248.90kg

Table 2. Specifications from literature survey

Maximum speed	1100 km/hr
altitude	15800m
Rate of climb	49 m/s
Aspect ratio	4
B/L	0.6
Takeoff weight	19800 kg
Wing loading	410 kg
Range	1250 m

Table 3.Weight estimation

Crew weight	75 kg
Payload weight	2248.90 kg
Overall weight	8919.17 kg
Empty weight	4786 kg
Fuel weight	2189 kg
Percentage of error	0.5%

Table 4. Airfoil selection specifications

Maximum co-efficient of lift	2.11
Co-efficient of drag	0.5658
Fuselage length	32.67 m
Root chord	4.63 m
Aerodynamic wing chord	2.462 m
Aerodynamic chord	1.532 m
Surface area of horizontal tail	3.309 sq.m
Surface area of vertical tail	0.5673 sq.m

Table 5. Shear force and bending moment calculations

Midpoint	Load at mid point(in magnitude)	Shear force (N)	Bending moment (N-m)
0	0	0	-248.1
0.5	238.8	2.1	-300.45
1.5	228.7	18.9	-197.76
2.5	220.7	46.5	-108.56
3.5	209.1	91.7	-42.0
4.5	176.8	136.82	-9.6
5.5	148.93	169.89	-0.4
6.5	106.56	153.71	0

Table 6. Lift coefficient calculation for sea level altitude

Velocity in m/s	C_L	Drag due to lift (N)	C_{DO}	Zero lift drag (N)
50	2.645	13876.40	0.0045	205546.78
100	0.6756	3345.98	0.0178	117845.45
150	0.3126	1456.97	0.0056	775023.76
200	0.1510	867.45	0.0034	407865.89
250	0.1089	557.23	0.0025	398675.12
300	0.0765	337.87	0.0015	456734.56

Table 7. Lift, drag, load factor and thrust-to-weight ratio for sea level altitude

Velocity in m/s	Lift(N)	Drag(N)	L/D	Load factor(n)	Thrust-to-weight ratio
50	231716	219536	1.0567	9.79991712	0.9456
100	231711	121337	1.8834	9.79963453	0.5643
150	231717	776594	0.2879	9.79999781	3.3245
200	231654	406678	0.5674	9.79723469	1.7854
250	231667	412467	0.5612	9.79785643	1.7712
300	231589	456734	0.4879	9.79453217	2.012

Table 8. Lift coefficient calculation for h= 4m altitude

Velocity in m/s	C_L	Drag due to lift(N)	C_{DO}	Zero lift drag (N)
50	0.411	23218.12	0.0067	12476.12
100	0.1056	5756.23	0.0023	74569.55
150	0.0456	2345.54	0.0012	473531.22
200	0.0234	1452.45	0.00023	213487.46
250	0.1567	890.34	0.00067	225478.13
300	0.0127	654.12	0.00021	256788.90

Table 9. Lift, drag, load factor and thrust-to-weight ratio for h=4m altitude

Velocity in m/s	Lift(N)	Drag(N)	L/D	Load factor(n)	Thrust-to-weight ratio
50	231716.6	35747.89	6.4978	9.79991734	0.1534903
100	231719.9	81245.16	2.8536	9.79453456	0.3246512
150	231708.34	466445.36	0.4566	9.79993424	2.0234561
200	231478.34	215578.98	1.0878	9.79454578	0.9134234
250	231456.89	226745.12	2.4546	9.73454333	0.9856123
300	231457.34	257856.78	5.4325	9.79445673	1.1234231

Table 10. Lift coefficient calculation for h= 8m altitude

Velocity in m/s	C_L	Drag due to lift(N)	C_{DO}	Zero lift drag (N)
50	0.7023	41236.90	0.1023	7011.97
100	0.1756	10896.34	0.0031	43251.67
150	0.0674	4623.89	0.0014	197834.78
200	0.0423	2547.87	0.0011	113423.12
250	0.0260	1645.36	0.0007	122123.18
300	0.0219	1132.56	0.0002	146723.23

Table 11. Lift, drag, load factor and thrust-to-weight ratio for h=8m altitude

Velocity in m/s	Lift(N)	Drag(N)	L/D	Load factor(n)	Thrust-to-weight ratio
50	231745.6	48673.48	4.7564	9.79923456	1.63344
100	231345.9	53214.89	4.2765	9.79452322	1.63344
150	231704.34	197823.18	1.1564	9.79923187	1.63344
200	231348.34	117223.89	1.9453	9.79455645	1.63344
250	231410.89	213678.32	1.6786	9.73231789	1.63344
300	231423.34	145343.87	1.4987	9.79324512	1.63344

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REVIEW ARTICLE

Toxicological Profiling of New Chemical Entity- A Review

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ABSTRACT

The main aim is to study about the toxicological studies of new chemical entity,a Toxicity testing of new drugs is essential for drug discovery process, in this study we are mainly saying about the methods of general toxicity,and we also seeing about the toxicological profiling of various diseases through *invitro,invivo* studies are covered in this review article.

Keywords: toxicological studies,drugdiscovery,new drugs,*invitro,invivo* studies**.**

INTRODUCTION

A New Chemical Entity (NCE) is a medication that doesn't contain any dynamic moiety that has been endorsed by the United States Food and Drug Administration (USFDA) with some other application. The makers of a pioneer drug for the most part foster a NCE during the early advancement phase of the item cycle. The NCE, then, at that point, goes through different clinical preliminaries to change into a medication item. A functioning moiety is a particle or particle, barring those attached segments of the atom that cause the medication to be an ester, salt (counting a salt with hydrogen or coordination bonds), or other noncovalent derivative(such as a complicated, chelate, or clathrate) of the atom, answerable for the physiological or pharmacological activity of the medication substance. Moreover, because of related dangers and results of the medication, the utilization of restorative items is completely controlled.. Around then, toxicological assessment prior to delivering a restorative item available to be purchased was not needed and the item was just tried organoleptic (appearance, scent and flavor).
Developing a new drug can be broadly divided into four main pillars:
1.Drug discovery,
2.preclinical development,
3.clinical studies,
4.marketing authorisation of the new chemical entity (NCE) with subsequent post-marketing drug-like molecules[1,2,3]

METHODS OF TOXICITY STUDIES

General toxicity studies

Acute toxicity studies

Separate single portion or intense poisonousness studies are not ordinarily viewed as fundamental any longer. Intense harmfulness can be surveyed from momentary portion running or portion heightening examinations. Moreover, these examinations can be directed as non-GLP (Good Laboratory Practice) considers. The primary objective of intense harmfulness studies is the recognizable proof of potential objective organs which are toxicologically affected by the managed substance. A computation of the middle deadly portion (LD50) is at this point not suggested. Expanded single portion harmfulness studies can be led to help exploratory clinical preliminaries (for the most part single portion human preliminaries). With this sort of study, boundaries like hematology, clinical science or histopathological information can be evaluated(5)

Repeated dose toxicity studies

This test portion poisonousness studies are led to describe toxicological profiles, for instance, to distinguish target organs and tissue toxicologically affected after rehashed organization of high dosages. One more point of this review type is to build up no-impact levels, similar to the non-noticed unfavorable impact level (NOAEL) filling in as security edges and to decide the most elevated portion for resulting toxicological examinations. studies must be led before first-in-man (FIM) preliminaries and in this manner support the lead of clinical preliminaries. The investigations must be directed as per great research facility pratice. It is for the most part needed to play out these examinations in two creature species whereby one ought to be a rat (for example rodent or mouse) and the other ought to be a non-rat animal types like primates, minipigs or canines. As to have the option to make the right inference and to make an interpretation of preclinical discoveries to patients in clinical preliminaries, the creatures are constantly needed to be the "most human-like creature species". The pharmacokinetic profile, pharmacodynamic impacts just as metabolic information ought to be pretty much as comparative as conceivable to information got in human preliminaries (6)

sub-chronic toxicity

This test was performed on 24 sound white hares of one or the other sex from 1200 to 1800 grams. All creatures were similarly isolated into three gatherings, one gathering viewed as control and other two got 20 and 60 mg/kg dosages of home grown definition for back to back 60 days through oral intubation tube. Portions were ready in DMSO anyway control bunch got DMSO orally equivalent to the volume of individual dosages as indicated by their body weight. Prior to organization of medication, actual strength of these creatures was seen during the molding time frame under the research center climate for seven days unequivocally seeing loss of hair, looseness of the bowels, edema, ulceration and absence of movement (7)

Sample Collection

Blood test of around 6 ml were gathered from these creatures via cardiovascular cut toward the finishing of dosing on 61st day to decide different biochemical and hematological boundaries (8)

Assessment of Toxicities

Physical Examination

The Gross poison levels were seen each one-week in the wake of giving home grown definition for 60 days unequivocally seeing skin ulceration, normal weight variety, loss of hair, loss of hunger, loss of action, hematuria, heaving, looseness of the bowels, edema, lacrimation, salivation, muscle tone, quake and forceful conduct. Dissection was performed after arbitrary choice, toward the consummation of dose and test assortment for biochemical tests (9)

Biochemical Evaluation

Blood tests were gathered from abstained creatures preceding necropsy. Around 7 ml of blood tests were gathered via cardio cut. Serum were quickly isolated by centrifugation for 10 min at 4000 rpm and was analyzed for the

accompanying boundaries inside 3 hours of test assortment on Humalyzer 3000 (GmBH Germany) at 37°C using reagents provided by Human Gmbh Germany (10,11)

Hematological Evaluation

Blood tests were gathered under 10% EDTA at 7.2 pH and hematological boundaries for example RBC, WBC, PLT, Hematocrit, and hemoglobin were investigated utilizing Humacount hematology analyzer GmbH 17400, a completely computerized cell counter with an underlying veterinary programming module (12)

Microscopic Examination

The blocks from various spaces of heart, liver and kidney were cut from each example in the wake of isolating all fat from particular organs. The squares were handled through Gilford 101 s programmed tissue processor. Tissue cuts of 3 - 4 micron were taken from the wax blocks by rotating microtome. The tissue cuts were mounted on slides and dried out delicately by squeezing with channel paper. The mounted slides were put basically for drying on a hot plate (45°C) for an hour and a half and afterward left in a hatchery at 37°C short-term to dry before infinitesimal assessment (13)

Statistical Analysis

Every one of the qualities for biochemical tests were expressed as the mean and standard blunder to the mean (S.E.M.) and were investigated by utilizing one way unstacked ANOVA and p esteems were noticed [30]. Results were considered critical if p esteem was under 0.05 and profoundly huge if p esteem was under 0.005 (14)

TOXICOLOGICAL PROFILING OF VARIOUS DISEASES ON NEW CHEMICAL ENTITIES:

Genotoxicity and carcinogenicity studies

This test generally done on rodents and mice for new medication testing. Genotoxicity depicts the property of a substance to initiate hereditary harm on DNA or chromosomal level by an assortment of components. Harm, for example, transformation in microorganism or substantial cells can prompt super durable heritable changes. Immediate or circuitous DNA harm which can't be forestalled by DNA fix or cell apoptosis is as often as possible viewed as fundamental for a multi-step course which at last can prompt the foundation of malignancy. Numerous in vitro and in vivo tests exist to inspect the genotoxic capability of conceivable medication applicants .

A depiction of the standard battery to be led for a thorough genotoxic assessment can be found in the ICH Guidance S2(R1). The universally supported portrayal of the potential examines also as standard test conventions can be found in the particular OECD (Organization for Economic Co-Operation and Development) rules. By directing a battery of in vitro and in vivo tests the capacity of identifying the majority of the genotoxic instruments in regards to potential new drugs increments. Genotoxicity tests ought to consistently be done by GLP. The main measure which ought to be directed is a quality change test in microorganisms. By and large, the bacterial converse change test (Ames1) utilizing S. typhimurium is utilized. This test makes it conceivable to dependably demonstrate significant hereditary distortions just as a large portion of the genotoxic cancer-causing agents in rodents and people . Further in vitro tests assessed in mammalian cells can follow and may distinguish quality or chromosomal harms. These incorporate, for example, the in vitro micronucleus test or the mouse lymphoma cell Tk (thymidine kinase) quality change examine (MLA). Ensuing in vivo tests can demonstrate or refute the primary outcomes got by the in vitro examines. Moreover, they ought to guarantee that substances not mutagenic in vitro(15)

Dose Descriptor and Risk Assessment of Carcinogens

It is for the most part perceived that a few synthetic substances (non-edge cancer-causing agents) will cause cancer-causing chances even at the littlest openness focus. For these synthetic compounds the traditional NOAEL and wellbeing factor way to deal with determine openness security principles isn't proper.

Two helpful portion descriptors for cancer-causing nature are T25 and BMD10. They might be gotten from two-year cancer-causing nature rat bioassay (in rodent or mice).
T25: The constant portion rate that will give 25% of the creatures' cancers at a particular tissue after adjustment for unconstrained frequency, inside the existence season of that species.
BMD10: inferred seat mark portion accepted to give 10% of the creatures' growths at a particular tissue after rectification for unconstrained rate, inside the existence season of that species (17)

Acute toxicity testing for inhalation

The toxicity Intense inward in halaing poisonousness testing is performed for spray like arrangements. Rodents are the most favored creature species. The creatures are adjusted to research center conditions (temperature ideally 22°C ± 2°C). They are kept up with in a wind stream of 12–15 air changes each hour with sufficient oxygen (19%/h). The creature is presented to the test substance for at least 4 h, and afterward it is checked for 14 days. Food is retained during the openness time frame, and water might be retained under specific conditions. During the perception time frame, the creature is noticed for quakes, spasms, salivation, the runs, dormancy, rest, and trance state. Mortality during the openness and perception period is noted. Dead creatures are analyzed for histological and neurotic changes. Toward the finish of the review, the creatures are forfeited, and neurotic changes are evaluated.(18)

Dose for inhalation

A typical portion reaction descriptor for intense poisonousness is the LD50 (Lethal Dose half). This is a measurably determined portion at which half of the people will be relied upon to bite the dust. For inward breath poisonousness, air focuses are utilized for openness esteems. In this way, the LC50 (Lethal Concentration half) is utilized.
The units of LD50 and LC50 are recorded as follows:
LD50: mg/kg/bw. mg/kg bw/d represents mg of substance per kg of body weight directed each day.
LC50:mg/L. mg/L is the assessed air convergence of a substance directed by means of inward breath course.
It will be noticed that LD50/LC50 from intense poisonousness reads are principally utilized for GHS intense harmfulness grouping, subjective danger evaluation and portion choice for rehashed portion harmfulness contemplates. They can't be utilized to infer No-noticed antagonistic impact level (NOAEL).[19]

Acute toxicity testing for topical preparations

The eye test and skin disturbance test are vital for effective arrangements. Dermal and ophthalmic arrangements can be tried utilizing Draize tests. The Draize eye irritancy test and the Draize skin irritancy test are utilized to quantify the hurtfulness of synthetics and drug substances in hares and guinea pigs. In the eye disturbance test, 0.5 ml of a test substance is controlled to a creature's eyes, and the creature is limited for 4 h. Redness, enlarging, release, ulceration, discharge, and visual deficiency are evaluated and checked for 14 days. The skin aggravation test, 0.5 g of a test substance is applied to the outer layer of a creature's skin. During the perception time frame (14 days), signs, for example, erythema and edema are evaluated. Some option in vitro testing techniques are accessible that can be utilized instead of the Draize eye irritancy test. Toward the finish of the review, the creatures are forfeited and obsessive changes are assessed on this test.(20).

Dose Descriptor for Skin/Eye Irritation

No Observed Adverse Effect LeveL (NOAEL) can't be acquired from skin/eye disturbance tests because of study plan. For skin/eye aggravation, the primary methodology ought to be the subjective danger portrayal dependent on strength arrangement (yes or no, destructive, solid, gentle) and afterward characterizing the proper danger the board measures (RMMs) (21).

Reproduction toxicity studies

This incorporate regenerative toxicology which examinations the danger for male and additionally female ripeness just as formative toxicology which inspects the poisonous impacts for infant and unborn in more detail. Conceptive disappointments in grown-up barrenness, premature delivery or birth deformities could show up. Unfriendly

impacts instigated during pregnancy like teratogenicity is another issue with respect to the posterity. For assessment of conceptive poisonousness mammalian species and strains previously utilized for other pharmacological and toxicological investigations ought to be utilized. This permits the likeness with the outcomes previously got. With information accumulated in rehashed portion harmfulness contemplates, significant data concerning ripeness, in extraordinary male fruitfulness can much of the time be given. For multiplication toxicology considers, the utilization of one rat and one non-rat creature animal categories is suggested. Rodents are the rat types of decision concerning reasons of practicability and a lot of information about these creatures. Hares are much of the time utilized as non-rat species for embryotoxicity considers . Other in vitro techniques like tissue, organs or cell frameworks might be utilized to build the information. As in the examinations previously referenced over, the course of organization ought to be equivalent to the expected course in people. The portion applied in propagation harmfulness studies ought to be painstakingly chosen dependent on the information from currently led considers. On the off chance that no information is accessible, fundamental investigations are recommended (22).

Portion Descriptor for Reproductive poisonousness commonly, a NOAEL or LOAEL can be gotten from conceptive/formative poisonousness considers. NOAEL/LOAEL can be additionally utilized for quantitative danger appraisal. No Observed Adverse Effect Level (NOAEL): The most elevated openness level at which there are no organically critical expansions in the recurrence or seriousness of antagonistic impact between the uncovered populace and its suitable control; a few impacts might be created at this level, however they are not viewed as unfriendly impacts. Most minimal Observed Adverse Effect Level (LOAEL): The least openness level at which there are organically critical expansions (23).

Subchronic oral toxicity testing (repeated dose 90-day oral toxicity testing)

Rodents and nonrodents are utilized to contemplate the subchronic poisonousness of a substance. The test substance is managed orally for 90 days, and week after week body weight varieties, month to month biochemical and cardiovascular boundaries changes, and social changes are noticed. Toward the finish of the review, the exploratory creatures are forfeited. Gross obsessive changes are noticed, and every one of the tissues are exposed to histopathological investigations. There ought to be minimal individual variety between the creatures, and the permitted weight variety range is ±20% (24).

Dose Descriptor for Repeated Dose Toxicity

Normally, a NOAEL or LOAEL can be acquired from rehashed portion poisonousness contemplates.

No Observed Adverse Effect Level (NOAEL): The most noteworthy openness level at which there are no organically critical expansions in the recurrence or seriousness of unfriendly impact between the uncovered populace and its suitable control; a few impacts might be delivered at this level, yet they are not viewed as antagonistic impacts.

Lowest Observed Adverse Effect Level (LOAEL): The least openness level at which there are naturally critical expansions in recurrence or seriousness of antagonistic impacts between the uncovered popular.

The units of NOAEL or LOAEL: mg/kg/bw/day or ppm. For inthalation course, the unit can be mg/L/6h/day **(25)**

Developmental toxicity/embryotoxicity studies

Embyrotoxicity can be concentrated on utilizing both in vivo and in vitro techniques. Rodents are liked for in vivo harmfulness screening. The compound is regulated between the 8thand 14thday of pregnancy, and embryolethal impacts are considered. Toward the finish of the review or on the 21st day of the review, a cesarean segment is performed and boundaries, for example, embryos with hemorrhagic bullae, appendage abnormalities, exencephaly, congenital fissures, open eyelids, and tail disfigurements just as the mortality and the quantities of dead and live little guys are noted. Embryotoxicity studies can be performed utilizing in vitro techniques, for example, the undeveloped foundational microorganism test (EST) for embryotoxicity, micromass embryotoxicity measure, and entire rodent incipient organism embryotoxicity examination.[26]

Laboratory analysis of toxins

Poisons might be assessed subjectively or quantitatively. Subjective examination gives data about the idea of poisons, however quantitative investigation gives data about the science of the poisons and their fixation. Vague instrumental investigations, for example, colorimetric and UV-apparent spectrophotometric examinations might be utilized for subjective examination of poisons. Modern strategies like infrared spectroscopy, gas chromatography, High Pressure Liquid Chromatography, and immunoassay procedures might be utilized to evaluate the poisons (27)

Euthanasia

An death means a delicate demise and ought to be viewed as a demonstration of a human technique for forfeiting a creature with at least physical and mental misery (28)

Euthanasia of experimental animals

Biomedical exploration needs creatures. This is generally clear in the event of in vivo creature tests. Notwithstanding, for other logical purposes, for example in vitro examines, natural material is likewise important to concentrate on catalysts, films, receptors, cells, tissues, or organs which are gotten from dead creatures. Consequently, creatures must be forfeited in biomedical research centers.

(i) During tests where penance of the creatures isn't essential for the concentrate yet should be done when agony, misery and enduring surpass adequate levels or then again in case it is logical for the creature to stay in torment or trouble after suspension of the trial.
(ii) To give organic material to *in vitro* (29)

Zebra fish

The zebra fish is a significant and generally utilized vertebrate model living being in logical examination, for instance in drug advancement, specifically pre-clinical turn of events. It is additionally prominent for its regenerative capacities, and has been changed by analysts to deliver numerous transgenic strain (30)

Zebrafish in cardiotoxicological studies

The physiological contrasts are apparent among zebra fish and mammalian heart, the zebra fish has turned into a decent choice to concentrate on heart improvement and heart recovery. The zebra fish has added to get estimations as activity potential box voltage planning, to decide cells coupling, and this reality along with calcium flagging, are significant for cardiomyocyte multiplication and separation.

The last decade's huge creatures, like mice, rodents and hares, have been generally used to concentrate on cardiotoxicity after drug administration], introducing a few limits. For example, rodents can be harsh toward mixtures' cardiotoxicity, especially when the endpoint estimation is left ventricular contractile capacity. This might be because of rodents' capacity to remunerate loss of myocytes by enrolling elective mechanisms. According to the United States government, rat and bunny poisonousness testing has been the norm for evaluating intense harmfulness since the 1950s. Notwithstanding, the interaction is exorbitant and tedious, which has prompted an accumulation in synthetic testing. Due to these restrictions, the requirement for utilization of other elective creature models has expanded.

The zebrafish is especially appropriate for this reason since it addresses a vertebrate animal varieties, its genome has been sequenced and an enormous number of simultaneously creating, straightforward incipient organisms can be delivered. Specifically, the zebrafish has a significant expense impact advantage and has turned into a significant instrument to assess Geno-cardiotoxicity, to concentrate on undeveloped organism improvement and general harmful. For example, a few compound screens, including some assessing drug-incited cardiotoxicity and others currently in preclinical preliminaries, have effectively tried medication impacts in zebrafish. The zebra fish heart is two-chambered, its key electrical properties are surprisingly like those of people. Zebrafish pulse and activity

potential are comparable to those of people; likewise it presents featured hereditary qualities and administrative organizations similitudes driving cell destiny equal those of higher vertebrates Moreover, heart execution in grown-up zebrafish can be identified by new noninvasive techniques. It tends to be surveyed by progressing traditional echocardiography with dot following investigations and changes in heart execution, and empowers profoundly delicate evaluation of local myocardial movement and twisting in high spatio-transient resolution. Then in vivo studies address a fundamental stage in drug improvement and poisonousness study, and the zebrafish cardiotoxicity test has been accounted for truly solid, depicting the expected harmfulness of medications to the human cardiovascular framework in cardiovascular system.[31]

Zebrafish in hepatotoxicity studies

Toxicology studies are expected to decide the appropriateness and results of medication organization in people. During the time spent medication revelation, one of the primary concerns is to assess drug hepatotoxicity, which is surveyed utilizing preclinical cell culture, creature models and clinical preliminaries. Notwithstanding, drug hepatotoxicity is hard to recognize before human use, restricting the disclosure and improvement of novel treatments utilizing regular models. Because of this reality, to foster new in vivo and in vitro models for adequacy and security testing is required. In this way, to set up better apparatuses to evaluate for drug-actuated liver injury (DILI) of enormous compound libraries in beginning phases of medication advancement will permitted to acquire a superior comprehension of hepatotoxicity. Being this reality the most widely recognized reason for drug withdrawal Zebrafish liver organogenesis begins at 3 days post preparation (dpf) and is completely practical by 5 dpf]. The tri-lobed liver of the zebrafish is like that of the warm blooded creature as to organic capacity, including the preparing of lipids, nutrients, proteins and sugars, just as the amalgamation of serum proteins. A few investigations propose that medications are used when presented to zebrafish incipient organisms by comparative responses to those in people. Zebrafish have a wide scope of cytochrome P450 proteins that permit metabolic responses including hydroxylation, formation, oxidation, demethylation and de-ethylation Following openness to a scope of hepatotoxic medications, the zebrafish liver creates histological examples of injury equivalent to those of mammalian liver, and biomarkers for liver injury can be evaluated in the zebrafish flow.

Since hepatotoxicity is gotten from metabolic cycles, zebrafish are helpful to concentrate on DILI with in vivo models. Boundaries like apoptosis, liver haziness or size, can be assessed in the zebrafish. The accessibility of explicit transgenic lines marking the liver, like fabp10:RFP, permits liver harm perception after the treatment. Investigation of fluorescent force can be educational as to measure or the quantity of hepatocytes. Notwithstanding, has been depicted the badly designed to work with the hatchlings, contending that the CYP framework, which assumes a fundamental part in drug digestion, isn't yet completely created in hatchlings and proposing that some CYPs have all the earmarks of being inadequate in the early zebrafish life. Moreover, zebrafish undeveloped organisms and hatchlings showed no or low biotransformation limit of four human CYP-explicit substrates, dextromethorphan, diclofenac, testosterone and midazolam. Conversely, has been accounted for the hatchling as a promising device equipped for recognizing hepatotoxic and non-hepatotoxic synthetic analogs, inferring that it very well might be applied as an evaluating model for DILI. Additionally a new report has fostered another exploratory method (ZeGlobalTox examine) that tends to the organ-explicit harmfulness of various medications on zebrafish hatchlings (up to 5 dpf). It allows the free examination of cardio-, neuro-, and hepatotoxicity impacts in a similar creature. The principle concern was that drug-instigated teratogenicity (formative poisonousness) or potentially mortality could veil conceivable organ-poison levels showing up later being developed (32).

CONCLUSION

We have studied on review of toxicological studies of new chemical entities, which is an drug development process, With the growing market of new medicine, the safety of new drug treatment has been raise due to insufficiency in toxicological studies. Increasing evidence showing the harmful effects related to new medicine further highlights the

demand and necessity in toxicological studies.although for all safety of the medication in animals,assesement in humans is the only accurate of establishing safety and efficacy of any drug prior to use in humans.

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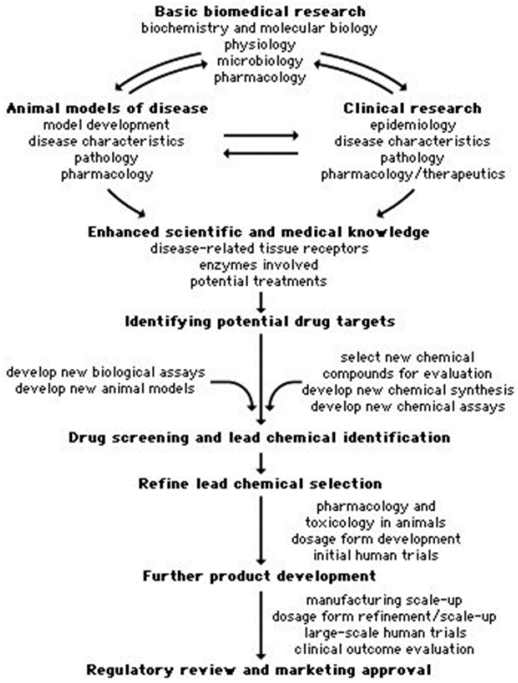

Flow Chart : 01 research and discovery processes used for drug development[4]

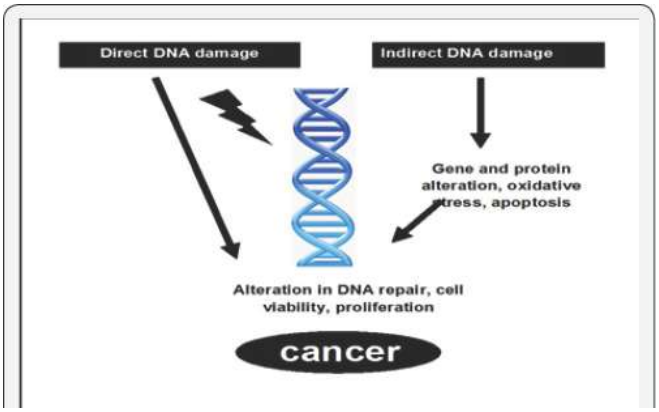

Fig no: 01 Relationship between carcinogenecity and Genotoxicity (16)

Fig no: 02 acute toxicity testing for topical preparations (20).

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RESEARCH ARTICLE

Development and Evaluation of Mouth Dissolving Tablets of Efonidipine using Solid-Dispersion

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ABSTRACT

Efonidipine hydrochloride ethanolate (EHE) is a new age group dipyridine derivative used to treat hypertension**.** Hence,this study wasperformed to develop mouth dissolving tablet (MDT) of EHE solid dispersion (SD) due to poor aqueous solubility& systemic availability. EHE SD were formulated by solvent evaporation and physical mixture (PM) methodsby using polyethylene glycol 6000 (PEG-6000), Polyvinyl pyrolidone K-90 (PVP K-90) and mannitol as water-soluble hydrophilic carriers into 6 formulations.The developed SD was evaluated for parameters like physical characterization, solubility, drug content and in-vitro drug release. The SD of EHE increases the solubility and dissolution of the EHEcompare to pure drug and PM.Mannitol based EHE SD was found to be optimized SD formulation, which was developedto Mouth Dissolving Tablets (MDT) were formulated by direct compression technique by takingCrosscarmllose sodium (CCS) as super disintegrant and camphor as subliming agent. The formulation FR 6 exhibited lowest disintegration time (DT), suitable drug concentration and highest in-vitro drug release was subjected for further studies. The drug release from MDT was best fitted with first order kinetics. Developed MDT exhibited good stability at accelerated condition 40^{o}C/75% RH for 3 months.From the present study results, it can be concluded that MDT of EHE SD can be successfully formulated which could result in higher solubility and dissolution of EHE and eventually proving higher systemic bioavailability through oral cavity.

Keywords: Efonidipine, Solid dispersion, mouth dissolving tablet, fast dissolving tablets, Polyvinylpyrrolidone.

INTRODUCTION

EHE is used for the treatment of hypertension (high blood pressure), it is a novel dihydropyridine calcium channel blocker that inhibits both L- and T-type calcium channels which eventually result in vasodilation and reduces the heart automaticity. The chemical name of EHE is 2-(*N*-benzylanilino)ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ^4-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4 dihydropyridine-3-carboxylate as shown below [1,2]

EHE has poor water solubility (0.000248 mg/ml) and dissolution (Biopharmaceutical classification system, BCS class II) because of that EHE exhibit poor in-vivo bioavaialability [3]. The water solubility of poorly water-soluble drugs can be increased by a popular technique called solid-dispersion (SD) [4-6]. SD technique increases the solubility by increasing wettability, solubilization of drugs by inert carriers and reduction of aggregation or agglomeration. In addition, transformation of the crystalline drug to the amorphous by SD increases the dissolution rate because no crystal lattice structure [7]. Oral route of drug administration is reported to be the most popular route of administration because of its several benefits like ease of drug ingestion and enhanced patient compliance [8]. However, some drawbacks are also associated with oral routes like difficulty in swallowing specially in pediatrics, geriatrics and mentally challenged patients leading to serious patients, non-compliance [9]. MDT can be used to replace traditional tablets to permit intra-oral absorption of drug and bypass the gastro-intestinal drug absorption through liver [10]. The MDT Tablets are uncoated, which rapidly disintegrate releasing the drug in oral cavity providing better bioavailability in comparison to conventional tablets [11]. Therefore, the current study was carried out to develop SD of EHE by using different water-soluble carriers to increase the solubility of the low soluble EHE. Further the developed SD of EHE was formulated to MDT tablets for providing better dissolution, absorption and bioavailability with enhanced patient compliance.

MATERIALS AND METHODS

MATERIALS

EHE drug was provided as kind gift sample by Zuventis healthcare Ltd (Mumbai, India). The water-solublepolymers polyethylene glycol 6000 (PEG-6000), Polyvinyl pyrrolidone -90 (PVP-K 30) and super disintegrant croscarmellose sodium (CCS)were procured from Gangawal Chemicals Pvt Ltd. (Mumbai, India). Mannitol, Potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and methanol were procured from CDH Fine chemicals Pvt. Ltd (Delhi, India). Lactose monohydrate, Talc, Magnesium stearate, Menthol, Aspartame and Camphor were procured from Akums Drugs and Pharmaceuticals Ltd. (Haridwar, UK). All other ingredients and solvents used were of analytical reagent grade quality.

METHODS

Solubility study of EHE

To determine the saturated solubility of EHE in various solvents, excessive amount of EHE was added to 20 ml of Dimethyl sulfoxide (DMSO), Methanol, Ethanol, N'N Dimethylformamide (DMF), 0.1N hydrochloric acid, phosphate buffer pH 4.5, phosphate buffer pH 6.8 and distilled water in a conical flask. The flasks were shaken in a mechanical shaker for period of 24 hours at room temperature [12]. After 24 hour,samples were filtered and analyzed by UV-VIS spectrophotometer at 330 nm after suitable dilution.

Differential scanning calorimeter (DSC) Study

To assess any drug-carrier interaction, DSC analyzes of pure EHE, SD, physical mixture (PMX) and drug mixed with all excipients of MDT (EHE-MDT) (in ratio of 1:1) was performed. Samples equivalent to 5 mg of drug was packed in aluminum pan and analyzed over temperature from 25 °C to 300 °C with 20 °C/min heating rate.

X-ray diffraction study (XRD)

The XRD study of SD and PMX was carried out by using powder diffractometer with CuK_{α} radiationin order to study the physical form of EHE (crystalline or amorphous). Thin layer of powdered sample was placed on normal cavity mount and XRD chromatogram were obtained using 2θ scan mode varying from 3° to 60° with rate of 0.5° per minutes.

Preparation of calibration curve of EHE

20 mg of EHE was dissolved in 20 ml of ethanol. Now from the this stock solution (primary), 1 ml was transferred in 10 ml volumetric flask and volume was make to 10ml with phosphate buffer pH 6.8 (second stock solution). From second stock solution 0.5, 1, 1.5, 2, 2.5 and 3 ml solution were taken and volume was made up to 10 ml with phosphate buffer pH 6.8 to get 5, 10, 15, 20, 25 and 30 µg/ml concentrations respectively. Absorbance of all solutions was determined spectrophotometrically at 330 nm and plotted against concentration.

Formulation of solid dispersion

EHE solid dispersion was prepared withPVP-K 30 and PEG-6000 by using two methods-Physical mixture (PMX) and Solvent Evaporation (SVP) [13,14].

Evaluation of prepared SD

Solubility of EHE in SD

The excess of prepared SD was added in 20 ml of distilled water present in a volumetric flask. The flasks were shaken on a mechanical shaker for period of 24 hours at room temperature. After 24 hour of shaking, samples were filtered and analyzed spectrophotometrically at 330 nm after suitable dilution [12].

Determination of EHE in SD

Accurately weighed SD corresponding to 20 mg of EHE was dissolved it in 50 ml of HPLC grade acetonitrile (ACN). The flask was shaken for 15 minute and made up to 100ml by HPLC grade ACN. Now 10 ml of the solution was centrifuged for 15 minutes and supernatant was filtered and determined by spectrophotometrically at 330 nm [15].

***In-vitro* Drug release of SD**

The dissolution study of developed SD of EHE was performed by using USP dissolution apparatus type II (Paddle)taking 900 ml phosphate buffer pH 6.8 as dissolution medium (maintained at 37°C ±1) which was stirred at 100 rpm. Sample of quantity 5 ml were withdrawn at 5, 10, 15, 20, 30,40 and 60 minutes and immediately replenish with same volumes of fresh dissolution medium. The samples were filtered and analyzed spectrophotometrically at 330 nm [16].

Development of MDT of EHE SD

From all SD prepared, depending upon the drug content, cumulative drug release (%) one optimized SD was selected for the formulation of MDT of EHE. The MDT of EHE was prepared by direct compression technique [17]. The composition of all MDT formulations given in Table 1.

Evaluation of Developed MDT

1) **Weight Variation**: From the batch, 10 tablets were selected randomly and weighed individually and deviation from average weight was estimated. The weight variation acceptance criteria were considered as per IP 2018 [18].
2) **Hardness**: The hardness of individual tablets was estimated by using Monsanto hardness tester in Kg/cm^2 units [22].
3) **Friability**: The friability of all developed MDT was determined by using Rochefriabilator at 100 revolutions on 20 tablets [18]. The acceptance criteria was considered as per IP 2018.

4) **Disintegration time (DT):** The DT of developed MDT was determined by using DT apparatus. Six tablets were dropped in each tube and apparatus was run till complete tablets disintegrate passed through mesh of tube [18]. Acceptance criteria are considered as per IP 2018.
5) **Drug contents**: Ten MDT tablets were taken and made to fine powder. Powder quantity equivalent to average weight of tablet was weighed and transfer to 100ml volumetric flask. A 50 ml of ACN was transferred to volumetric flask, it was shaken for 15 minutes, and then volume was made up to 100 ml with ACN. Now 10 ml of the solution was centrifuged for 15 minutes and supernatant was filtered. The content of EHE in SD was determined by spectrophotometrically at 330 nm [19].
6) ***In-vitro* dissolution and release kinetics**: The in-vitro dissolution study of Marketed formulation of EHE (EFNOCAR-20mg) and developed MDT was carried out by using USP dissolution apparatus type II (Paddle). 900 ml of phosphate buffer pH 6.8 was usedas dissolution medium (maintained at 37^{o}C ±1) which was rotated at 100 rpm speed. 5 ml of samples weretaken out at 5, 10, 15, 20, 30,40 and 60 minutes and immediately replenish with same volumes of fresh dissolution medium [20]. Samples were analyzed spectrophotometrically at 330 nm.In-vitro drug release data of developed MDT was fitted to different release kinetic models such zero-order, first order, second order, Higuchi model and best fit was determined.
7) **Accelerated Stability studies:** The MDT formulation which showed good drug content and in-vitro drug release was subjected for accelerated stability (40°C/75% RH) studies for 3 months as per ICH guidelines.The tablets were wrapped in Aluminum blister pack and place in accelerated stability chamber; the samples were withdrawn at regular intervals. The withdrawn samples were analyzed for any variation in physical parameters and drug release studies [21].

RESULTS AND DISCUSSION

Solubility

The solubility data of EHE showed that it is poorly soluble in water (0.000248 mg/ml) and soluble in organic solvents like dimethyl sulfoxide (DMSO), methanol, ethanol and DMF. Further EHE showed more solubility in phosphate buffer pH 6.8 in comparison to 0.1 N HCL and phosphate buffer pH 4.5 which suggest pH dependant solubility of EHE. It is highly soluble in DMF.

Compatibility studies

DSC chromatogram of pure EHE exhibited a sharp endothermic peak at 151.6 °Cwhich correspond to its melting point and indicating EHE crystalline nature[22]. Drug mixed with all excipients of MDT (EME-MDT) in ratio of 1:1 (lactose monohydrate, CCS, magnesium stearate, talc) also showed a peak near to EHE endothermic peak (156.4 °C) suggesting no interaction of EHE with all excipients used for MDT development (Fig 1). However, DSC chromatogram of SD (SD9) and PMX (PMX 9) did not show any peak at or near 151.6 °C, which indicated solubilization of drug in hydrophilic carrier during testing or conversion of crystalline nature of EHE to amorphous form due formation of SD.

XRD study

To examine the physical state of EHE in developed SD and their respective PMX, XRD study was performed.XRD chromatogram of EHE, mannitol, SD of EHE and mannitol (1:2 ratio) and their PMX are shown in Fig 2. The EHE exhibited sharp peak at 2θ of 8°, 10°, 12°, 14°, 16°, 21° and 24°. The DSC chromatogram of mannitol exhibited number of characteristic peaks indicating its crystalline nature. The DSC chromatogram of PMX's and SD's did not show any characteristic peaks of EHE except those at 8° and 24° with very low intensity (Fig 2).

Formulation of SD

In present study SD were formulated by solvent evaporation method and by physical mixing which are widely used for development SD as reported by earlier studies.SD of EHE has been successfully achieved in present study by both the methods (Table 1).

Solubility of EHE in SD

The solubility of different prepared SD's and PMX's was determined in distilled water.The SD's and PM's showed more solubility of EHE as compared to pure drug**.** Further all SD's exhibited more solubility of EHE as compared to their respective PM's this might be due to more amorphous form of EHE achieved in SD[23]. In present study, SD prepared with mannitol observed with highest solubility of drug in comparison to SD with PEG-6000 and PVP K-30 with same drug and hydrophilic carrier ratio [24].

Content of EHE in SD

The drug content of SD prepared by mannitol in ratio of 1:2 (SD3) observed with drug content 99.89% which is slight higher than SD1 (1:0.5 ratio) and SD2 (1:1 ratio), while Drug and PEG-6000 in 1:2 (PMX3) showed poor drug content (95.84%). However, there was insignificant difference observed in drug contents of all developed SD's prepared from all hydrophilic polymers [12].

***In-vitro* drug release**

The EHE showed very less drug release (38.67%) after 60 minutes of in-vitro drug release due to its poor solubility (Fig 3a). In-vitro drug release of EHE was observed more in SD's than their respective PMX's at same ratio irrespective of the hydrophilic carrier used (Fig 3a, 3b and 3c) [19,23,24]. In addition, SD's showed more solubility of the drug in comparison to PMX's thereby resulting more drug release. *In-vitro* drug release with varying sorbitol ratios (1:0.5, 1:1, and 1:2) showed the highest drug release at 1:2 ratio in both SD and PMX (95.23% and 58.76% respectively) as shown. Similar kind of observation were also reported with other two hydrophilic polymer i.e. PVP K-30 and PEG 600 where increase in drug to polymer ration increase the drug release as above [25;26]. The results of *in-vitro* drug release study further indicated that rate of dissolution of EHE from its SD's and PM's were followed the order of Mannitol > PEG 6000 > PVP K-30. This might be due to the difference in hydrophilic character of these polymers [27]. Mannitol being more soluble than other two polymers thereby provided more drug release.

Development and Evaluation of MDT Tablets

The SD of Mannitol in ratio of 1:3 to EHE (SD9) showed good drug concentration and highest in-vitro drug release hence was considered for development of MDT. The composition various MDT formulations for 200mg tablets are given: 6 formulations were evaluated for pre-compression parameters. Results observed with blends were shown were within acceptable limits [18].

***In-vitro* Drug release and kinetic of drug release**

The *In-vitro* drug release of pure drug (EHE) and all developed MDT were conducted and their release profile is given in Fig 4. The release of drug from all MDT tablets was found to more than marketed formulation (EFNOCAR-20mg) (Table 4). This might be due to solubility enhancement effect of SD present in MDT. Usually in-vitro drug releases among MDT formulations are compared at 15 minutes [28]. The in-vitro release profiles of all MDT showed that FR6 MDT formulation showed highest drug release (90.65%± 1.65 %) after 15 minutes. The concentration of CCS was directly proportional to drug release due to the presence of superdisintegrant[29]. The use of camphor as subliming agent resulted in decreased DT and faster dissolution, because sublimation of camphor increased dissolution medium penetration [30].

The *in-vitro* drug release data of all developed MDT formulation were fitted to various release kinetic models and it was found that all the formulations (FR1 to FR6) were best fitted to first order kinetics.

Stability studies

The optimized formulation with highest in-vitro drug release and good drug content (F6) was packed in aluminum blister pack and subjected for stability study for 3 months at accelerated stability condition (40°C/75%) as per ICH. There was no significant change was reported in evaluation parameters and drug release studies of formulation FR 6 after 3 months (Table 5).

CONCLUSION

The present study showed that increased dissolution of poorly water-soluble drug EHE could be achieved by preparing its SD with PVP K-30, PEG 600 and mannitol as water-soluble carrier**.** SD prepared by solvent evaporation method in ratio of 1:2 for drug and carrier showed faster dissolution when compared with pure drug**.** MDT of EHE SD was prepared with varying concentrations of CCS and combination of CCS with subliming agent (camphor). The drug release from MDT increased in higher concentration of CCS and presence of subliming agent. All MDT showed higher drug release than marketed formulation (EFNOCAR-20mg). MDT formulation FR6 with highest in-vitro drug release, shortest DT and good drug content was selected as optimized formulation. The storage of FR 6 formulation at 40°C/75% for 3 months showed no significant change in physical and drug release parameters from their initial values. Hence, based on present study it may be concluded that MDT of EHE prepared using SD with CCS as super disintegrant and camphor as subliming agent could be an ideal approach for improving dissolution and thereby bioavailability of poorly water soluble EHE.

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Table 1: Composition of different SD

Code	Composition	Ratio
Physical Mixture (PMX)		
PMX1	Drug: PEG 6000	1:0.5
PMX2	Drug: PEG 6000	1:1
PMX3	Drug: PEG 6000	1:2
PMX4	Drug: PVP K-30	1:0.5
PMX5	Drug: PVP K-30	1:1
PMX6	Drug: PVP K-30	1:2
PMX7	Drug: Mannitol	1:0.5
PMX8	Drug: Mannitol	1:1
PMX9	Drug: Mannitol	1:2
Solvent Evaporation Method		
SD1	Drug: PEG 6000	1:0.5
SD2	Drug: PEG 6000	1:1
SD3	Drug: PEG 6000	1:2
SD4	Drug: PVP K-30	1:0.5
SD5	Drug: PVP K-30	1:1
SD6	Drug: PVP K-30	1:2
SD7	Drug: Mannitol	1:0.5
SD8	Drug: Mannitol	1:1
SD9	Drug: Mannitol	1:2

Table 2: Composition of all developed MDT

Ingredients	Formulations					
	FR1	FR2	FR3	FR4	FR5	FR6
SD 9	80	80	80	80	80	80
Lactose	112.16	92.16	72.16	52.16	45.16	38.16
CCS	-	20	40	60	60	60
Camphor	-	-	-	-	7	14
Aspartame	3.6	3.6	3.6	3.6	3.6	3.6
Menthol	1.24	1.24	1.24	1.24	1.24	1.24
Talc	2	2	2	2	2	2
Magnesium stearate	1	1	1	1	1	1

Table 3: Pre-compression blend physical properties

Code	Angle of repose (°)	Bulk Density (g/cc)	Tapped Density (g/cc)	Carr's Index (%)	Hausner's Ratio
FR1	26.43±0.02	0.24± 0.02	0.31± 0.05	21.34± 1.05	1.19± 0.05
FR2	24.56± 0.03	0.27± 0.02	0.39± 0.02	20.78± 1.25	1.18± 0.02
FR3	25.89±0.03	0.29± 0.03	0.41± 0.03	20.89± 2.06	1.17± 0.03
FR4	23.78±0.02	0.37± 0.04	0.52± 0.04	19.98± 2.45	1.14± 0.04
FR5	24.56±0.01	0.41± 0.03	0.55± 0.02	19.08± 1.15	1.12± 0.03
FR6	22.04±0.03	0.45± 0.05	0.61± 0.03	18.94± 2.65	1.09± 0.02

Table 4: Compression parameters of developed MDT

Code	Weight variation (mg)	Hardness (kg/cm²)	Friability (%)	DT (seconds)	Drug content (%)
FR1	200.34±1.02	3.7±0.12	0.45±0.08	22.19±0.19	99.48±0.98
FR2	200.45±1.12	3.6±0.14	0.39±0.04	14.24±0.18	98.12±1.03
FR3	199.78±0.98	3.5±0.11	0.53±0.07	10.45±0.25	96.64±0.89
FR4	198.89±1.92	3.8±0.09	0.52±0.05	8.65±0.18	97.34±1.12
FR5	201.09±2.02	3.6±0.08	0.46±0.04	5.34±0.28	98.78±1.25
FR6	199.32±2.12	3.6±0.10	0.42±0.03	4.22±0.16	99.64±0.95

Table 5: Accelerated stability observation of FR6 formulation packed in Aluminum blister pack

Evaluation Variables	Stability condition 40C/75%RH	
	Initial	After 3 months
Weight (mg)	199.32±2.12	200.22±2.02
Hardness (kg/cm²)	3.6±0.10	3.5±0.24
DT (seconds)	4.22±0.16	5.72±0.18
Friability (%)	0.42±0.03	0.58±0.04
Drug content (%)	99.64±0.95	98.54±1.05
% Drug release (after 15 minutes)	90.65%± 1.65	89.15%± 1.55

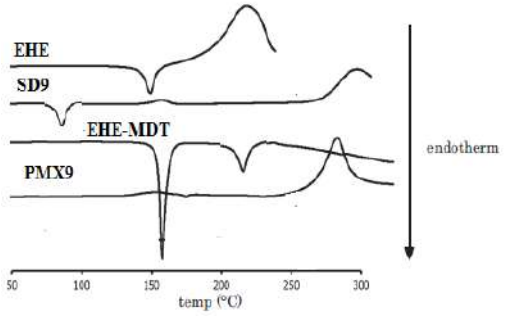

Fig 1: DSC chromatogram of EME (pure drug), SD, PMX and EME mixed with excipients of MDT tablets (EME-MDT) in ratio of 1:1

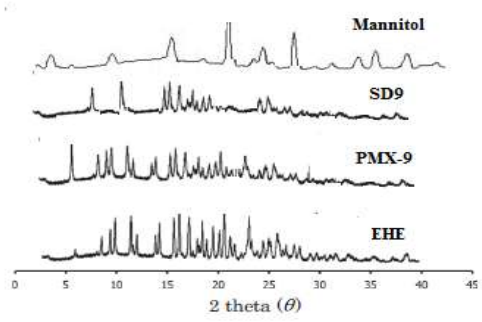

Fig 2: XRD chromatogram of EME (pure drug), SD, PMX and Mannitol

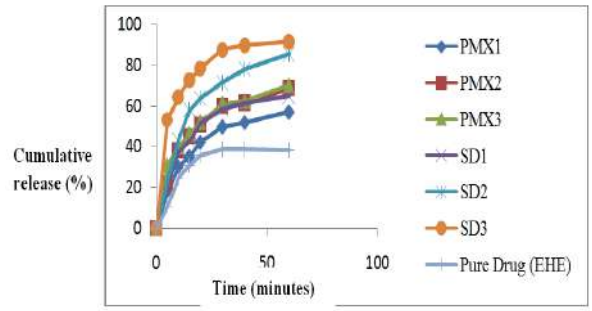

Fig 3a: *In-vitro* drug release from PMX's and SD's with PEG 600

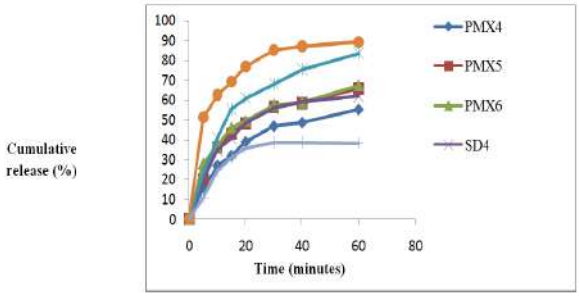

Fig 3b: *In-vitro* drug release from PMX's and SD's with PVP K-30

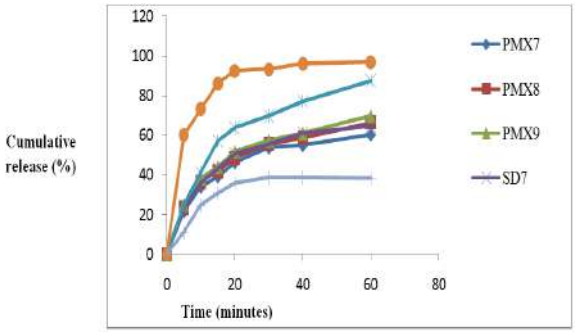

Fig 3c: *In-vitro* drug release from PMX's and SD's with Mannitol

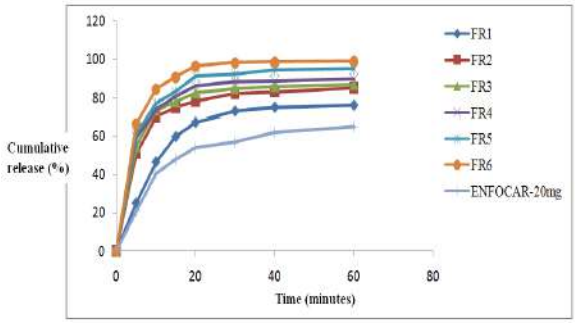

Fig 4: *In-vitro* drug release of developed MDT and marketed formulation.

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RESEARCH ARTICLE

GST- Boon or Bane for Various Business Segments: A Study Assessing the Impact of GST on Manufacturer, Trader and Service Provider

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ABSTRACT

Indirect taxes as one of the major sources of revenue contributes approximately 49 percent of total taxes collected. To overcome the deficiencies in old tax regime like cascading effect of tax, lack of uniformity in tax rates at national level, multiple compliances and registrations under multiple laws, all previous taxes has been replaced by a new "one nation one tax" known as "Goods & Services Tax". Implementation of GST has been affected all the business segments. An attempt to analyse the impact of GST statistically on various business segments

Keywords: Goods & Services tax, Manufacturer, Trader, Service provider

INTRODUCTION

In Indian taxation system, taxes are majorly collected through two sources direct and indirect. Direct tax is directly contributed by the person on his earnings and liability of paying indirect tax is of the supplier but collects tax from the final consumer. So we can say that direct tax is charged on income but indirect tax is charged on expenditure. Direct tax is progressive in nature as earning increases, government revenue also increases but indirect tax is regressive in nature and all consumers equally bears the burden irrespective of their level. Indirect taxes are one of the important sources of income for the government. Appx 49 percent of total revenue from taxes are collected through indirect taxes. Under old tax regime, some of the indirect taxes like excise duty, central sales tax and custom duty were collected by the central and value added tax, entertainment tax, entry tax were collected by the states.

In earlier tax regime, at different point of time, different taxes were imposed. At the time of manufacturing excise duty (CENVAT) were levied and on intra state sales, value added tax were levied on total of both basic value and excise duty. As far as utilization of credit is concerned, credit of one could not be utilized to set off against another as CENVAT was levied at the central level and VAT was levied at the states level. There was overlapping of taxes at many places that increased ultimately the burden of the consumer. Moreover there was lack of uniformity in tax rates at national level as different rate of VAT were levied by states.

Multiple laws require multiple compliances and registrations with no inter-linkages amongst them leading to silos being created. In earlier tax regime person was required to register separately for each type of taxes to be paid and ultimately created confusion. There was huge administrative cost for government also. In old tax regime, there was dilemma existed regarding classification of goods or services. Service tax was levied on all services other than the services either exempted or included in negative list of services and CST or VAT was levied on sale of goods. Some transactions lie somewhere in between having elements of goods as well as service and difficult to place on a scale with pure goods being on one side and services on the other side. There were deficiencies existed in earlier tax regime and to overcome all shortcomings, indirect tax structure in India has witnessed a paradigm shift on July 01, 2017, with a new comprehensive tax structure covering both goods and services and a unified tax subsumed all previous central and state taxes- Goods and Services Tax (GST).

Goods & Service Tax

Article 366(12A) of the Constitution as amended by 101st Constitutional Amendment Act, 2016 defines "Goods and services tax" means any tax on supply of goods, or services or both except taxes on the supply of the alcoholic liquor for human consumption and petroleum products. It is a value added tax and levied on manufacture, sale and consumption of goods and services and allowed tax credit at every point of supply from producer or service provider to the retailers. The supplier at each stage is allowed to set off the GST to be paid on the output from the GST paid by him on inputs. Thus the burden of GST is finally bears by the consumer only. Since, only the value added at each stage is taxed under GST, it completely eliminates the cascading effect of tax.

India has adopted dual GST model where both centre and states are simultaneously imposing tax. Integrated Goods and Service Tax (IGST) is levied and collected by central government on inter-state supply of taxable goods and services wherein Central Goods and Service tax (CGST) and State Goods and service tax (SGST) levied and collected by State Governments/Union Territories with State Legislatures/ Union Territory Goods and Service Tax (UTGST) levied and collected by union territories without state legislature on interstate supply of goods or services.

The very important feature of GST is that it is a consumption based tax and tax revenue would accrue to the state where goods are consumed. It helps in poor states to gain. To make simple classification of goods and services, Harmonised System of Nomenclature (HSN) code is used to classify goods and Service Accounting code (SAC) is used to classify services. To provide a single common platform for all types of services and uniform interface for tax payers and authorities, GST electronic portal has been set by the government. For registration requirements on GSTN portal, limit of aggregate turnover is fixed by the government. In case of supplier of goods, if aggregate turnover exceeds Rs. 40 lakhs (Rs.10 lakhs in Special Category States) and in case of service provider exceeds Rs. 20 lakhs in a financial year is required to register on GSTN portal.

For providing relief to small businesses (making only intrastate supply) having an aggregate turnover not more than Rs. 1.5 crore (Rs. 75 Lakhs in Special Category Notified States) from complying with the requirement of paying tax on the value addition by maintaining the detail of inputs and outputs, an option of composition scheme is provided to them. A composition dealer is required to file quarterly return instead of monthly return and rate of GST is also very low compared to other registered taxpayers.

Genesis of GST in India

France was the first country in the world who implemented one nation one tax GST in the year 1954. Because of unique feature of capability of raising revenue in most transparent and impartial manner, 160 countries worldwide have adopted GST during the period of 62 years, The idea of one nation one tax was not new in India and started way back in 2000 during tenure of Vajpayee government. An empowered committee was set up for discussion on GST. In 2004, Kelkar Task Force strongly recommended nation-wide GST. While presenting central budget (2007-08), Union Finance Minister Mr. P Chidambaram announced introduction of GST from 1st April, 2010. After that GST missed quite a few deadlines and gained drive again in year 2014 when NDA government presented the constitution (122nd Amendment) Bill, 2014 on GST in the Parliament on 19th December, 2014.On 6th May, 2015 bill passed in Lok Sabha and on 3rd August 2016, passed in Rajya Sabha. Consequent to approval of the bill by quite 50% of the states, Constitution (122nd Amendment) Bill, 2014 received the assent of the President on 8th September, 2016 and have become Constitution (101st Amendment) Act, 2016, which cemented the way for preamble of GST in India.

Literature Review: Few studies have been considered here for the purpose of review.

Bhavna Binwani (2019) studied the perception of small business persons about GST. Her research was primary data based and data were collected through structured questionnaire. Results of the findings showed that most of the small business persons had wrong insight about GST but same time dissatisfied with the efforts of the government regarding awareness and training about GST. Gautami (2018) in her research tried to analyse the impact of GST on micro, small and medium enterprises. Stratified random sampling was used to collect data from three divisions of Chittor district. Results of the findings revealed that 70 percent of the business operators had sufficient knowledge about challenges arising from the implementation of GST. The respondents also admitted that they are facing problems regarding filing of returns, claiming input tax credit.

Sanjay, Diksha (2018) in their research investigated the difference between the perception and satisfaction level of traders and manufacturers towards GST implementation. Primary data were collected from 200 respondents through questionnaire in three regions i.e. Rohtak, Gurgaon and Faridabad of Haryana state. The findings of the research came out with the facts that 73 percent respondents were happy with the way GST implemented. Suggestions were also given to Government regarding conduct of more awareness and training programs, improvement of digital infrastructure, reduction of filing formalities for successful implementation of GST. Rohit, et.al. (2018) in his paper statistically tested the Impact of GST on the Working of Rural India. A small survey has been conducted in the region of lower Himachal Pradesh, including different locations in the districts of Una, Bilaspur, Mandi, Hamirpur, and Dharamshala covering 100 merchants, vendors and 25 auditor / financial and legal advisors. Factor analysis and descriptive analysis techniques has been used for analysing the data. Results suggest that there is an urgent need for educating business sectors toward the act to prevent myth and afraid reaction and for smooth conduct of business.

Mukesh, Suniti (2019) in their study tried to find out the level of awareness and impact of GST on small business owners in the Mandsaur city of State of Madhya Pradesh. Structured questionnaire was used for collection of responses. Results of the analysis revealed that though businessman were aware about GST implementation and agreed about the fairness of the tax system but same time opined that act requires more clarity, simplify procedures and less formalities.

Research Gap

From the literature it is eminent that GST is a very emerging issue for research purposes. Even after efforts of government for successful implementation of the act, various business segments including manufacturer, traders and service providers are facing issues regarding its processes and execution. Few studies have been done for analysing the impact of GST on small business persons. In this paper we attempted to analyse the impact of GST on all business segments including manufacturer, traders and service providers.

RESEARCH METHODOLOGY

This research is based on primary data and responses are collected from merchants of various business segments of Delhi region through questionnaire with 20 statements, rated on a Likert scale. For scale reliability, cronbach alpha is used. Anova is carried out to analyse the means for the category of respondents for 4 variables.

Data analysis

After an extensive review of literature, a questionnaire with 20 statements, rated on a Likert scale was developed. The statements were categorised into 4 variables which were based on experience of various business segments related to perception for GST, Convenience in using GST, challenges faced, and Changes required in GST laws. The cronbach alpha, a measure of internal consistency was found to be .705 which was in the acceptable limit. Responses were taken from the merchants associated to manufacturing sector, service sector and trading sector. The frequency distribution of the three respondent categories is given in table 2. The above table shows frequency distribution of different respondent categories. Frequency percentage shows that number of respondents was almost equal in all categories. Approximately same percentage of respondents from the three business segments categories – Manufacturing, Services and Trading were the respondents of the survey. Frequency of statements for the 4 variables-Perception, Convenience, Challenges faced, Changes required were studied to understand the respondent's views on GST. As per table 3, 81% of the respondents were of the opinion that input tax mechanism did have a major role under GST. It was also found that 83 % of respondents perceived that pre GST regime offered more convenience. Whether introduction of GST resulted in more profitability had 66 % agreeing while 28 % disagreed. The respondents felt that introduction of GST did affect the demand of product/service (66%) and affected the price of goods/service (68%). Table 4 shows that respondents (66%) felt that the registration process for GST was smooth. The GST portal (63%) felt was user friendly. Views were not found to be in agreement to smooth functioning during claim of refund where 67% showed their disagreement. However, 76% respondents felt that composite scheme is beneficial for small business entities From the above table 5, it is inferred that while 41 % respondents agreed, an approximately same percentage (47%) disagreed that billing has been affected after implementation of GST. Similarly mixed responses (47% agreement, 54 % disagreement) were obtained from respondents when asked about whether product pricing being affected posts GST. 62 % felt that supply was not affected post GST. However, respondents (58%) felt that payment through GST is time consuming and difficult. Table 6 shows that respondents want changes in laws related to refund (53%), returns (69%), Documentation process (66%), place of supply (66%) and ITC (57%).

Anova Test

Anova was carried out to analyse the means for the category of respondents for 4 variables –Perception, Convenience, Challenges faced, Changes required

H0: There is no significant difference in the perception of various business segments

H1: There is significant difference in the perception of various business segments

H0 is 0.649 >0.05 that means null hypo is accepted. Thus there is no significant difference felt in perception by various business segments

H0: There is no significant difference in convenience in using GST among the 3 categories of business segments

H1: There is significant difference in convenience in using GST among the 3 categories of business segments

H0 is 0.021<0.05 that means null hypo is rejected. Thus there was significant difference felt in convenience by the 3 categories of respondents which are from Manufacturing, Service and Trade. To further identify among which 2 groups the difference was observed, post hoc test was conducted.

The post hoc test (Tukey) scores revealed that the respondents from manufacturing and service sector had difference of opinion (.019<.05). The reasons for difference in convenience felt may be due to the difference in styles of operation, different product categories handled by the two, and the difference in level of professionalism required by the manufacturing and service sector.

H0: There is no significant difference in the 3 categories of business segments regarding challenges faced
H1: There is significant difference in the 3 categories of business segments regarding challenges faced
H0 is 0.832>0.05 that means null hypo is accepted. Thus there is no significant difference felt by the 3 categories of business segments regarding challenges faced
H0: There is no significant difference in the views of 3 categories of business sectors for making changes in GST law to make it user friendly
H1: There is significant difference in the views of 3 categories of business sectors for making changes in GST law to make it user friendly
H0 is 0.193>0.05 that means null hypo is accepted. Thus there is no significant difference felt by the 3 categories of business sectors for making changes in GST law

CONCLUSION

GST, the biggest reform in Indian taxation system after independence has been benefitted all business segments like manufacturing, trading and service providers as it overcome all the deficiencies in earlier tax system. Successful implementation of GST was a huge challenge for the government in a country like India where reforms are not easily accepted. Government made all its efforts to create awareness, educate and train merchants before and after implementation of GST. Our analysis also shows that perceptions of merchants of various business segments are positive towards GST but same time they are facing issues and challenges regarding process and execution part of GST. For smooth functioning of business, government also from time to time takes suggestions from various business segments and making changes in GST law accordingly. Our analysis also suggests that there is need to change in law regarding return and refund.

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Table 1: Cronbach Alpha

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.705	.721	20

Table 2: Frequency Distribution of Respondent Categories

Category	Frequency	Percent
Manufacturing	34	34.0
Services	33	33.0
Trading	33	33.0

Table 3: Frequency of Responses for Statements related to Perception

	Strongly agree	Agree	Neutral	Disagree	Strongly Disagree	Median	Standard Deviation
Input tax mechanism plays a major role under GST	28	53	7	6	6	2	1.065
GST regime is more convenient than pre GST regime	51	32	4	9	4	1	1.120
Introduction of GST has resulted in better profitability of the business	12	54	6	22	6	2	1.140
Introduction of GST in India has affected the demand for the product or service	32	34	12	20	2	2	1.169
Implementation of GST has made the prices of your goods or services cheaper	34	34	20	4	8	2	1.1484

Table 4: Frequency of Responses for statements Related to Convenience

	Strongly agree	Agree	Neutral	Disagree	Strongly Disagree	Median	Standard Deviation
The procedure for registration under GST is smooth	39	27	10	18	6	2	1.306
The GST portal is user friendly	18	45	3	26	8	2	1.27
There is smooth functioning in claiming refund under GST regime	2	30	1	41	26	4	1.223
Composite scheme is beneficial for the small business entities	33	43	0	10	14	2	1.387

Table 5: Frequency of Responses for Statements Related to Challenges Faced Post GST

	Strongly agree	Agree	Neutral	Disagree	Strongly Disagree	Median	Standard Deviation
Billing has been affected after the implementation of GST	26	15	12	20	27	3	1.578
Product pricing has been affected after the implementation of GST	25	22	9	25	19	3	1.498
Supply has been affected after the implementation of GST	12	21	5	36	26	4	1.387
Payment of GST is time consuming	16	23	14	31	16	3	1.353
Payment of GST through GSTN portal is difficult	28	30	5	20	17	2	1.490

Table 6 Frequency of Responses for Statements related to Changes Required in GST Law

	Strongly agree	Agree	Neutral	Disagree	Strongly Disagree	Median	Standard Deviation
GST rate	14	28	3	22	33	4	1.517
Refunds	9	21	17	35	18	4	1.246
ocumentation	2	27	5	38	28	4	1.212
Return	23	46	10	17	4	2	1.129
Place of supply	2	10	22	44	22	4	0.981
ITC	15	42	5	21	17	2	1.378

Table 7: ANOVA for Respondent's Views on Perception for GST

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.388	2	3.694	0.434	0.649
Within Groups	825.772	97	8.513		
Total	833.16	99			

Table 8 ANOVA for Convenience among Respondents

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.999	2	30	4.046	0.021
Within Groups	719.241	97	7.415		
Total	779.24	99			

Table 9: Multiple comparisons, Dependent variable: Tukey HSD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.958	2	3.479	0.185	0.832
Within Groups	1827.152	97	18.837		
Total	1834.11	99			

*. The mean difference is significant at the 0.05 level.

Table 10: ANOVA for Respondent's Views on Challenges Faced

(I) Category	(J) Category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Manufacturing	Services	-1.83957*	0.66541	0.019	-3.4234	-0.2557
	Trading	-1.29412	0.66541	0.132	-2.878	0.2897
Services	Manufacturing	1.83957*	0.66541	0.019	0.2557	3.4234
	Trading	0.54545	0.67036	0.695	-1.0502	2.1411
Trading	Manufacturing	1.29412	0.66541	0.132	-0.2897	2.878
	Services	-0.54545	0.67036	0.695	-2.1411	1.0502

Table 11: ANOVA for Respondent's Views on Changes Required to Make GST Law User Friendly

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.053	2	28.026	1.672	0.193
Within Groups	1626.057	97	16.763		
Total	1682.11	99			

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REVIEW ARTICLE

Review on List of Drugs Failed in Clinical Trial

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INTRODUCTION

Clinical Research provides data on dosage, safety, and effectiveness [1]. They can only be performed after obtaining approval from the health authority/ethics committee of the country/region requesting approval of the treatment. These authorities are responsible for verifying the balance of benefits and risks of the research-their approval does not mean that the treatment is "safe" or effective. Clinical trials of new drugs are generally divided into five stages. Each stage of the drug approval process is considered a separate clinical study. The drug development process usually goes through stages I-IV for many years. If the drug successfully passes phases I, II, and III, it will usually be approved by the national regulatory agency for use in the general population [2] IV . The studies are carried out after drugs, diagnoses or approved devices are recently introduced into the market and provide risk assessments, benefits or better use [2].

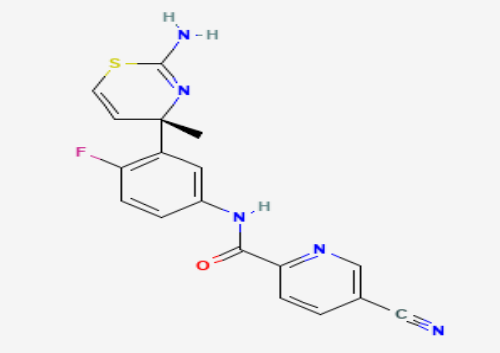
LIST OF DRUGS FAILED IN CLINICAL TRIALS(2018) [4,5]

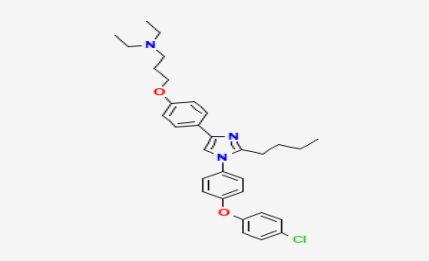
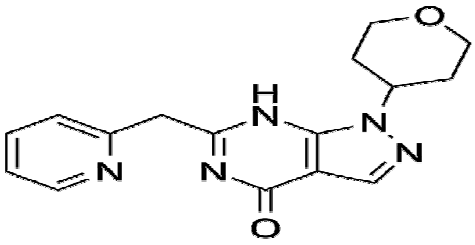
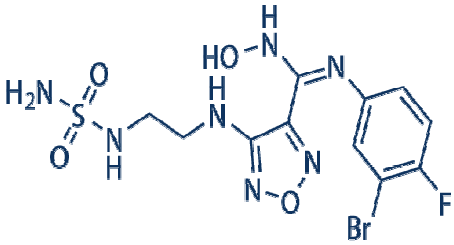
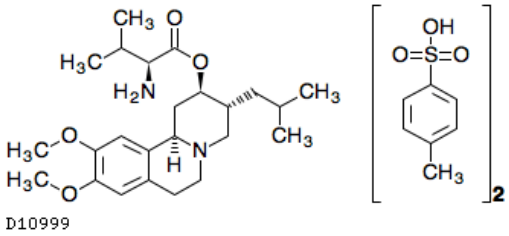
S.NO	DRUG NAME	PHASE	REASON	USES	COMPANY NAME
1	**ATABECESTAT**	**Phase II**	**Failure due to"serious" elevations of liver enzymes in some study participants who received the drug.**	**Alzheimer's disease, BACE inhibitor**	Janssen, Shionogi Pharma
2	**AXALIMOGENE FILOLISBAC**	**Phase I / II**	**A Phase I/II combination trial with AstraZeneca's Imfinzi® was halted in March after a patient death, then**	Cervical + head and neck cancerTargete	**Advaxis**

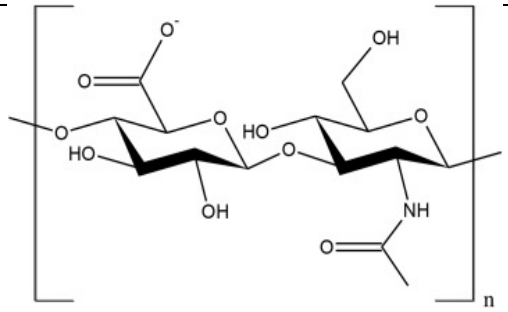
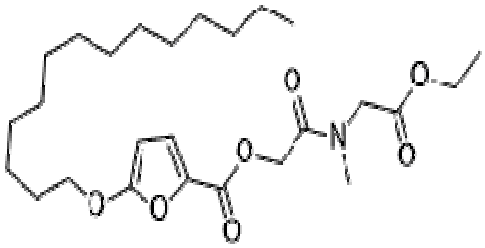
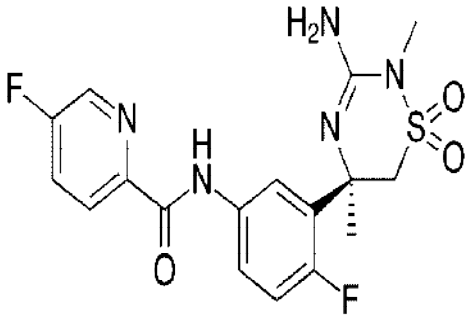
			resumed in July after C-level overhaul.	d Lm-based immunotherapy	
3	**AZELIRAGON**	Phase III	Shares plunged 71% after vTv halted azeliragon studies following an initial readout failure in Part A of the Phase III STEADFAST study. However vTv identified a subpopulation (Alzheimer's patients with type 2 diabetes) showing statistically significant benefit	**Alzheimer's disease** **RAGE inhibitor**	vTv Therapeutics
4	**B1 409306**	**Phase II**	**BoehringerIngelheim shifted BI 409306's development focus to ongoing schizophrenia studies after missing efficacy end points in two Phase II trials in patients with cognitive impairment and memory dysfunction in Alzheimer's and schizophrenia**	**Alzheimer's disease** **PDE9 inhibitor**	
5	**EG – 1962**	Not Revealed	Not Revealed	Not Revealed	Not Revealed
6	**EPACADOSTAT**	**Phase III**	**Incyte downsized its epacadostat clinical program after a combination with Merck & Co.'s Keytruda® (pembrolizumab) failed the Phase III ECHO-301/ KEYNOTE-252 trial.**	**Unresectable or metastatic melanoma IDO1 inhibitor**	**Incyte**
7	**HTL0018318**	**Phase II**	**The companies said Phase II studies in Lewy body dementia and other dementias, including Alzheimer's, would be delayed at least 6 months. The companies cited the need to investigate an unexpected toxicology finding that occurred in a single animal study involving nonhuman primates.**	**Lewy body dementia** **Muscarinic M1 receptor agonist**	Sosei Group Allergan
8	**Ingrezza**	Phase II	**Neurocrine began 2019 assessing Ingrezza's future in Tourette syndrome after its third trial failure in that indication, missing the primary endpoint in the Phase IIb T-Force GOLD Study.**	**Tourette syndrome Selective VMAT2 inhibitor**	**Neurocrine Biosciences**
9	**Keyzilen**	**Phase II**	**Auris has been weighing Keyzilen's future since March 2018, when the drug missed its primary endpoint in the TACTT3 trial of significant Tinnitus Functional Score**	**Acute inner ear tinnitus** **NMDA receptor**	**Auris Medical Holding**

			improvement vs. placebo - Keyzilen's second Phase III failure in 19 months.	**antagonist**	
10	**MM-141**	**Phase II**	**Merrimack scrapped MM-141 after missing primary and secondary endpoints in the Phase II CARRIE trial, assessing the drug plus nab-paclitaxel in patients with previously untreated metastatic pancreatic cancer and high serum levels of free insulin-like growth factor-1.**	**Pancreatic cancer Tetravalent monoclonal antibody**	**Merrimack Pharmaceuticals**
11.	**OLUMACOSTAT GLASARETIL**	**Phase III**	**Dermira effectively scrapped olumacostatglasaretil after it failed to reduce lesion counts or show 2+ grade IGA improvement in two Phase III trials. The drug was once projected to generate $250M in annual sales.**	**Acne vulgaris Topical sebum inhibitor**	**Dermira**
12.	**VERUBECESTAT**	**Phase III**	**Merck & Co. dropped verubecestat from its late-stage pipeline after the drug failed the Phase III APECS study in February 2018—the drug's second late-stage failure. The first came in February 2017 in the EPOCH study.**	**Prodromal Alzheimer's disease BACE1 inhibitor**	**Merck & Co.**
13	**VR475**	**Phase III**	**Vectura ended VR475 development in November after it failed a Phase III trial in adults and adolescents with severe uncontrolled asthma.**	**Asthma Drug-device combo with jet nebulizer**	**Vectura Group**

DRUG NAME AND ITS STRUCTURE(2018)

S.NO	DRUG NAME	STRUCTURE	IUPAC NAME /CHEMICAL NAME
1	**ATABECESTAT**		**N-[3-[(4S)-2-amino-4-methyl-1,3-thiazin-4-yl]-4-fluorophenyl]-5-cyanopyridine-2-carboxamide**

2	**AXALIMOGENE FILOLISBAC**	**Not Revealed**	**Not Revealed**
3	**AZELIRAGON**		**Not Revealed**
4	**B1 409306**		**Not Revealed**
5	**EG – 1962**	**Not Revealed**	**Not Revealed**
6	**EPACADOSTAT**		**1,2,5-Oxadiazole-3-carboximidamide, 4-((2-((Aminosulfonyl)amino)ethyl)amino)-N-(3-bromo-4-fluorophenyl)-N'-hydroxy-, (C(Z))**
7	**HTL0018318**	**Not Revealed**	**(ethyl (3-endo)-3-(3-oxo-2,8-diazaspiro [4.5] dec-8-yl)-8-azabicyclo [3.2. 1] octane-8-carboxylate hydrochloride)**
8	**Ingrezza**	 D10999	**1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-yl ester, 4-methylbenzenesulfonate**

9	**Keyzilen**		**Not Revealed**
10	**MM-141**	**Not Revealed**	**Not Revealed**
11	**OLUMACOSTAT GLASARETIL**		**2-((2-ethoxy-2-oxoethyl)(methyl)amino)-2-oxoethyl 5-(tetradecyloxy)furan-2-carboxylate**
12	**VERUBECESTAT**		*N*-[3-[(5*R*)-3-amino-2,5-dimethyl-1,1-dioxo-6*H*-1,2,4-thiadiazin-5-yl]-4-fluorophenyl]-5-fluoropyridine-2-carboxamide
13	**VR475**	**Not Revealed**	**Not Revealed**

LIST OF DRUGS FAILED IN CLINICAL TRIAL 2019

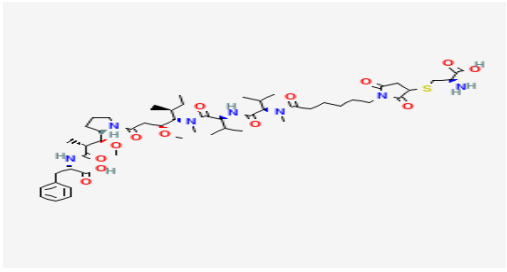
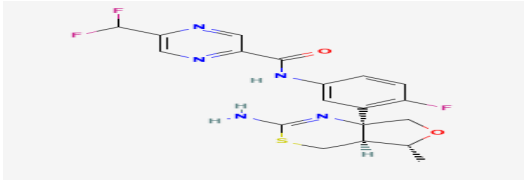
S.NO	DRUG NAME	PHASE	REASON	USES	COMPANY NAME
1	**Depatux-M**	phase 3	High-grade gliomas (HGGs) are one of the toughest tumors to treat, Depatux-M—is one of at least three drugs that failed to hit the mark in this highly aggressive form of brain cancer in 2019.Depatux-M (depatuxizumabmafodotin) flunked out in the phase 3 INTELLANCE-1 trials[6].	**Not Revealed**	AbbVie
2	**Elenbecestat**	phase III	Eenbecestat was designed to block an enzyme involved in the formation of amyloid beta peptides that collect into the	**Not Revealed**	Biogen, Eisai

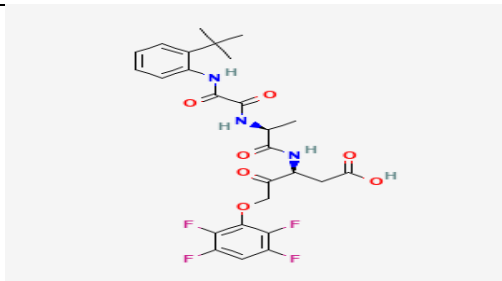
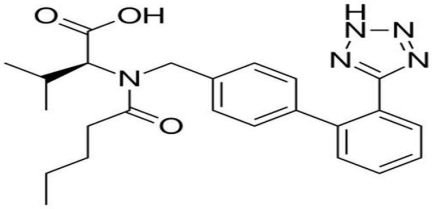
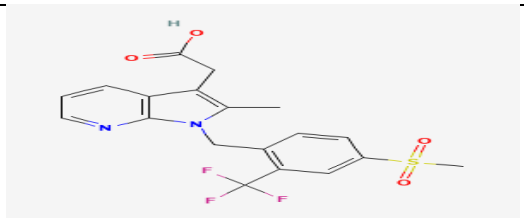
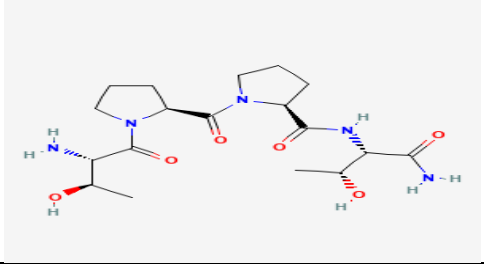
			characteristic amyloid plaques that are a hallmark of Alzheimer's, and its failure was yet another example among dozens of amyloid-targeting drugs and was dropped after an "unfavorable risk-benefit ratio" seen in an interim look at data from the phase 3 MISSION AD trials, so safety seems to be main issue once again[7].		
3	**Emricasan**	phase II	In the phase 2b ENCORE-LF trial, emricasan wasn't able to best placebo on a primary endpoint that looked at whether patients died, suffered new decomposition events or experienced liver disease progression at either of two doses tested. At the same time, another study found that emricasan was no better than placebo at improving mean hepatic venous pressure gradient in compensated NASH cirrhosis patients [8].	Not Revealed	Conatus, Novartis
4	**Entresto**	**Phase III**	Novartis has announced that its heart failure with reduced ejection fraction (HFrEF) drug Entresto (sacubitril/valsartan) has shown further efficacy in two Phase IV trials, but the drug failed to meet its primary endpoint in the Phase III PARAGON-HF study of heart failure patients with preserved ejectionfraction[9].	Heart failure	Novartis
5	**Fevipiprant**	PhaseII I	The drug's star fell in October when the Basel-based company announced it had failed a pair of trials in moderate asthmatic patients. Now, fevipiprant has flopped in two additional studies in moderate-to-severe patients, spelling the end to its development for asthma[10].	oral treatment for asthma	Novartis
6	**Opdivo**	Phase III	BMS' Opdivo fails in Phase III glioblastoma trial .Bristol-Myers Squibb (BMS) has announced that its immunotherapy drug Opdivo (nivolumab) has failed to meet its primary endpoint of overall survival (OS) in Phase III CheckMate-498 trial. Credit: AFIP Atlas of Tumor Pathology[11].	Treat people with a type of advanced stage lung cancer (called non-small cell lung cancer)	Bristol-Myers Squibb

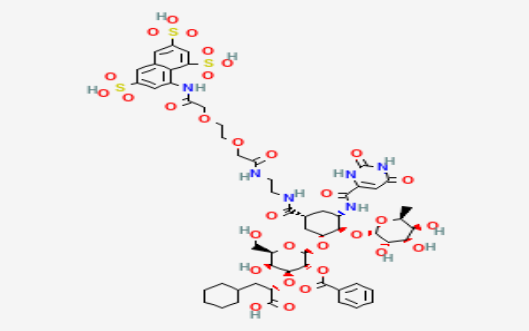
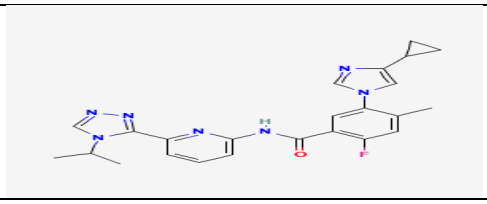
7	**Pexa-Vec**	**Phase III**	SillaJen seems determined to press on with Pexa-Vec, arguing that the failure of the trial was down to an imbalance in the proportion of patients who received salvage therapies, including chemotherapy, which skewed the results in favor of the control group. Transgene said last month however that it had also decided to abandon a phase 1/2 trial evaluating Pexa-Vec in combination with Opdivo in first-line HCC[12].	**Not Revealed**	Transgene, SillaJen
8	**Praliciguat**	phase II	The company announced topline data from its Phase II proof-of-concept trial of praliciguat in diabetic nephropathy. Praliciguat is a once-daily, oral sGC stimulator. The trial did not meet statistical significance on its primary endpoint of reduction in albuminuria from baseline compared to placebo[13].	**Not Revealed**	Cyclerion
9	**Rapastinel**	**Not Revealed**	The Phase III RAP-MD-01,-02,-03 trials compared the safety, efficacy, and tolerability of rapastinel to placebo, both in combination with antidepressant therapy (ADT), in patients with major depressive disorder (MDD)[14].	**Not Revealed**	Allergan
10	**ResVax**	PhaseIII	On 28 February, Novavax announced that its experimental respiratory syncytial virus (RSV) vaccine, ResVax, did not meet its primary endpoint in a Phase III study evaluating its safety and efficacy for the prevention of medically significant RSV-associated lower respiratory tract infections (LRTIs) in infants via maternal immunisation [15].	**Not Revealed**	Novavax
11	**Rivipansel**	Phase III	Pfizer Inc. (NYSE:PFE) announced today that the Phase 3 Rivipansel (GMI-1070): Evaluating Safety, Efficacy and Time to Discharge (RESET) pivotal study did not meet its primary or key secondary efficacy endpoints. The objective of the trial was to evaluate the efficacy and safety of rivipansel in patients aged six and older with sickle cell disease (SCD) who were hospitalized for a vaso-occlusive crisis (VOC) and required treatment with intravenous (IV) opioids[16].	**Not Revealed**	GlycoMimetics, Pfizer
12	**Rova-T**	phase III	Rova-T as first-line maintenance therapy for advanced small-cell lung cancer (SCLC), failed to demonstrate a survival benefit following a pre-planned interim analysis. [17].	**Not Revealed**	AbbVie

13	**SB-913**	**Not Revealed**	The actions in the field of biotechnology were hit hard after lead-based therapy could not move the needle to the study of 1/2 phase samples that patients involved with a rare genetic disease of type II mucopolysacáridosis (MPS II), or Hunter syndrome. It was a great disappointment in the first data to produce with genome editing in vivo in man[18].	**Not Revealed**	Sangamo Biosciences
14	**Selonsertib**	Phase III	Gilead announced that one of its key NASH candidatesselonsertib, a ASK1 inhibitor, failed a crucial phase 3 test, after the drug was unable to improve fibrosis without worsening of NASH, in those with bridging fibrosis (F3). Selonsertib had already failed another phase 3 test two months prior, that involved advanced NASH patients (F4) whose disease had progressed into compensated cirrhosis[19].	**Not Revealed**	Gilead Sciences
15	**Toca 511/Toca FC**	Phase III	Treatment with Toca 511 and Toca FC did not improve overall survival compared with standard therapy in patients with recurrent high-grade glioma undergoing resection, missing the primary endpoint of the phase III Toca 5 trial [20].	**Not Revealed**	Tocagen

DRUG NAME AND ITS STRUCTURE(2019)

S.NO	DRUG NAME	STRUCTURE	IUPAC NAME /CHEMICAL NAME
1	**Depatux-M**		Depatuxizumabmafodotin
2	**Elenbecestat**		N-[3-[(4aS,5R,7aS)-2-amino-5-methyl-4,4a,5,7-tetrahydrofuro[3,4-d][1,3]thiazin-7a-yl]-4-fluorophenyl]-5-(difluoromethyl)pyrazine-2-carboxamide

3	**Emricasan**		(3S)-3-[[(2S)-2-[[2-(2-*tert*-butylanilino)-2-oxoacetyl]amino]propanoyl]amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid
4	**Entresto**		**Hexasodium;4-[[(2S,4R)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl)pentan-2-yl]amino]-4-oxobutanoate;(2S)-3-methyl-2-[pentanoyl-[[4-[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-dien-5-yl)phenyl]phenyl]methyl]amino]butanoate;pentahydrate**
5	**Fevipiprant**		CC1=C(C2=C(N1CC3=C(C=C(C=C3)S(=O)(=O)C)C(F)(F)F)N=CC=C2)CC(=O)O
6	**Opdivo**	**Not Revealed**	**Not Revealed**
7	**Pexa-Vec**	**Not Revealed**	**Not Revealed**
8	**Praliciguat**	**Not Revealed**	**1,1,1-trifluoro-3-[(5-fluoro-2-{1-[(2-fluorophenyl)methyl]-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl}pyrimidin-4-yl)amino]-2-(trifluoromethyl)propan-2-ol**
9	**Rapastinel**		**1,1,1-trifluoro-3-[(5-fluoro-2-{1-[(2-fluorophenyl)methyl]-5-(1,2-oxazol-3-yl)-1H-pyrazol-3-yl}pyrimidin-4-yl)amino]-2-(trifluoromethyl)propan-2-ol**
10	**ResVax**	**Not Revealed**	**Not Revealed**

11	**Rivipansel**		(2*S*)-2-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3-benzoyloxy-2-[(1*R*,2*R*,3*S*,5*R*)-3-[(2,4-dioxo-1*H*-pyrimidine-6-carbonyl)amino]-5-[2-[[2-[2-[2-oxo-2-[(3,6,8-trisulfonaphthalen-1-yl)amino]ethoxy]ethoxy]acetyl]amino]ethylcarbamoyl]-2-[(2*S*,3*S*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxycyclohexyl]oxy-5-hydroxy-6-(hydroxymethyl)oxan-4-yl]oxy-3-cyclohexylpropanoic acid
12	**Rova-T**	**Not Revealed**	(2R)-3-[(3R)-1-[3-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[(2S)-1-[[(2S)-1-[4-[[(6S,6aS)-3-[5-[[(6aS)-2-methoxy-8-methyl-11-oxo-6a,7-dihydropyrrolo[2,1-c][1,4]benzodiazepin-3-yl]oxy]pentoxy]-6-hydroxy-2-methoxy-8-methyl-11-oxo-6a,7-dihydro-6H-pyrrolo[2,1-c][1,4]benzodiazepine-5-carbonyl]oxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-3 oxopropoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-3-oxopropyl]-2,5-dioxopyrrolidin-3-yl]sulfanyl-2-aminopropanoic acid.
13	**SB-913**	**Not Revealed**	**Not Revealed**
14	**Selonsertib**		5-(4-cyclopropylimidazol-1-yl)-2-fluoro-4-methyl-*N*-[6-(4-propan-2-yl-1,2,4-triazol-3-yl)pyridin-2-yl]benzamide
15	**Toca 511/Toca FC**	**Not Revealed**	**Not Revealed**

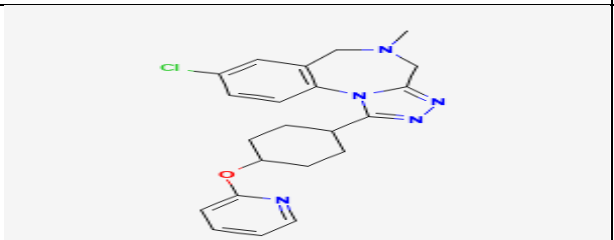
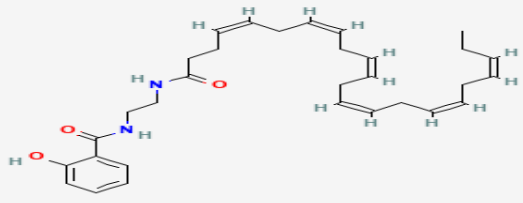
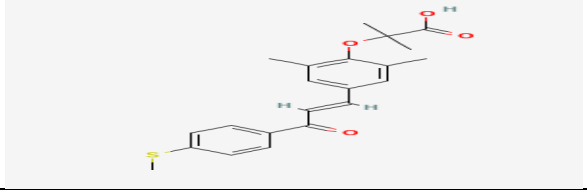
LIST OF DRUGS FAILED IN CLINICAL TRILAS 2020

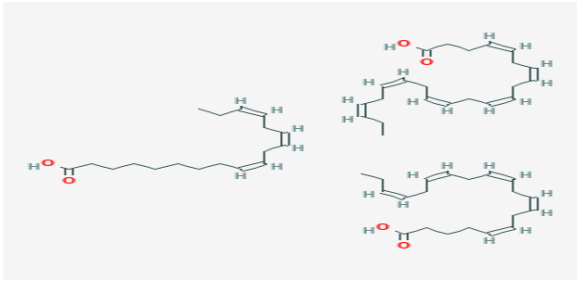
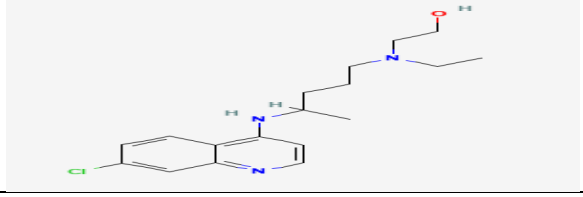
S.NO	DRUG NAME	PHASE	REASON	USES	COMPANY NAME
1	**ALVAC-HIV/AIDSVAX B/E**	**Not Revealed**	The phase 2b/3 clinical trial—called HVTN 702—tested the combination in around 5,400 sexually active men and women aged 18 to 35. One piece of the combo was a canarypox vector-based vaccine developed by Sanofi Pasteur called ALVAC-HIV. The other was a two-component, gp120 protein subunit vaccine with an adjuvant developed by GSK[21].	**Not Revealed**	GlaxoSmithKline and Sanofi
2	**Balovaptan**	**Not Revealed**	According to the Centers for Disease Control and Prevention (CDC), about 1 in 59 children has been identified with ASD, with accompanying difficulties in social interaction and communication, as well as other symptoms like repetitive behaviors[22].	**Not Revealed**	Genentech,Chugai Pharmaceutical Co.
3	**Edasalonexent**	Phase III	There was more than one late-stage disappointment among companies developing drugs for the muscle-wasting disease Duchenne muscular dystrophy in 2020, but the demise of Catabasis' edasalonexent was particularly keenly felt. The program was already high-risk after edasalonexent failed to move the needle in a mid-stage trial reported in 2017[23].	**Not Revealed**	Roche
4	**Elfibranor**	Phase III	2020 proved once again how tough a challenge non-alcoholic steatohepatitis (NASH) is for drug developers, with Genfit'selafibranor unable to improve patient outcomes in the phase 3 study RESOLVE-IT study reported in July. Elafibranor fell short of its primary endpoint—resolving NASH without worsening fibrosis scarring compared to placebo—and also missed its secondary targets in what turned out to be a comprehensive fail for the drug[24].	Not Revealed	Genfit
5	**Epanova**	Phase III	With its fish oil-derived drug Epanova, AstraZeneca was looking to follow in the footsteps of Amarin's blockbuster	Not Revealed	AstraZeneca

			hopeful Vascepa and show that it could cut heart risks among patients with high blood lipid levelsa category it used to dominate with its Crestor statin before it lost patent protection. That hope came crashing down in January, when the company decided to ditch its phase 3 STRENGTH study of Epanova (omega-3 carboxylic acids) as an add-on to statins for lowering cardiovascular risks among patients with mixed dyslipidemia, a disease marked by elevated triglyceride levels[25].		
6	**HYDROXYCHLOROQUINE**	Not revealed	Hydroxychloroquine, allowed to be used in hospitals to fight the pandemic, but the idea that it could be a quick, low-cost medicine for coronavirus infections came crashing down when multiple studies found no benefit from the drug as either post-exposure prophylaxis or as a COVID-19 treatment. [26].	Not Revealed	Multiple
7	**Iw3718**		At one time, Ironwood claimed its lead candidate, IW-3718, had the potential to become a $2 billion-a-year product, but that promise evaporated in September when it failed a phase 3 trial in refractory gastroesophageal reflux disease (GERD) and was canned[27].	Not Revealed	Ironwood
8	**sarscov2 Vaccine**	Not Known	The holdup comes after a phase 1/2 trial in 440 patients showed that the vaccine stimulated an immune response similar to that of patients who recovered from COVID-19 in adults aged 18 to 49 years, but it didn't seem effective in older people—apparently because of "an insufficient concentration of the antigen [28].	Not Revealed	Sanofi, GlaxoSmithKline
9	**Soremab**	Phase II	With anti-amyloid drugs repeatedly failing to make any headway in Alzheimer's disease, targeting tau protein has emerged as a new focus for dementia drug developers. However, a setback in September with one of the leading candidates in the anti-tau field—Roche and AC Immune's semorinemab—suggests this target may be equally challenging [29].	Not Revealed	Roche / AC Immune

10	**Tecentriq**	Not Revealed	There was jubilation among patients with triple negative breast cancer (TNBC) when Roche's checkpoint inhibitor Tecentriq (atezolizumab) was approved to treat the disease in March 2019, becoming the first immuno-oncology option for the highly aggressive tumor—and breast cancer in general. In the follow-up IMpassion131 trial, Roche tested the PD-L1 inhibitor with regular, generic paclitaxel—one which, unlike Abraxane, isn't bound to albumin—in the same previously-untreated, PD-L1-posiitve TNBC population[30].	Not Revealed	Roche

DRUG NAME AND ITS STRUCTURE(2020)

S.NO	DRUG NAME	STRUCTURE	IUPAC NAME /CHEMICAL NAME
1	**ALVAC-HIV/AIDSVAX B/E**	**Not Revealed**	
2	**Balovaptan**		8-chloro-5-methyl-1-(4-pyridin-2-yloxycyclohexyl)-4,6-dihydro-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine.
3	**Edasalonexent**		*N*-[2-[[(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoyl]amino]ethyl]-2-hydroxybenzamide
4	**Elafibranor**		2-[2,6-dimethyl-4-[(*E*)-3-(4-methylsulfanylphenyl)-3-oxoprop-1-enyl]phenoxy]-2-methylpropanoic acid

5	**Epanova**		(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid;(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoic acid;(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid
6	**Hydroxychloroquine**		2-[4-[(7-chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol
7	**IW-3718**	**Not Revealed**	**Not Revealed**
8	**SARS-CoV-2 vaccine**	**Not Revealed**	**Not Revealed**
9	**Semorinemab**	**Not Revealed**	**Not Revealed**
10	**Tecentriq**	**Not Revealed**	**Not Revealed**

CONCLUSION

In spite of difficulties in the clinical trials for the new drug entity, the great effort is to make ensure the safety and efficacy of the new medicament to the consumer. During their work, there are discontinuation in their further studies due to various reasons like shrinking clinical research workforce, the difficulties of navigating administrative and regulatory requirements, and the recruitment and retention of patients. Once the molecule has promising therapeutic effect, then there are ample of studies have to be done by the clinical trial professionals to scrutinize the safety and monitor the side effects if any. The list of clinical trial failed drugs with the possible reasons provides some knowledge about the clinical trials which could be useful for the professionals who opt this particular career.

REFERENCES

1. Rohilla, Ankur, et al. "PHASES OF CLINICAL TRIALS: A REVIEW." International Journal of Pharmaceutical, Chemical & Biological Sciences 3.3 (2013).
2. Rohilla, A., Singh, R. K., Sharma, D., Keshari, R., &Kushnoor, A. (2013). PHASES OF CLINICAL TRIALS: A REVIEW. International Journal of Pharmaceutical, Chemical & Biological Sciences, 3(3).
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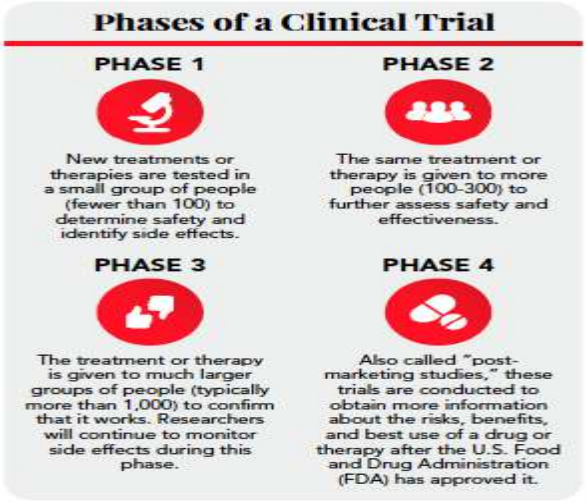
TABLE 1 [3] Phases of a clinical trial



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RESEARCH ARTICLE

Mother Tongue as the Medium of Instruction: Towards Effective Implementation of NEP 2020

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ABSTRACT

While there are many aspects that are involved in providing a good basic education, language is unquestionably important for expression of ideas and comprehension in interactive process of teaching learning. Despite the fact that most of the emerging Nations are marked by social multilingualism, the education sector continues to be dominated by a single foreign language. This practice of putting learners in water without teaching them how to properly swim is termed as submersion. Submersion makes learning and teaching incredibly difficult, as it can often lead to the teacher not being able to effectively communicate with one another. This is also compounded by the lack of proper teaching and learning materials. The paper focuses on the challenges of practical implementation of teaching in mother tongue as envisaged in New National Education Policy 2020. The paper focuses on reading and writing skills, as well as academic content, to be taught in mother tongue which is learners' first language. The second or foreign language should be taught in a systematic manner so that the learners can gradually transfer their skills to the new one. Although both the English and bilingual models have their own unique characteristics, one thing they all have in common is the need for children to learn and develop their reading skills in the mother tongue.

Keywords: Language, Mother Tongue, Multilingualism, Policy, Instruction

INTRODUCTION

India, as the world's largest multilingual federation, is facing a lot of debates on language in education. The Union government's new education policy has reignited one of the most debated issues in the country: Should Indian students be taught in whichever language they learn?

The New Education Policy recommends that "wherever possible, the medium of instruction until at least Grade 5, but preferably till Grade 8 and beyond, will be the home language/mother-tongue/local language/regional language" and the media has vehemently attacked this idea.

Due to the importance of language in modern society, it is not surprising that debate about the use of language elicits passionate reactions. But, despite the controversy, there is a scientific consensus that teaching kids in their own language is the most effective method of learning.

Out of the three domains of Bloom's Taxanomy of educational objectives, the affective domain is strengthened by the use of parent tongue instruction. This method increases levels of motivation and creativity. Unlike passive and dead walls of schools, where children are forced to sit like a ghost in passive mode or mechanically keep on repeating and cram, mother tongue interactions in the classrooms allow children to develop their personalities and potentials to their full development which substantially reduce frustration, repetition, failure and dropout.

The first twelve years of a child's life are the most crucial years of his or her life. It is during this period that they develop their personal attitudes and abilities. "It is critical that children are provided with the necessary care and guidance during their early years to ensure their well-being."(NEP, 2020)

Since 1953, the United Nations Educational, Scientific, and Cultural Organization has advocated for "every effort should be made to provide education in the mother tongue". In a report published half a century later, Carole Benson, a researcher at Stockholm University's Centre for Research on Bilingualism, explained, "Mother tongue-based bilingual schooling is rarely questioned on the basis of its pedagogical reasoning."

"To be taught in a language different than one's own has a negative effect on learning," UNESCO stated in its Global Education Monitoring Report in 2016. The research supports that 40 percent of the global population usually study in the language they do not understand in the formative years of education. (Kevin & Benson, 2019) UNESCO also recommends "At least six years of mother tongue education should be provided in ethnically diverse communities to ensure those speaking a different language from the medium of instruction do not fall behind."

Conceptual Framework

The importance of teaching children in their native tongue should be recognised in national education policies. Language diversity is rarely reflected in educational policies. According to a study of 40 countries' education policies, only about 50% of them understand the necessity of incorporating the mother tongue language in teaching learning process, especially in the formative stages. The New Education Policy 2020 has ushered a new era in Education by laying emphasis on the Indian Centric Education and holistic development by giving due importance to the use of mother tongue in early education of children. Using the child's native language in the early years of school promotes attendance, academic results, and the ability to learn new languages. It also enhances classroom participation, lowers dropout rates, and eliminates grade repetition, according to studies from throughout the world. Prof. Krishna Kumar, the former Director, NCERT observes that this has been a widely explored area for decades. "It's such a self-evident statement that it can't be contested. The ideal place to start a child's education is with his or her mother tongue" he commented.

In the 1970s, a swing of empirical research began to emerge that scientifically supported what the current NEP 2020 has proposed: The multilingual education with mother tongue being the medium of instruction in early educational stages followed by introducing English as the child grows older. Aliu Fafunwa (1970), an educator from Nigeria, initiated a project in which experimental groups of pupils were taught in their native language (Yoruba) during their early years of schooling. Nigeria is a bilingual country with a similar history of British colonialism as India, therefore the dominance of English in the educational system of Nigeria is also prevalent. The findings of the study revealed that the students who were taught in their native tongue performed better than those who were taught in English

medium in early years. There is a negative influence on test scores when home and school languages are different. The percentage of students who attained an international minimum learning standard in reading during an assessment is referred to as the score. (Global Education Monitoring Report, 2016). Schooling that begins with the mother tongue is so effective that it even aids in the acquisition of a second language. According to Jim Cummins, a professor at the University of Ontario in Canada, when children study a second language later in life, they can use the abilities they learned when they were younger and were taught in their mother tongue.

Research Questions

The following research questions are framed with regard to use of mother tongue in early years of life of a child

1. What is to be done?
2. How it is to be done?
3. What levels it will be done?
4. What resources will be required?
5. How it be monitored and reviewed?

METHODOLOGY

The purpose of this study was to see the challenges faced by the primary teachers in using mother tongue as a language of instruction in primary schools in three blocks of Samba district of J&K as proposed by National Education Policy 2020. The research was carried out in three blocks viz. Samba, Vijaypur and Purmandal. The participants were 40 teachers from primary schools who were chosen at random from three blocks. The investigation was conducted using a survey method. Data was collected from teachers using a questionnaire and was analyzed using qualitative methods.

Variables of the study

Dependent Variable: Use of mother tongue

Independent Variables: Teachers' capacity building and training, Instructional material in Mother tongue, Support of parents and school management.

DISCUSSION OF THE RESULTS

Table 1 show that the majority of teachers suggested the following

- teachers and head teachers should be provided with training and capacity building opportunities ;
- parents should be counseled and educated to promote their child's early education in mother tongue ;
- Instructional material and text books need to be developed in regional languages; and
- school curriculum needs revamping

Solutions Proposed by the Teachers

The questionnaire on probable solutions towards effective implementation of use of mother tongue as the pedagogical strategy was administered on the respondents in second stage. The below mentioned table shows the results:

Primary school teachers are aware of the advantages of using one's mother tongue as a medium of instruction. Many schools, however, feel conflicted about adopting mother language as a medium of education due to the following factors:

- The teachers lack in the training in using mother tongue effectively in the classrooms.
- There is lack of text books and instructional material in native and regional languages. So need is to develop the transactional material and teaching aids in the native language/s.

- Lack of requisite funding for developing instructional materials is also one of the main causes for the lack of culturally relevant resources.
- Some school principals are also reluctant to use mother tongue because: Parental pressure; they believe it wastes time; they believe English language as status symbol.
- Another reason why head teachers oppose the use of mother tongue is that it will undermine parents' faith in the administration's abilities, and parents will be less likely to send their children to school if mother tongue is used.
- When employing mother tongue as a medium of education, teachers encounter numerous problems. Parents have negative views against using mother tongue as a language of teaching; there is a dearth of culturally relevant materials; teachers have difficulty translating some concepts into mother tongue; and there is a lack of reference materials, to name a few. Teachers suggested that in order to overcome the obstacles, they need more opportunities for mother tongue training; parents should be educated about the benefits of mother tongue; adequate culturally relevant materials should be available; and mother tongue should be included in the school curriculum.
- The majority of parents oppose the use of mother language as a medium of instruction because they fear that learning will be hindered and that it may result in poor academic performance. Other factors contributing to a lack of parental support include fears that their children would drop out of school and parents' satisfaction with their children being taught in English.

RECOMMENDATIONS AND CONCLUSION

All the stakeholders should focus on the effective implementation of usage of mother tongue at primary level. The researcher has suggested the planning in phased manner as follows:

Phase I	**Phase II**		**Phase III**	**Phase IV**
Need Assessment Phase: Region wise area mapping and area scouting should be done for the need assessment	Capacity Building of Inservice Teachers	Training of Pre-service teachers	(i)Guidance and Counselling workshops for parents	Implementation in schools
	(i)Reframing text books with local flavour (ii)Culturally relevant instructional material		(ii)Sensitization of parents and community regarding advantages of using mother tongue towards better learning	Feedback

Institutional mechanism has to be developed in terms of establishment of advisory bodies to specific local and regional needs, resources and nature of inputs to be offered under domain and active engagement of the existing institution mechanism, CRC, BRCs, DIETs, SCERTs, and NCERT in to the process of stream-line teachers education.

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Table 1: Challenges Perceived by Teachers

Items	f	%	Items	f	%
Children from different socio –economic backgrounds	19	**47.5**	Children from different Linguistic backgrounds	30	**75**
complicatedness in translation of certain concepts into mother tongue	11	**27.5**	Fear of less enrolment due to non usage of English	25	**62.5**
Examinations not set in mother tongue	25	**62.5**	Lack of relevant books	27	**67.5**
Lack of culturally relevant materials	21	**52.5**	Difficulty in adoption of policy in totality	11	**27.5**
Parents' negative attitudes towards mother tongue	26	**65**	Lack of interest in children	18	**45**
Need of training	20	**50**	Boosting self confidence	24	**60**
No allotment of time for Mother tongue	13	**32.5**	Increase in scores	19	**47.5**
Difficulty in articulation	22	**55**	Better learning outcomes	13	**32.5**
Change in accent	19	**47.5**	negative attitude of management towards using mother tongue	16	**40**
Lack of support by state and centre	10	**25**	Difficulty in understanding concepts	9	**22.5**
Negative attitudes by colleagues	10	**25**	Lack of capacity building	11	**27.5**

Table 2: Solutions Proposed by the Teachers towards Challenges in Using Mother Tongue as a Language of Instruction

Items	f	%
Implementation of the policy in true spirit	34	**85**
Capacity building and training for teachers and heads	32	**80**
Sensitization of the parents towards the long term benefits	38	**95**
Reframing culturally relevant materials	38	**95**
Exam in mother tongue	24	**60**
More research should be done in the area	30	**75**
Inclusion of mother tongue in the school curriculum	31	**77.5**
Provision of reference materials	34	**85**
Training of teachers	33	**82.5**
More capacity building trainings	34	**85**
Revamping Curriculum	31	**77.5**

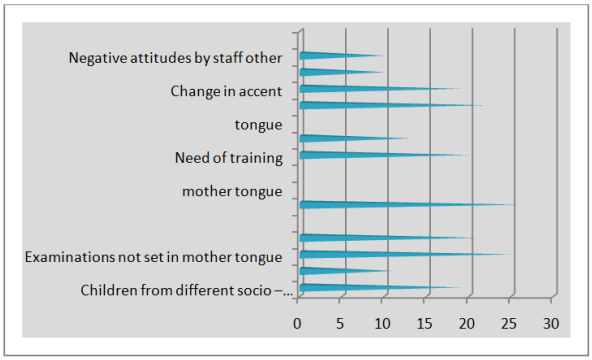

Figure 1: Showing Challenges Perceived by Teachers

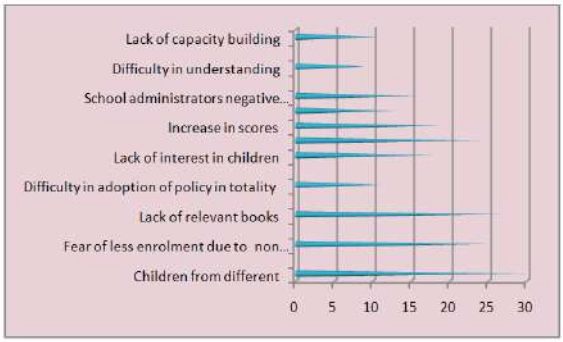

Figure 2: Showing Challenges Perceived by Teachers Using Mother Tongue as a Language of Instruction

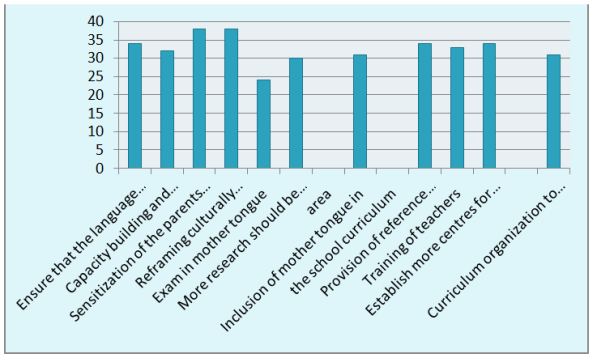

Figure 3: Showing proposed solutions towards effective implementation of use of mother tongue as medium of instruction

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REVIEW ARTICLE

A Review on Clinical Trails

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ABSTRACT

A clinical test can be a studies have a look at in human volunteers to reply particular fitness questions. Carefully carried out medical trials are quickest and most secure way to locate remedy that employment in human beings and way to enhance health. Investigational trials decide whether or not experimental or new methods of the usage of recognized remedies are secure and powerful under managed environment. Observational trials deal with fitness problems in massive businesses of human beings or populace in herbal settings. Developers of medicine , biologicals, and clinical gadgets have to make certain product protection, exhibit clinical advantage in human beings, and mass produce the merchandise . Preclinical improvement starts earlier than medical trials and consequently the principle desires are to exercise session protection and effectiveness of the intervention. If preclinical research display that the remedy is secure and powerful, medical trials are started. Clinical trial levels are steps inside the studies to exercise session if an intervention might be useful or adverse to people and encompass Phases 0, I, II, III, IV, and V clinical studies.

Keywords: Clinical Trials, Preclinical Studies, Clinical studies, Clinical Phase Trials, regulatory agencies.

INTRODUCTION [1-20]

A medical take a look at can be a studies examine that assessments a substitute clinical remedy or a substitute manner of the usage of an present remedy to envision if it is going to be a miles higher way to save you and display screen for diagnose or deal with a disease. For any new drug to go into in medical take a look at, it ought to by skip preclinical research. Preclinical research contain in vitro (i.e. take a look at-tube or Laboratory) research and trials on animal populations. Wide variety of dosages of the examine drug is given to animal topics or to an in-vitro substrate

soon get initial efficacy, toxicity and pharmacokinetic facts; Developers of drugs, biologicals, and clinical gadgets ought to make sure product protection, reveal clinical advantage in people, and mass produce the merchandise . Preclinical improvement begins off evolved earlier than medical trials and consequently the primary dreams are to exercise session protection and effectiveness of the intervention. Research might also additionally encompass pharmacodynamics, pharmacokinetics, absorption, distribution, metabolism and excretion research, and toxicity checking out. During preclinical research, in vitro and in vivo checking out is performed. Toxicity consists of research of which organs are centered and long-time period carcinogenic outcomes or outcomes on mammalian reproduction .Two species of animals are usually applied in drug improvement research. Choice is determined on which animal offers the handiest correlation to human research. Medical gadgets are typically studied in large animal species. No Observed Adverse Effect Level (NOAEL), the volume of publicity at which there may be no biologically or statistically sizable growth in the frequency or severity of any negative outcomes with inside the uncovered populace in contrast to its suitable control, is mounted supported preclinical trials. These are wont to decide preliminary phasel clinical trial medical trial dosage tiers on a mass active pharmaceutical ingredient (API) in line with mass affected person basis. If preclinical research display that the remedy is secure and effective, medical trials, described as A scientifically managed research of the safety and effectiveness of a healing agent the usage of consenting human subjects â€Â, are started.

The 4 feasible results are: 1) the brand new remedy functions a big useful impact and is advanced to standard remedy; 2) the brand new remedy is like trendy remedy 3) the brand new remedy is neither truely advanced nor truely inferior to standard remedy; or 4) a substitute remedy is inferior to standard remedy. The (FDA) function starts off evolved after preclinical assessment for protection and effectiveness. These potential research are designed to reply unique questions about biomedical or behavioral interventions and ought to adhere to the concepts of extraordinary medical practices (GCP) Classification of the trial might also additionally mirror how the researchers behave (observational as opposed to interventional medical trials), via way of means of their purpose (prevention, screening, diagnostic, remedy, first-class of life, or increased get right of entry to medical trials), or whether or not the trial layout lets in adjustments supported statistics amassed at some stage in the trial (constant as opposed to adaptive medical trials). Ten regions which can be cautiously assessed in those medical research are safety of human topics, sampling, diploma of masking, randomization, purpose to deal with analysis, choice of interventional and contrast groups, choice of quit points, interpretation of results, trial duration, and choice of conventional as opposed to equivalence checking out. Randomized managed trials (RCT) are the gold trendy and are regularly wont to assess the efficacy or effectiveness of assorted forms of clinical intervention and must offer facts approximately negative out comes efficacy or effectiveness of numerous kinds of clinical intervention and might offer facts approximately negative outcomes Classifications of RCT's encompass examine layout (parallel-group, crossover, cluster, or factorial), final results of interest (efficacy as opposed to effectiveness), and assessment of a hypothesis (superiority, non inferiority, or equivalence)The human beings being studied are randomly allotted to one of the one of a kind remedies which are below take a look at.

The best randomization method maximizes statistical power, minimizes choice bias, and minimizes allocation bias Clinical trial stages are steps within side the studies to decide if an intervention could be useful or adverse to people and encompass Phases 0, I, II, III, IV, and V medical research During Phase 0, pharmaco dynamics and pharmacokinetics are determined. Safety research are evaluated throughout Phase I, efficacy throughout Phase II, and affirmation of protection and efficacy throughout Phase III. Sentry research are executed in Phase IV and comparative effectiveness studies and community-primarily based totally studies in Phase V. Although this sounds smooth and straightforward, definitions and functions of the one of a kind stages end up muddied and research to decide if a remedy must be used within side the well known populace of sufferers can be complicated and consequences hard to interpret. Clinical trials may fit so incorrect that unplanned adjustments within side the populace studied, cease factors or evaluation plan have to be made Understanding the idea of medical trial stages will assist researchers plan and put into effect medical take a look at protocols and, via way of means of doing so, enhance the variety of healing procedures coming to marketplace for sufferers. Clinical drug improvement is a time-

eating and complicated method that takes round 6–15 years The price of growing a brand new drug, from studies and improvement to advertising and marketing approval, is about USD 2.6 billion Approximately 85% of healing procedures fail thru early medical improvement, and best 1/2 of of these accomplishing section three are permitted Over two-thirds of the overall price, in each cash and time, of the invention and improvement of a brand new drug is embedded with inside the medical-checking out section Patient recruitment is the unmarried largest reason of medical trial delays, and 30% of section three take a look at terminations are because of enrollment difficulty. Approximately 80% of trials fail to satisfy the preliminary enrollment goal and timeline .These delays can bring about as much as USD eight million in keeping with day in misplaced sales for pharmaceutical companies. Additionally, almost USD 6 billion yearly are spent on affected person recruitment. Moreover, best 2% of the eligible populace with inside the USA take part in medical trials, and people who do take part attend a mean of eleven visits on the trial web web page in 6 months Virtual medical trials (VCT) are a distinctly new and underutilized approach of accomplishing medical studies the use of technologies (apps, electronically tracking devices, etc.) and on line social engagement systems.

Making accurate diagnoses primarily based totally on snap shots and affected person symptomatology has continually been a part of the dermatologist's routine. The visible nature of dermatological conditions, the relative ease in comparing pores and skin illnesses virtually, and the truth that pores and skin illnesses regularly aren't lifethreatening and infrequently require complicated examinations make VCT very appealing for dermatological studies. Thus, VCT are in lots of methods made for dermatology. Herein we talk benefits and demanding situations of VCT and description the results of VCT for dermatological studies come the demanding situations confronted in traditional medical trials like many lengthy appointments throughout running hours. In traditional medical trials contributors are recruited thru sanatorium visits, clinical clinics, or the use of media which include newspaper/radio/tv ads. Moreover, the goal populations are regularly restricted via way of means of their geography. In VCT recruitment is centered without delay to the affected person via way of means of web-primarily based totally systems (e.g., Google seek engine) and social media (e.g., Facebook, Instagram), without geographical limitation, accomplishing ability eligible sufferers worldwide. Patients can signal up, upload extra records, and solution questionnaires approximately demographics, ailment history, and geographical vicinity on particular websites. To fulfil the inclusion standards and to verify the prognosis a few on line recruitment systems require photograph add of goal lesions, i.e., images of frame components affected, for example, via way of means of acne, atopic dermatitis, or psoriasis.

This type of recruitment initiative may be very attractive as 80% of net customers are searching for healthcare records and inside eczema on my own there are 4,343,000 searches/month (searches on Google, USA, November 2018). Furthermore, jogging on line campaigns in comparison to newspaper/radio/tv ads, allows: flexibility as possible flip a marketing campaign on or off at a moment's notice, right monitoring in location can in particular goal real leads (i.e., atopic dermatitis searches best), and it could be price green with a decrease price in keeping with affected person than conventional media. Informed consent is given remotely if allowed via way of means of the national/ country moral evaluation board An on line questionnaire can check the contributors' know-how of the knowledgeable consent. In addition to the web records the contributors have the possibility to invite questions and talk applicable subjects with the investigator thru a telecell smartphone or on line name earlier than giving the consent Furthermore, a restricted variety of take a look at webweb sites are worried in VCT. There is regularly best a unmarried webweb page, or one webweb page in every u.s .in worldwide research, led via way of means of a most important investigator whose crew evaluation all of the facts as they're suggested in actual time to reveal the fitness and protection of the contributors. Studies are controlled centrally via way of means of a far off take a look at coordination middle facilitating all studies activities. This are different from conventional clinical trials with many study teams and sights teams which contribute to the increased expense. VCT are always allow data to collection from the multiple sources of reporters, e.g., mobile devices, apps, watch, electronic patient-reported outcomes, and e-diaries this is often in contrast to plain clinical trials where the info collection is formed by the study team.

PHASE 21,22,23,24,25,26,27

Phase zero is a current designation for exploratory, first-inhuman trials performed according with the (U.S.) United states and (FDA)Food and drug administration in 2006 Guidance on Exploratory. Investigational New Drug (IND) Studies Phase zero trials are designed to hurry up the improvement of promising tablets or imaging retailers via way of means of organizing very early on whether or not the drug or agent behaves in human topics as become predicted from preclinical research. Distinctive capabilities of Phase zero trials encompass the management of unmarried sub healing doses of the observe drug to a small wide variety of topics (10 to 15) to acquire initial information at the agent's pharmacokinetics (how the frame tactics the drug) and pharmacodynamics (how the drug works withinside the frame). In September 2003, the National Institutes of Health (NIH) introduced a sequence of tasks to cope with the developing disaster in shifting new fundamental technological know-how discoveries to the marketplace wherein they're to be had for affected person use. One of the goals become strengthening scientific studies infrastructure. This become observed via way of means of an FDA document issued in March 2004 reading the "Challenge and Opportunity at the Critical Path to New Medical Products" US Pharmaceutical R & D Spending and the NIH Budget had elevated dramatically among 1993 and 2003, however foremost drug and organic product submissions to the FDA decreased. Investment required for one a hit drug release elevated from $1.1B in 1995-2000 to $1.7B in 2000-2002. The essential direction, which starts whilst candidate merchandise are decided on for improvement, become challenging, inefficient, and costly. Clinical failure blanketed protection issues and shortage of effectiveness.

The challenge become stagnation and declining innovation with a widening hole among know-how and scientific use. A drug coming into Phase I trials in 2000 become now no longer much more likely to return back to marketplace than one coming into Phase I trials in 1985 Improvement in prediction of failure throughout early scientific trials saves in improvement prices and time to marketplace The idea of exploratory research new drug (IND) research become a end result of this FDA evaluation and might assist with figuring out whether or not a described mechanism of motion also can be located in humans, offer records on pharmacokinetics, pick promising merchandise from a set of candidates, and examine biodistribution. The reason of those research is to assist withinside the pass as opposed to no-pass choice making technique of a drug's destiny early withinside the improvement technique the use of human fashions as opposed to counting on animal information. Exploratory IND research (additionally referred to as Phase zero research) are performed early in scientific segment research and contain confined human publicity and haven't any healing or diagnostic intent. Doses are subtherapeutic and sufferers are monitored via way of means of the scientific researcher and contain approximately 10 observe sufferers. Duration of a affected person's participation is normally much less than 1 week. Phamacodynamics and pharmacokinetics are studied.

These trials are earlier than the conventional dose escalation, protection, and tolerance research, do now no longer update the Phase I scientific trials and do now no longer suggest whether or not a remedy has a tremendous effect at the centered pathology. These research assist in getting rid of candidate treatments earlier than they attain Phase I research These trials had been evolved to shorten the essential direction for drug improvement, to discover pharmacokinetic and pharmacodynamic profiles of IND's in humans, to assist in accelerating identity of promising tablets, and to lessen improvement time and prices. Limitations of those trials encompass loss of healing intent, motivation of sufferers to participate, can also additionally postpone or exclude sufferers from different scientific trials which could have healing intent, microdosing pharmacokinetics and dating to healing dose, and availability of touchy analytical strategies Attrition fees are excessive and best approximately 8% come to marketplace.

PHASE 1 27-43

Phase I trials are the primary level of checking out in human topics. Normally, a small (20-80) organization of wholesome volunteers may be selected. This segment consists of trials designed to evaluate the protection (pharmacovigilance), tolerability, pharmacokinetics, and pharmacodynamics of a drug. These trials are frequently carried out in an inpatient clinic, in which the situation may be located through full-time staff. The situation who gets the drug is generally located till numerous half-lives of the drug have passed. Phase I trials additionally commonly

consist of dose-ranging, additionally known as dose escalation, research in order that the proper dose for healing use may be found. The examined variety of doses will generally be a fragment of the dose that reasons damage in animal checking out. Phase I trials most usually consist of wholesome volunteers. However, there are a few instances whilst actual sufferers are used, together with sufferers who've end-level disorder and absence different remedy options. This exception to the guideline of thumb most usually takes place in oncology (cancer) and HIV drug trials. Volunteers are paid an inconvenience charge for his or her time spent within side the volunteer center. Pay levels from a small amount of cash for a quick length of residence, to a bigger quantity of as much as approx £4000 relying on duration of participation. Phase I research are regularly undertaken with regular wholesome men and on occasion with sufferers, e.g., oncology drugs. A Phase I scientific trial evaluates the first-class manner to manage a drug, its frequency and dose, the most tolerated dose (MTD), and facet results. Tolerability, pharmacokinetics, and pharmacodynamics are evaluated. These research decide, maximum importantly, if the remedy is safe.

Trials generally consist of 20 to one hundred sufferers and are monitored through the scientific researcher. Doses are elevated if there aren't yet any intense facet results and sufferers are examined to decide if she or he is responding to the therapy. These escalation dose research are used to decide the first-class and most secure dose that may be administered and is a fragment of the dose that prompted damage in the course of animal checking out. Unnecessary publicity of topics to sub therapeutic doses at the same time as keeping protection and speedy accrual is the number one purpose of Phase I trials Subjects, in maximum cases, are wholesome volunteers despite the fact that sufferers with a sure disorder can be required. Contract studies businesses generally behavior those research and stipends can be given. Testing is generally sequential with facts being reviewed after each affected person or small organization of sufferers. Dose-toxicity and dose-efficacy curves are decided in the course of this segment and consist of unmarried ascending dose trials (Phase IA), a couple of ascending dose trials (Phase IB), and meals impact research. Dose escalation strategies can be rule-primarily based totally or model-primarily based totally. Rule-primarily based totally designs do now no longer stipulate any previous assumption of the dose-toxicity curve and permit escalation and de-escalation of the dose with diminishing fractions of the previous dose relying on presence or absence of toxicity. They are smooth to enforce and do now no longer require unique software. The conventional three + three layout proceeds with cohorts of three sufferers. The beginning dose is primarily based totally on extrapolation from animal toxicological facts.

Increasing dose stages were constant earlier and generally comply with a changed Fibonacci collection wherein the dosing increments turn out to be smaller because the dose will increase If not one of the sufferers revel in a dose-restricting toxicity, three greater sufferers may be dealt with at the following better dose. If 1 of the sufferers reviews a dose-restricting toxicity, the equal dose is repeated in three greater sufferers. Dose escalation maintains till at rent 2 sufferers from a cohort of three to six revel in dose-restricting toxicities. Recommended dose for Phase II trials is described because the dose stage simply beneath the poisonous dose stage. Alternate rule-primarily based totally dose escalation strategies consist of the “2 + 4,” “3 + 3 + 3,” and “3 + 1 + 1” (“first-class of five”) rule study, a 3rd cohort of three sufferers is brought if 2 of 6 sufferers within side the first 2 cohorts have a dose-restricting toxicity. If at the least three of nine sufferers revel in a dose-restricting toxicity, the have a look at is terminated. The “first-class of 5” layout calls within for that 1 extra affected person is brought if 1 or 2 dose-restricting toxicities will located within side the first three sufferers. Another affected person is brought if 2 dose-restricting toxicities are visible a number of the four dealt with sufferers. Escalation is sustained if no dose-restricting toxicities are visible of 3, 1 of 4, or 2 of 5 sufferers. If 3 or greater dose-restricting toxicities are visible, the trial is stopped.

Accelerated titration designs integrate versions of the 3 + 3 layout and the version-primarily based totally layout. Patient task to doses is primarily based totally on pre exact rules. Pharmacologically guided dose escalation is a variant of the 3 + 3 layout technique. This assumes that animal version research correctly replicate dose-restricting toxicities primarily based totally on plasma drug concentrations. In the primary stage, plasma publicity is extrapolated from preclinical statistics. Pharmacokinetic statistics are then acquired for every affected person to decide next dosing Theisotonic regression version assumes toxicity is non reducing with dose and suits an isotonic

regression to accrued statistics. The dose given is that with predicted toxicity idea closest to the most tolerable toxicity The "rolling six layout" lets in for accrual of two to six sufferers simultaneously onto a dose degree primarily based totally at the variety of sufferers enrolled and evaluable, the variety having dose-restricting toxicity (DLT), and the variety nonetheless liable to growing DLT This layout is meant to shorten the take a look at period wherein there may be previous statistics approximately the dose variety and is beneficial in pediatric populations. The "biased coin up-and-down layout" calls for that the remedy reaction or the toxicity assessment is discovered quickly, allocates a dose to every affected person primarily based totally at the toxicity statistics of the final finished situation and lets in more than one sufferers to be simultaneously studied Another rule-primarily based totally layout lets in next sufferers to be assigned to doses consistent with the toxicity consequences on the contemporary dose via way of means of calculating the toxicity possibility c language below the beta-binomial version Model-primarily based totally designs use statistical fashions that search for a dose degree that produces a possibility of dose- restricting toxicity via way of means of the use of toxicity statistics from all enrolled sufferers to compute a greater specific dose-toxicity curve. Bayesian fashions are generally used.

These fashions require an estimation of θ (characterizes the form of the dose-toxicity curve.) Occurrence of toxicity outcomes in adjustment of θ primarily based totally on Bayes' theorem. These designs offer a self assurance language for encouraged doses for Phase II scientific trials. The persistent reassessment technique turned into the primary Bayesian version-primarily based totally technique utilized in Phase I scientific trial designs The preliminary estimate of θ is sought from specialists acquainted with the preclinical statistics or who've enjoy with comparable drugs. Patients are handled on the dose idea to be closest to the MTD and estimation of the possibility of a dose-restricting toxicity is calculated for every new affected person who enters the take a look at till a prespecified situation is met at which period the trial is stopped. Modifications of this technique have protected treating the affected person at the bottom began out dose degree, growing the dose via way of means of most effective one pre specified degree at a time, now no longer permitting dose escalation for the instant subsequent affected person if a preceding affected person skilled a dose-restricting toxicity, treating numerous sufferers on the identical dose degree and increasing the cohort of sufferers Escalation with overdose manipulate turned into recommended as an opportunity Bayesian method to conquer the challenge of sufferers being uncovered to excessive poisonous doses Other version-primarily based totally designs encompass time-to-occasion endpoint and the efficacy and toxicity strategies. These version-primarily based totally strategies bring about true estimations of the goal possibility of DLT on the encouraged dose for Phase II scientific trials without treating too many sufferers at a suboptimal dose.

Dose-escalation techniques for trials of mixtures of dealers have protected change escalation of the dealers withinside the collection of dose levels, simultaneous escalation of each dealers, escalation of 1 agent to the encouraged dose for Phase II trials whilst conserving the alternative agent at a set dose, and escalation of 1 agent to the encouraged dose for Phase II trials whilst conserving the alternative agent at a low dose. In a review and associates decided that 88% of the rigors had a conventional or changed three + three dose escalation layout used. The calculated median DLT charge turned into 6%. The authors encouraged that the beginning doses, dose levels, and dose-escalation steps should make certain affected person safety, deal with as few sufferers as viable at sub healing doses, and become aware of greatest drug mixtures for in addition assessment No particular scientific trial designs had been formulated for molecularly centered dealers which have a tested applicable goal and a confirmed technique for measuring goal inhibition.

HERE ARE DIFFERENT KINDS 0F PHASE I TRAILS

SAD

Single Ascending Dose research are the ones wherein small agencies of topics are given a unmarried dose of the drug even as they're discovered and examined for a duration of time. If they do now no longer showcase any unfavorable aspect outcomes, and the pharmacokinetic information is more or less in step with expected secure values, the dose is escalated, and a brand new organization of topics is then given a better dose. This is sustained till pre calculated

pharmacokinetic protection tiers are reached, or insupportable aspect outcomes begin displaying up at which factor the drug is stated to have reached the Maximum tolerated dose (MTD).

MAD

Multi Ascending Dose research are carried out to higher apprehend the pharmacokinetics & pharmacodynamics of more than one doses of the drug with complaince.

PHASE 244-50

Once the preliminary protection of the look at drug has been showed in Phase I trials, Phase II trials are finished on large groups (20-300) and are designed to evaluate how properly the drug works, in addition to maintain Phase I protection checks in a bigger organization of volunteers and sufferers. When the improvement system for a brand new drug fails, this generally happens at some point of Phase II trials whilst the drug is determined now no longer to paintings as deliberate, or to have poisonous effects.

Phase II research are once in a while divided into Phase IIA and Phase IIB. Phase IIA is specially designed to evaluate dosing requirements (how a whole lot drug have to be given), while Phase IIB is specially designed to look at efficacy (how properly the drug works on the prescribed dose(s)). Some trials integrate Phase I and Phase II, and check each efficacy and toxicity. Phase I /II dose locating research decide the maximum a hit dose (MSD) that is the dose which maximizes the made from the chance of seeing no toxicity collectively with the chance of seeing a healing response. While a Phase I scientific look at makes a speciality of figuring out the MTD, Phase II research examine capability efficacy andcharacterizes remedy advantage for the ailment in a powerful manner. The intervention isn't presumed to have any healing impact whatsoever. These research are finished on large groups (one hundred to three hundred subjects) and are designed to evaluate how properly the drug works and to maintain protection checks. Therapeutic doses which have been decided at some point of Phase I are administered and sufferers are monitored via way of means of the scientific researcher. Trials are frequently carried out in a multi-group setting. Phase II can be divided into Phase IIA that are pilot scientific trials to assess efficacy and protection in decided on populations with the ailment or situation to be treated, identified or prevented (targets can be on dose-response, form of affected person, frequency of dosing, or different identifiers of protection and efficacy) and Phase IIB that are the maximum rigorous trials designed to illustrate efficacy.

The improvement system generally fails at some point of this Phase II whilst the drug is determined now no longer to paintings as deliberate or to have poisonous effects. The Phase II layout relies upon at the pleasant and adequacy of Phase I research. A susceptible factor of each levels is the form of affected person enrolled. Patients in Phase II trials normally have greater exclusion standards than the ones in Phase III trials. Case collection and randomized scientific trial designs had been used. Single degree and multi-degree Phase II scientific trial designs are frequently advanced on the idea that one endpoint is of interest. A normally used Phase II layout is primarily based totally at the paintings of Gehan, a model of a two-degree layout Other designs have greater levels or a sequential factor. Hybrid designs had been used to enhance efficiency. In an update, Gehan reviewed statistical components of plans for Phase II most cancers scientific trials which include a minimal quantity of sufferers plan, a two-degree selection idea approach, a confined affected person accrual plan, a predictive chance plan, and a one-pattern a couple of trying out manner plan. The writer makes pointers concerning the plan that nice suits the wishes of the look at Adaptive scientific trial designs primarily based totally on gathered facts at intervening time have additionally been utilized in Phase II scientific trials due to flexibility and efficiency.

This layout can also additionally permit the researcher to adjust or redecorate the trial even as the look at is ongoing. However, researchers have hesitated of their use-there may be confusion with appreciate to definition, controversy concerning pattern length re-estimation techniques, and logistical boundaries in the usage of adaptive designs inside current trial frameworks In 2010, The FDA categorized adaptive designs into “properly under- stood” and “much less properly understood” categories“ Well understood” designs had been in use for years with corresponding

statistical techniques which have been properly hooked up and the FDA is acquainted with the look at designs thru the assessment of submissions the usage of them. In the "much less properly understood" designs, relative deserves and obstacles have now no longer been absolutely evaluated, legitimate statistical techniques have now no longer been advanced, and the FDA does now no longer have lots of enjoy with submissions the usage of the look at designs. Chow et al. deliver a broader definition of adaptive layout-one which permits diversifications in trial strategies and/or statistical strategies after initiation of the trial without undermining the validity and integrity of the trial. Adaptive scientific trial designs encompass an adaptive randomization layout, an adaptive organization sequential layout, a bendy pattern length re-estimation layout, a drop-the-losers layout, an adaptive dose-locating layout, a biomarker-adaptive layout, an adaptive remedy- switching layout, an adaptive-speculation layout, a Phase I/II or II/III adaptive seamless trial layout and a couple of adaptive layout.

PHASE 3 49,51,52,53,54,47

Phase III research are randomized managed multicenter trials on huge affected person groups (300–3,000 or extra relying upon the disease/scientific circumstance studied) and are aimed toward being the definitive evaluation of the way powerful the drug is, in evaluation with current 'gold wellknown' remedy. Because in their length and relatively lengthy duration, Phase III trials are the maximum highly-priced, time-eating and hard trials to layout and run, mainly in treatment plans for continual medicalconditions. It is not unusualplace exercise that positive Phase III trials will retain even as the regulatory submission is pending at the suitable regulatory agency.

While now no longer required in all cases, it's far usually predicted that there be at the least a hit Phase III trials, demonstrating a drug's protection and efficacy, that allows you to achieve approval from the suitable regulatory agencies (FDA (USA), TGA (Australia), EMEA (European Union), etc.). Once a drug has proved first-rate after Phase III trials, the trial consequences are typically blended right into a huge record containing a complete description of the techniques and consequences of human and animal research, production procedures, system details, and shelf life. This series of statistics makes up the "regulatory submission" this is supplied for overview to the suitable regulatory authorities in one-of-a-kind countries.

Most capsules present process Phase III scientific trials may be advertised beneathneath FDA norms with right guidelines and guidelines, however in case of any destructive results being pronounced anywhere, the medicine want to be recalled right away from the market. While maximum pharmaceutical businesses chorus from this exercise, it isn't unusual to peer many capsules present process Phase III scientific trials withinside the market. Phase III trials are the entire scale assessment of remedy and are designed to examine efficacy of the brand new remedy with the usual remedy. These are the maximum rigorous and vast form of medical scientific research of a brand new remedy. This is the "pre-advertising phase" of scientific trials. These are typically the maximum highly-priced and time-eating of the trials. The trials can be hard to layout and run. Large groups (one hundred to 3000 subjects) are recruited and trial designs have blanketed randomized managed trials (parallel layout), out of control trials (unmarried remedy), historic controls, no-randomized concurrent trials, factorial designs, and institution sequential designs.

Patients are monitored with the aid of using the scientific researcher and private physician. Phase III scientific trials can be divided into Phase IIIA that are trials finished after efficacy of the remedy is verified however earlier than regulatory submission of a New Drug Application (NDA) or different file and Phase IIIB that are performed after submission of an NDA or different file however earlier than approval and launch. During the 1980's, the FDA posted steering files that efficacy ought to be verified with the aid of using prolonga- tion of life, advanced health-associated exceptional of life, or a longtime surrogate for certainly considered one among those. If the brand new remedy consequences in a statistically giant improvement, the brand new remedy is typically permitted for scientific use. Traditional endpoints for trials have blanketed normal survival, time to tumor progression, normal reaction fee, time to remedy failure and affected person-pronounced outcomes. Overall survival has been the gold wellknown for the demonstration of scientific benefits. Subpart H permits for extended approval of medicine for extreme and life-

threatening sicknesses in which the drug demonstrates a bonus over to be had remedy. This is primarily based totally on a surrogate endpoint that probably predicts scientific advantage. While randomized Phase III scientific trials were the gold well-known proof for the approval of latest capsules, issues related to drug improvement have blanketed restricted scientific advantage in huge RCT's, prediction of a a hit Phase III trial from Phase II statistics, dedication of toxicity, layout of research with drug combinations, and price of the trial. Ocana and co-workers advise that adaptive designs in decided on prescreened populations should lessen the limitations. Statistical techniques for the layout and evaluation of adaptive designs started withinside the 1990's However, the various designs aren't well known and relate simplest to the utility being considered. The revel in of sponsors and regulators in planning, undertaking and deciphering consequences the usage of those designs is restricted and interplay with regulating government early is crucial. In Europe, the European Medicines Agency gives builders of medicine and healing gadgets medical recommendation and protocol help In 2010, the FDA posted steering on adaptive layout scientific trials Evaluators of adaptive scientific trial research solution the subsequent 6 questions: 1) Is there an awesome purpose and feature opportunity designs been considered? 2) Does the inspiration suit nicely withinside the context of the improvement application and the statistics with a view to be to be had for the advertising authorization utility? 3) Can the inspiration be carried out without critical harm to trial integrity? 4) Is the sort I mistakes fee managed? 5) Has the capacity bias of remedy impact estimates been evaluated? 6) Is the inspiration realistic and viable These questions also are requested of different designs.

The European Organization for Research and Treatment of Cancer apprehend that those designs may be advantageous, however warn that they have to save you bias that would be uncontrollable. Recommended strategies encompass randomization, blinding, prospectively deliberate diversifications and in advance implementation of the technique and firewalls had to make sure restrained get right of entry to meantime evaluation consequences and blinding of body of workers worried in every day trial proceedingsPhase III research are randomized managed multicenter trials on huge affected person groups (300–3,000 or extra relying upon the disease/scientific circumstance studied) and are aimed toward being the definitive evaluation of the way powerful the drug is, in evaluation with current 'gold wellknown' remedy. Because in their length and relatively lengthy duration, Phase III trials are the maximum highly-priced, time-eating and hard trials to layout and run, mainly in treatment plans for continual medical conditions. It is not usualplace exercise that positive Phase III trials will retain even as the regulatory submission is pending at the suitable regulatory agency.

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PHASE 455,56,57,53,58,59,60,61,62,65

Phase IV trial is likewise called Post Marketing Surveillance Trial. Phase IV trials contain the protection surveillance (pharmaco vigilance) and ongoing technical aid of a drug after it gets permission to be sold. Phase IV research can be required via way of means of regulatory government or can be undertaken via way of means of the sponsoring business enterprise for competitive (locating a brand new marketplace for the drug) or different reasons (for example, the drug won't were examined for interactions with different tablets, or on positive populace organizations which includes pregnant women, who're not going to problem themselves to trials).

The protection surveillance is designed to locate any uncommon or long-time period damaging outcomes over a far large affected person populace and longer term than changed into feasible in the course of the Phase I-III scientific trials. Harmful outcomes observed via way of means of Phase IV trials might also additionally bring about a drug being now no longer sold, or restrained to positive uses: current examples contain cerivastatin (emblem names Baycol and Lipobay), troglitazone (Rezulin) and rofecoxib (Vioxx) Upon authorization via way of means of the FDA, treatments decided to have verified protection, efficacy and first-rate can be made to be had to the overall populace. Patients and their physicians have expectancies of benefit. However, now no longer all protection or efficacy troubles were decided. The FDA calls for persevered assessment after launch to assess protection symptoms and symptoms

which can have an effect on the benefit-threat ratio. These Phase IV research encompass "all research (apart from recurring surveillance) done after drug approval and associated with the accredited indication" These are post-advertising and marketing surveillance research. The cognizance of the rigors is on how tablets paintings withinside the actual world. Anyone searching for remedy from their medical doctor can be dealt with the remedy. Their private medical doctor video display units the consequences of remedy. Efficacy and detection of uncommon or long-time period damaging outcomes over a far large affected person populace and longer term are evaluated, healthcare charges and results are decided, and pharmacogenetics are studied.

New scientific warning signs for a drug can be mounted and big wide variety of sufferers and physicians are worried The FDA might also additionally require that a developer behavior in a Phase IV trial as a stipulation on drug approval. Less than 1/2 of of research are finished or maybe initiated via way of means of developers Phase IV trials might also additionally bring about a drug being eliminated from the marketplace or restrained to positive warning signs. Initially, those trials had been run just like Phase III research and had been carried out for advertising and marketing purposes. Studies had been achieved at establishments with investigators acquainted with scientific trials and had inclusion and exclusion standards much like the ones of Phase III research. Results did now no longer mirror what might take place beneathneath regular conditions. As a result, revolutionary research had been designed to contain everyday physicians in naive studies communities. Goals were broadened and encompass assessment of particular pharmacological outcomes, organizing the occurrence of damaging reactions, figuring out outcomes of long-time period management of a remedy, organizing a brand new scientific indication for the remedy, assessment of the remedy in better threat populations, etc.

A major difficulty of situation is the combine of scientific studies and scientific exercise Reported critical damaging drug reactions submitted to the FDA's Med Watch application have accelerated from 150,000 in 2000 to 370,000 in 2009 Physician and clients or drug producers publish those reports. Criticisms have covered reliance on voluntary reporting of damaging events, agree with in drug producers to collect/evaluate/file drug protection facts which can threat economic interests, and dependence on one authorities frame to approve a drug after which require research that would result in withdrawal from the marketplace Proffered answers have covered big-scale easy RCTs with few eligibility and remedy standards preplanned me taanalyses of a sequence of associated trialsand established order of a country wide fitness facts community to assess post-advertising and marketing surveillance unbiased of the FDA-approval process.

PHASE 545

This translational studies is designed to "flow from bench to bedside". Phase V scientific trials talk over with comparative effectiveness studies and community-primarily based totally studies. Research is carried out on information collected. All suggested makes use of are evaluated. Patients aren't monitored. Its predominant consciousness is to decide integration of a brand new remedy into huge unfold scientific practice. Filed under: cornell cooperative extension, evidence-primarily based totally living, policy, the getting to know middle tagged with: cooperative extension programs, evaluation, evidence-primarily based totally programs, studies methods, studies translation.

TYPES OF CLINICAL TRIALS 55

1. Treatment trials

Test experimental treatments, new combinations of drugs, or new approaches to surgery or radiation therapy.

2. Prevention trials

Look for better ways to prevent disease in people who have never had the disease or to prevent a disease from returning. These approaches may include medicines, vitamins, vaccines, minerals, or lifestyle changes.

3. Diagnostic trials

Conducted to find better tests or procedures for diagnosing a particular disease or condition.

4. Screening trials Test the best way to detect certain diseases or health conditions.

5. Quality of Life
Trials (or Supportive Care trials) explore ways to improve comfort and the quality of life for individuals with a chronic illness

OVERVIEW OF CLINICAL DESIGN66-76

Clinical trials, of their purest form, are designed to study consequences of human topics under "experimental" situations managed via way of means of the scientist. This is contrasted to non interventional have a look at designs (ie, cohort and case-manipulate studies), wherein the investigator measures however does now no longer influence the publicity of interest. A scientific trial layout is regularly preferred as it allows randomization of the intervention, thereby successfully disposing of the choice bias that effects from the imbalance of unknown/immeasurable confounders. Within this inherent electricity is the potential to unveil causality in an RCT. Randomized managed trials, however, nevertheless stay difficulty to barriers along with misclassification or data bias of the final results or publicity, co-interventions (wherein one arm gets an extra intervention extra often than another), and contamination (wherein a share of topics assigned to the manipulate arm acquire the intervention outdoor of the have a look at). Execution of a sturdy scientific trial calls for the choice of the correct have a look at populace.

Despite all individuals voluntarily consenting for the intervention, the enrolled cohort can also additionally probably vary from the overall populace from which they had been drawn. This sort of choice bias, called "volunteer bias," can also additionally rise up from such elements as have a look at eligibility standards, inherent difficulty attributes (eg, geographic distance from the have a look at site, fitness status, attitudes and beliefs, education, and socioeconomic status), or subjective exclusion via way of means of the investigator due to bad predicted enrollee compliance or normal prognosis Although RCTs are trying to find to attain inner validity via way of means of enrolling a enormously homogeneous populace in line with predefined characteristics, slim inclusion and exclusion standards can also additionally restrict their outside validity (or "generalizability") to a broader populace of sufferers with rather conventional comorbidities that might not be protected withinside the pattern cohort. This subject underscores why an experimental remedy's "efficacy" (ie, a degree of the achievement of an intervention in an artificial setting) might not translate into its "effectiveness" (ie, a degree of its fee implemented withinside the "actual world").

Attempts to enhance affected person recruitment and generalizability the use of unfastened scientific care, financial payments and advanced conversation strategies are taken into consideration moral so long as the incentives aren't unduly coercive .In order to evaluate the efficacy of an intervention in the context of a scientific trial, there need to be planned manipulate of all recognized confounding variables (inclusive of comorbidities), thereby requiring a homogeneous institution of individuals. However, the proof furnished via way of means of a nicely-designed and done scientific trial will don't have any fee if it can't be implemented to the overall populace. Thus, designers of scientific trials need to use subjective judgment (inclusive of scientific, epidemiological, and biostatistical reasoning) to decide on the outset how tons trade-off they're inclined to make among the inner validity and generalizability of a scientific trial. A "surrogate endpoint" is regularly selected in location of a number one endpoint to beautify have a look at efficiency (ie, much less price and time, advanced measurability, and smaller pattern length requirement). Ideally, the surrogate must absolutely seize the impact of the intervention at the scientific endpoint, as officially proposed via way of means of Prentice.

Blood strain is a nicely set up surrogate for cardiovascular-associated mortality due to the fact its normalization has been related to clinically beneficial consequences, along with fewer strokes, and much less renal and cardiac complications. However, one need to use warning whilst counting on surrogates, as they will be erroneously implicated withinside the direct causal pathway among intervention and authentic final results. A often described, clinically logical, however fl awed use of a surrogate endpoint changed into untimely ventricular contractions (PVCs) to evaluate whether or not antiarrhythmic capsules decreased the prevalence of surprising loss of life after a myocardial infarction withinside the Cardiac Arrhythmia Suppression Trial (CAST). Despite proof of the affiliation

among PVC sand early arrhythmic mortality, pharmacologic suppression of PVCs suddenly elevated the very event (mortality) that it changed into imagined to remedy. As surrogates are typically hired in segment I–II trials, it's far rather probably that a excessive share of clinically powerful therapeutics are discarded due to false-terrible effects the use of such endpoints. This is exemplified withinside the trial via way of means of the International Chronic Granulomatous Disease (CGD) have a look at institution, wherein the surrogate markers of superoxide manufacturing and bactericidal efficiency had been to start with implemented to evaluate the efficacy of interferon-γ for remedy of CGD. For motives outdoor the scope of this review, the authors determined a priori to increase the have a look at length so as to thoroughly discover the scientific endpoint of interest (recurrent critical infections) in place of the firstly proposed surrogate markers (superoxide manufacturing and bactericidal efficiency).

Treatment with interferon-γ changed into tremendously successful, because the charge of recurrent critical infections changed into rather decreased. However, there has been no observable impact on superoxide manufacturing and bactericidal activity. Had the number one endpoint now no longer been changed, the firstly proposed surrogate biomarkers might have masked the clinically applicable efficacy of this remedyThese examples illustrate the significance of validating surrogates as dependable predictors of scientific endpoints the usage of meta-analyses and/or herbal records research of huge populace cohorts, along side making sure organic plausibility For an ordeal to effectively cope with the "number one question(s)" of interest, a sufficient pattern length is needed to have sufficient energy to come across a capacity statistical distinction. Traditionally, energy is defined as having at the least an 80% risk of finding a statistically significant distinction among the effects of two interventions whilst a clinically significant distinction exists. The effects or endpoints of the investigation, whether or not objective (eg, death) or subjective (eg, pleasant of life), need to usually be dependable and significant measures. Statistical analyses usually used to research effects consist of logistic regression for dichotomous endpoints (eg, occasion occurred/did now no longer occur), Poisson regression for rates (eg, range of activities according to person-years), Cox regression for time-to-activities (eg, survival analysis), and linear regression for non-stop measures (eg, weight).

OVERWIEW IN DRUG DEVELOPNMENT 76

The preferred avenue to drug improvement and approval has been defined and controlled via way of means of the United States Food and Drug Administration (FDA) for decades. Safety has traditionally been its number one focus, accompanied via way of means of efficacy. If a drug seems promising in pre-medical studies, a drug sponsor or sponsor-investigator can put up an investigational new drug (IND) application. This special suggestion incorporates investigator qualifications and all pre-medical drug records and data, and a request for exemption from the federal statutes that limit interstate delivery of unapproved drugs. After approval, the drug is studied (section I–III trials, defined below) and if established secure and efficacious within side the supposed populace, the drug sponsor can then put up a New Drug Application (NDA) to the FDA. After an intensive overview via way of means of the FDA that regularly includes a advice via way of means of an outside committee, the FDA determines whether or not the healing may be granted a demonstration and marketed. After final approval, the drug can remain studied in section IV trials, wherein protection and effectiveness for the indicated populace is monitored. To facilitate assessment and endorsement of overseas drug data, efforts were made to harmonize this approval manner throughout the United States, Europe, and Japan thru the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use in international conference of harmonization (ICH).

PRE-CLINICALTRAILS 79-85

Re-medical research contain in vitro (i.e., take a look at tube or laboratory) research and trials on animal populations. Wide ranging dosages of the examine drug are given to the animal topics or to an in-vitro substrate if you want to reap initial efficacy, toxicity and pharmacokinetic facts and to help pharmaceutical corporations in identifying whether or not it's far profitable to head beforehand with in addition trying out. Results of animal biomedical experiments have massively didn't translate into human medical trials which might be commonly attributed due to variations within side the underlying biology among human beings and animals to shortcomings withinside the experimental layout or to bias withinside the reporting of consequences from animal research Animal research were

commented broadly to be methodologically vulnerable which possibly keeps to offer the organic foundation for epidemiological research however large development is wanted in the way it needs to be performed and synthesized to enhance the predictability of animal research for the human condition. Ideas for implementation of consensus practices and results concerning medical translation aren't in reality understood which could offer greater comprehensive, transparent, proof primarily based totally and theoretically knowledgeable motive for evaluation of preclinical research With appreciate to medical research, it relies upon at the examine protocols which are designed after evidential facts generated from preclinical research. The software of this statistics to the human research is of predominant problem in which the paramount significance lies with the capacity to recognize the preclinical examine consequences earlier than arriving at any end To understand this scenario, there have been three hundred poor research posted in selectively recognized SCI listed Indian Journals out of which best 19 research had animal topics. Majority of animal poor research become located to be calculated with out the inclusion of energy and pattern length.

There are occurrences in which under-reporting of numerous parameters become noticeable. Criticism become strongly raised at the Ethics Committee which become the motive for the discount in pattern length Sample length is a extreme attention for animal research because the much less quantity of the animals might misread the importance withinside the examine and it might be wastage of assets along side moral problems to cope with markedly greater quantity of animals Regarding the wastage of assets, it become remarked with the aid of using Mandaletal. that animal trying out wastes time and assets with the aid of using deceptive researchers. Dr. Albert Sabin, who mounted Oral polio vaccine has said in his testimony that his paintings had a put off due to the "inaccurate concept of the character of the human ailment primarily based totally on deceptive experimental fashions of the ailment in monkeys". There is a different testimony with the aid of using Dr. Richard Klausner who has stated that "the records of most cancers studies has been a records of curing most cancers withinside the mouse. We have cured mice of most cancers for many years and it genuinely didn't paintings in human beings, we want to renowned the reality that use of animals will now no longer make us higher scientists, however sour scientists".

CLINICAL TRAILS OVER SIGHT 86-88

Historic abuses and cutting-edge day tragedies spotlight the significance of Institutional Review Boards (IRBs) and Data and Safety Monitoring Boards (DSMBs) in making sure that human studies conforms to neighborhood and countrywide requirements of protection and ethics Under the Department of Health and Human Services Title forty five Part forty six of the Code of Federal Regulations (CFR), IRBs are charged with shielding the rights and welfare of human topics worried in studies performed or supported with the aid of using any federal branch or agency. In order to make certain compliance with the stern and specified tips of the CFR, participants of IRBs (one in every of whom should be a non-scientist, and one in every of whom should be unbiased of the board's domestic institution) are legal beneathneath the "Common Rule" to approve, require modification to, or reject a studies activity. Based at the perceived chance of the study, IRBs have some of degrees of evaluation from exempt for "minimum chance" studies (defined with the aid of using the "Common Rule" as dangers which are no more thatones encountered in each day existence or ordinary scientific examinations or tests) to the greater prolonged and worried complete board critiques for higher-chance studies. General standards for IRB approval include:

1) dangers to topics are minimized, and are affordable with regards to benefits
2) choice of topics is equitable
3) knowledgeable consent is sought
4) sufficient provisions for statistics tracking exist to preserve topics' protection
5) good enough mechanisms are in location to make certain problem confidentiality And
6) rights and welfare of prone populations are protected.

GOOD CLINICAL PRACTICE 89

Clinical trial behavior is particularly inspired via way of means of a well-set up report known as Good Clinical Practice (GCP), a fixed of suggestions meant to standardise scientific trial behavior. It defines roles and obligations for trial staff, and protects the rights, protection and wellbeing of trial topics. The International Conference on Harmonization (ICH) affords the global general, primarily based totally at the Declaration of Helsinki, even though different corporations have evolved their personal comparable hints. The guiding principle affords a unified general for the EU, Japan and the USA, which assists the mutual reputation of scientific information via way of means of regulatory government in those jurisdictions. The thirteen middle standards of ICH GCP hints for scientific trials are:

1. Clinical trials must be carried out according with the moral standards of the Declaration of Helsinki, and steady with Good Clinical Practice and the ideal regulatory requirement(s).
2. A trial must best be carried out if the capability dangers and inconveniences are outweighed via way of means of the predicted gain for the trial challenge and society.
3. The rights, protection and wellbeing of trial topics are the maximum crucial issues and must succeed over the pastimes of technological know-how and society.
4. Non-scientific and scientific records approximately a brand new intervention (specifically an investigational medicinal product) must be used to justify the proposed trial.
5. A scientific trial must be scientifically sound, and defined in a clean and sufficiently particular protocol.
6. A proposed trial and its protocol need to have approval from an unbiased ethics committee. Researchers must comply with the protocol while accomplishing the trial.
7. Trial topics must be the duty of a certified clinician (or dentist), who will make choices approximately the scientific care.
8. All researchers concerned in accomplishing a tribulation must be certified via way of means of education, education and enjoy applicable to their tasks.
9. All human topics must provide knowledgeable consent earlier than they take part in a tribulation.
10. Clinical trial recorded to treated and saved in a manner that permits its to be corrected, reporting, interpretation and verification.
11. Data must be saved private and protected, specifically while it identifies a selected challenge. The guidelines that govern privateness and confidentiality must be followed, wherein required.
12. Investigational medicinal merchandise must be manufactured, treated and saved according with Good Manufacturing Practice and used as detailed withinside the trial protocol.

Thirteen. Systems for assuring the first-rate of the trial behavior and information must be in place. The standards of GCP can be carried out to any scientific studies research that could effect upon the protection and wellbeing of human topics.

ROLE OF PHARMACIST IN CLINICAL TRAILS 89

Pharmacists have an energetic position to play in studies and scientific trials first of all, we offer the important centers required for correct garage of the investigational medicinal products (IMPs), both within side the refrigerator or at managed room temperature. Regular temperature tracking is ensured and recorded. It is likewise the pharmacist's responsibility to make certain there may be consistent deliver of IMPs in any respect times, and that they're allotted to sufferers accordingly. Patients are counselled on the perfect use of the IMPs similarly to any written facts this is provided, consisting of, Informed Consent Form or the Patient Information Leaflet. IMPs returns from sufferers are counted and documented to decide compliance to the treatment. For inject capin a position IMPs, pharmacists can even make certain that they're organized according to the specs stipulated within side the trial, and that they're administered appropriately.

Besides coping with scientific trials, oncology pharmacists regularly run studies tasks which can be geared toward enhancing results in sufferers who obtain medicinal drugs, consisting of chemotherapy or different supportive

capsules like anti-emetics, blood increase thing injections, etc. Drug Utilization Evaluations (DUEs) are studies tasks which can be typically carried out through pharmacists. These tasks goal to facilitate rational use of medication inside our sufferers. Essentially, offering insights on how capsules are utilized in sufferers and looking at prescribing styles through our physicians. DUEs are once in a while taken into consideration as drug audits due to the fact pharmacists are making sure the use of drugs is appropriate. In addition, pharmacists additionally behavior observational surveys which can be geared toward investigating sufferers' or physicians' views and attitudes closer to medicinal drugs. Results acquired from surveys are used to enhance the offerings that we offer to our sufferers. Currently, NCC's oncology pharmacy is carrying out surveys. They are geared toward investigating sufferers' use of complementary and opportunity medicinal drugs and on sufferers' attitude on secure managing of oral anti-most cancers capsules. Very regularly, pharmacy college students who're thoroughly skilled to behavior studies are assigned to survey the sufferers. We would really like to take this possibility to thank all our sufferers who've consented to take part within side the survey.

CONCLUSION

A clinical trial for any new drug follows under the guidelines of ICH and GCP, clinical trial are conducted in human volunteers for confirmation of useful properties of new drug. After preclinical development, investigational new drug passes through clinical phases I, II, III and IV. By improving developmental strategies and studies, time to availability to the general public with resulting benefit should result in better patient outcomes and fewer morbidities.

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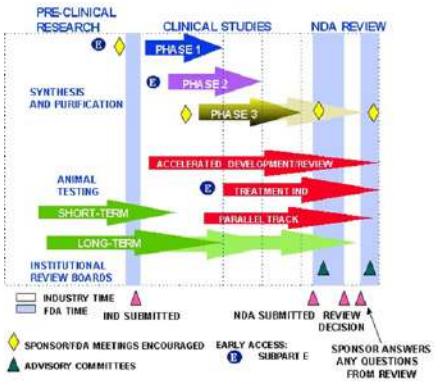

Fig.1 Classification

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RESEARCH ARTICLE

A Descriptive Study on FDC Drugs by Prescription Auditing in General Medicine Department of Tertiary Care Hospital using Anatomical Therapeutic Classification and Defined Daily Dose Concept

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ABSTRACT

Prescription audit is a systematic and critical analysis of a prescription to ensure the quality of medical care. Fixed Dose Drug Combinations are defined by WHO as a combination of two or more active ingredients in a fixed ratio of doses. A retrospective study was carried out to find out the rationality of the different fixed dose drug combinations prescribed by physicians and also to evaluate the prescriptions on the basis of FDCs recommended by WHO in its essential drug list. The study was conducted in general medicine department of VMKVMC&H Salem for a period of Six months from October 2019 to March 2020.In this study 1000 prescriptions were collected from the department of general medicine and found major diseases patterns such as cardiovascular system, respiratory system, digestive system, central nervous system, circulatory system, endocrine system, renal system. Our study proved that majority of FDC Drugs prescribed is found to be rational. That shows the prescriptions were found to be prescribed in the generic name which is rational and better patient care. Inspired by this interest, a system named anatomical therapeutic chemical (ATC) classification was developed by the

Norwegian Medicinal Depot. Norwegian researchers also developed a technical unit of measurement called the defined daily dose (DDD) to be used in drug utilization studies.

Keywords: Prescription, FDC, ATC, DDD, Disease pattern .

INTRODUCTION

Prescription is an important intervention by the physician and legal duty of the practitioner. Errors can arise from choice of drugs, dose, route, frequency, duration of treatment. This leads to errors in dispensing and administration. A Patient detail plays an important role in the prescription and also the record keeping purposes. WHO developed prescription indicators to evaluate the services provided to the population in regards to medications [1].

WHO Core Prescribing Indicators

Average number of drugs per prescription was also counted as one drug. This helps to indicate adverse drug reaction and drug –drug interaction. Percentage of drugs prescribed by generic name enables the investigator to calculate the number of prescriptions in which the drugs are prescribed. Percentage of antibiotics per prescription - This indicator evaluates the use of antibiotics in excess which contributes to bacterial dissemination and resistance. Percentage of injection per prescription, this indicators helps to evaluate the injectable in excess administration. Percentage of drugs prescribed from the essential drugs list helps in measuring the degree to which practice conform to the current national drug policy (NDP) of October 1998 [2].

Fixed Dose Combination

Fixed dose drug combinations (FDCs) are defined by WHO as a combination of two or more active ingredients in a fixed ratio of doses, they offer many advantages such as ease of administration or intake by a patient in condition where multiple drugs have to be taken.

Types

First group includes one or more active ingredients is a new drug. That is to be approved for marketing data required for any new drug. Second group includes active ingredients already approved / marketed individually are combined for the first time. Third group includes those which are already marketed to change the ratio of active ingredients or to make a new therapeutic claim. Fourth group includes whose individual active ingredients have been widely used in a particular indication. Fixed-dose combination products (FDCs) they are also called as “Fixed Ratio Combinations”. Two or more pre-approved active substances are presented on the market in a single product, in fixed-doses [3-5].

ATC/DDD

The Anatomical Therapeutic Chemical (ATC) classification system and the Defined Daily Dose (DDD) as a measuring unit are recommended by the WHO for drug utilization monitoring and research. The system is widely used internationally and the number of users is increasing. The purpose of preparing guidelines is to make information about the ATC/DDD system available to the users. Drug consumption can be expressed in cost, number of units, number of prescriptions or by the physical quantity of drugs. However, these variables can vary between regions and countries over time. These limits comparisons of drug consumption at an international level. To address this, a technical unit of measurement, the Defined Daily Dose (DDD) was created. The assumed average maintenance doses per day for a drug used for its main indication in adults. DDDs are only assigned for medicines given an ATC codes. The DDDs are allocated to drugs by the WHO Collaborating Centre in Oslo, working in close association with the WHO International Working Group on Drug Statistics Methodology. Only one DDD is assigned per ATC code and route of administration (e.g., oral formulation). The DDD is sometimes a dose that is rarely or never prescribed because it is an average of two or more commonly used doses [6-7].

MATERIAL AND METHODS

A retrospective study was conducted in general medicine of a tertiary care hospital, Salem, Tamilnadu, to analyze the prescribing pattern of FDC drugs and calculate the DDD for selected category patients for a period of six months from October 2019 to March 2020. A total of 1000 cases were collected from medical record department, all the relevant and necessary data is collected from patient case sheets. Informed consent was obtained from the patients undergoing the study, after providing all the required information of the study in their local language. The study was approved by the institution ethical committee of VMKVMC&H, Salem. The demographic data including in patient IP number, name, age, gender, address, date of admission, date of surgery, date of discharge, history of associated diseases, physical examination, laboratory data's, chief complaints, and diagnosis were analyzed and recorded. The details which include choice of the drug, dosage, duration of therapy and route of administration were also recorded.

RESULTS

Table No. 1 Distribution based on Disease Pattern

S. No.	Disease pattern	No. of Prescription (n= 1000)	Percentage (%)
1	Cardiovascular System	156	15.6
2	Respiratory System	81	8.1
3	Gastro Intestinal Tract	178	17.8
4	Central Nervous System	77	7.7
5	Endocrine System	245	24.5
6	Renal System	101	10.1
7	Others	162	16.2
Total		**1000**	**100.0**

Table 2: Distribution based on Class of Drugs

S. No	Category of drugs	No. of FDC Prescriptions (n=450)	Percentage (%)
1	NSAIDs	31	6.37
2	PPI + Antiemetic	43	9.55
3	Xanthine's	33	7.33
4	Multivitamin, Nutrient Supplements	61	13.55
5	Antihistamines	27	6.0
6	Expectorants + Bronchodilators + Nebulisers	36	8.0
7	Antidiabetic	7	1.55
8	Antiplatelet + Anticoagulant	20	4.44
9	Penicillin Antibiotics	39	8.66
10	Cephalosporin Antibiotics	20	6.44
11	Others	131	28.11
Total		**450**	**100**

Table 3: Distribution based on Class of FDC drugs Prescribed

1	FDC drugs Prescribed in Cardiovascular system	No. of FDC drugs Prescribed	Percentage (%)
	Antiplatelets	18	36
	Salicylates	19	38

	Anticonvulsants	8	16
	ACE Inhibitors	5	10
2	**FDC drugs Prescribed in Respiratory system**		
	Bronchodilators	15	21
	Xanthines	38	54
	Expectorants	11	15
	Respiratory stimulants	7	10
3	**FDC drugs Prescribed in Digestive system**		
	Proton pump inhibitor	16	35
	Antacids	12	26
	Laxatives	14	30
	Antidiarrheals	4	9
4	**FDC drugs prescribed as Antibiotics**		
	Penicillin	39	59.09
	Cephalosporins	20	30.31
	Beta lactamase	4	6.06
	Quinolone	3	4.54

Table 4: Distribution based on Total number of FDC drugs prescribed per prescription

S.No	No. of FDC drugs Prescribed per prescription	No. of prescription	Percentage (%)
1	1	75	37.33
2	2	58	28.85
3	3	39	19.40
4	4	21	10.44
5	5	4	1.99
6	6	4	1.99
Total		**201**	**100**

Table 5: Prescribing Frequency of Chosen Drugs acting on System

1	**Prescribing Frequency of Chosen Drugs acting on CVS**	**Drugs Prescribed in No. of Prescriptions (n=1000)**	**Percentage (%)**
	Tab. Amlodipine	188	18.8
	Tab. Atenolol	137	13.7
	Tab. Metoprolol	149	14.9
2	**Prescribing Frequency of Chosen Drugs acting on Respiratory System**		
	Inj. Aminophylline	56	5.6
	Tab. Salbutamol	127	12.7
	Neb. Ipratropium	28	2.8
3	**Prescribing Frequency of Chosen drugs acting on GIT**		
	Tab. Ranitidine	235	23.5
	Inj. Pantoprazole	198	19.8
	Cap. Omeprazole	156	15.6

4	**Prescribing Frequency of Chosen Drugs acting on CNS**		
	Tab. Amitriptyline	32	3.2
	Inj. Lorazepam	6	0.6
	Tab. Chlordiazepoxide	12	1.2
5	**Prescribing Frequency of chosen Drugs acting on Endocrine System**		
	Tab. Metformin	203	20.3
	Tab. Glimepiride	164	16.4
	Tab. Voglibose	84	8.4
6	**Prescribing Frequency of Chosen Drugs acting on Renal System**		
	Tab. Furosemide	87	8.7
	Inj. Torsemide	5	0.5
	Tab. Hydrochlorothiazide	49	4.9
7	**Prescribing Frequency of Chosen Anti-Bacterial drugs**		
	Inj. Ceftriaxone	266	26.6
	Inj. Cefperazone+ Sulbactam	163	16.3
	Tab. Ciprofloxacin	120	12.0

Table No. 6 Distribution Based on Co-Morbidities

S. No.	Co-Morbidities	Number of Prescriptions (n= 1000)	Percentage (%)
1	DM	98	9.8
2	DM with SHTN	56	5.6
3	SHTN	61	6.1
4	Hypotension	32	3.2
5	Dyslipidemia	81	8.1
6	SHTN with Dyslipidemia	42	4.2
7	Anaemia	19	1.9
Total		**389**	**38.9**

Table No.7 Distribution based on Combined Class of Drugs in Prescriptions

S. No	Prescription Types	Combined Class of Drugs Prescribed in No. of Prescriptions (n=1000)	Percentage (%)
1	With Combined Class of Drugs	406	40.6
2	Without Combined Class of Drugs	594	59.4
Total		**1000**	**100.0**

Table No. 8 ATC Code and DDD/1000 patients/day

S. No	Drug	ATC Code	WHO DDD	DDD/ 1000 patients/ day
1	Tab. Amlodipine	C08CA01	5.0	188.0
2	Tab. Atenolol	C07AB03	75.0	58.4

3	Tab. Metoprolol	C07AB02	0.2	60.3
4	Inj. Aminophylline	R03DA05	0.6	26.0
5	Tab. Salbutamol	R03CC02	12.0	0.6
6	Neb. Ipratropium	R01AX03	0.2	0.2
7	Tab. Ranitidine	A02BA02	0.3	75.7
8	Inj. Pantoprazole	A02BC02	40.0	101.5
9	Cap. Omeprazole	A02BC01	20.0	133.6
10	Tab. Amitriptyline	N06CA01	75.0	5.3
11	Inj. Lorazepam	N05BA06	2.5	11.0
12	Tab. Chlordiazepoxide	N05BA02	30.0	2.8
13	Tab. Metformin	A10BA02	2.0	87.8
14	Tab. Glimepiride	A10BB12	2.0	80.7
15	Tab. Voglibose	A10BF03	0.6	2.6
16	Tab. Furosemide	C03CA01	40.0	7.7
17	Inj. Torsemide	C03CA04	15.0	0.8
18	Tab. Hydrochlorothiazide	C03AA03	25.0	11.8
19	Inj. Ceftriaxone	J01DD04	2.0	97.3
20	Inj. Cefperazone+ Sulbactam	J01DD62	4.0	39.5
21	Tab. Ciprofloxacin	J01MA02	1.0	35.0

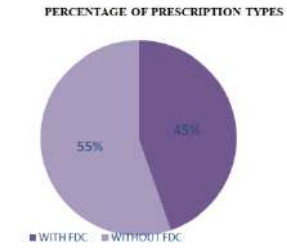

Figure 1: Distribution based on Prescription types

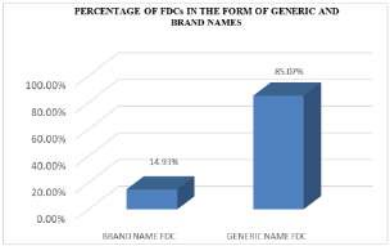

Figure 2: Distribution based on Brand and Generic Names

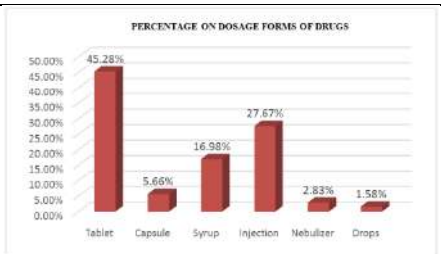

Figure 3: Distribution based on Dosage forms

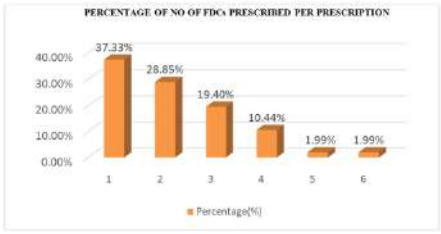

Figure 4: Distribution based on total number of FDCs drugs prescribed per prescription

DISCUSSION

The audit of the 1000 prescriptions was collected from general medicine department of tertiary care hospital for a period of six months from October- March 2020. Gender wise distribution was made among 1000 prescriptions. Male patients were 579 (57.9%) and Female patients were 421 cases (42.1%) and the patients classified according to age

group, such as 0-20, 21-40, 41-60, 61-80, 81 & above. The majority of the patients 452 (45.2%) were in 41- 60 age group and rest all the other categories are less compare to 41- 60 group. Among 1000 patients, based on month wise distribution the most affected patients were found to be highest in the month of December 310 (31%) cases, and followed by in November 245 (24.5%) cases, in October 189 (18.9%) cases, in January 164 (16.4%) cases, in February 65 (6.5%) cases and the least in the month of March 27 (2.7%) cases

During the study period prescriptions were collected and incidence of Polypharmacy were assessed. 235 prescriptions were with 9 (23.5%) drugs which is the highest percentage, 13 & above drugs were prescribed in 9 (0.9%) prescriptions and 1 drug was prescribed in 6 (0.6%) prescriptions which is the least percentage. The total prescriptions were categorized as Prescription with FDC drugs and without FDC drugs, in which number of prescriptions with FDC drugs were found to be 450 (45%) and without drugs were found to be 550 (55%). Distribution were made based on brand and generic names prescriptions with FDC drugs, among 450 prescriptions 68(15.11%) prescriptions were in brand name and 382 (84.89%) prescriptions were in generic name.

CONCLUSION

Rational combinations can be of immense help to the health care system; these combinations may improve the quality of life for many people. Such combinations (i.e., Antitubercular, Respiratory, Antiplatelet) are used frequently for many diseases. However, the irrational fixed dose combination products are considered to be equally harmful and our study proved that majority of FDC drugs prescribed are found to be rational. According to the WHO Expert Committee, Fixed dose ratio combination products are acceptable only when the dosage of each ingredient meets the requirements of a disease and our study revealed that each ingredient meets the requirements, and also most of the prescriptions were found to be prescribed in the generic name which is found to be rational and better patient care. From 1000 collected prescriptions, distributed based on ATC Code and DDD/ 1000/ patients/day were calculated for selected Anti- Hypertensive, Bronchodilators, Anti- Ulcerative, Sedatives and Hypnotics, Anti-Diabetics, Diuretics and Anti- Bacterial drugs with each category with WHO DDD Value. This study was carried out in a tertiary care hospital which gives insight into the day-to-day functioning status of our health care delivery system and this study showed that valuable information can be obtained by using the DDD methodology on a retrospective basis. Because DDD calculations are independent of dosage form, the calculation of DDDs makes it possible to study national and international data respectively. It can be concluded from that the DDD methodology is a useful technique to measure and compare drug consumption data nationally and internationally.

Conflict of Interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

A Review on Pharmacological Models and Traditional Herbs for the Treatment of Mood Disorders

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ABSTRACT

The most devastating psychiatric illnesses are mood disorders, particularly profound depression and bipolar disorder. Significant progress has been made in the neuroscience of emotion, cognition, and behaviour, and numerous studies in clinically ill populations have been conducted; yet, we are still a long way from applying most of these results in the clinical context. We have a reasonable grasp of the circuity driving negative emotion, reward, fear, anxiety, cognition, and behaviour based on neuroimaging research, and we have identified many anomalies in mood disorders. Various herbs and pharmacological models have supporting role in designing the newer drugs for the treatment profile of depression like *Centella asiatica Linn, Clitoria ternatea, Aegle marmelos, Cassia occidentalism and Melissa officinalis* .There are plenty of herbs having antidepressant activity that are enriched with phytoconstituents and by carring out futher investigational studies better drugs can be established in the market for the treatment of mood diosrders with less adverse effects .

Keywords: Aegle marmelos, mood disorders , neuroimaging and antidepressants

INTRODUCTION

"I cry out to you, God, but you do not answer; I stand up, but you merely look at me". It's a weeping of Job in Bible towards the god, which shows the depressed feeling and agony that he suffers in his life. Mood disorder or depression is the state of feeling despaired or sad due to many reasons. It can be due to anatomical, physiological, social and genetic variations .Depression is a term for extreme lows. or mania (hypomania or mania). (1) Mood disorders are classified as bipolar disorders and depressive disorders in the Diagnostic and Statistical Manual of

Mental Disorders, Fifth Edition (DSM-5). Bipolar disorders are further divided into bipolar I, bipolar II, cyclothymic disorder, and bipolar with another medical condition .Hypomania is a non-psychotic, moderate, or subthreshold manic condition that lasts for at least four days and is not associated with significant social or vocational damage. (2) It necessitates a high mood or an irritable mood (with four or more of the following symptoms) - increased goal-directed activity, grandiosity, decreased sleep needs, distractibility, racing thoughts, increased/pressured speaking, and reckless behaviours.(Mood disorders in children and adolescents, such as severe depression, dysthymia, and bipolar disorder, have been found to be very common. (3) The emotional and behavioural dysfunction associated with various mood disorders can affect functioning in a variety of domains, including academic and social settings. Suicide is a major public health issue, with suicide accounting for 1.4 percent of all fatalities globally. The majority of suicides are caused by psychiatric illness, (4) with depression, substance abuse, and psychosis being the most common risk factors. However depression plays a major role in suicide tendency and severe mortality among the patients if not treated or identified correctly .A variety of unfavourable adult health outcomes, including mental disorders and suicide mortality, have been linked to adverse childhood experiences (ACE). Only ACE, as well as female gender and depressive symptoms, was able to distinguish between first-time and repeat self-harm. The most common diagnosis among suicide victims are depression and substance use disorders, primarily alcoholism. (5) Furthermore, comorbid diseases are linked to a higher risk of suicide.

Since the state of depression has created a big transformation in the life of human being that leads to various mortality and morbidity in the current era of human society. There are many pharmacological and medical remedies to tackle the serious after effects of depression. We have modern system of medicine which makes less relapse in the stages of depression with lots of adverse effects.

Etiology of mood disorder

The amygdala and orbitofrontal cortex are the parts of the brain that control our moods and emotions. On brain imaging, patients with mood disorders had an enlarged amygdala, confirming the conviction that anomalies in these areas cause mood disorders. Ventricular expansion is also one of the brain anatomical changes that reflects the signs of depression. Apart from this various factors like – Biological factors such as neurotransmitters plays an a important role in depression .Serotonin and norepinephrine are neurotransmitters that play a key role in mood disorders and are reduced during depressive episodes. The chemical serotonin is most typically linked to depression. Dopamine has also been linked to mood disorders, with studies indicating that it is lower in depression and higher in mania. Apart from this (6) genetic, hormonal, psychological and neuroimmunological factors plays an important role the cause of depression.

Pharmacological models in the treatment of mood disorder

Basic scientific research of disease causes and pre-clinical studies of possible therapeutics rely heavily on animal models of human disease. (7) Rapid advances in animal modelling have led to a better knowledge of the core disease mechanisms of many CNS illnesses, including early cell death and brain degeneration. I a) Swim stress-induced 'behavioural despair' test: The apparatus used was a modified version of that described, Porsolt et al (1978). Individual rats were forced to swim in a polypropylene vessel (45 ern x 40 cm x 30 cm) with a water level of 20 cm, ensuring that the animals foot should not touch the bottom of vessel and the rat cant climb the or jump out of it .. After allowing the rat to swim for 10 minutes, the total period of immobility, defined as complete cessation of swimming with the head floating slightly above the water level, was recorded for the next 5 minutes. (8) After initial frantic attempts to flee, the immobility stage is thought to represent 'behavioural despair' as an experimental model of endogenous depression (Porsolt et al., 1978; Vogel and Vogel, 1997)

'Learned helplessness' test

The rats were subjected to footshock (60 scrambled shocks, 15 s duration, 0.8 rnA, every min) in a two-compartment jumping box (Techno) with the escape door to the adjoining unelectrified 'safe' compartment closed. The exercise continued for 1 h. 48 h later, The rats were given avoidance training with the identical apparatus, but the escape

route was left available. The rats were placed in an electric chamber for 5 minutes to acclimate before being treated to 30 avoidance trials with a 30 second inter-trial interval during this avoidance training.. During the first 3 s of the trial, a buzzer stimulus (conditioned stimulus,) was sounded followed by electroshock (unconditional stimulus; 0.8 rnA) delivered via the grid floor for the next 3s. The avoidance response was characterized by escape to the adjoining 'safe' chamber during UCS within 15 s and was assessed as 'escape failure', which is postulated to represent despair or depression (Vogel and Vogel, 1997; Bhattacharya et al., 1999). WSG, or the vehicle, was administered for 5 days before testing for avoidance and escape, whereas IPM was administered once 30 min prior to the test (10).

Tail suspension test (TST)

The TST has been widely utilised as an antidepressant medication screening assay. The mice were suspended by their tails with an elastic band that was adhered to the tail with adhesive tape and hooked on a horizontal rod. Dark-colored cardboard was used to visually segregate each mouse from the rest of the room, and it was placed at least 150 mm away from the mice.. The distance between the heads of the mice and the floor was approximately 200 mm. (11) The behaviour was recorded for a 6 min period with a digital camera, and the duration of immobility of the mouse limbs within the last 4 min was measured. The depressant activity was measured by the immobility of animal

Open-field test

The activity level of animals treated with the extract was evaluated in the open-field test to see if there was a link between immobility in the tests and changes in motor activity. The open field arena was built of acrylic (clear walls and black floor, 30 cm x 30 cm x 15 cm), and was divided into nine equal-sized squares. The open-field was utilised to assess the animal's exploration activity. The mice were placed separately in the centre of the arena and allowed to explore it freely after 21 days of treatment with vehicle (10 mL/kg, p.o.) and Ext (1.0 g/kg, p.o.). (12) Ambulation (the number of squares crossed with all four paws) and amount of grooming and rearing events were observed for 5 min. Each test group consisted of 10 mice. The walls and floor surfaces were thoroughly cleaned with 10% ethanol between the tests.

Elevated Plus maze

In summary, the apparatus consisted of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 cm) with a 40 cm high wall organised so that the arms of the same kind were opposite each other with a central square of 10 cm to make a plus sign. A single central support raised the wooden equipment to a height of 50 cm above the floor level. (13) A tiny raised edge on the open arms (0.25 cm) provided greater grip for the animals, and testing in a poorly illuminated area promoted open arm activity even more. The trial lasted from 9 a.m. until 4 p.m.. To facilitate adaptation to new surroundings, rats were transported to the laboratory at least 1 h prior to testing.The testing began with an animal being placed on the maze's middle platform, facing an open arm. The maze was carefully cleaned between subjects and utilised for a standard 5-minute test. The rats were divided into three groups at random: vehicle control, positive control: diazepam (DZ; 1 mg/kg po), and extracts (mg/kg po). The animal was positioned in the centre of the maze, facing an open arm. Between subjects, the maze was meticulously cleaned before being used for a regular 5-minute test. The experiments were carried out using a third party who was unaware of the rats' treatment in the room.. In this test, the following parameters are traditionally measured: Arm visits: frequency and duration (s), separately for open and closed arms. When a mouse's four paws were on the arm, it was thought to have entered it. Open and closed arm entries as a fraction of all arm entries (open or closed arm entries/total arm entries 100; percent open or closed arm entries)are used as traditional indices of the anxiety. The latency time, or the time spent at the maze's centre, was also recorded. In addition, the head dip count, raring, foci bolus (stool ball), and latency time (time spent at the maze's centre) were all recorded.

Herbs in Mood Disorder

Traditional medicine has a long history in India. India's materia medica contains a wealth of knowledge on folklore and traditional characteristics of therapeutically useful natural ingredients. Ayurveda, Siddha, and Unani are some of the systems used in Indian traditional medicine.(15) The evaluation of these medications is generally based on

phytochemical, pharmacological, and related methodologies, which include chromatography, microscopy, and other instrumental techniques. Although each of these traditional Indian medical systems is distinct, there is a similar thread running through their essential concepts and practises. Here we discuss about various herds involved in the treatment of depression . A variety of molecular mechanisms are involved in stress reactions mediated by the CNS at the cellular and whole-organism level. As a result, a variety of molecules may be active against a variety of targets, all of which contribute to the observed impact. Given the complexity of mental diseases (such as depression, anxiety, or insomnia), treating the patient with a single neurotransmitter may not be as effective as treating the patient with many neuroendocrine systems. The expanding body of (16) positive studies using supplementary combinations of therapies such as various herbs to improve efficacy in mood disorders attests to this. Mechanisms of action for herbal medicines used for treatment of psychiatric disorders primarily involve modulation of neuronal communication, via specific plant metabolites binding to neurotransmitter/neuromodulator receptors and via alteration of neurotransmitter synthesis and general function. Bellow we discuss some of the antidepressant activities of some selected medicinal plants

Glycyrrhiza uralensis

The open field test, forced swimming test, and tail suspension test were used to determine the antidepressant effect. Radioimmunoassay was used to determine the level of serum corticosterone. The neurogenesis protecting actions were studied using 5'-Bromo-2'-deoxyuridine (BrdU) labelling assays. In the open field test of the CUS (chronic unpredictable stress model of depression) in rats, flavonoids can enhance the total of line crosses and number of rears while decreasing the quantity of faecal boli generated. Flavonoids can also reduce immobility time in the forced swim test and the tail suspension test. Furthermore, flavonoids can lower CUS rats' serum corticosterone levels while increasing the amount of newly formed BrdU p. positive progenitor cells in the hippocampus's sub granular zone (SGZ) of the dentate gyrus (DG). The findings showed that extracting total flavonoids from cultivated Glycyrrhiza uralensis Fisch. could have an anti-depressive impact.(17)

Sipho campylus verticillatus

In two mouse models of depression, the antidepressant-like activity of a hydroalcoholic extract derived from aerial portions of *Sipho campylus verticillatus,* a Brazilian medicinal plant, was examined. When tested in an open area, the extract (dose range 100–1000 mg/kg, i.p.) considerably reduced immobility periods in the forced swimming test (FST) and the tail suspension test (TST), (18) without causing alterations in ambulation. The extract was similarly efficient in lowering the immobility time in the TST when given orally. The efficacy of the extract in the TST was comparable to that of imipramine (15 mg/kg, i.p.) and fluoxetine (32 mg/kg, i.p.).

Hypericum glandulosum

In mice, the methanol extracts of several Hypericum species were studied, particularly in depression animal models. (19) The butanol and chloroform fractions of both species examined were shown to considerably reduce immobility time in the forced swimming test, while having no effect or just a modest depression on spontaneous motor activity when measured in a photocell activity metre. In this sense, the chloroform extract from *Hypericum glandulosum* Ait. (500 mg/kg p.o.) was equivalent to the tricyclic antidepressant imipramine (50 mg/kg p.o.) in the forced swimming test.

Tinospora cordifolia

With related to the assessment of depression (20) in mice, a petroleum ether extract of Tinospora cordifolia (Wild.) Miers. Swiss young albino mice were given the extract (50, 100, and 200 mg/kg, p.o.) for 14 days in a row. In both the tail suspension and forced swim tests, petroleum ether extract had a strong antidepressant-like effect at all three dosages, with efficacies equivalent to imipramine (15 mg/kg, p.o.) and sertraline (20 mg/kg, p.o.). The extract had the most potent impact when given at a level of 50 mg/kg. And its works by GABA negic system.

II e) Polygala Sabulosa

In the tail suspension test and forced swimming test, scopoletin, a coumarin from Polygala sabulosa, had an effect. Furthermore, the capacity of scopoletin to counteract depression-like behaviour in mice generated by immobility stress was tested in a forced swimming test.In the tail suspension test, Scopoletin (10–100 mg/kg, p.o.) reduced immobility time, but not in the forced swimming test.. In the forced swimming and tail suspension tests, fluoxetine (positive control) reduced immobility time (20 mg/kg p.o. and 10 mg/kg p.o., respectively).(21) In the forced swimming test, immobility stress increased immobility time (depression-like behaviour), which was reversed by scopoletin.

Mitragyna speciosa

The role of mitragynine found in *Mitragyna speciosa* in psychiatric illnesses like depression has not been studied thoroughly. As a result, the current study assesses mitragynine's antidepressant effect in the mouse forced swim test (FST) (22) and tail suspension test (TST), two models predictive of antidepressant activity. Assessing corticosterone concentration in mice exposed to FST and TST, as well as the influence of mitragynine on the neuroendocrine system of the hypothalamic-pituitary-adrenal (HPA) axis.The open-field test (OFT) was performed to see if there was a link between immobility in the FST and TST and alterations in motor activity in mitragynine-treated mice.More over Mitragynine, at doses of 10 mg/kg and 30 mg/kg, significantly reduced corticosterone secretion in mice exposed to FST and TST. Overall, mitragynine possesses antidepressant characteristics in an animal behavioural model of depression, according to the current study (FST and TST).

Tagetes lucida Cav

In Mexican traditional medicine, *Tagetes lucida* (Asteraceae)(has been used to treat a variety of central nervous system (CNS) illnesses, primarily depression. This study looked into the antidepressant-like impact of *Tagetes lucida* extract in rats, as well as its potential detrimental consequences (23) on male sexual behaviour (MSB).The forced swimming test (FST), motor activity in the open-field test, and MSB were used to study antidepressant action in sexually experienced guys. *Tagetes lucida* aqueous extract was given orally in doses of 5, 10, 50, 100, and 200 mg/(kg/day)(-1) for 14 days, and the results were analysed on day 14. Based on the duration of immobility, the results show that *Tagetes lucida* has antidepressant activity.

Valerianawallichii patchouli

The roots essential oil of Valerianawallichii patchouli alcohol chemotype had an antidepressant-like effect in both acute and chronic treatment studies utilising a forced swim test (FST). On the 14th day after the behavioural testing, the neurotransmitter levels in the mouse brain were measured. (24) The 20 mg/kg dose resulted in a significant increase in norepinephrine (29%) and serotonin (19%) levels (p 0.05), but the 10 and 40 mg/kg doses had no effect. The nitric oxide pathway is important in mediating the antidepressant-like effect of this chemotype's essential oil, according to these studies.

Mimosa Pudica

To treat depression, aqueous extracts from dried *Mimosa pudica* leaves are used. The behavioural effects of aqueous extracts of *M. pudica* at various doses were investigated in this study. The forced swimming test and the elevated plus maze were used to assess the anxiolytic efficacy of *M. pudica* extracts over a 30-day period. (25) *M. pudica* (6.0 mg/kg and 8.0 mg/kg, I.R) reduced immobility in the forced swimming test, indicating that *M. pudica* has an antidepressant-like profile similar to two tricyclic antidepressants. However, in the open arm of the Elevated plus maze, the animal were less exposed this reflects that was having *Mimosa pudica* has less anxiolytic activity.

Areca catechu Nut.

The antidepressant efficacy of Areca catechu nut ethanol (26) extract and its various fractions was investigated utilising behavioural (acute and sub-chronic forced swim tests) and biochemical (monoamines and their metabolite levels using high performance liquid chromatography).. In both acute and sub-chronic forced swim tests, the areca nut ethanol extract and its aqueous fraction showed antidepressant efficacy (IC50 ~ 50 and 20 mg/kg, respectively),

which was further supported by unaltered locomotor (horizontal and vertical) activities of rats in the activity cage. Saponins found in the areca nut may be the active ingredient in its antidepressant properties, according to phytochemical study. And the aqueous fraction were less toxic .

DISCUSSION AND CONCLUSION

Even though the mood disorders are creating lots of mortality and morbidities in the current era, the modern medicine has a bunch of therapies to overcome the episodes of depression. But almost all chemical entities have a specific reaction on a particular receptor like 5HT and DA (Dopamine) receptors in post synaptic neurons. But in herbs the medicine may make relevant confirmational changes, but apart from that they produce additional supplementary actions that's boost the levels of neurotransmitters in different parts of brain. One of the important hidden supplementary action provided by the herbs are antioxidant activity ie to scavenge the ROS which may leads to various permanent disorders in the brain like amygdala, hippocampus and hypothalamus. Among the pharmacological models TST (Tail suspension test) and FST (Force swim test) plays an important role in identify the real medicinal herbs that can be used for the treatment of depression or mood disorders. The assessment of the extracts in the respective models are by using immobility exhibited by the mice or rats. Elevated plus maze helps in identify the exploratory nature of the herbs that supports that whether the herbal extracts having anxiolytic activity or not. Earth is composed of various types of herbs which enriches different phytoconstituents, different phytoconstituents having different activities like alkaloids, flavonoids and phenolic compounds having various antioxidant activities and supplementary activities that boost the mood disorders . By doing further investigation and clinical analysis the above-mentioned herbs can be converted to newer and effective drugs in the market that having less adverse effects of antidepressants.

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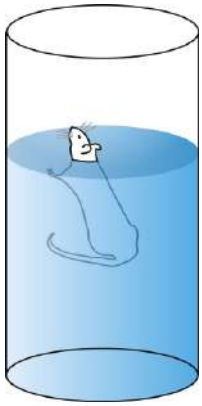

Figure 1. The forced swim (Porsolt) test is used to imitate depression in rodents as a test of behavioural despair.(9)

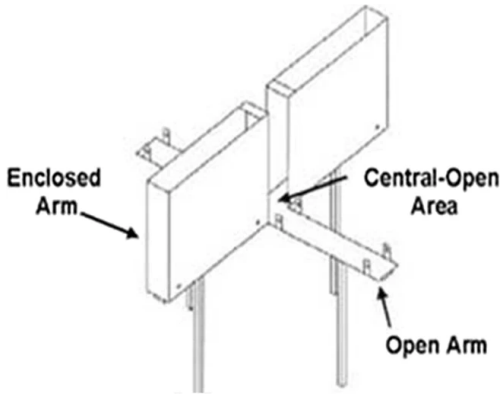

Fig .2 Schematic representation of an elevated-plus maze. (14)

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RESEARCH ARTICLE

Knowledge Regarding Utilization of Hand Sanitizer among Housewife in Selected Community, Salem.

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ABSTRACT

A descriptive study with cross sectional survey approach was under taken to assess the knowledge regarding utilization of hand sanitizer among housewife in selected community, Salem. Semi structured interview schedule was used to collect the data from 50 housewives. The collected data was analysis by inferential statistics. Highest (47%) percentage of housewives were in the age group between 20-30 years & Hindus. 33% of them had high school, 50% of housewives belongs to nuclear family and 35% of them had family monthly income between Rs.3001-5000. Higher percentage (53%) of them had source of information from friends & relatives and 64% of them had average knowledge regarding utilization of hand sanitizer. Highest percentage (60%) of housewives responded correctly to the item that what are the components used the hand sanitizer and which one kills bacteria and fungi faster and more effectively utilization of hand sanitizer.

INTRODUCTION

In 2010 WHO produced a guide for manufacturing hand sanitizer. Hand sanitizer is also called as hand antiseptic or hand rub, hand rub agent applied to the hands for the purpose of removing common pathogen. Hand sanitizers typically come in foam, gel or liquid form. Their uses is recommended when soap and water are not available for

hand washing or when repeated hand washing compromises the natural skin barrier. Although the effectiveness of hand sanitizer is variable, it is employed as a simple means of infection control in a wide variety of settings from day-care centers and schools to hospitals and health care clinics and from supermarkets to cruise ships (R.Babeluk, 2014).

Statement of the problem

"A study to assess the knowledge regarding utilization of hand sanitizer among housewife in selected community, Salem"

Objective

To assess the knowledge regarding utilization of hand sanitizer among housewife.

Research Design and Approach

A descriptive research design with cross section survey approach

Study Setting

The study was conduct in Veerapandi village, Salem district.

Population

The study population comprised of the entire individual with the housewives living in Veerapandi village, Salem.

Sampling

The study samples were housewives living in Veerapandi village, Salem who fulfilled the inclusive criteria.

Sampling Technique

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

Sampling Size

50 housewives living in Veerapandi village, Salem.

Tool used

Closed-ended questionnaire was used to collect the data regarding the knowledge regarding utilization of hand sanitizer among housewives.

RESULT AND DISCUSSION

50 housewives were selected by convenient sampling technique and data were collected by using questionnaire method. The collected data was analysis by inferential statistics. Demographic characteristics reveals that highest (47%) percentage of housewives were in the age group between 20-30 years & Hindus. 33% of them had high school, 50% of housewives belongs to nuclear family and 35% of them had family monthly income between Rs.3001-5000. Higher percentage (53%) of them had source of information from friends & relatives and 64% of them had average knowledge regarding utilization of hand sanitizer. Highest percentage (60%) of housewives responded correctly to the item that what are the components used the hand sanitizer and which one kills bacteria and fungi faster and more effectively utilization of hand sanitizer. Percentage wise distribution of level of knowledge score regarding utilization of hand sanitizer among house wives shows that highest percentage (64%) of them had average knowledge and 23% of them had poor knowledge. Lowest percentage(13%) of them had good knowledge. Hence, it can be interpreted that highest percentage (64%) of the housewives had average knowledge regarding utilization of hand sanitizer.

CONCLUSION

In the present study it can be concluded that the housewives had average knowledge regarding utilization of hand sanitizer. Hence, it can be interpreted that the investigator needs to conduct experimental study to assess the knowledge regarding utilization of hand sanitizer among housewives.

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Percentage wise distribution of knowledge score regarding utilization of hand sanitizer among housewives.

S.No	Level of knowledge	Maximum Score	Number	Percentage (%)
1	Poor	0-10	07	23
2	Average	11-20	19	64
3	Good	21-30	04	13
	Total	**30**	**30**	**100**

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RESEARCH ARTICLE

An Experimental Investigation and Analysis of AA2024 by GRA Method

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ABSTRACT

Friction stir welding (FSW) is a process which joins two material, it need solid state welding method, it is mostly works in same type and also in different types of welding as like Al, Mg, Cu, Ti, and their alloys. In this experimental test, friction stir welding of two aluminum alloys AA2024 has taken place with many number of sets of tool rotation speed, feed rate and axial pressure. In this test work FSW process has carried out for AA 2024and the analysis of the work piece were pointed out for maximum tensile strength values. Taguchi's L_4 orthogonal array was utilized for three parameters – tool rotational speed (TRS), traverse speed (TS), and axial force (AXF) with two levels. Many tests has carried out with Taguchi method of grey relational tests. At the time of investigation the resulted highest tensile strength value fourth sample 60.887 N/mm^2 and lowest hardness strength value second sample 31HRB and bead appearance found very best surface achieved at fourth test plates at the same time angle distortion has also very fine in the fourth test plate. The final conclusion has found for both ultimate tensile strength and hardness value. The test of grey relational grade has changed from 0.804 to 0.892, it has the highest value received throughout this experimental results. It has mentioned that the many responses of FSW process has improved with this test method.

Keywords: FSW, TRS, TS, AXF.

INTRODUCTION

The FSW method has some special process that makes the advantages of solid state type of welding for fabrications such as continuous linear welds, the most similar type of welding configurations that has generally maintained by arc welding processes in now a days work. So the fusion welding always gives the weld property degradation, FSW may result in a weld with mechanical properties similar or better than base metal.[1][2]. The outline of this work is too create make the FSW process to a manufacturing technology where it may be utilized over on-site construction of big, complex and critical thick section type shapes which has both high performance and high temperature materials (such as high-strength steels, Titanium alloys and super alloys). [3]. It initiates to convert the FSW a super welding method to a useful application potential over a huge number of companies, so reducing energy, environmental pollution and economic advantages are more useful. Hence to get a good successful project it needs both innovation process concepts and less engineering efforts to compensate the fundamental problems which are there in FSW technology. With all these points FSW is used to develop a field deployable friction stir welding system with the flexibility and affordability for difficult structural components. This field deployable FSW system works as the base for a concerted effort in this case, to integrate relevant new process concepts to improve field welding techniques.[4][5][6]

LITERATURE SURVEY

RajKumar.Va, [1]et.al proved that the characters of FSW dissimilar AA2024, AA5052 and AA6082, the Tensile test and hardness tests has conducted to achieve the mechanical characters of the materials. It resultsthat ductility is good when using low weld feed rate, hence good weld is achieved by observing mechanical characters and metallurgical characters. Sadeesh Pa,[2]et.al states that accurate parameters has received to joints by the help ofstatistical methods. Good efficiency and less defects were the results by changing the parameters. The reason to choose this is the difference of ratio between the tool shoulder diameter and the pin diameter.The readings of microscopy structural analysis shows that a material placed over the advancing side dominates the nugget area. The output results of hardness test values over the HAZ of AA2024 andAA6082 was lesser, so as the areas of welded joints were damaged at the time of tensile tests. R. K. Kesharwania, [3] et.al states that the preferred parameters which damaged the quality of weld in the FSW butt weld. With this test results the experimental data, empirical relations of parameters compared to all output which gives with normal regression type method. Good set of parameters were identified with the help of GRA. M. Ilangovan [4] et.Al were identified the welding of two different grades of aluminum alloys which were need in several less weight military products. The hardness increased results in changed the set of perfect grains and other intermetallic grains in stir zone, with this great tensile properties were generated by reducing the size of weaker regions such as TMAZ and HAZ regions. K. Kimapong [5] et.alproved that speed of pin rotation, axis of pin and its position along with diameter of pin on the tensile strength and microstructure of the joint. A small quantity of intermetallic compound was created at the interface between the steel fragments and the aluminum matrix. So the region where the intermetallic compounds created looks to be fracture region in a joint. M Senthil kumar [6] et.al proved that for both ultimate tensile strength and hardness value the test of grey relational grade has changed from 0.704 to 0.792, it has the highest value received throughout their experimental results.

TOOL TYPES AND ITS PROCESS

The FSW tool has a probe shaped pin and one shoulder area. The pin plunges into the joining place of the materials it creates the friction similarly deformational heating, afterwards it softens the work piece by connecting with shoulder along with the material increase the material heat afterwards it expands the zone, after that softs the material by constrains the aligned materials. The work piece must have the properties such as easy availability, machinability, thermal fatigue resistance and wear resistance. Some o the materials like aluminum, magnesium and aluminum matrix and its composites are always joined with steel tools. AISIH13 was made by chromium molybdenum, which was a hot worked air hardening steel and it can be generally used anywhere.

The different types of tools are i) Fixed ii) Adjustable and iii) Bobb in type tool. PCBN tool produces in higher strength and hardness the materials higher temperatures with high temperature stability. Hence this is generally used as a tool material for FSW process for hard alloys like steels and Titanium alloys .Because of its low coefficient of friction a smooth weld surface can be achieved. Similarlya very high temperature and pressure were need in the method of manufacturing for PCBN tool, hence the generation amount for the tool is always very high.

MATERIALS AND METHODS-AA2024

Al 2024 has the characters such as smoothly finishing of surface, great resistance for corrosion, also quickly and easily available for welding and it has the tendency of anodized. At the T4 condition the formability shows good output. Friction stir welding operation has done by the help of Czechoslovakian vertical milling machine which is shown in Figure 1. The quality of the were ascertained by visual inspection of weld bead and defected free joints are along the weld region. The experimental setup has to be done by friction stir welding which is shown in the Figure 2 The welding parameters with the stages were shown in the table 1

FINITE ELEMENT ANALYSIS WORK

Finite Element analysis work of a 3-D model has been carried out with three main steps. 3 different types of Al composite is analyzed by ANSYS workbench.

- Pre Processing stage
- Solution stage
- Post Processing stage

The preprocessing stage has engineering values and data, geometry and discretization values with all this data a simple 3-D design was designed. The solution stage shows the difference in analysis, location of forces and fixation of parts. The post processing stage contain checking of data files resulted by the ansys software at the time of solution stage. The figure 3 and figure 4 illustrates the stress levels of Square pin and Taper cylindrical pin. The advantage of FSW tools helps to give a detailed of contact stresses. Such range of contact stress and deformed were utilized in choosing of work piece in several areas. The main use of different range types of tools in FSW tools results in contact stresses. This region of contact stress and deformation were helpful in selection of FSW tools in several areas. The obtained results of FSW WITH TAPER CYLINDRICAL PIN is showing good results. Similarly deformation, stress, strain were monitored by ANSYS 19. FSW WITH TAPER CYLINDRICAL PIN has been picked for the production.

RESULT AND DISCUSSION

The above shown details are welded in FSW process by LML KODI 40machine, also with these work Hardness tests, Tensile test and Elongation tests were also conducted and it is also used in consideration for analyzing mechanical behavior of the work piece. The output vales of Hardness test and the Tensile strength test for the material were find out and tabulated in the box.

GREY RELATIONAL ANALYSIS

This different type of analysis method was based on the factor named as grey system, which was helped in getting several problems of interfered problems in a responses with very and easy effective way. Grey relational is a special method, some of the data were known and some of the data were not known. Hence this method were widely utilized in the development of FSW work with different tool profiles involved during the welding process with multiple responses. The first stage of the GRA process was data preprocessing, because the range and unit in one data sequence will be varied with the others sequence. Data preprocessing stage states that transferring the original order to a new

comparable order. Based on the characteristics of data orders, there are several methods of data preprocessing were available for such type analysis work. In this test a very fine finish has come as at test plate-4 parameter of speed-1100RPM, tool traverse-35mm/min and axial force-10KN. In test plate-5 GRA (speed-900RPM, tool traverse-35mm/min and axial force-10KN) has a smooth bead appearance, no crack & porosity was there. Hence the Friction stir speed increases the fine holes obtained on the top side of the weld bead

CONCLUSION AND FUTURE SCOPE

Many of the scholars and researchers went for the work on changing a particular parameter at a time and with no consideration was given to interaction effect of two or more parameters. Similarly many of them used cylindrical tool but taper cylindrical tool, taper threaded tool and square type of tool profile were rarely used for such tests, hence in this research work the taper cylindrical and square tool were used and this is to be compared with all the other tools and find out the tool profile which one has higher tensile strength and good bead properties. With those results of the FEA, the obtained results was, FSW with Taper cylindrical Pin having less deformation, stress, strain were analyzed by ANSYS 19. At the time of the investigation the resulted highest tensile strength value fourth sample 67.97 N/mm^2 and lowest hardness strength value second sample 31 HRB. Then the bead appearance were found that it was a very best surface occurred fourth test plates at the same time angle distortion was also very fine in the fourth test plate. The experimental results were varying from 0.804 to 0.892, which has the highest value resulted in all the other test results. It states that a multi-responses in the FSW process was increase by using this kind of techniques. In future these parameters were taken into consideration and Grey grade value can also be improved by changing the properties of work piece.

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Table 1: Processing range and their stages

STAGE	Processing range		
	Speed of rotation RPM	Tool movement Mm/min	Tool design
1	900	25	TP
2	1100	35	SQ

Table 2: Result of tool selection

TOOL SHAPES	Total deformation (m)		Equivalent Stress pascal		Equivalent elastic strain (mm)	
	Minimum	Maximum	Minimim	Maximum	Minimum	Maximum
Square	0	$3.8\ e^{-6}$	$7.8\ e^{5}$	$6.3\ e^{7}$	$4.3\ e^{-6}$	$2.9\ e^{-4}$
Taper cylindrical	0	$3.3\ e^{-6}$	$7.9\ e^{5}$	$5.9\ e^{7}$	$4.6\ e^{-6}$	$2.9\ e^{-4}$

Table 3: Welding parameters for Al2024

Speed of rotation (Rpm)	Tool movement (mm/min)	Axialforce applied(KN)
900	25	10
900	35	10
1100	25	10
1000	35	10

Table 4: Hardness output

TEST	Speed of rotation (Rpm)	Tool movement (Mm/min)	Axialforce applied (KN)	Hardness value HRB
T_1	900	25	10	38
T_2	900	35	10	31
T_3	1100	25	10	40
T_4	1100	35	10	33

Table 5: Tensile strength output

Test	Speed of rotation (Rpm)	Tool movement (mm/min)	Axialforce applied (KN)	Tensile Strength value (N/mm^2)
T_1	900	25	10	44.83
T_2	900	35	10	52.86
T_3	1100	25	10	61.28
T_4	1100	35	10	67.97

Table 6: Grey grade value

SL.NO	Normalization value		Sequence value		Grey relational Co-efficient value		Gg Value
	Hardness value (HRB)	Tensile Strength value (N/mm^2)	Hardness value (HRB)	Tensile Strength value (N/mm^2)	Hardness value (HRB)	Tensile Strength value (N/mm^2)	
1	0.322	1.100	0.878	0.000	0.491	1.000	0.796
2	0.989	0.753	0.211	0.447	0.918	0.690	0.804
3	0.400	0.389	1.100	0.811	0.433	0.513	0.473
4	1.100	0.000	0.000	1.000	1.000	0.433	0.767

Table 7: Weld appearances

SL.NO	Speed of rotation (Rpm)	Tool movement (mm/min)	Load applied (KN)	Output
T_1	900	25	10	It has a coarse bead appearance,hence no crack & porosity
T_2	900	35	10	It has a coarse bead appearance, hence no crack & porosity
T_3	1100	25	10	It has a coarse bead appearance, hence no crack & porosity but angle deviation was higher than others
T_4	1100	35	10	It has a very smooth bead appearance, similarly no crack & porosity was there.
T_5 GRA SAMPLE	900	30	10	Very smooth bead appearance, no crack & porosity

Figure 1: Vertical Milling Machine Setup

Figure 2: Friction stir welding machine

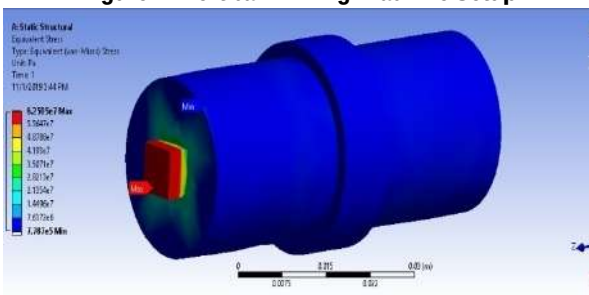

Figure 3: Stress Distribution of FSW Tool with Square Pin

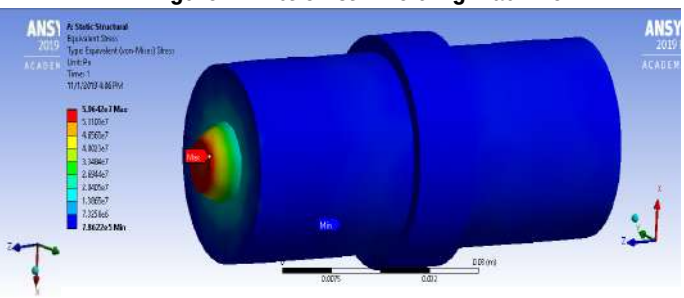

Figure 4: Stress Distribution of FSW Tool with Taper cylindrical Pin

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RESEARCH ARTICLE

Antimicrobial Activity, Enzyme Production and Plant Growth Promoting Activity of *Bacillus megaterium* Isolated from Lichen *Parmotrema perlatum*

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ABSTRACT

Parmotrema perlatum is a lichen commonly known as black stone flower, used as spice in India. Lichens are the self-supporting mutualistic associations between bacteria and fungi. Lichen symbiosis have been recently begun to be studied often by culture-independent techniques. Lichens are externally and internally colonized by bacterial communities. However, lichens are used to produce many primary and secondary products. In accordance with this information our study was to improve the recovery of bacteria associated with lichens by using novel isolation and culture approaches. The result obtained in this study shows strong antimicrobial action on *Pseudomonas sp.,* in bacteria and minimum activity showed in fungi compare with control. In this study we also produce amylase enzyme from *Bacillus megatarium* using potato peel as a substrate. The concentration of the protein and sugar is estimated. *Bacillus megatarium* isolated from lichen *Parmotrema perlatum* used as a biofertilizer for plant growth and their results of growth were compared. The purpose of this study was to increase plant growth with no hazardous to plants and consumers. This study was to elaborate isolation protocols to increase the recovery of endo-lichenic culturable bacterial populations associated with lichen and to use a cheap raw material for amylase enzyme production for industrial purposes.

Keywords: *Parmotrema perlatum,* lichen associated bacteria, antimicrobial activity, amylase enzyme, plant growth promotion.

INTRODUCTION

Parmotrema perlatum is lichen which belongs to Parmeliacea family. It is commonly known as black stone flower used as spice in India (Shanu Hoda *et al.,* 2015). Lichens are known as singular symbiotic associations, also harbour other microorganisms such as non-photosynthetic bacteria, which increasingly considered as integral components of the lichen thallus. The presence of such bacteria within lichen thalli have been known reveal their high diversity and abundance as well as some possible roles in lichen symbiosis (Rithika *et al.,* 2013). To date, only few studies have reported the isolation of heterotropic bacteria from the reduced number of lichen species due to difficulties in isolating and culturing them. Since lichens are externally and internally colonized by bacterial communities (Bates *et al.,*2011). One purpose of this study was to elaborate isolation protocols to increase the recovery of endo-lichenic culturable bacterial populations associated with lichens.

Antibiotics are widely known as the agents that fight against pathogens. However, the exploration of the microbes and their metabolic products are source of therapeutically useful compounds (Pannapa *et al.,* 2017). Antibiotic producing microbes found in nature are not only useful for medical purposes, but very useful in agricultural disease management, enzyme production etc., Lichens are the traditionally used as the natural antimicrobial drugs due to the presence of various secondary metabolites such as phenolic compounds, di-benzofurans, depsidones, usnic acid, lactones, depsones, pulvunic acid, derivatives and quinines (Ranganathan *et al.,* 2015). This study is to identify antimicrobial activity of the *Bacillus megaterium* isolated from *Parmotrema perlatum* against various clinical pathogens. And this study also, about the amylase enzyme production by using potato peels as the cheap substrate for industrial purposes. For optimum plant growth, nutrients must be available in sufficient and balanced quantities. Biofertilizers are products containing living cells of different types of microorganisms. These potential biofertilizers would play key role in productivity of soil and also protect the environment as eco-friendly and cost-effective inputs for the farmers (Priyanka *et al.,* 2016).

MATERIALS AND METHODS

Collection of Sample

The Lichen sample was collected from the local supermarket.

Preparation of Lichen for Isolation of Bacteria

Surface sterilization was done using saline. (Bates *et al*., 2011). Then the sample was serial diluted using 10mM phosphate buffer with ph 7.0 (Noah fierrer *et al.,* 2011).After serial dilution, pipette out 0.1ml from the appropriate desired dilution series onto the centre of the surface of an agar plate and spread the sample using sterile L-rod. Incubate and count the colonies.

Identification of Bacteria

- Gram staining
- Motility
- Spore staining
- Biochemical test
- IMVC
- Catalase

Microbial Strains used

Escherichia coli, Pseudomonas sp., Klebsiella sp., Staphylococcus aureus, Streptococcus pyogens bacterial samples and *Fusarium sp., and Penicillium sp.,*fungal samples were collected from Kovai Medical Centre and Hospital, Coimbatore.

Antimicrobial activity of isolated bacteria well diffusion method: (Chauhan *et al., 2013)*
The sterile Muller Hinton Agar (MHA) plates were prepared and the plates were lawn cultured with the test organisms. The inoculated plates were allowed to dry and a standard cork-borer was used to cut uniform wells on the surface of the MHA and different concentration of 25µl,50µl, and75µl of sample was introduced into the well. Streptomycin (10µl) is used as the positive control. The plates were incubated at 37ºc and zone of inhibition were measured.

Antifungal Activity by Poisoned Food Technique: (Mohana and Raveesha, 2007)
The antifungal efficacy of the sample was determined by poisoned food technique. Fungi test cultures.SDA plates were prepared. The plate along with the test culture was kept as control. The fungal discs of 9mm size was cut from seven days old culture were incubated at the centre of the petri plates and incubated at room temperature (28ºc ±2) for 5 to 7 days. The diameter of the mycelial growth (mm) pathogens were measured and recorded after the incubation.

Production of amylase enzyme by bacteria isolated from lichen using potato peel as substrate: (Maria Ghani *et al.,* 2013).
For production of amylase, 500ml of flasks were taken and 300ml of potato peel medium. The flasks containing media was autoclaved, and then inoculated with 1% inoculum size under aseptic conditions. After inoculation, the flasks were incubated at 30ºc with agitation speed of 140rpm for 24hrs. At the end of the fermentation time (24hrs) flasks were subjected to centrifuge for 20 min at 10,000g at 4ºc. The clear supernatant without bacterial growth obtained was used as crude amylase enzyme source.

Protein Estimation by Lowry et al Method
The protein estimation was determined by the Lowry's method (Lowry *et al*., 1951) using bovine serum albumin as the standard. Optical density of the reaction mixture was observed at 660nm against the blank with 0.1ml of buffer.

Determination of Reducing Sugar by Di-Nitro Salicylic Acid method
The reducing sugar was assayed by adding o.5ml of supernatant to 0.5ml of soluble starch in 100mM glycine NaOH buffer, Ph 10 and keeps it in boiling water bath for 10 to 15 mins. The reaction was stopped by adding 1ml of DNS reagent and the absorbance was measured at 550nm. (Miller, 2020)

Effect on Plant Growth: (Hayat *et al.,* 2010)
Macrotyloma uniflorum, *Vigna radiata, Vigna mungo* seeds were used. All seeds are weight about 1g and soaked in a liquid sample for 1hr. Then after soaking, all the seeds are planted in the pots and then record the growth of each seeds. Without liquid sample seeds are used as a positive control.

RESULTS

Identification of Bacterial Isolates
The bacterial strain was identified on the basis of morphological and biochemical characteristics (data not shown here) and it's confirmed as *Bacillus megatarium.*

Antibacterial activity of bacteria isolated from lichen
The antibacterial potential of the bacteria isolated from lichen was assessed against different bacteria by well diffusion method in comparison with standard antibiotic streptomycin. The zone of inhibition against test organisms ranged between 2.5-3 cm highest in *Pseudomonas sp*., and lowest ranged between 2-2.5 cm in *Escherichia coli.* The activity was recorded from different concentration of bacteria against various pathogens. (Table: 1). The different

concentration of bacteria, isolated from lichen, has zone of inhibition in the concentration of 75µl in *Pseudomonas* sp., among various pathogens compared to positive control streptomycin. (Fig.1).

Antifungal activity of bacteria isolated from lichen

The antifungal potential of the bacteria isolated from lichen was assessed against different fungi by poisoned food technique in comparison with control fungal plate. (Table 2). The diameters of the mycelial growth of different fungi are *Fusarium* sp., (3mm), *Penicillium sp*., (2mm), (Fig. 2). The average diameter of mycelium of test fungi in poisoned food plates is **less** in both *Fusarium* sp., and *Penicillium sp.,* compared to the control fungal plate.

Production of amylase enzyme by isolated bacteria using potato peels as substrate

Screening of starch hydrolysis activity

A clear zone was observed after adding, iodine pellets indicates that the organism is capable to hydrolyse starch. The result is positive. (Fig.3)

Protein estimation by lowry *et al.,* method

The amount of protein present in the amylase enzyme was found to be 40µg/ml.

Sugar analysis by DNSA method

The amount of sugar present in the amylase enzyme was found to be **150µl/ml.**

Effect on plant growth

Root and shoot length of *Macrotyloma uniflorum, Vigna radiata, Vigna mungo*which is soaked in the liquid broth of bacteria isolated from lichen is higher compared to the control. (Table 3 & Fig. 4).

CONCLUSION

The lichen associated bacteria *Bacillus megarium* has potential antibacterial activity against Pseudomonas sp., and capable of producing amylase enzyme using potato peel as substrate. Our study also shows that the *Bacillus megatarium* has the capability to act as natural biofertilizer and induce the plant growth. Further studies needed for the isolation of metabolites from lichen associated bacteria.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to publish this research article in this journal.

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Table 1: Antibacterial activity of isolated bacteria tested against various pathogens using well diffusion method. Results are shown in cm

S.NO	ORGANISMS	CONC. 25µL (cm)	CONC. 50µL (cm)	CONC. 75µL (cm)	CONTROL Streptomycin (cm)
1.	*Pseudomonas sp.,*	2.5	2.9	3	2.7
2.	*Escherichia coli*	2.1	2.2	2.5	2
3.	*Klebsiella sp.,*	2	2.2	2.5	2.5
4.	*Staphylococcus aureus*	2.5	2.7	2.9	2.5
5.	*Streptococcus pyogenes*	2.2	2.6	2.9	2.9

Table 2: Antifungal activity of isolated bacteria tested against various fungal pathogens using poisoned food technique. Results are shown in mm

S.No.	Organisms	Mycelial Growth(mm)	Control (mm)
2.	*Fusarium sp.,*	3	6
3.	*Penicillium sp.,*	2	6

Table 3: Effect on plant growth on *Macrotyloma uniflorum, Vigna radiate and Vigna mungo*

Growth of plant	*Bacillus megatarium*	Control	Growth of plant	*Bacillus megatarium*	Control	Growth of plant	*Bacillus megatarium*	Control
Root length (cm)	10.3	6	Root Length (cm)	6	3.8	Root length (cm)	1.5	2
Shoot length (cm)	10	4	Shoot length (cm)	17.5	15	Shoot length (cm)	6.5	4

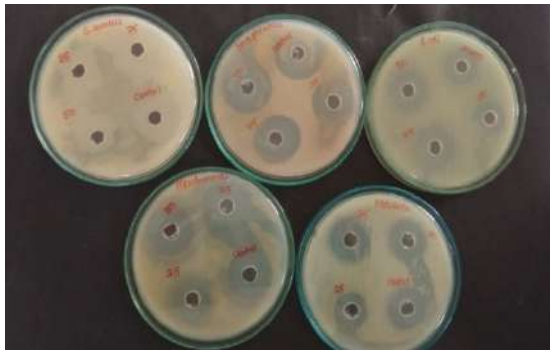

Fig 1: Antibacterial activity of bacteria against various pathogen. Control: Streptomycin

Fig 2: Antifungal activity of bacteria against various fungal pathogens.

Fig 3: Starch hydrolysis activity of Isolated Bacteria

Fig 4: Effect on plant growth

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RESEARCH ARTICLE

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Cetrizine Hydrochloride, Phenylephrine Hydrochloride, Paracetamol from Bulk and Formulation

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ABSTRACT

The plethora subscribed in this research is directed towards the development and validation of stability indicating RP-HPLC method for the estimation Cetirizine hydrochloride, Phenylephrine Hydrochloride, Paracetamol from its bulk and formulations and its forced degradation studies under different conditions of pH, temperature, oxidation etc. The HPLC method developed as per the guidelines prescribed under ICHQ2 guidelines. The results obtained for validation of developed method are in the limit as per the ICH guidelines. Hence the method found to accurate, linear and reproducible.

Keywords: Cetirizine hydrochloride, Phenylephrine Hydrochloride, Paracetamol, RP-HPLC, Validation

INTRODUCTION

The combination of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol treatment in anti-allergic, as a nasal decongestant to relieve stuffy nose or nasal congestion & antipyretic. Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol are a potential combination for us. Further investigations to assess the potential effect on the evaluation of drug resistance, disease transmission, and safety of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol are warranted.Cetirizine hydrochloride is a second generation antihistamine drugs and HPLC methods have been reported for analysis of cetirizine individually and in combination with several other drugs.[1-3]**.** Phenylephrine hydrochloride is an ingredient used in prescription and drug products s used to relieve nasal discomfort caused by colds, allergies, and hay fever. It is also used to relieve sinus congestion and pressure. Phenylephrine will relieve symptoms but will not treat the cause of the symptoms or speed recovery. Analytical Technique to determine Phenylephrine in pharmaceuticals has been generally used GC and HPLC method individually and in combination with other drugs. Paracetamol is acetanilide derivative having analgesic, antipyretic and weak anti-inflammatory action.[4-5]

The analytical research is carried out on cetirizine hydrochloride i.e. high-performance Liquid chromatographic determination of cetirizine in human plasma, urine, serum and Phenylephrine hydrochloride high-performance liquid chromatographic determination of phenylephrine hydrochloride in tablet,multicomponants formulations and analysis paracetamol in tablets by HPLC. These three drugs analytical methods are already reported in the market individually and combination of several other drugs. We study RP-High Performance Liquid Chromatographic method for the analysis of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol drugs that is a ternary combination which is simple sensitive and new method and not yet reported in the market.[6]

MATERIAL AND METHODS

MATERIAL

The required chemicals were purchased from local market of Nanded and are of HPLC grade.

METHODS

Selection of Analytical Wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200-400 nm and the spectra were overlain. Figure no. 01.

SELECTION OF MOBILE PHASE [7]

The solutions of Cetirizine hydrochloride, Phenylephrine hydrochloride, Paracetamol working standards were injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water and acetonitrile, buffer in different proportions were tried and finally Phosphate buffer pH4.0 and Acetonitrile in the ratio of (85:15) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Cetirizine hydrochloride, Phenylephrine hydrochloride, Paracetamol.

PREPARATION OF MOBILE PHASE

Phosphate buffer pH4.0 and Acetonitrile in the ratio of (85:15) was prepared, filtered through 0.2 μm membrane filter and sonicated on ultra-sonic bath.

PREPARATION OF STANDARD STOCK SOLUTION:-

1) Cetirizine hydrochloride standard stock solution :- (50 μg/ml) [8,9]

5 mg cetirizine hydrochloride weighed accurately and transferred in to 100 ml volumetric flask. Drug was dissolve in 50 ml Acetonitrile with shaking 10 min and then volume was made up mark so as to get the concentration 50 μg/ml. stock solution was filter through 0.2 μm membrane filter paper, for the preparation work standard, suitable aliquots of stock solution were pipetted out and volume were made up to the mark with mobile phase.

2) Phenylephrine hydrochloride standard stock solution :- (100 μg/ml)

10 mg Phenylephrine hydrochloride weighed accurately and transferred in to 100 ml volumetric flask. Drug was dissolve in 50 ml Acetonitrile with shaking 10 min and then volume was made up mark so as to get the concentration 100 μg/ml. stock solution was filter through 0.2 μm membrane filter paper, for the preparation work standard, suitable aliquots of stock solution were pipette out and volume were made up to the mark with mobile phase.

3) Paracetamol standard stock solution :- (1000 μg/ml)

500 mg Paracetamol weighed accurately and transferred in to 100 ml volumetric flask. Drug was dissolve in 50 ml Acetonitrile 10 min and then volume was made up mark so as to get the concentration 1000 μg/ml. stock solution was filter through 0.2 μm membrane filter paper, for the preparation work standard, suitable aliquots of stock solution were pipette out and volume were made up to the mark with mobile phase.

A mixed standard solution was prepared from these stock solutions by transferring 10 mL of each of the stock solution to a 100 mL volumetric fl ask and diluting with acetonitrile to get a solution of 10, 500 and 5 μg/ml of PHE, PAR and CET respectively.

Optimized chromatographic conditions

a) Column : Kinetex-C18 (4.6,150 mm, 5 mm)
b) Mobile phase : Phosphate buffer pH4.0 and Acetonitrile (85:15)
c) Flow rate : 1.5 ml/min
d) Detection Wavelength : 220 nm
e) Sample injector : 20 µl loop
f) Temperature : Ambient

ANALYSIS OF TABLET FORMULATION

20 tablets (Oncet-CF ® Tab) were weighed and powdered finely. Tablet powder equivalent to 10 mg of PHE, 500 mg of PAR and 5 mg CET was transferred to a 100 ml volumetric flask and dissolved in acetonitrile up to the mark. The solution was ultra sonicated for 15 min and filtered through 0.45 micron membrane filter. The solutions were further diluted to obtain resultant concentration of 10 μg/ml of PHE, 500 μg/ml of PAR, 5 μg/ml of CET the resultant mixture was subjected to HPLC analysis in developed chromatographic conditions.

VALIDATION OF DEVELOPED METHOD [10]

LINEARITY

For linearity and range studies 5 concentrations were chosen ranging from 50 % to 150 % of the target analyte concentrations in formulations. Hence the linearity dilution concentrations were Phenylephrine hydrochloride 5–15 μg/ml, Paracetamol 250–750 μg/ml and Cetirizine Hydrochloride 2.5–7.5 μg/ ml. All the solutions were prepared by diluting in acetonitrile. Each concentration of standard mixture solutions was injected in triplicate and the mean value of peak area was taken for the calibration curve. Calibration graph was obtained by plotting Peak area vs. concentration of standard drugs. The results obtained are shown in Table no. 02. One set of three different concentrations of mixed standard solutions of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol were prepared. All the solutions were analyzed thrice, in order to record any Intraday variations in the results. The result obtained for intraday variations. For Inter day variations study three different concentrations of the mixed standard were analyzed up to three days. The result obtained for Inter day variations .

ACCURACY

Accuracy studies were done by standard addition method to previously analyzed tablet powder (Onset cold ® ,). Results of accuracy studies were expressed as % recovery of the standard spiked into test sample of tablet. The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 80 %, 100 %, and 120 % each of the labeled claim and injected in developed chromatographic conditions in triplicate.

SPECIFICITY

A blend of commonly used tablet excipients was treated as per developed procedure and the chromatogram showed no interfering peaks at retention time of the three drugs.

ROBUSTNESS

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and ambient temperature were altered. Variation of mobile phase ratio is seen to have greater impact on resolution than other parameters and hence should be meticulously controlled.

Mobile ratio changes

Phosphate buffer pH4.0 and Acetonitrile in the ratio of (83:17)

The retention times for the three drugs were found to be:

Cetirizine hydrochloride	: 2.30
Phenylpropanolamine hydrochloride	: 2.31
Paracetamol	: 3.50

Forced Degradation Study

Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol were subjected to variety of stress conditions to affect degradation up to about 5-20%. The drugs were stressed under a variety of stress conditions like acid, alkali, effect by oxidation, light and dry heat. The stressed samples were subjected to chromatographic separation to resolve the drug from any potential degradation products. Stress degradation of analytes was performed. For study 10mg of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol were accurately weighed, transferred to separate 10ml volumetric flask, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol 1ml of above solution transferred in 10ml of volumetric flask and the volume was made with diluents. The concentration of both drug is 100µg/ml. All of above samples were stressed with acid, base, and peroxide stress solutions and kept aside for required time. After completion of stress, acid/alkali solutions were neutralized and volume made up with mobile phase up to the marks. These solution were used for acid, alkali, oxidation and photolytic degradation. For thermal degradation oven heated drugs were used to make equivalent concentration.

Acid/ alkali hydrolysis

For acid/ alkali hydrolysis, 2ml of 0.1N HCL and 0.1N NaOH was added to the solutions. These solutions were kept aside for 1hr at 60ºC. Resultant solutions were injected in to system after neutralization and chromatogram were recorded to access stability.

Oxidation Degradation

For oxidation degradation 3ml of 2% hydrogen peroxide (H2O2) was added and kept aside for 24 hr at 60ºC and injected in system and chromatogram were recorded.

Photo Degradation:

For photo degradation solutions were exposed to near UV light for 24hr and resultant solutions were injected in chromatographic system and compared with standard drug solution.

Thermal Degradation

Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol were transfer into petri plate separately and kept in a hot air oven at 70ºc for 12hrs. from the above stressed sample, 10 mg was weighed accurately and transferred to 10 ml volumetric flask separately and volume was made up to the mark with the mobile phase to get the concentration 1000µg/ml of both drug solution.5 ml of above solution transferred in 10 ml volumetric flask and the volume was made with diluents.

RESULT AND DISCUSSION

Selection of wavelength

The wavelength selected for the analysis was 220 nm for Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol showed considerable absorbance

VALIDATION OF DEVELOPED METHOD

LINEARITY

Excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

ACCURACY

The percentage recoveries were calculated by measuring differences in peak areas obtained for spiked and unspiked solutions and subjecting the values to the slope and Y-intercept of the calibration curve. The recovery data for accuracy studies is presented in the results obtained are shown in Table no. 11

Forced Degradation study:

Acid /Alkali hydrolysis:

For Acid/Alkali hydrolysis, 2ml of 0.1M Hydrochloric acid (HCL) / 2ml of 0.1N Sodium hydroxide (NaOH) was added to solutions. These solutions were kept aside for 1hr at 60^{0}C. Resultant solutions were injected in to system after neutralization and chromatograms were recorded to access stability.

Thermal degradation

Cetirizine hydrochloride, Phenylephrine hydrochloride and Paracetamol were transferred to petri plate separately and kept in a hot air oven at 70°C for 12hrs. From the above stressed sample, 10mg was weighed accurately and transferred to 10ml volumetric flask separately and volume was made up to the mark with the methanol to get the concentration of 1000µg/ml of both drug solution. 5ml of above solution transferred in 10 ml volumetric flask and volume was made with diluents.

CONCLUSION

Development and validation of RP-HPLC method was found to be linear, accurate, precise, specific and robust according to acceptance criteria. The results show that the HPLC method presented here can be considered suitable for the analytical determination of Paracetamol, Cetirizine hydrochloride and Phenylephrine hydrochloride in bulk and tablet dosage form. The developed method was validated. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The method was successfully applied to the available marketed formulation without any interference due to the excipients and can have an application in the industry.

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Table no. 01 : Analysis of Tablet Formulation

Sr. No.	Amount present in (mg/tab)			Amount found in * (mg/tab)			% of Label claim*		
	CET	PHE	PAR	CET	PHE	PAR	CET	PHE	PAR
1	5	10	500	4.97	24.95	499.74	99.83	99.81	99.96

* Denotes average of five determinations.

Table no. 02 : Linearity of Cetirizine hydrochloride

Standard Concentrations ⇨	2.5 µg/ml	5.0 µg/ml	7.5µg/ml
Replicates ⇩	Peak Area		
1	90883.55	186786.32	274765.61
2	90650.30	183564.21	277128.25
3	90130.15	188551.23	278189.50
Mean	90544.67	186300.58	276694.45
SD	402.24	2528.74	1752.68
% RSD	0.444	1.357	0.633

Regression Equation y = 37230x - 1636.5
Coefficient of correlation: 0.9997

Table no. 3: Linearity of Phenylephrine Hydrochloride

Standard Concentrations ⇨	5 µg/ml	10 µg/ml	15 µg/ml
Replicates ⇩	Peak Area		
1	86829.5	198492.42	317758.33
2	86728.7	192482.62	313448.43
3	86226.7	193597.85	316768.25
Mean	86594.9	194857.63	315991.67
SD	322.88	3196.82	2257.45
% RSD	0.372	1.640	0.714

Regression Equation : y = 22940x - 30249
Coefficient of correlation: 0.999

Table no. 4: Linearity of Paracetamol

Standard Concentrations ⇨	250 µg/ml	500 µg/ml	750 µg/ml
Replicates ⇩	Peak Area		
1	3178836	7357672	12715344
2	3130738	7337873	12564960
3	3085975	7325575	12326928
Mean	3131849	7340373	12535744
SD	46440.48	16193.92	195849.24
% RSD	1.48	0.22	1.56

Regression Equation : y = 18808x - 2E+06

Coefficient of correlation: 0.9963

Table no. 05: Intra-day variability of cetirizine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
2.5 µg/ml	91883.55	90083.25	89889.85	90618.88	1099.49	1.2133
5.0 µg/ml	184786.42	176786.32	180016.22	181196.32	3169.34	1.7491
7.5 µg/ml	269895.81	274765.61	271755.68	272139.03	2457.42	0.9030

Table no. 06: Intra-day variability of Phenylephrine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
5 µg/ml	85829.5	86329.5	84899.8	86352.93	1283.04	1.4858
10 µg/ml	189895.42	193687.82	187578.52	190387.25	3084.20	1.6199
15 µg/ml	307958.83	317758.73	307756.88	311158.14	5717.16	1.8373

Table no. 07: Intra-day variability of Paracetamol

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
250 µg/ml	3175836	3080838	3085985	3114219.6	53423.33	1.715
500 µg/ml	7357672	7217273	7322575	7299173.3	73066.33	1.001
750 µg/ml	12715344	12564960	12326928	12322410.67	93370.77	0.757

Table no. 08: Inter-day variability of cetirizine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
2.5 µg/ml	91883.25	91083.45	90889.33	91285.34	526.82	0.5771
5.0 µg/ml	183786.48	186786.52	188896.12	186489.70	2567.71	1.3768
7.5 µg/ml	267885.81	274765.65	271755.58	271469.01	3448.86	1.2701

Table no. 09 : Inter-day variability of Phenylephrine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
5 µg/ml	85729.5	86329.5	86899.8	86319.6	585.21	0.6779
10 µg/ml	192895.42	199687.82	197578.52	196720.58	3476.52	1.7672
15 µg/ml	317958.83	317758.73	308756.88	314824.81	5255.93	1.6694

Table no. 10 : Inter-day variability of Paracetamol

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
600 µg/ml	2412345.90	2416789.87	2413543.61	2413543.61	2299.33	0.095
800 µg/ml	3012967.90	3101567.89	3115345.76	3076627.20	55559.30	1.805
1000 µg/ml	4012345.89	4015671.12	4030453.98	4019490.30	9639.27	0.239

Table no. 11 : Recovery Studies

Conc. (µg/ml)	Std added	Amount added mg	Mean recovery ± %RSD (n=3)	Mean Recovery
80 %	Phenylephrine Hydrochloride	8	8.12 ±1.4	101.57
	Paracetamol	400	405 ±2.2	100.5
	Cetirizine	4.0	3.9 ±2.6	98.25
100 %	Phenylephrine Hydrochloride	15	15.2 ±2.5	100.2
	Paracetamol	500	507.5 ±2.5	100.5
	Cetirizine	5	5.10 ±1.5	102.3
120 %	Phenylephrine Hydrochloride	18	17.90 ±2.4	99.44
	Paracetamol	600	605 ±2.5	101.2
	Cetirizine	6	6.05 ±2.1	100.8

Table no. 12: Summary of degradation data for Cetirizine hydrochloride.

Stress Condition	Retention Time	Area of Peak	Degradation (%)	API after degradation %
Std. Drug	4.152	2563824	-	-
Acidic (0.1N HCL)	4.048	2189745	83.25	16.75
Alkaline (0.1 N NaOH)	4.176	1562897	64.38	35.62
Oxidation (3% H2O2)	3.987	1971038	77.28	22.72
Photolytic (UV)	3.879	2345687	96.87	3.13
Thermal	4.123	2483764	99.05	0.95

Table no. 13 : Summary of degradation data for Phenylephrine hydrochloride

Stress Condition	Retention Time	Area of Peak	Degradation (%)	API after degradation %
Std. Drug	5.752	1082574	-	-
Acidic (0.1N HCL)	4.578	758648	83.45	16.55
Alkaline (0.1 N NaOH)	4.950	710587	65.48	34.52
Oxidation (3% H2O2)	4.986	744825	72.84	27.16
Photolytic (uv)	5.188	954876	88.67	11.33
Thermal	5.846	895204	85.48	14.52

Table no. 14 : Summary of degradation data for Paracetamol

Stress Condition	Retention Time	Area of Peak	Degradation (%)	API after degradation %
Std. Drug	5.755	1048527	-	-
Acidic (0.1N HCL)	4.943	753845	83.54	16.46
Alkaline (0.1 N NaOH)	4.975	706548	67.24	32.76
Oxidation (3% H2O2)	4.986	744214	72.54	27.46
Photolytic (uv)	5.644	954245	88.64	11.36
Thermal	5.624	899458	82.95	17.05

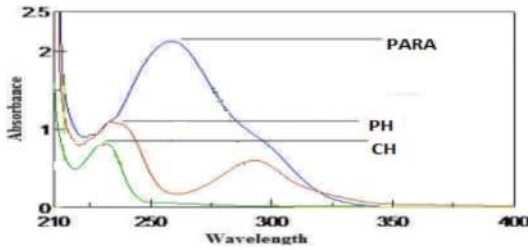

Fig. no. 01 : Overlain spectra of Cetirizine Hydrochloride, Phenylephrine Hydrochloride and Paracetamol

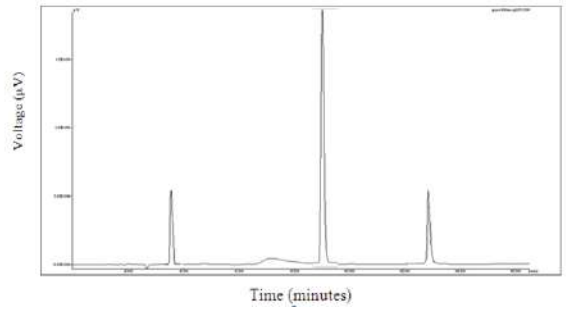

Fig. no. 02 : Chromatogram of working standard mixture of Phenylephrine, Paracetamol and Cetirizine Hydrochloride

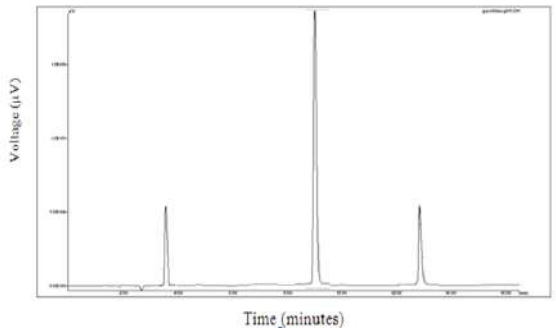

Fig. no. 03: Chromatogram of sample consisting Phenylephrine Hydrochloride (3.50 min), Paracetamol (9.63 min) and Cetirizine Hydrochloride (13.30 min)

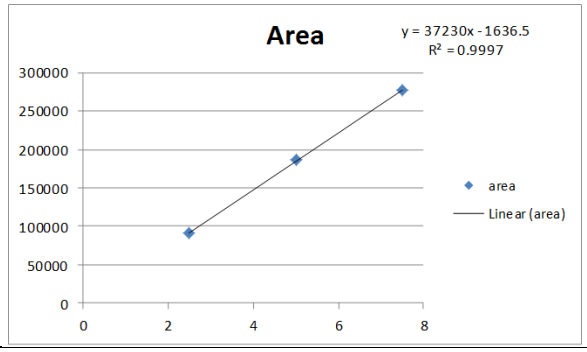

Fig no. 04 : Calibration Curve of Cetirizine Hydrochloride.

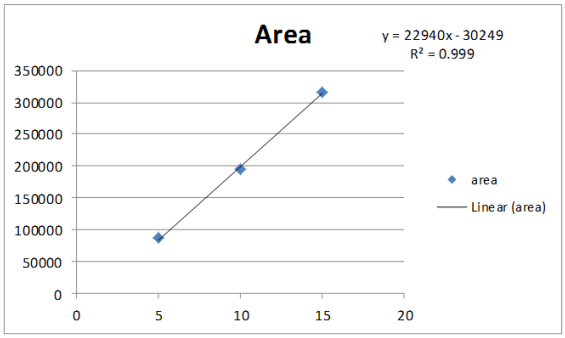

Fig no. 05 : Calibration Curve of Phenylephrine Hydrochloride

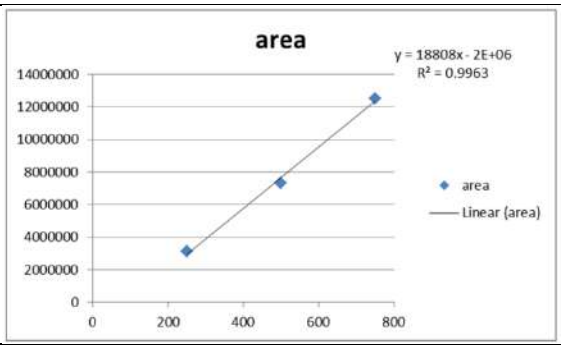

Fig no. 06 : Calibration Curve of Paracetamol.

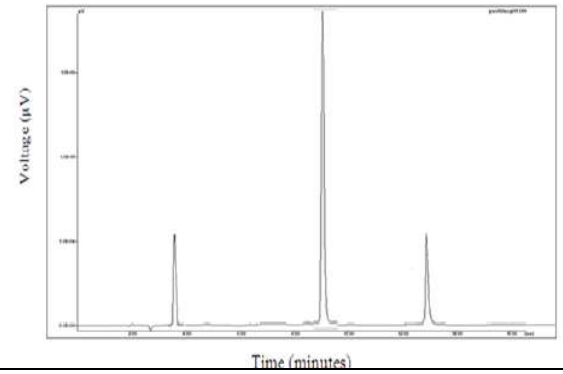

Fig. no. 07 : No Interfering Chromatogram of sample consisting Phenylephrine Hydrochloride (3.53 min), Paracetamol (9.65 min) and Cetirizine Hydrochloride (13.40 min)

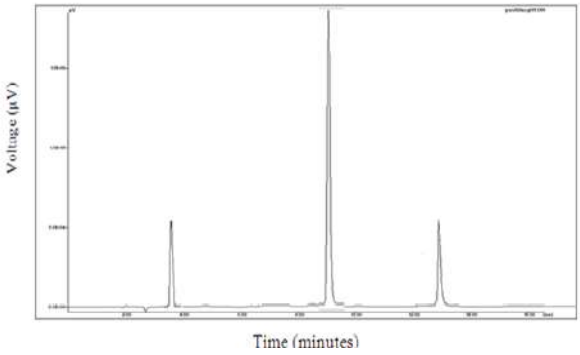

Fig no. 08 : Mobile ratio changes Chromatogram of sample consisting Phenylephrine Hydrochloride (3.49 min), Paracetamol (9.62 min) and Cetirizine Hydrochloride (13.37 min)

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RESEARCH ARTICLE

Influence on Compost and Industrial By-Products on Yield of Maize, Soil Microbial Population and Dehydrogenase Activity

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ABSTRACT

Maize (Queen of Cereals) is the third most important cereal crops in the world after wheat and rice. Field experiment was conducted with conventional organic sources *viz.,* biocompost (2.5 and 5 t ha^{-1}), non-conventional organic sources *viz.,* municipal solid waste compost and industrial by-products *viz.,* bagasse ash (5 and 10 t ha^{-1}) and rice husk ash (5 and 10 t ha^{-1}) with recommended dose of fertilizers. The soil was coarse loam with pH (8.1), EC (0.16 dSm^{-1}), with taxonomical classification of *Typic ustropept.* Regarding the available nutrient status it was 116 kg ha^{-1} (low) in alkaline $KMnO_4$–N, 15 kg ha^{-1} (low) in Olsen-P, 235 kg ha^{-1} (medium) in NH_4OAC-K. The design followed was randomized block design and replicated thrice. The application of 75% RDF + Municipal solid waste compost @ 10 t ha^{-1} registered highest grain yield (10.09 t ha^{-1}) and stover yield (11.55 t ha^{-1}). The NPK uptake of grain recorded as 135.02, 23.6 and 26.29 kg ha^{-1}. The post harvest soils were analysed for dehydrogenase and soil microbial population and the application of 75% recommended dose of fertilizers + Municipal solid waste compost (T_3) recorded maximum dehydrogenase (10.2 μTPF g^{-1} soil h^{-1}), bacterial population (60.3 × 10^6 CFU g^{-1} soil), fungi population (16.8 × 10^4 CFU g^{-1} soil) and actinomycetes population (12.1 × 10^2 CFU g^{-1} soil).

Keywords: Maize, Municipal solid waste compost, Bagasse ash, Grain yield, Soil microbial population.

INTRODUCTION

Maize (*Zea mays*) is a cereal crop grown all around the world. It serves as the third important cereal next to rice and wheat. Even though the maize consumption by human as food is minimum when compared to other cereals, it gains the important as poultry feed. Waste generation rates will be doubled over the next twenty years in lower income countries. Waste management costs have been increased about five times in low-income countries and our times in lower-middle income countries. These ever-growing large amounts of wastes are associated with environmental and

public health problems, and odour from the landfills. The reuse of wastes for agricultural purpose to improve soil properties and increase crop yield is a good solution for minimizing these problems. Nowadays, with increasing demand to conserve natural resources and energy, recycling of wastes assumes major importance (Padmavathiamma *et al.,* 2008). Pressmud as biocompost used to maintain soil fertility and to enhance crop production, because it is rich in sugar and contains appreciable amount of essential plant nutrients *viz.,* organic carbon, nitrogen, phosphorus, potassium, calcium and magnesium along with traces of micronutrients *viz.,* Zn, Fe, Cu and Mn (Banulekha, 2007). So the beneficial effects of this biocompost for enhancing the soil fertility and thereby improving the crop productivity is well established (Laird *et al.,* 2001).

Bagasse is an important agro-industrial waste by-product that is generally used as a fuel in sugar industry. The resulting boiler ash is usually sent to landfill or accumulated in uncultivated areas close to the plants and no particular use is made of this by-products (Pita, 2009). The ash is an alkaline material, nearly free of nitrogen (N), but containing other elements, such as potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P), which are required for plant nutrition (Augusto *et al.* 2008). Rice husk is a by-product of the rice milling. About 100 millions of tons of husk per year are produced worldwide (Alhassan *et al.,* 2007). The objective of the study is to evaluate the microbial count and dehydrogenase enzyme activity.

MATERIALS AND METHODS

Municipal solid waste is collected from the garbage in Punjai Puliampatti municipality and composting is carried out starting with partial segregation of plastics, metals, rubbers *etc.,* from the waste before taking it to the composting yard. A heap of manually separated mixed municipal solid waste of 4′ height, 8′ long was placed on paved ground on composting windrow type and manually every 3-5 days for the first six weeks of composting cycle. From the seventh week, the moisture was allowed to drop when optimum bio-solids decomposition was achieved. The process was completed in about 8-9 weeks. After this period the compost was allowed to cure for additional three weeks without turning. The reduction in weight was more significant in the first week. It may be done to maximum microbial activity during this period. Composting of municipal solid waste during summer season required 4-8 weeks, where more than 70% weight loss was recorded, earthy smell of the material after one week cleanly indicated the maturity of compost. The finished compost was then screened out and weighed. The chemical composition of municipal solid waste compost was given in Table 1.

Bio-Compost

Bio-compost was produced by composting pressmud from Bannai Amman sugars received from cane juice filtration and spent wash taken from distilleries. Pressmud is stored in triangular shaped rows known as windrows. Spent wash is sprayed on it at specific intervals. The windrows are then turned. During the composting process, the temperature gone up to 65-70°C. Due to turning of the pressmud and spent wash mixture., it gets good aeration and increase bacterial activity thereby accelerating the composting process and watered regularly to maintain the moisture content 50-60%. The composting process takes typically 60 days to complete the cycle. The bio-compost was collected from the Bannari Aman Sugars near Sathyamangalam. The chemical composition of bio-compost was given in Table 4.

Bagasse Ash

The bagasse ash is an organic industrial by-product obtained from the sugar industries in the process of sugar production. The bagasse obtained from crushing of sugarcane is also used up as a fuel to produce energy in most sugar industries and ash produced during the combustion of bagasse comes out as the ash. Disposing this wastes is one of the constrains so to make use of it's chemical compositions, which are a source of nutrients to plants and also enables the waste management and utilization. The bagasse ash was collected from the Bannari Amman Sugar near Sathyamangalam. The properties and composition of bagasse ash used is furnished in Table 1.

Rice Husk Ash

Rice mills produce lot of rice husk is milling process and the husk are used as burning fuel to the boilers in the rice mill. Even though the ash is only 1/4th of weight of rice husk, it still a concern for its proper disposal. The low carbon content in ash enables it's application on agriculture as a nutrient source to the crops. It is low in nitrogen but it is having other nutrients like phosphorus, potassium and micronutrients such as iron, manganese, zinc etc. It was collected from a rice mill near Nambiur, Erode district of Tamil Nadu. The properties and composition of rice husk ash used is given in Table 1.

Sampling of Organic Amendments

The organic sources were collected, mixed thoroughly and made into heaps. The home homogenous samples from heaps were drawn by means of a scoop from different parts *viz.,* front, middle and back and at different depths. Reduced the bulk tone kilogram level by quartering, final homogeneous samples were subjected to various analysis (Table 2).

Field Experiment

A field experiment was carried out to study the effect of soil application of RDF as control, 100% RDF + Municipal solid waste compost @ 5 t ha^{-1} and 75% RDF with municipal solid waste compost @ 10 t ha^{-1}, 100% RDF with bio-compost @ 2.5 t ha^{-1} and 75% RDF with bio-compost @ 5 t ha^{-1}, 100% RDF with bagasse ash @ 5 t ha^{-1} and 75% RDF with bagasse ash @ 10 t ha^{-1}, 100% RDF with rice husk ash @ 5 t ha^{-1} AND 75% RDF with rice husk ash @ 10 t ha^{-1} on growth, yield and nutrient uptake of maize as well as response level of soil application of above conventional, non-conventional organic source sources and industrial by-products to maize. The experiment was conducted in randomized block design (RBD).

Location of the Experiment Site

The field experiment was conducted at the farmer's field Karapadi village, Sathyamangalam taluk, Erode district, located in Western Zone of Tamil Nadu at 11°20′47.7″N latitude 77°11′53.6″E longitude and at an altitude of 577.6 meters above mean sea level.

Treatment Details of Field Experiment

T_1 – Control – 100% RDF
T_2 –100% RDF + Municipal solid waste compost @ 5 ha^{-1}
T_3 –75% RDF + Municipal solid waste compost @ 10 ha^{-1}
T_4 –100% RDF + Bio-compost @ 5 ha^{-1}
T_5 –75% RDF + Bio-compost @ 10 ha^{-1}
T_6 –100% RDF + Bagasse ash @ 5 ha^{-1}
T_7 –100% RDF + Bagasse ash @ 10 ha^{-1}
T_8 –100% RDF + Rice husk ash @ 5 ha^{-1}
T_9 –75% RDF + Rice husk ash @ 10 ha^{-1}

Details of the Field Experiment

Location	:	Farmer's field at Karapadi village, Sathyamangalam taluk of Erode district
Crop	:	Maize
Variety	:	KAVERI 25K55
Duration	:	105 days
Design	:	Randomized block design (RBD)
Treatments	:	Nine
Replications	:	Three
RDF	:	150:75:75 N: P_2O_5:K_2O kg ha^{-1}

Analysis of Soil Sample

Physical and Chemical Properties

Soil samples were collected just before the start of the field experiment and at harvest to determine the various physico-chemical characteristics and nutrient status of the soil. The collected soil samples were air dried in shade, ground with wooden mallet, passed through 2 mm sieve and stored in polythene bags. These samples were analysed for pH, EC, organic carbon, available NPK. The post-harvest soil samples were analyzed for organic carbon, available NPK. The details of procedure followed for the analyzed of soil samples are listed in Table 3.

Biological Properties

The changes in the biological properties of soil *viz.,* bacteria, fungi and actinomyctes population, and dehydrogenase activity due to application of conventional, non-conventional, organic sources and industrial by-products were studied for the samples collected at post-harvest stage following the standard procedures detailed below.

Estimation of Soil Microbial Population

To estimate the number of soil micro flora, counts were calculated on the basis of serial 10 fold dilution technique, using the pour plate methods and replicate of 10 g soil samfples, and an appropriate dilution as described by Johnson and Curl (1972). 10 g of air dried soil was taken from each soil samples and were sieved properly to discard all the foreign particles and added with 100 ml of sterilized distilled water to make a dilution of 10^{-1}, from this dilution 10 ml of the aliquot was transferred to 90 ml of sterilized distilled water to make dilution of 10^{-2}. Likewise, the soil samples were serially diluted (six fold series). Aliquots of 1 ml from dilution 10^{-5} were spread on nutrient agar medium for total bacterial and from 10^{-4} on rose Bengal gram for fungi and ken knights medium for actinomycetes. Each dilution was spread onto five replicate. After microbial colonies are readily visible was counted at 2-7 days for bacteria fungi and 7-14 days for actinomycetes, after incubation at 32±2°C for bacteria and 25±2°C for fungi and actinomycetes. The number of colonies on each plates were counted and colony forming units per g of soil (CFU g^{-1}) was calculated using the equation.

$$\text{CFU } g^{-1} = \frac{\text{No. of colonies}}{\text{Volume plated (ml)}} \times \text{Dilution factor}$$

Dehydrogenase Activity

The dehydrogenase activity was detrmined by the procedure as given by Casida *et al.* (1964). One g of fresh soil was taken in a test tube (15 ml capacity) and added 0.2 ml of 2,3,5-triphenyl tetrazolium chloride (TTC) solution (3%) and 1.0 ml of 1.0% glucose solution to each of the tubes and gently tapped the bottom to drive out all trapped oxygen. The test tubes were cotton plugged and incubated at 28±1°C for 48 h. After incubation 10 ml methanol was added to each tube and mixed vigorously. It was allowed to stand for 6 h for colour development. The clear pink/red cooured supernatant was withdrawn and readings with spectrophotometer at a wave length of 485 nm and the results were expressed as μg TPF h^{-1} g^{-1}.

RESULTS AND DISCUSSION

Physico-Chemical Properties Of Experiment Soil

The composite soil at 0-15 cm collected from the farmer's field in Karapadi village, Sathyamangalam taluk, Erode district were analyzed for various physico-chemical properties (Table 4). The textural composition of soil was coarse loamy. The experimental soil of Karapadi comes under the taxonomical classification *Typic Ustropept.* The soil pH was 8.1 with EC of 0.160 dSm^{-1}. The cation exchange capacity was 18 cmol (P^+) kg^{-1}. The organic carbon content was 2.7 g kg^{-1}. The available nitrogen, phosphorus and potassium content of the soil were 116.0, 15.0 and 235.0 kg ha^{-1} respectively recording low, low and medium status in soil fertility. The dehydrogenase activity records as 7.2 ⊚g TPF g^{-1} soil h^{-1}.

The bacterial population (14.2 × 10^6 cfu g^{-1} soil), fungi population (8.4×10^4 cfu g^{-1} soil) and actinomyctes population (6.3× 10^2 cfu g^{-1} soil) were registered in the initial soil.

Microbial Population

Bacteria

The result revealed that amongst the microbes, bacterial population was the highest compared to fungi and actinomycetes in soil. The highest bacterial population (60×10^6 cfu g^{-1} soil) was recorded in the treatment T_3 (75% RDF + Municipal solid waste compost @ 10 t ha^{-1}) (Table 5). The organic addition coupled with NPK fertilization exerted a stimulating effect influence on the preponderance of bacteria in soil. This indicated the importance of easily degradable compounds for the proliferation of bacterial population in the soil (Hanene Cherif *et al.,* 2009).

Fungi

The results showed maximum fungal population with treatment T_3 (75% RDF + Municipal solid waste compost @ 10 t ha^{-1}) (Table 5). Among the organic sources, performance of municipal solid waste compost stimulates fungal growth were of higher order which is mainly attributed to vegetable wastes available from municipal solid waste compost (Guerrero *et al.,* 2000).

Actinomycetes

The highest actinomycetes population of 12.2×10^2 cfu g^{-1} g^{-1} soil was registered in the treatment T_3 receiving 75% RDF + Municipal solid waste compost @ 10 t ha^{-1} (Table 5). It is known that municipal solid waste compost stimulates microbial population and biological activity in soil. The number of colony forming units of actinomycetes in soil increases when municipal solid waste compost is added. Soil biological activity and microbial growth increased when municipal solid waste compost is added. Application of municipal solid waste compost increases soil actinomycetes and organic matter increases the amount of carbon (C)to soil as well as the availability of C and nitrogen (N) sources that can be used by soil actinomycetes significantly (Sonia Mokni-Tili *et al.,* 2009).

CONCLUSION

The combined application of 75%RDF +Municipal solid waste compost @ 10tha-1 recorded highest dehydrogenase activity and microbial count (bacteria, fungi and actinomycetes population)

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Table 1. NPK composition of materials

Materials	Organic carbon (g kg^{-1})	Total N (%)	Total P (%)	Total K (%)
Municipal solid waste compost	270	1.13	2.92	0.53
Biocompost	201	1.2	2.87	1.7
Bagasse ash	7.1	0.015	0.0048	0.022
Rice husk ash	–	–	0.09	0.92

Table 2. Methods of analysis of organic manures and industrial by-products

S. No.	Parameters	Methods	References
Municipal solid waste compost, bio-compost, Rice husk ash and bagasse ash			
1	Total nitrogen	Micro-kjedhal method (Diacid extraction H_2SO_4:$HClO_4$ in 5:1 ratio)	Humphries (1956)
2	Total phosphorus	Vanado molybdate yellow colour method (Triple acid extraction, HNO_3, H_2SO_4:$HClO_4$ in 9:2:1 ratio)	Jackson (1973)
3	Total potassium	Flame photometer (Triple-acid extract)	Jackson (1973)

Table 3. Methods of soil analysis

S. No.	Parameters	Methodology	References
Physico-chemical properties			
1	Textural fractions	International pipette method	Piper (1966)
2	Bulk density, particle density and pore space	Measuring cylinder method	Sree Ramulu (2003)
3	Soil colour	Munsell soil colour chart	U.S. Dept. of Agriculture Hand Book (2000)
4	Soil reaction, pH	Potentiometry (1:2.5 soil: suspension)	Jackson (1973)
5	Electrical conductivity, EC	Conductometry (1:2.5 soil: suspension)	Jackson (1973)
6	Cation exchange capacity, CEC	Neutral normal ammonium acetate method	Jackson (1973)
7	Organic carbon	Chromic acid wet digestion method	Walkley and Black (1934)
8	Available nitrogen ($KMnO_4$-N)	Alkaline permanganate method	Subbiah and Asija (1956)
9	Available phosphorus (Olsen-P)	Ascorbic acid blue method (spectrophotometry)	Watanabe and Olsen (1965)
10	Available potassium (NH_4OAC-K)	(Neutral 1N NH_4OAC extract) Flame photometer	Stanford and English (1949)
Biological properties			
11	Dehydrogenase activity (µg TPF g^{-1} soil h^{-1})	TTC dehydrogenase technique	Casida *et al.* (1964)
Microbial population			
12	Bacterial population (X 10^6 cfu g^{-1} soil)	Pour plate method	Johnson and Curl (1972)
13	Fungi population (X 10^3 cfu g^{-1} soil)	Pour plate method	Johnson and Curl (1972)
7	Actinomycetes population (X 10^3 cfu g^{-1} soil)	Pour plate method	Johnson and Curl (1972)

Table 4. Physico-chemical properties of the experimental soil

A.	Mechanical Properties	Content
1	Clay (%)	16
2	Silt (%)	12
3	Sand (%)	72
4	Textural classification	Coarse loamy
5	Taxonomical classification	*Typic Ustropept*
B	Physical Properties	
1	Bulk density (Mg m^{-3})	1.54
2	Particle density (Mg m^{-3})	2.53
3	Pore space (%)	42

C	Physico-chemical properties	
1	pH	8.1
2	EC (dSm^{-1})	0.16
3	CEC [$cmol(P^{+})\ kg^{-1}$]	18
D	CHEMICAL PROPERTIES	
1	Organic carbon ($g\ kg^{-1}$)	2.7 g kg^{-1} (Low)
2	Available nitrogen ($kg\ ha^{-1}$)	116 (Low)
3	Available phosphorus ($kg\ ha^{-1}$)	15 (Low)
4	Available potassium ($kg\ ha^{-1}$)	235 (Medium)
E	Biological Properties	
1	Dehydrogenase activity (⌾g TPF g^{-1} soil h^{-1})	7.2
F	Microbial population	
1	Bacteria population (X 10^{6} cfu g^{-1} soil)	14.2
2	Fungi population (X 10^{4} cfu g^{-1} soil)	8.4
3	Actinomycetes population (X 10^{2} cfu g^{-1} soil)	6.3

Table 5. Effect of conventional, non-conventional organic sources and industrial by-products on dehydrogenase activity, bacterial, fungal and actinomycetes count soil

Treatments	Dehydrogenase activity (⌾g TPF g^{-1} soil h^{-1})	Bacteria (x10^{6} cfu g^{-1})	Fungi (x10^{4} cfu g^{-1})	Actinomyctes (x10^{6} cfu g^{-1})
T_1 – Control – 100% RDF	5.1	15.2	8.3	6.4
T_2 –100% RDF + Municipal solid waste compost @ 5 ha^{-1}	9.7	50.4	15.2	10.6
T_3 –75% RDF + Municipal solid waste compost @ 10 ha^{-1}	10.2	60.3	16.8	12.2
T_4 –100% RDF + Bio-compost @ 5 ha^{-1}	6.8	44.2	12.2	9.4
T_5 –75% RDF + Bio-compost @ 10 ha^{-1}	7.0	48.4	13.8	11.4
T_6 –100% RDF + Bagasse ash @ 5 ha^{-1}	6.5	20.2	10.4	7.2
T_7 –100% RDF + Bagasse ash @ 10 ha^{-1}	6.7	21.4	11.2	8.2
T_8 –100% RDF + Rice husk ash @ 5 ha^{-1}	5.4	17.2	9.2	7.4
T_9 –75% RDF + Rice husk ash @ 10 ha^{-1}	5.9	18.4	10.3	7.2
Mean	7.03	32.85	11.93	8.88
S.Ed.	0.31	1.57	0.51	0.38
CD (P=0.05)	0.6	3.3	1.1	0.8

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RESEARCH ARTICLE

Phytomass-Derived Activated Carbon: A Potential Material for Antibacterial Resistance

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ABSTRACT

Recently, antimicrobial resistance seems to cause a universal setback on the medical ground. At the same time, it is noticed that the development of effective and wide spectrum antibacterials has reached a saturation stage because of the expression of newer bacterial strains, high epidemic and pandemic activity of bacteria (and viruses). In this situation, charcoal or activated carbon (AC) is playing a vital role as an antibacterial and antimicrobial agent in the ancient Indian and Chinese medicines. Hence connecting these information, the present investigation has been so intended to produce AC derived from the blades of sabai grass (*Eulaliopsis binata*) and further to prove its antibacterial efficacy against select human pathogens viz., *B. subtilis* & *S. pyogenes* (Gram Positive bacteria) and *E. coli* & *P. aeruginosa* (Gram Negative bacteria) by agar well diffusion method. Sabai grass plant is abundantly available in nature, free of cost and renewable too. Hence it is considered to be the material of our study making them more economically viable for producing a carbon based antimicrobial owing to the involvement of green precursor. % phytomass carbon yield, phase purity, ultimate elemental analysis, particle morphology, and surfacial organic moities were investigated on the phytomass carbon mass produced. Minimal Inhibition Concentration-MIC was also evaluated. Likely and probable mechanisms of action of the carbon against the pathogens are also presented. We have shown that pyrolysis of phytomass could provide eco-friendly active carbon materials by adopting the abundantly available sabai grass phytomass as the source to produce sulphuric acid activated carbon (SGAC) and evaluated its antibacterial efficacy against Gram Positive and Gram Negative bacteria by well-diffusion method. The MIC for the chosen bacterial strains has been evaluated as 80 µg/mL. Studies hint that the antibacterial activities of the SGAC-DMSO extracts increased linearly with increase in concentration of extracts (µg/ml) and the results further discovered that in the extracts for bacterial activity, *S. pyogenes* & *E.*

coli were more sensitive as compared with *B. subtilis* & *P. aeruginosa* based on the inhibition zone developed. SGAC has thus bestowed itself as a potential antimicrobial representative and could therefore be used for the prevention of microbial contamination in human being by topical application. As the results are complimentary, the investigation would prove an interesting and novel research topic in optimizing activated carbons for the targeted pharmaceutical and medical applications.

Keywords: activated carbon, antibacterial resistance, pathogens, phytomass, *Eulaliopsis binata*, sabai grass, well diffusion method.

INTRODUCTION

Our Nature has been an incessant resource of rich and diversified biomass which could be exploited for generating many value-added products for the benefit of the society but not at the cost of the biotic and abiotic ecosystems. One such value-added, high-end, low-cost and advanced medicinal and engineering material is the activated carbon (AC) that too obtained from zero-cost phytomass. Despite extensive research with phytomass derived carbon, further advancement in carbon materials is still considered necessary since carbon and AC are a class of popular material bestowing several indispensable spread of applications in medicinal, scientific and technological grounds, from alleviating environmental pollution i.e. adsorption of obnoxious gases, liquids and solids, treating wastewater i.e. water purification to intensifying non-conventional energy sources i.e. electrode materials for supercapacitors, batteries etc.

Directing the plant wastes and agro-industrial wastes for conversion into activated carbon may add economic value to a large quantity of wastes that are generally discarded. Plentiful agricultural wastes and various unused plant parts offer an inexpensive and renewable additional source of ACs. ACs obtained from agricultural by-products has many advantages but not limited to, as a low-cost replacement for many types of carbon-based materials obtained from non-renewable sources and costly-cum-elaborate processes. Thus we thought the focal point should be to develop low-cost carbon, especially from wastes, with inspiring properties for many promising applications. These have become a reality only because of the high surface area of the AC employed [1-3] Besides high surface area, it integrates numerous other key advantages related to their structural composition (porosity) and functional composition (presence of rich and varieties of surface organic functional groups) which are the inherent features that add to the performance of the AC powders. Important factor that contributes to the above-said features is the nature of the raw materials (precursors) and the methodology adopted for producing AC powders. Generally production of AC can be tedious, costly and taxing on our environment [4]. Nevertheless, several recent researchers of carbon material scientists have indicated that low-cost yet high performing AC carbons could efficiently be produced from various precursors including residues from agricultural and forestry space. It should also be noted by the way that the precursors used for the preparation of AC should be rich in carbon content [4]. The readers of this article could have a good view on the production of activation carbon from ref. [5]. Almost all lignocellulosic matters can be used as a starting material for deriving ACs [6].

Further, phytomass derived ACs may have sulphur and nitrogen along with carbon, hydrogen and oxygen. Compounds containing sulphur and nitrogen are interesting owing to their significant antifungal, antibacterial and anticancer activity [7]. Scanning of literature of past 6 years about biological activities of phytomass derived AC gives not many articles yet the authors of this communication summarizes the following important reports. Kitchen soot was once applied as an antimicrobial with a special name of "*old woman's remedy* [8]. Antimicrobial activity of carbon nanoparticles isolated from natural sources against pathogenic Gram negative and Gram positive bacteria was studied by Sheena et. al. [8]. Yallappa et. al. [9] have reported a work using groundnut shell and likewise a review on activated carbon nanoparticles from biowaste as new generation antimicrobial agents was done by Lakshmi et. al. [10]. Karthik et. al. [11] have prepared AC from *Tribulus terrestris* and have shown activity against *E.*

coli, *B. subtilis*, *S. aureus* and *K. pneumoniae*. Sekaran et. al. [12] have reported the production of mesoporous AC from rice husk by precarbonization at 400 ^{o}C, chemical activation using phosphoric acid and have immobilized *Bacillus sp.* in this mesoporous AC for the degradation of sulphonated phenolic compounds in waste water samples. Shamsi et. al. [13] have reported a inhibition of carbon nanoparticles derived from sandalwood bark against *E. coli*, *B. cereus*, *C. violeceum* and *P. notatum*. Dheepan et. al. [14] have reported antibacterial efficacy using AC prepared from *Passiflora foetida* against a few pathogenic strains. Further, in the recent report by the authors of the present communication [15], *Vitex negundo* plant leaves were utilized to produce AC and proved the antibacterial potential against a few bacterial pathogens. Thus it can be seen that activated carbon materials produced from plant biomass still has enormous potential for acting against various strains bacteria. It is worth mentioning here that one of the authors of the present article had investigated a dozen of biomass wastes for producing AC for the possible application as electrodes in for energy storage [16] and as electrocatalyst support for hydrogen gas generation through water electrolysis [17]. The present work is yet another preliminary investigation to prove the value of a waste as an antimicrobial. The purpose of the present work was thus to investigate the possibility of producing AC derived from the blades of sabai grass (*Eulaliopsis binata*) and further to assess its antibacterial efficacy against pathogens viz., *B. subtilis* & *S. pyogenes* (Gram Positive bacteria) and *E. coli* & *P. aeruginosa* (Gram Negative bacteria) by agar well diffusion method.

Sabai grass is a plant which is locally available; abundant in nature at free of cost. Hence sabai grass plant leaves have been the material of our study leading to a value added product namely AC. Sabai grass, like many other grass plant, is benign both from environmental and health perspectives. It is a bush with natural fibres that brings social and economic benefits. These fibres mainly enrich the textile industries. Sabai grass is a perennial plant, belonging to the family *Poaceae*, and is known to grow in many Asian countries. As the blades of sabai grass appear slender, long, and flexible and possess superior-quality strong fibre, it is utilized as a major material for the paper as well as textile industries in India since 1870 [18]. Thus sabai grass possessing multi-fold applications and also socio-economic values has been chosen as a zero-cost precursor for the present study for converting in to AC for evaluating its antibacterial efficacy of this sabai grass derived activated carbon (SGAC). It is expected that the study reveal a new path for producing low-cost antibacterial in the pharmaceutical and medicinal fronts.

EXPERIMENTAL METHODS

All the chemical reagents used for the preparation of SGAC were of analytical grade and were procured from Merck, India. Double Distilled water was collected from in-house water plant built in-house. Grass was collected from a farm land near our MKU Campus and specimens with voucher No. PK/MADU/CHEMDDEMKU/027/03/2019-20 were deposited and preserved at The Dept. of Botany of one of the reputed Institutes, Tamil Nadu, India and was identified and authenticated by a Taxonomist/Botanist of the same Institute.

Preparation of sulphuric acid activated sabai grass derived activated carbon (SGAC)

Blades from sabai grass bunches were collected from our Institute, plucked flowers and stems off and washed several times with distilled water to remove soil, dust and dirt and were dried under sun. The completely dried grass were crushed into pieces with hand and finally with a blender to a fine powder. Activation was done by soaking a known mass of the grass powder with conc. H_2SO_4 in the ratio of 1:1(w/v) for 24h at room temperature (30^{o}C). On adding acid to the grass powder enormous fumes (as a result of aggressive action of the acid on the grass resulting in decomposition/charring of the various chemical constituents of the grass) evolved with concomitant generation of heat. Hence of acid was added under cold water and carefully mixed until the heat subsided. The resulting black colored mass was left to rest for the whole day after which time the mass has crumbled in to a loose or coarse powder. The mass was thermally activated at 800^{o}C of 1h under continuous N_2 flow. After activation, the carbon sample was washed several times with hot distilled water and ensured the neutrality in pH and absence of sulphate ions. Further, low conductivity of the washings ensured thorough washing of the carbon sample. The

powder was finally oven dried and ground. The powder now designated as SGAC, was stored in an air tight vials for further studies.

Physio-chemical characterization of SGAC

Physio-chemical characterization of SGAC includes burn off, ultimate elemental analysis, X-ray Diffractometry (XRD), Fourier Transform Infrared spectroscopic studies, (FTIR) and Scanning Electron Microscopic studies (SEM). Elemental analysis of the sample was made with Vario ELIII CHNS/O elemental analyzer. X-ray diffraction pattern of SGAC was recorded between 10 to 80^{o} on X'Pert Pro X-ray diffractometer with CuK_{α} radiation source. The morphology of SGAC was imaged with Hitachi S-4700 model scanning electron microscope (SEM). A thin layer of gold was sputtered on the sample surface for charge dissipation during imaging. The sputtering was done using 6mA current in argon atmosphere for 4min. Organic functional groups on the surface of the SGAC were ascertained using FTIR spectrometer (Model # Nexus 670) from 4000 to 400cm^{-1}. The ultimate analysis of the activated carbon was carried out using Vario ELIII CHNS/O Analyzer. The specific surface area of SGAC was studied by N_2 adsorption at 77K with an ASAP 2020 model instrument (Micromeritics, USA). The surface area (S_{BET}) of SGAC was calculated from the isotherms using BET equation.

Minimal Inhibition Concentration (MIC)

Minimal Inhibition Concentration (MIC) is the regular procedure followed for determining the vulnerability of the pathogens to an antimicrobial. MIC is defined as "the lowest concentration of an antibiotic (antibacterial in this study) that will inhibit the visible growth of bacteria/pathogens after overnight incubation" [19]. Method of dilution was followed for verifying the MIC in this case. In a typical practice, an extract was prepared by dispersing 50 to 90µg of SGAC in 1 mL of 5% DMSO-water mixture with sterile nutrient broth in sterilized glass test tubes by sonicating for 30 min. By means of a standard and sterilized wire loop, purchased from Merck, India, a loop full (10 µL) of, for e.g., *B. subtilis* culture, was inoculated into the test tubes containing 1 mL of the various concentrations of the SGAC mixture in the nutrient broth. Similar procedure was followed for the other strains. The test tubes were incubated at 38 °C for 24 h and observed through unaided eye for any growth or turbidity.

Antibacterial efficacy of SGAC by agar well diffusion method

Antimicrobial efficacy of SGAC was investigated by agar well diffusion method [20] against four human pathogenic bacterial strains *viz., Bacillus subtilis* (*MTCC 441*) *and Streptococcus pyogenes (MTCC 442)* (Gram positive bacteria), *Pseudomonas aeruginosa* (*MTCC 424*) *and Escherichia coli (MTCC 443)* (Gram negative bacteria) pathogens and have been selected on a trial basis only for exploring the efficacy of SGAC, which could even provide details related to formulation or improving the features of SGAC. Antibacterial efficacy of SGAC was examined using 5% DMSO (Dimethyl sulfoxide) as a solvent.

Culture and maintenance of microorganisms

The media, bacterial strains and other chemicals used in the study were the products of Himedia Laboratories Pvt, Ltd., India. Clean Borosil glass wares were used. The cleaned glasswares, swabs and well cutter were sterilized in an autoclave at 120°C for 15 min. The pure bacterial cultures were maintained in nutrient agar medium. Each bacterial culture was further maintained by sub-culturing regularly on the same medium and stored at 4°C until use.

Preparation of SGAC for antibacterial studies

The SGAC powder was dispersed in 5% DMSO in water by following the method described in ref. [9]. To test the antibacterial efficacy of SGAC, 10 different concentrations of SGAC ranging from 100 to 1000 µg/ml were prepared by dispersing100 µg to 1000 µg of the powder in 1ml of 5% DMSO in water mixture by sonicating for 30min.

Microbiological screening, media preparation and sterilization

Antibacterial activity of the GAC against different pathogens was observed by the well established Agar Well Diffusion method [19]. For agar well diffusion method, antibacterial susceptibility was tested on nutrient agar by

growing the bacterial colony. Nutrient agar was prepared using 0.5% peptone, 0.3% beef extract 0.5% sodium chloride, 1.5% Agar and finally the media pH was adjusted to 6.8. All the components prepared for sterilization was autoclaved at 120°C for 15 minutes and the media was poured in to the sterilized petri plates.

Agar well diffusion method

Under sterilized conditions, required number of nutrient agar plates were swabbed using sterile cotton swabs with 12h old-broth culture of the respective bacterial strain. Required numbers of wells of 6mm diameter were punched into the agar on each plate using a sterile cork borer. Into the wells on the petri plates, 10µl of the sample extracts of different concentrations ranging from 100µg/ml to 1000µg/ml was added using a sterile micropipette and the setup were allowed to diffuse at ambient temperature for 2hrs. In one well, 20µl of 5% DMSO in water which is the blank was placed. The plates were incubated at 37ºC for 24h. Inhibition zone test was carried out to qualitatively investigate the antibacterial property of the SGAC powder. It is a known fact that if the growth of the bacteria was inhibited, a clear zone, called the zone of inhibition, around the wells will be observed and measurement of this zone will give an idea of extent of inhibition. Hence usually diameter of the zone of inhibition is measured and the values are presented in millimeters (mm). Activity of the SGAC sample against the four pathogens was compared. SGAC is considered to be inactive against a pathogen if the corresponding zone of inhibition value is lesser than 8mm or no zone was developed around the wells.

Analysis of data

The zone of inhibition data are presented as a mean of the biological triplicate measurements±Standard Error of Mean (SEM). Statistical Package for Social Sciences-SPSS version 20.0 software was used for the analysis of data. The statistical difference of the mean zone of inhibition due to the four pathogens was independently carried out by one-way analysis of variance (ANOVA) at a significant level of $p<0.05$.

RESULTS AND DISCUSSION

AC sample produced from sulphuric acid activation of the sabai grass blades was assessed by different techniques and the important and significant results achieved are elaborated in the sections to follow.

Physio-chemical features of SGAC

Yield and Burn-off

While yield is the ratio of the weight (w) of the AC to that of the dry and powdered biomass (w_0) and is given by the following formula, Yield (%) = $(w/w_0) \times 100$, burn-off, on the other hand, is the % of weight loss due to the activation step. The yield of SGAC was calculated as 60% and thus burn-off as 40%, which means nearly 40% of the precursor mass has been lost during acid and thermal activation steps and also in washings.

Elemental (CHNS) analysis

The SGAC sample was subjected to ultimate analysis and the result has been presented in fig. 1. Significant % of N & O in the samples shows the presence of various organic functional moieties. The analysis has indicated the absence of S content in SGAC sample. The authors consider this as an importance result because the presence or absence of S &/or N contents in the sample could provide clues regarding the mechanism of action or activity in killing or inhibiting the growth of the pathogens chosen of the study. The presence of various organic groups has also been corroborated through FTIR studies and thus is rigorously considered in explaining the mechanism of biocidal activity of the SGAC powder.

Phase analysis by X-ray diffractometry (XRD)

XRD pattern of SGAC is shown in fig. 2. The broad reflection between 23° & 30° indicates (002) diffraction peak, attributed to the amorphous and low graphitization features of the SGAC. The broad shape is also indicative of the

present of highly disordered structure in SGAC [21]. As of now, the authors reserve investigation of correlating the biological activity and the extent of graphitization in SGAC for the future.

Morphology by Scanning Electron Microscopy (SEM) and surface area measurement

Surface morphology of the SGAC particles has been depicted in fig. 3 and suggests that the crystallites appear as near square and rectangles and individual crystallites to be agglomerated together and are compacted together with a good network of all particles and porosity is also observed. H_2SO_4 being very active has worked aggressively on the cellular structure of the sabai grass imparting porous nature to SGAC. It is needless to stress that the morphology of the biomass carbon depends on the activation conditions. The porous structure and hydrophilic character of the carbon is presumed to be useful for the attachment of microorganisms for growth inhibition.

Specific surface area of SGAC; S_{BET} was found to be 1003 m^2/g, which is relatively a high value amongst the reported biomass derived AC. High surface area could mean more available active attractive sites for action on pathogens.

Fourier Transform Infrared (FTIR) vibrational studies

It is well documented that plant biomass contains active principles such as amino acids, proteins, triterpenoids saponin, steroids, glycosides, anthraquinone and flavonoids out of which tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms. It is to be understood that thermal processing of the phytomass would result in the decomposition of the various chemical constituents and develops into various functional organic moieties on the surface of the SGAC as demonstrated from FTIR analysis. In addition to various physical features said above, the anti bacterial activity of the carbon depends also on the chemical structure and nature of the functional moieties present on the carbon surface. Hence FTIR data was obtained for qualitative information on the surface organic functionality that may be present on the SGAC sample in a way correlate the bacterial activity of the AC with the pathogens studied. Fig. 4 represents the complex FTIR of SGAC. The interpretation and assignments of various FTIR peaks are as follows.

It is evident that from fig. 4 that SGAC shows four strong peaks in the region of 3500~1000 cm^{-1}. The peaks could respectively be recognized to be due to the stretching vibration of –OH groups appearing around 3754cm^{-1} and of C=O bond between 1720cm^{-1} & 1621cm^{-1} [22]. The small peak around 1840cm^{-1} justifies the asymmetric stretching vibration of C=O of carbonyl groups [21]. Strong signature between 1600 & 1510 cm^{-1} may be due to stretching vibration of benzene ring skeleton. Very less intense peaks around 1650 – 1575cm^{-1} could be due to NH bending and/or O-H bending vibrations of adsorbed water along with some carboxyl groups [23]. The peak at 1091cm^{-1} may be endorsed due to the vibration of C–O bonds.

The FTIR spectrum of SGAC also shows a characteristic broad peak around 3336cm^{-1} which is ascribed to the N-H stretching of amino group [24]. A peak around 2250 –2220cm^{-1} may be due to –C≡N stretching [24]. Peak at 1627cm^{-1} could be due to the C=C ring skeletal stretching vibration of aromatic carbons [25] & IR signature at 1375cm^{-1} could be assigned to the C–H in plane bending vibration of allylic carbon [26]. The band around 1370cm^{-1} may be due to N–H stretching. Peak at 1228cm^{-1} may represent C–O–C stretching vibration of ether [22]. Moderate intense peaks at 1375 cm^{-1} &1315cm^{-1} might be due to C-F stretching vibration. A band registered from 800-600cm^{-1} has been attributed to C-H out of plane bending [21]. Thus FTIR spectroscopic studies confirms the presence of hydroxyls, carbonyls, carboxyls, amines and alcoholic functional groups, in addition to other oxygen containing organic moieties rendering hydrophilicity to SGAC, governing the surface chemistry [27, 28]. These results indicate the possible involvement of these organic functional moities in the bactericidal properties and efficacy.

Minimal Inhibition Concentration (MIC)

MIC is that concentration of antibacterial which exhibited no turbidity or bacterial growth after the first 24 hours of incubation show signs of turbidity after the addition of equal volumes of the sterile nutrient broth and further 24 hours of incubation. Data on MIC has presented in table 1.

Antibacterial activity studies

Each antibacterial assessment was performed in biological triplicates and the mean values, which represents the inhibitory effect of SGAC±SEM is presented in table 2. The antimicrobial activity of the extracts of *SGAC* were studied in different concentrations (100 to 1000µg/ml) against four pathogenic bacterial strains *viz.,* two Gram positive (*P. subtilis* & *S. pyogenes*) and two Gram negative (*E. coli* & *P. aeruginosa*). Antibacterial potential of the SGAC extracts were assessed in terms of zone of inhibition of bacterial growth and the qualitative results of the antibacterial activities are presented in table 2. These observations have been presented *vide* figs. 5(a) & (b) also, representing one Gram positive and one Gram negative bacteria.

Minimal Inhibition Concentration (MIC)

Data in table 1 indicates that all bacterial strains showed turbidity after incubation in nutrient broth with different SGAC concentrations ranging from 50 through 80 µg/mL and thereafter, turbidity was not observed in all bacterial strains chosen and indicates no further bacterial growth. Hence 80 µg/mL has been thought of as the MIC for all the select bacterial strains.

Antibacterial activity studies of SGAC on select bacterial strains

Table 2 gives the susceptibility results of the various pathogens to growth inhibition by 5% DMSO extracts of SGAC. xThe zone of minimum inhibition concentration was measured in a range of 7mm in 100µg/ml for *E. coli* & *P. aeruginosa* and it was only 5.5mm±SEM and 6.9mm±SEM respectively with *B. subtilis* & *S. pyogenes* at a slightly higher concentration of 300µg/ml. It is evident from the studies that the pathogens tested were sensitive to almost all tested concentrations of SGAC sample, which was confirmed from the size of the zone of inhibition. Further, it is observed that the effect of antibacterial activity was low when the concentration was 100µg/ml and activity increased as the concentration of SGAC was increased. Also the zone of inhibition of SGAC at 1000 µg/mL is not significantly different from the value at 900 or 800 µg/mL but it is significantly different at 700 µg/mL and also at 100 µg/mL against the tested bacteria at $p<0.05$ significant level. That is to say, there was no significant improvement in the activity after700 µg/mL. Thus it is concluded from table 2 that the SGAC particles possess better bactericidal activity against both gram negative and positive bacterial strains. Nevertheless, SGAC shows better antibacterial activity against *S. pyogenes & E. coli* when compared to the other strains studied.

A comparison of the previous work of the authors with *Vitex negundo* leaves (VNLAC) [15] and the present work leaves at least four factors to investigate further. Firstly, the S_{BET}; secondly, the type, structure and bonding of hetero elements; and finally % of these elements. $S_{BET, SGAC}$ is observed to be higher than $S_{BET, VNLAC}$ and as mentioned earlier, higher surface area could mean more sites available for activity. So the slightly higher observed activity of SGAC could be envisaged in terms of S_{BET}. It is clear from the elemental analysis that SGAC has no sulphur containing groups and that % N is equal in both the samples. While in VNLAC, %C is very high & %O is low and vice-versa in the case of SGAC. Nevertheless, in the absence of S, N & O may have an important role in offering the observed antibacterial activity in the ACs investigated. It is to be noted that the presence of heteroatoms containing groups on the surface of the SGACs would also enhance the surface polarity and makes it more hydrophilic, the factors which predominantly attracts the pathogens towards the AC's surface. C provides mass to the sample and besides provides anchorage to various organic functional groups and hence all these factors seem to be competing with each other and hence a thorough investigation is invited for an effective phytomass derived AC based antibacterial or bactericidal to be developed. In other words, qualitative as well as quantitative studies on the hetero elements in ACs would prove an interesting and novel research topic in optimizing AC for the targeted applications.

Having put forth relevant physio-chemical inputs on the antibacterial activity of AC from a chemist's point of view, it is very pertinent to consider the various probable mechanisms by which the carbon particles act on the microorganisms to exhibit bactericidal properties. One of the mechanisms is that carbon particles adheres to the cell wall of the microorganisms, which contains a thin layer of peptidoglycan, through strong Lifshitz-van der Waals forces and the inhibition of bacterial growth is thus due to the interaction of the SGAC particles with this membrane

and sometimes particles may also permeate through the bacterial cell wall and tend to cleave the cell wall finally leading to bacterial cell death. The cell membrane damage causing changes in the membrane potential is a well-known mechanism of antimicrobial activity. Membrane potential of the bacterial cells can be studied using $DioC_2(3)$ (3,3′-Diethyloxacarbocyanine, iodide) fluorescence, which has been reserved for the future. In principle, $DioC_2(3)$ is a green fluorescent dye and forms red fluorescent aggregates with increasing membrane potential. The observed spectral shift will be then considered to measure the membrane potential ratiometrically [29].

Though the molecular and biochemical aspects of the antimicrobial activity of the carbon particles may be complicated and not fully understood it can be stated that carbon particles when introduced in to microorganisms are presumed to have activity against bacteria due to the affinity for proteins present in the bacteria and also the capability of the carbon particles to affect and modify the intracellular matters, enzymes, oxygen metabolism, membrane protein structures, and molecular metabolism. Further, it is generally a well known fact that antimicrobials reduce the growth rate and population of microorganism by extending the lag phase of microbes or inactivating them [30]. It is understood from the reports of Moranes et. al. [31] on the bactericidal effects of silver nanoparticles that bactericidal behavior is presumed to be attributed to the presence of electronic effects that are brought about as a result of change in local electronic structure of the surface of carbon particles due to smaller particle sizes [32]. The same explanation can also be given for SGAC also. These effects are considered to be contributing towards enhancement of reactivity of SGAC particle surface. Also carbon particles have several surface organic functional groups that might interact strongly with the thiol groups of vital enzymes of the bacterial cells to inactivate them and ultimately that DNA may probably lose its replicating ability when the bacteria are treated with SGAC [31]. Carbon particles may also tend to destabilize plasma membrane potential and depletion of levels of intracellular adenosine triphosphate by targeting bacterial membrane resulting in bacterial cell death finally.

Regardless of the incredible pace of introduction of novel nanoparticles, carbon based nanostructures or nanometal loaded AC, relatively only a few reports, for e.g., [33, 34] are available on the effects of the surface chemistry and size effects of carbon particles on the interaction with the bacterial cell resulting in cellysis to explain the bactericidal properties of the carbon particles. Thus it is essential to understand the sequence of cell death defined in terms of their structure and function when they interact with carbon particles *in-vivo*. Moreover, carbon particle-mediated cell death needs an in-depth understanding about the consequence of proteins conformation (denaturation), their changes and biological functions when particles like activated carbon penetrate through cell wall of the microbes and interact with them and how the microorganisms collapse by disrupting the cell membranes. Finally, we can say that imprudent use of antibiotics has resulted in the multitude of microorganisms to develop resistance to the commonly used antimicrobials. Consequently this necessitates the R & D of novel and high-potent antimicrobials. Therefore, novel technological developments towards the design and synthesis of carbon based antimicrobial or bactericidal agents from phytomass wastes which have the potential in reducing the microbial resistance and infection burden should be seriously looked into while the whole world in facing the COVID-19 pandemic.

It may be interesting to the readers of this communication to note that quite recently, carbon dots (CDs) have also gained impetus in this area and hence the authors present here a few pertinent literature reports on CDs to show that phytomass (derived) could be an excellent choice towards CDs in addition to activated carbons. Dong et. al. [35] have reported that carbon dots have enormous antibacterial activity at a Minimal Inhibitory Concentration (MIC) of 64mg/ml against *E. coli*. Gum arabic carbon dots synthesized using microwave assisted method and have been tested against two Gram negative bacterial strains of *E. coli* & *P. aeruginosa* at a concentration of 80mg/ml and it was found that 90% of bacterial cells are in live condition after incubation and shows minimum activity against both the bacteria [36]. CDs obtained from vitamin C was shown to be better against *E. coli* at a range of 75mg/ml [37]. The above studies would pave way for a new or next generation antibacterials.

CONCLUSIONS AND FUTURE DIRECTIONS

An ever escalating human population and industrialization have produced microbial pollutants. These pollutants are of societal concern and obviously with unbelievable antimicrobial resistance too. We also notice a constant multiplication and spread of bacterial resistant to several external factors and so pathogens tend to rapidly develop resistant against the commercially existing antiseptics and/or antibiotics. As a solution to this issue, we have reported in this paper that pyrolysis of phytomass could provide environmentally benign multifunctional carbon materials by adopting the zero-cost sabai grass phytomass as the source to produce sulphuric acid activated carbon (SGAC) and evaluated its antibacterial efficacy against two Gram Positive and two Gram Negative bacteria by well-diffusion method. Studies showed that the antibacterial activities of the SGAC-DMSO extracts increased linearly with increase in concentration of extracts (µg/ml). The results further revealed that in the extracts for bacterial activity, *S. pyogenes & E. coli* were more sensitive as compared with *B. subtilis* & *P. aeruginosa* based on the clear inhibition zone developed. SGAC has projected itself as a potential antimicrobial agent and could therefore be used for the prevention of microbial contamination by topical application.

However, further studies are to be extended to completely evaluate the effectiveness of the SGAC extracts as antimicrobial agents. It is believed that the present results will however form the basis for selection of phytomass for researching further leading to the discovery of new natural bioactive carbon based materials for the said application. Further studies which aim at correlating qualitative and quantitative effects of O, N, S heteroatoms and S_{BET} with antimicrobial activities would be carried out. Future studies would also be concentrated on utilizing SGAC for light-induced bactericidal effects and mechanisms. Yet our data allow suggesting SGAC as a promising starting point for the development of carbon inspired antibacterials.

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List of Abbreviations

AC – Activated carbon
B. subtilis – Bacillus subtilis
S. pyogenes – Streptococcus pygogenes
E. coli - Escherichia coli
P. aeruginosa – Pseudomonas aeruginosa
B. cereus – Bacillus cereus
C. violeceum – chromobacterium violeceum
P. notatum – Penicillium natatum
H_2SO_4 - sulphuric acid
w/v – weight by volume
SGAC – sabai grass derived activated carbon
MIC - Minimal Inhibition Concentration
XRD – Powder X-ray Diffractometry
FTIR - Fourier Transform Infra-Red spectroscopy
SEM - Scanning Electron Microscopy, Standard Error of Mean
CHNS/O – carbon, hydrogen, nitrogen, sulphur/oxygen
µg – micro gram
µL – micro litre

DMSO – dimethyl sulphoxide
DNA – Deoxyribo Nucleic acid
$DioC_2(3)$ - (3,3′-Diethyloxacarbocyanine
COVID-19 – corona virus disease–2019
CD - carbon dots

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spectrum antibacterial activity. ACS Appl Mater Interfaces, 24, 10(32):26936-26946. https://doi.org/10.1021/acsami.8b08832

Table 1. Inhibition of bacterial strains using different concentrations of SGAC in broth after incubation for 24 h at 37 °C.

Bacterial strains	Turbidity in broth concentration of SGAC (µg/mL)				
	50	60	70	80	90
B. subtilis	+	+	+	+	-
S. pyogenes	+	+	+	+	-
E. Coli	+	+	+	+	-
P. aeruginosa	+	+	+	+	-

+ indicates growth; - indicates no growth.

Table 2. Susceptibility of the pathogens to growth inhibition by 5% DMSO extracts of SGAC against bacterial test organisms

S. No.	Concentration (µg/ml)	Zone of Inhibition±SEM (in diameter; in mm)*				
		B. subtilis	*S. pyogenes*	*E. coli*	*P. aeruginosa*	Control (5% DMSO in water)
1	100	-	-	7.0±0.14	6.0±0.11	No zone
2	200	-	-	9.0±0.14	6.5±0.13	No zone
3	300	5.5±0.14	6.9±0.17	10.0±0.13	7.0±0.13	No zone
4	400	6.5±0.14	8.2±0.17	12.0±0.15	8.5±0.12	No zone
5	500	5.0±0.15	8.8±0.16	13.0±0.14	8.0±0.11	No zone
6	600	6.1±0.11	9.2±0.15	15.0±0.14	8.0±0.14	No zone
7	700	6.5±0.12	9.7±0.14	16.0±0.15	8.5±0.12	No zone
8	800	7.0±0.14	10.4±0.13	18.0±0.14	10.0±0.14	No zone
9	900	8.5±0.12	10.9±0.14	18.0±0.12	10.0±0.11	No zone
10	1000	8.5±0.12	11.5±0.15	18.5±0.12	10.0±0.12	No zone

***** Values are reported as mean±SEM (n=3); values <8 mm were considered in active against pathogens.

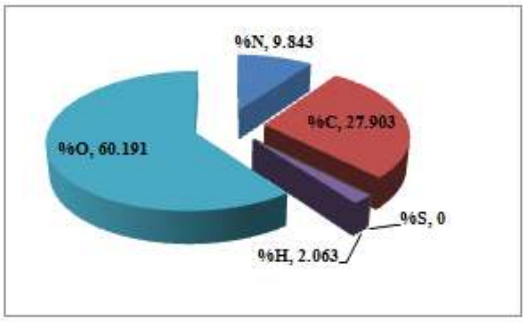

Fig. 1. Ultimate analysis of SGAC.

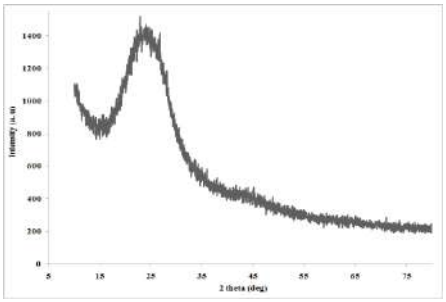

Fig. 2. PXRD of SGAC.

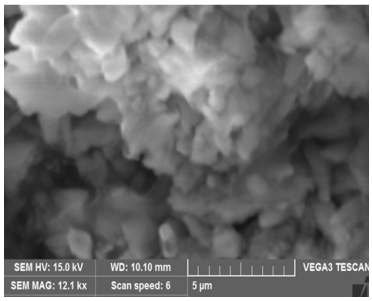

Fig. 3. SEM of SGAC.

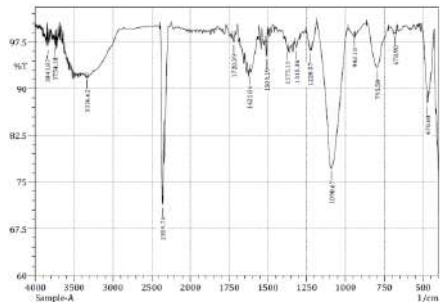

Fig. 4. FTIR spectra of SGAC.

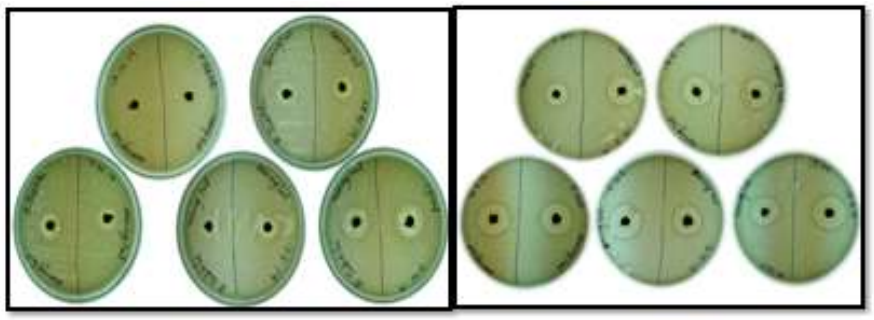

Fig. 5. Representative images for antimicrobial activities in agar plates showing SGAC and zone of inhibition against (a) *B. subtilis* & (b) *E. coli*

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RESEARCH ARTICLE

The Application of the Queuing Theory in a Vehicular Traffic Intersection Point, for Estimating and Optimization of Traffic Congestion in Dibrugarh, Assam

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ABSTRACT

Overcrowding on the highways is aggravating and bothersome. In recent years, the number of traffic has been rapidly increasing. This shift could be attributed to various factors, including population growth, limited resources, everyone's desire for their own vehicle, urbanization, etc. Well-functioning transportation infrastructure is critical in meeting the needs of individuals daily. Transportation is a critical aspect for urban incorporation at the individual level since it provides access to economic activities, facilitates family life, and aids in forming social networks. The number of vehicles has expanded in tandem with the expansion of our Indian economy, resulting in a significant increase in traffic congestion in road traffic. Due to the ever-increasing automotive volume, long and inconvenient traffic bottlenecks occur at most of the traffic intersections point in most of the towns in India. This work uses queuing theory and statistical experiments to analyze the traffic circumstances of an intersection in a particular city, create a mathematical model, and compare it to the actual data. A traffic intersection point in the Thanachariali area in Dibrugarh town, Assam, India, was chosen for analysis in this study. The majority of the data for the junction point simulation came from observations. The outcome demonstrates the application of queuing theory to the study of intersection traffic flow. Using Queuing Theory, we simulate and estimate traffic congestion on Thanachariali traffic intersection points. This strategy aims to

figure out what factors cause traffic congestion and then works to improve traffic flow. A Poisson process is considered to govern the traffic flow.

Keywords: Queuing theory, Traffic Congestion, Traffic Management.

INTRODUCTION

Traffic congestion is becoming a significant concern in many metropolitan areas, increasing travel time, air pollution, carbon dioxide (CO2) emissions, and fuel consumption. A competent traffic management system is critical for a country's economy as well as security. Traffic control issues are complex and becoming increasingly significant; these issues should be tackled scientifically. Simulation effectively addresses such complicated problems since it is based on well-developed statistical theories[1]. Many parameters (such as arrival time, departure time, waiting time, and so on) can be examined to the necessary level of precision. Traffic congestion also has a spillover effect from congested main routes to secondary roads and side streets as alternative routes are sought [2]. Such spillover effect results in delays which in turn leads to late arrivals for meetings and business activities. The study of intersection traffic flow is practical since intersection capacity directly impacts the efficiency of the highway network. Queuing theory takes its origin from the research credited to Agner Krarup Erlang, who created models to describe the Copenhagen telephone exchange [3]. The idea has been extended to applications such as telecommunication, traffic engineering, computing, and the design of factories, shops, offices, and hospitals. The paper explains how queuing theory can be used to tackle the problem of optimizing traffic congestion problems. Queuing theory is used broadly to cover a variety of problems, usually for economic balance and optimization involving waiting and delay in serving people or servicing machines and equipment [4]. In order to eliminate road delays in Dibrugarh town, Assam, this article aims to model vehicular traffic flow and investigate how vehicular traffic could be minimized using queuing theory. As a result of the influx of school students and market products delivery around the Thanachariali area, it is more dominant and severe throughout the morning and afternoon hours. Delays in the supply of products and services, excessive fuel use and pollution, annoyance, and the inability to anticipate journey time are all consequences of traffic congestion [5,6]. Using queuing theory, this research adds to the forecast of road traffic intensity in that location. The paper's approach defines traffic intensity as a performance measure for predicting the extent of queue build-up at traffic signal junctions in the chosen area. The prediction could help with efficient traffic management and avoid unnecessary delays [7,8]. This work tries to investigate road congestion problems and predict and provide information that may engender free traffic flow. The investigation is based on the traffic intensity of the Thanachariali intersection. The investigation can be extended to densely populated areas in India for comparison.

The traffic intersection points and the crossing areas

Four crossing areas, namely A, B, C, and D, can be identified in the traffic intersection point. Crossing area, A is in over bridge to Phoolbagan direction, B is in the railway station to Naliapool direction, c is in Phoolbagan to over bridge direction and D is in Naliapool to railway station direction. Later in our discussion, we will use the crossing as A, B, C, and D.

Literature review

According to the literature on queuing, waiting in line or queue results in inconvenience and economic consequences for individuals and organizations. Hospitals, airline businesses, banks, and manufacturing enterprises, for example, strive to decrease their clients' total waiting time and service costs. As a result, service speed is increasingly becoming a critical competitive factor [9]. According to Davis et al. (2003), managers have increasingly focused on offering ever-faster service with the ultimate goal of having no client waiting time. To start, in more developed countries with higher living standards, time becomes a more important commodity, and as a result, customers are less willing to wait for service. Second, firms increasingly realize that how they treat their consumers today has a significant impact on whether or not they will stay loyal customers in the future. Finally, technological advancements

such as computers and the internet have enabled businesses to provide faster services. For these reasons, hospital administrators, clinicians, and managers are constantly looking for ways to improve service delivery, assuming that waiting will harm post-service evaluation [10].

Additionally, analyzing and addressing hospital inefficiencies is critical for making health care policy and financing decisions. It was suggested that increased operational efficiency of the hospital is likely to help limit the cost of medical services, allowing for more inexpensive care and increased public access. Solving queuing problems requires making a trade-off between the expense of consumer wait time and the cost of delivering speedier service [11].According to researchers, service wait times can be reduced using one of two techniques: operations management or perception management [12]. The operation management part is coordinating patients (clients), lines, and servers in order to offer the best service possible at the lowest feasible cost. Patients' evaluation of service quality is affected by the actual waiting time and the perceived waiting time. The act of waiting has a significant impact on patients' satisfaction. The number of time customers must spend waiting can significantly influence their satisfaction [13]. Additionally, research has shown that customer happiness is influenced not only by wait time but also by customer expectationsor attributions of the waiting's causes. [14]. As a result, one of the difficulties in queue management is not just the actual duration of the customer's wait but also the customer's impression of that delay. Clearly, there are two ways to improve customer satisfaction with waiting times: by reducing actual wait times and by improving the customer's waiting experience [15]. Apparently, there are two ways to improve customer satisfaction with waiting times: by reducing actual wait times and by improving the customer's waiting experience[16,17]. Queuing theory is a mathematical tool for examining waiting lines in the field of operations management [18]. In queuing system arrivals create a demand on a resource with a restricted capacity. In the instance of the ante-natal care unit, pregnant women appear or seek treatment at random. The purpose of queuing analysis and its use in health organizations is to "minimize costs" - both tangible and intangible - to the organization. The rising cost of health care is due to a variety of causes, including an aging population, increased use of expensive and advanced treatment procedures, and inefficiencies in health delivery. The application of queueing theory aims to reduce the cost of providing health care services by minimizing system inefficiencies and delays [19]. Queuing theory involves the use of queueing models or mathematical models and performance measurements to evaluate and ideally enhance the flow of consumers through a queuing system [20]. A good patient flow results in little patient queuing, whereas a poor patient flow results in significant patient queuing delays [19]. Queuing theory is extremely versatile and is commonly used in the service business [10,19].

METHODOLOGY

Components of a Queuing System [20,21,22,23,24]
A queuing system, in general, consists of two components: the queue and the service facility.
Several fundamental components of the queuing system include the following:

Input or arrival Process
A queue can be avoided if arrivals and services are strictly scheduled.
However, this does not occur in practice. The majority of the time, arrivals are the result of external ***circumstances.***

Service process
The service mechanism is unclear in terms of the number of servers, the number of consumers served at any given time, and the duration and manner of operation. Queue networks are made up of multiple servers that are connected in series or parallel.

System Capacity

At most, the number of consumers who can wait in a queueing system at one time is a crucial element to consider. If the waiting room is huge, one can **reasonably conclude that it is limitless.**

Service pattern

All other criteria pertaining to the queue's norms of conduct can be grouped under this section. One of these is the policy that the server adheres to when admitting customers for service. In this context, norms such as first-come, first-served, First-Served (FCFS), Last-Come, First-Served (LCFS), and Random Selection for Service (R.S.) are self-explanatory. Ozigbo[19] argues that the fundamental components of a queuing system are arrival, servers, and waiting for lines. The analysis of queues is based on the construction of a mathematical model that represents the process by which an item enters the queue, the rules that govern their acceptance into service, and the time required to serve.

Characteristics of a Queuing Model [24,25,26,27,28,29,30,31]

The Arrival Process of Customers

Typically, this is based on the assumption that the arrival times are independent and have a common distribution. In many real-world scenarios, clients arrive in a Poisson stream (i.e., exponential inter-arrival times).Customers may arrive individually or in groups. A case in point of batch arrivals is the customs office at the border, where bus passengers' travel documents must be verified.

The Customers Behaviour

Customers may be patient and accommodating (for a long time). Alternatively, consumers may become impatient and exit after a short period of time.

The Service Times

Typically, this is based on the assumption that service times are uniformly distributed and independent of inter-arrival pattern.

Definition of Model Parameters

Pr= Probability of r customers in the entire system.
n = total customers in the entire system.
Ls= Expected number of customers in the entire system.
Lq= number of customers in the queue.
Ws= customers Waiting time of in the system
Wq= customers Waiting time in the queue.
λ=The arrival rate.
μ= The service rate

Statistical inference

This part mainly sets up the mathematical model based on data solves the problem by the appropriate method of Queuing theory and achieves the rationalization of the queuing system.

Poisson Service Times

The theory can be classified into two types: single-channel queuing systems and multi-channel queuing systems. The M / M /1 system is a single-channel queuing system. Assume that clients arrive at random. In an M/M/1 queueing system, we suppose that service times for customers are similarly negative exponentially distributed (i.e., generated by a Poisson process). Regrettably, this assumption is not as universal as the dispersion of arrival times. A queuing discipline determines the manner in which the exchange handles calls from customers. The most common queue discipline is "first- come, first served," abbreviated as FCFS; in some inventory

applications, the same rule is called "first in-first out" and abbreviated as FIFO. Additionally, this idea benefits customers one at a time. Additionally, this idea benefits customers one at a time. The customer with the shortest wait time, on the other hand, will be serviced first. The equations describing an M/M/1 queuing system are fairly straightforward and easy to use. To begin, we define traffic intensity(sometimes called occupancy).It is calculated by dividing the average arrival rate (λ) by the average service rate (μ). The average servicing rate should always be greater than the average arrival rate in order to maintain a stable system.

Mean performance parameters

a) Traffic intensity

$$\rho = \frac{\lambda}{\mu}$$

b) The mean number of customers in the system (N): can be found using the following equation

$$N = \sum_{i=0}^{\infty} i\rho_i = \frac{\rho(1-\rho)}{(1-\rho)^2} = \frac{\rho}{1-\rho} or \;\; N = \frac{\lambda}{\mu - \lambda}$$

c) Average number of customers in the queue (prior to service)

$$N_q = \sum_{i=0}^{\infty} (i-1)\rho_i = \frac{\rho}{(1-\rho)} - (1-(1-\rho)) = \frac{\rho^2}{(1-\rho)}$$

d) ***The total (mean) waiting time (including the service time):***

$$T = W = \frac{N}{\lambda} = \frac{\rho}{(1-\rho)\lambda} = \frac{1}{\mu(1-\rho)} = \frac{1}{\mu - \lambda} \quad \text{or} \quad T = W_q + \frac{1}{\mu}$$

e) Mean time spent waiting in queue (prior to service)

$$T_q = W_q = \frac{\rho}{\mu(1-\rho)}$$

Extraction of OSM file of the Thanachariali traffic intersection point

The OSM file obtained from the open street map is one of the advanced techniques in the traffic simulation study. OSM file extracted from the Open Street map can be used as one of the inputs in some advanced traffic simulation software like PTV vision SUMO etc. The OSM file can accurately capture both the longitude and latitude of the selected place. Thus it facilitates the measurement of various traffic parameters like velocity, distance covered, inter-arrival time, etc. It reduces the layout preparation time of the study area as compared to the other manual method of the traffic study. It contains street map information and saves as XML data in the form of node, way, and relations (between the street and other objects. This is open-source licensed, and it can be used as input in some open-source traffic simulation software like SUMO. The OSM file of the Thanachariali traffic intersection point has extracted the coordinates (94.90538-94.91037) and (27.47975-27.48232).

Observation and data collection using drone camera and static camera

Traffic movements have been recorded in that traffic point for 28 numbers of days during the month of December 2020. In this month total of 4 hours of data per day was captured, 1 hour in the morning, 2 hours in the afternoon,

and 1 hour in the evening each day. These data were mainly collected using two static video recorders continuously placed at two different locations to cover the entire point simultaneously and with a drone camera to cover the axial view of the traffic point.

Data Analysis

This recorded video was analyzed both manually as well as automatically**.**

For automatic or computerized data extraction purposes, the Good Vision Insight software was found to be useful.

Various traffic parameters were calculated using Queuing theory

Arrival Rate λ = 1387/3600 Cars/second
= .3852 Cars/second

Service Rate μ = 1387/1920 Cars/second
= .7223 Cars/second

Traffic Intensity $\varrho = \lambda / \mu$
= .3852/.7223
=.533

Mean time spent in the system $w = \frac{1}{(\mu - \lambda)}$
= 1/ (.7223-.3852)
= 2.96 second

Meantime spent waiting in queue w_q $= \frac{\varrho}{\mu(1-\varrho)}$

$= \frac{.533}{.7223(1-.533)}$
= *1.58*

Mean number of cars in the system N $= \frac{\lambda}{(\mu - \lambda)}$
$= \frac{.3852}{(.7223 - .3852)}$
=1.14

The mean number of cars in the queue (prior to service) N_q $= \frac{\varrho * \varrho}{(1-\varrho)}$
$= \frac{.533 * .533}{(1-.533)}$
= 0.6

RESULT AND DISCUSSION

From the result, it has been clear that Most of the crossing suffers a high traffic intensity in the morning 10 AM-11 AM as in this period most of the commercial as well as school vehicles are under operation. The traffic intensity at the afternoon session is somehow less than morning and evening sessions. The traffic intensity is at peak condition during the evening between 4 PM to 5 PM, i.e., in all the crossing, it ranges from 0.52 to 0.73. The maximum traffic intensity occurs at crossing no D in the evening. It can be predicted that if the car's arrival rate in crossing no D increases to more than 0.596 in the evening between 4 to 5 PM, the traffic intensity ϱ will be > 1, which will lead to infinite Queue formation in that crossing. As the number of cars is increasing day by day, it is expected that the cars arrival rate will increase in the near future. For keeping ϱ below 1, we have to either reduce λ or increase μ. λ can be decreased by providing extra lanes or by constructing over-bridge in those areas, which requires huge capital and human resources investment, but μ can be increased with optimum utilization of the existing resources. Finding the optimum timing of red traffic lights and green lights is one of the major aspects of the traffic simulation study. The increase in green light timings will increase the μ value of a particular crossing, but it will affect the μ value of other crossings. It has been observed that the average traffic intensity at crossing C is less than crossing D in the evening. Thus, increasing green light timing in crossing D by reducing the same amount of time from C will improve the

traffic situation in crossing D. This simulation will help in controlling the traffic intensity of a particular crossing in an optimum range. This study is important in controlling the traffic intensity of any crossing ϱ and service rate μ.

Future scope

The prediction of future arrival rate (increase in the car) and its effect on the present traffic system will be interesting. Keeping the ϱ<1 will be the main challenge in the near future. Construction of a new lane or overbridge as per the requirement of the traffic situation is also one of the significant future aspects of this study.

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Table 1. Traffic Movement Counts Report

DATE CREATED	17 Dec 2020, 11:15:34
CAMERA GROUP	Test Group
CAMERA	THANACHARIALI
MONITORED INTERVAL	17 Dec 2020, 14:27:01 - 17 Dec 2020, 14:32:30
TIME UNIT	1 minute
SELECTED OBJECT CLASSES	CAR, BUS, MOTORCYCLE, VAN, TRUCK, HEAVY TRUCK
ID	ea07bef1

Table 2. Overall traffic data Extracted from the Drone video by the good vision insight software.

APPROACH	**A**				**B**			
MOVEMENT	**A -> B**	**A -> C**	**A -> D**	**total**	**B -> A**	**B -> C**	**B -> D**	**total**
17 Dec 2020, 14:27:01 - 17 Dec 2020, 14:28:01	3	15	5	23	5	5	7	17
17 Dec 2020, 14:28:01 - 17 Dec 2020, 14:29:01	8	8	4	20	2	7	5	14
17 Dec 2020, 14:29:01 - 17 Dec 2020, 14:30:01	5	11	6	22	8	8	6	22
17 Dec 2020, 14:30:01 - 17 Dec 2020, 14:31:01	8	9	3	20	7	7	7	21
17 Dec 2020, 14:31:01 - 17 Dec 2020, 14:32:01	8	8	3	19	6	8	6	20

17 Dec 2020, 14:32:01 - 17 Dec 2020, 14:33:01	2	5	2	9	1	3	1	5
SUM	**34**	**56**	**23**	**113**	**29**	**38**	**32**	**99**

APPROACH	**C**				**D**			
MOVEMENT	**C -> A**	**C -> B**	**C -> D**	**total**	**D -> A**	**D -> B**	**D -> C**	**total**
17 Dec 2020, 14:27:01 - 17 Dec 2020, 14:28:01	9	9	4	22	0	5	5	10
17 Dec 2020, 14:28:01 - 17 Dec 2020, 14:29:01	6	7	4	17	1	4	4	9
17 Dec 2020, 14:29:01 - 17 Dec 2020, 14:30:01	7	3	6	16	13	11	5	29
17 Dec 2020, 14:30:01 - 17 Dec 2020, 14:31:01	7	5	4	16	9	10	5	24
17 Dec 2020, 14:31:01 - 17 Dec 2020, 14:32:01	16	8	3	27	5	12	7	24
17 Dec 2020, 14:32:01 - 17 Dec 2020, 14:33:01	13	3	2	18	1	1	0	2
SUM	**58**	**35**	**23**	**116**	**29**	**43**	**26**	**98**

Table 3. Detail analysis of the movement from A to B Extracted from the Drone video by the good vision insight software.

A -> B	**CLASS**						
INTERVAL	**CAR**	**BUS**	**MOTORCYCLE**	**VAN**	**TRUCK**	**HEAVY TRUCK**	**TOTAL**
17 Dec 2020, 14:27:01 - 14:28:01	2	0	1	0	0	0	3
17 Dec 2020, 14:28:01 - 14:29:01	2	0	4	0	2	0	8
17 Dec 2020, 14:29:01 - 14:30:01	1	0	4	0	0	0	5
17 Dec 2020, 14:30:01 - 14:31:01	2	0	5	0	1	0	8
17 Dec 2020, 14:31:01 - 14:32:01	2	0	6	0	0	0	8
17 Dec 2020, 14:32:01 - 14:33:01	1	0	1	0	0	0	2
TOTAL	10	0	21	0	3	0	34
CLASS %	29.41%	0.00%	61.76%	0.00%	8.82%	0.00%	100.00%
A -> C	**CLASS**						
INTERVAL	**CAR**	**BUS**	**MOTORCYCLE**	**VAN**	**TRUCK**	**HEAVY TRUCK**	**TOTAL**
17 Dec 2020, 14:27:01 - 14:28:01	1	0	11	0	3	0	15
17 Dec 2020, 14:28:01 - 14:29:01	1	0	5	0	2	0	8
17 Dec 2020, 14:29:01 - 14:30:01	4	0	5	0	2	0	11
17 Dec 2020, 14:30:01 - 14:31:01	2	0	4	0	3	0	9
17 Dec 2020, 14:31:01 - 14:32:01	4	0	4	0	0	0	8
17 Dec 2020, 14:32:01 - 14:33:01	0	0	5	0	0	0	5
TOTAL	12	0	34	0	10	0	56
CLASS %	21.43%	0.00%	60.71%	0.00%	17.86%	0.00%	100.00%
A -> D	**CLASS**						
INTERVAL	**CAR**	**BUS**	**MOTORCYCLE**	**VAN**	**TRUCK**	**HEAVY TRUCK**	**TOTAL**
17 Dec 2020, 14:27:01 - 14:28:01	0	0	5	0	0	0	5
17 Dec 2020, 14:28:01 - 14:29:01	1	0	2	0	1	0	4
17 Dec 2020, 14:29:01 - 14:30:01	1	0	5	0	0	0	6

17 Dec 2020, 14:30:01 - 14:31:01	1	0	1	0	1	0	3
17 Dec 2020, 14:31:01 - 14:32:01	0	0	0	0	3	0	3
17 Dec 2020, 14:32:01 - 14:33:01	0	0	1	0	1	0	2
TOTAL	3	0	14	0	6	0	23
CLASS %	13.04%	0.00%	60.87%	0.00%	26.09%	0.00%	100.00%

Table 4.Various types of vehicles were observed during the manual observation.

Sl. No.	Type/name	Length average In meters	Breadth average In meters	Average Speed In km/hour	Average number/hour
1	Bus	9.1	2.3	18.1	6
2	Truck	7.2	2.3	16.3	12
3	Car	3.9	1.5	20.5	160
4	Auto riskswa, Tempo	2.5	1.2	8.3	90
5	Motor cycle	2	2	7.2	170
6	others moving objects (cycle, man, cattle, etc.)	1	1.5	5.2	930

Table 5. Various results and outcomes of the study. Average traffic parameter of the crossings.

crossing	time	AVERAGE Arrival Rate (λ)	Average Service Rate (μ)	TRAFFIC INTENSITY (λ/μ)	w	w_q	N	N_q
A	10 AM to 11 AM	0.385	0.72239583	0.53333333	2.96387774	1.58203728	1.14109293	0.60952381
	12 PM to 1 PM	0.415	0.82239583	0.50462318	2.45461519	1.23865572	1.0186653	0.51404212
	2 PM to 3PM	0.315	0.79239583	0.39752859	2.0946978	0.83270227	0.65982981	0.26230121
	4 PM to 5 PM	0.435	0.77239583	0.56318274	2.96387774	1.66920478	1.28928682	0.72610408
B	10 AM to 11 AM	0.372	0.68239583	0.54513815	3.22169273	1.75626761	1.1984697	0.65333155
	12 PM to 1 PM	0.265	0.71239583	0.37198421	2.23515716	0.83144317	0.59231665	0.22033244
	2 PM to 1 3PM	0.225	0.64239583	0.35025134	2.39580734	0.83913473	0.53905665	0.18880531
	4 PM to 5 PM	0.335	0.61239583	0.54703181	3.60495682	1.97202605	1.20766053	0.66062873
C	10 AM to 11 AM	0.375	0.613	0.61174551	4.20168067	2.5703593	1.57563025	0.96388474
	12 PM to 1 PM	0.282	0.72	0.39166667	2.28310502	0.89421613	0.64383562	0.25216895
	2 PM to 1 3PM	0.285	0.67	0.42537313	2.5974026	1.10486528	0.74025974	0.31488661
	4 PM to 5 PM	0.311	0.588	0.52891156	3.6101083	1.90942803	1.12274368	0.59383212
D	10 AM to 11 AM	0.384	0.666	0.57657658	3.54609929	2.04459779	1.36170213	0.78512555
	12 PM to 1 PM	0.278	0.565	0.4920354	3.48432056	1.71440905	0.96864111	0.47660572
	2 PM to 1 3 PM	0.31	0.53	0.58490566	4.54545455	2.65866209	1.40909091	0.82418525
	4 PM to 5 PM	0.41	0.556	0.73741007	6.84931507	5.05075392	2.80821918	2.07080911

Figure-1: - Satellite view of some important traffic intersection points of Dibrugarh along with the study area

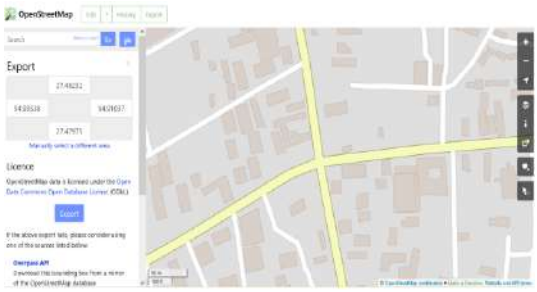

Figure-2: Openstreet map of the study area with longitude and altitude

Figure-3 The main crossings of the intersection, A, B, C, and D

Figure-4 :- Use of Open Street map in traffic simulation

Figure-5 use of a static camera for observation

Figure-6 use of Drone camera for observation

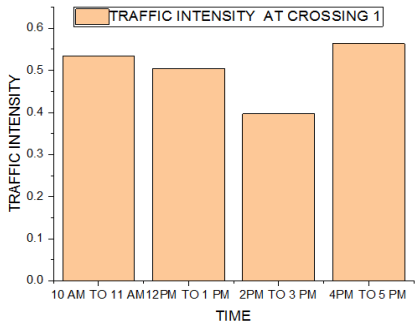

Figure-7 a AVERAGE TRAFFIC INTENSITY AT CROSSING A

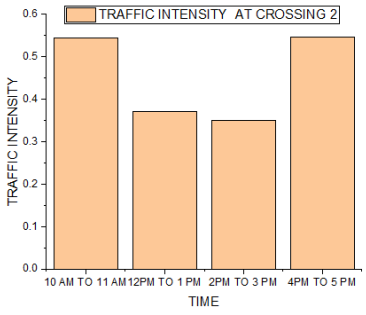

Figure-7 b AVERAGE TRAFFIC INTENSITY AT CROSSING B

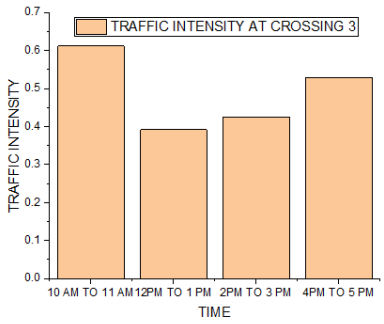

Figure-7 c AVERAGE TRAFFIC INTENSITY AT CROSSING C

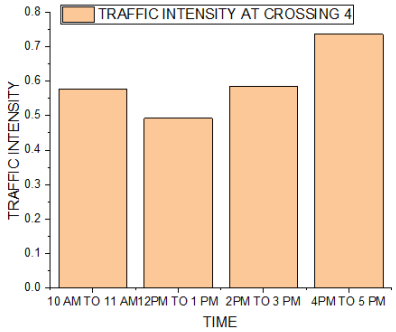

Figure-7 c AVERAGE TRAFFIC INTENSITY AT CROSSING D

Annexure 1. Manual Data observation sheet

			GREEN AND YELLOW	A	RED	B	A+B	(A+B)/75	(A+B)/40	
START TIME	END TIME	TOTAL TIME	LIGHT ON	VEHICLE ARRIVED	LIGHT ON	VEHICLE ARRIVED	TOTAL NUMBER OFVEHICLE	Arriv al Rate (λ)	Servic e Rate (μ)	
10:00:00	10:01:15	75	40	20	35	18	38	0.506666667	0.95	
10:01:15	10:02:30	75	40	17	35	18	35	0.466666667	0.875	
10:02:30	10:03:45	75	40	21	35	12	33	0.44	0.825	
10:03:45	10:05:00	75	40	16	35	12	28	0.373333333	0.7	
10:05:00	10:06:15	75	40	8	35	8	16	0.213333333	0.4	
10:06:15	10:07:30	75	40	14	35	13	27	0.36	0.675	
10:07:30	10:08:45	75	40	23	35	21	44	0.586666667	1.1	
10:08:45	10:10:00	75	40	12	35	13	25	0.333333333	0.625	
10:10:00	10:11:15	75	40	15	35	12	27	0.36	0.675	
10:11:15	10:12:30	75	40	16	35	11	27	0.36	0.675	
10:12:30	10:13:45	75	40	19	35	13	32	0.426666667	0.8	
10:13:45	10:15:00	75	40	21	35	12	33	0.44	0.825	
10:15:00	10:16:15	75	40	22	35	18	40	0.533333333	1	
10:16:15	10:17:30	75	40	23	35	16	39	0.52	0.975	
10:17:30	10:18:45	75	40	24	35	19	43	0.573333333	1.075	
10:18:45	10:20:00	75	40	21	35	17	38	0.506666667	0.95	
10:20:00	10:21:15	75	40	13	35	16	29	0.386666667	0.725	
10:21:15	10:22:30	75	40	18	35	14	32	0.426666667	0.8	
10:22:30	10:23:45	75	40	16	35	17	33	0.44	0.825	
10:23:45	10:25:00	75	40	18	35	16	34	0.453333333	0.85	
10:25:00	10:26:15	75	40	22	35	16	38	0.506666667	0.95	
10:26:15	10:27:30	75	40	21	35	14	35	0.466666667	0.875	
10:27:30	10:28:45	75	40	22	35	14	36	0.48	0.9	
10:28:45	10:30:00	75	40	16	35	12	28	0.373333333	0.7	
10:30:00	10:31:15	75	40	12	35	9	21	0.28	0.525	
10:31:15	10:32:30	75	40	13	35	11	24	0.32	0.6	
10:32:30	10:33:45	75	40	14	35	10	24	0.32	0.6	
10:35:00	10:36:15	75	40	17	35	9	26	0.346666667	0.65	
10:36:15	10:37:30	75	40	16	35	12	28	0.373333333	0.7	
10:37:30	10:38:45	75	40	12	35	14	26	0.346666667	0.65	
10:38:45	10:40:00	75	40	12	35	12	24	0.32	0.6	
10:40:00	10:41:15	75	40	14	35	13	27	0.36	0.675	
10:41:15	10:42:30	75	40	8	35	11	19	0.253333333	0.475	
10:42:30	10:43:45	75	40	13	35	13	26	0.346666667	0.65	
10:43:45	10:45:00	75	40	15	35	13	28	0.373333333	0.7	
10:45:00	10:46:15	75	40	11	35	14	25	0.333333333	0.625	
10:46:15	10:47:30	75	40	16	35	11	27	0.36	0.675	
10:47:30	10:48:45	75	40	12	35	14	26	0.346666667	0.65	
10:48:45	10:50:00	75	40	13	35	16	29	0.386666667	0.725	
10:50:00	10:51:15	75	40	12	35	14	26	0.346666667	0.65	
10:51:15	10:52:30	75	40	12	35	8	20	0.266666667	0.5	
10:52:30	10:53:45	75	40	14	35	11	25	0.333333333	0.625	
10:53:45	10:55:00	75	40	13	35	6	19	0.253333333	0.475	
10:55:00	10:56:15	75	40	12	35	9	21	0.28	0.525	
10:56:15	10:57:30	75	40	12	35	14	26	0.346666667	0.65	
10:57:30	10:58:45	75	40	13	35	12	25	0.333333333	0.625	
10:58:45	11:00:00	75	40	17	35	12	29	0.386666667	0.725	
				759		628	1387	0.385277778	0.722395833	0.533333333
				TOTAL		TOTAL	TOTAL	AVARAGE	AVARAGE	AVARAGE (λ/μ)

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RESEARCH ARTICLE

Knowledge on Multiple Cystic Ovarian Syndrome among Adolescent Girls in Selected Rural Areas, Salem

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ABSTRACT

A descriptive design with cross sectional survey approach was undertaken to assess the knowledge on multiple cystic ovarian syndrome among adolescent girls 18-19 years in selected rural areas, Salem. 150 adolescent girls were selected by using convenient sampling technique and data were collected by Semi-structured Interview schedule. Finding revealed that highest percentage (60%) adolescent girls were in the age group of 18-19 years and almost 89% belongs to Hindu. Highest percentage (64%) of the adolescent girls living in the rural area, 51% of them belongs to joint family, 68% of them were mixed diet, 53% of them got knowledge from friends & family members and highest percentage (74%) of them had good knowledge. Overall mean was (19.19 ± 51.33) which was 41% of the maximum score shows that the adolescent girls had average knowledge on multiple cystic ovarian syndrome.

INTRODUCTION

Adolescence period is the cross road period in life's development, characterized by a steady progression of psychological and social adaptations push the adolescent to learn and develop coping mechanisms that will be carried throughout the life. Hormone imbalances are becoming increasingly common due to changes in diet and other environmental factors. Today, more adolescent girls are showing signs of hormone imbalance. For a adolescent girl, problems associated with a hormone imbalance can be particularly disturbing and embarrassing. (Allahabadia .G.N, 2019). Gynecological problems of adolescents occupy a special space in the spectrum of gynecological disorders of all ages. Multiple cystic ovarian syndrome an endocrine disorder among the adolescence who can be seen from the adolescent period with clinical features including menstrual irregularities, obesity, acne and hirsutism occurs in about 6-10% of all adolescent girls, later it may lead to infertility. Keeping the adolescent girls as sample in the study, they can be taught how to manage the disease (Diane M. Fraser, 2019).

Statement of the Problem
A study to assess the knowledge on multiple cystic ovarian syndrome among adolescent girls in selected rural areas at Salem, Tamilnadu.

Objectives
To assess the knowledge on multiple cystic ovarian syndrome among adolescent girls.

Research Design and Approach
A descriptive research design with cross section survey approach

Study Setting
The study was conduct in Thumpalpatty village, Salem district.

Population
The study population comprised of the entire individual with the adolescent girls living in Thumpalpatty village, Salem.

Sampling
The study samples were adolescent girls living in Thumpalpatty village, Salem who fulfilled the inclusive criteria.

Sampling Technique
Convenient sampling was used as a sampling technique for the present study.

Sampling Size
150 adolescent girls living in Thumpalpatty village, Salem.

Tool used
Closed-ended questionnaire was used to collect the data regarding the knowledge onmultiple cystic ovarian syndrome among adolescent girls.

RESULT AND DISCUSSION

150 adolescent girls were selected by convenient sampling technique and data were collected by using questionnaire method. The collected data was analysis by inferential statistics. Demographic characteristics reveals that highest percentage (60%) adolescent girls were in the age group of 18-19 years and almost 89% belongs to Hindu. Highest percentage (64%) of the adolescent girls living in the rural area, 51% of them belongs to joint family, 68% of them were mixed diet, 53% of them got knowledge from friends & family members and highest percentage (74%) of them had good knowledge. Overall mean was (19.19 ± 51.33) which was 41% of the maximum score shows that the adolescent girls had average knowledge on multiple cystic ovarian syndrome. Percentage wise distribution of level of knowledge score regarding multiple cystic ovarian syndrome among adolescent girls shows that and highest percentage (74%) of them had good knowledge and 16% of them had average knowledge. Lowest percentage (10%) of them had poor knowledge. Hence, it can be interpreted that highest percentage (74%) of the adolescent girls had good knowledge on multiple cystic ovarian syndrome.

CONCLUSION

In the present study it can be concluded that the adolescent girls had good knowledge on multiple cystic ovarian syndrome. Hence, it can be interpreted that the investigator needs to conduct experimental study to assess the knowledge on multiple cystic ovarian syndrome among adolescent girls.

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Percentage wise distribution of knowledge score regarding multiple cystic ovarian syndrome among adolescent girls.

S.No	Level of knowledge	Maximum Score	Number	Percentage (%)
1	Poor	0-10	03	10
2	Average	11-20	5	16
3	Good	21-30	22	74
	Total	**30**	**30**	**100**

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RESEARCH ARTICLE

Tramadol Induced Inhibition of the Testicular Activities in Male Albino Rats

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ABSTRACT

In the current study, effect of Tramadol induced inhibition of testicular activities like gravimetric changes, histological and biochemical analysis has been evaluated. Three groups of healthy adult male albino rats having six rats in each group were taken. The rats of groups II and III were administered Tramadol at the dose level 1 and 3 mg/100 g body weight respectively in intraperitoneal mode everyday between 10:00 and 11:00 am for 21 days and group I maintained as control. After the experimental periods, the rats were sacrificed and the gravimetric, histological and biochemical study of testicles were carried out. In the results, testicular weights of the rats of group II and III showed significant reduction, histological and biochemical changes also inhibited by marked cytotoxicity in the testicular physiology. Histrometric changes were observed in testicular parameters like diameter and surface epithelial cell height were reduced significantly. Biochemical changes are parallel to the gravimetric changes, the protein and cholesterol contents are elevated significantly with respective administration of graded dose of Tramadol. Although, the gravimetric analysis of accessory organs such as epididymis, vas deferens, seminal vesicle and prostate gland were decreased significantly due to the administration of Tramadol.

Keywords: Tramadol, Testicles, Gravimetric, Biochemical, Accessory organs, Rats

INTRODUCTION

Tramadol is an analgesic drug exhibiting dual mode of action in CNS mechanism as an opioid and non-opioid component. It acts as a agonist with the opioid receptors, additionally, it inhibits the hormone serotonin and norepinephrine and also observed reuptake of these hormones inhibit the pain conduction in the spinal cord [1]. It is

pharmaceutically prescribed for the treatment of pain killing agent in different intensity of acute level to chronic condition [2]. The several side effects were reported of Tramadol overuse, which includes constipation, dizziness, headache, nausea, pruritus, somnolence, sweating and also over stimulates to central nervous system [3]. Tramadol efficacy has been considered low plasma protein binding drug (20%) and found maximum distribution among the tissues. It is extensively metabolized in the liver by O- and N-desmethylation and by conjugation reactions to form glucuronides and sulfate metabolites, eliminated as O-desmethyl Tramadol and partially through the renal system. O-desmethyl Tramadol has been recognized as greater affinity with opioid receptors than the parent drug and also act as potential analgesic. The drug half-live exhibited from 4.5 to 9.5 hours to react the all active ingredients and elimination of Tramadol from total plasma is in higher concentration (600 ml/min). The Tramadol interaction with other drugs noticed very negligible level due to the disposition action [4]. Hence, it is considered as potential analgesic drug instead of drug abuse, even though there are reports on the abuse, addiction and withdrawal effect [5]. Over intake of Tramadol concentration may lead to accumulation of toxic metabolites, increase the risk of drug-drug interactions and reduced elimination level from the body to induce chronic level of toxicity [6]. Hence, it has been noticed from several reports for the adverse affect on male reproductive system. As an a evidence of Safarinejad et al. [7] reports on chronic reproductive toxicity, like nucleic acid damage exhibited and its oxidative damage in the testis.

The objectives of the present work are to evaluate the testicular activities like gravimetrical, histological, histometrical, biochemical and induced changes in accessory organs by chronic intraperitoneal treatment at the graded concentration of Tramadol in male albino rats.

MATERIALS AND METHODS

Healthy male albino rats of Wistar strain from inbred colony, weighing 150 - 180gm, of 60 - 90 days old were maintained at room temperature of 20 ± 28 ºC with lighting schedule of 12 h light and 12 h darkness. They were maintained in individual cages and divided in groups each containing six animals and fed with balanced diet as described by CFTRI (Central Food and Technological Research Institute) Mysore, Karnataka, India and water ad libitum. The acclimatization of the animals lasted for 7 days before Tramadol administration for the experiments. It was carried out in accordance with ethical regulations for the animal care and use of laboratory animals. The study parameters like gravimetrical, histological, histometrical, biochemical and statistical analysis were carried out of testis by inducing graded dose of Tramadol through intraperitoneal.

Drugs

Tramadol (Trambax OD) generic is an opioid analgesic), wase purchased commercial product of Ranbaxy Laboratories, India from local drug houses.

Experimental Groups

The animals were divided into following groups

Group-I: Received 0.2 ml saline/100 g body weight i.p. for 21 days and served as control.

Group-II: Received 1 mg Tramadol/100 g body weight i.p. for 21 days in 0.2 ml saline.

Group-III: Received 3 mg Tramadol/100 g body weight i.p. for 21 days in 0.2 ml saline.

All the animals were sacrificed by cervical dislocation after 24 h of the last injection. The testis were dissected out immediately and separated from adherent tissue, weighed up to the nearest mg on electronic balance to determine gravimetry. Organs from one side of each animal were fixed in Bouin's fluid for histological studies. They were embedded in paraffin, sectioned at 5 μ, stained with Ehrlich hematoxylin and Eosin. The micrometric measurements like diameter of testis, its epithelial cell height were made from randomly chosen 20 sections appearing round at cross sections from each group using ocular and stage micrometers [8]. Spermatogenic elements count was made from randomly chosen 20 round cross section taken from the middle part of the testis [9]. Organs from other side

were used for biochemical estimations of the protein content [10], total cholesterol [11] and glycogen [12] of testicular tissues were estimated.

Statistical Analysis

All the values were statistically analysed by Student's-'*t*' test using SPSS (19.0.1.). Data are expressed as the Mean + S.E. Statistical significance was set at $p<0.05$ and $p<0.01$ [13].

RESULTS

Changes in the Testis and Accessory Organs

Gravimetric Changes in Testis (Table: 1 & 2)

Treatment of the 1mg/100g b.w. of Tramadol decreased ($P<0.05$) the weight of testis significantly, whereas 3mg/100g b.w. has reduced the testis weight significantly ($P<0.01$), when compared to control rats.

Gravimetric Changes in Accessory Organs

Treatment of the 1mg/100g b.w. of Tramadol decreased ($P<0.05$) the weight of epididymis (caput & cauda), vas deferens, seminal vesicle, prostate gland significantly, whereas 3mg/100g b.w. has reduced the all the studied organ weight highly significant ($P<0.01$), when compared to control rats.

Changes in the Sperm Count

Treatment of the 1mg/100g b.w. of Tramadol decreased ($P<0.05$) the sperm count significantly, whereas 3mg/100g b.w. has reduced highly significant ($P<0.01$), when compared to control rats.

Histological Changes (Figure 1)

In the histological sections of the testis, a significant reduction in the number of spermatogonia, spermatocytes and spermatids were observed. Necrosis in tubular epithelium, shrinkage of sertoli cells was recorded. No spermatozoa were observed in the lumen of seminiferous tubules in the Tramadol treated rats. The Leydig cells in the both the dose received groups were significantly degenerated.

Histometric Changes (Figure 1)

Moderate significant ($P<0.05$) reduction in the diameter of testis due to the treatment of 1mg/100g b.w. whereas, 3mg/100g b.w. reduced the diameter of testis significantly ($P<0.01$). Similarly the diameter of seminiferous tubules was reduced significantly in a moderate level with the dose 1mg/100g b.w. ($P<0.05$) and highly significant ($P<0.01$) with 3mg/100g b.w. of Tramadol treatment, when compared to control rats. The diameter of Leydig cell nucleus is reduced significantly in moderate level with 1mg/100g b.w. and highly significant ($P<0.01$) with 3mg/100g b.w. of Tramadol treatment, when compared to control rats.

Biochemical Changes (Table 3)

There is significant ($P<0.05$) decrease in the protein and glycogen content of testis due to the 1mg/100gm Tramadol, whereas cholesterol content is increased significantly ($P<0.05$) in 1mg/100gm b.w. treatment. The treatment of Tramadol at 3mg/100g b.w. decreased highly significantly ($P<0.01$) protein and glycogen content of testis and in total cholesterol content is highly significant ($P<0.01$) increase, when compared to control.

Sperm Morphology and Number (Table 1)

The cauda epididymal sperms of normal rat shows sickle shaped head and straight tailpiece. But in Tramadol treated rats the sperms were abnormal as their head region reduced and the tail is wrinkled or coiled. A significant reduction in sperm population were observed in both the doses of Tramadol treatment, whereas in the sperm count of cauda

epididymis with 1mg Tramadol was almost significant and exhibited highly significant ($P < 0.01$) reduction with 3mg Tramadol treatment.

DISCUSSION

The testis function completely depends on the supportive male accessory reproductive organs and they play an significant role in the fertility, motility, formation of semen and sperm maturation [14]. In the present study Tramadol induced inhibition of the testicular activities revealed in the form of antispermatogenic activity due to the indication of the significant reduction in weight of testis and their diameter, number of spermatogonic elements like spermatogonia, spermatocytes and spermatids were decreased, suggesting indirectly the inhibition or non availability of pituitary gonadotrophins, specifically follicle stimulating hormone, which is essential for spermatogenesis [15, 16].

Significant increases in the cholesterol level indicates the non utilization of these precursors for steroidogenesis which may be due to an inhibition in the availability of gonadotrophins such our ICSH/LH or FSH that are necessary to stimulate the germinal epithelium [17]. The glycogen level in the cells indicates the energy storage in sertoli cells and spermatogonia often contain glycogen, secrete substrates from the blood and provide source of reserve carbohydrates for seminiferous tubules, and the glycogen level has been found to be directly proportional to the steroid hormones [18]. The decreased glycogen content of the testis after the administration of Tramadol drug might be correlated with decreased spermatogenic number due to reduced energy source for spermatogenic activity. Moreover, testosterone plays a pivotal role in sexual maturation, behavior and maintenance of accessory sex organs [19]. As the administration of Tramadol has caused significant reduction in the spermatogenesis, steroidogenesis and androgen production it may alter the sexual behavior and may cause antifertility. Among the two dose of Tramadol, found highly significant at the dose level of 3mg/100g body weight is more effective in causing antispermatogenic and antisteroidogenic activities. Similar to our findings with various pharmaceutical drugs and plant extracts reported for their antifiertility recently, such relationship were concluded by Vijaykumar *et al.,* [20-22] and Londonkar *et al.,* [23-24] in their treatments with various extracts and drugs in male albino mice and rats.

CONCLUSION

In the conclusion we suggest that long term exposure of Tramadol exhibit chronic physiological functions and deleterious toxic effects on the reproductive system of male albino rats. Hence, it is advisable that Tramadol should be used with precaution with appropriate concentration and dose monitoring to avoid its significant side effects on fertility as well as other contradictory physiological mechanism of action.

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Conflict Of Interest

The authors declare no conflict of interest.

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Table 1: Effect of Tramadol on the weight of testis and accessory reproductive organs in mature rats.

Groups	Testis weight and accessory organ weight mg/100g body weight						
	Testis (g/100g body wt)	Epididymis		Vas deferens	Seminal Vesicle	Prostate gland	Sperm Count (million/ml)
		Caput	Cauda				
Control	1.185±0.15	388.08±1.05	292.93±1.21	82.20±2.10	275.14±2.34	108.03±3.85	52.48±3.76
1mg	1.163*±0.08	366.71*±2.01	281.43*±1.96	68.59*±2.17	245.28*±1.86	96.61*±4.19	42.55*±4.58
3mg	1.043**±0.16	366.09**±3.06	260.16**±2.15	44.18**±2.35	182.32**±2.40	74.46**±1.95	40.63**±3.77

Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.

Table 2: Effect of Tramadol on histometric changes in the testis of male rats.

Groups	Diametr of testis		
	Testis (µm)	Seminiferous tubules (µm)	Leydig cells (µm)
Control	6150.00 ± 10.00	310.12 ± 4.12	6.88 ± 0.54
1mg	6067.00*± 5.50	301.14* ± 4.32	5.90* ± 0.53
3mg	5910.00** ± 11.30	290.35** ± 1.34*	5.70** ± 0.06

Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.

Table 3: Effect of Tramadol on biochemical changes in the testis of male rats.

Groups	Biochemical changes		
	Protein (µg/mg)	Cholesterol (µg/mg)	Glycogen (µg/mg)
Control	66.42 ± 3.44	7.64 ± 1.41	1.52 ± 1.75
1mg	50.40* ± 2.21	9.28* ± 2.16	0.83* ± 0.88
3mg	40.82** ± 3.33	12.15** ± 3.32	0.59** ± 0.12

Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.

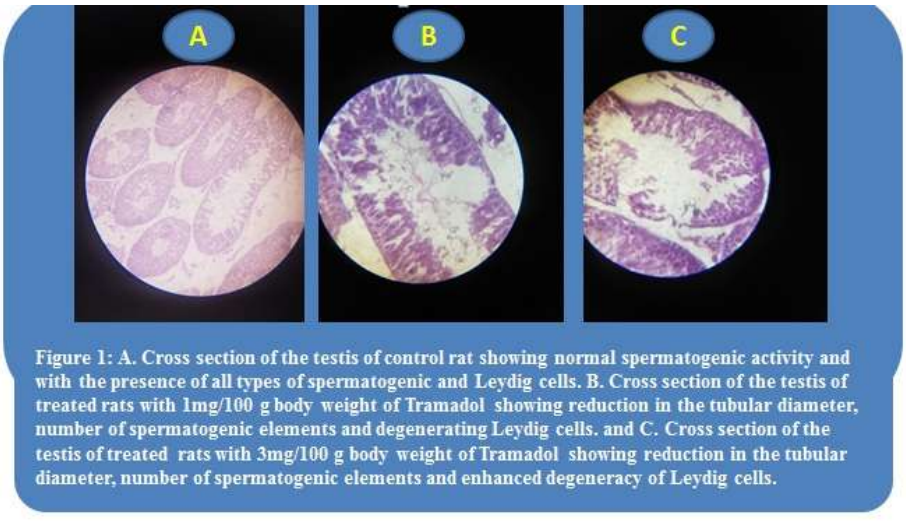

Figure 1: A. Cross section of the testis of control rat showing normal spermatogenic activity and with the presence of all types of spermatogenic and Leydig cells. B. Cross section of the testis of treated rats with 1mg/100 g body weight of Tramadol showing reduction in the tubular diameter, number of spermatogenic elements and degenerating Leydig cells. and C. Cross section of the testis of treated rats with 3mg/100 g body weight of Tramadol showing reduction in the tubular diameter, number of spermatogenic elements and enhanced degeneracy of Leydig cells.

Fig.1. Histological Changes

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RESEARCH ARTICLE

Mathematical and Stochastic Growth in Business and Industries

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ABSTRACT

Development marvels are universal and inescapable in science and the clinical sciences, yet in addition in financial matters, advertising and sociological studies. While on a superficial level deceivingly basic, the perplexing co-operations that administer development render the undertaking of delivering reliable numerical models for such wonders exceptionally testing, without a doubt. Of course, the research network has committed a considerable lot of merited thoughtfulness regarding creating Mathematical and Statistical growth models that can anticipate and clarify the degree of development observe in different zones of human undertaking. Plenty of mathematical development models proposed in the writing falls into two general classes: deterministic development models and stochastic development models. In this paper we emphasized portrayal on stochastic development in business and enterprises. The ideas and methods engaged with taking care of stochastic models are not any more perplexing than the deterministic case, in spite of the fact that state spaces will be in general extend as the estimations of stochastic stuns enter the state space. This paper enumerated with five sub-parts and have conclusive information to the suitable evidence of statement of the problem.

Keywords: Mathematical, Stochastic development, growth, business, enterprises, models.

INTRODUCTION

Over many centuries, the assignment of understanding the elements of different development wonders saw in nature and society had caught the enthusiasm of researchers and savants. Except for D. Bernoulli's effort to show the episode of a smallpox pandemic, T. R. Malthus' populace development model and P. Verhulst definition of the calculated development model, a large portion of the early endeavors, including Gompertz's human mortality model, were experimental, the information accessible was exceptionally questionable and the overall feeling of proof was missing by the present principles. This were to change in 1874 when Watson and Galton embraced the principal precise effort to get development and annihilation wonders saw in the sociologies that, in any case, couldn't be convincingly clarified. Their work was among the most punctual orderly efforts to enroll the assistance of likelihood hypothesis in demonstrating, and in this manner, understanding, the elements of populace development. While their spearheading work had zeroed in on a somewhat thin issue, specifically that of representing the discount annihilation of family names in Great Britain, their numerical techniques (and, specifically, the at this point exemplary Galton-Watson measure) ended up being shockingly amazing and general. Shockingly, Galton and Watson's work and their mathematical model was dismissed for a long time, more accurately until 1924, when Yule applied comparative probabilistic apparatus to the investigation of the elements of the expansion of new species and genera. Yule's commitment, a straight unadulterated birth measure, was rediscovered, a couple of years after the fact by W. H. Fuzzy with regards to electron physics and by Feller and Lotka in populace biology.

The major significant milestone in modelling growth model was given by Kolmogorov and Dimitriev's original work where they summed up the Galton-Watson model, proposing powerful measures as an incredible displaying instrument for a huge class of development marvels, that as it turned out, sub-added a great part of the past work referred to above and make way for a methodical glance at development models. Of course, in the next many years a plenty of mathematical development models expected to catch the substance of common and social marvels going from the sociologies to hereditary qualities, to science, to the study of disease transmission, to material science, to stargazing, to software engineering and full scale financial aspects have been proposed in the writing. A portion of these models are planned to catch the embodiment of powerful, unhindered development as seen, for instance, in molecule material science and cosmology (e.g.the Big Bang theory). Conversely, the vast majority of the development wonders that we encounter in science and medication, financial aspects and the sociologies include a nearby communication between the marvel under examination and its general climate. For instance in financial matters, the merger of organizations is dependent upon inner boosts and to outside weight (hindrance) originating from the commercial center and rivalry. In the natural sciences, when assets are copious and ecological conditions fitting, microscopic organisms populace can increment quickly. However, in many occasions resources are not boundless and natural conditions are a long way from ideal. Atmosphere, food, natural surroundings, water accessibility, and other comparative variables scheme to hold population development under wraps. Surely, the climate can just help a set number of people in a populace before some asset runs out and imperils the very endurance of those people. Populace models are used to choose most noteworthy assemble for cultivating, to fathom the components of natural interruptions, and have different environmental conservation recommendations. Populace models are moreover used to grasp the spread of parasites, contamination, and ailment. The acknowledgment of our reliance on natural well being has made a need to comprehend the dynamic collaborations of the earths flora and fauna. Strategies in populace displaying have significantly improved our comprehension of biology and the characteristic world.

Stochastic analysis of economic growth:

Gross domestic product is a broadly picked marker for assessing the monetary conduct of a nation, since it shows pay created by various financial operators. It likewise gauges the expense of merchandise and enterprises creation in the economy, which is estimated regarding factor installments and items delivered in each financial area. Accordingly, pay and use are identical at a macroeconomic level. The differentiation between Gross Domestic Product at trade regard and at factor cost is interpreted by unusual costs (Startz, Fischer, and Dornbusch, 2004).

Financial development could be utilized as a measured variable as it empowers governments to give finer open merchandise and enterprises, for example, schooling, health assistance and foundation (Mankiw, 2012;Acemoglu and Robinson, 2012).Gross domestic product is the market estimation of every final good and service delivered in a nation during a given period(Dornbuschet.al., 2004). The Gross Domestic product adds various types of items together to get the estimation of monetary movement at trade costs. Its motivation is to incorporate all things created in the economy and sold available. In any case, certain items are overlooked, for example, those that are delivered and sold illegally and hand crafted merchandise that don't arrive at the trade. The measurement is presented once every three months all together on examine drifts, and is occasionally acclimated to represent occasional creation changes intrinsic to certain products and ventures (Jones, 2015, Dornbusch et al., 2004,).

Investment is otherwise called gross capital development, which is made out of uses on stable resources of the economy in addition to alterations in inventories (Dornbusch et al., 2004). Fixed resources incorporate property upgrades, structures, hardware and gear buys, just as development of streets, railroads, and comparable foundation. Inventories are supplies of merchandise held by enterprises to meet impermanent or unforeseen variances underway or deals. Consequently, changes in inventories speak to the contrasts among anticipated and contemporary use in the economy. In like manner, gross capital development adds to development via genuine speculation, estimated corresponding to GDP, which mirrors the actual amount advancement of capital and yield (BalcerzakSimionescu, Dobeš, Sopková, and Lazányi, 2017; Miller, Doppelhofer, and Sala-I-Martin, 2004; Acemoglu and Robinson, 2015).

Introduction to GNP

Gross National Product (GNP), absolute market esteem of the final commodity and enterprises designed and developed by the nation's economy during a period of time frame (generally a annum), sorted before compensation is done for the deterioration or application of capital used during the time spent development. It is recognized from Gross Domestic Product (GDP), which is registered after the kind of an investment is made. The GNP is practically indistinct from that point, the last gets eject the compensation working to a nation's inhabitants from hypotheses abroad (less the compensation acquired in the local economy gathering to non nationals from abroad). GNP is a useful pointer of the level of economical development.

A model of firms development elements

A dominating stream of composing since the referred Gibrat's example has shown the improvement of enterprises as self-ruling exemplary ways made by a comparable stochastic cycle. One of the huge disadvantages of such a system is clearly the presumption of self-rule between the recorded setting of various enterprises, in this way excusing any resistance cycle through which they associate. A somewhat elective definition, at first explained by Herbert Simon and later reformulated by John Sutton, expects the presence of a restricted game plan of advancement "openings" (or, similarly, a reliable appearance of new possibilities) and enterprises' improvement adjusted by the amount of chances each firm can get. The model which follows shares with the last custom the fundamental depiction of a stochastic genuine cycle, yet operates the framework through which "openings" are consigned in a substitute way.

This epic strategy grants us to address self-fortifying parts whereby the possibility for an offered enterprise to get another open entryway determinedly depends upon the amount of chances recently got. Definitely, as opposed to tolerating that the errand of each event to a given enterprise is a free capacity with consistent possibility, we present the chance of "competitiveness among objects whose market accomplishment is total or self-fortifying". These "business openings" can be thought of as the wellspring of scaled down stocks affecting the size of firms. This is a genuinely ordinary answer for such a model, and returns to the early works of Simon. We make no presumptions on the genuine thought of these stocks and we have to relate "openings" to "advancement" in the most effortless way.

Significant adapted certainty in the evolution of business is determinent as growth variances. Industrious vacillations, this sense like growth changes that are dependable. Clearly, this is a stun to GNP is steady when those belongings don't disperse soon and GNP doesn't show a critical inclination to re-visitation of its pattern standard.

Post work by Nelson and Plosser in 1982, the overarching view on diligence is that the impacts of a stun keep going forever and, accordingly, GNP has a main root. Thus, after the decrease in GNP today, gauges of GNP were brought down over any conceivable skyline. Such outcomes are broadly affirmed utilizing information from various nations. For instance, Campbell and Mankiw in 1989 show that 1/4th GNP, here these gathering of G-7 nations, exceptionally diligent. For every one of these nations, a 1% decrease in growth today, brings down the long-standing esteem of growth significantly over 1%. Their assessments of ingenuity show, in any case, enormous contrasts across nations. All nations, except for the United Kingdom, show variances that last longer than in the US. For instance, diligence in Japan is somewhere in the range of 2 and multiple times bigger than in the US. These outcomes are additionally affirmed by Cogley in 1989 who shows, for a comparable example of nations, impressive contrasts in the fluctuation of the perpetual segment of growth.

Stochastic trends

The points by Plosser and Nelson in 1982 tested conventional strategy for estimating business evolution as impermanent stability of growth from the determined pattern. Despite the fact that they supposed to open discussion upon the presence of the accurate unit root in growth, this is a wide understanding that variances are profoundly determined; GNP shows for all intents and purposes no inclination to return to its pattern level after an aggravation. Subsequently, the idea of a deterministic pattern has been commonly surrendered for the thought of a stochastic pattern and, for instance, filtering through recurrent parts by utilizing log contrasts, which assumes the presence of a stochastic pattern, is current practice.

What are the repercussions of the presence of a stochastic example in GNP for budgetary showcase? The presence of a stochastic example is related with the chance of stochastic new development. Models that fall into this name can be amassed into two unquestionable groupings. Regardless, there are models where the wellspring of segments (exogenous suffering profitability paralyze) is the sole mindful of the presence of a stochastic model. Second, there are models where the stochastic idea of headway is the result of the impacts that insecurities have on progress. Both of such models have close to observational longings yet their suspicions about the reason behind improvement changes and their administration help proposals can be ordinarily surprising.

Exogenous Permanent Productivity Shocks

The fundamental explanation to the dauntlessness of GNP instabilities was given by the authentic business cycle writing. In the standard RBC model, changes are deviations from a reliable state answer for a neoclassical improvement model. In its most un-complex structure, GNP per capita follows a sporadic walk around a buoy, where the buoy is exogenously constrained by the movement of work growing mechanical progression. Besides, simply little deviations around a predictable state are examined and impermanent advancement components are basically ignored. In this game plan, unending exogenous developments in the creation work are the fundamental possible wellspring of the inventiveness of development changes.

This model was the benchmark to decipher observational decay of GNP into a lasting and a temporary segment. These deterioration distinguish low-recurrence growth changeability with exogenous innovation stocks, while high recurrence developments are considered as request stocks. Thus, the observational commitment of the lasting segment of growth is viewed as a proportion of the size and recurrence of innovation stocks comparative with request stocks. All of these papers affirm the huge commitment of the lasting segment of GNP in any case, they give next to no consideration to the observed cross-country contrasts. Consider the example, Cogley (1990) considers the changeability of the low-recurrence segment of growth in an example of 9 nations and shows that there are huge contrasts among them, the US having the most steady low-recurrence segment of the test. He reasons that growth vacillations, at any rate inside his example, are not all similar. If we somehow managed to utilize the standard RBC model as a benchmark to clarify these cross-country contrasts, we would need to expect contrasts in the hidden stochastic cycle that drives innovation shocks. Hence, we propose to join in the investigation the homogeneity of efficiency development so as to comprehend its potential co-operations with the ingenuity of monetary variances.

Endogenous Growth and Stochastic Trends

At the point when profitability development or mechanical advancement are the result of cognizant choices made by monetary operators, the idea of persevering vacillations may have an altogether different translation. Ruler, Plosser and Rebelo (1988) and Stadler (1989) saw that, inside the setting of an endogenous development model, there are numerous kinds of aggravations, unique in relation to perpetual movements in the creation work, that can deliver tireless changes. All the more explicitly, any impermanent unsettling influence causes lasting consequences for the degree of growth as long as it produces brief changes in the measure of assets apportioned to development. The development component includes the brief deviations to prompt a unit-root arrangement of growth. For this situation, constancy can't be utilized any longer to recognize shocks and, as an outcome, the accentuation of the examination shifts from the beginning to the transmission of the shocks. A result is that unsettling influences, for example, total interest shocks, generally considered as brief, can affect the degree of monetary movement.

Endogenous vs. Exogenous Stochastic Trends

The two arrangements of models depicted above offer the presence of a stochastic pattern and, thus, they can deliver constant yield vacillations. However, the stochastic properties of the pattern are exogenously expected in the primary case while they are the consequence of the reaction of efficiency to repetitive changes in the subsequent one. Moreover, their suggestions as far as the reason and welfare costs of monetary changes can be very unique. It is all things considered hard to configuration tests to experimentally recognize the two clarifications as their expectations may be, much of the time, practically indistinguishable.

Measuring Persistence

Let yt be the log of output and assume that it has the accompanying World portrayal

$$\Delta y_t = D(L)\,\epsilon_t$$

$D(L) = d_0 + d_1 L + d_2 L^2 + d_3 L^3 + \cdots$ is a log polynomial. At that point, the coefficients dj measure the effect of a shock t on the development pace of GNP in period t + j. In the event that we include these coefficients we can discover the effect of a given shock fair and square of GNP. When all is said in done,,

$$P^J = \sum_{j=0}^{j=J} d_j$$

represents the effect of a shock t on the level of yield at t + J. The boundless entirety of all dj coefficients, gauges the perpetual effect of a given shock fair and square of yield, left P alone this sum,

$$P = \lim_{J\to\infty} P^J = D(1)$$

A second proportion of diligence proposed by Cochrane (1988), is a proportion of fluctuations that can likewise be composed as a weighted total of auto correlations

$$V^J = \frac{(1/J)\,\mathrm{var}(y_t - y_{t-J})}{\mathrm{var}(y_t - y_{t-1})} = 1 + 2\sum_{j=1}^{J-1}(1 - j/J)\,\rho_j$$

where ϱj is the j–th auto correlation of the development pace of yield. Taking the limit of this articulation as J tends for infinity, we get a proportion of since quite a while ago run determination,

$$V = \lim_{J\to\infty} V^J$$

Both V and P take value 0 for a pattern fixed arrangement and worth 1 for a arbitrary walk. For some other series,

$$V = |P|^2 \frac{\mathrm{var}(\epsilon)}{\mathrm{var}(\Delta y)}$$

CONCLUSIONS

There is an agreement among macro economists that growth changes are exceptionally tenacious and that drawn out development isn't as steady as a deterministic pattern would propose. Experimental appraisals of diligence show, be that as it may, huge and critical contrasts across nations. We have demonstrated that these distinctions can be completely clarified by contrasts in long haul development rates. Nations that become quicker have more constant business cycles. All in all, current business cycle models don't foresee any connection betwee these two factors as they treat one of the two, long haul development rates, as an exogenous variable. For instance, on the off chance that all nations followed an arbitrary stroll with a float, at that point the level of tirelessness would be the equivalent in all nations regardless of whether the size of the float differed. We have taken a gander at the idea of diligence inside the setting of an endogenous development model and indicated that, in this setting, brief aggravations become tenacious as they have impacts in the measure of assets dispensed to development.

In addition, the noticed positive relationship of constancy and progression is a trademark about these models. Our outcomes propose that stochastic progression is fundamental to comprehend imperative highlights of the transmission of business cycles. Stochastic improvement can't be basically lessened to the presence of exogenous interminable advancements in the creation work, as it is regularly expected in RBC models, the dull lead of the favorable circumstances doled out to progression should be considered. Unmistakably, if we will probably take a gander at highlights of the business cycle, for example, confirmation, the utilization of models where changes are deviations around a consistent state answer for a neoclassical (Solow-type) improvement model can be dumbfounding.

Concluding all the suggestions that the endogeneity of advancement has for business cycles is an open zone for future exploration. One could imagine that, in these models, the transmission of shocks can provoke budgetary fluctuations that are one of a kind corresponding to the ones made by a model where improvement is treated as exogenous. The components are possibly more excessive and could speak to a segment of the specific observations that are correct now unexplained by business cycle models.

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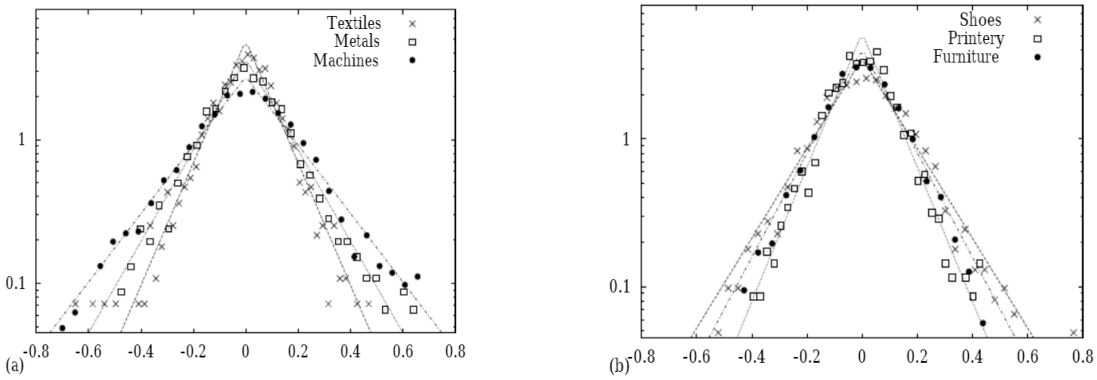

Fig. 1. (a,b) Binned empirical densities of the annual growth rates for six different manufacturing sectors: textiles (181 firms), treatment of metals and metal coating (182 firms), special purpose machines (424 firms), shoes (245 firms), not publishing printery (199 firms) and furniture (444 firms). Both the plots draws upon the MICRO.1 databank developed by the Italian Statistical Office (ISTAT) that contains longitudinal data on a panel of several thousands of Italian manufacturing firms, with 20 or more employees, over a decade

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RESEARCH ARTICLE

Optics of Crystals in Finsler Space

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ABSTRACT

This paper has been devoted to the study on Optics of Crystal in Finsler space. In this paper we have studied the application of Finsler geometry to physics is crystal optics and studied the symmetry properties all crystals can be divided into seven crystal systems as written triclinic, monoclinic, orthorhombic, trigonal, tetragonal, hexagonal and cubic. But transparent crystals fall into only three distinct classes from the point of view of their optical properties, biaxial, uniaxial and isotropic crystals. Here we have studied only two classes: biaxial and uniaxial, since the isotropic crystals behave optically as amorphous bodies they have no optical anisotropy and correspond to Euclidean geometry. Also we have studied Uniaxial Crystals in Finsler space. After these observations we have generalization to higher algebraic orders introducing Finsler spaces of type (α, β_m), $m = 1,2,\ldots$, and it is easy to see that the indicatrix of the mth-order Kropina space is an algebraic surface of order 2m. For $m > 2$ and a non-vanishing b-tensor these spaces cannot be Riemannian space.

Keywords: Crystal Optics, Uniaxial, Biaxial, optical anisotropy, Kropina space.

INTRODUCTION

Many researchers Born, M. and Wolf [1], Born, M. [2], P. L. Antonelli, R. S. Ingarden and M. Matsumoto[3] and *C. W.* Bunn [8] are studied on Crystal Optics in Finsler space. Boguslavsky, G. Yu [6] studied theory of Locally Anisotropic Space-Time. Born and Wolf [1], studied the Optical theory is based on two way: Maxwell's equations and Material equations. The Maxwell's equations are given by

(1.1) $curl\, H - \frac{1}{c}\dot{D} = \frac{4\pi}{c} j$,

(1.2) $curl\, E + \frac{1}{c}\dot{B} = 0$,

Where dot denote the differentiation with respect to time, j is electric current density, D is electric displacement and H is magnetic vector.

The material equations in an isotropic medium are given by

(1.3) $j = \sigma E$,

(1.4) $D = \varepsilon E$,

(1.5) $B = \mu H$,

Here σ is specific conductivity, ε is dielectric constant and μ is magnetic permeability.

In dealing with crystals we have generalized these later equations in the view of anisotropy. We consider that the medium is homogeneous, non-conducting, and magnetically isotropic, there are also magnetic crystals, but as the effect of magnetization on optical phenomena is small, the magnetic anisotropy may be neglected (Boguslavsky, G. Yu [6]). We consider substances whose electrical excitations depend on the direction of the electric field. The equation (1.4) we assume the relation between D and E to have the simplest form which can account for anisotropic behavior, which each component of D is linearly related to the components of E, we can written as

(1.6) (a) $D_x = \varepsilon_{xx}E_x + \varepsilon_{xy}E_y + \varepsilon_{xz}E_z$,

(b) $D_y = \varepsilon_{yx}E_x + \varepsilon_{yy}E_y + \varepsilon_{yz}E_z$

(c) $D_z = \varepsilon_{zx}E_x + \varepsilon_{zy}E_y + \varepsilon_{zz}E_z$

The nine quantities $\varepsilon_{xx}, \varepsilon_{xy}, \varepsilon_{xz}, \ldots \varepsilon_{zz}$ are constants of the medium, and constitute the dielectric tensor, the vector D is equal to the product of this tensor with E.

We shall write equation (1.6) in shorter form as

(1.7) $D_k = \sum_l \varepsilon_{kl}E_l$

where *k* stands for one of the three indices x, y, and z, and l stands for each of *x, y* and z in turn in the summation.

Crystal Optics in Finsler Spaces

The most direct and simple application of Finsler geometry to physics is crystal optics. In crystals the electric vector $E = E_i$, $i = 1,2,3$ is not in general parallel to the electric displacement vector $D = D_i$, the set of equation (1.7) can be written as (*C. W.* Bunn [8])

(2.1) $D_i = \varepsilon_{ij}E_j$

Where ε_{ij} is the dielectric tensor, here we use Einstein's summation convention, but we do not distinguish contravariant and covariant indices since coordinates are orthogonal Cartesian, we have to distinguish two directions of light propagation,

(2.2) $s = \frac{D\times B}{|D\times B|}$

where $B = \mu H$ (B is magnetic induction vector, μ is magnetic permeability(scalar), H is magnetic vector), and that of energy flow

(2.3) $t = \frac{S}{|S|}$, where $S = \frac{c}{4\pi}(E \times H)$

c is the light velocity (Gaussian units). For the simplicity we shall only consider the wave normal directions S, t is obtained from B by a simple geometrical construction (Born, M. and Wolf [1]). We have studied the symmetry properties all crystals can be divided into 7 crystal systems as written in table (1) below: Here, C is axis with colour dispersion (change of axis with frequency of light), F is axis fixed in direction (no colour dispersion), R is freely rotatable or indeterminate axis. We have studied only two classes: biaxial and uniaxial, since the isotropic crystals behave optically as amorphous bodies they have no optical anisotropy and correspond to Euclidean geometry *(C. W.* Bunn [8]). We have studied some concepts as in Table (1). The dielectric axes are those corresponding to the eigen vectors and eigen values of tensor ε_{ij} in (1) which is assumed to be real and symmetric.

(2.4) $D_i = \varepsilon_i E_i$, $v_i = \frac{c}{\sqrt{\mu\varepsilon_i}}$, $(i = 1, 2, 3)$.

Where v_i is velocities of propagation of light in the crystal (R. Courant [7]). The ellipsoid of wave normal is formed the expression for electric energy density in the coordinate system of the dielectric axes (Born, M. and Wolf [1]),

(2.5) $C^2 = 8\pi\omega_e = E.D = \frac{{D_1}^2}{\varepsilon_1} + \frac{{D_2}^2}{\varepsilon_2} + \frac{{D_3}^2}{\varepsilon_3}$,

If we take $x = \frac{D_1}{C}$, $y = \frac{D_2}{C}$, $z = \frac{D_2}{C}$ then we can write

(2.6) $\frac{x^2}{\varepsilon_1} + \frac{y^2}{\varepsilon_2} + \frac{z^2}{\varepsilon_3} = 1$

Here, we construct the directions of vibrations of the D vectors belonging to a wave normal s as axes of the section through the origin normal to s and the directions of optical axes of the crystal as normals to spherical sections of the ellipsoid through the origin. Since ellipsoid can be triaxial, biaxial or uniaxial, and the optical axes can be one or two,(we see all the bases of Table(1)). The distinguish between phase velocity v_p in direction s and the ray velocity v_r which is the velocity of energy transport in direction t, (Born, M. and Wolf [1]) we have

(2.7) $v_p = \frac{c}{n} = v_r\, t.s = v_r\, cos\alpha = \frac{|S|}{\omega} cos\alpha$, $\omega = \omega_e + \omega_m = 2\omega_e$,

where n is the refraction index, and fig.1, it can be shown that as a condition of solvability of the problem one obtains the Fresnel equation of wave normals ${s_1}^2 + {s_1}^2 + {s_1}^2 = 1$, $s = s_i$ (Born, M. and Wolf [1]),

(2.8) $${s_1}^2\left({v_p}^2 - {v_2}^2\right)\left({v_p}^2 - {v_3}^2\right) + {s_2}^2\left({v_p}^2 - {v_3}^2\right)\left({v_p}^2 - {v_1}^2\right) + {s_3}^2\left({v_p}^2 - {v_1}^2\right)\left({v_p}^2 - {v_2}^2\right) = 0.$$

Now we have studies the case of an optically uniaxial crystal. Let us assume that the optical axis is in the three directions, $v_1 = v_2 = v_0$ for ordinary velocity and $v_3 = v_e$ for extraordinary velocity. Then the set of equation (2.8) can be written as

(2.9) $\left({v_p}^2 - {v_0}^2\right)\left({v_p}^2 - {v_e}^2\right)sin^2\theta + \left({v_p}^2 - {v_0}^2\right)cos^2\theta = 0$,
where (2.10) ${s_1}^2 + {s_2}^2 = sin^2\theta$, ${s_3}^2 = cos^2\theta$,
(2.11) ${v'_p}^2 = {v_0}^2$,
(2.12) ${v''_p}^2 = {v_0}^2\, cos^2\theta + {v_e}^2 sin^2\theta$

We have obtained two shells of the normal surface in Fig.2. One is a sphere of radius v_0 and corresponds to ordinary wave with a velocity independent of the direction of propagation. The other is a surface of revolution of the fourth order, and corresponds to the extraordinary wave with velocity depending on the angle between the direction of the wave normal and the optical axis. The two velocities are only equal if the wave normal is in the direction of the optical axis i.e. $\theta = 0$. From fig.2. shown that both waves are linearly polarized with the direction of polarization D orthogonal one to the other (Born and Wolf [1]). Such situation is called transparent solid monocrystals. Optical axes are same direction in all points of the material. In fluid crystals the optical axes are fixed in space i.e. an external electric or magnetic field and can be different in different points in a smooth way.

Uniaxial Crystals in Finsler Space

Using Okubo [4] method, we have studied the equation of the extraordinary normal surface (2.12) of the previous section in orthogonal Cartesian coordinates. We have written the well known relations between spherical and rectilinear orthogonal coordinates (Born and Wolf [1])

(3.1) $x = rsin\theta cos\emptyset$, $y = rsin\theta sin\emptyset$, $z = rcos\theta$

(3.2) $r^2 = x^2 + y^2 + z^2$, $cos\theta = \frac{z}{\sqrt{x^2+y^2+z^2}}$ and $sin\theta = \frac{\sqrt{x^2+y^2}}{\sqrt{x^2+y^2+z^2}}$.

Put these relations in the set of equation (2.12), we can get ($v_0 = a$, $v_e = b$)

(3.3) $x^2 + y^2 + z^2 = a^2\frac{z^2}{x^2+y^2+z^2} + b^2\frac{x^2+y^2}{x^2+y^2+z^2}$, $a \neq b$, $a, b \neq 0$.
Multiplying by $(x^2 + y^2 + z^2)$ in (3.3) we have

(3.4) $(x^2 + y^2 + z^2)^2 - a^2z^2 - b^2(x^2 + y^2) = 0$, $x, y, z \neq 0$.
Using Okubo [4] method,

(3.5) $1^i = \frac{y^i}{L(x,y)}$, $i = 1, 2, 3$,

substitute this for x, y, z in (3.4) and solving for L we have

(3.6) $L(x, y) = \frac{(y^1)^2+(y^2)^2+(y^3)^2}{\sqrt{a^2(y^3)^2+b^2[(y^1)^2+(y^2)^2]}}$, $y \neq 0$, $a, b \neq 0$.

Here, if $a = b = 1$, we get the Lagrangian of Euclidean space, for $a \neq b$, $a, b \neq 0$.
Putting $L = 1$ for the indicatrix in (3.6), we have,

(3.7) $[(y^1)^2 + (y^2)^2 + (y^3)^2]^2 - a^2(y^3)^2 - b^2[(y^1)^2 + (y^2)^2] = 0$, $y^i \neq 0$.
The set of equation (3.6) is written in special coordinates adjusted to the symmetry of our example. The Lagrangian of the type

(3.8) $L(x, y) = \frac{a_{ij}(x)y^iy^j}{\sqrt{b_{ij}(x)y^iy^j}}$, $a_{ij}(x) \neq b_{ij}(x)$,

where we assumed both quadratic forms $\alpha^2 = a_{ij}(x)y^iy^j$ and $\beta^2 = b_{ij}(x)y^iy^j$ are different and positive definite. We see that the Kropina metric $L = \frac{\alpha^2}{\beta}$, $\beta = b_i(x)y^i = \beta_1$. $L = \frac{\alpha^2}{\beta}$ in (3.8) is essentially different. A Kropina metric is singular (for $\beta \neq 0$), while metric (3.8) is regular.
Now we have defined the (α, β_2) type in (3.8), can be written as

(3.9) $L(x, y) = \alpha + \beta_2 = \sqrt{a_{ij}(x)y^iy^j} + \sqrt{b_{ij}(x)y^iy^j}$, $a_{ij}(x) \neq b_{ij}(x)$.

Which is an analogy with metric of the Randers space, but essentially different.
The indicatrix of (3.9) is the 4th -order surface

(3.10) $[(a_{ij} - b_{ij})y^iy^j]^2 - 2(a_{ij} + b_{ij})y^iy^j + 1 = 0$.

This space is also non-C-reducible. The case $a_{ij}(x) = b_{ij}(x)$ with in (3.8) to the Riemannian space. The essential difference between cases (3.8) and (3.9) is that in the equation for indicatrix (3.10) there is a free term which is taking in the corresponding equation for (3.8),

(3.11) $[(a_{ij}(x)y^iy^j]^2 - b_{ij}(x)y^iy^j = 0$, $y^i \neq 0$.

Here we can say that (3.8) is second order Kropina space, and (3.9) is second order Randers space since β_2 is a second order form. Now we have generalization to higher algebraic orders introducing Finsler spaces of type (α, β_m), m = 1,2,…,

where

(3.12) $\beta_m = b_{i_1 i_2 i_3 \ldots i_m}(x) y^{i_1} y^{i_2} y^{i_3} \ldots . y^{i_m}$,

and α has the same meaning as before. In particular, the metric function

(3.13) $L(x, y) = \frac{\alpha^2}{\beta_m} = \frac{a_{ij}(x) y^i y^j}{\sqrt[m]{b_{i_1 i_2 i_3 \ldots i_m}(x) y^{i_1} y^{i_2} y^{i_3} \ldots . y^{i_m}}}$,

defined the mth -order Kropina space, while

(3.14) $L(x, y) = \alpha + \beta_m = \sqrt{a_{ij}(x) y^i y^j} + (b_{i_1 i_2 i_3 \ldots i_m}(x) y^{i_1} y^{i_2} y^{i_3} \ldots . y^{i_m})^{1/m}$

defined the mth order Randers space. It is easy to see that the indicatrix of the mth-order Kropina space is an algebraic surface of order 2m. For m > 2 and a non-vanishing b-tensor these spaces cannot be Riemannian space. (3.14) generalizes the well-known m-root Finsler spaces of Shimada [5]. We have to distinguish between the m-order Kropina spaces $\frac{\alpha^2}{\beta_m}$ and the generalized m-Kropina metric $\alpha^{m+1}\beta^{-m}$ defined.

CONCLUSION

This paper has been divided into three sections of which the first section is introductory. In the second section we have studied Crystal Optics in Finsler spaces. In this section we have studied the application of Finsler geometry to physics is crystal optics and the symmetry properties all crystals can be divided into seven crystal systems as written: triclinic, monoclinic, orthorhombic, trigonal, tetragonal, hexagonal and cubic. But transparent crystals fall into only three distinct classes from the point of view of their optical properties, biaxial, uniaxial and isotropic crystals. Here we have studied only two classes: biaxial and uniaxial, since the isotropic crystals behave optically as amorphous bodies they have no optical anisotropy and correspond to Euclidean geometry. Also we have studied some concepts as in Table (1). In this way we have studied the case of an optically uniaxial crystal and assume that the optical axis is in the three directions, $v_1 = v_2 = v_0$ for ordinary velocity and $v_3 = v_e$ for extraordinary velocity. We have obtained two shells of the normal surface in Fig.2. One is a sphere of radius v_0 and corresponds to ordinary wave with a velocity independent of the direction of propagation. The other is a surface of revolution of the fourth order, and corresponds to the extraordinary wave with velocity depending on the angle between the direction of the wave normal and the optical axis. The two velocities are only equal if the wave normal is in the direction of the optical axis i.e. θ = 0. From fig.2. shown that both waves are linearly polarized with the direction of polarization **D** orthogonal one to the other. Such situation is called transparent solid monocrystals. Optical axes are same direction in all points of the material. In fluid crystals the optical axes are fixed in space i.e. an external electric or magnetic field and can be different in different points in a smooth way. In the third section we have studied Uniaxial Crystals in Finsler space. In this section we have studied using the method Okubo [4]. But first of all we have studied the equation of the extraordinary normal surface (2.12) of the previous section in orthogonal Cartesian coordinates. After these observations we have generalization to higher algebraic orders introducing Finsler spaces of type (α, β_m), m = 1,2,…,. We can say that (3.13) is mth order Kropina space, and (3.9) is mth order Randers space. Also it is easy to see that the indicatrix of the mth-order Kropina space is an algebraic surface of order 2m. For m > 2 and a non-vanishing b-tensor these spaces cannot be Riemannian space. (3.14) generalizes the well-known m-root Finsler spaces of Shimada [4]. We have to distinguish between the m-order Kropina spaces $\frac{\alpha^2}{\beta_m}$ and the generalized m-Kropina metric $\alpha^{m+1}\beta^{-m}$ defined.

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Table 1: Crystal Systems

Crystal System	Dielectric Axis	Ellipsoid of Wave Normals	Optical Classification
1. Triclinic	CCC 	Triaxial Ellipsoid	Biaxial
2. Monoclinic	CCF 	Triaxial Ellipsoid	Biaxial
3. Orthorombic	FFF 	Triaxial Ellipsoid	Biaxial
4. Trigonal 5. Tetragonal 6. Hexagonal	FRR 	Biaxial Ellipsoid(Spheriod)	Uniaxial
7. Cubic	RRR 	Sphere	Isotropic

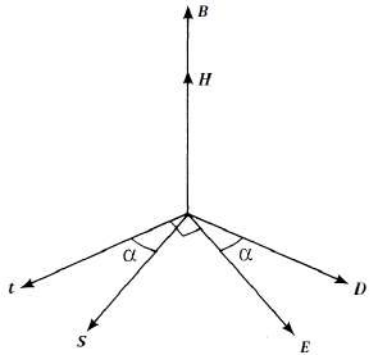
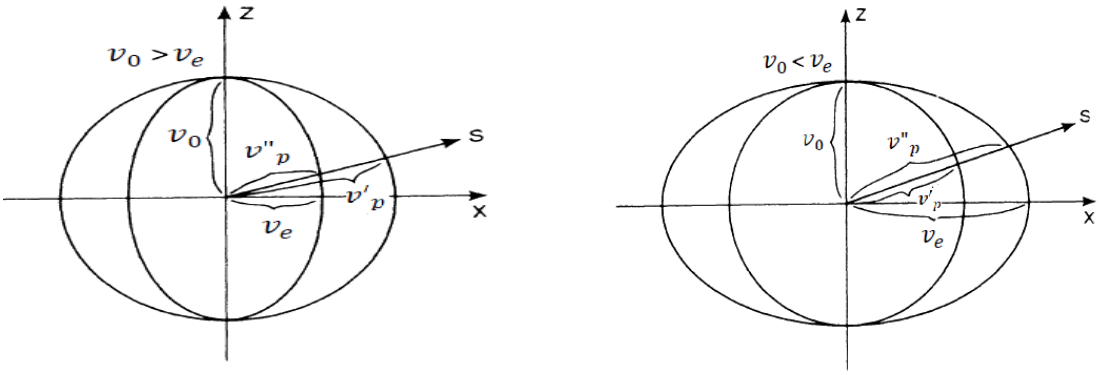


Fig 1: Directions of the wave normal of field vectors and of the energy flow in an anisotropic crystals.



(a) Positive uniaxial crystal (b) Negative uniaxial crystal

Fig 2: Normal surfaces of a uniaxial crystal

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RESEARCH ARTICLE

Bipolar Intuitionistic Fuzzy Matrices

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ABSTRACT

In this paper the concept of bipolar intuitionistic fuzzy matrices (BIFM) is introduced. Some results on transitive closure and power convergence. Some operations on BIFM are defined and illustrated by numerical examples.

Keywords: Bipolar intuitionistic fuzzy matrices, Operations on BIFM power convergence, Numerical examples.

INTRODUCTION

The concept of fuzzy set was introduced by Zadeh [15] and generalized as IFS by Atanassov [1, 2, 3, 4]. Hashimoto [5] decomposed transitive matrix as the sum of nilpotent and symmetric matrices and obtained a canonical form of transitive matrix. Kim et al. [6] studied determinants of fuzzy square matrices. Kolodziejczyk [7] extended the convergence property of max min to transitive fuzzy matrices. Shyamal et al. [10] defined two new binary fuzzy operators for fuzzy matrices. Thomason [11] established convergence of powers of a fuzzy matrix. Xin [12, 13] defined the controllable convergence of power of fuzzy matrices. Khan et al. [8] developed the concept of IFM. The concept of bipolar fuzzy set was first introduced by Zhang [14]. Pal et al. [9] defined the concept of BFM. Motivated by these theories, bipolar intuitionistic fuzzy matrices, operations of BIFM and convergence of BIFM has been developed.

Bipolar Intuitionistic Fuzzy Matrices
This section deals with operations on BIFM and some properties.

Definition 2.1: A BIFM of order $(r \times s)$ is denoted by M_{rs} and that of order $(r \times r)$ that is square BIFM is denoted by M_{rr}. $A=[(-\mu_A^n(a_{ij}),\mu_A^p(a_{ij})),(-\nu_A^n(a_{ij}),\nu_A^p(a_{ij}))]$, where $\mu_A^n(a_{ij}),\mu_A^p(a_{ij}),\nu_A^n(a_{ij}),\nu_A^p(a_{ij}) \in [-1,1]$ and satisfies $-1 \le \mu_A^n(a_{ij})+\nu_A^n(a_{ij}) \le 0, 0 \le \mu_A^p(a_{ij})+\nu_A^p(a_{ij}) \le 1$ $-1 \le \mu_A^n(a_{ij})+\nu_A^p(a_{ij}) \le 0$ and $0 \le \mu_A^p(a_{ij})+\nu_A^n(a_{ij}) \le 1$

Operations on BIFM

Definition 3.1: Consider the BIFM $A=[(-\mu_A^n(a_{ij}),\mu_A^p(a_{ij})),(-\nu_A^n(a_{ij}),\nu_A^p(a_{ij}))] \in M_{rs}$ and $B=[(-\mu_B^n(b_{ij}),\mu_B^p(b_{ij})),(-\nu_B^n(b_{ij}),\nu_B^p(b_{ij}))] \in M_{rs}$. Their operations are defined

$$(i)A+B=[-\max(\mu_A^n(a_{ij}),\mu_B^n(b_{ij})),\max(\mu_A^p(a_{ij}),\mu_B^p(b_{ij}))$$
$$-\min(\nu_A^n(a_{ij}),\nu_B^n(b_{ij})),\min(\nu_A^p(a_{ij}),\nu_B^p(b_{ij}))]$$

$$(ii)A\cdot B=[-\min(\mu_A^n(a_{ij}),\mu_B^n(b_{ij})),\min(\mu_A^p(a_{ij}),\mu_B^p(b_{ij}))$$
$$-\max(\nu_A^n(a_{ij}),\nu_B^n(b_{ij})),\max(\nu_A^p(a_{ij}),\nu_B^p(b_{ij}))]$$

$$(iii)A\odot B=\sum_{k=1}^{m}[a_{ik}.b_{kj}]$$
$$=[-\max[\min(\mu_A^n(a_{ik}),\mu_B^n(b_{kj}))],\max[\min(\mu_A^p(a_{ik}),\mu_B^p(b_{kj}))]$$
$$-\min[\max(\nu_A^n(a_{ik}),\nu_B^n(b_{kj}))],\min[\max(\nu_A^p(a_{ik}),\nu_B^p(b_{kj}))]]_{r\times s}$$

$$(iv)A\otimes B=\prod_{k=1}^{m}[a_{ik}+b_{kj}]$$
$$=[-\min[\max(\mu_A^n(a_{ik}),\mu_B^n(b_{kj}))],\min[\max(\mu_A^p(a_{ik}),\mu_B^p(b_{kj}))]$$
$$-\max[\min(\nu_A^n(a_{ik}),\nu_B^n(b_{kj}))],\max[\min(\nu_A^p(a_{ik}),\nu_B^p(b_{kj}))]]_{r\times s}$$

Theorem 3.2: Let *A,B,C* be BIFMs, then

(i) *A+B=B+A,*
(ii) *A.B=B.A,*
(iii) *A+(B+C)=(A+B)+C,*
(iv) *A.(B.C)=(A.B).C,*
(v) *A.(B+C)=(A.B)+(A.C),*
(vi) *A+(B.C)=(A+B).(A+C),*
(vii) *A+0=0+A=A,*
(viii) *A.0=0.A=A,* where 0 is the zero matrix with appropriate order.

Proof:

$$(i)A+B=[-\max(\mu_A^n(a_{ij}),\mu_B^n(b_{ij})),\max(\mu_A^p(a_{ij}),\mu_B^p(b_{ij}))$$
$$-\min(\nu_A^n(a_{ij}),\nu_B^n(b_{ij})),\min(\nu_A^p(a_{ij}),\nu_B^p(b_{ij}))]$$

$$= [-\max(\mu_B^n(b_{ij}), \mu_A^n(a_{ij})), \max(\mu_B^p(b_{ij}), \mu_A^p(a_{ij}))$$
$$-\min(\nu_B^n(b_{ij}), \nu_A^n(a_{ij})), \min(\nu_B^p(b_{ij}), \nu_A^p(a_{ij}))] = B + A$$

$$(ii)\, A \cdot B = [-\min(\mu_A^n(a_{ij}), \mu_B^n(b_{ij})), \min(\mu_A^p(a_{ij}), \mu_B^p(b_{ij}))$$
$$-\max(\nu_A^n(a_{ij}), \nu_B^n(b_{ij})), \max(\nu_A^p(a_{ij}), \nu_B^p(b_{ij}))]$$
$$= [-\min(\mu_B^n(b_{ij}), \mu_A^n(a_{ij})), \min(\mu_B^p(b_{ij}), \mu_A^p(a_{ij}))$$
$$-\max(\nu_B^n(b_{ij}), \nu_A^n(a_{ij})), \max(\nu_B^p(b_{ij}), \nu_A^p(a_{ij}))] = B + A.$$

Similarly (iii) and (iv) holds.

$$(v)\, B + C = [-\max(\mu_B^n(b_{ij}), \mu_C^n(c_{ij})), \max(\mu_B^p(b_{ij}), \mu_C^p(c_{ij}))$$
$$-\min(\nu_B^n(b_{ij}), \nu_C^n(c_{ij})), \min(\nu_B^p(b_{ij}), \nu_C^p(c_{ij}))]$$
$$A \cdot (B + C) = [-\min(\mu_A^n(a_{ij}) - \max(\mu_B^n(b_{ij}), \mu_C^n(c_{ij}))), \min(\mu_A^p(a_{ij}), \max(\mu_B^p(b_{ij}), \mu_C^p(c_{ij})))$$
$$-\max(\nu_A^n(a_{ij}) - \min(\nu_B^n(b_{ij}), \nu_C^n(c_{ij}))), \max(\nu_A^n(a_{ij}), \min(\nu_B^p(b_{ij}), \nu_C^p(c_{ij})))] \quad (3.1)$$
$$(A \cdot B) + (A \cdot C) = [-\max(-\min(\mu_A^n(a_{ij}), \mu_B^n(b_{ij})), -\min(\mu_A^n(a_{ij}), \mu_C^n(c_{ij}))),$$
$$\max(\min(\mu_A^p(a_{ij}), \mu_B^p(b_{ij})), \min(\mu_A^p(a_{ij}), \mu_C^p(c_{ij})))$$
$$-\min(-\max(\nu_A^n(a_{ij}), \nu_B^n(b_{ij})), -\max(\nu_A^n(a_{ij}), \nu_C^n(c_{ij})))$$
$$\min(\max(\nu_A^p(a_{ij}), \nu_B^p(b_{ij})), \max(\nu_A^p(a_{ij}), \nu_C^p(c_{ij})))]$$
$$= [-\min(\mu_A^n(a_{ij}) - \max(\mu_B^n(b_{ij}), \mu_C^n(c_{ij}))),$$
$$\min(\mu_A^p(a_{ij}), \max(\mu_B^p(b_{ij}), \mu_C^p(c_{ij})))$$
$$-\max(\nu_A^n(a_{ij}) - \min(\nu_B^n(b_{ij}), \nu_C^n(c_{ij}))),$$
$$\max(\nu_A^n(a_{ij}), \min(\nu_B^p(b_{ij}), \nu_C^p(c_{ij})))] \quad (3.2)$$

By (3.1) and (3.2) (v) holds.

$$(vi)\, B \cdot C = [-\min(\mu_B^n(b_{ij}), \mu_C^n(c_{ij})), \min(\mu_B^p(b_{ij}), \mu_C^p(c_{ij}))$$
$$-\max(\nu_B^n(b_{ij}), \nu_C^n(c_{ij})), \max(\nu_B^p(b_{ij}), \nu_C^p(c_{ij}))]$$
$$A + (B \cdot C) = [-\max(\mu_A^n(a_{ij}) - \min(\mu_B^n(b_{ij}), \mu_C^n(c_{ij}))), \max(\mu_A^p(a_{ij}), \min(\mu_B^p(b_{ij}), \mu_C^p(c_{ij})))$$
$$-\min(\nu_A^n(a_{ij}) - \max(\nu_B^n(b_{ij}), \nu_C^n(c_{ij}))), \min(\nu_A^n(a_{ij}), \max(\nu_B^p(b_{ij}), \nu_C^p(c_{ij})))] \quad (3.3)$$
$$(A + B) \cdot (A + C) = [-\min(-\max(\mu_A^n(a_{ij}), \mu_B^n(b_{ij})), -\max(\mu_A^n(a_{ij}), \mu_C^n(c_{ij}))),$$
$$\min(\max(\mu_A^p(a_{ij}), \mu_B^p(b_{ij})), \max(\mu_A^p(a_{ij}), \mu_C^p(c_{ij})))$$
$$-\max(-\min(\nu_A^n(a_{ij}), \nu_B^n(b_{ij})), -\min(\nu_A^n(a_{ij}), \nu_C^n(c_{ij})))$$
$$\max(\min(\nu_A^p(a_{ij}), \nu_B^p(b_{ij})), \min(\nu_A^p(a_{ij}), \nu_C^p(c_{ij})))]$$

$$=[-\max(\mu_A^n(a_{ij})-\min(\mu_B^n(b_{ij}),\mu_C^n(c_{ij}))),$$
$$\max(\mu_A^p(a_{ij}),\min(\mu_B^p(b_{ij}),\mu_C^p(c_{ij})))$$
$$-\min(\nu_A^n(a_{ij})-\max(\nu_B^n(b_{ij}),\nu_C^n(c_{ij}))),$$
$$\min(\nu_A^n(a_{ij}),\max(\nu_B^p(b_{ij}),\nu_C^p(c_{ij})))] \quad (3.4)$$

By (3.3) and (3.4) (vi) holds. When 0 is a zero matrix, (vii) and (viii) always hold for BIFMs.

Numerical Example

Consider the BIFMs

$$A=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.15,0.75)(-0.18,0.12)\\ (-0.4,0.5)(-0.3,0.17) & (-0.15,0.68)(-0.42,0.1)\end{bmatrix}$$
$$B=\begin{bmatrix}(-0.6,0.7)(-0.28,0.2) & (-0.25,0.4)(-0.2,0.06)\\ (-0.17,0.52)(-0.7,0.08) & (-0.3,0.7)(-0.6,0.2)\end{bmatrix}$$

$A+B=B+A=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.15,0.75)(-0.2,0.06)\\ (-0.17,0.52)(-0.7,0.08) & (-0.15,0.7)(-0.6,0.1)\end{bmatrix}$

$A.B=B.A=\begin{bmatrix}(-0.6,0.7)(-0.28,0.2) & (-0.25,0.4)(-0.18,0.12)\\ (-0.4,0.5)(-0.3,0.17) & (-0.3,0.68)(-0.42,0.2)\end{bmatrix}$

Theorem 3.3: Let *A,B* be BIFMs, then

$(i)A\odot B\neq B\odot A,$

$(ii)A\otimes B\neq B\otimes A$

Proof: From Definition $A\odot B$ and $A\otimes B$ are possible if the other of *A,B is r×t* and *t×s,* respectively. The number of columns of *A*= number of rowsof *B. Then* $B\odot A$ and $B\otimes A$ do not exist. To verify consider the following example. Let

$$A=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.15,0.75)(-0.18,0.12) & (-0.35,0.7)(-0.55,0.3)\\ (-0.4,0.5)(-0.3,0.17) & (-0.15,0.68)(-0.42,0.1) & (-0.3,0.8)(-0.6,0.2)\\ (-0.6,0.7)(-0.28,0.2) & (-0.25,0.4)(-0.2,0.06) & (-0.6,0.5)(-0.3,0.4)\end{bmatrix}$$

$$B=\begin{bmatrix}(-0.2,0.9)(-0.8,0.1) & (-0.85,0.25)(-0.15,0.75) & (-0.65,0.3)(-0.25,0.5)\\ (-0.4,0.5)(-0.3,0.25) & (-0.85,0.32)(-0.15,0.68) & (-0.7,0.2)(-0.15,0.6)\\ (-0.8,0.2)(-0.15,0.6) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.25)\end{bmatrix}$$

$$A\odot B=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.2)\\ (-0.4,0.5)(-0.3,0.17) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.25)\\ (-0.4,0.7)(-0.28,0.2) & (-0.75,0.6)(-0.25,0.4) & (-0.6,0.5)(-0.3,0.4)\end{bmatrix} \quad (3.5)$$

$$B\odot A=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.2,0.75)(-0.2,0.12) & (-0.35,0.7)(-0.55,0.3)\\ (-0.4,0.5)(-0.3,0.25) & (-0.4,0.5)(-0.18,0.25) & (-0.4,0.5)(-0.3,0.3)\\ (-0.6,0.5)(-0.28,0.25) & (-0.4,0.6)(-0.25,0.25) & (-0.6,0.6)(-0.3,0.4)\end{bmatrix} \quad (3.6)$$

$A\odot B\neq B\odot A.$ Similarly to find the BIFMs $A\otimes B$ and $B\otimes A$.

$$A\otimes B=\begin{bmatrix}(-0.35,0.7)(-0.3,0.3) & (-0.35,0.7)(-0.18,0.3) & (-0.35,0.7)(-0.18,0.25)\\ (-0.3,0.68)(-0.42,0.2) & (-0.4,0.5)(-0.3,0.2) & (-0.35,0.68)(-0.42,0.2)\\ (-0.6,0.5)(-0.3,0.4) & (-0.6,0.4)(-0.2,0.2) & (-0.4,0.4)(-0.2,0.25)\end{bmatrix} \quad (3.6)$$

$$B \otimes A = \begin{bmatrix} (-0.6,0.5)(-0.28,0.2) & (-0.25,0.4)(-0.25,0.1) & (-0.6,0.5)(-0.3,0.4) \\ (-0.6,0.5)(-0.28,0.2) & (-0.25,0.4)(-0.1,0.12) & (-0.6,0.5)(-0.3,0.4) \\ (-0.4,0.6)(-0.3,0.2) & (-0.25,0.5)(-0.18,0.12) & (-0.4,0.5)(-0.3,0.3) \end{bmatrix} \quad (3.7)$$

$A \otimes B \neq B \otimes A.$

Theorem 3.4: The BIFMs *A,B,C* satisfies the following laws

$(i) A \odot (B \odot C) = (A \odot B) \odot C,$

$(ii) A \otimes (B \otimes C) = (A \otimes B) \otimes C,$

$(iii) A \odot I = I \odot A = A,$

$(iv) A \otimes I = I \otimes A = A,$ *where I is identity matrix with appropriate order*

Proof: Let $A = [(-\mu_A^n(a_{ij}), \mu_A^p(a_{ij})), (-\nu_A^n(a_{ij}), \nu_A^p(a_{ij}))] \in M_{qr}$,

$B = [(-\mu_B^n(b_{ij}), \mu_B^p(b_{ij})), (-\nu_B^n(b_{ij}), \nu_B^p(b_{ij}))] \in M_{rt}$ and

$C = [(-\mu_C^n(c_{ij}), \mu_C^p(c_{ij})), (-\nu_C^n(c_{ij}), \nu_C^p(c_{ij}))] \in M_{ts}$ and also consider the matrix with $\odot$ operation.

$(B \odot C) = [(-\mu_D^n(d_{ij}), \mu_D^p(d_{ij})), (-\nu_D^n(d_{ij}), \nu_D^p(d_{ij}))] \in M_{rs}$. Then the jth entries of $(B \odot C)$ is

$$[(-\mu_D^n(d_{ij}), \mu_D^p(d_{ij})), (-\nu_D^n(d_{ij}), \nu_D^p(d_{ij}))] = \sum_{k=1}^{m} [(-\mu_B^n(b_{ik}), \mu_B^p(b_{ik})), (-\nu_B^n(b_{ik}), \nu_B^p(b_{ik}))] \odot [(-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))]$$

Therefore, the ij[th] entries of $A \odot (B \odot C)$ is

$$\sum_{q=1}^{r} [(-\mu_A^n(a_{iq}), \mu_A^p(a_{iq})), (-\nu_A^n(a_{iq}), \nu_A^p(a_{iq}))] \odot [(-\mu_D^n(d_{qj}), \mu_D^p(d_{qj})), (-\nu_D^n(d_{qj}), \nu_D^p(d_{qj}))]$$

$$= \sum_{q=1}^{r} [(-\mu_A^n(a_{iq}), \mu_A^p(a_{iq})), (-\nu_A^n(a_{iq}), \nu_A^p(a_{iq}))] \odot \left(\sum_{k=1}^{s} [(-\mu_B^n(b_{qk}), \mu_B^p(b_{qk})), (-\nu_B^n(b_{qk}), \nu_B^p(b_{qk}))] \odot [(-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))] \right)$$

$$= \sum_{q=1}^{r} \sum_{k=1}^{s} [(-\mu_A^n(a_{iq}), \mu_A^p(a_{iq})), (-\nu_A^n(a_{iq}), \nu_A^p(a_{iq}))] \odot [(-\mu_B^n(b_{qk}), \mu_B^p(b_{qk})), (-\nu_B^n(b_{qk}), \nu_B^p(b_{qk}))] \odot [(-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))]$$

$$= \sum_{k=1}^{s} \sum_{q=1}^{r} [(-\mu_A^n(a_{iq}), \mu_A^p(a_{iq})), (-\nu_A^n(a_{iq}), \nu_A^p(a_{iq}))] \odot [(-\mu_B^n(b_{qk}), \mu_B^p(b_{qk})), (-\nu_B^n(b_{qk}), \nu_B^p(b_{qk}))] \odot [(-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))]$$

$$= \sum_{k=1}^{s} \left(\sum_{q=1}^{r} [(-\mu_A^n(a_{iq}), \mu_A^p(a_{iq})), (-\nu_A^n(a_{iq}), \nu_A^p(a_{iq}))] \odot [(-\mu_B^n(b_{qk}), \mu_B^p(b_{qk})), (-\nu_B^n(b_{qk}), \nu_B^p(b_{qk}))] \right) \odot [(-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))]$$

$$= \sum_{k=1}^{s} [(-\mu_E^n(e_{ik}), \mu_E^p(e_{ik})), (-\nu_E^n(e_{ik}), \nu_E^p(e_{ik})) \odot (-\mu_C^n(c_{kj}), \mu_C^p(c_{kj})), (-\nu_C^n(c_{kj}), \nu_C^p(c_{kj}))]$$

Therefore $A \odot (B \odot C) = (A \odot B) \odot C$ holds. The proof of (ii) is similar.

Numerical Example

Let

$$A = \begin{bmatrix} (-0.2,0.9)(-0.6,0.1) & (-0.15,0.75)(-0.18,0.12) & (-0.35,0.7)(-0.55,0.3) \\ (-0.4,0.5)(-0.3,0.17) & (-0.15,0.68)(-0.42,0.1) & (-0.3,0.8)(-0.6,0.2) \\ (-0.6,0.7)(-0.28,0.2) & (-0.25,0.4)(-0.2,0.06) & (-0.6,0.5)(-0.3,0.4) \end{bmatrix}$$

$$B = \begin{bmatrix} (-0.2,0.9)(-0.8,0.1) & (-0.85,0.25)(-0.15,0.75) & (-0.65,0.3)(-0.25,0.5) \\ (-0.4,0.5)(-0.3,0.25) & (-0.85,0.32)(-0.15,0.68) & (-0.7,0.2)(-0.15,0.6) \\ (-0.8,0.2)(-0.15,0.6) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.25) \end{bmatrix}$$

$$C = \begin{bmatrix} (-0.4,0.5)(-0.2,0.3) & (-0.5,0.6)(-0.3,0.1) & (-0.7,0.3)(-0.2,0.1) \\ (-0.7,0.3)(-0.1,0.2) & (-0.4,0.7)(-0.1,0.2) & (-0.4,0.5)(-0.1,0.2) \\ (-0.6,0.2)(-0.1,0.4) & (-0.3,0.5)(-0.2,0.1) & (-0.4,0.5)(-0.3,0.1) \end{bmatrix}$$

$$A \odot B = \begin{bmatrix} (-0.2,0.9)(-0.6,0.1) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.2) \\ (-0.4,0.5)(-0.3,0.17) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.25) \\ (-0.4,0.7)(-0.28,0.2) & (-0.75,0.6)(-0.25,0.4) & (-0.6,0.5)(-0.3,0.4) \end{bmatrix}$$

$$(A \odot B) \odot C = \begin{bmatrix} (-0.4,0.5)(-0.2,0.3) & (-0.4,0.6)(-0.3,0.1) & (-0.5,0.5)(-0.3,0.1) \\ (-0.4,0.5)(-0.2,0.3) & (-0.4,0.6)(-0.3,0.17) & (-0.5,0.5)(-0.3,0.17) \\ (-0.4,0.5)(-0.2,0.3) & (-0.5,0.6)(-0.28,0.2) & (-0.6,0.5)(-0.3,0.2) \end{bmatrix} \quad (3.9)$$

$$B \odot C = \begin{bmatrix} (-0.4,0.5)(-0.2,0.3) & (-0.5,0.6)(-0.3,0.1) & (-0.65,0.3)(-0.25,0.1) \\ (-0.4,0.5)(-0.2,0.3) & (-0.5,0.5)(-0.3,0.25) & (-0.7,0.32)(-0.2,0.25) \\ (-0.6,0.3)(-0.15,0.34) & (-0.4,0.6)(-0.2,0.25) & (-0.5,0.5)(-0.3,0.25) \end{bmatrix}$$

$$A \odot (B \odot C) = \begin{bmatrix} (-0.4,0.5)(-0.2,0.3) & (-0.4,0.6)(-0.3,0.1) & (-0.5,0.5)(-0.3,0.1) \\ (-0.4,0.5)(-0.2,0.3) & (-0.4,0.6)(-0.3,0.17) & (-0.5,0.5)(-0.3,0.17) \\ (-0.4,0.5)(-0.2,0.3) & (-0.5,0.6)(-0.28,0.2) & (-0.6,0.5)(-0.3,0.2) \end{bmatrix} \quad (3.10)$$

From (3.9) and (3.10) $A \odot (B \odot C) = (A \odot B) \odot C$.

$$A \otimes B = \begin{bmatrix} (-0.35,0.7)(-0.3,0.3) & (-0.35,0.7)(-0.18,0.3) & (-0.35,0.7)(-0.18,0.25) \\ (-0.3,0.68)(-0.42,0.2) & (-0.4,0.5)(-0.3,0.2) & (-0.35,0.68)(-0.42,0.2) \\ (-0.6,0.5)(-0.3,0.4) & (-0.6,0.4)(-0.2,0.2) & (-0.4,0.4)(-0.2,0.25) \end{bmatrix} \quad (A \otimes B) \otimes C =$$

$$\begin{bmatrix} (-0.35,0.7)(-0.18,0.3) & (-0.35,0.7)(-0.18,0.2) & (-0.35,0.7)(-0.18,0.2) \\ (-0.4,0.5)(-0.3,0.2) & (-0.4,0.68)(-0.3,0.2) & (-0.4,0.5)(-0.3,0.2) \\ (-0.6,0.5)(-0.2,0.4) & (-0.5,0.5)(-0.2,0.2) & (-0.6,0.4)(-0.2,0.2) \end{bmatrix} \quad (3.11)$$

$$B \otimes C = \begin{bmatrix} (-0.7,0.3)(-0.15,0.4) & (-0.4,0.5)(-0.15,0.2) & (-0.5,0.4)(-0.15,0.2) \\ (-0.7,0.2)(-0.15,0.4) & (-0.4,0.5)(-0.15,0.2) & (-0.5,0.4)(-0.15,0.2) \\ (-0.7,0.5)(-0.2,0.3) & (-0.5,0.5)(-0.25,0.2) & (-0.7,0.3)(-0.2,0.2) \end{bmatrix}$$

$$A \otimes (B \otimes C) = \begin{bmatrix} (-0.35,0.7)(-0.18,0.3) & (-0.35,0.7)(-0.18,0.2) & (-0.35,0.7)(-0.18,0.2) \\ (-0.4,0.5)(-0.3,0.2) & (-0.4,0.68)(-0.3,0.2) & (-0.4,0.5)(-0.3,0.2) \\ (-0.6,0.5)(-0.2,0.4) & (-0.5,0.5)(-0.2,0.2) & (-0.6,0.4)(-0.2,0.2) \end{bmatrix} \quad (3.12)$$

From (3.11) and (3.12) $A \otimes (B \otimes C) = (A \otimes B) \otimes C$.

(iii) For the square matrix

$A = [(-\mu_A^n(a_{ij}), \mu_A^p(a_{ij})), (-\nu_A^n(a_{ij}), \nu_A^p(a_{ij}))] \in M_{rr}$ and the identity matrix.

$I = [(-\mu_E^n(e_{ij}), \mu_E^p(e_{ij})), (-\nu_E^n(e_{ij}), \nu_E^p(e_{ij}))] \in M_{rr}$ of the same order, then ijth entry of

$A \odot I = [(-\mu_B^n(b_{ij}), \mu_B^p(b_{ij})), (-\nu_B^n(b_{ij}), \nu_B^p(b_{ij}))] \in M_{rr}$. Where

$[(-\mu_B^n(b_{ij}),\mu_B^p(b_{ij})),(-\nu_B^n(b_{ij}),\nu_B^p(b_{ij}))]$

$$= \sum_{k=1}^{m} [(-\mu_A^n(a_{ik}),\mu_A^p(a_{ik})),(-\nu_A^n(a_{ik}),\nu_A^p(a_{ik})) \odot (-\mu_E^n(e_{kj}),\mu_E^p(e_{kj})),(-\nu_E^n(e_{kj}),\nu_E^p(e_{kj}))]$$

Therefore

$$\begin{aligned}
&[(-\mu_B^n(b_{ij}),\mu_B^p(b_{ij})),(-\nu_B^n(b_{ij}),\nu_B^p(b_{ij}))] \\
&= [(\mu_A^n(a_{i1})\cdot\mu_E^n(e_{1j}))+(\mu_A^n(a_{i2})\cdot\mu_E^n(e_{2j})) \\
&+\cdots(\mu_A^p(a_{i1})\cdot\mu_E^p(e_{1j}))+(\mu_A^p(a_{i2})\cdot\mu_E^p(e_{2j})) \\
&+\cdots(\nu_A^n(a_{i1})\cdot\nu_E^n(e_{1j}))+(\nu_A^n(a_{i2})\cdot\nu_E^n(e_{2j})) \\
&+\cdots(\nu_A^p(a_{i1})\cdot\nu_E^p(e_{1j}))+(\nu_A^p(a_{i2})\cdot\nu_E^p(e_{2j})) \\
&+\cdots(\mu_A^n(a_{im-1})\cdot\mu_E^n(e_{m-1j}))+(\mu_A^n(a_{im})\cdot\mu_E^n(e_{mj})) \\
&+\cdots(\mu_A^p(a_{im-1})\cdot\mu_E^p(e_{m-1j}))+(\mu_A^p(a_{im})\cdot\mu_E^p(e_{mj})) \\
&+\cdots(\nu_A^n(a_{im-1})\cdot\nu_E^n(e_{m-1j}))+(\nu_A^n(a_{im})\cdot\nu_E^n(e_{mj})) \\
&+\cdots(\nu_A^p(a_{im-1})\cdot\nu_E^p(e_{m-1j}))+(\nu_A^p(a_{im})\cdot\nu_E^p(e_{mj}))] \\
&= A = [(-\mu_A^n(a_{ij}),\mu_A^p(a_{ij})),(-\nu_A^n(a_{ij}),\nu_A^p(a_{ij}))]
\end{aligned}$$

Thus $A \odot I = A$. Similarly $I \odot A = A$. $A \otimes I = I \otimes A = A$, can also be proved in a similar way.

Theorem 3.5: Let *A,B,C* be BIFMs, satisfies the following laws,

$(i) A \odot (B \otimes C) \neq (A \odot B) \otimes (A \odot C)$,

$(ii) A \otimes (B \odot C) \neq (A \otimes B) \odot (A \otimes C)$.

Proof: Let $A = [(-\mu_A^n(a_{ij}),\mu_A^p(a_{ij})),(-\nu_A^n(a_{ij}),\nu_A^p(a_{ij}))] \in M_{qr}$,

$B = [(-\mu_B^n(b_{ij}),\mu_B^p(b_{ij})),(-\nu_B^n(b_{ij}),\nu_B^p(b_{ij}))] \in M_{rt}$ and

$C = [(-\mu_C^n(c_{ij}),\mu_C^p(c_{ij})),(-\nu_C^n(c_{ij}),\nu_C^p(c_{ij}))] \in M_{ts}$ and also consider the matrix with $\odot$ operation. ($B \otimes C$

$)=[(-\mu_D^n(d_{ij}),\mu_D^p(d_{ij})),(-\nu_D^n(d_{ij}),\nu_D^p(d_{ij}))]\in M_{rs}$. Then the jth entries of $(B\odot C)$ is

$$[(-\mu_D^n(d_{ij}),\mu_D^p(d_{ij})),(-\nu_D^n(d_{ij}),\nu_D^p(d_{ij}))]=\sum_{k=1}^{s}[(-\mu_B^n(b_{ik}),\mu_B^p(b_{ik})),(-\nu_B^n(b_{ik}),\nu_B^p(b_{ik}))]$$
$$\otimes[(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]$$

Therefore, the ij[th] entries of $A\odot(B\otimes C)$ is

$$\sum_{q=1}^{r}[(-\mu_A^n(a_{iq}),\mu_A^p(a_{iq})),(-\nu_A^n(a_{iq}),\nu_A^p(a_{iq}))]\odot[(-\mu_D^n(d_{qj}),\mu_D^p(d_{qj})),(-\nu_D^n(d_{qj}),\nu_D^p(d_{qj}))]$$
$$=\sum_{q=1}^{r}[(-\mu_A^n(a_{iq}),\mu_A^p(a_{iq})),(-\nu_A^n(a_{iq}),\nu_A^p(a_{iq}))]\odot\left(\sum_{k=1}^{s}[(-\mu_B^n(b_{qk}),\mu_B^p(b_{qk})),(-\nu_B^n(b_{qk}),\nu_B^p(b_{qk}))]\otimes[(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]\right)$$
$$=\sum_{q=1}^{r}\sum_{k=1}^{s}[(-\mu_A^n(a_{iq}),\mu_A^p(a_{iq})),(-\nu_A^n(a_{iq}),\nu_A^p(a_{iq}))]\odot[(-\mu_B^n(b_{qk}),\mu_B^p(b_{qk})),(-\nu_B^n(b_{qk}),\nu_B^p(b_{qk}))]\otimes[(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]$$
$$=\sum_{k=1}^{s}\sum_{q=1}^{r}[(-\mu_A^n(a_{iq}),\mu_A^p(a_{iq})),(-\nu_A^n(a_{iq}),\nu_A^p(a_{iq}))]\odot[(-\mu_B^n(b_{qk}),\mu_B^p(b_{qk})),(-\nu_B^n(b_{qk}),\nu_B^p(b_{qk}))]\otimes[(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]$$
$$=\sum_{k=1}^{s}\left(\sum_{q=1}^{r}[(-\mu_A^n(a_{iq}),\mu_A^p(a_{iq})),(-\nu_A^n(a_{iq}),\nu_A^p(a_{iq}))]\odot[(-\mu_B^n(b_{qk}),\mu_B^p(b_{qk})),(-\nu_B^n(b_{qk}),\nu_B^p(b_{qk}))]\right)\otimes[(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]$$
$$\neq\sum_{k=1}^{s}[(-\mu_E^n(e_{ik}),\mu_E^p(e_{ik})),(-\nu_E^n(e_{ik}),\nu_E^p(e_{ik}))\otimes(-\mu_C^n(c_{kj}),\mu_C^p(c_{kj})),(-\nu_C^n(c_{kj}),\nu_C^p(c_{kj}))]$$

Therefore $A\odot(B\otimes C)\neq(A\odot B)\otimes(A\odot C)$ holds.

Numerical Example

$$B\otimes C=\begin{bmatrix}(-0.7,0.3)(-0.15,0.4) & (-0.4,0.5)(-0.15,0.2) & (-0.5,0.4)(-0.15,0.2)\\ (-0.7,0.2)(-0.15,0.4) & (-0.4,0.5)(-0.15,0.2) & (-0.5,0.4)(-0.15,0.2)\\ (-0.7,0.5)(-0.2,0.3) & (-0.5,0.5)(-0.25,0.2) & (-0.7,0.3)(-0.2,0.2)\end{bmatrix}$$

$$A\odot(B\otimes C)=\begin{bmatrix}(-0.4,0.5)(-0.15,0.2) & (-0.4,0.5)(-0.15,0.2) & (-0.4,0.5)(-0.15,0.2)\\ (-0.4,0.5)(-0.15,0.2) & (-0.4,0.5)(-0.15,0.2) & (-0.4,0.5)(-0.15,0.2)\\ (-0.5,0.5)(-0.25,0.2) & (-0.5,0.5)(-0.25,0.2) & (-0.5,0.5)(-0.25,0.2)\end{bmatrix}\quad(3.13)$$

$$A\odot B=\begin{bmatrix}(-0.2,0.9)(-0.6,0.1) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.2)\\ (-0.4,0.5)(-0.3,0.17) & (-0.75,0.6)(-0.25,0.4) & (-0.4,0.5)(-0.3,0.25)\\ (-0.4,0.7)(-0.28,0.2) & (-0.75,0.6)(-0.25,0.4) & (-0.6,0.5)(-0.3,0.4)\end{bmatrix}$$

$$A\odot C=\begin{bmatrix}(-0.4,0.5)(-0.2,0.2) & (-0.35,0.7)(-0.3,0.1) & (-0.4,0.5)(-0.3,0.1)\\ (-0.4,0.5)(-0.2,0.2) & (-0.3,0.68)(-0.3,0.17) & (-0.4,0.5)(-0.3,0.17)\\ (-0.6,0.5)(-0.2,0.2) & (-0.4,0.6)(-0.28,0.2) & (-0.4,0.4)(-0.3,0.2)\end{bmatrix}$$

$(A\odot B)\otimes(A\odot C)=$

$$\begin{bmatrix}(-0.4,0.5)(-0.25,0.2) & (-0.4,0.6)(-0.3,0.2) & (-0.4,0.5)(-0.3,0.2)\\ (-0.4,0.5)(-0.25,0.2) & (-0.4,0.6)(-0.3,0.2) & (-0.4,0.5)(-0.3,0.2)\\ (-0.6,0.5)(-0.25,0.2) & (-0.4,0.6)(-0.3,0.2) & (-0.4,0.5)(-0.3,0.2)\end{bmatrix}\quad(3.13)$$

Therefore $A\odot(B\otimes C)\neq(A\odot B)\otimes(A\odot C)$. Similarly $A\otimes(B\odot C)\neq(A\otimes B)\odot(A\otimes C)$.

Convergence of BIFMs

In this section the concept of convergence and power of convergence of a BIFM is defined. A sequence of matrices $R_1, R_2, \ldots, R_m, R_{m+1} \ldots$ that is the matrix $[R_m]$is said to converge to a finite matrix R if $\lim_{m\to\infty}[R_m] = R$.

Power of convergence of a BIFM

A least positive integer m is said to be power of convergence of a BIFM R in binary composition * if $R^{m+n}=R^{m+n-1}=R^{m+n-2}=\cdots=R^{m+1}=R^m$ where $n \in N$ and $R^2=R*R, R^3=R*R*R=R^2*R$ and so on. The number m is called the index of R and is denoted by $i(R_1)$.

Definition 4.1: Let $R_1, R_2, R_3 \in M_m$. The BIFM R_1 is said to be transitive, If $c \leq R1$. The BIFM R_2 is said to be transitive closure of matrix $R1$ if $R2$ is transitive, $R1 \leq R2$ and $R2 \leq R3$ for any transitive matrix R_3, satisfying $1 \leq R3$. The transitive closure of R_1 is denoted by $t(R_1)$.

Theorem 4.2: Let $R1 \in Mm$ be a BIFM. Then the transitive closure of R is given by $t(R) = \sum_{k=1}^{m} R^k$.

Proof: Let $R_2 = \sum_{k=1}^{m} R^k$, obliviously $R1 \leq R2$ since Mm is idempotent under addition, we ha $R_2^2 = \sum_{k=2}^{2m} R^k \leq \sum_{k=2}^{2m} R^k$ *or* $R_2^2 \leq R_2 \sum_{k=m+1}^{2m} R^k R_1^k \leq \sum_{\rho=1}^{2m} R_1^{\rho} = R_2$ as $k>m$. Hence $R_2^2 \leq R_2$. If there is a matrix $R3$ such that $R1 \leq R3$ and $R_3^2 = R_1$, then $R_3 \leq R_1 R_1 \leq R_3^2 \leq R_3$ and by induction $R_1^k \leq R_3^k \leq R_3$ for all positive integers k. Hence $R2 \leq R3$. Thus by the definition of transitive closure $R_2 = t(R_1) = \sum_{k=1}^{m} R^k$.

Numerical Example

Let $R = \begin{bmatrix} (-0.4,0.5)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.8)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix}$

$$R^2 = R \odot R = \begin{bmatrix} (-0.4,0.5)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.8)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix} \odot$$

$$\begin{bmatrix} (-0.4,0.5)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.8)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix} R^2 = \begin{bmatrix} (-0.4,0.6)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.7)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix} \neq R$$

$$R^3 = R^2 \odot R = \begin{bmatrix} (-0.4,0.6)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.7)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix} \odot$$

$$\begin{bmatrix} (-0.4,0.5)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.8)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix}$$

$$R^3 = \begin{bmatrix} (-0.4,0.6)(-0.3,0.2) & (-0.5,0.6)(-0.3,0.1) \\ (-0.3,0.7)(-0.25,0.2) & (-0.2,0.7)(-0.4,0.1) \end{bmatrix} = R^2$$

Hence, the matrix R power converges to the power $k=2$. i.e., $i(R)=2$. The matrix R of order 2 in the above example is both row and column diagonally dominant and $i(R)=2=3-1$. If all entries of a matrix R are same, then the BIFM is power convergence to 1. Also for the matrix R, $R \leq R^2$. R converges to $t(R)=R+R^2+R^2=R^2$ [since $R^3 = R^2$].

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RESEARCH ARTICLE

Validated RP-HPLC Method for the Determination of Fluticasone in Bulk and Tablet Dosage Form

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ABSTRACT

The novel, a simple and flexible economical method of high performance liquid chromatography (RP-HPLC) is designed to measure Fluticasone in quantity and tablet measurement form with greater accuracy and greater accuracy. Separation was reached in the Hypersil C18 column (250X4.6mm id, 5µm) in top mode using Methanol, Water and Triethylamine at a rate of 68:30: 02 (v / v / v) as part of a cell, enclosed in a column with a flow rate of 1.0 ml / min and the intake of eluent from the column was performed using a UV wavelength wave detector at 262 nm. The total working time was 6 min and the column was kept at a dry temperature. Fluticasone's final time was 3,490 minutes. Typical lines were equivalent to a concentration range of 20 - 80 µg / ml at R2 0.999 and the LOD and LOQ values of Fluticasone were 0.017 µg / ml and 0.052 µg / ml, respectively. Percentage detection was found to be 97 - 103%,% RSD was found to be 0.056. The percentage of manufacture of Fluticasone pills sold was 100.1%. This method is verified as ICH guidelines. Verification studies have shown that the proposed RP-HPLC method is simple, clear, fast, reliable and productive. The proposed method can be used in the general quality control analysis of Fluticasone in bulk and forms on tablet scales.

Keywords: Fluticasone, RP-HPLC, Method Development, Validation, ICH guidelines

MATERIALS AND METHODS

Instrumentation

Waters HPLC Auto Detector equipped with Empower-2 Software HPLC, equipped with Hypersil C18 250 x 4.6 mm, 5µ, Waters 2469 UV-Visible detector was used in the study. A 20 µl Hamilton injection was used with a sample injection. Data acquisition is done using the Empower 2 software.

The termination of the cell phase was performed using a spectra lab model DGA 20A3 ultrasonic bath sonicator. The electronic balance of shimadzu AUX 200 was used to measure equipment. Class 'A' Borosil glassware was hired for volumetric purpose and general purpose in the study.

Drugs

The reference sample and formulations of the drug was supplied by a local hospital.

Chemicals and solvents

Methanol - HPLC grade (Qualigens)
Acetonitrile - HPLC grade (Qualigens)
Tri ethyl amine – HPLC grade (Qualigens)
Water - HPLC grade water prepared using Millipore Milli Q system

Preparation of the mobile phase

The mobile Phase was prepared by mixing Methanol, water and triehylamine in a ratio of 68:30: 02 v / v / v. The cellular phase is then powered by the use of Ultra-Sonicator to remove impurities and dissolved gases, as they can lead to unwanted peaks in the chromatogram.

Standard solution Preparation

Standard solutions were prepared by accurately dosing 10 mg of Fluticasone Propionate and transferred to two pure volumetric clear bottles. Approximately 7mL of Mobile Phase is added to each flask and given the power to dissolve the powder completely. Final values adjusted to mark per Mobile Phase (per drug concentration was 1000ppm). From Stock 0.4 ml solutions Fluticasone Propionate solutions were transferred to 10ml volumetric bottles and diluted and labeled with the same diluent (concentration per drug was 40ppm).

Preparation of sample solution

Properly measured in 20 tablets, the average weight is taken. A dose equal to Fluticasone Propionate 100mg was accurately measured and taken in a 100 ml volumetric bottle with an additional 50 ml diluent of. The mixture was then given to a sonicator for 20 minutes with a stirring process between complete disinfection, filtered with whatman filter paper and lowered to room temperature and the solution was made to mark methanol .From the filtrate pipette above came 0.4 ml to a. 10 ml volumetric flask and diluted with diluents (drug concentration was 40ppm).

CHROMATOGRAPHIC CONDITIONS AND METHOD DEVELOPMENT

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. The following studies were conducted for this purpose

The mobile phase and the flow rate

In order to obtain a high degree of sharpness and line separation of objects, the author conducted several experiments on the variations of the most commonly used solvents, their composition and their flow rate.

To make a good separation of the drug under theological conditions, the most widely used chemical compounds such as water, methanol and acetonitrile with different or non-composite particles were tested as moving phases in the C18 phase. The combination of Methanol, water and tri ethyl amine at a ratio of 68:30: 02 V / V has been shown to be the most suitable for all compounds since the chromatographic peaks found were better defined and resolved and almost no tail. Stage flow rate of 1.0 ml / min. found suitable for a study range of 0.5 -15 ml / min.

Detection wavelength

The UV absorption spectrum of the drug was taken in methanol and the λ max found to be at 262 nm. Hence detection of the drug was made at 262 nm.

Retention time of Fluticasone Propionate

A model chromatogram showing the separation of Fluticasone Propionate is presented in Fig 1. Under the above optimized conditions a retention time of 4.2 min. was obtained for lamotrigine. After a thorough study of the various parameters the following optimized conditions mentioned in Table 2 were followed for the determination of Fluticasone Propionate in bulk samples and pharmaceutical formulations.

Linearity and Construction of calibration curve

The equal determination of the drug is achieved in the usual external way. The cell section was filtered through a membrane filter of 0.45µ before use. The flow rate of the mobile phase was changed to 1mL / min. The column is measured in the mobile section at least 30 min before injecting the drug solution. Column temperature was maintained at 37 ± 10 C throughout the study. The proportion of the upper extremity response was determined by taking six repeated measures in seven concentration areas. The therapeutic efficacy of fluticasone propionate at 20 - 80 µg / mL was adjusted by taking the appropriate solution concentrations in 10 volumetric volumes 10 mL and diluted up to the mark per cellular phase. Twenty microlitres of dilution are regularly added to the column with a flow rate of 1.0 mL / min. Each cleansing was injected six times in a column. The drug in eluents was monitored at 262 nm and consistent chromatograms were detected. From chromatograms, high altitudes were observed and a concentration structure was built at very high altitudes. The deflection of a building is calculated at least in the form of a square reversal. A relative correlation was found to be 20 - 80 µg / mL between Fluticasone Propionate concentration and high-dose response. This regression figure was used to estimate the amount of Fluticasone Propionate in drug dosage forms. The linear structure is shown in Fig. 2. and linearity data and linear statistical parameters are reported in Tables 2 and 3.

VALIDATION OF THE PROPOSED METHOD

This approach has been verified in accordance with ICH guidelines. The following parameters are limited to verification.

Specificity

The specificity of the method was tested by comparing the chromatograms obtained from the drug with the most commonly used excipient mixture with those obtained from the empty solution. The empty solution was fixed by mixing the connecting elements in the mobile unit without the tree. The drug in the excipient rate used was similar to that in commercial construction. The most widely used ingredients in the formulation such as lactose, starch, microcrystalline cellulose, ethyl cellulose, hydroxypropyl methylcellulose, magnesium stearate and colloidal silicon dioxide were used in the study. The mixtures were filtered through a membrane filter of 0.45µ before injection. A look at chromatograms indicates the absence of desirable peaks near the peak of the drug during the study period. This indicates that the method is specified.

Accuracy

Specification is the degree of repetition of the analysis method under normal operating conditions. The accuracy of the method was studied in terms of duplication (intra-day assay) and intermediate accuracy (inter-day assay). The repetition of the method was learned by repeating the test six times on the same day with internal accuracy of the days and the median accuracy was learned by repeating the test on three different days, three times each day (accuracy of certain days). Individual and intermittent duration of Fluticasone Propionate dosage was performed at three different concentration levels of 20, 40, 60 µg / ml. The variance values of the variables as presented in Table 4 indicate that the method provides acceptable variables (<2) within and between days.

Accuracy

The accuracy of this method is checked by the standard addition. The amount of pure drug solution at three different concentration levels was added to the pre-analysis dilution (20µg / mL) of the drug. Sample solutions were analyzed three times at each level according to the proposed method. Percentage return per cent and% RSD recovery per level calculated. Results are observed (Table 5). Satisfactory earnings from 99.90 to 99.98 received in the proposed manner indicate that this method is accurate.

Robustness

Research has been done to determine the effect of deliberate variations in chromatographic conditions such as Mobile Phase, flow rate, column oven temperature and cell pH. System appropriateness parameters compared to changes made to keep all other components running smoothly. Trial, tailing factor and number of theoretical plates tested. Results are found within the approved limits indicating that the method is specified.

Variations in the mobile phase composition

The effect of the percentage variation of natural content on the cellular phase was assessed by altering the structure of living organisms in the cellular phase by ± 20%. The tailing factor and number of theoretical plates showed small changes and changes in the cellular structure. Prices shown in Table 6.

Variations in flow rate of the mobile phase

A study was conducted to determine the effect of flow rate variability. System fit parameters were tested at 0.8 mL / min. and 1.2 mL / min. The results were within the acceptance process. Therefore the permissible variation of flow rate is 0.8 mL / min to 1.2 mL / min.

Variations in column oven temperature

A study was conducted to determine the effects of column temperature variations at 25^{0}C and 30^{0}C. System efficiency results were found within the temperature limits of both columns.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of the analyte that provides a measurable response. The LOD is determined based on the three-dimensional sound rating (S / N) standard for HPLC methods. The quantification limit (LOQ) is defined as the lowest concentration that can be reliably calculated with a certain degree of accuracy and precision. It is a very low condition where the specificity expressed by the RSD is less than 2%. In this study the analyst's response was ten times greater than the noise response. In this study six analyst responses at the lowest level in the rating scale were measured and rated. The LOD and LOQ of Fluticasone Propionate obtained by the proposed method were 0.017 and 0.0528.

System precision and System suitability

System precision and system suitability are done by injecting six repetitions of the standard solution and calculating% CV of the found area. The details as presented in Table 7. establish the reproductive function of the tool. The system adequacy parameters are given in Table 8.

ASSAY OF THE DRUG FROM TABLET DOSAGE FORMS:

Satisfactory results obtained with the method development for the assay of Fluticasone Propionate has attempted its applicability for the estimation of the drug from its dosage forms.

Preparation of sample solution and recovery study

Twenty Fluticasone Propionate tablets are weighed and powdered in the same size in mud and pestle. This calculates the average weight of the tablet. The precise weight from the 10, 20 mg and 50mcg powder of Fluticasone Propionate was transferred to a 100 mL volumetric flask containing 70 mL of mobile components. The contents of the

bottle are created for about 15 minutes to achieve complete dissolution of the drug. The mixture was then filtered through a membrane of 0.45 μ and filtered into a cellular phase. From the above solution (dilution within the linearity range) was prepared using a cellular phase. The above solution (20 μL) was then injected three times into the column. The highest concentrations of the sample and standard were calculated and the drug content in the formulation was calculated by method modification. The results are presented in Table 9.

CONCLUSION

In the process, efforts are being made to provide a new, more sensitive, simpler, more accurate and lower cost of HPLC used successfully in determining Fluticasone Propionate in pharmaceutical preparations without interruption of other structural components. In the HPLC method, HPLC conditions are designed for detection, sufficient separation of eluted compounds. Initially, a variety of mobile class songs were tried to get the best results. Mobile cell selection and flow rate were based on high parameters (height, suspension, theoretical plates, volume factor), operating time etc. System Methanol: Water: Tri ethylamine (68:30:02 v / v) with 1 ml / Minute flow rate is very stable. The maximum detection time was 262 nm when a better detection of the drug detector was obtained. The average storage time for Fluticasone Propionate was found to be 3.49 minutes. System suitability testing is an important part of the chromatographic approach. They are used to ensure the reproduction of the chromatographic system. To ensure its effectiveness, system validity tests are performed on newly adjusted stock solutions. The dosage was estimated at a concentration level of 20 - 80 μg / ml with regression 1, find 107.0 and slope 20057 for Fluticasone Propionate. Low prices for% R.S.D. show that this method is accurate and precise. The mean earnings were obtained in the range of 97% - 103%.

Sampling for precision and accuracy was tested using three samples of five and three different concentrations respectively, which were prepared and analyzed on the same day. Day-to-day variability was assessed using three focused analyzes on three different days, over a three-day period. These results indicate the accuracy and reproduction of the experiment. The complexity of the proposed methods is determined by analyzing aliquots from the same area as different analysts, using the same operating and environmental conditions% RSD reported was found to be less than 2% .The proposed method was confirmed in accordance with ICH limits of commercial drugs. There is therefore no significant difference in the results obtained by the proposed method.

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Table 1. Optimized Chromatographic Conditions

S.No.	Parameter	Value
1	Column	HypersilC_{18}(250 x 4.6 mm, packed with 5µm
2	Mobile Phase	Methanol, Water and Tri ethylamine in proportion of (68:30:02 v/v)
3	Flow Rate	1.0 mL/min
4	Run Time	6 min
5	Column Temperature	37±1^0 C
6	Volume of Injection	20 µL
7	Detection Wave Length	262 nm
8	Retention time of the drug	3.490 min.

Table 2. Calibration data of the proposed HPLC Method

Concentration of Fluticasone Propionate (µg/mL)	Mean Peak Area (n=6)
20 µg/ml	401430
30 µg/ml	601551
40 µg/ml	802349
50 µg/ml	1003478
60 µg/ml	1203234
70 µg/ml	1404427
80 µg/ml	1604367

Table 3. Regression characteristics of the linearity curve

Parameter	Value
Linearity Range (µg)	20 – 80
Slope (a)	20055
Intercept (b)	218
Correlation coefficient	1
Regression equation y = ax+b	y=20055x + 218

Table 4. Precision of the proposed method

Concentration of Fluticasone Propionate (µg/mL)	Intra-day Precision			Inter-day Precision		
	Mean (n=3)	SD	% RSD	Mean (n=3)	SD	% RSD
20	401430	30	0.1%	401290	50	0.1%
40	802469	66.5	0.1%	801488	67	0.1%
60	1203267	90.1	0.1%	1119879	80.1	0.1%

Table 5. Accuracy data (Triplicate values at different concentration levels)

S. No	Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
1	50% Injection 1	20	19.99	99.98	**MEAN**	100.04
2	50% Injection 2	20	20.01	100.07	**SD**	0.0519
3	50% Injection 3	20	20.01	100.07	**%RSD**	0.0519
4	100 % Injection 1	40	40.02	100.06	**MEAN**	99.98
5	100 % Injection 2	40	39.98	99.95	**SD**	0.0635
6	100% Injection 3	40	39.98	99.95	**%RSD**	0.0635
7	150% Injection 1	60	59.98	99.98	**MEAN**	99.97
8	150% Injection 2	60	59.98	99.97	**SD**	0.0057
9	150% Injection 3	60	59.98	99.97	**%RSD**	0.0057

Table 6. Results of the robustness study

S No	Parameter	Fluticasone Propionate		
		R_t	Area	Tailing Factor
1.	Initial sample	3.490	802065	1.184
2.	Variation in flow rate	3.488	802918	1.169
	0.8ml/min 1.2ml/min	2.988	746948	1.137
3.	Variation in temperature	3.488	802918	1.124
	25°c 30°c	3.518	802421	1.153
4.	Variation in mobile phase	3.505	802636	1.150
	50% organic phase 70% organic phase	3.598	811978	1.167

Table 7. System precision

Injection No.	Peak area
1	803557
2	802022
3	803601
Mean	805601
SD	899.2035
% RSD	**0.112**

Table 8. System suitability parameters

Parameters	Values
Theoretical plates (n)	9389
Tailing factor (T)	1.18
LOD	0.017
LOQ	0.0528

Table 9. HPLC analysis of tablets formulations

S. No	Fluticasone Propionate	
	Sample area	Standard area
01	803557	802918
02	802022	802064
03	803601	802731
AVG	805601	802571
STDEV	899.2035	448.9198
%RSD	0.112	0.056

Table 10. Assay Results

S.no	Fluticasone Propionate Assay results	
1	Sample Area	805601
2	Standard Area	802571
3	Standard weight	10mg
4	Sample weight	20mg
5	Label claim	50mcg
6	Standard Purity	99.80%
7	Assay	100.006%

Table 11. Significant difference

S. No	Parameter	Results
1	Linearity range (µg/mL)	20-80 µg/ml
2	Correlation coefficient	1
3	Theoretical plates (N)	9389
4	Tailing factor	1.164
5	LOD (µg/mL)	0.0429
6	LOQ (µg/mL)	0.0528
7	Assay	100.006%
8	Percentage Recovery	99.95-100.06%

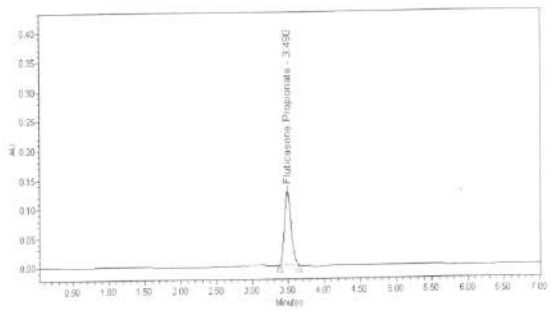

Fig. 1. A Model Chromatogram showing the separation Fluticasone Propionate

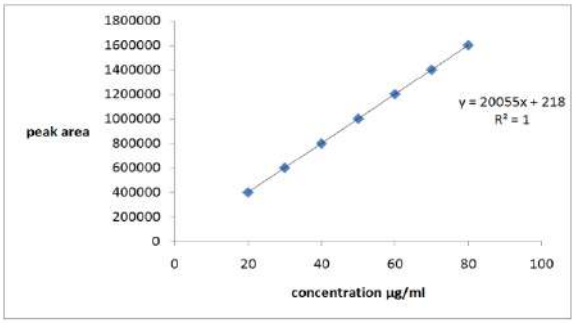

Fig. 2. Linearity Plot for the proposed method

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RESEARCH ARTICLE

Assessment of Disease Incidence and Virulence of *Sclerotium rolfsii* Sacc. Incited by Stem Rot of Groundnut (*Arachis hypogae* L.)

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important edible leguminous oilseed crop. The cultivation of groundnut is hindered by several biotic and abiotic stresses. Stem rot disease incited by *Sclerotium rolfsii* Sacc. is one of the destructive diseases of peanut and it deteriorate the quality and quantity of peanut. An average yield loss goes up to 80% in severe cases. A survey was conducted in major groundnut growing districts of Tamil Nadu, to study thedisease incidence of groundnut stem rot. The overall disease severity ranges from 10.78 to 39.03 percent and the highest disease incidence 39.03 per cent was noticed in the Thoradipattu village (isolate SrTRP), whereas the least incidence 10.78 per cent was recorded in Nedungur village (isolate SrNDR).The morphological studies on the pathogen showed variation among different isolates. Twenty isolates of *Sclerotium rolfsii* collected from different geographical locations were found varied in their mycelial growth rate, colony character, pattern and distribution, number and size of the sclerotia. Isolate SrTRP showed profuse cottony white mycelium with maximum mycelial growth. The virulence was conducted to all the twenty isolates and the isolate SrALR exhibited the maximum disease incidence and proved to be more virulent.

Keywords: Groundnut, Stem rot, *Sclerotium rolfsii*, Morphological characters, Virulence.

INTRODUCTION

Groundnut is an important edible oilseed crops, grown extensively in various parts of the country in both Kharif and Rabiseasons. It is popularly known as goober pea, goober, manila nut, pygmy nut, pignut and monkey nut (15). It considered as "King of Oilseeds". It is belonging to the family Fabaceae, Sub family Papilionaceae and it believed to

be a native of Brazil (South America). It contains the valuable source of all nutrients; the kernel contains 40 to 50 per cent oil and 25 to 30 per cent protein. It also contains 18 per cent carbohydrates and minerals like Ca, Mg and Fe in higher levels in an available form, vitamins B1, B2 and niacin are present in a considerable level. In Worldwide, the crop is raised on 26.4 million hectares with a total production of 37.1 million MT. The average productivity is 1400 kg/ha (10).Globally, India ranks first in area and second in production of 8.94 million tons from 4.89 million hectares with the productivity of 1825 kg/ha (9). In India, the major growing states are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharastra, Rajasthan, Madhya Pradesh, Odissa and Uttar Pradesh. In Tamil Nadu,major groundnut cultivating districts are Tiruvannamalai, Cuddalore, Kallakurichy, Villupuram, Vellore, Ariyalur, Perambalur, Thanjavur, Salem and Dharmapuri.

Generally, the cultivation of groundnut is hindered by several biotic and abioticstresses. Among the biotic stresses, soil borne and foliar diseases account for the deterioration of quality and quantity of groundnut. Nearly 67 fungal diseases were reported, among the fungal diseases stem rot disease caused by *Sclerotium rolfsii* Sacc. is one of the major concerns causing huge economic losses in Peanut (6).It poses a great threat to the groundnut farming community. The average yield losses due to stem rot disease are known to 25% which goes up to 80% in severe cases (8). *Athelia rolfsii* (Curzi) C. C. Tu & Kimbr., well recognized by its anamorph synonym *Sclerotium rolfsii* Sacc., is an economically important soil borne fungal pathogen that can cause disease on more than 500 plant species, including field and horticultural crops (13). It is a soil inhabitant pathogen, very aggressive nature, attacks at soil-line. The infection leads to the drying of lower leaves and wilts ultimately results in death of the plant. The stem rot of groundnut may occur at any growth stage of the plant (2).High temperature of 30°C, dense planting, frequent irrigation, moist conditions favor infection and fungal mycelial spread within and between plants.

Infection of *S. rolfsii* in host plants leads to production of abundant white mycelia and formation of appressoria with tissue necrosis and later on advanced mycelial growth causes tissue death (18).Circular and light-tantobrownclustersofseed-likebodieslessthan1/10thofaninchindiametercalled sclerotia form on the mat of fungal growth on the soil surface, decaying stems and podsand other crop debris. These sclerotia enable the fungus to survive for extensive periods on plant debris and the soil (14). The presence of melanin in the rind cells of the sclerotia may be responsible for their ability to survive for long durations and avoid degradation by chemicals and microorganisms(5). Tounder stand the occurrence, ecology, severity and evolutionary potential aspects of the *S. rolfsii*, it is important to study the cultural, morphological and pathogenic variability of the *S. rolfsii*. Therefore, the present study was undertaken with a view to study the morphological, cultural and pathogenic variabilities among isolates of *S. rolfsii* collected from different major groundnut growing areas Tamil Nadu.

MATERIALS AND METHODS

Survey and collection of groundnut stem rot infected plants from different localities of Tamil Nadu

A field survey was carried to assess the stem rot incidence of groundnut from different districts of Tamil Nadu such as Cuddalore, Perambalur, Kallakurichy, Ariyalur, Villupuram and Tiruvannamalai districts. Twenty villages were selected for this study from these districts.In each village, four fields were selected and mean disease incidence was assessed from four plots in each field. Percentage of disease incidence was calculated using the following formula:

$$PDI = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Isolation and identification of pathogen

The pathogen was isolated from the infected groundnut plants showing typical symptoms of stem rot disease by tissue segment method (16). The infected portion of the stem was cut into small bits, surface sterilized in 0.1 percent sodium hypochlorite solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto Potato Dextrose Agar medium in sterilized Petri dishes. The plates were incubated in room temperature 28±2°C for five days and were observed for the fungal growth. The fungal isolates were purified by single hyphal tip method. The purified isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics such as mycelial growth, colony colour, mycelial dispersion, shape, colour, number of sclerotia and sclerotial arrangement on surface media.

Inoculum preparations

The pathogen *Sclerotium rolfsii* was multiplied on sand maize medium. Maize and river sand were added proportionately(1:9) and thoroughly mixed with desired quantity of water was added, then it was transferred to open mouthed bottles and closed with a cotton wool plug. The bottles were sterilized at 15 lbs pressure for 2 h for 2 successive days. After sterilization, the bottles were inoculated with 9mm size mycelial discs were taken from the seven days old culture of *S. rolfsii* and the bottles were incubated for 15 days at 28±2°C for proper mycelial growth (3).

Assessing the susceptible stage of the groundnut stem rot disease

To identify the susceptible stage for the groundnut stem rot incidence, an experiment was conducted under pot culture. Five stages i.e., 0, 15, 30, 45and 60 days old of the groundnut plants were taken for their susceptible reaction against stem rot incited by pathogen *Sclerotium rolfsii*. These plants were maintained in the plastic pots and filled with sterilized soil. In each pot 5 seeds of groundnut cultivar VRI 2 was shown and fertilizer dose applied as per recommended. After raising all the respective stages, the sand maize inoculums were added at the collar region up to 10 to 15 g on each pot. The whole experiment was replicated thrice. Inoculated pots were kept in open place for observation and the pots were irrigated with sterilized water. The plants were observed for stem rot incidence at 15, 30, 45 and 60 days after inoculation at respective stages and per cent disease incidence was calculated using formula

$$PDI = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

The disease severity was calculated according to the scale given by BeKriwala TH (3) with some slight modifications (table 1)

Assessing the virulence of *Sclerotium rolfsii* isolates

The pot mixture was prepared by thoroughly mixing sand, clay loam soil and farm yard manure at the ratio of 1:1:1respectively. A total of twenty isolates were multiplied in sand maize medium. The inoculum of each isolate of *Sclerotium rolfsii* collected from different locations were separately mixed with soil in pots @ 10 g Kg-1 of soil and filled in 30 cm dia. earthen pots ten days before sowing. The groundnut seeds were surface sterilized with 0.1% sodium hypochlorite solution for 30 sec. followed by two washings in sterile water. Plants in pots without inoculum served as control. Three replications were maintained and the groundnut cultivar VRI-2 was used in this study. Soil moisture was sustained at 25 per cent moisture holding capacity of soil by adding sterilized water on weight basis throughout the period. After 30 days of inoculation, observe the typical wilting symptoms on the plants. Re-isolation was made from such affected portion of the plant tissue and compared with that of original isolate for conformity.

RESULT AND DISCUSSION

Survey and collection of groundnut stem rot infected plants from different localities of Tamil Nadu

A field survey was carried out in major groundnut growing districts of Tamil Nadu viz., Cuddalore, Perambalur, Kallakurichy, Ariyalur, Villupuram and Tiruvannamalai districts to collect and measure the disease incidence of groundnut stem rot and the data were presented in the table 2. The disease incidence was recorded on different groundnut cultivars viz., VRI 2, TMV 7 and some other locally grown varieties etc., The stem rot incidence ranged from 10.78 to 39.03 percent. Among the different localities, Thoradipattu (Isolate SrTRP) village registered maximum disease incidence of 39.03 percent followed by Perampattu (Isolate SrPRT) village recorded 35.85 percent disease incidence and the minimum stem rot incidence of 10.78 percent was reported in Nedungur village (Isolate SrNDR).

The survey conducted by Divya rani *et al*. (7) revealed that the stem rot incidence ranged from 4 % (Lingala mandal) to 12.8 % (Ramachandrapur mandal). Similarly, In Anantapur stem rot incidence ranged from 6% (Singanamala mandal) to 11.1% (Mudigubba mandal) in 24 villages spread over in six mandals of the district in Andhra Pradesh. Variations observed in stem rot incidence and intensities of the disease infection occurring in the conventional groundnut growing areas of Mathur and Sivapuri areas of Cuddalore district recorded the maximum disease incidence of 34.43 % and 32.73 % respectively (12). The highest disease incidence was due to continuous cultivation of groundnut crop which results in buildup of pathogen inoculum over time. The variation in the extent of the disease incidence might be due to the occurrence of the isolates of the pathogen differing in their virulence and environmental factors as observed in the present study.

Cultural and Morphological characteristics of different *Sclerotium rolfsii* isolates

Mycelial characteristics

The morphological variability among the twenty isolates of *S. rolfsii* was studied and exhibited the variation in their colony characteristics as fluffy white mycelium, dense cottony white mycelium, light cottony white mycelium and dull white profuse mycelium represented in table 3. Among the twenty different isolates, SrTRP showed profuse cottony white mycelium with maximum mycelial growth of 90mm, whereas least mycelial growth of 75mm was observed in the isolate of SrNDR. Karthik Pandi *et al*. (11) have collected eight isolates of *S. rolfsii*, among these three isolates namely SFSR2, SFSR3 and SFSR6 have produced fluffy, dull white colonies, while other isolates produced compact and dull white colonies. The variation in the morphological characters observed in this study mainly due to the different strains of *S. rolfsii*.

Sclerotial characteristics

Sclerotial formation

Sclerotial formation was first appeared on 8 days after inoculation in SrALR isolate and 15 days in SrPGM and SrMLR isolates. All other isolates produced sclerotia in 9 to 14 days after inoculation.

Sclerotial number

All the twenty isolates of *S. rolfsii* were produced Sclerotial bodies and represented in table 3. The maximum sclerotial number of 328 / plate,316 / plate obtained from the isolates SrTRP, SrPRT respectively, whereas the minimum sclerotial number of 77 was observed in isolate SrNDR. Asish Mahato and Mohan Kumar Biswas (1), stated that isolates of *S. rolfsii* varied in their sclerotial variation, the maximum sclerotial number, 395.00 no/plate of sclerotia production was recorded in SRPM-1 isolate and lowest sclerotial number of 154.00 no./ plate was recorded in SRBR-1 isolate.

Sclerotial shape and color

The sclerotial shape differs from round, oval, spherical and irregular which were arranged in central, peripheral and scattered of 90mm pertidish. Sclerotial color was categorized into brown, dark brown, chocolate brown and light brown. Savita Ekka *et al*. (17) reported the sclerotial characters of *S. rolfsii*, mustard seed like sclerotia produced which were deep brown or brownish black, shiny, hard and spherical and irregular in shape.

Identification of susceptible stage of the groundnut crop

To identify the susceptible stage of the groundnut to stem rot disease development. A trial was conducted in pot conditions and the results are presented in the table 4. The results revealed that the significant difference in disease severity percentage among the different stage of plant. In this study observed that *Sclerotium rolfsii* can infects all the stages of the crop**.** Higher percent of 69.33 % wilting was recorded in plants at 45 days after emergence, which recorded most susceptible stage of the crop compared to rest of treatments. The 15 days and 30 days old plants recorded the disease severity of 57.67% and 61.26 % respectively. An experiment was conducted in groundnut plants to find out the susceptible stages of the crop. Forty-five-day old plant had maximum 79.04% disease severity was recorded followed by30- and 15-days old plants with 74.45% and 69.36% disease severity, respectively. Least disease severity was recorded in 0 days old plants with 25.71% whereas; 60 days old plant had 49.68% disease severity (3).

Virulence of *Sclerotium rolfsii* isolates under pot culture condition

Twenty isolates of *S. rolfsii* were tested to evaluate the ability to cause disease in groundnut plant under pot culture experiment. The result represented in the table 5 revealed that the virulence varied significantly among the isolates. Among the different isolates, the isolate SrALR collected from Aalathur village reported the maximum disease incidence of 40.81 per cent and was identified as virulent isolate followed by SrPNR which recorded 39.26 per cent from Pinnalur village. The isolate SrTPL collected from T-Palur village was the least virulent isolate which recorded the minimum stem rot incidence of 16.37 per cent.

Bekriwala TH *et al*., (3)proved Pathogenicity on 15 days old groundnut plants (cv. GJG-9) under pot conditions. The initial symptoms observed were water soaked brown to dark brown spots at basal portion of plants and the leaves of infected plants gradually turned yellow and dried 4 days after of inoculation. Pathogenicity reactions were observed for all the 10 isolates *S. rolfsii*, the isolate Sr9 exhibited maximum disease incidence of 100% followed by Sr7 (90.67%). The lowest disease incidence of 46.33% was recorded in Sr6 isolate under pot culture experiments (4).

CONCLUSION

The incidence of groundnut stem rot revealed significant variations from different parts of Tamil Nadu. The disease incidence was higher in areas where mono cropping of groundnut was in practice. It may be due to the buildup of pathogen inoculum in larger amounts over time. So, it's always better to practice crop rotation with non-host crops in order to reduce the magnitude of disease. The assessment of virulence among different isolates, revealed that the isolate SrALR collected from Aalathur reported the maximum disease incidence of 40.81 per cent and was identified as virulent isolate and these variations could be due to varying in virulence among the isolates and environmental conditions. The variation in cultural and morphological characters may be due to presence of different strains and biotypes of the pathogen. Although one major drawback with these kinds of studies is that the virulence of the isolates changes from place to place and also from one variety of crop to another. Molecular studies have to be done to see the molecular variability among the virulent and non-virulent isolates.

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Table 1. Assessing the susceptible stage of the groundnut stem rot disease

Disease rating	Treatment (Days)	Description
0	0	Healthy
1	15	Lesions on stem only
3	30	Up to 25% of the plant symptomic (wilt, dead or dying)
5	45	26% to 50% of the plant symptomic
7	60	>50% of the plant symptomic

Table 2. Survey on the stem rot incidence in major groundnut growing areas of Tamil Nadu

Sl. No	Districts	Locality	Isolates	Soil type	Variety	Disease Incidence (%)
1.	Cuddalore	Perampattu	SrPRT	Clay loam	VRI 2	35.85^{b} (36.78)
2.		Bhuvanagiri	SrBVG	Red loam	LOCAL	18.41^{kl} (25.40)
3.		Pinnalur	SrPNR	Red loam	VRI 2	29.37^{def}(32.82)
4.		Vadakuthu	SrVDK	Red loam	LOCAL	13.51^{mn}(21.57)
5.	Perambalur	Veppanthatti	SrVPT	Black soil	VRI 2	28.89^{ef} (32.51)
6.		Nedungur	SrNDR	Red loam	VRI 2	10.78^{n} (19.17)
7.		Aalathur	SrALR	Red loam	LOCAL	28.20^{ef} (32.08)
8.	Kallakurichy	Parigam	SrPGM	Red loam	TMV 7	32.77^{bc} (34.92)
9.		Kadathur	SrKDR	Clayey loam	TMV 7	23.91^{hi} (29.27)
10.		Thoradipattu	SrTRP	Red loam	VRI 2	39.03^{a} (38.66)
11.		Kondiyanatham	SrKYM	Clayey loam	TMV 2	16.33^{lm}(23.83)
12.	Ariyalur	Mallur	SrMLR	Red loam	VRI 2	27.59^{efg}(31.68)
13.		T- Palur	SrTPL	Red loam	VRI 2	30.30^{cde}(33.40)
14.		Nachiyarpettai	SrNYP	Red loam	VRI 2	22.18^{ij} (28.10)
15.	Villupuram	Melpadai	SrMPD	Red sandy loam	JL 24	25.57^{fgh}(30.38)
16.		Illangadu	SrIGD	Sandy loam	JL 24	22.64^{ij} (28.41)
17.	Tiruvannamalai	Mambattu	SrMBT	Clayey loam	VRI 2	29.95^{cde}(33.18)
18.		Sengam	SrSGM	Red loam	TMV 2	20.72^{jk} (27.08)
19.		Thurinjapuram	SrTJP	Red loam	LOCAL	24.92^{ghi}(29.95)
20.		Melpennathur	SrMPT	Red loam	VRI 2	32.14^{cd} (34.54)

Mean of three replications

Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Table 3. Morphological identification of *Sclerotium rolfsii* isolates in different localities of Tamil Nadu

Isolates	Mycelial characters		Sclerotial character				
	Colony character	Mycelial growth (5DAI)	Sclerotial formation (No of days)	No of Sclerotia (15DAI)	Color of Sclerotia	Shape of Sclerotia	Sclerotial Arrangement
SrPRT	Dense cottony white mycelium	89	12	316	Chocolate brown	Oval	Central
SrBVG	Fluffy white mycelium	77	9	97	Dark Brown	Irregular	Scattered
SrPNR	Dense cottony white mycelium	88	11	303	Brown	Spherical	Central
SrVDK	Light cottony white mycelium	76	13	83	Chocolate brown	Round	Central
SrVPT	Dull white profuse mycelium	83	13	204	Brown	Spherical	Peripheral
SrNDR	Fluffy white mycelium	75	10	77	Dark brown	Oval	Scattered
SrALR	Dense cottony white mycelium	87	8	180	Brown	Spherical	Peripheral
SrPGM	Fluffy white mycelium	88	15	287	Chocolate brown	Round	Peripheral
SrKDR	Cottony profuse mycelium	80	12	132	Brown	Irregular	Central
SrTRP	Profuse cottony white mycelium	90	10	328	Dark brown	Round	Scattered
SrKYM	Light cottony white mycelium	76	9	86	Light brown	Irregular	Scattered
SrMLR	Fluffy white mycelium	82	15	176	Brown	Round	Central
SrTPL	Cottony profuse mycelium	85	9	246	Chocolate brown	Round	Peripheral
SrNYP	Profuse cottony white mycelium	78	14	120	Light brown	Spherical	Central
SrMPD	Profuse cottony white mycelium	81	11	152	Chocolate brown	Oval	Scattered
SrIGD	Cottony profuse mycelium	79	10	124	Dark brown	Round	Scattered
SrMBT	Dull white profuse mycelium	84	9	218	Brown	Round	Central
SrSGM	Light cottony white mycelium	78	14	109	LightBrown	Spherical	Central
SrTJP	Cottony profuse mycelium	80	11	136	Dark brown	Oval	Peripheral
SrMPT	Fluffy white mycelium	87	13	271	Chocolate brown	Spherical	Scattered

Table 4. Identification of susceptible stage of the groundnut crop

Treatment No	Treatment (By days)	Percent Disease incidence (PDI)
0	Zero stage	0.00
1	15 days crop	57.67^{c}(49.60)
3	30 days crop	61.26^{b} (51.35)
5	45 days crop	69.33^{a} (56.17)
7	60 days crop	42.93^{d} (40.98)
	Control	0.00

Mean of three replications
Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

Table 5. Virulence of *Sclerotium rolfsii* isolate sunder pot culture condition

Sl. No	Isolates	Stem rot incidence (%)				Mean
		30 DAS	60 DAS	90 DAS	At harvest (105 DAS)	
1	SrPRT	24.73^{f} (29.80)	32.34^{e} (34.63)	38.46^{e} (38.29)	45.18^{de}(42.19)	35.18
2	SrBVG	18.49^{i} (25.40)	28.67^{h} (32.33)	32.20^{i} (34.57)	41.65^{g} (40.16)	30.25
3	SrPNR	29.03^{b} (32.58)	34.41^{b} (35.91)	43.69^{b} (41.32)	49.90^{b} (44.94)	39.26
4	SrVDK	22.83^{fg} (28.52)	30.05^{g} (33.21)	35.31^{fg}(36.45)	43.08^{f} (40.98)	32.82
5	SrVPT	17.31^{j} (24.58)	26.98^{i} (31.24)	31.45^{ij} (34.08)	40.30^{gh}(39.41)	29.01
6	SrNDR	09.24^{pq}(17.66)	17.63^{n} (24.80)	22.04^{p} (27.97)	31.19^{ms}(33.89)	20.03
7	SrALR	31.10^{a} (33.89)	35.26^{a} (36.39)	45.51^{a} (42.42)	51.31^{a} (45.74)	40.81
8	SrPGM	14.69^{m} (22.46)	23.86^{jk} (29.19)	27.92^{l} (31.88)	36.27^{j} (36.99)	25.69
9	SrKDR	16.74^{k} (24.12)	25.45^{ij} (30.26)	30.09^{j} (33.21)	38.67^{i} (38.41)	27.74
10	SrTRP	23.14^{fg} (28.73)	31.78^{ef} (34.27)	36.93^{f} (37.41)	44.43^{e} (41.78)	34.07
11	SrKYM	28.78^{bc} (32.39)	34.17^{bc}(35.73)	42.86^{bc}(40.86)	48.45^{c} (44.08)	38.57
12	SrMLR	25.61^{e} (30.39)	33.03^{cd}(35.06)	40.16^{d} (39.29)	46.67^{d} (43.05)	36.37
13	SrTPL	05.32^{s} (13.31)	13.26^{p} (21.30)	18.74^{r} (25.62)	28.17^{o} (32.01)	16.37
14	SrNYP	20.96^{h} (27.20)	29.14^{gh}(32.65)	33.99^{h} (35.61)	42.28^{fg}(40.41)	31.59
15	SrMPD	15.26^{l} (22.95)	24.07^{j} (29.33)	29.63^{jk} (32.96)	37.36^{ij} (37.64)	26.58
16	SrIGD	10.63^{p} (19.00)	19.36^{m} (26.28)	23.74^{o} (29.13)	32.87^{l} (34.94)	21.65
17	SrMBT	12.74^{no}(20.88)	21.45^{kl} (27.56)	25.09^{n} (30.00)	33.67^{kl}(35.43)	23.24
18	SrSGM	13.27^{n} (21.30)	22.18^{k} (28.04)	26.03^{m} (30.66)	34.47^{k} (35.91)	23.99
19	SrTJP	08.07^{qr} (16.43)	15.72^{o} (23.34)	20.49^{q} (26.85)	30.24^{n} (33.34)	18.63
20	SrMPT	26.47^{d} (30.92)	33.92^{bc}(35.60)	41.56^{cd}(40.11)	47.18^{cd}(43.34)	37.28

Mean of three replications
Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT)

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RESEARCH ARTICLE

Genetic Traits & Chromosomal Approaches in Anxiety Disorders

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ABSTRACT

Anxiety Disorders are complex disorders which can be due to several genetic and environmental factors. Generalized anxiety disorder (GAD), panic disorder (PD) & Phobias are various types of anxiety disorders. The prevalence of anxiety disorders estimated in U.S is 19.3%. Anxiety is showing genetic correlation with depression & insomnia, CAD as per the epidemiological studies. The most vulnerable gene involved in AD is Serotonin transporter gene and it is highlighting the pleiotropic hypothesis regarding anxiety. Recently in 2017, studies concluded that GAD can be inherited and other associated conditions are linked to various genes. The 5-HT2A & T102C polymorphism has a role in development of SAD. Oxytocin hormone controls several physiologic functions and also it can stimulate the neurons expressing oxytocin receptors. Hence OXT is also involved in stress response and stress-related behaviour. European studies suggest that the pathology of anxiety disorders includes inherited variation in catecholamine metabolism. The frequency of polymorphism of MAO-A gene is three times greater in females. The intronic rs1709393 minor C allele on chromosomal band 3q12.3 of an uncharacterised non-coding RNA locus is associated with lifetime diagnosis of AD. Studies also revealed a significant intronic hit for rs35855737 minor C allele on chromosome 3q14.1. The frequency of polymorphism of serotonin transporter gene is higher in patients with GAD. The patients with bipolar disorder showed higher frequency of COMT Met 158 genotypes. Anxiety disorders are highly complex and inheritable. The susceptibility risk of anxiety disorders has been explained by various acquired genetic trait.

Keywords: Anxiety Disorders, COMT gene, Serotonin transporter genes, gene polymorphism, Panic Disorder, Generalised Anxiety Disorder

INTRODUCTION

Anxiety disorder is a mental disorder characterised b change in emotions such as tension, worry, extreme mood changes, tiredness etc. All of these can lead to mental instability which is a reason for anxiety disorders. Physical, intellectual, environmental, vocational, social, emotional and spiritual health are seven dimensions of mental health. Mental health refers to cognitive, behavioural and emotional well-being.

METHODOLOGY

The American Journal of Psychiatry, PubMed and research papers were searched using keywords anxiety, its types, genes and chromosomal polymorphism etc. The aetiology and risk factors of anxiety disorder was also searched in. Most papers were found relevant and are based on clinical trials and pre-clinical trials. Got the valid information and discrepancies were cleared by inputs given by the authors.

TYPES OF ANXIETY DISORDER

i) Generalised Anxiety Disorder: Chronic anxiety, exaggerated worries and tensions are termed as Generalised Anxiety Disorder (GAD). ii) Obsessive Compulsive Disorder: It is another type of anxiety disorder characterised by unwanted thoughts, obsessions, compulsions etc. The repetitive behaviours done by OCD patients include washing hands repeatedly, counting and cleaning often. iii) panic disorder: Patients with panic disorder show physical symptoms such as chest pain, heart palpitations, shortness of breath, dizziness or abdominal distress. iv) post-traumatic stress disorder (PTSD): This condition occurs after exposure to a terrifying event or any physical harm had occurred to the patient or the patient was threatened. The traumatic events including personal assaults, natural or human caused disasters, accidents etc can trigger Post Traumatic Stress Disorder. v)Social Phobia: It is a condition with overwhelming anxiety and excessive self-consciousness. Social phobia can be limited to only one type of situation – fear of speaking, eating or drinking in front of others. This condition is called as social anxiety disorder. vi) Specific Phobias: It can be due to intense fear of object or heights or situations. It includes the following: a. Agoraphobia: It is an intense fear of place where it seems hard to escape.b. Separation anxiety: It is feeling of anxiety or fear when someone loses the loved ones.c. Selective Mutism: it is seen among young children and they don't speak in public but talk normally with their family. d. Claustrophobia: it is an anxiety disorder characterised by intense fear of enclosed spaces. This condition can be due to dysfunction in amygdala. e. Medication induced anxiety disorder: Use of illegal drugslike corticosteroids used in condition of asthma, arthritis, allergies or bronchitis can cause symptoms of anxiety. Eg: Dexamethasone, cortisone and prednisone. Patients taking caffeine who are already prone to anxiety can heighten the anxiety symptoms.

AETIOLOGY &RISK FACTORS OF ANXIETY DISORDERS

White race, family history of anxiety disorder, female gender, disturbed family environment, early parental loss, childhood sexual abuse, conduct disorder, traumatic events and low self-esteem are the various risk factors for developing anxiety disorders. [1]. In case of Generalized Anxiety Disorder, the risk factors include: Female gender because of their hormone functions or cultural expectations. Substance abuse of alcohol, drug, smoking increases the risk of GAD. People with chronic illness are also prone to GAD. History of physical or emotional traumatic events, low education level, childhood neglect, poor or oppressed etc can also enhance the risk of GAD condition. Fear, uneasiness, feelings of down or danger, sleeping problems, not being able to stay calm, cold, sweaty, numb or tingling hands or feet, shortness of breath, breathing faster, hyperventilation, heart palpitations, dry mouth, nausea, tense muscles, dizziness, thinking repeatedly about a problem(rumination), inability to concentrate, obsessively or intensely avoiding feared objects or places etc are the common symptoms of anxiety disorders.

The genetics plays a crucial role in causing anxiety disorder. Brain chemistry is associated with faulty circuit in the brain that is linked to fear and emotions. The childhood abuse, witnessing a violence, loss of loved ones etc can lead

to environmental stress. The sudden withdrawal of some drugs or misuse of certain drugs can increase the anxiety symptoms. Diseases or ailments associated with heart, lungs, brain, thyroid etc. can cause anxiety disorder symptoms. In the biological aspect, the serotonin and dopamine, the two neurotransmitters get disrupted leading to various types of anxiety condition.

There are several neurotransmitters like norepinephrine, serotonin, dopamine and gamma-aminobutyric acid which are found to be significant mediators of anxiety disorders. The symptoms of anxiety disorders are mediated by sympathetic nervous system. As in fig.1.the female gender (may be related to hormonal factors, less internal focus of control), Genetics (positive family history) and other biological factors lead to predisposition to anxiety. The imbalance or abnormal functioning of these neurotransmitters play a significant role in anxiety disorders. It can be due to death of neurons in the hippocampus caused by chronic activation stress hormones. This leads to shrinkage of hippocampus which further dysregulate mood and cause memory impairment. The ability of hippocampus to normally integrate environmental stimuli is further compromised. The Brain-Derived Neurotrophic Factor(BDNF) value correlates with the degree of neuronal loss in the hippocampus. In case of anxiety there can be measurable decrease in BDNF. The hippocampus and cingulate Gyrus abnormally process threat. In the prefrontal cortex, the modulation of amygdala gets impaired. Amygdala activates fear response which in turn causes activation of hypothalamus pituitary-adrenal cortex axis and autonomic nervous system & adrenal medulla. This together can lead to increased release of both cortisol and epinephrine. The interaction of stress hormones with brain and body is through various complicated mechanisms. This ultimately leads to anxiety disorder characterised by fear, unpleasant tension and sense of foreboding or apprehension.

The prevalence of anxiety disorders globally was found to be 5.3% in Africa cultures and 10.4% in Euro cultures. [3]. Anxiety disorders are affecting around 40 million adults in US with age above 18 years. Anxiety disorders is considered as one of higher prevalent illness by the ECA. According to World Mental Health Surveys (WMHS), the prevalence of anxiety disorder varies from country to country. The potential reasons for this cross-national valuation can be due to variation across countries in attitudes towards mental illness [5]. It can also be due to errors in epidemiological surveys. WMHS findings suggest that i) certain anxiety disorders are more prevalent whereas others are less prevalent (eg: Agoraphobia), ii)anxiety disorders have an earlier age of onset when compared to agoraphobia and Generalised Anxiety Disorders.iii) anxiety disorders have socio demographic correlates across the globe i.e., female sex is more prone to anxiety conditions. iv) anxiety disorders are highly comorbid with other mental disorders; v) first treatment of anxiety disorders usually do not occur until at least a decade after onset [5],[6]. As in fig.2. there are variations in the incidence rate of various types of anxiety disorders which include Agoraphobia, Social phobia, OCDr and TAD. While comparing all the three age groups the occurrence of agoraphobia is more among 18-24 years age group with a prevalence rate of 6% while other two age groups are having 5.5% and 4% respectively. The incidence of social phobia is high among 18-24 years age group with 4%. Obsessive-compulsive disorder is also having a prevalence rate of 1.5% among the first age group. The highest incidence rate of TAD is 8% among people with 45-64 years of age whereas 2% and 5.5% are the occurrence rate of TAD among 18-24 and 25-44 years of age group respectively.

As represented in table.1, according to the data of prevalent cases of anxiety disorders worldwide in 2018 based on gender, United States, China, Brazil and India comes in the first four positions respectively. 30.7% and 45.2% of men and women was having the anxiety disorder in US. In India, around 13.6 % of men was battling with anxiety. The prevalence of anxiety disease among women in India was around 18.5% [4].

GENETIC APPROACHES

There are several genes involved in anxiety disorders. Recently in 2017, studiesconcluded that here anxiety disorders can be inherited or it can be linked to various genetic traits. Around 30% of Generalised Anxiety Disorders, 48% of panic disorders, 51% of social phobia and 59%-67% of agoraphobia are inherited [8]. According to molecular genetic association studies, several genes such as 5-HTT, MAO-A, COMT, CCK-B, ADORA2A, CRHRI, FKBP5, ACE, RGS2/7

and NPSRI are linked to anxiety disorders. Anxiety is genetic which can also be influenced by environmental factors. In twin studies, identical twins since having the same set of genesare more prone to mental illness.

SEROTONIN TRANSPORTER GENES

The most vulnerable gene involved in the anxiety disorder is serotonin transporter gene and it is highlighting the pleiotropic hypothesis regarding anxiety. The serotonin receptors regulate the neurotransmission of serotoninthere by terminating its action in the synapse. The serotonin transporter gene is the site of action of the Selective Serotonin Reuptake Inhibitors (SSRIs), that are used in the treatment of different types of anxiety. The susceptibility towards stress can be enhanced, if there is an interaction between the serotonin transporter (SERT) linked polymorphic region (5-HTTLPR) and adverse early life stressing (ELS) events. This leads to increased risk of developing mental illness like anxiety, aggressiveness and depression. Functional polymorphism in the human serotonin transporter (SLC6A4) promoter has been linked to two sub dimensions of Health Anxiety also called as hypochondria (a condition which usually develops during adulthood). They are Anticipatory worry (HA1) and Fear of Uncertainty (HA2) [11]. Serotonin Transporter (SERT) can be found within central and peripheral nervous stem. Serotonin is a biochemical messenger which has been obtained from the essential amino acid L-TRYPTOPHAN. When there is a deficiencyof essential amino acid tryptophan, there can be decreased production of serotonin levels.Lowserotonin levels in the body which canlead to psychiatric disorders. The human serotonin gene (SLC6A4) is mapped to chromosome 17q 11. 1-17q12 [10]. The transporter encoded by SLCA4 gene halt signalling by taking 5-HT back into neurons from synapse. Selective serotonin reuptake inhibitors (SSRIs) work by inhibiting the serotonin transporter thereby promoting 5-HT to remain in synapse for long time. The polymorphism of promoterregion 5-HTTLPR in SLCA64 gene influence the production of SERT with the long (L) allele and the short (S) allele [12]. The long (L) allele is associated with twice the basal expression whereas the short allele is associated with the reduced transcriptional activity of the serotonin transporter. The key protein for neurotransmission in the brain is encoded by SERT gene. Elevated anxious mood can be experienced in individuals with at least one copy of S or L allele at 5-HTTLPR. Thepolymorphism in 5-HTTLPR is associated with psychic anxiety, muscular tension, psychasthenia, and lack of assertiveness [15].

COMT GENE

According to European studies, the inherited variation in catecholamine metabolism is having a vital link in pathology of anxiety disorder. High frequency of COMT Met 158 genotypes is shown b patients with anxiety disorder. The catecholamine neurotransmitter like dopamine, epinephrine and norepinephrine is metabolised by comt gene. In humans, the gene encoding for COMT is found on q11 band of chromosome 22. The COMT mRNA transcription encodes: cytoplasmic membrane bound COMT and also total COMT polypeptide in the human brain. The COMT plays a significant role in dopamine catabolism which is owing in the prefrontal cortex. This provides strong association with cognitive and emotional processes. The COMT gene polymorphism regulate COMT enzyme by changing in the amino acid from valine to methionine at codon 158. There is a positive correlation between the number of met158 alleles and the BOLD response. In patients with anxiety disorder, due to exposure to unpleasant stimuli, there can be enhance activation of limbic and prefrontal areas in brain which leads to increased levels of dopamine and norepinephrine. COMT (Catechol-O-methyl transferase) is one of several enzymes that degrade catecholamines, catecholestrogens, various drugs and substances having a catechol structure. There can be down regulation of COMT gene transcription by oestrogen, so there may be variation in the studies of COMT 158 Met polymorphism in with anxiety related traits due to sex differences [16]. There can be greater activation of amygdala in response to emotional faces among valine carriers. The functional polymorphism of COMT gene regulate the substitution of methionine (Met) for valine (Val) at codon 158 respectively. The polymorphism of the COMT gene is associated with two traits that are over and under expressed respectively in anxiety disorder: neuroticism and extraversion [17]. There are two possible COMT polymorphism. The nonsynonymous single nucleotide polymorphisms are: i) rs4680 that generate valine to methionine substitution (Val158 Met) and ii) rs737865. Two of the single nucleotide polymorphisms (SNPs) of rs4680 ('Val/met') and rs737865 has been linked with (low) extraversion and high neuroticism [18].

The MAO-A (Monoamine Oxidase A) is another enzyme which can catalyse the catecholamine catabolism. The MAO enzymes are present in the outer membrane of the mitochondria and thus in brain, primarily in nerve terminals and glia. The frequency of the MAO-A gene polymorphism was significantly higher in female patients suffering from anxiety disorders, specifically panic attacks and generalised anxiety disorders [20]. The MAO-A gene is found at the Xp 11.4-Xp 11.3 region. A functional VNTR has been found in the promoter region of the MAO-A gene. The polymorphism consists of 2, 3, 3.5, 4 and 5 30 bp repeats, among which 3 and 4 alleles more common, has been associated with neuropsychiatric disorders and also plays avital role in the molecular mechanism [21].

OXYTOCIN RECEPTOR GENES

pituitary gland stimulates neurons expressing oxytocin receptors. Oxytocin is associated with modification of stress related behaviours, anxiety and social interactions. The individuals with the A allele showed high stress reactivity compared to individuals with G allele. The symptoms of anxiety disorder are highly reported in females who were heterozygous for oxytocin receptor gene rs2254298 polymorphism. Amygdala is a part of brain which connects other regions of brain associated with fear such as anterior cingulate cortex and medial prefrontal cortex. There is a positive correlation between the anxiety disorder and decreased baseline oxytocin plasma levels. DNA methylation in the promoter region of the oxytocin receptor gene reflects a downregulation of the oxytocin system associated with regulation of fear-related responses through the amygdala [23]. Oxytocin receptor gene hypomethylation can result in increased OXTR mRNA expression, and also it can result in overall decreased oxytocin tone which is associated with the enhanced responsiveness of amygdala to social anxiety disorder. Oxytocin administration also resulted in attenuating the activation of amygdala in response to socially relevant or fear-conditioned emotional stimuli in healthy subjects as well as in patients with social anxiety disorder [22]. Oxytocin receptor gene are expressed both centrally and peripherally and perform both peripheral and central functions. OXTRgene is located on human chromosome 3p25. As in fig.3. oxytocin receptor gene (OXTR) rs53576 is a single nucleotide polymorphism. A common variant (rs53576) of the oxytocin receptor gene has been linked to anxiety related behaviour. The variation of the OXTR gene may affect an anxiety related temperamental tract in females via modulating prefrontal amygdala functional connectivity [24]. The OXTR rs53576 genotype expression in the amygdala varies in distribution in both sexes, i.e., it is more in men with GG genotype and women with an AA genotype. The oxytocin carryout flexible regulation of oxytocin system by playing central role in methylation and multiple psychopathologies. [26]. Human studies exhibit two oxytocin receptor single nucleotide polymorphisms, rs53576 and rs2254298 which has been linked to stress reactivity. The social recognition has been associated with significant structural and functional differences in amygdala, hypothalamus and cingulate gyrus [26]. The higher the OXTR methylation, higher will be the neuronal activity in certain areas of brain which is known for emotion regulation. The DNA methylation is also having a negative impact on connectivity between amygdala and brain thus becoming highly responsive to stimuli like anger and fearful expressions.

MANAGEMENT OF ANXIETY DISORDERS

Mindfulness, counselling, cognitive therapy, relaxation techniques, breathing techniques, dietary adjustments, exercise, learning to be assertive, building self-esteem, medication etc. are several options for managing anxiety disorders. Mindfulness helps the patient to bring back the attention to present moment. Relaxation therapy helps to reduce the tension by releasing the muscle tone. Abdominal breathing is an effective way for progressive muscle relaxation. Hyperventilation raises the oxygen levels and thereby reducing the carbon dioxide level in the blood. Hence, hyperventilation can trigger the physical symptoms of anxiety. Cognitive therapy includes self-take, attention trainings, challenging the fears and beliefs to overcome that. In behaviour therapy; it helps the patient to overcome the danger or fear aspect of the situation. The dietary management is also essential in the anxiety disorders. The vitamin B, calcium, magnesium, cereals, leafy vegetables, low fat diet can be included in the diet. The mineral intake helps in relaxing the muscle tissues. Avoid the nicotine, caffeine, salt etc. which can cause the release of adrenaline, which is a stress chemical. Exercise can burn up stress chemicals and promote relaxation. Learning to behave assertively helps to develop a strong self-esteem. Breaking down a problem into its components and deciding the course of action on it help to manage various type of anxiety disorders. Various medications are provided for

preventing and some for curing the disease. The antidepressants are first category of drugs given for such patients. The selective serotonin reuptake inhibitors (SSRIs) are such as citalopram, escitalopram, fluoxetine, venlafaxine etc. and SNRIs (serotonin and norepinephrine reuptake inhibitors) are the classes of drugs used for treating chronic anxiety. Tricyclic antidepressant like Tofranil and novel antidepressants like mirtazapine also helps to relieve anxiety. Anticonvulsant drugs like gabapentin and pregabalin, antihistamines like hydroxyzine and beta-blockers such as propranolol help in case of performance anxiety or social anxiety disorder. The most prominent anti-anxiety drugs are benzodiazepines which include alprazolam, clonazepam, chlordiazepoxide, diazepam and lorazepam. But these drugs can cause drowsiness, irritability, memory and physical dependence. Buspirone is another anti-anxiety drug with fewer side effects.

CONCLUSION

Anxiety is a feeling of nervousness or worry that is normal in human life. Now, anxiety has become the cause of suffering for people across the world. The rate of prevalence of anxiety disorder is increasing due to lifestyle, social factors and environmental aspects. So, it is better to follow the management tips which helps to resolve some of the anxiety related problems. Anxiety is a highly subjective human emotion which should be taken into consideration.

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Table 1. Prevalent cases of Anxiety disorders worldwide in 2018[4].

COUNTRY	MEN	WOMEN
United states	31%	45%
China	16%	29%
Brazil	15%	28%
India	14%	19%

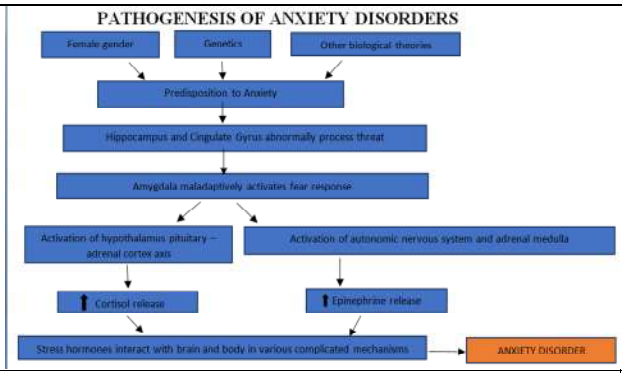

Figure 1: Pathophysiology of anxiety disorder [9]

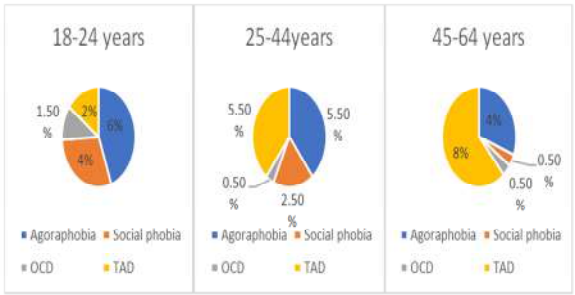

Figure 2. Age-specific prevalence rates of anxiety disorders [7]

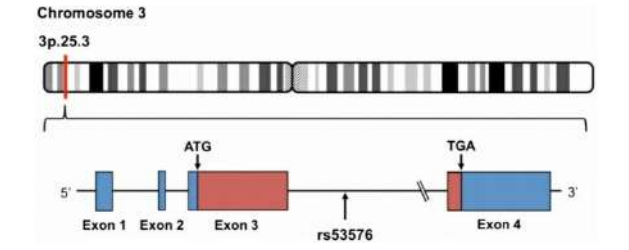

Figure 3. Location of oxytocin receptor gene (OXTR) rs53576 [25].

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RESEARCH ARTICLE

A Study on Work-life Balance in Hotel Industry with Special Reference to Ahmedabad

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ABSTRACT

Covid-19 pandemic has altered the way the work is done throughout the world. With the shift on online platform, a demanding situation has emerged wherein good balance in personal and professional life needs to be achieved. Many times the change in the way of doing work creates a stressful situation and the mounting pressure of work at home and office leads to tensed life. This requires the organizations to integrate work and non-work roles of employees in such a way that job dissatisfaction is minimized or avoided. The focus should be on increasing employee productivity by assuring appropriate work life balance among employees. The purpose of this paper is to establish the influence of work life balance on employees productivity in hotel industry with special reference to Ahmedabad city. The study targets employees at corporate hotels like Marriott Courtyard, TGB and Taj Skyline of Ahmedabad. This study is exploratory & descriptive research based on primary data collected through structured interview in person with 108 employees of Marriott Courtyard, TGB and Taj Skyline & supported by available secondary data too. The research methodology deployed for research work is collection of primary data through questionnaire.

Keywords: Work life Balance, Job Satisfaction, Hospitality, Productivity

INTRODUCTION

The Hospitality industry is primarily concerned with provision of food services and accommodations at hotels, resorts, and theme parks etc. It is a part of the tourism industry. With the increase in tourism a tremendous growth has been noticed in hospitality industry. This has lead to increase in demand for various services offered by hospitality industry like vacation planning, business trip planning, travel arrangements, accommodation, food & beverages, leisure activities like spa, gym in hotels and resorts. The term 'Hospitality' means warm welcome of guest. It is the relationship between a guest and host. The hospitality industry is a service industry. In the hospitality business, it is extremely important to assure customer satisfaction for long term sustainability of the organization. Moreover, it deals with the emotions of customers and emotions being very sensitive needs to be tackled very systematically. A happy employee can serve customers happily considering the same employee work life balance becomes an important element to be considered by the organizations.

Theoretical Framework

Work life balance is attained on management of multiple responsibilities at office and home appropriately. It is extremely important for the organizations and employees. In the present pandemic scenario all the organizations are struggling for increasing productivity and need employees with improved work-life balance as an employee with better work-life balance will contribute more meaningfully towards the organizational growth and success. This issue is recognized due to multitude of changes in the work place, in employee demographics and in the family sphere. Due to rapidly changing business environment, the organization are not able to provide secure employment and that is why the attitudes and values of people in work are also changing. Demanding work culture at office and home leads to imbalance in ones life. The youth believes in working hard and partying harder. They love to travel at remote and unexplored places and take up adventurous sports trips. Sometimes on not getting enough time to enjoy these activities, they get unhappy and disturbed. The work life balance of individuals is disturbed due to changesin family spheres which includes nuclear families, single parent households, and dual earning parents, parents working at different locations and increasing household work. This has made very difficult to meet the family demands. Now a days women are also found to be career oriented. So, they are playing a dual role.This leads to increase in their primary responsibility of household and demands a lot of adjustments from them to accommodate work pressures.

Significance of the Study

The present study focuses on understanding the level of Work Life Balance in Hospitality Industry. This industry being the part of service industry requires its employees to serve the guests with level of courtesy. The employees in this industry work 24 X 7 and their working hours may extend up to 12 hours or even more. This makes it extremely difficult to maintain a balance between work and personal life. The present study provides conceptual framework of work life balance in hospitality industry. The study presents the relation of work life balance with other major aspects of human resource management i.e. job satisfaction, job stress and employee turnover. The purpose of the study is to deepen the understanding of the concept of quality of work life, as well as to gain an insight into the mindset and characteristics of employees in the hospitality industry. This paper additionally aims at identifying factors that positively contribute to an improved quality of work life.Especially the paper is intended to address the following problems:

a) What is work culture of Hospitality Industry?
b) Are the employees satisfied with work-life balance?
c) What actually does these employees look for in terms of work-life balance?

The present study mainly focuses on quality of work life and personal life. It states relationship between work hours and its effect on their personal life. It also takes into consideration the working schedule, working place, co-workers, family and friends and the role of management in balancing their life.

RESEARCH METHODOLOGY

The research methodology proposed examines work life balance of employees at corporate hotels likeMarriott Courtyard, TGB and Taj Skyline of Ahmedabad. This study is exploratory & descriptive research based on primary data collected through structured interview in person with 108 employees of Marriott Courtyard, TGB and Taj Skyline & supported by available secondary data too. The research methodology deployed for research work is collection of primary data through survey of 108 employees. The convenience sampling method is used for data collection. The collected & analyzed data is presented with the help of graphs & charts for more clarification.

Limitation of the Study

The study is time consuming and poses a challenge of data collection. Considerable care needs to be taken for collecting unbiased data. However adequate time and interest could not be realized in the case of many respondents because of their lack of interest. The inferences of the study are thus subject to inherent limitations in both the primary and secondary data. Apart from this, the results of the study are applicable to corporate hotels at Ahmedabad and cannot be generalized for the entire hospitality sector.

Objectives of the Study

The study approaches the problem from the viewpoint of both the employees and the employers to attain the work life balance. The main objective of the study is to know how the employees are balancing their work and life by which they attain the Quality Work Life. The main objectives of the study are as follows:

1. To identify major factors influencing Work Life balance.
2. To study the work life balance and its effect on productivity
3. To evaluate how work affect the family life and productivity
4. To come up with the strategies to improve the quality of Work Life of employees.
5. To understand the satisfaction level of the employees in hotel industry

Scope of the Study

The study on work life balance highlights the importance of balance to be maintained by the employees between their work and lifestyle. Workand personal life demands are two most important priority of anyperson and problems arise when there is an imbalance between these two. If work and life is not properly balanced it can adversely affect each other and can increase job dissatisfaction, affect family relations, may lead to development of stress related diseases and also affect adversely productivity of the firm. The study can help the organization in improving the work life balance of employees and thereby augment their overall performance and productivity. In case the organization takes appropriate care about the work life balance of the employees, they may improve their performance leading to increased efficiency of the organization. Also the employees needs to understand importance of balance between professional working and personal life making it important to study work life balance of employees.

Sampling Universe

Population or Universe is the aggregate of all elements possessing certain specified characteristics which need to be studied and defined prior the sample population. The universe can be finite or infinite. In this research, the sampling universe comprises of employees at corporate hotels like Marriott Courtyard, TGB and Taj Skyline of Ahmedabad.

Sampling Method Used

In this research, Stratified Random Sampling method is used for data collectionby diving respondents into stratum based on their designation. The respondents are stratified into 3 categories; Managerial level, Administrative level, and Executive staff members. Respondents are further selected from these three strata in equal proportion. I.e. 12 employees from each strata consisting of 36 employees from each hotel.

Sample Size

In this research the sample size is 108 employees of corporate hotels like Marriott Courtyard, TGB and Taj Skyline of Ahmedabad.Respondents are further selected from these three hotels in equal proportion. I.e.36 employees from each hotel. They are further stratified into 3 categories; Managerial level, Administrative level, and Executive staff members& respondents are selected in equal proportion from these strata. Respondents are selected from three different strata to avoid influence and biased opinions of particular level of management.

Tools Used For Data Collection

Data collection is most important part of research. Inaccurate data ultimately leads to invalid results. In this research, data is collected through questionnaire. This is the most commonly used method of data collection.

Statistical Tools Used For Analysis

For the purpose of analysis different statistical tools are used, they are as follow:

- Graphs and Chart
- Percentage Analysis
- Factor Analysis
- Chi-square Test

Factor Analysis

Factor analysis is generally used for data reduction & summarization. Factor analysis is an interdependence technique wherein an entire set of interdependent relationship is examined without making distinction between independent & dependent variables. In this study the various factors considered for the study are salary, working environment, working hours, overtime, incentives, target/ deadline pressure, attitude of supervisor, provision of work from home, availability of amenities, working on holidays, working autonomy, frequent office work trips, performance appraisal, career growth prospect, conflicts at work place, organizational change, family support and management support.

One of the major objectives of the research is to identify major factors influencing Work Life balance& factor analysis is conducted for the same as below:

Kaiser-Meyer-Olkin (KMO) measures sampling adequacy. It is measure used to examine appropriateness of factor analysis. Its high value between 0.5 & 1 indicates factor analysis is appropriate. Its value below 0.5 implies that factor analysis is not appropriate. In this case KMO is 0.673. This indicates that factor analysis is appropriate & acceptable.

Bartlett's test of Sphericity is test statistics used to examine hypothesis that variables are uncorrelated in population.

The hypothesis tested accordingly is as follows:

Ho: Population correlation matrix is identity matrix where each variable correlates perfectly with itself only & has no correlation with other variables

Ha: Population correlation matrix is identity matrix where each variable does not correlates with itself only & has correlation with other variables.

Significance value is 0.000 which is lesser than 0.5. Thus, Ha is accepted i.e. Population correlation matrix is identity matrix where each variable does not correlates with itself only & has correlation with other variables. This ensures reliability of the test.

Total Variance Explained tells about number of factors extracted by SPSS. Reading has come for first three variables only that mean SPSS has converted 18 original variables into three new factors. These three variables explains 80.58% of variance i.e. 81% approximately which is a good result as more than half of the data is explained by these factors (Extraction method used is principal component analysis)

Determination based on Eigen Value: Eigen value represents the amount of variance associated with the factor**.** In this approach, only factors with Eigen value greater than one are retained. Hence, only factors with a variance

greater than one are included. Factors with Eigen value less than one are no better than a single variable, since due to standardization each variable has a variance of one & factors with Eigen value below one does not explain great deal of variance, in this case first three variables have Eigen values of one approximately so only first three factors are drawn for further analysis.

Principal Component Analysis (PCA) extraction method is used to extract these three factors. This method is recommended when primary concern is to determine minimum number of factors that will account for maximum variance in data for use in subsequent multivariate analysis. These factors are called Principal Components. But Component Matrix does not give clear idea about which variables are going into which factor. This identification of factors clearly comes from Rotated Component Matrix. Thus, as Component matrix does not give clear classification about which variable is going into only one factor so rotation with Varimax method is performed to get the required clarity.

Rotation means rotating axis of factors in such a way that variables are clearly going towards one or another factor & clear cut distinction between factors emerges. Also the factors become consistent within themselves & very different from each other & no variable has high loading with more than one factor whereas Varimaxminimizes the number of variables with high loading on a factor for enhancement of the interpretability of factors. Thus, variables which are very closely matching & from customer point of view are only one factor

Thus, the most important factors determining work-life balance in hotel industry are
1. Monetary gains
2. Organizational Work Climate
3. HR Policy

Thus, It is observed from factor analysis that monetary gains is the most important factor influencing employees work life balance as it directly affects the employees motivation level to continue the job. Second most important factor influencing work life balance of employees is found to be organizational work climate. It is observed that most of the employees finds it very irrational working for office from home also. It is found to be hindering their personal life. It is followed by deadline/target pressures. Employees finds it very hard every time to stand at par of challenging targets. Salary and career growth prospects which are part of monetary gains highly influence work life balance. Salary being the biggest reason for working of an employee, it is required to be appropriately paid for assuring higher level of job satisfaction for the employee. The individual thinks about continuing the job for long term only in case if he is assured about bright career prospect. Thus, promising career growth prospects assures employee retention which in turn helps in achieving work life balance. Amicable working environment, working hours, overtime, incentives, working autonomy, family & management support are also found to be influencing work life balance. Moreover, certain factors like attitude of supervisor, availability of amenities, frequent traveling for work trips etc are not found to be highly influential factors contributing to work life balance.

Testing Of Hypothesis

Ho: Employeesare not satisfied with their work life balance
H1: Employeesare satisfied with their work life balance
O = Observed Value
E = Expected Value
E = Column total * Raw total / Grand total
Degree of Freedom = (Number of rows – 1) (Number of column – 1)
= (2-1)(5-1)= 4

Interpretation

Calculated Chi-Square value is 1.48 lesser than the table value 9.49, thus, Ho is accepted. It indicates that employees are not satisfied with their work life balance and the management needs to initiate with motivational measures for assuring effective & efficient working of employees.

Findings and Discussions

Majority of the hospitality industry employees are male and married. Employees are found to be working for 6 to 10 hours. Most of the employees works for at least 6 hours and not more than 10 hours most of the time. Employees are taking care of completion of official working hours. Only few are working over time. Most of the employees are coming from nearby places which take less than one hour to travel. Employees coming from far distance are very few & some have moved from their home town for job purpose. Most of the employees feels that they are unable to attend social family gathering because of their busy work schedules. They experience sometimes even the guilt of not sparing enough of the time with their family members specifically along with their kids. Employees are expecting flexible working hours to ease their tension about reaching on time as well as to adjust along with their family related requirements. Most of the employees are not satisfied with the leave policy & expect great changes to be incorporated in it. They find long working hours affects their productivity. Work pressure is also realized by many employees and some are found to be suffering from stress related health issues. Employees have experienced that work pressure affects their working efficiency at home also. Lack of good growth career prospects leads to dissatisfaction among employees resulting as the major cause of their attrition. Moreover, monetary gains, organizational working climate & HR policy are found to be most important factors influencing employees work life balance as it directly affects the employees motivation level to continue the job. Significant influence of employees' family size on work satisfaction is not observed. Work place support and financial assistance serves as a significant predictor for personal life satisfaction of employees working in hospitality sector. Family support and self-management serves as the variables of work satisfaction of employees in hospitality sector.

Suggestions

The work life balance strategies differ for single, married and women with children. Based on the findings of the study the following suggestions are drawn.Counseling programs regarding proper work life balance can be conducted for employees who cannot manage work and lifestyle since work life balance is an important factor in an employees life. Women employees playing dual role of handling household as well as office if possible are not suppose to be considered for overtime working. This will assure their long term continuity with the organization. Also the time schedules could be fixed in such a manner that it is not affecting anyone's work life balance. ◎ The employees could be encouraged to work productively without wasting much of their time gossiping with each other. Hotels could be providing staff members with free accommodation facility. Saving upon the commutation time & expense would even lead to increased working efficiency. Also sometimes job rotation can be practised. This would help in reducing boredom caused due to monotonous working of the employees. Most importantly leave request should be handled cautiously by considering them favorably if possible. Unnecessary delays in sanctioning leaves should be avoided.

The provision of flexitime could be incorporated. This allows easy achievement of work-life balance and organization of work around their other personal errands and commitments. Some organizational initiatives like Part-time, Job sharing, Shift work, Staggered hours, Compressed hours etc could be introduced for maintaining work life balance. Most importantly respectful treatment of employees at all levels, compensation/pay and benefits which are the key drivers of job satisfaction are supposed to be incorporated in the organization. Some motivational programmes could be arranged for employees. Frequent social functions, achievement and reward function celebrations along with the family members can also play a role of motivator. Some of the individual level initiatives could also be initiated for maintaining work life balance. Its important to attain a balanced mind approach for the employees. Employees should take nutritious & healthy diet on time for assuring working efficiency with required

level of attention. In fact if possible, hotels should provide free meals or meals at very subsidized rates to its staff members. Exercise is a very good medicine and it helps us in keeping ourselves stress free so proper exercise, yoga, meditation can help in maintaining better work-life balance.

CONCLUSION

Employees should prioritize his or her work they should decide themselves that what thing is more important for them and then work accordingly. One should make effort for assessing one's values and then set the priorities accordingly. Whenever perfect balance is unattainable in life, employees should take care about it by designating some time for sleep, exercise and relaxation. Even employees should talk with friends, relatives, coworkers & should try to change the situation which is found to be frustrating. Employees should also find a mentor at the work place who can give regular or timely advice on career development, time management etc. An individual must manage the time consumed on various rolesand responsibilities of life by assuring that time and energy support Work-life balance. Human body also require recharging as a battery through recreation being off work,nice time with family etc. Moreover it is important to understand that every person is responsible for his/her own work-life balance and no one else can be much effective in attaining a WLB than the individual himself or herself. As said by experts that our emotions are the product of our own thoughts, we alone can control our thoughts and emotions also. So having and cultivating a faith and confidence in oneself can be the strong tool for maintaining work-life balance.

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Table 1 : Variables Extracted

Factor	Variables extracted	Factor Name
1	High loading with factors like Salary, Incentives, Performance Appraisals, Career Growth	Monetary Gains
2	High loading with factors like target, Attitude of Supervisor, Provision of Work from Home, Conflict at Work Place	Organizational Work Climate
3	High loading with factors like working Environment, Working hours, Overtime, Availability of Amenities, Working on Holidays, Working Autonomy, Frequent office work trips, Organizational Change, Family & Management Support,	HR Policy

Table 2: Employees level of satisfaction

	Highly Satisfied	Satisfied	Neutral	Dissatisfied	Highly Dissatisfied	Total
Male	10	15	06	14	15	60
Female	12	13	04	9	10	48
Total	22	28	10	23	25	108

Table 3: Chi-Square Analysis

Observed Value	Expected Value	$(O-E)^2$	$(O-E)^2/E$
10	12.22	4.93	0.40
15	15.56	0.31	0.02
6	5.56	0.19	0.03
14	12.78	1.49	0.12
15	13.89	1.23	0.09
12	9.78	4.93	0.50
13	12.44	0.31	0.02
4	4.44	0.19	0.04
9	10.22	1.49	0.15
10	11.11	1.23	0.11

Table 4: Chi-Square Test Statistics

Level of Significance	Degree of Freedom	Calculated Value	Table Value	Result
5%	4	1.48	9.49	Accept H0

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RESEARCH ARTICLE

A Design of Two Sided Modified Complete Chain Sampling Plans TSMCChSP-1 using Fuzzy Parameter

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ABSTRACT

This study develops TSMCChSP-1 using fuzzy numbers. Tables are constructed for FOC values for this plan. The sample size is obtained such that it satisfies both the conditions of risks. Sum of the risks is minimized for best possible value of sample size. Examples are given for the Specified plan. This plan gives further safety to the purchaser whereas gives mild pressure to the manufacturer to sustain the value of items.

Keywords: Acceptance Sampling, Operating characteristic (OC) curve, Modified Complete Chain Sampling Plan, Fuzzy number, Trapezoidal fuzzy number.

INTRODUCTION

A measurable procedure is utilized to control, improve and keep up the quality to tackle quality issue is known as Statistical Quality Control [12]. In many industries SQC method is involved for improvement of the quality of the materials [14]. In traditional methods crisp values are involved to find probability of acceptance and proportion defective. [16] While solving real life problems using traditional methods there are some inaccuracy and uncertainty present. [17] The powerful mathematical tool is used to solve these imprecision and ambiguity is fuzzy set theory. Dodge [5] presented chain sampling inspection plans. Clark [2] developed OC curves for ChSP-1, chain sampling plans. Frishman and Fred [8] extended chain sampling plan. Dodge and Stephens [6] developed a general family of chain sampling plan. Govindaraju and Lia [9] developed modified chain sampling plans. MChSP-1 plan requires a smaller sample size than the zero acceptance number plan. Deva Arul and Edna [3] designed two sided complete

chain sampling plan. Here the new chaining rule is applied on the current lot. Edna [13] contributed quality measures based on acceptance sampling plans. Deva Arul and Vijila [4] presented two sided modified complete chain sampling plan. It is probably used where the inspections involving destructive or expensive while testing.

The improvement of chain sampling plan for a different operating procedure is given for two sided modified complete chain sampling plans MCChSP (C_1, C2, i, j). These TSMCChSP -1 plan can be applied to any type of quality control units in industries. It gives more safety to the customer. Milky Mathew and Rajeswari [11] compared operating characteristic (OC) function of TSMCChSP-1 and TSCChSP-1 plans under Poisson distribution. Fuzzy acceptance sampling plans are developed by Kahraman and Kaya [10]. Jamkhaneh and Sadeghpour Gildeh [1] created a (ChSP-1) chain sampling scheme using fuzzy probability theory. Turanoglu, Kaya and Kahraman [15] have studied OC curve using fuzzy parameters in acceptance sampling. Basic definitions of fuzzy number, trapezoidal fuzzy number, operating procedure for TSMCChSP-1 and its flow chart are included in this work. Then FOC Curve or band values are intended using fuzzy number. The specified plan for the given $\breve{\wp}_{1f}$ and $\breve{\wp}_{2h}$ value is determined to satisfy the inequality conditions and also to minimize the risks. The results are presented in tables.

Definitions

Fuzzy Number (Zadeh [16] and Dubis & Prade [7]): " Fuzzy set that are characterized on the arrangement of real numbers having the structure $\breve{E}$: R tends to [0,1] are known as fuzzy number. A fuzzy number $\breve{E}$ will be a fuzzy set in the real line that fulfills the state of both normal and convexity".

Trapezoidal fuzzy number (Zadeh [16] and Dubis & Prade [7]):"If trapezoidal fuzzy numbers (TrFNs) are $\breve{E}$ = (e_1, e_2 , e_3 , e_4) then its membership function is as follows"

$$\text{TrFN} = \begin{cases} 0 & , \quad otherwise \\ \frac{y-e_1}{e_2-e_1} & , \ e_1 \le y \le e_2 \\ 1 & , \quad e_2 \le y \le e_3 \\ \frac{e_4-y}{e_4-e_3} & , \ e_3 \le y \le e_4 \\ 0 & , \quad otherwise \end{cases} \qquad \ldots\ldots\ldots (1)$$

"The interval of confidence of trapezoidal fuzzy number defined by γ cuts can be written as follows"

$$\breve{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma] \qquad \ldots\ldots\ldots (2)$$

Operating procedure of TSMCChSP-1

According to Deva Arul and Vijila [4] and Milky Mathew and Rajeswari [11] as

Step 1: Draw a random sample of size n from the lot and count the number of defectives.

Step 2: If d =0 are found in the immediately preceding i samples and succeeding j samples then accept the current lot.

Step 3: Accept the current lot if in the current lot d =0 and any one of the preceding i samples and the succeeding j samples contains only one defective. And also the rest of (i-1) and (j -1) samples are free from nonconforming units.

Step 4: Reject the current lot if d>0.

Fuzzy Proportion of defective and Fuzzy Probability of Acceptance

As per Baloui Jamkhaneh and Sadeghpour-Gildeh [1] fuzzy probability of acceptance is calculated using binomial distribution. γ cut of trapezoidal fuzzy number is used to solve TSMCChSP-1such that $\breve{\wp}_s$ = ($s, e_2 + s, e_3 + s, e_4 + s$) Where $e_j = b_i - b_1$, i = 2,3,4 and $s \in [0,1 - e_4]$ $\breve{\wp}_s[\gamma] = [s + e_2\gamma,\ e_4 + s - (e_4 - e_3)\gamma]$ and taking γ=0,1 then we get fuzzy interval of proportion defective $\breve{\wp}_s[\gamma] = [\breve{\wp}_s^{lb}, \breve{\wp}_s^{ub}]$ and interval value of fuzzy probability of acceptance $\mathcal{L}(\breve{\wp}_s)[\gamma] = [\mathcal{L}(\breve{\wp}_s^{lb}), \mathcal{L}(\breve{\wp}_s^{ub})]$. Deva Arul and Vijila [4] and Milky Mathew and Rajeswari [11] given probability of acceptance. Here using fuzzy parameters for TSMCChSP-1 was changed as follows.

Case (i) For i = j

$$\mathcal{L}(\tilde{\wp}_s^{lb})[\gamma] = \min\left\{ (1-\breve{\wp})^{2i\eta} \left((1+\breve{\wp})^{\eta} + \frac{2i\eta\breve{\wp}}{(1-\breve{\wp})}\right)\right\} \ldots\ldots\ldots (3)$$

$$\mathcal{L}(\tilde{\wp}_s^{ub})[\gamma] = \max\left\{ (1-\breve{\wp})^{2i\eta} \left((1+\breve{\wp})^{\eta} + \frac{2i\eta\breve{\wp}}{(1-\breve{\wp})}\right)\right\} \ldots\ldots\ldots (4)$$

$$\mathcal{L}(\tilde{\wp}_s)[\gamma] = \begin{bmatrix} (1-\tilde{\wp}_s^{ub})^{2i\eta}\left(\left(1+\tilde{\wp}_s^{ub}\right)^{\eta} + \frac{2i\eta\tilde{\wp}_s^{ub}}{\left(1-\tilde{\wp}_s^{ub}\right)}\right), \\ (1-\tilde{\wp}_s^{lb})^{2i\eta}\left(\left(1+\tilde{\wp}_s^{lb}\right)^{\eta} + \frac{2i\eta\tilde{\wp}_s^{lb}}{\left(1-\tilde{\wp}_s^{lb}\right)}\right) \end{bmatrix} \ldots\ldots\ldots (5)$$

Case (ii) For i ≠ j

$$\mathcal{L}(\tilde{\wp}_s^{lb})[\gamma] = \min\left\{ (1-\breve{\wp})^{\eta(i+j)} \left((1+\breve{\wp})^{\eta} + \frac{\eta\breve{\wp}(i+j)}{(1-\breve{\wp})}\right)\right\} \ldots\ldots (6)$$

$$\mathcal{L}(\tilde{\wp}_s^{ub})[\gamma] = \max\left\{ (1-\breve{\wp})^{\eta(i+j)} \left((1+\breve{\wp})^{\eta} + \frac{\eta\breve{\wp}(i+j)}{(1-\breve{\wp})}\right)\right\} \ldots\ldots (7)$$

$$\mathcal{L}(\tilde{\wp}_s)[\gamma] = \begin{bmatrix} (1-\tilde{\wp}_s^{ub})^{\eta(i+j)}\left(\left(1+\tilde{\wp}_s^{ub}\right)^{\eta} + \frac{\eta\tilde{\wp}_s^{ub}(i+j)}{\left(1-\tilde{\wp}_s^{ub}\right)}\right), \\ (1-\tilde{\wp}_s^{lb})^{\eta(i+j)}\left(\left(1+\tilde{\wp}_s^{lb}\right)^{\eta} + \frac{\eta\tilde{\wp}_s^{lb}(i+j)}{\left(1-\tilde{\wp}_s^{lb}\right)}\right) \end{bmatrix} \ldots\ldots (8)$$

The fuzzy probability of acceptance value and fuzzy proportion defective are calculated for various values and is provided in Table 1 and Table 2. One can observe that when the parameter 's' value is very small or nearer to zero then the acceptance value of fuzzy probability is approximately equal to unity.

Example 1

Suppose $\tilde{\wp}_s$ = (0.005,0.006,0.007,0.008) where n=20 and i = j =1. From Table 1 fuzzy proportion defective is obtained as $\tilde{\wp}_s[\gamma = 0]$ = [0.005 0.013] and $\tilde{\wp}_s[\gamma = 1]$ = [0.011 0.012] and fuzzy probability of acceptance is calculated as $\mathcal{L}(\tilde{\wp}_{as})[\gamma = 0]$ = [0.9047 0.7682] and $\mathcal{L}(\tilde{\wp}_{as})[\gamma = 1]$ = [0.8008 0.7844].

Example 2

Let us consider $\tilde{\wp}_s$ = (0.005,0.006,0.007,0.008) where n=20 , i=1 and j =2. Then from Table 1 we get $\tilde{\wp}_s[\gamma = 0]$ = [0.005 0.013] and $\tilde{\wp}_s[\gamma = 1]$ = [0.011 0.012] as fuzzy proportion defective and fuzzy probability of acceptance is calculated as $\mathcal{L}(\tilde{\wp}_{as})[\gamma = 0]$ = [0.8928 0.7115] and $\mathcal{L}(\breve{\wp}_{as})[\gamma = 1]$ = [0.7564 0.7339].

Fuzzy Operating characteristic (FOC) curve or band

From the above Figures, fuzzy proportion defective is plotted against fuzzy probability of acceptance for γ=0 and γ=1. OC Curve we have upper band and lower band therefore it is called as FOC band or curve. When γ value increases from zero to one then FOC band value becomes closer for both the cases where i = j=1 and i = 1& j=2.

Fuzzy probability of acceptance when sample size varies

Let us assume that $\tilde{\wp}_s$ = (0.002,0.003,0.004,0.005) and the sample size n varies from 5 to 50 then γ cut of trapezoidal fuzzy number is used to calculate fuzzy proportion defective. The interval is obtained as $\tilde{\wp}_s[\gamma = 0]$ = [0.002 0.007] , $\tilde{\wp}_s[\gamma = 1]$ = [0.005 0.006]. Table 3, reveals that when the sample size values decreases the width of FOC curve decreases.

Determination of sample size

"Let us consider that Accepting quality level $(\widetilde{AQL})$ as $\widetilde{\wp}_{1f}$ and Limiting quality level $(\widetilde{LQL})$ as $\widetilde{\wp}_{2h}$. Then producer's risk $\widetilde{\alpha_f}$ and consumer's risk $\widetilde{\beta_h}$ relates to acceptance sampling plan where rejecting the good lot is called producer's risk and accepting the bad lot is called Consumer's risk. Here two sided modified complete chain sampling plan is used to design the parameter sample size n to satisfy the following two inequalities for $\mathcal{L}(\widetilde{\wp}_{1f})$ and $\mathcal{L}(\widetilde{\wp}_{2h})$ simultaneously.

$\mathcal{L}(\widetilde{\wp}_{1f}) \geq 1 - \widetilde{\alpha_f}$ and $\mathcal{L}(\widetilde{\wp}_{2h}) \leq \widetilde{\beta_h}$, $\widetilde{\alpha_f}$ = 0.05 and $\widetilde{\beta_h}$ = 0.10 is fixed so that the interval of fuzzy probability of acceptance satisfies the conditions $\mathcal{L}(\widetilde{\wp}_{1f}) \geq 0.95$ and $\mathcal{L}(\widetilde{\wp}_{2h}) \leq 0.10$ for different sample sizes".

Case(i) For i = j

$$\mathcal{L}\left(\breve{\wp}_{1f}\right) = \left\{ (1-\breve{\wp}_{1f})^{2i\eta} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{1f}}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\} \geq 0.95 \ldots\ldots (9)$$

$$\mathcal{L}\left(\breve{\wp}_{2h}\right) = \left\{ (1-\breve{\wp}_{2h})^{2i\eta} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{2h}}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\} \leq 0.10 \ldots\ldots\ldots (10)$$

Case(ii) For i ≠ j

$$\mathcal{L}\left(\breve{\wp}_{1f}\right) = \left\{ (1-\breve{\wp}_{1f})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{\eta\breve{\wp}_{1f}(i+j)}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\} \geq 0.95 \ldots\ldots\ldots (11)$$

$$\mathcal{L}\left(\breve{\wp}_{2h}\right) = \left\{ (1-\breve{\wp}_{2h})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{\eta\breve{\wp}_{2h}(i+j)}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\} \leq 0.10 \ldots\ldots\ldots (12)$$

Minimizing the sum of risks

The mathematical expression to minimize the sum of risk is $\widetilde{\alpha_f} + \widetilde{\beta_h} = 1 - \mathcal{L}(\breve{\wp}_{1f}) + \mathcal{L}(\breve{\wp}_{2h})$. The sum of risks is obtained as interval of fuzzy. The sample size is calculated so as to minimize the sum of the risks for two sided modified complete chain sampling plan and its values are displayed in Table 6 and Table 7.

Case (i) for i = j

$$\mathcal{L}\left(\breve{\wp}_{1f}\right) = \left\{ (1-\breve{\wp}_{1f})^{2i\eta} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{1f}}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\} \ldots\ldots\ldots (13)$$

$$\mathcal{L}\left(\breve{\wp}_{2h}\right) = \left\{ (1-\breve{\wp}_{2h})^{2i\eta} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{2h}}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\} \quad \ldots\ldots\ldots (14)$$

$$\widetilde{\alpha_f} + \widetilde{\beta_h} = 1 - \mathcal{L}(\breve{\wp}_{1f}) + \mathcal{L}(\breve{\wp}_{2h})$$

$$= 1 - \left\{ (1-\breve{\wp}_{1f})^{2i\eta} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{1f}}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\} + \left\{ (1-\breve{\wp}_{2h})^{2i\eta} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{2i\eta\breve{\wp}_{2h}}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\}$$

Case(ii) For i ≠ j

$$\mathcal{L}\left(\breve{\wp}_{1f}\right) = \left\{ (1-\breve{\wp}_{1f})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{\eta\breve{\wp}_{1f}(i+j)}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\} \ldots\ldots (15)$$

$$\mathcal{L}\left(\breve{\wp}_{2h}\right) = \left\{ (1-\breve{\wp}_{2h})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{\eta\breve{\wp}_{2h}(i+j)}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\} \ldots\ldots (16)$$

$$\widetilde{\alpha_f} + \widetilde{\beta_h} = 1 - \mathcal{L}(\breve{\wp}_{1f}) + \mathcal{L}(\breve{\wp}_{2h})$$

$$= 1 - \left\{ (1-\breve{\wp}_{1f})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{1f}\right)^{\eta} + \frac{\eta\breve{\wp}_{1f}(i+j)}{\left(1-\breve{\wp}_{1f}\right)} \right) \right\}$$

$$+ \left\{ (1-\breve{\wp}_{2h})^{\eta(i+j)} \left(\left(1+\breve{\wp}_{2h}\right)^{\eta} + \frac{\eta\breve{\wp}_{2h}(i+j)}{\left(1-\breve{\wp}_{2h}\right)} \right) \right\}$$

CONCLUSION

TSMCChSP-1 is designed using fuzzy parameters. Binomial distribution is used for TSMCChSP-1. The interval value of fuzzy proportion defective and the fuzzy probability of acceptance is calculated for trapezoidal fuzzy number. By fixing the quality levels, the optimum value of n is calculated such that inequality conditions are satisfied and simultaneously minimizing the sums of the risks.

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Table 1 Fuzzy probability of acceptance with n=20 and i = j =1

$\widetilde{\wp}_s = (s, e_2+s, e_3+s, e_4+s)$	$\widetilde{\wp}_s[\gamma=0]$	$\mathcal{L}(\widetilde{\wp}_{as})[\gamma=0]$	$\widetilde{\wp}_s[\gamma=1]$	$\mathcal{L}(\widetilde{\wp}_{as})[\gamma=1]$
(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9371]	[0.001 0.002]	[0.9796 0.9586]
(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9796 0.8928]	[0.003 0.004]	[0.9371 0.9151]
(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9586 0.8476]	[0.005 0.006]	[0.8928 0.8703]
(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9371 0.8019]	[0.007 0.008]	[0.8476 0.8248]
(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9151 0.7564]	[0.009 0.010]	[0.8019 0.7791]
(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.8928 0.7115]	[0.011 0.012]	[0.7564 0.7339]
(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.8703 0.6674]	[0.013 0.014]	[0.7115 0.6893]
(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.8476 0.6246]	[0.015 0.016]	[0.6674 0.6458]
(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.8248 0.5831]	[0.017 0.018]	[0.6246 0.6037]
(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.8019 0.5433]	[0.019 0.020]	[0.5831 0.5630]
(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.7791 0.5051]	[0.021 0.022]	[0.5433 0.5240]
(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.7564 0.4687]	[0.023 0.024]	[0.5051 0.4867]
(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.7339 0.4342]	[0.025 0.027]	[0.4687 0.4512]
(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.7115 0.4015]	[0.027 0.028]	[0.4342 0.4176]
(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.6893 0.3707]	[0.029 0.030]	[0.4015 0.3858]
(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.6674 0.3417]	[0.031 0.032]	[0.3707 0.3559]
(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.6458 0.3145]	[0.033 0.034]	[0.3417 0.3279]
(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.6246 0.2890]	[0.035 0.036]	[0.3145 0.3015]
(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.6037 0.2653]	[0.037 0.038]	[0.2890 0.2770]
(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.5831 0.2432]	[0.039 0.040]	[0.2653 0.2540]

Table 2 Fuzzy probability of acceptance with n=20, i =1 and j =2

$\widetilde{\wp}_s = (s, e_2+s, e_3+s, e_4+s)$	$\widetilde{\wp}_s[\gamma=0]$	$\mathcal{L}(\widetilde{\wp}_{as})[\gamma=0]$	$\widetilde{\wp}_s[\gamma=1]$	$\mathcal{L}(\widetilde{\wp}_{as})[\gamma=1]$
(0.000, 0.001, 0.002, 0.003)	[0 .000 0.003]	[1.0000 0.9418]	[0.001 0.002]	[0.9802 0.9608]
(0.001, 0.002, 0.003, 0.004)	[0.001 0.005]	[0.9802 0.9047]	[0.003 0.004]	[0.9418 0.9231]
(0.002, 0.003, 0.004, 0.005)	[0.002 0.007]	[0.9608 0.8690]	[0.005 0.006]	[0.9047 0.8867]
(0.003, 0.004, 0.005, 0.006)	[0.003 0.009]	[0.9418 0.8344]	[0.007 0.008]	[0.8690 0.8515]
(0.004, 0.005, 0.006, 0.007)	[0.004 0.011]	[0.9231 0.8008]	[0.009 0.010]	[0.8344 0.8174]
(0.005, 0.006, 0.007, 0.008)	[0.005 0.013]	[0.9047 0.7682]	[0.011 0.012]	[0.8008 0.7844]
(0.006, 0.007, 0.008, 0.009)	[0.006 0.015]	[0.8867 0.7366]	[0.013 0.014]	[0.7682 0.7523]
(0.007, 0.008, 0.009, 0.01)	[0.007 0.017]	[0.8690 0.7059]	[0.015 0.016]	[0.7366 0.7211]
(0.008, 0.009, 0.01, 0.011)	[0.008 0.019]	[0.8515 0.6760]	[0.017 0.018]	[0.7059 0.6908]
(0.009, 0.010, 0.011, 0.012)	[0.009 0.021]	[0.8344 0.6470]	[0.019 0.020]	[0.6760 0.6614]
(0.010, 0.011, 0.012, 0.013)	[0.010 0.023]	[0.8174 0.6188]	[0.021 0.022]	[0.6470 0.6328]
(0.011, 0.012, 0.013, 0.014)	[0.011 0.025]	[0.8008 0.5915]	[0.023 0.024]	[0.6188 0.6050]
(0.012, 0.013, 0.014, 0.015)	[0.012 0.027]	[0.7844 0.5649]	[0.025 0.027]	[0.5915 0.5781]
(0.013, 0.014, 0.015, 0.016)	[0.013 0.029]	[0.7682 0.5392]	[0.027 0.028]	[0.5649 0.5520]
(0.014, 0.015, 0.016, 0.017)	[0.014 0.031]	[0.7523 0.5143]	[0.029 0.030]	[0.5392 0.5266]
(0.015, 0.016, 0.017, 0.018)	[0.015 0.033]	[0.7366 0.4902]	[0.031 0.032]	[0.5143 0.5021]
(0.016, 0.017, 0.018, 0.019)	[0.016 0.035]	[0.7211 0.4668]	[0.033 0.034]	[0.4902 0.4784]
(0.017, 0.018, 0.019, 0.02)	[0.017 0.037]	[0.7059 0.4443]	[0.035 0.036]	[0.4668 0.4555]
(0.018, 0.019, 0.02, 0.021)	[0.018 0.039]	[0.6908 0.4225]	[0.037 0.038]	[0.4443 0.4333]
(0.019, 0.02, 0.021, 0.022)	[0.019 0.041]	[0.6760 0.4016]	[0.039 0.040]	[0.4225 0.4120]

Table 3: Fuzzy probability of acceptance for different sample size where i = j and i ≠ j

n	i = j	$\mathcal{L}(\tilde{\wp}_{as})[\gamma=0]$	$\mathcal{L}(\tilde{\wp}_{as})[\gamma=1]$	i ≠ j	$\mathcal{L}(\tilde{\wp}_{as})[\gamma=0]$	$\mathcal{L}(\tilde{\wp}_{as})[\gamma=1]$
5		[0.9851 0.9561]	[0.9657 0.9609]		[0.9848 0.9535]	[0.9641 0.9588]
10		[0.9705 0.9140]	[0.9325 0.9232]		[0.9693 0.9043]	[0.9263 0.9153]
15		[0.9560 0.8735]	[0.9003 0.8868]		[0.9533 0.8534]	[0.8873 0.8704]
20		[0.9418 0.8344]	[0.8690 0.8515]		[0.9371 0.8019]	[0.8476 0.8248]
25	i =1, j=1	[0.9277 0.7966]	[0.8385 0.8173]	i =1, j=2	[0.9206 0.7507]	[0.8075 0.7790]
30		[0.9138 0.7600]	[0.8089 0.7842]		[0.9039 0.7002]	[0.7676 0.7337]
35		[0.9001 0.7246]	[0.7801 0.7520]		[0.8871 0.6511]	[0.7280 0.6891]
40		[0.8866 0.6904]	[0.7519 0.7207]		[0.8701 0.6037]	[0.6891 0.6457]
45		[0.8732 0.6574]	[0.7245 0.6904]		[0.8530 0.5582]	[0.6511 0.6037]
50		[0.8600 0.6254]	[0.6978 0.6609]		[0.8359 0.5150]	[0.6140 0.5632]
5		[0.9843 0.9493]	[0.9614 0.9554]		[0.9836 0.9436]	[0.9578 0.9508]
10		[0.9672 0.8894]	[0.9167 0.9032]		[0.9645 0.8703]	[0.9042 0.8874]
15		[0.9490 0.8244]	[0.8680 0.8463]		[0.9432 0.7890]	[0.8436 0.8165]
20		0.9298 0.7574	[0.8169 0.7872]		[0.9201 0.7057]	[0.7796 0.7426]
25		0.9097 0.6907	[0.7648 0.7276]		[0.8956 0.6243]	[0.7148 0.6691]
30	i =2,	0.8890 0.6259	[0.7128 0.6689]	i =2,	[0.8699 0.5475]	[0.6511 0.5982]
35	j =2	0.8677 0.5641	[0.6616 0.6119]	j =3	[0.8433 0.4764]	[0.5896 0.5312]
40		0.8460 0.5060	[0.6119 0.5575]		[0.8162 0.4120]	[0.5312 0.4690]
45		0.8240 0.4519	[0.5642 0.5060]		[0.7887 0.3543]	[0.4766 0.4121]
50		0.8018 0.4021	[0.5187 0.4578]		[0.7610 0.3033]	[0.4259 0.3605]
5		0.9827 0.9365	[0.9533 0.9451]		[0.9816 0.9282]	[0.9480 0.9383]
10		0.9611 0.8478	[0.8891 0.8688]		[0.9570 0.8228]	[0.8719 0.8477]
15		0.9361 0.7493	[0.8154 0.7825]		[0.9278 0.7070]	[0.7844 0.7457]
20		0.9084 0.6503	[0.7381 0.6939]		[0.8950 0.5940]	[0.6941 0.6433]
25		0.8787 0.5565	[0.6612 0.6078]		[0.8596 0.4906]	[0.6062 0.5467]
30	i =3,	0.8475 0.4709	[0.5871 0.5271]	i =3,	[0.8224 0.3999]	[0.5239 0.4590]
35	j =3	0.8152 0.3948	[0.5175 0.4533]	j =4	[0.7843 0.3224]	[0.4488 0.3816]
40		0.7823 0.3285	[0.4533 0.3871]		[0.7456 0.2577]	[0.3817 0.3147]
45		0.7491 0.2715	[0.3950 0.3286]		[0.7069 0.2044]	[0.3227 0.2578]
50		0.7159 0.2231	[0.3426 0.2775]		[0.6686 0.1612]	[0.2713 0.2100]

Table 4: Optimum parameter n, when $\mathcal{L}(\tilde{\wp}_{1f}) \geq 0.95$ and $\mathcal{L}(\tilde{\wp}_{2h}) \leq 0.10$

i = j	$(\tilde{A}\tilde{Q}\tilde{L})$	$(L\tilde{Q}L)$	n
1	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	38
		(0.06,0.061,0.062,0.063)	35
		(0.07,0.071,0.072,0.073)	25
		(0.08,0.081,0.082,0.083)	23
		(0.09,0.091,0.092,0.093)	20
	(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	22
		(0.09,0.091,0.092,0.093)	22
2	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	27
		(0.06,0.061,0.062,0.063)	25
		(0.07,0.071,0.072,0.073)	23
		(0.08,0.081,0.082,0.083)	15
		(0.09,0.091,0.092,0.093)	10
	(0.002,0.0021,0.0022,0.0023)	(0.05,0.051,0.052,0.053)	19
		(0.06,0.061,0.062,0.063)	17
		(0.07,0.071,0.072,0.073)	15
		(0.08,0.081,0.082,0.083)	13
		(0.09,0.091,0.092,0.093)	10
	(0.003,0.0031,0.0032,0.0033)	(0.06,0.061,0.062,0.063)	11
		(0.07,0.071,0.072,0.073)	10
		(0.08,0.081,0.082,0.083)	9
		(0.09,0.091,0.092,0.093)	8
	(0.004,0.0041,0.0042,0.0043)	(0.09,0.091,0.092,0.093)	10

Table 5: Optimum parameter , when $\mathcal{L}\left(\tilde{\wp}_{1f}\right) \geq 0.95$ and $\mathcal{L}(\tilde{\wp}_{2h}) \leq 0.10$

i = j	$(\tilde{A}\tilde{Q}\tilde{L})$	$(\tilde{L}\tilde{Q}\tilde{L})$	η
i =1, j =2	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	38
		(0.06,0.061,0.062,0.063)	35
		(0.07,0.071,0.072,0.073)	25
		(0.08,0.081,0.082,0.083)	23
		(0.09,0.091,0.092,0.093)	20
	(0.002,0.0021,0.0022,0.0023)	(0.08,0.081,0.082,0.083)	22
		(0.09,0.091,0.092,0.093)	22
i =2, j =3	(0.001,0.0011,0.0012,0.0013)	(0.05,0.051,0.052,0.053)	27
		(0.06,0.061,0.062,0.063)	25
		(0.07,0.071,0.072,0.073)	23
		(0.08,0.081,0.082,0.083)	15
		(0.09,0.091,0.092,0.093)	10
	(0.002,0.0021,0.0022,0.0023)	(0.05,0.051,0.052,0.053)	19
		(0.06,0.061,0.062,0.063)	17
		(0.07,0.071,0.072,0.073)	15
		(0.08,0.081,0.082,0.083)	13
		(0.09,0.091,0.092,0.093)	10
	(0.003,0.0031,0.0032,0.0033)	(0.06,0.061,0.062,0.063)	11
		(0.07,0.071,0.072,0.073)	10
		(0.08,0.081,0.082,0.083)	9
		(0.09,0.091,0.092,0.093)	8
	(0.004,0.0041,0.0042,0.0043)	(0.09,0.091,0.092,0.093)	10

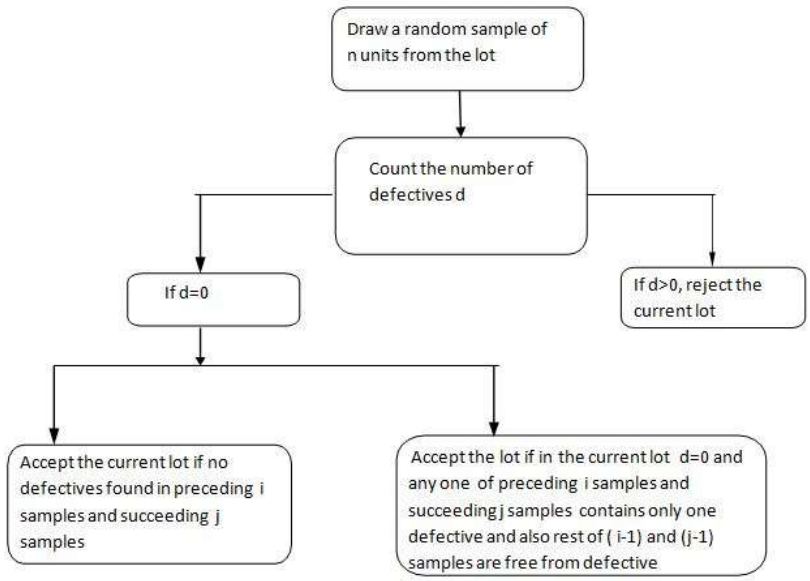
Table 6: Optimum parameter n for i = j and minimum sum of risks $When\ \widetilde{\alpha_f} \cong 0.05\ and\ \widetilde{\beta_h} \cong 0.10$

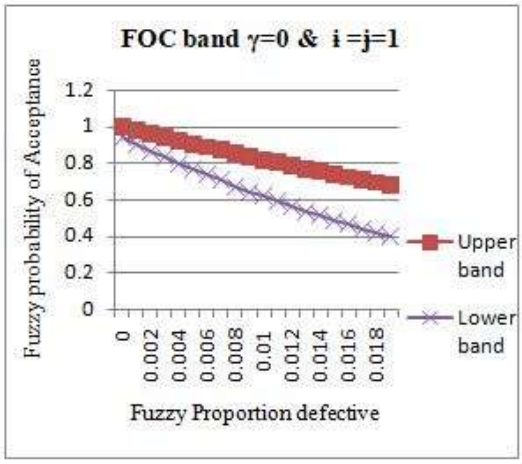
i=j	η	$\tilde{\wp}_{1f}$ [Y = 0]	$\mathcal{L}(\tilde{\wp}_{1f})$[Y = 0]	$\tilde{\wp}_{2h}$ [Y = 0]	$\mathcal{L}(\tilde{\wp}_{2h})$[Y = 0]	$\widetilde{\alpha_f} + \widetilde{\beta_h}$
1	38	[0.001 0.0013]	[0.9627 0.9518]	[0.05 0.053]	[0.0840 0.0698]	[0.1213 0.1180]
	35	[0.001 0.0013]	[0.9656 0.9555]	[0.06 0.063]	[0.0603 0.0506]	[0.0947 0.0951]
	25	[0.001 0.0013]	[0.9753 0.9680]	[0.07 0.073]	[0.1043 0.0924]	[0.1290 0.1244]
	23	[0.001 0.0013]	[0.9773 0.9706]	[0.08 0.083]	[0.0895 0.0799]	[0.1122 0.1093]
	20	[0.001 0.0013]	[0.9802 0.9743]	[0.09 0.093]	[0.0945 0.0855]	[0.1143 0.1112]
	22	[0.002 0.0023]	[0.9570 0.9507]	[0.08 0.083]	[0.1017 0.0913]	[0.1447 0.1406]
	22	[0.002 0.0023]	[0.9570 0.9507]	[0.09 0.093]	[0.0706 0.0631]	[0.1136 0.1124]
2	27	[0.001 0.0013]	[0. 9707 0.9611]	[0.05 0.053]	[0.0233 0.0175]	[0.0526 0.0564]
	25	[0.001 0.0013]	[0.9730 0.9642]	[0.06 0.063]	[0.0136 0.0103]	[0.0406 0.0461]
	23	[0.001 0.0013]	[0.9753 0.9673]	[0.07 0.073]	[0.0090 0.0069]	[0.0337 0.0396]
	15	[0.001 0.0013]	[0.9843 0.9793]	[0.08 0.083]	[0.0315 0.0215]	[0.0472 0.0422]
	10	[0.001 0.0013]	[0.9787 0.9865]	[0.09 0.093]	[0.0999 0.0902]	[0.1212 0.1037]
	19	[0.002 0.0023]	[0.9576 0.9506]	[0.05 0.053]	[0.0888 0.0735]	[0.1312 0.1229]
	17	[0.002 0.0023]	[0.9624 0.9563]	[0.06 0.063]	[0.0698 0.0587]	[0.1074 0.1024]
	15	[0.002 0.0023]	[0.9672 0.9619]	[0.07 0.073]	[0.0624 0.0534]	[0.0952 0.0915]
	13	[0.002 0.0023]	[0.9719 0.9673]	[0.08 0.0 83]	[0.0636 0.0556]	[0.0917 0.0883]
	10	[0.002 0.0023]	[0.9787 0.9753]	[0.09 0.093]	[0.0999 0.0902]	[0.1216 0.1149]
	13	[0.003 0.0033]	[0.9564 0.9516]	[0.07 0.073]	[0.0988 0.0868]	[0.1424 0.1352]
	12	[0.003 0.0033]	[0.9601 0.9557]	[0.08 0.083]	[0.0830 0.0734]	[0.1229 0.1177]
	11	[0.003 0.0033]	[0.9637 0.9597]	[0.09 0.093]	[0.0742 0.0662]	[0.1105 0.1065]
	10	[0.004 0.0043]	[0.9552 0.9516]	[0.09 0.093]	[0.0999 0.0902]	[0.1447 0.1386]

Table 7: Optimum parameter n for i ≠ j and minimum sum of risks $When\ \widetilde{\alpha_f} \cong 0.05\ and\ \widetilde{\beta_h} \cong 0.10$

i≠j	n	$\breve{p}_{1f}[\gamma = 0]$	$\mathcal{L}(\breve{p}_{1f})[\gamma = 0]$	$\breve{p}_{2h}[\gamma = 0]$	$\mathcal{L}(\breve{p}_{2h})[\gamma = 0]$	$\widetilde{\alpha_f} + \widetilde{\beta_h}$
i =1 j =2	38	[0.001 0.0013]	[0.9627 0.9518]	[0.05 0.053]	[0.0840 0.0698]	[0.1213 0.1180]
	35	[0.001 0.0013]	[0.9656 0.9555]	[0.06 0.063]	[0.0603 0.0506]	[0.0947 0.0951]
	25	[0.001 0.0013]	[0.9753 0.9680]	[0.07 0.073]	[0.1043 0.0924]	[0.1290 0.1244]
	23	[0.001 0.0013]	[0.9773 0.9706]	[0.08 0.083]	[0.0895 0.0799]	[0.1122 0.1093]
	20	[0.001 0.0013]	[0.9802 0.9743]	[0.09 0.093]	[0.0945 0.0855]	[0.1143 0.1112]
	22	[0.002 0.0023]	[0.9570 0.9507]	[0.08 0.083]	[0.1017 0.0913]	[0.1447 0.1406]
	22	[0.002 0.0023]	[0.9570 0.9507]	[0.09 0.093]	[0.0706 0.0631]	[0.1136 0.1124]
i =2 j =3	27	[0.001 0.0013]	[0. 9707 0.9611]	[0.05 0.053]	[0.0233 0.0175]	[0.0526 0.0564]
	25	[0.001 0.0013]	[0.9730 0.9642]	[0.06 0.063]	[0.0136 0.0103]	[0.0406 0.0461]
	23	[0.001 0.0013]	[0.9753 0.9673]	[0.07 0.073]	[0.0090 0.0069]	[0.0337 0.0396]
	15	[0.001 0.0013]	[0.9843 0.9793]	[0.08 0.083]	[0.0315 0.0215]	[0.0472 0.0422]
	10	[0.001 0.0013]	[0.9787 0.9865]	[0.09 0.093]	[0.0999 0.0902]	[0.1212 0.1037]
	19	[0.002 0.0023]	[0.9576 0.9506]	[0.05 0.053]	[0.0888 0.0735]	[0.1312 0.1229]
	17	[0.002 0.0023]	[0.9624 0.9563]	[0.06 0.063]	[0.0698 0.0587]	[0.1074 0.1024]
	15	[0.002 0.0023]	[0.9672 0.9619]	[0.07 0.073]	[0.0624 0.0534]	[0.0952 0.0915]

Flow chart for operating procedure of TSMCChSP-1





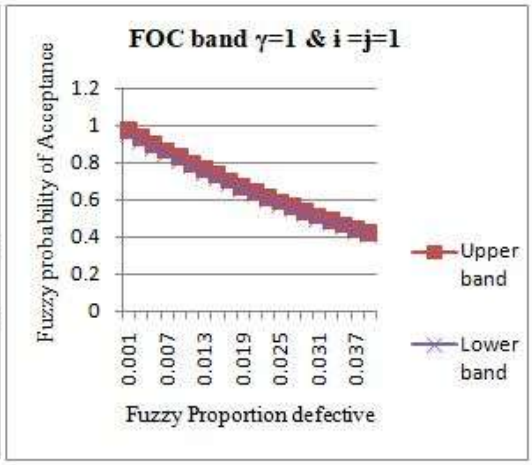
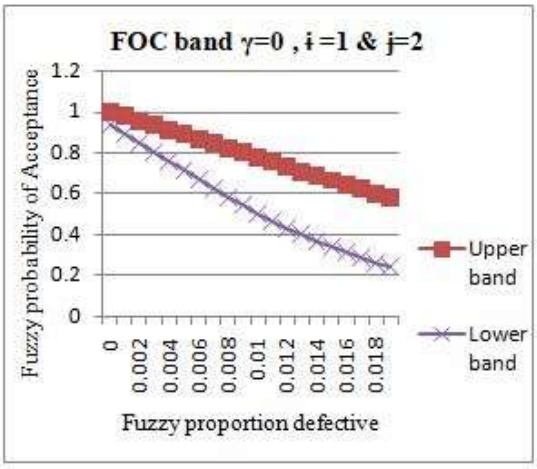


Figure 1 & Figure 2 Fuzzy Operating Characteristic curve for TSMCChSP-1 Plan



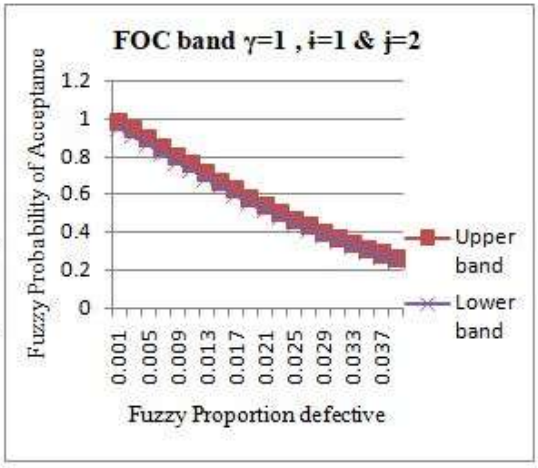


Figure 3 & Figure 4 Fuzzy Operating Characteristic curve for TSMCChSP-1 Plan

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RESEARCH ARTICLE

To Study of the Different Influences of Color on Aspects of Human Behavior

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ABSTRACT

The meaning of color varies depending on one's culture, race, gender and even age .So, it isn't just the selection of color in general but also which color to use with your target customers. For instance ,white is often associated with wedding in north America and evokes the feeling of innocence in eastern cultures, white also signifies death .An exporter of white wedding gown to china would go broke in no time .It seems hard to believe that colors can have an impact on our body and mind .However ,scientists know colors can influence our body's physiology and mental states .The science of color has been used by market researchers to determine how to apply this knowledge to influence human businesses ,psychology and health .The power of color stimulate our nerves system and evokes emotional states. The color of over environment travels through our eyes to our brain causing various hormonal releases. Artists and interior designers have long understood how colors can dramatically affect moods, feelings and emotions. It is a powerful communication tool and can be used to signal action, influence mood and cause physiological reactions. Certain colors can raise blood pressure, increase metabolism or cause eyestrain .Of course, your feeling about colors can also be deeply personal and are often rooted in your own experience or culture .Color is such a powerful force in our lives, our bodies and minds.

While perception of color are somewhat subjective ,these are some color effects that have universal meaning .Colors in the red area of the color spectrum are known as warm colors and include red ,Orange ,yellow .These color evoke emotions ranging from feelings of warmth and comfort to feelings of anger and hostility .Color on the blue side of the spectrum are known as cool colors and include blue ,purple and green. These colors are often described as calm, but can also call to mind feelings of sadness or indifference. The reason behind it is that the awareness of the consumer regarding this subject is less than the affect of astrological symbols or color choice on human behavior. The above facts depict the specialties of every color along with color choice. The textile designer, fashion designer, and architect

have constantly been working on these colors, the researchers have attempted to study he different aspects of human behavior and the affect of colors in astrology through their brief research work.

Key words: - color, human behavior, psychological aspects, color therapy, astrological symbol.

METHODOLOGY

Respondents Selection:
This study was conducted on Indore ,Jaipur ,and surat .These study sites are relatively more developed cities in the country .The cities are also thought to represent population of business class, services class and literate class, it also they include literate section of the society , All type of random selected samples are included .

Study design
A cross sectional, random sampling method were selected, interview and survey questionnaires are also used.

Sampling
Two different approaches, systematic and purposive sampling, were used to select the different working, none working respondent selected for the study.

Sample size
Based on the available local evidence we assumed that 87% respondents agree for color choice effect by symbolized astrology and their impact on human behaviors .This procedure involved a total sample size of 500 study subjects (working and non working).

Instrument
One questionnaire was used in three sections to detect the color impact on psychological behavior, business, success, luck and astrological symbological .In the first stage, the self reporting questionnaire for preference of color choice, belief in astrological symbols and color was depicted. In the second stage ,the diagnostic interview for responded was used for confirmatory diagnostic interviews on those screened effect of the astrologic symbols and color on different aspects of human behavior .In the section third that were meant to pick affect of certain color and human nature and habits .

Data collection
Data collection by using those questionnaires and interview method .Collect psychologist short guideline on how to administer the questionnaire was prepared and closely monitored day to day data collection activities and checks the completeness of the questionnaire in the different aspects of study.

Data processing
Data analysis used by the mean values, percentage, and deviation for continuous variables and proportion for categorical variables were used as descriptive summary measure and Result and discussion define by different tabulation. Astrological effects on the living aspects of human beings:- The first point to group is the distinction between color as transmitters of astrological influences ,and astrological factor as signification of color .These sound the same ,but they are not ,In the first case ,we have color used for its effect on man .Thus the color therapist interior designers and magicians all used red color to produce a sense of energy and worth .We all explore the color meaning of four different group of color .
Cool color meaning (calming) blue, green turquoise
Worm color meaning (exciting) red, pink, yellow

Mixed color –purple, lavender, green purple, turquoise
Neutral color meaning – (unifying) brown, beige, gray, black.

A total of 2000 respondents ,1000 service class and 1000 business class families were selected and the respondents aged 25-35 year were interviewed based on yes or no questions .Answer collected are related and they show their belief in astrological symbols and color effect . Data was shown in table no 1.

About 90% (1796) respondents believe in the effect of astrological symbols .The views of 114 respondents were based on yes or no. According to them, sometime there is effect of these symbols. 30 respondents said that they don't believe in such effects and they have not paid any attention on it. Astrological symbolic Preference based on: - Questionnaire of three questions birth date, birth name and common official name related to the preference of astrological symbols was put before the respondents and positive reply was found. .The collected data is presented in table no 2.

Table no-2 shows that 48% respondent prefers astrological symbols according to birthday and 26.28% prefer according to common official name and 25% prefer according to birth taba(Horoscope) name.
Relationship between color choice or Astrological symbols:- On the study of respondents it was observed that daily life of humans is affected by the choice of color and astrological symbols .The data related to it is mentioned .The affect of color choice can be seen in about 76% of people but it is reported to affect only their beauty ,cheerfulness mood ,like disliking and attractiveness .About 24%the respondents admitted that they themselves don't have enough knowledge of these astrological symbols. Therefore it is essential to perform research work on the topic on the basis of the obtained data .It is also necessary because the respondents show considerable curiosity about the knowledge of astrological symbols and also very less research work has been done in India.

Effects of different Colors in Astrology

Choice of color of a person reflects the status of his soul, body and mind. Many times we see that by seeing a particular color we feel very happy and sometimes we get very irritated. On the other hand we can see that a person can react negatively to the favorite color of other person. If we analyze it in depth we will find that a particular color has a particular wave length because of which our mind feels vibration and when this vibration synchronize with our mind we feel good and when it does not synchronize with our mind we feel irritation. Every color has a particular quality and is governed by a specific planet. As we use other remedies for a particular planet in the same way we can also use this color therapy for planet remedies. By use of this color therapy we can increase the effect of planets and by ignoring a particular color we can protect our self from the malefic influence of an evil or unfavorable planet.

Colors associated with planets are as follows –

RED COLOUR

Red can be used in treating irregularities in blood circulation, but it is not very conducive to people who suffer from mental maladies. If your natural choice is the red color, you are the outgoing type. You are not only impulsive, but also prone to abrupt mood swings. You have a lot of compassion for fellow human beings and can be easily persuaded. Though you have an optimistic approach to life, you don't hesitate to grumble and complain as well. Your spontaneous nature is assertive; you freely voice your opinions. You have a strong sex drive and are likely to end up having extra-marital affairs, unless your strong sense of duty restrains you from indulging in wild fantasies.

ORANGE COLOUR

Orange, being the color of many fruits and vegetables, stands for nourishment and also represents the attractive force between elements. The choice of this color shows that you are basically good-natured and loyal. You are sociable but at the same time you tend to be swayed by the opinions of other people. You are a generous soul, sincere at heart.

Your gestures are friendly, and inspired by goodwill. More often than not, you are overtaken by feelings of wanton elation.

YELLOW COLOUR

Yellow fruits and vegetables generally act as laxatives to the bowel and also soothe the nerves. If you have preference for yellow, it shows that you have a vivid imagination and lots of nervous energy. Your thoughts are clear and well organized. You do harbor a need to help the world, but you won't get the dirt under your fingernails doing that. Deep down you are a shy person and a loner; perhaps that's what makes you a reliable friend. You may not show it, but you would actually love to be respected and admired for your prudence.

GREEN COLOUR

While yellow-green stimulates generous feelings, spring green heralds new life. Preference for the color green shows that you are a dutiful citizen. You are not only aware but also sensitive to social customs, and bear a good name in your community. Your choice also indicates your honesty and straightforwardness. You have a normal sex drive and are very emotionally attached to your family. You have the potential to be an excellent teacher.

BLUE COLOUR

Too much of blue on the other hand could be a depressing influence. If your personal color is blue, you are introspective and purposeful by nature. You hold conservative beliefs and under stressful conditions, prefer to withdraw into gentler surroundings. You seem to have a lot of control over your passions and desires, but are sensitive to the needs and feelings of others, nonetheless. You are a loyal friend and would prefer to lead a sober life.

PURPLE COLOUR

If your choice is purple, then you are intelligent and quick-witted. You have a keen eye for detail, things which are normally overlooked by people. You are infuriated at the slightest provocation. You tend to be extremely effusive in your expressions of grief. You are a creative person and an egotist of sorts. You seem to possess a cultivated taste for the subtle in life, while recognizing the magnificent.

BROWN COLOUR

Interestingly, this dull shade is also thought of as representing the plane from which beauty is born, and thus serves as an excellent backdrop for art objects. A personal choice of the color brown implies that you are meticulous when it comes to work, thrifty when it comes to money, and adamant in your beliefs. You are a very reliable and composed person, not impulsive at all. You can rival a seasoned horse trader in your talent for striking the best bargain. Practical consideration are also involved ,blue was listed infrequently ,since good blue dye was expensive ,and the colors also had to reflect the fashion of the times .It is hardly surprising that such lists show variations they need to be worked out afresh for every generation and nation modern astrologers ,such as spherical continue to devised them ,usually in connection with attempts to predict the winning owners color in a horse race .
Colors associated with planets are as follows –

Color used according to Astrological symbol to improve our luck and success:- Randomly selected respondents were asked to note down the uses of color related accessories ,their effect on their luck and success and the main events of life according to their chosen color. When they were asked to recall particular colored dress or the uses of color of any other objects or their particular passed time, it was observed that there is a similarity between the astrological symbols of respondents and the remembered events. Wit. Johnson in his research study has also proved that the astrological symbols according to the color choice are responsible for a good, mood, emotional stability and it also has a positive effect on their confidence and will power. The presentation of a person is rendered effective due to the positive reaction of all these factors. Due to which we get maximum success and their luck remains in favor. A chart is provided by wit Johnson on horoscope color for luck and successes which the researcher has coded. Good luck

usually follows when we align with our true selves, so wearing or surrounding ourselves with the colors that are best for our sun sign can really help!

ARIES: March 21-April 19: Think of fiery hues–red, hot pink, yellow.

TAURUS: April 20-May 21: Think of a garden in early summer–soft greens, rose-pinks, pale turquoise.

GEMIN: May 22-June 20: Think of inspiring early-spring airiness–white, silver, yellow, spring green, pale gray.

CANCER: June 21-July 22: Think of moonlit seascapes–pale blue, silver, pearl, glistening white, emerald green.

LEO: July 23- Aug 22: Think of sunny and positive tones–gold, yellow, orange, vermilion, copper, blood red.

VIRGO: Aug 23-Sept 22: Think of colors of nature in summer and early Fall–pastel shades of blue, gold, peach, yellow, and amethyst; jade green, autumn hues.

LIBRA: Sept 23-Oct 22: Think of goddess-like dignity–royal blue, cerulean blue, rose-pink, amethyst, violet.

SCORPIO: Oct 23-Nov 21: Think of mysterious and occult shades–deep, dark shades of red, russet-brown, shadow-black, stone-gray.

SAGITTARIUS: Nov 22-Dec 21: Think of stained-glass colors–lilac, mauve, purple, amethyst, violet, indigo, vermilion, midnight blue.

CAPRICORN: Dec 22-Jan 19: Think of neutral and earthy shades–black, gray, violet, dark brown, earth colors.

AQUARIUS: Jan 20-Feb 18: Think of neon rainbow colors–electric and ultramarine blue, electric green, deep violet.

PISCES : Feb 19-March 20: Think of marine tones–purple, violet, amethyst, sea-green, turquoise.

Color effect on mood: - Claire named (inner tradition, 1994) color can have a significant effect on our mood and behavior, and on the ways in which other perceive us. When our color choice was aligned with a deeper. ,more authentic self rather than with what is merely fashionable ,we radiate an aura of confidence grounded in who we are .find out which colors are in tune with your sun sign so you can truly dress for success ,or decorate your home to reflect more of your deeper self . The assignments are presumably based on the impressions which the items would make and consequently there are many overlaps, the choice after depending on shade and texture. Marsilio also suggested that rich shades of purple are associated with Jupiter and the sun with Venus and the moon.

Color effect on business: - As a small business owner, you have a meaningful understanding of the color of money but what about the color of your business? Learn whether the colors of your business puts your customers in a buying mood or creates the wrong perception of your company .The science of color has been used by market researcher to determine how best to apply this knowledge to influence customers perceptions of businesses. It seems hard to believe that colors can have an impact on our body and mind. However scientists know colors can influence our body's physiology and mental states. The powers of color stimulate our nervous system and evoke emotional states. The color of our environment travels through our eyes to brain causing various hormonal releases.

Consider the meaning of the following color on your business marketing:

Consider the meaning of the following color on your business marketing. Several large brand name companies are associated with their corporate colors. IBM- Big Blue signifies stability and conservatism. UPS- Brown symbolizes

longevity and reliability. A color can be connected to a product like Tide; in the bold orange box, evoking the feeling of vibrancy.

White: Pure, Clean, Youthful, It's a neutral color that can imply purity in fashion and sterilization in the medical profession.

Black: Power, Elegant, Secretive, The color black can target your high-end market or be used in youth marketing to add mystery to your image.

Red: While red generally signifies everything from love to anger, from power to danger. Red is the color of attention, Passion. Excitement causing the blood pressure and heart rate to rise. Use red to inject excitement into your brand.

Orange: Vibrant, Energy, Play, Add some fun to your company if you want to create a playful environment for your customers.

Yellow: Happy, Warm, Alert, Yellow can be an attractor for your business with a relaxed feeling.

Green: Natural, Healthy, Plentiful, to create a calming effect or growth image choose green. Go green go.

Purple: Royalty, Wise, Celebration, Maybe add some purple tones to your look for your premium service business.

Blue: Loyal, Peaceful, and Trustworthy. Blue is the most popular and neutral color on a global scale. A safe choice for a business building customer loyalty.
Turquoise Colors- A mix of blue and green, turquoise has a sweet feminine feel while the darker teal shades add lively sophistication

Color choice and personality:-A study of the different behavioral aspects of about 500 respondents was conducted The data was collected on the different aspects such as behavioral habits ,emotions, psychology ,way of thinking , locking ,living standard ,social relationship, habits ,like-dislike, on comparative study of the collected data and their color choice .It was observed that there were certain similarities in the color choice and behavior of about 86.7%respondents .During this study ,the most common color and their closely related shades have a deadening affect on the behavioral characteristics as is mentioned in the table shown:

The behavioral properties of maximum respondents preferring the above colors is shown in the table .Through these results the analysis of the properties of particular color was done which is made by color psychologist and it also matches with it about 98%.Therefor it is proved that there is a relation between color choice and behavioral quality .It can also be emphasized that the affect of company is always there.

FINDINGS

1. In table no 1 it was found that 90% people believe in the different effects of astrological symbols.
2. In table no 2 it was found that about 48 % people believe that the birthdates are according to the astrological symbols.
3. Horoscope is prepared according to birthdates & the stars mentioned in the horoscopes are of peculiar colors which affects our luck and success.
4. The affects of particular color choice can be clearly seen in the different aspects of our behavior.

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Table 1. Astrological symbols and color effect

Yes	no	I don't know
1796(90%)	114(8.3%)	30(1.5%)

Table 2. Questionnaire

Taba name	Birth date	Common official name
466(25%)	858(47.7%)	472(26.28%)

Table 3. Colors associated with planets

S. No	Stars	Color
1	Moon	Blue/ Pale white cream
2	Mercury	Blue, Green
3	Venus	Light blue/Green/pink
4	Sun	Yellow, Orange
5	Mars	Red
6	Jupiter	Green/red
7	Saturn	Black/Dark blue

Table 4. Turquoise Colors

S.No	Colors	Symbolized	Characteristics
1	Black	Night, death, magic	Formal, conventional, dignified
2	White	Purity, femininity	Precise, critical, sincere
3	Red	Masculinity, life, warmth, danger	Active daring, passionate, optimistic, comfort, healing
4	Orange	More spiritual	Produce detachment, reduce depression,
5	Yellow	Intellectual, communicative, sum	Reduce stress, sympathetic, steadfast, restrained, balances in possessiveness.
6	Green	Life, growth, rebirth	Steadfast, restrained, balances in possessiveness, reduce stress, sympathetic
7	Cyan turquoise	Life, growth, rebirth, spiritual	Charming, self observed, self confidence, calms, refreshes.

8	Blue	More spiritual (Light), more sociable (dark)	Idealistic, rational, honest, tranquil
9	Purple, violet	Dignity, dermatologist	Grand, idealistic, sensitive, lack self –criticism, maturity, disturbing
10	Brown	Boldness, rough	Practical, earthy, obstinate, conscientious
11	Grey	Nobler, more spiritual	Calming, convey, lack of commitment.

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RESEARCH ARTICLE

Study of Various Rare Earth Doped $SrBi_2Ta_2O_9$ Ceramic for Device Applications

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ABSTRACT

Aurivillius Structured materials, also known as bismuth layer structured ferroelectrics (BLSFs), have recently gained a lot of attention due to its potential use in technical devices like NVRAM (FE-RAM). $SrBi_2Ta_2O_9$ (SBT) has gotten a lot of attention in the scientific community because of its fatigue-free features, which make it a good candidate for use in the FE-RAM. It has excellent fatigue resistance as well as a low switching voltage. In these bismuth-layered structures, a variety of investigations on the influence of rare-earth (RE) ion substitution have been carried out. The Curie temperature was lowered in the majority of the RE doped SBT. The transition temperature, commonly known as the Curie temperature (T_C), is the temperature at which ceramics change from ferroelectric to paraelectric. The ferroelectric, dielectric, piezoelectric, optical, and photocatalytic characteristics of SBT ceramics can also be improved by substituting rare earths. The properties of Y-doped SBT, Sm-doped SBT, Ce-doped SBT, and Eu-doped SBT are described in this paper.

Keywords: Dielectric parameters; Transition temperatures, Bismuth layered structure, Rare earths

INTRODUCTION

Memory devices (nonvolatile), high-power electronic transducers, Energy harvesting, and other applications utilize ferroelectric materials [1]. Industrially produced ferroelectric materials, on the other hand, contain toxic materials like lead, which is poisonous and harmful to the environment [2]. A number of ferroelectric systems are being investigated as possible replacements for $PbZr_xTi_{(1-x)}O_3$ and PZT. Perovskites materials with Bismuth layer-structured have been a major topic of research in recent years as an alternate material for various ferroelectric

devices. $SrBi_2Ta_2O_9$ (SBT) is a bi-layered pseudo perovskite oxide that shows ferroelectric activity at room temperature and belongs to the Aurivillius family of bi-layered pseudo perovskite oxides. In the previous few years, hundreds of research articles have been published on ferroelectricity in SBT, which was discovered in the early 1960s [3]. Rae and colleagues [4] were the first to look into its crystal structure.

The primitive cell has 28 atoms and is orthorhombic (space group $A2_1am$) at ambient temperature. The standard unit cell's lattice parameters are a = 5.531□, b = 5.534□, and c = 24.984□. It is found that, the perovskite-type $[SrTa_2O_7]^{2-}$-unit and $[Bi_2O_2]^{2+}$ layer are stacked alternately with double TaO_6 octahedral layers along the pseudo tetragonal c-axis [5, 6]. The Bi_2O_2 layers and TaO_6 octahedral layer are significantly deformed, and spontaneous polarization occurs as a result of atomic displacements along the a-axis [7]. As a result, it is envisaged that its electrical characteristics will be very anisotropic. SBT has excellent polarization fatigue-free performance, a low tendency to imprint, and a low leakage current, all of which contribute to its usefulness in memory devices. This material may also be deposited in exceedingly thin films without losing any of its bulk properties [8]. SBT is used commercially for nonvolatile memory applications due to its advantageous features (ferroelectric random access memories). SBT single crystals, ceramics, and thin films have all received a lot of attention [9–13]. SBT, on the other hand, has a serious difficulty with small 2Pr of around 14 $\mu C/cm^2$ [14]. The composition of SBT has been altered in order to improve polarization properties, primarily for a bigger 2Pr [15-17]. Without affecting the fundamental properties of the $[(ATa_2O_7)^{2-}$ $(Bi_2O_2)^{2+}]$ structure, the divalent Sr cation positioned between the corner-sharing TaO_6 octahedra can be completely or partially substituted by other cations, most often Ca, La, or Ba. In these bismuth-layered structures, a number of investigations on the influence of rare-earth (RE) ion substitution have been done [21-23]. These findings imply that defect-related alteration associated with Sr-ion substitution by smaller rare-earth ions (such as La, Nd, Pr, and Sm, among others) improves ferroelectric characteristics. The goal of this review is to look at the features of rare earth that could help improve the many useful qualities of SBT and to see how doping affects them.

Eu-doped SBT

Coondoo et al. [24] investigated europium substituted samples of $Sr_{(1-x)}Eu_xBi_2Ta_2O_9$ produced by solid-state reaction technique in 2009. The average grain size grows as the Eu content rises, according to microstructural investigations. With increasing europium concentrations, an increase in remnant polarization and d_{33} values has been seen. In the sample with x= 0.20, the maximum 2Pr~ 14 $\mu C/cm^2$ is observed. This could be due to donor doping, which introduces cation vacancies into the lattice structure. In comparison to the pure sample, which had a residual polarization of ~5 $\mu C/cm^2$, Eu doping resulted in an increase in remnant polarization. For reliable FRAM devices, materials with high Pr values but poor conductivity can be advantageous. The ceramic's piezoelectric coefficient d_{33} improves with Eu doping, reaching a peak of (20 pC/N) for the sample with x= 0.20, compared to 13 pC/N for the undoped-sample.

Later in 2019, Zhonga et al. [25] reported the solid-state synthesis of a new Eu^{3+}-doped $SrBi_2Ta_2O_9$ red-emitting phosphor. The properties of $SrBi_2Ta_2O_9$'s luminescence have been studied in depth. The phosphor may emit intense red light at 615 nm under stimulation of = 395 nm and = 465 nm due to the $^5D0 \rightarrow {}^7F2$ transition. The thermal stability and appropriate doping concentration are examined. The Commission International de L'Eclairage (CIE) coordinates of the red phosphor $SrBi_2Ta_2O_9$:$0.10Eu^{3+}$ are significantly closer to the standard of the National Television System Committee than published commercial $Y_2O_2S:Eu^{3+}$, $Y_2O_3:Eu^{3+}$ (NTSC). The colour purity value is 97 percent. $SrBi_2Ta_2O_9:Eu^{3+}$ has its luminescence lifetime measured. Furthermore, InGaN can be used to create white light emitting diodes (w-LEDs). The combined w-chromaticity LED's coordinates are (0.327, 0.350), which is close to the equal energy point (0.333, 0.333). In addition, an excellent CCT of 5242 K and a decent Ra of 80 were obtained. These findings show that the phosphor $SrBi_2Ta_2O_9:Eu^{3+}$ can be employed in w-LEDs.

Ce-doped SBT

V. Senthil et al[26] investigated cerium doped bismuth layered ferroelectric $SrBi_2Ta_2O_9$ (SBT) with the general formula $SrBi_{(2-x)}Ce_{3x/4}Ta_2O_9$ (x=0, 0.025, 0.05, 0.075 and 0.1). The mixed oxide method is used to make these ceramics. The electrical characteristics of SBT ceramic as a function of cerium content are investigated. With a rise in Ce

concentration, the temperature dependent dielectric analysis demonstrates a diffuse phase transition with a linear drop in transition temperature and dielectric constant. With an increase in the doping concentration of Ce content results in a rise in dielectric diffuseness. With a rise in Ce concentration, the ferroelectric property is examined using a P–E hysteresis loop, which indicates a drop in coercive field and an increase in remnant polarisation.

This group discussed the photocatalytic activity of these compounds later in 2016 [27]. The bandgap of Ce doped SBT ferroelectrics was calculated using the diffusive reflectance spectrum, and the values were raised by increasing the substitution concentration. The photocatalysts' conduction band is made up of Ta5d Bi6p(Ce4f)O2p orbitals, whereas the valence bands are made up of Ta5d Bi6s(Ce4d) O2p orbitals. Under ultraviolet irradiation, the water splitting photocatalytic activity of these photocatalysts was examined, and it was discovered that they have photocatalytic activity for H_2 and O_2 evolution from aqueous solutions containing sacrificial reagents. The photocatalytic activity samples demonstrated that ferroelectric dipole moments boost photo separated electrons and holes spontaneously, improving the photocatalytic activity of SBCT ferroelectric ceramics. As a result, the cerium modified multilayer perovskite Aurivillius compound has been discovered to be new water splitting photocatalysts for the formation of H_2/O_2.

Sm-doped SBT

Zhonga et al. [28] recently disclosed a new orange-red $SrBi_2Ta_2O_9:Sm^{3+}$ phosphor produced in an air atmosphere by a high-temperature solid-state method. In the photoluminescence (PL) spectrum, the phosphor has a high excitation peak at 403 nm and produces vivid orange-red light corresponding to wavelength at 564, 600, 647 and 701 nm. Due to the $4G_{5/2} \rightarrow 6\ H_{7/2}$ transition of Sm^{3+}, the intense peak is found at 600 nm. Sm^{3+}'s critical transfer distance is estimated to be 26.31Å. The ideal concentration for doping has been calculated to be around 2 mol%. The thermal stability of the temperature-dependent emission spectra is outstanding. The $SrBi_2Ta_2O_9:xSm^{3+}$ (0.005 x 0.30) coordinates of the Commission International de L'Eclairage (CIE) are in the reddish orange zone. These findings suggest that the $SrBi_2Ta_2O_9:Sm^{3+}$ phosphoremitting orange-red light could be employed as a red phosphor alternative in white light emitting diodes (w-LEDs). The above findings suggest that $SrBi_2Ta_2O_9:Sm^{3+}$ is a viable red phosphor for w-LED applications.

Y doped SBT

Bismuth-layered compounds$Sr_{1-x}Y_{2x/3}Bi_2Ta_2O_9$ prepared by conventional solid state route with compositions x = 0, 0.05, 0.075, and 0.1 have also been reported [29]. By X-ray diffraction, the lesser size of ionic radii of Y^{3+} in Y doped SBT decrease the lattice parameters and enhance the orthorhombic distortion (b/a) value, confirming that there is mismatch in the lattice in perovskite structures. The transition from ferroelectric to paraelectricoccurs at temperature more than 270 ^{0}C in a temperature-dependent dielectric research, and donor replacement reduces dielectric loss. The expression for switching dipoles polarisation (P) dependency of the electric field (E) is used to fit the P-E loop using a ferroelectric capacitor model, and it yields the best agreement of the theoretical data with the experimental. In the presence of sacrificial reagents, the water splitting photocatalysis of $Sr_{1-x}Y_{2x/3}Bi_2Ta_2O_9$ ferroelectrics for H_2/O_2 evolution from water is also investigated. In the presence of ultraviolet irradiation (350WHg lamp), the greatest rate of H_2/O_2 evolution obtained for these Y doped ferroelectric materials is around 1.7 times that of pure $SrBi_2Ta_2O_9$. These materials have the potential to make significant changes in water splitting for production of hydrogen. The rotation of the octahedron is enhanced by a-site vacancy, and defect charge neutrality is responsible for better ferroelectric properties.

CONCLUSION

It was discovered that by doping RE into SBT ceramic, the dielectric behaviour can be improved by lowering the transition temperature. This has a significant impact on ferroelectric behaviour (increased residual polarisation and loe Ec). By substituting rare earth ions, photocatalytic and optical characteristics are also generated.

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RESEARCH ARTICLE

Biosynthesis and Physicochemical Characterisation of Zinc Oxide Nanoparticles

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ABSTRACT

The biosynthesis of clean, non-toxic, and environmentally friendly metal nanoparticles is a highly recommended method. Determining nanoparticles synthesis and physicochemical properties and exploring their structure-function relationships is a critical challenge for scientists today. In this, we attempt to synthesize a novel Zinc oxide nanoparticle coated with Ellagic acid (EA-ZnONPs) obtained from Pomegranate using a simple method and explore its characterization accurately. This in-vitro study assessed the optical and physicochemical description of Ellagic acid coupled Zinc oxide nanoparticles (EA-ZnONPs) using various standardized analytical techniques. The peak at 367.85 nm (0.291 AU) in EA has a shift in the peak observed for Zinc oxide nanoparticles at 357.95 nm (0.606 AU), which indicates the participation of EA in the synthesis of Zinc oxide nanoparticles using UV-Visible Spectrometer analysis. Dynamic Light Scattering (DLS) analysis revealed that EA-ZnONPs were in nanosize (123.8 nm) with less polydispersity index (0.288). The Zeta potential confirmed that this newly synthesized nanoparticle was negatively charged (-0.279). Scanning Electron microscope determination confirmed the spherical-shaped morphology of this nanoparticle ranging from 72.89nm to 114.4 nm in diameter. The FTIR spectra revealed EA-ZnONPs exhibited different functional groups, indicating their functionality in biological environments.

Keywords: Capping agents; Ellagic acid; Reducing agents; Nanoparticles; Zinc oxide.

INTRODUCTION

Nanobiotechnology aims to develop novel biosynthetic and eco-friendly nanomaterials that find applications in various domains. Nanomedicine attempts to solve the puzzle of numerous known human disease problems at the nanoscale level. Among the many nanoparticles being explored and identified, Zinc oxide nanoparticles (ZnONPs) are promising due to their non-toxic and biocompatible nature. Hence it finds applications as a drug carrier, cosmetics, and filling material in dentistry (1). Production of ZnONPs at a large scale used many chemical and physical methods, necessitating a large sum of manpower, money, and material. In addition, waste management of these processing methods was cumbersome (2). To overcome the issues, biosynthesis of nanoparticles was attempted by researchers using plant resources. Evidence suggested that plants of various oxygen were eligible candidates for enormous production of metal nanoparticles in a faster phase in an environmentally safe mode (3). Many plants products are being explored for their nanoscience application. One such plant is Pomegranate.(4) It belongs to the pumiciace family, which grows up to 6-10m in height that can be grown (5) Native of Iran, this magical herb is harvested worldwide, with the annual production accounting for 20 lakh tonnes (6). Ellagic acid is a natural phytochemical that is one of the various key constituents of Pomegranate. This compound is reported to have multiple medicinal characteristics in medicine ranging from antimicrobial properties to anticarcinogenic effects. Though many clinical studies have been conducted, little is known about ellagic acid's physiological, genetic, and ecological aspects (7). To our knowledge, none of the studies have synthesized ZnONPs using ellagic acid as a capping agent. Determining the physicochemical and characterization is a critical factor in any new nanomaterial identified. The structure-function relationship is essential to provide the comprehensive origin of nanoparticle function and behavior from the laboratory into real-world applications. Hence the present study aimed to evaluate nanostructural characterization of ellagic acid-coated zinc-oxide using various physiochemical analytical techniques.

MATERIALS AND METHODS

All chemicals were used in this in-vitro study procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. The Ellagic acid (EA) has been obtained from St. Louis, MA, USA. Analyses, qualitative and quantitative measurements involved the use of the following tools and systems: UV-Visible Spectrometer (Lambda 365, PERKIN ELMER, USA), Particle Size Analysers (Nano Plus – Micro metrics, USA), Scanning Electron Microscope (EVO-18, CarelZeiss, USA), Fourier Transform Infra-Red Spectrometers (Spectrum Two, PerkinElmer, USA).

Synthesis of Nanoparticles

Synthesis of Zinc oxide nanoparticles carried out using Ellagic acid and 1 mM Zinc acetate solution in double distilled water. Zinc acetate and Ellagic acid solution were mixed together in a ratios of 5:5, 6:4, 7:3, 8:2 and 9:1. The reaction mixture was heated below the boiling point and continuously stirred at 800 rpm using magnetic stirrer. The mixture turned yellowish in color within 1 h. The whole reaction was carried out in the dark. The obtained suspension was centrifuged at 15,000 rpm for 15 min. The pellet containing Zinc oxide nanoparticles was washed 3–4 times with deionized water to remove impurities. The precipitated Zinc oxide nanoparticles were lyophilized. Lyophilized nanoparticles were stored in a cool, dry, and dark place and further their characterization was carried out (8). The prepared zinc oxide nanoparticles were tested for the following their optical and nanostructural properties (i) first to analyze the size of the Ellagic acid using UV-Visible Spectrometer (ii) Secondly to determine the hydrodynamic size and its dispersity using Dynamic light scattering and Zeta potential (iii) thirdly to quantify the average size of EA-ZnONPs by using SEM (iv) lastly to identify the functional groups by using FTIR method.

RESULTS

The absorption spectrum of EA shows three corresponding peaks at 252.60 nm (0.443 AU), 367.85 nm (0.291 AU), and 397.55 nm (0.285AU) shown in figure 1. The maximum absorption peak of ellagic acid shifted from Zinc oxide nanoparticles exhibited a gradual decrease in absorbance, accompanied by a shift in the wavelength from 430 nm to

357.95 nm (0.606 AU). The peak at 367.85 nm (0.291 AU) in EA has a shift in the peak observed for Zinc oxide nanoparticles at 357.95 nm (0.606 AU), which indicates the participation of EA in the synthesis of Zinc oxide nanoparticles shown in figure 2. The UV–Vis spectrum shows the role of Zinc acetate and the presence of active atoms in EA for forming zinc oxide nanoparticles by the appearance of a yellow color spectrum. At 5:5 concentration of *EA,* the energetic particles present in EA are responsible for fabricating Zinc oxide nanoparticles (EA-ZnONPs).

Dynamic light scattering (DLS-Particle Size Analyzer) measurements were used to determine the synthesized nanoparticles' size. The pattern obtained was displayed in figure-3. The average length of the Zinc oxide nanoparticles and the statistical distribution of the measure were found to be 123.8nm with a polydispersity index of 0.288. Further Zeta potential obtained for these novel NPs is -0.279. On observing Scanning electron micrographs, the size of EA-ZnONPS in varied sizes from 72.89nm to 114.4 nm was observed with surface texture suggesting spherical shape (Figure 4). Fourier Transform Infrared Spectroscopy is a technique used to identify the functional groups involved in the stabilization of synthesized nanoparticles. The EA, EA-ZnONPs were analyzed using FTIR spectra of EA and EA-ZnO. For EA, the band was observed at 3557.13 cm^{-1} shown in figure 5. There is a decrease in the O-H stretching frequency from 3600.00 cm^{-1} to 3557.13 cm^{-1}. The broadening of the same band indicates the intermolecular hydrogen bond between the oxygen and hydrogen present in EA and water molecules. The bands at 1695.43 and 1111.61 cm^{-1} are due to C=O stretching and aromatic C= O trying of >C=O carbonyl group. The band observed at 1617.76 cm^{-1} is due to aromatic C=C stretching. The narrow bands obtained at 1396.63 and 1337.54 cm^{-1} are due to –C- H bending. A band at 1056.39 cm^{-1} is may be due to the C-O stretching of primary alcohol. The bands observed at 882.07 and 811.45 cm^{-1} are due to ring oxygen present in the aromatic ring. 923.52 cm^{-1} band is due to aromatic C-C stretching. The intense band appeared at 3070.55 cm^{-1} are due to aromatic C-H stretching.

For Zinc oxide nanoparticles, the band was observed at 3556.92 $cm^{-1,}$ shown in figure 6. There is a decrease in the O-H stretching frequency from 3600.00 cm^{-1} to 3556.92 $cm^{-1,}$ and the broadening of the same band indicates the presence of an intermolecular hydrogen bond. The band at 1699.47 cm^{-1} is due to the C=O stretching of >C=O carbonyl group. The band observed at 1619.07 cm^{-1} is due to aromatic C=C stretching. The narrow bands obtained at 1397.07 and 1340.96cm^{-1} are due to –C- H bending. A band at 1058.03 cm^{-1} is may be due to the C-O stretching of primary alcohol. The band observed at 882.03 cm^{-1} is due to ring oxygen present in the aromatic ring. There is a disappearance of a band for aromatic C-C stretching. The intense band appeared at 3082.57 cm^{-1} is due to aromatic C-H stretching. 923.74 cm^{-1} band is due to aromatic C-C stretching. The corresponding band for Zinc oxide is observed at 578.62 cm^{-1}.

DISCUSSION

Inorganic nanoparticles, in particular, Zinc Oxide, have a promising role with multifaceted advantage in various domains of nanomedicine such as imaging, diagnostics, drug delivery, and anti-cancer therapies.(9,10). The recent era encourages core competencies among material scientists to develop environmentally friendly techniques for synthesizing nanoparticles. In the current scenario, many methods of producing ZnO for marketing are being carried out.(11,12). The most common methods employed are physical and chemical methods. Each method has its other side of the coin, with physical methods requiring the high expenditure of energy and chemical processes releasing toxic gases into the atmosphere (13). We employed naturally occurring Ellagic acid as a capping agent to produce Zinc oxide nanoparticles in an environmentally friendly manner. Evidence revealed properties of any new zinc oxide nanoparticles are different from their elemental Zinc characteristics, necessitating understanding the characterization of any novel ZnO nanoparticle identified about their size and morphology. Hence, the present work attempted to analyze the biological components responsible for the stability and functionality of this novel ZnO capped with ellagic acid. Almost the entire property of the nanoparticle is determined by its size. Hence exploring the scope of this novel nanoparticle synthesized is highly recommended. UV- spectroscopy is a widely accepted technique to assess the size of the nanoparticle. The shift in the maximum absorbance spectrum of Ellagic acid zinc oxide nanoparticles in this study showed a sharp drop in wavelength compared to its pure ellagic acid counterpart.Hence this indicates particle size of ZnO EA NPs decreased as the absorption peak moved towards blue shift. This shows

the incorporation of both the raw material used at the nanoscale level.

A sharp absorption of ZnO observed in Figure 2 indicated the monodispersed phase of ellagic acid due to its surface plasmon resonance, which confirmed ZnO NPs.(14). Further, this study's absorbance peak correlated with the results of ZnO NPs synthesized using A. hydrophila and O. europea, as reported byJayaseelan et al. (15). Similarly, another study was done by Zhang et al. (16) on aliphatic alcohol extract corroborated with the findings reported by our work. EA- ZnO.NPS exhibited a characteristic broad absorption peak between 330–460 nm(17), as found in the study done by Sangeetha et al., The UV region's excellent absorption of the ZnO-NPs implies its applicability in medical applications such as sunscreen protectors or antiseptic ointments.(18) The bandgap and catalytic activity of metal oxide nanoparticles were reported to play a crucial role in their cytotoxic response to biological systems (19). Dynamic light scattering was carried out to assess the hydrodynamic behavior of these novel SNPs. This will indirectly give a clue of its behavior in biological fluid. We found that lesser particle size was reported by Jan et al. (131nm)(20) and Modena et al(21). The negative zeta potential further confirmed the controlled release of this new nanoparticle, qualifying its use in in vivo settings. we have more negative binding affinity compared to a study done by Jan et al .(20). In this study, SEM analysis (figure 4) revealed the evenly dispersed spherical nanoparticles of different sizes in the order of 72.89nm to 114.4 nm. Nagarajan et al. conducted a study that showed that typical SEM micrographs image of the ZnO nanoparticles size 96-110 nm obtained by the biosynthesis method.(17)Another study done by Mahamuni et al(22) showed that the average particle size of ZnONPs in SEM analysis was in the range of 15 to 100 nm. Within the study's limits, the smallest particles of metal oxide nanoparticles obtained with ellagic acid capping make it a superior candidate for antimicrobial activity in biological environments. However, further in vivo studies are needed to substantiate this. FTIR is a reliable technique for identifying various chemical groups that may have contributed to the potentiation of ZnO- NPs due to the presence of metabolites bound to the ZnO-NP, such as polyphenols flavonoids, alkaloids, and carboxylic acids. The study was correlated with broadband observed at 3100 cm^{-1} corresponds to the O–H stretching mode of hydroxyl groups as reported by Vijayalakshmi et al.(23). FTIR spectra confirmed the successful capping of biomolecules on the NP surface, thus providing stability and dispersion capacity as needed in biological fluids to ZnO-NPs.

CONCLUSION

Ellagic acid coated ZnO nanoparticles have been prepared using a simple method and were characterized by UV-vis absorption, DLS with Zeta potential, SEM, FTIR. All analytical studies confirmed the highly desirable nano-characterization with spherical particles with controlled release in an aqueous medium, making it a perfect piece of the puzzle to be experimented with and implied for natural settings in various fields.

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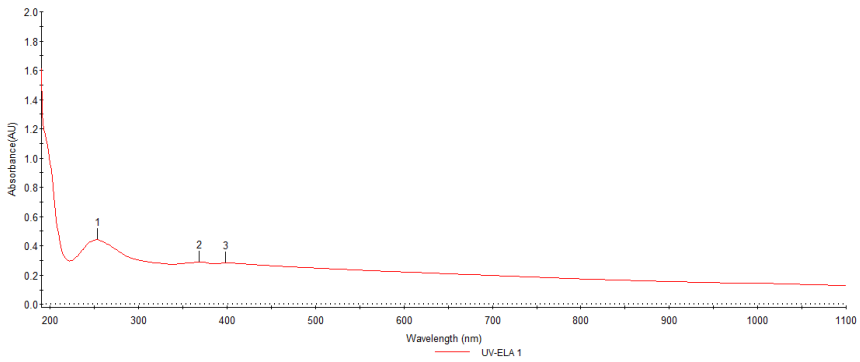

Figure1: UV–vis spectra Ellagic acid (EA)

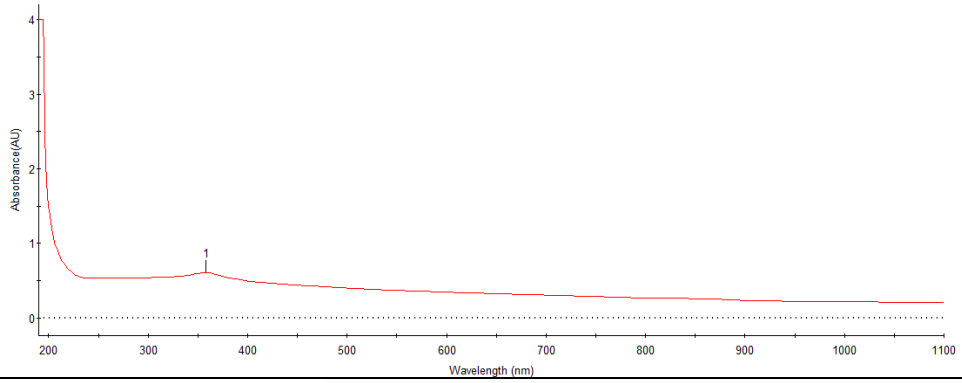

Figure 2: UV–vis spectra of Zincoxide nanoparticles synthesised from Ellagic acid (EA-ZnONPs)

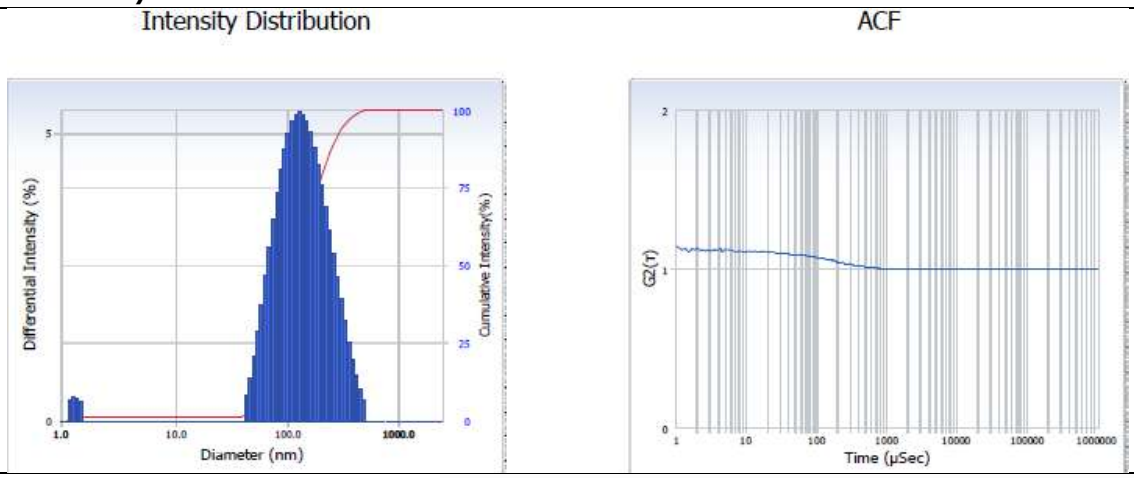

Figure 3: Dynamic Light Scaterring of Zincoxide nanoparticles synthesised from Ellagic acid (EA-ZnONPs)

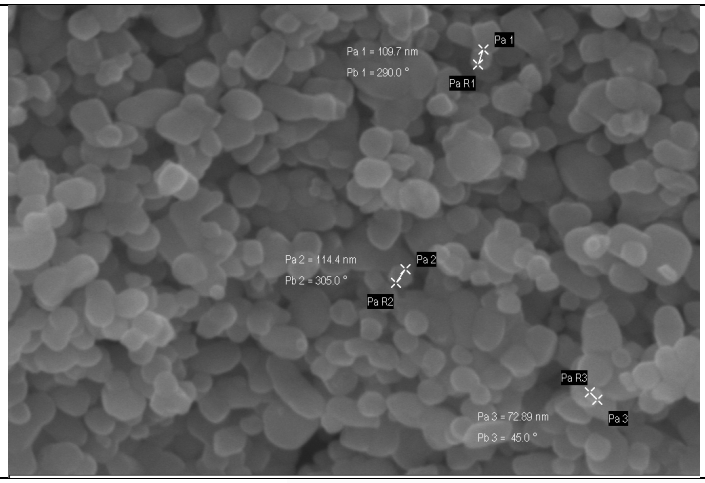

Figure 4: Scanning Electron Microscope of ZnO nanoparticles synthesised from Ellagic acid (EA-ZnONPs)

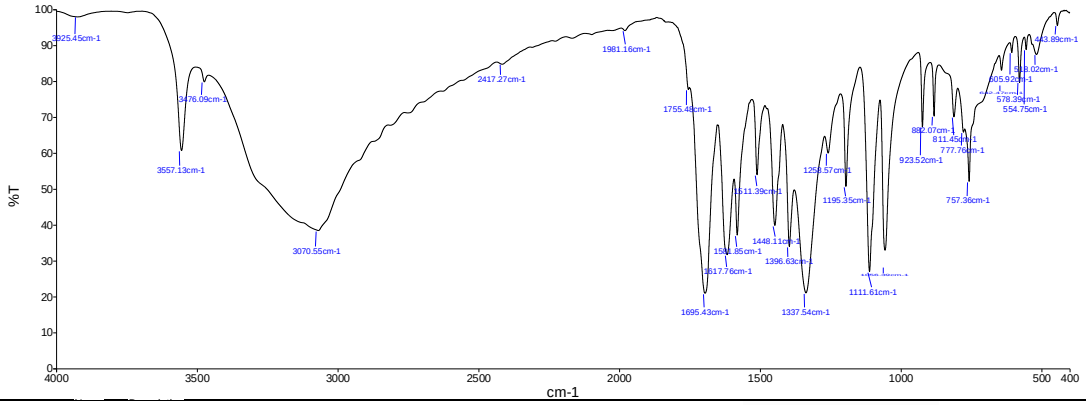

Figure 5: Fourier Transform infrared Spectroscopy Ellagic acid (EA)

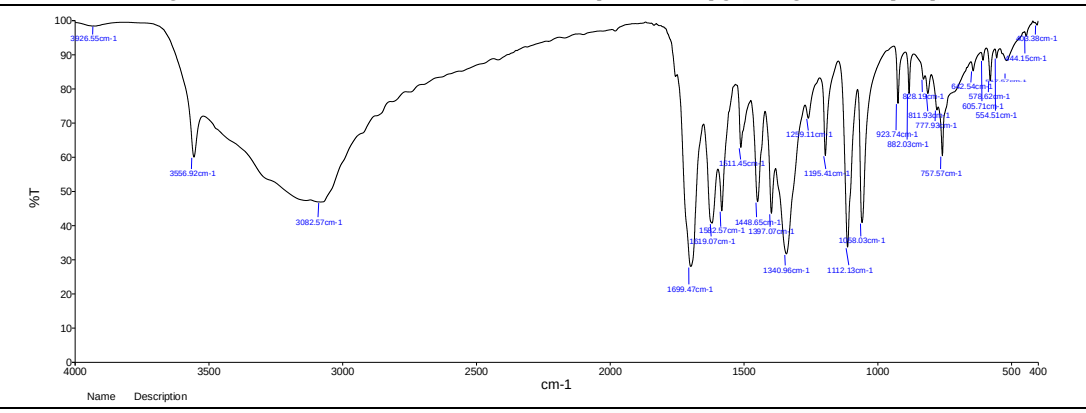

Figure 6: Fourier Transform infrared Spectroscopy of ZnO nanoparticles synthesised from Ellagic acid (EA-ZnONPs)

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RESEARCH ARTICLE

Human Wildlife Conflict Reduction Technology using YOLO Machine Learning Model

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ABSTRACT

Human–wildlife conflict has developed as the central jargon to strike a balance between resource demands of wildlife and human. There is a quite huge increase in the population of both wildlife and humans leading to overlap the usage of the geographical areas. And hence, there is a physical conflict between them. It is one of the main reasons behind endangering animals in the world and as of now needs serious attention. Therefore, our idea is to develop a security system that undermines all the disadvantages. In this paper, we propose to integrate an IP camera, Raspberry Pi and deep learning technology which will detect the presence of an animal in the photo taken by the camera and then raise an alarm which will help to keep the animal away from that area.

Keywords: Animal Identification, Human Wildlife, Technology, Machine Learning, YOLO.

INTRODUCTION

Human-wildlife conflicts is a serious matter to be dealt with to avoid problems causing due to negative interactions between the wildlife and human and we can isolate them [1]. This includes tourism, industry, air travel, wind power, agriculture and many more. The way to increase the lifetime of wildlife is the main focus of the wildlife damage management [2]. It is very vital to effectively manage wildlife to cut back the negative impact of human wildlife conflicts. Already available methods and devices effectiveness is extremely varying due to the dynamic change of habituation. This problem can be addressed by Deep Learning which is a subset of Artificial Intelligence. Artificial intelligence (AI) is a broad area of computing that highlights on the utilization of a computer to model intelligent behavior [3]. It focuses on the creation of intelligent machines that resembles like humans in thinking. AI has become an important part of the technology trade and analysis related to AI is extremely technical and specialized. The various applications in which artificial intelligence plays an important role are problem solving, reasoning and

robotics. Machine Learning (ML) is a division of Artificial Intelligence. ML is a science of designing and applying algorithms [4] that are able to learn things from previous experience. Humans generally learns from their experience. Similarly, ML uses this aspect of human. But when it receives a new data, wherein such data is not already seen by that algorithm, there comes the real challenge of ML. Many research was done to solve it. More complex algorithms were developed to solve such problems. These algorithms studies a pattern from the data and match the new data according to that pattern [5]. They utilize computer science and statistics to predict rational outputs.

Deep learning is a part of machine learning where artificial neural networks, algorithms galvanized by the human brain, learn from large amounts of data [6]. Like human, deep learning algorithm tries to enhance the outcome based on its previous experience. 'Deep Learning' is used because there are many deep layers in the neural network to extract the feature vectors i.e., the neurons which memorize certain activities. Here, in this paper, we have used deep learning for object detection.

Related Work

Predicting the location of the object along with the class is called Object Detection. Predicting the class to which the object belongs to is called as Object Recognition. Object Detection and recognition [7] is a classification problem. A window of fixed size is scanned across the whole image at all locations which is then fed to the image classifier. Each window of image is fed to the classifier to predict to which class it belongs to through which we find the object type. Predicting the location of an animal in digital images and videos is called Animal Detection [8]. It is a subset of object detection which focuses on the semantic object Animal. Animal detection in a given frame is a binary pattern classification task. There are various research work done in the literature.

Handmann Uwe and Thomas Kalinke used data fusion of edge and texture information to detect object [9]. A self-organizing Kohonen map is used as the coupler element of the various representations. There needs to occur some noise and background removal over a period which needs to be stabilized. This approach is mainly used for vehicles. Tuli Shreshth et al proposed a system which harness edge and cloud resources to provide better service quality for various applications [10]. This framework is not generic enough to support many applications. The people detection process is generated from video frames which had varied objects such as people, sky, automobiles, trees and roads [11]. Some types of animals cannot be covered using this approach. A deep neural network based approach for automatically identifying, counting and describing wild animals is proposed based on the SS dataset of wild animals [12]. The trained model is very big and it is not feasible to use it inside a microcontroller like Raspberry pi. The state of the art object detection algorithms are improved using Composite Backbone Network (CBNet) [13]. It has a series of backbones having same network structure and creates new composition of connections to connect the backbones. The speed and memory requirements increase with increase in the number of backbones making it inefficient.

The work presented in [14] uses thermal imaging to automate animal detection and classification. They used images in top view which are taken manually from a lift. The system has a lot of expensive components and requires high maintenance. In [15] an animal detection and annotation system based on deep convolutional neural network (DCNN) and Fully Convolutional Neural Network (FCNN) is proposed. This work involves a five component pipeline in a computer vision-based animal recognition system. Model size is directly proportional to the number of animals to be supported, leading to large model size, which cannot be fit inside a microcontroller. From the literature, we can infer that each has its own advantage and disadvantage. We have developed a system which detects the presence of animal and the name of it and intimate it to the concerned authorities by raising an alarm by using deep learning algorithm.

PROPOSED METHODOLOGY

In this work, we propose an early warning system to prevent human-wildlife conflict. This conflicts often ends in complication when there exist an overlap between humans and wildlife territory. The proposed system uses a

camera to capture images and process them using a deep learning model to detect the presence of a wild animal as shown in Fig.1. In case a wild animal is present, an alarm is raised to notify the presence of the animal.

YOLOv3: An incremental improvement

You only look once (YOLO) [16] is a current, well designed real-time object detection system. YOLO makes use of darknet, which is an open source neural network framework. YOLO actually scans the image just one time and then divides the whole image 13x13 cells. Each of these cells predicts five bounding boxes in it. A bounding box is nothing but a rectangle box which surrounds the object. YOLO lays a bounding box across each object in the image and provides with a confidence score of how much does this box enclose an object. And also, it predicts to which class does the object belongs to. The architecture of YOLOv3 is a 53 layer convolutional neural network called as Darknet-53. The model has four stages: a. Bounding box Prediction b. Class Prediction c. Predictions across Scales d. Feature Extractor. Tiny yolo is a smaller model for constrained environments like a raspberry pi. It is much faster but less accurate the normal YOLO model. It can handle up to 200 frames per second in a GPU enabled system. It consists of 9 convolutional layers and 6 pooling layers. This implementation uses the tiny version of yolo as it is run on raspberry pi.

Previous work on the same topic has been done with the use of a back-end server to process the captured image. But this system focuses on processing the image in the raspberry pi remotely without the need for a back-end server. By doing so, the portability of the system is enhanced and sending an image from a raspberry pi is avoided, eliminating the need for a Wi-Fi module.

Bounding Box Prediction

In a very clever way, YOLO actually looks at the image just once. YOLO splits the image into a grid of 13 by 13 cells as shown in Fig. 2. Each of these cells is responsible for predicting five bounding boxes [17]. The predicted bounding box/boxes may look something like in Fig. 3 where higher the confidence then fatter the box.

Class Prediction

The object will be surrounded by a bounding box which predicts the label to which it belong to using multi-label classification [18]. A softmax (normalized exponential function) function is not used as it affects the performance of the system. Instead independent logistic classifiers are used. There can be complex situation where there can be overlapping labels such as man, person. But actually, softmax assumes to have a single box for each box which is not the actual scenario. Hence, multi label approach is used to for this scenario of complex situation. So, each bounding box predicts a class and provides a probability over all other classes.

Predictions using scales

YOLOv3 predicts boxes at 3 different scales. Our system extracts features from those scales using a related concept to feature pyramid networks. Several convolutional layers are added to the base feature extractor. The last of these predicts a 3-d tensor encoding bounding box, object, and class predictions.

Feature Extractor

Darknet-53 is used for performing feature extraction. This new network Darknet-53 consists of 53 convolutional layers. Darknet-53 employs a hybrid approach between YOLOv2, Darknet-19, YOLOv2 and that innovative residual network stuff. Darknet-53 performs at the same level with the other classifiers. It uses only higher floating point operations and operates at more speed. The highest measured floating point operations per second is achieved by Darknet-53. This makes efficient utilization of the GPU to operate faster. Darknet-53 is better than ResNets as ResNets have too many layers which make them inefficient.

Image Capturing

Image capturing is done with the help of raspberry pi camera module, using Python and pi camera [19]. It can be used to take still pictures, record video, and apply image effects.

Steps to capture an image using pi camera module

i. Locate the camera port and connect the camera module in the right way as in Fig 4.
ii. Start up the Pi and ensure the software is enabled by opening Raspberry Pi Configuration Tool from main menu and enable camera software.
iii. Use the capture method of the Pi camera which is a pure Python interface to the Raspberry Pi's camera module, to capture an image, after including sleep operation of at least 2 seconds. Specify the path where the image captured has to be stored as a parameter to the capture method.

The camera can also be programmed to take multiple pictures by using a loop. The image/images captured will be stored in the pi itself.

Animal Detection and Recognition

To perform animal Detection and recognition we propose to use the YOLO model. The image which has been captured by camera module is first resized to 416 x 416 pixels and then given as an input to the YOLO model which is also run in the raspberry pi. YOLO then describes the bounding boxes for the grid cells. Since there are 13x13 = 169 grid cells and there are 5 bounding boxes predicted by each cell, so totally we acquire 845 bounding boxes at the end. Many of the boxes with no objects and only with background will get low confidence scores. The boxes with a score of more than 30% will be kept for further processing. The confidence score along with the class prediction is used to generate the final score which gives the probability of type of object in the box. The confusion matrix is calculated by running the model for a new unseen set of images. Accuracy, precision, recall and F1 score are found using the values in the confusion matrix. Once an animal is detected, a suitable alarm is raised by the system.

RESULTS AND DISCUSSIONS

In this section we present the implementation details and results of this work. We show the working of pi camera to capture an image and detection in day time (normal images) and detection in night time (infra-red images) using YOLO model.

Image Capturing

The camera has been programmed to capture images in time intervals which is determined based on the degree of infestation of animals in an area. It is then installed in a suitable position where it can capture images of animals when required. Sometimes, the image captured will have a greater resolution than expected. In general, resizing can be done through the libraries but it could be further more efficient to have Pi's GPU to do resizing while capturing the image itself through the resize() method.

Animal Detection and Recognition

During day time with ample light: As the next step, we perform detection and recognition on the captured image. Given an input, on running the classifier, it produces the output as in the Fig.5. During night time with less light: We perform detection and recognition on the captured infra-red image. Given an input, on running the classifier, it produces the output.

Performance Measurement

The well known performance metrics are considered for evaluating the system. The testing was done on 400 images.

CONCLUSION

To conclude, the detailed design and related algorithms to implement animal detection and recognition for prevention of human-wildlife conflict is designed and developed. Since a Raspberry Pi is used to execute, for the sake of better portability, we are constrained by limited processing capacity. In order to make the best use of it, we intend to use Intel Movidius to enhance the processing power of the raspberry pi. The developed model has given accuracy of 62%. There is still scope of improvement. The feature extraction can still be drilled down and more activation functions can be added to improve the performance of the model.

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Table 1: Experimental Results

Performance Metrics	Values Obtained
Accuracy	0.625
Precision	0.615
Recall	0.444
F1 Score	0.516

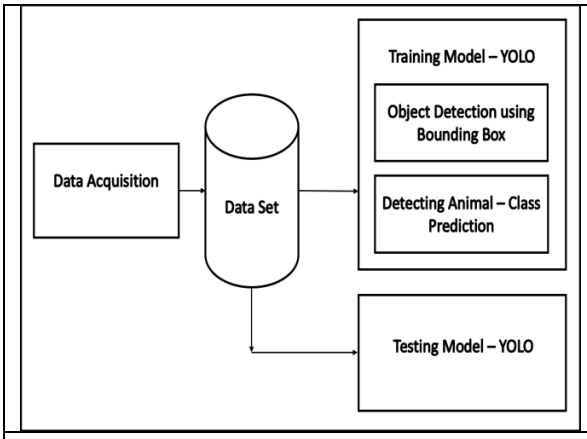

Figure 1. Proposed Architecture Diagram

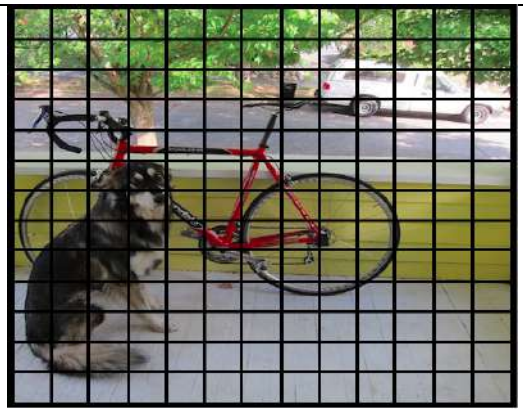

Figure 2. Image with Grids

Figure 3. Predicted Bounding Boxes

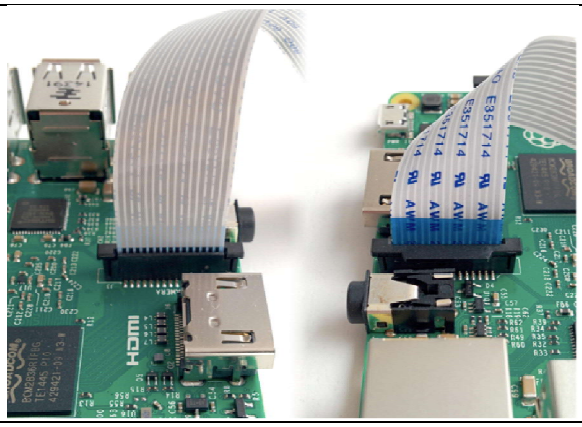

Figure 4. Connected Camera Module

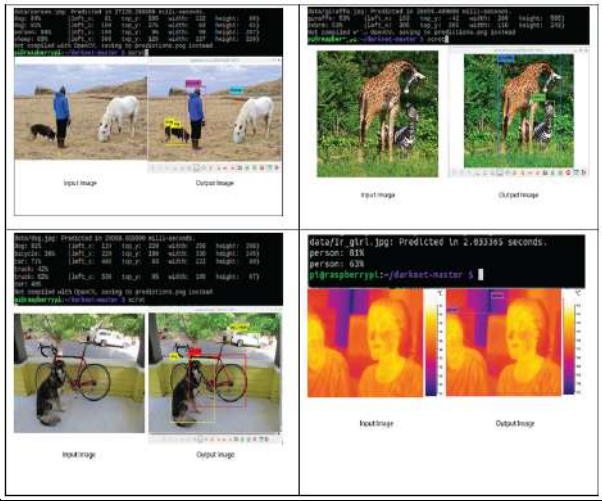

Figure 5. Output: Samples

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RESEARCH ARTICLE

Various Types of Somewhat Functions using δP_S-Open Sets

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ABSTRACT

This paper aims to introduce and study various somewhat δP_S-continuous functions and various somewhat δP_S-open functions by using δP_S-open sets in topological spaces. Some properties and characterizations of these functions are obtained.

INTRODUCTION

In 1963, Levine [7] introduced semi-open sets in topological spaces, a weaker form of open sets. Veliko [12] introduced a more robust form of closed sets namely δ-open sets, in 1968. Munshi [9] initiated and studied the concept of super-continuous mappings in 1982. Ekici [5] introduced δ-semi-continuity in 2005. Gentry [6] introduced and explored the idea of somewhat continuous functions and somewhat open functions in the year 1971. These ideas are closely related to weakly equivalent topologies, which were first introduced in 2010.In 1993, Rayachaudri [10] studied on δ-almost and δ-preopen sets. In 2012, Balasubramanian [1,2] introduced and studied various types of somewhat functions. In 2019, Zanyar[15] introduced and studied various forms of irresolute functions. In 2010 Benchalli et al[3] introduced a type of somewhat continuous and somewhat open functions.

In 2020 Vidhyapriya et al. [13] introduced a new class of open sets namely δP_S-open setscombining the concepts of δ-preopen and semi-closed sets. This class of sets lies between the classes of Ps-open and δ-pre-open sets. Further, Vidhyapriya et al. [14] introduced the notion of δP_S-continuous functions in topological spaces. Inspired by these developments, somewhat δP_S-continuous, somewhat almost δP_S-continuous, somewhat δP_S-irresolute, somewhat δP_S-open and somewhat almost δP_S-open functions are introduced. The properties and interrelations between newly introduced functions are obtained in this article.

Preliminaries

Some important definitions and results needed for the research work are collected and given in this section.

Definition 2.1

A subset A of a topological space (X,τ) is called a

a) Regular open set [11] if A = Int (Cl(A))
b) Semi-open [7] if A ⊆ Cl (Int(A))
c) δ-open set [10] when A is a union of regular open sets.
d) δ-preopen [15] if A ⊆ Int(δCl(A))

The complement of the above mentioned open sets are their respective closed sets. The intersection of all regular closed (resp. semi closed, δ-closed and δ-pre-closed) subsets of (X,τ) containing A is called the regular closure (resp. semi closure, δ-closure and δ-pre closure) of A and is denoted by rcl(A) (resp. scl(A), δ-cl(A) and δ-pcl(A)).

Definition 2.2[13]

A δ- preopen subset A of a space X is called a δP_S-open set if for each x ∈ A, there exists a semi-closed set F such that x ∈ F⊆ A. The complement of a δP_S-open - set is called δP_S-closed.

Definition 2.3

A subset A of a topological space (X,τ) is called δP_S-dense if every point x in X either belongs to A or is aδP_S-limit point of A(i.e.,) δP_S-cl(A)=X.

Definition 2.4

A function $f: (X,\tau) \to (Y,\sigma)$is called

a) δ* -almost-continuous [9] if$f^{-1}(V) \in \delta PO(X,\tau)$for each$V \in \delta PO(Y,\tau)$.
b) preirresolute [15] if$f^{-1}(V) \in PO(X)$ for every$V \in PO(Y)$.
c) Complete continuous [6] if the inverse image of each open subset of Y is regular open in X.
d) Super continuous [7]if the inverse image of every open set in (Y,σ) is δ-open in (X,τ).
e) δ-semi continuous[5]if the inverse image of every open set in $(Y,\sigma)$is δ-semi open in (X,τ).
f) δP_S- continuous [14]A function $f: (X,\tau) \to (Y,\sigma)$ is called δP_S- continuous at a point x ∈ X if for each x ∈ X and each open set V of Y containing f (x), there exists a δPs-open set U of X containing x such that f (U) ⊆ V. If f is δPs-continuous at every point of X, then it is calledδP_S-continuous.
g) Somewhat continuous [6] if for $U \in \sigma$ and $f^{-1}(U) \neq \phi$, there exists $V \in \tau$ such that $V \neq \phi$ and $V \subseteq f^{-1}(U)$.

Proposition 2.5[13]

Let (Y,τ_Y) be a subspace of a space (X,τ). If$A \in \delta P_S O(Y,\tau_Y)$ and$Y \in RO(X,\tau)$, then$A \in \delta P_S O(X,\tau)$.

Lemma 2.6[4]

Let A be a subset of a topological space (X, τ). Then the following properties hold:
(1) If A is preopen in (X, τ), then it is δ-preopen in (X, τ),
(2) A is δ-preopen in (X, τ) if and only if it is preopen in (X, τ_s),

3.1 Somewhat δP_S-Continuous Functions

Definition 3.1.1: A function f is said to be somewhat δP_S-continuous if for $U \in \sigma$ and $f^{-1}(U) \neq \phi$, there exists a non-empty δP_S-open set V in (X,τ) such that $V \subseteq f^{-1}(U)$.

The following two examples are established to show the existence of somewhat δP_S-continuous function, and not all functions are somewhat δP_S-continuous.

Example 3.1.2: Let $X = Y = \{a,b,c\}$ with $\tau = \{X,\phi,\{a\},\{a,b\},\{a,c\}\}$ and $\sigma = \{Y,\phi,\{a\},\{a,b\}\}$. Let $f: (X,\tau) \to (Y,\sigma)$ be defined by $f(a) = a, f(b) = a, f(c) = b$. Then f is somewhat δP_S-continuous.

Example 3.1.3: Let $X = Y = \{a,b,c\}$ with $\tau = \{X,\phi,\{a\},\{a,b\},\{a,c\}\}$ and $\sigma = \{Y,\phi,\{a\},\{a,b\}\}$. Let $f: (X,\tau) \to (Y,\sigma)$ be the identity functions. Then f is not somewhat δP_S-continuous.

Proposition 3.1.4: Every δP_S-continuous function is somewhat δP_S-continuous function.
Proof. Let $f:(X,\tau)\to(Y,\sigma)$ be δP_S-continuous. Let $x\in X$ and $U\in\sigma$ and $f^{-1}(U)\neq\phi$.
Therefore, there exists $x\in f^{-1}(U)=\phi\Rightarrow f(x)\in U$. Since f is δP_S-continuous, there exists a δP_S-open set V in (X,τ) such that $f(V)\subseteq U\Rightarrow V\subseteq f^{-1}(U)$. Hence f is somewhat δP_S-continuous.

Remark 3.1.5: The converse of above proposition is not true, in general.
The following example exhibits somewhat-δP_S-continuous function need not be δP_S-continuous.

Example 3.1.6: Let $X=Y=\{a,b,c,d\}$ with $\tau=\{X,\phi,\{a\}\}$ and $\sigma=\{Y,\phi,\{a\},\{c\},\{a,b\},\{a,c\},\{a,b,c\},\{a,c,d\}\}$. Let $f:(X,\tau)\to(Y,\sigma)$ be defined by $f(a)=d,f(b)=a,f(c)=c,\ then\ f^{-1}(c)=\{c\},f^{-1}(a,b)=\{b\},f^{-1}(a,c)=\{c\},f^{-1}(a,b,c)=\{b,c\},f^{-1}(a,c,d)=\{a,c\}$. Therefore f is somewhat δP_S-continuous. Since $\delta P_S O(X)$ = {X, Φ, {b}, {c}, {d}, {b, c}, {b, d}, {c, d}{b, c, d}} but not δP_S-continuous as $f^{-1}(a,c,d)$ is not δP_S-open.

Note 3.1.7: The composition of two somewhat δP_S-continuous functions need not be somewhat δP_S-continuous functions. In general, which is shown in the following example.

Example 3.1.8. Let $X=Y=Z=\{a,b,c\}$ with $\tau=\{X,\phi,\{a\},\{a,b\},\{a,c\}\},\sigma=\{Y,\phi,\{a\},\{a,b\}\}$ and $\eta=\{Z,\phi,\{a\},\{a,b\}\}$ Let $f:(X,\tau)\to(Y,\sigma)$ be defined by $f(a)=b,f(b)=a,f(c)=a$ and $g(a)=b,g(b)=a,g(c)=a$. Then the functions f and g are somewhat δP_S-continuous but their composition $g\circ f:(X,\tau)\to(Z,\eta)$ is not somewhat δP_S-continuous.

Proposition 3.1.9: If f is somewhat δP_S-continuous and g is continuous, then $g\circ f$ is somewhat δP_S-continuous.
Proof. Let $f:(X,\tau)\to(Y,\sigma)$ be a somewhat δP_S-continuous function and $g:(Y,\sigma)\to(Z,\eta)$ be a g-continuous function. Let U be open in (Z,η) then $g^{-1}(U)$ is open in (Y,σ) since g is complete continuous. Since f is somewhat δP_S-continuous, $f^{-1}(g^{-1}(U))$ will contain a non-empty δP_S-open set in (X,τ). Therefore $g\circ f$ is somewhat δP_S-continuous.

Corollary 3.1.10: If f is somewhat δP_S-continuous and g is super continuous (complete continuous), then $g\circ f$is somewhat δP_S-continuous.
Proof. Follows from the definition of super continuity (complete continuity) and every δ-open set (regular open) is open. The proof follows as in Proposition 3.1.9.

Proposition 3.1.11: A subset A of (X,τ) is δP_S-dense in (X,τ) if there is no proper δP_S-closed set C in (X,τ) such that $A\subseteq C\subseteq X$.
Proof. Suppose there exists C, δP_S-closed set in X such that $A\subseteq C\subseteq X$ → (1)
Since A is δP_S-dense, $\delta P_S\ cl(A)=X$.
$\delta P_S cl(A)=\cap\{M|A\subseteq M$ and M is δP_S-closed in $X\}$ → (2)
(1) and (2) $\Rightarrow C$ is one set in theabove intersection. Therefore $\delta P_S cl(A)\subseteq C$,
$X\subseteq C\Rightarrow C=X$.

Proposition 3.1.12: For a surjective function f, the following statements are equivalent:
a) f is somewhat δP_S-continuous.
b) If C is a closed subset of (Y,σ) such that $f^{-1}(C)\neq X$, then there is a proper δP_S-closed subset D of (X,τ) such that $f^{-1}(C)\subseteq D$.(Equivalently, if $\mathcal{C}$ is an open subset of (Y,σ) such that $f^{-1}(C)\neq X$, then there exists a proper δP_S-open subset D of (X,τ) such that $f^{-1}(C)\subseteq D$).
c) If A is a δP_S-dense subset of (X,τ), then $f(A)$ is a dense subset of (Y,σ).

Proof.$(a) \Rightarrow (b)$ Let C be a closed subset of (Y,σ) such that $f^{-1}(C) \neq X$. Then $Y\backslash C$ is an open set Y such that $f^{-1}(Y\backslash C) = X\backslash f^{-1}(C) \neq \phi$. By (a), there exists a non-empty subset $U \in \delta P_S O(X,\tau)$ such that $U \neq \phi$ and $U \subseteq f^{-1}(Y\backslash C) = X\backslash f^{-1}(C)$. This means that $f^{-1}(C) \subseteq X\backslash U$ and $X\backslash U = D$ is a proper δP_S-closed set in (X,τ).
$(b) \Rightarrow (a)$ Let $U \in \sigma$ and $f^{-1}(U) \neq \phi$ then $Y\backslash U$ is closed and $f^{-1}(Y\backslash U) = X\backslash f^{-1}(U) \neq X$. By (ii), there exists a proper δP_S-closed set D such that $f^{-1}(Y\backslash U) \subseteq D,\ X\backslash f^{-1}(U) \subseteq D$. This implies that $X\backslash D \subseteq f^{-1}(U)$ and $X\backslash D$ is δP_S-open and $X\backslash D \neq \phi$.
$(b) \Rightarrow (c)$Let A be a δP_S-dense set in (X,τ). Suppose that $f(A)$ is not dense in Y. Then there exists a proper closed set C in Y such that $f(A) \subseteq C \subseteq Y$. Clearly $f^{-1}(C) \neq X$. By (b), there exists a proper δP_S-closed set D such that $A \subseteq f^{-1}(C) \subseteq D \subseteq X$. This is a contradiction to the fact that A is δP_S-dense in (X,τ).
$(c) \Rightarrow (b)$Suppose (ii) is not true there exists a closed set C in Y such that $f^{-1}(C) \neq X$ but there is no proper δP_S-closed set D in (X,τ) such that $f^{-1}(C) \nsubseteq D$. This means that $f^{-1}(C)$ is δP_S-dense in (X,τ). But by (c), $f(f^{-1}(C)) = C$ must be dense in Y, which is a contradiction to the choice of C.

Definition 3.1.13: If X is a set and τ and σ are topologies on X, then τ is said to be δP_S**-equivalent** to σ provided if $U \in \sigma$and U ≠ ∅, then there is a non-empty δP_S-open set V in (X, τ) and $V \subseteq U$.

Proposition3.1.14: Let X be a set, τ and σ are δP_S -equivalent topologies on X. When f is identity then $f:(X,\tau) \to (Y,\sigma)$ and $f^{-1}:(Y,\sigma) \to (X,\tau)$ are somewhat δP_S-continuous. Conversely, if the identity function f is somewhat δP_S-continuous in both directions, then τ and σ are δP_S-equivalent.
Proof. Let τ and σ be two δP_S -equivalent topologies on X. Let $f:(X,\tau) \to (Y,\sigma)$be an identity function. Then for an open set C in $Y, f^{-1}(C) = C$ in (X,τ) such that $D \subseteq C = f^{-1}(C)$. By Proposition 3.1.12,$f$ is somewhat δP_S continuous function. Conversely, Retracing the steps the converse can be proved.

Proposition3.1.15: Let $f:(X,\tau) \to (Y,\sigma)$be a surjective somewhat δP_S -continuous function and τ* be a topology for X, which is δP_S-equivalent to τ. Then $f:(X,\tau^*) \to (Y,\sigma)$ is somewhat δP_S-continuous.
Proof. Let $V \in \sigma$ such that $f^{-1}(V) \neq \phi$. Since $f:(X,\tau) \to (Y,\sigma)$ is somewhat δP_S-continuous, there exists a non-empty δP_S-open set U in (X,τ) such that $U \subseteq f^{-1}(V)$. But by hypothesis,τ^* is δP_S-equivalent to τ. Therefore, there exists a δP_S-open set $U^* \in (X,\tau^*)$ such that $U^* \subseteq U$. But $U \subseteq f^{-1}(V)$ then $U^* \subseteq f^{-1}(V)$, hence $f:(X,\tau^*) \to (Y,\sigma)$ is somewhat δP_S -continuous.

Proposition 3.1.16: Let $f:(X,\tau) \to (Y,\sigma)$ be a surjective somewhat δP_S-continuous function and σ^* be a topology for Y, which is equivalent to σ. Then $f:(X,\tau) \to (Y,\sigma^*)$ is somewhat δP_S -continuous.
Proof. Let $V^* \in \sigma^*$ such that $f^{-1}(V^*) \neq \phi$. Since σ^* is equivalent to σ, there exists a non-empty open set V in(Y,σ) such that V ⊆ V*. Now ∅ ≠ f-1(V) ⊆ f-1(V*). Since $f:(X,\tau) \to (Y,\sigma)$ is somewhat δP_S -continuous, there exists a non-empty δP_S -open set U in (X, τ) such that $U \subseteq f^{-1}(V)$. Then $U \subseteq f^{-1}(V^*)$, hence $f:(X,\tau) \to (Y,\sigma^*)$ is somewhat δP_S-continuous.

3.2. Somewhat Almost δP_S- Continuous Functions

Definition 3.2.1: A function f is said to be somewhat almost δP_S-continuous if for every $U \in \delta PO(Y,\sigma)$ and $f^{-1}(U) \neq \phi$ there exists a non-empty $V \in \delta P_S O(X,\tau)$ such that$V \neq \phi$ and $V \subseteq f^{-1}(U)$.

Proposition 3.2.2: Every somewhat almost δP_S-continuous map is a somewhat δP_S-continuous.
Proof. Let $f:(X,\tau) \to (Y,\sigma)$ is a somewhat almost δP_S-continuous. Consider $U \in \sigma$ such that $f^{-1}(U) \neq \phi$. Then $U \in \delta PO(\sigma)$. Since f is somewhat almost δP_S-continuous there existsa δP_S-open set V in (X,τ) such that $V \subseteq f^{-1}(U)$.
$\Rightarrow f$ is somewhat δP_S-continuous.

Example 3.2.3: Let $X = Y = \{a,b,c\}$ with $\tau = \{X,\phi,\{a,b\}\}, \sigma = \{Y,\phi,\{a\}\}$ and $f:(X,\tau) \to (Y,\sigma)$ such that $f(a) = b, f(c) = c$ and $f(c) = a$ Then $\delta P_S O(\tau) = \{X,\phi,\{c\}\}$ and $\delta P_S O(\sigma) = P(X)$. Hence f is somewhat δP_S but not somewhat almost δP_S-continuous.

Remark 3.2.4: δP_S continuous and somewhat almost δP_S-continuous are independent.
The following examples shows that both are independent.

Example 3.2.5: Let $X, Y, \tau, \sigma$and f be as in Example 3.2.3. It is δP_S-continuous but not somewhat almost δP_S-continuous.

Example 3.2.6: Let$X = Y = \{a, b, c, d\}$ with$\tau = \{X, \Phi, \{a\}, \{b\}, \{a, b\}\}$ and $\sigma = X, \Phi, \{a\}, \{a, b\}, \{a, b, c\}, \{a, b, d\}\}$. Then $\delta PO(X) = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}, \{a, b, d\}\}$, where the inverse images of f is in g. Hence f is somewhat almost δP_S-continuous but not δP_S-continuous.

Proposition 3.2.7: The composition of somewhat almost δP_S-continuous functions is somewhat almost δP_S-continuous.
Proof. Let $f: (X, \tau) \to (Y, \sigma)$ and $g: (Y, \sigma) \to (Z, \eta)$ be somewhat almost δP_S-continuous. Let $U \in \delta PO(Z, \eta)$ such that $(g \circ f)^{-1} U \neq \phi \Rightarrow f^{-1}(g^{-1}(U)) \neq \phi \Rightarrow g^{-1}(U) \neq \phi$. Since g is somewhat almost δP_S-continuous, there exists a non-empty $V \in \delta P_S O(Y, \sigma)$ such that $V \neq \phi$ and $V \subseteq g^{-1}(U)$. Since every δP_S-open set is δP-open set, we get $V \in \delta PO(Y, \sigma)$ and since f is somewhat almost δP_S-continuous, there exists $W \in \delta P_S O(X, \tau)$ and $W \neq \phi$ and $W \subseteq f^{-1}(V) = f^{-1}\left(g^{-1}(U)\right) = (g \circ f)^{-1}(U)$. Hence $g \circ f$ is somewhat almost δP_S-continuous.

Remark 3.2.8: If f is somewhat almost δP_S-continuous and g is δ^*continuous, then $g \circ f$ is somewhat almost δP_S-continuous.
Proof. Since g is an δ^* almost continuous functions, by 2.4(i), for everyδP_S-open set of $V, g^{-1}(V)$ isδP_S-open in(Y, σ). Now f is somewhat almost δP_S-continuous implies there exists a δP_S-open set U in (X, τ) such that $U \subseteq f^{-1}\left(g^{-1}(V)\right) = (g \circ f)^{-1}(V)$. Hence $g \circ f$ is somewhat almost δP_Scontinuous function.

Proposition 3.2.9: If$f: (X, \tau) \to (Y, \sigma)$ is somewhat almost δP_S-continuous and$g: (Y, \sigma) \to (Z, \eta)$ is pre-irresolute, then $g \circ f: (X, \tau) \to (Y, \sigma)$ is somewhat almost δP_S-continuous function.
Proof. Since $\delta PO(Y, \sigma) = PO(Y, \sigma_S)$ and $\delta PO(Z, \eta) = PO(Z, \eta_S)$by Lemma 2.6 and g is pre-irresolute, we get for every $V \in \delta PO\left(Z, \eta\right), g^{-1}(V) \in \delta PO(Y, \sigma)$**.** Now f is somewhat almost δP_S-continuous function and hence $f^{-1}(g^{-1}(V)$ contains a δP_S-open set W in (X, τ)
(i.e.,) $W \subseteq (g \circ f)^{-1}(V) \Longrightarrow g \circ f$ is somewhat almost δP_S- continuous function.

Proposition 3.2.10: For a surjective function f, the following statements are equivalent:
(a) f is somewhat almost δP_Scontinuous.
(b) If C is a δ-semi closed subset in (Y, σ) such that $f^{-1}(C) \neq X$, then there is a non-empty $D \in \delta P_S \mathcal{C}(X, \tau)$ and$f^{-1}(C) \subseteq D$.
(c) If A is a δP_S-dense subset of (X, τ), then f(A) is a δP-dense subset of (Y, σ).
Proof.(a) ⇒ (b)Let C be a δ-preclosed subset of (Y, σ) such that $f^{-1}(C) \neq X$. Then Y \ C is a δ-preopen set in (Y, σ) such that $f^{-1}(Y \setminus C) = X \setminus f^{-1}(C) \neq \emptyset$. By (a), there exists a non-empty $V \in \delta P_S O(X, \tau)$ and $V \subseteq f^{-1}(Y \setminus C) = X \setminus f^{-1}(C)$. Thus, $f^{-1}(C) \subseteq X \setminus V$ and $X \setminus V = D$ is a proper δP_S-closed set in (X, τ).
(b) ⇒ (a)Let $U \in \delta PO(Y, \sigma)$ and $f^{-1}(U) \neq \emptyset$ then $Y \setminus U \in \delta PC(Y, \sigma)$ and $f^{-1}(Y \setminus U) = X \setminus f^{-1}(U) \neq X$. By (b), there exists a proper $D \in \delta P_S \mathcal{C}(X, \tau)$ such that $f^{-1}(Y \setminus U) \subseteq D$. This implies that $X \setminus D \subseteq f^{-1}(U)$ and $X \setminus D$ is δP_S-open and $X \setminus D \neq \emptyset$.
(b) ⇒ (c)Let A be a δP_S-dense set in (X, τ). Suppose that f(A) is not δP_S-dense in (Y, σ). Then there exists a proper subset $C \in \delta PC(Y, \sigma)$ such that $f(A) \subseteq C \subseteq Y$. Clearly $f^{-1}(C) \neq X$. By (b), there exists a proper subset $D \in \delta P_S \mathcal{C}(X, \tau)$ such that $A \subseteq f^{-1}(C) \subseteq D \subseteq X$. This is a contradiction to the fact that A is δP_S-dense in (X, τ).
(c) ⇒ (b)Suppose (b) is not true there exists $C \in \delta PC(Y, \sigma)$ such that $f^{-1}(C) \neq X$ but there is no proper subset $D \in \delta P_S \mathcal{C}(X, \tau)$ such that $f^{-1}(C) \subseteq D$. Thus $f^{-1}(C)$ is δP_S-dense in (X, τ). But by (c), $f(f^{-1}(C)) = C$ isδP-dense in (Y, σ), which contradicts the choice of C.

Proposition 3.2.11: Let f be a function and X = A∪B, where A, B∈RO(X, τ). Then, if the restriction functions $f|_A$ and $f|_B$ are somewhat almost δP_S-continuous, then f is somewhat almost δP_S-continuous.

Proof. Let U ∈ δPO(Y, σ) such that $f^{-1}(U) \neq \phi$. Then $(f|_A)^{-1}(U) \neq \emptyset$ or $(f|_B)^{-1}(U) \neq \emptyset$ or both $(f|_A)^{-1}(U) \neq \emptyset$ and $(f|_B)^{-1}(U) \neq \emptyset$. Suppose $(f|_A)^{-1}(U) \neq \emptyset$, since $f|_A$ is somewhat almost δP_S-continuous, there exists a δP_S-open set V in A such that $V \neq \emptyset$ and $V \subseteq (f|_A)^{-1}(U) \subseteq f^{-1}(U)$. Since V is δP_S-open in A and A is Regular open in (X, τ), V is δP_S-open in (X, τ), by Proposition 2.5. Thus, f is somewhat almost δP_S-continuous.

The proof of other cases is similar.

Proposition 3.2.12: Let $f: (X, \tau) \to (Y, \sigma)$ be a somewhat almost δP_S-continuous surjective and τ^* be a topology for X, which is δP_S-equivalent to τ. Then f: $(X, \tau^*) \to (Y, \sigma)$ is somewhat almost δP_S-continuous.

Proof. Let V ∈ δPO(σ) such that $f^{-1}(V) \neq \emptyset$. Since f is somewhat almost δP_S-continuous, there exists a non-empty δP_S-open set U in (X, τ) such that $U \subseteq f^{-1}(V)$. But by hypothesis τ^* is δP_S-equivalent to τ. Therefore, there exists $U^* \in \delta P_S O(X, \tau^*)$ such that $U^* \subseteq U$. But $U \subseteq f^{-1}(V)$ then $U^* \subseteq f^{-1}(V)$, hence $f: (X, \tau^*) \to (Y, \sigma)$is somewhat almost δP_S-continuous.

Definition 3.2.13: If X is a set and τ and τ^* are topologies on X, then τ is said to be δ-pre-equivalent to τ^* provided if $U \in \delta PO(X, \tau)$ and $U \neq \phi$ then there exists $U^* \in \delta PO(X, \tau^*)$ such that $U^* \neq \phi$ and $U^* \subseteq U$ and if $U \in \delta PO(X, \tau^*)$ and $U \neq \phi$ then there exists $V \in \delta PO(X, \tau)$ such that $V \neq \phi$ and $U \supseteq V$.

Proposition 3.2.14: Let $f: (X, \tau) \to (Y, \sigma)$be a somewhat almost δP_S-continuous surjective and σ^* be a topology for Y, which is δ-pre-equivalent to σ. Then f: $(X, \tau) \to (Y, \sigma^*)$ is somewhat almost δP_S -continuous.

Proof. Let $V^* \in \delta PO(Y, \sigma^*)$ such that $f^{-1}(V^*) \neq \emptyset$. Since σ^* is δ-pre-equivalent to σ, there exists a non-empty δ-preopen set V in (Y, σ) such that $V \subseteq V^*$. Now$\phi \neq f^{-1}(V) \subseteq f^{-1}(V^*)$. Since f is somewhat almost δP_S-continuous, there exists a non-empty δP_S-open set U in (X, τ) such that $U \subseteq f^{-1}(V)$. Then $U \subseteq f^{-1}(V^*)$, hence $f: (X, \tau) \to (Y, \sigma^*)$ is somewhat almost δP_S-continuous.

3.3 Somewhat δP_S - Irresolute Functions

Definition 3.3.1: A function $f: (X, \tau) \to (Y, \sigma)$ is said to be somewhat δP_S-irresolute if for $U \in \delta P_S O(Y, \sigma)$ and $f^{-1}(U) \neq \emptyset$, there exists a non-empty δP_S-open set V in (X, τ) such that $V \subseteq f^{-1}(U)$.

Example 3.3.2: Let X = Y = {a,b,c} with τ = {X, ∅, {a,b}} and σ = {Y, ∅, {a}, {a,b}, {a,c}}. Let $f: (X, \tau) \to (Y, \sigma)$be the identity function. Then f is somewhat δP_S-irresolute but not somewhat irresolute as for the semi open set {a} in (Y, σ), $f^{-1}\{a\} = \{a\}$ doesn't contain any non-empty semiopen set in (X, τ).

Example 3.3.3: Let X = Y = {a,b,c} with τ = {X, ∅,{a},{b},{a,b}} and σ = {Y, ∅, {a}, {a,b}, {a,c}}. Let $f: (X, \tau) \to (Y, \sigma)$be defined by f(a) = b, f(b) = c, f(c) = a. Then f is not somewhat δP_S-irresolute as $f^{-1}\{a\} = \{c\}$ contains no non-empty δP_S-open set in (X, τ), but f is somewhat irresolute.

Proposition 3.3.4: If f and g are somewhat δP_S-irresolute functions from $f: (X, \tau) \to (Y, \sigma)$ and $g: (Y, \sigma) \to (Z, \eta)$ then $g \circ f$ is δP_S-irresolute.

Proof. Let $W \in (Z, \eta)$ be $\delta P_S O(\eta)$. Then, since g is somewhat δP_S-irresolute then there exists $V \in \delta P_S O(\sigma)$ such that $V \subseteq g^{-1}(W)$. Since f is somewhat δP_S-irresolutethere exists$U \in \delta P_S O(\tau)$ such that $U \subseteq f^{-1}(V)$.

$$\Rightarrow U \subseteq f^{-1}(V) \subseteq f^{-1}\left(g^{-1}(W)\right) = (g \circ f)^{-1}(W)$$

$\therefore g \circ f$ is somewhat δP_S-irresolute.

Proposition 3.3.5: For a surjective function f, the following statements are equivalent:

(a) f is a somewhat δP_S-irresolute function.

(b) If C is a δP_S-closed subset of (Y, σ) such that $f^{-1}(C) \neq X$, then there is a proper δP_S-closed subset D of (X, τ) such that $f^{-1}(C) \subseteq D$.
(c) If A is a δP_S-dense subset of (X, τ), then f(A) is a δP_S-dense subset of (Y, σ).
Proof. (a) ⇒ (b) Let C be a δP_S-closed subset of (Y, σ) such that $f^{-1}(C) \neq X$. Then $Y \setminus C$ is a δP_S-open set in (Y, σ) such that $f^{-1}(Y \setminus C) = X \setminus f^{-1}(C) \neq \emptyset$. By (a), there exists a non-empty δP_S-open set V in (X, τ) and $V \subseteq f^{-1}(Y \setminus C) = X \setminus f^{-1}(C)$. This means that $f^{-1}(C) \subseteq X \setminus V$ and $X \setminus V = D$ is a proper δP_S-closed set in (X, τ).
(b) ⇒ (a) Let $U \in \delta P_S O(Y, \sigma)$ and $f^{-1}(U) \neq \emptyset$ then $Y \setminus U$ is δP_S -closed and $f^{-1}(Y \setminus U) = X \setminus f^{-1}(U) \neq X$. By (b), there exists a proper δP_S-closed set D such that $f^{-1}(Y \setminus U) \subseteq D$. This implies that $X \setminus D \subseteq f^{-1}(U)$ and $X \setminus D$ is δP_S-open in (X, τ) and $X \setminus D \neq \emptyset$.
(b) ⇒ (c) Let A be a δP_S-dense set in (X, τ). Suppose that f(A) is not δP_S-dense in (Y, σ). Then there exists a proper δP_S-closed set C in (Y, σ) such that $f(A) \subseteq C \subseteq Y$. Clearly $f^{-1}(C) \neq X$. By (b), there exists a proper δP_S-closed set D such that $A \subseteq f^{-1}(C) \subseteq D \subseteq X$. This is a contradiction to the fact that A is δP_S-dense in (X, τ).
(c) ⇒ (b) Suppose (b) is not true, there exists a δP_S-closed set C in (Y, σ) such that $f^{-1}(C) \neq X$ but there is no proper δP_S-closed set D in (X, τ) such that $f^{-1}(C) \subseteq D$. This means that $f^{-1}(C)$ is δP_S-dense in (X, τ). But by (c), $f(f^{-1}(C)) = C$ must be δP_S-dense in (Y, σ), which is a contradiction to the choice of C.

Proposition 3.3.6: Let f be a function and $X = A \cup B$, where $A, B \in RO(X, \tau)$. Then, if the restriction functions $f|_A: (A; \tau|_A) \to (Y, \sigma)$ and $f|_B: (B; \tau|_B) \to (Y, \sigma)$ are somewhat δP_S-irresolute, then f is a somewhat δP_S-irresolute function.
Proof. Let $U \in \delta P_S O(Y, \sigma)$ such that $f^{-1}(U) \neq \emptyset$. Then $(f|_A)^{-1}(U) \neq \emptyset$ or $(f|_B)^{-1}(U) \neq \emptyset$ or both $(f|_A)^{-1}(U) \neq \emptyset$ and $(f|_B)^{-1}(U) \neq \emptyset$. Suppose $(f|_A)^{-1}(U) \neq \emptyset$, since $f|_A$ is somewhat δP_S-irresolute, there exists a δP_S-open set V in A such that $V \neq \emptyset$ and $V \subseteq (f|_A)^{-1}(U) \subseteq f^{-1}(U)$. Since A is regular open in (X, τ) and V is δP_S-open in (X, τ), by Proposition 2.5. Thus, f is somewhat δP_S-irresolute.
The proof of other cases is similar.

Proposition 3.3.7: If f is the identity function on a set X. The two topologies on X, τ and σ are δP_S-equivalent. Then f and f^{-1} are somewhat δP_S-irresolute functions. Conversely, if the identity function is somewhat δP_Sirresolute in both directions, then τ and σ are δP_S-equivalent.
Proof. Let τ and σ are δP_S-equivalent.
For every $U \in \delta P_S O(X, \tau)$ there exists $V \in \delta P_S O(X, \sigma)$ such that $V \subseteq U \rightarrow (1)$
and for every $U \in \delta P_S O(X, \sigma)$, there exists $V \in \delta P_S O(X, \tau)$ such that $U \supseteq V \rightarrow (2)$
If $f: (X, \tau) \to (X, \sigma)$, Let $U \in \delta P_S O(X, \sigma)$ then $f^{-1}(U) = U$ since f is identity. By (2), there exists $V \in \delta P_S O(X, \tau)$ such that $V \subseteq U = f^{-1}(U)$. Therefore, f is somewhat δP_S-irresolute.
If $f^{-1}: (X, \tau) \to (X, \sigma)$, Let $U \in \delta P_S O(X, \tau)$ then $(f^{-1})^{-1}(U) = U$ since f is identity. By (1), there exists $V \in \delta P_S O(X, \sigma)$ such that $V \subseteq U = f(U)$. Therefore, f is somewhat δP_S-irresolute.

Proposition 3.3.8: Let $f: (X, \tau) \to (Y, \sigma)$ be a somewhat δP_S-irresolute surjective and τ^* be a topology for (X, τ), which is δP_S-equivalent to τ. Then $f: (X, \tau^*) \to (Y, \sigma)$ is somewhat δP_S-irresolute.
Proof. Let $V \in \delta P_S O(Y, \sigma)$ such that $f^{-1}(V) \neq \emptyset$. Since $f: (X, \tau) \to (Y, \sigma)$is somewhat δP_S-irresolute, there exists a non-empty δP_S-open set U in (X, τ) such that $U \subseteq f^{-1}(V)$. Since τ^* is δP_S-equivalent to τ. By (2) in the above Proposition, there exists $U^* \in \delta P_S O(X, \tau^*)$ such that $U^* \subseteq U$. But $U \subseteq f^{-1}(V)$ then $U^* \subseteq f^{-1}(V)$, hence $f: (X, \tau^*) \to (Y, \sigma)$ is a somewhat δP_S-irresolute function.

Proposition 3.3.9: Let $f: (X, \tau) \to (Y, \sigma)$ be a somewhat δP_S-irresolute surjective function and σ^* be a topology for Y, which is equivalent to σ. Then $f : (X, \tau) \to (Y, \sigma^*)$ is somewhat δP_S-irresolute.
Proof. Let $V^* \in \delta P_S O(Y, \sigma^*)$ such that $f^{-1}(V^*) \neq \emptyset$. Since σ^* is equivalent to σ, by (1) inProposition3.3.7, there exists a non-empty $V \in \delta P_S O(Y, \sigma)$ such that $V \subseteq V^*$. Now $\emptyset \neq f^{-1}(V) \subseteq f^{-1}(V^*)$. Since f is somewhat δP_S-irresolute, there exists a non-empty δP_S-open set U in (X, τ) such that $U \subseteq f^{-1}(V)$. Then $U \subseteq f^{-1}(V^*)$, hence $f : (X, \tau) \to (Y, \sigma^*)$ is a somewhatδP_S-irresolute function.

3.4 Somewhat δP_S-Open Functions

Definition 3.4.1: A function f is said to be somewhat δP_S-open provided that if $U \in \tau$ and $U \neq \emptyset$, then there exists a non-empty δP_S-open set V in (Y, σ) such that $V \subseteq f(U)$.

The following function f is both somewhat open and somewhat δP_S-open.

Example 3.4.2: Let X = Y = {a,b,c} with τ = {X, ∅, {a}, {a,b}} and σ = {Y, ∅,{a},{b},{a,b}}. Let $f: (X, \tau) \to (Y, \sigma)$ be the identity function. Then f is somewhat δP_S-open and somewhat open.

Remark 3.4.3: The following examples exhibits somewhat openness and somewhat δP_S-openness are independent.

Example 3.4.4: Let X = Y = {a, b, c} with τ = {X, ∅,{a},{b},{a,b},{a,c}} and σ = {Y, ∅, {a}}. Then $f: (X, \tau) \to (Y, \sigma)$is neither somewhat open and nor somewhatδP_S- open.

Example 3.4.5: Let X = Y = {a, b, c} with τ = {X, ∅,{a},{b},{a,b}} and σ = {Y, ∅, {a},{b},{a,b},{a,c}}. Then the identity function$f: (X, \tau) \to (Y, \sigma)$is somewhat open but not somewhatδP_S- open.

Example 3.4.6: Let X = Y = {a, b, c} with τ = {X, ∅,b},{c},{b,c}} and σ = {Y, ∅, {a}}. Then the identity function$f: (X, \tau) \to (Y, \sigma)$is not somewhat open but it is somewhatδP_S- open.

Remark 3.4.7: The composition of two somewhat δP_S-open functions is not somewhat δP_S-open.

Example 3.4.8: Let X = Y = Z = {a,b,c} with τ = {X, ∅, {a}, {a,b}}, σ = {Y, ∅, {a,b}} and η = {Z, ∅,{a},{b},{a,b}}. Let $f: (X, \tau) \to (Y, \sigma)$ be defined by$f(a) = c, f(b) = a, f(c) = b$ and $g: (Y, \sigma) \to (Z, \eta)$ such that $g(a) = g(b) = a$ and $g(c) = c$. Then the functions f and g are somewhat δP_S-open but their composition$g \circ f: (X, \tau) \to (Z, \eta)$is not somewhat δP_S-open function.

Proposition3.4.9: Let f be an open function and g be somewhat δP_S-open. Then g ◦ f is somewhat δP_S-open.

Proof. Let U be open in (X, τ). Since f is an open function, f(U) is open in (Y, σ) and since g is somewhat δP_S-open, g(f(U)) contains non-empty somewhat δP_S-open set in (Z, η). Hence g ◦ f is somewhat δP_S-open.

Proposition3.4.10: For a bijective function f, the following are equivalent:

(a) f is somewhat δP_S-open

(b) If C is a closed subset of (X, τ), such that f(C) ≠ Y, then there is a δP_S-closed subset D of (Y, σ) such that D ≠ Y and f(C) ⊆ D.

Proof.(a) ⇒ (b)Let C be any closed subset of (X, τ) such that f(C) ≠ Y. Then X \ C is open in (X, τ) and X \ C ≠ ∅ as f is bijective. Since f is somewhat δP_S-open, there exists a δP_S-open set V ≠ ∅ in (Y, σ) such that V ⊆ f(X \ C). Put D = Y \ V. Clearly, D is δP_Sclosed in (Y, σ) and we claim D ≠ Y. If D = Y, then V = ∅, which is a contradiction. Since V ⊆ f(X \ C), D = Y \ V ⊇ (Y \ f(X \ C)) = f(C).

(b) ⇒ (a)Let U be any non-empty open subset of (X, τ). Then C = X \ U is a closed set in (X, τ) and since f is bijective, f(X \ U) = f(C) = Y \ f(U) implies f(C) ≠ Y. Therefore by (b), there is a δP_S-closed set D of (Y, σ) such that D ≠ Y and f(C) ⊆ D. Clearly V = Y \ D is a δP_S-open set and V ≠ ∅. Also, V = Y \ D ⊆Y \ f(C) = Y \ f(X \ U) = f(U).

Proposition3.4.11: f is somewhat δP_S-open if and only if for a non-empty open subset A⊆ (X, τ), δP_Sint(f(A)) ≠ϕ.

Proof. Let A ≠ϕ in (X, τ) and A is open. Since f is somewhat δP_S-open, there exists a non-empty δP_S-open set V of (Y, σ) such that V ⊆ f(A).

⇔ That is δP_Sint(V) = V ≠ϕ

⇔ Now δP_Sint(V) ⊆δP_Sint(f(A))

⇔δP_Sint(f(A)) ≠ϕ.

Proposition3.4.12: The following statements are equivalent:

(a) f is somewhat δP_S-open

(b) If A is a δP_S-dense subset of (Y, σ), then f^{-1}(A) is a dense subset of (X, τ).

Proof.(a) ⇒ (b) Suppose A is a δP_S-dense set in (Y, σ). If f^{-1}(A) is not dense in (X, τ), then there exists a closed set B in (X, τ) such that f^{-1}(A) ⊆ B ⊆ X. Since f is somewhat δP_S-open and X \ B is open, there exists a non-empty δP_S-open set C in Y such that C⊆ f(X \ B). Therefore C ⊆ f(X \ B) ⊆ f(f^{-1}(Y \ A)) ⊆ Y \ A. That is A ⊆Y \ C ⊆ Y. Now,Y \ C is a δP_S-

closed set and A ⊆ Y \ C ⊆ Y. This implies that A is not a δP_S-dense set in (Y, σ), which is a contradiction. Therefore f^{-1}(A) is a dense set in (X, τ).
(b) ⇒ (a)Suppose A is a non-empty open subset of (X, τ). To show f is somewhat δP_S-open, using the above lemma, we want to show that δP_Sint(f(A))≠ ∅. Suppose δP_Sint(f(A)) = ∅. Then,δP_Scl(f(A)) = Y. Therefore by (b),f^{-1}(Y \ f(A)) is dense in (X, τ). But f^{-1}(Y \ f(A)) ⊆ X \ A. Now, X \ A is closed. Therefore f^{-1}(Y \ f(A)) ⊆ X \ A gives X = cl[f^{-1}(Y \ f(A))] ⊆ X \ A. This implies that A = ∅, which is a contradiction to A ≠ ∅. Therefore δP_Sint(f(A)) ≠ ∅. Hence f is somewhat δP_S-open.

Proposition3.4.13: Let f be somewhat δP_S-open and A be any open subset of (X, τ). Then $f|_A$: (A, $\tau|_A$) → (Y, σ) is somewhat δP_S-open.
Proof. Let U ∈ $\tau|_A$ such that U ≠ ∅. Since U is open in A and A is open in (X, τ), U is open in (X, τ) and since by hypothesis f is somewhat δP_S-open function, there exists a δP_S-open set V in (Y, σ), such that V⊆ f(U). Thus, for any open set U of A with U ≠ϕ, there exists a δP_S-open set V in (Y, σ) such that V ⊆ f(U). Hence $f|_A$ is a somewhat δP_S-open function.

Proposition3.4.14: Let f be a function and X = A∪B, where A, B ∈ δO(X, τ). Then, if the restriction functions $f|_A$ and $f|_B$ are somewhat δP_S-open, then f is somewhat δP_S-open.
Proof. Let U be any open subset of (X, τ) such that U ≠ ∅. Since X = A∪B, either A∩U ≠ ∅ or B∩U ≠ ∅. Since U is open in (X, τ), $A \cap U$ is open in A and $B \cap U$ is open in B.
Case (i)If A∩U ≠ ∅. Since $f|_A$ is somewhat δP_S-open, there exists $V \in \delta P_S O(Y, \sigma)$ such that V ⊆ f(U∩A) ⊆ f(U), which implies that f is somewhatδP_S-open.
Case (ii)If B∩U ≠ ∅. Since $f|_B$ is somewhat δP_S-open, there exists W∈δP_SO(Y, σ) such that W⊆ f(U∩B) ⊆ f(U), which implies that f is somewhat δP_S-open.
Case (iii)If both A∩U ≠ ∅ and B∩U ≠ ∅. Then, by case (i) and (ii), f is somewhat δP_S-open.

Proposition 3.4.15: Two topologies τ and τ^* for X are δP_S-equivalent if and only if the identity functions i_τ : (X, τ) → (X, τ^*) and i_{τ^*} : (X, τ^*) → (X, τ) are both somewhat δP_S-open.
Proof. Let i_τ and i_{τ^*} be both somewhat δP_S-open
To Prove: τ is δP_S-equivalent to τ^*.
Consider $U \in \tau^*$ such that $U \neq \phi$, then since i_{τ^*} is somewhat δP_S-open. Then there exists $V \in \tau$ such that $V \neq \phi$ such that $V \subseteq i_{\tau^*}(U) = U$.
∴ τ is δP_S-equivalent to τ^*.
Similarly, for all $A \in \tau$ such that $A \neq \phi$, since i_τ is somewhat δP_S-open, there exists $B \in \tau^*$ such that $B \neq \phi$and $B \subseteq i_\tau(A) = A$
∴ τ^* is δP_S-equivalent to τ. Hence τ and τ^* are δP_S-equivalent.

3.5 Somewhat Almost δP_S- Open Functions

Definition 3.5.1: A function $f: (X, \tau) \to (Y, \sigma)$ is said to be somewhat almost δP_S-open provided that if U ∈ δPO(X, τ) and U ≠ ∅, then there exists a non-empty δP_S-open set V in (Y, σ) such that V ⊆ f(U).

Example 3.5.2: Let X = {a,b,c}, τ = {X, ∅, {a}, {b}, {a,b}} and σ = {Y, ∅, {c}}. The identity function $f: (X, \tau) \to (Y, \sigma)$ is somewhat almost δP_S-open and somewhat δP_S-open.

Proposition 3.5.3: Every somewhat almost δP_S-open function is a somewhat δP_S-open function.
Proof. Let f be a somewhat almost δP_S-open function. Consider $U \in \tau$ such that $U \neq \phi$. Then U is δPO in τ. Since f is somewhat almost δP_S-open, there exists a non-empty δP_S-open set V in σ such that $V \subseteq f(U)$,
⇒ f is a somewhat δP_S-open function.

Example 3.5.4: Let $X = Y = \{a, b, c\}$, $\tau = \{X, \phi, \{a, b\}\}$ and $\sigma = \{X, \phi, \{a\}\}$. Let $f: (X, \tau) \to (Y, \sigma)$ be an identity function. Then f is somewhat δP_S-open but not somewhat almost δP_S-open function.

Proposition 3.5.5: The composition of two somewhat almost δP_S-open functions is a somewhat almost δP_S-open function.

Proof. Let f and g be somewhat almost δP_S-open function.

To Prove: $g \circ f$ is somewhat almost δP_S-open function

Let $U \in \delta PO(\tau)$. Since f is somewhat almost δP_S-open such that $V \in \delta P_S O(\sigma)$ such that

$V \subseteq f(U)$ ⟶ (1)

Since, $\delta P_S O(\sigma) \subseteq \delta PO(\sigma)$, here V is $\delta PO(Y, \sigma)$ and moreover g is somewhat almost δP_S-open, then there exists $W \in \delta P_S(\eta)$ such that $W \subseteq g(V)$

(i.e.,) $W \subseteq g(V) \subseteq g(f(U)) = (g \circ f)(U)$

$\Rightarrow g \circ f$ is somewhat almost δP_S-open functions.

Proposition 3.5.6: For a bijective function $f: (X, \tau) \to (Y, \sigma)$, the following are equivalent:

(a) f is a somewhat δP_S-open function

(b) If C is a δ-preclosed set in (X, τ), such that f(C) ≠ Y, then there is a δP_S-closed subset D of (Y, σ) such that D ≠ ∅ and f(C) ⊆ D.

Proof. (a) ⇒ (b) Let C be any δ-preclosed subset of (X, τ) such that f(C) ≠ Y. Then X \ C is δ-preopen in (X, τ) and X \ C ≠ ∅. Since f is a somewhat almostδP_S-open function, there exists a δP_S-open set V ≠ ∅ in (Y, σ) such that V ⊆ f(X \ C). Put D = Y \ V. Clearly, D is δP_S-closed in (Y, σ) and D ≠ ∅. If D = Y, then V ≠ ∅, which is a contradiction. Since V ⊆ f(X \ C), D = Y \ V ⊇ (Y \ f(X \ C)) = f(C).

(b) ⇒ (a) Let U be any non-empty δ-preopen subset of (X, τ). Then C = X \ U is a δ-preclosed set in (X, τ) and f(X \ U) = f(C) = Y \ f(U) implies f(C) ≠ Y. Then by (b), there is a δP_S-closed set D of (Y, σ) such that D ≠ ∅ and f(C) ⊆ D. Clearly V = Y \ D is a δP_S-open set and V ≠ ∅. Also, V = Y \ D ⊆ Y \ f(C) = Y \ f(X \ U) = f(U).

Proposition 3.5.7: If $f: (X, \tau) \to (Y, \sigma)$is somewhat almost$\delta P_S$-open then for a subset A $\in \delta PO(X, \tau)$, δP_Sint(f(A)) ≠ϕ.

Proof. The proof is same as Proposition 3.4.10.

Proposition 3.5.8. The following statements are equivalent:

(a) $f: (X, \tau) \to (Y, \sigma)$is a somewhat almost δP_S-open function

(b) If A is a δP_S-dense subset of (Y, σ), then f^{-1}(A) is a δ-pre-dense subset of (X, τ).

Proof.(a) ⇒ (b)Let A is a δP_S-dense set in (Y, σ). If f^{-1}(A) is not δ-pre-dense in (X, τ), then there exists a δ-preclosed set B in (X, τ) such that f^{-1}(A) ⊆ B ⊆ X. Since f is somewhat almost δP_S-open and X \ B is δ-preopen in (X, τ), there exists a non-empty δP_S-open set C in Y such that C ⊆ f(X \ B). Now X \ B ⊆ X \ f^{-1}(A). Therefore C⊆ f(X \ B) ⊆ f(X \ f^{-1}(A)) ⊆Y \ A. That is A ⊆ Y \ C ⊆ Y. Now, Y \ C is a δP_S-closed set and A ⊆ Y \ C ⊆ Y. This implies that A is not a δP_S-dense set in (Y, σ), which is a contradiction. Therefore f^{-1}(A) is a δ-pre-dense set in (X, τ).

(b) ⇒ (a)If A is a non-empty δ-pre-open subset of (X, τ). To show f is somewhat almost δP_S-open function usingProposition3.5.7, Also we want to show that δP_Sint(f(A)) ≠ ∅. Suppose δP_Sint(f(A)) = ∅. Then, δP_Scl{(Y \ f(A))} = Y. Then by (b), f^{-1}(Y \ f(A)) is δ-pre-dense in (X, τ). But f^{-1}(Y \ f(A)) ⊆ X \ A. Now, X \ A is δ-pre-closed in (X, τ). Therefore f^{-1}(Y \ f(A)) ⊆ X \ A gives X = δ-pcl{(f^{-1}(Y \ f(A)))}⊆ X \ A. Thus A = ∅, which contradicts A ≠ ∅. Therefore $\delta P_S int(f(A)) \neq \phi$. Hence f is a somewhat almost δP_S-open function.

Proposition 3.5.9: Let f be a function and X = A∪B, where A, B ∈ δPO(X, τ).Then if $f|_A$ and $f|_B$ are somewhat almost δP_S-open, then f is somewhat almost δP_S-open.

Proof. Let U be any δ-pre-open subset of (X, τ) such that U ≠ ∅. Since X = A∪B, either A∩U ≠ ∅ or B∩U ≠ ∅ or both A∩U ≠ ∅ and B∩U ≠ ∅. Since U is δ-pre-open in (X, τ), $A \cap U$ is δ-pre-open in A and $B \cap U$ is δ-preopen in B.

Case (i)Now A∩U ∈ δPO(A) such that A∩U ≠ϕ. Since f|A is somewhat almost δP_S-open, there exists V ∈δP_SO(Y) such that V ⊆ f(U∩A) ⊆ f(U), which implies that f is somewhat almost δP_S-open.
Case (ii)Now B∩U∈δPO(B) such that B∩U ≠ϕ. Since f|B is somewhat almost δP_S-open, there exists V ∈δP_SO(Y) such that V ⊆ f(U∩B) ⊆ f(U), which implies that f is somewhat almost δP_S-open.
Case (iii)If both$A \cap B \neq \phi$ andB ∩ U ≠ ∅. Then, by case (i) and (ii), f is somewhat almost δP_S-open.

Proposition 3.5.10: Two topologies τ and σ for X are said to be δP_S-equivalent if and only if the identity function $f: (X, \tau) \to (X, \sigma)$is somewhat almost δP_S-open in both directions.
Proof. The proof is same as Proposition 3.4.15.

CONCLUSION

In general, the composition of continuity holds good. But for weaker and stronger forms of continuous functions it may not hold. ForδP_S-continuous functions, the composition fails by Vidhyapriya et al [14]. In this paper it proved that somewhat δP_S-continuous functions and somewhat δP_S-openness functions fails to hold composition whereas somewhat almost δP_S-continuous and somewhat almost δP_S-opennes satisfy composition property which is given below. For all irresoluteness the composition holds good which is true for somewhat δP_S-irresolute.

Functions	Composition
Somewhat δP_S-continuous	✗
Somewhat almost δP_S-continuous	✓
Somewhat δP_S-irresolute	✓
Somewhat δP_S-open	✗
Somewhat almost δP_S-open	✓

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REVIEW ARTICLE

Status of Women Empowerment and Gender Equality in India- Challenges & Issues

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ABSTRACT

Empowering women is a debatable subject. It was the strong urge of Indian constitution and national leaders just after Independence that women should enjoy equal status with men in society. Although today, in all walks of the fields, we have seen women holding respectable roles. Yet, some prejudice and violence against women' in society has not been fully abolished. Number of women are very less who have been able to determine their ability. Gender inequalities and prejudice are still observed today in India. A century ago, Mahatma Gandhi pioneered in this sector, paving the path for women's emancipation and advancement. An attempt has been made in the article to analyze status of women's & gender equality in India. It is believed that women have equal rights to participate in the activities and has identical freedom than male in today society.

Keywords: Gender equality, Women empowerment etc.

INTRODUCTION

Gender equality can be determined by the engagement of both genders equally in the power distribution and control system in society; empowering women is a crucial area to promote gender equality, concentrating on detecting and resolving power inequalities, and granting women greater authority in managing their own lives. Gender equality in all spheres of life refers to a society in which men and women have equal opportunities, performance, rights, and duties. Women's empowerment is essential condition for long-term progress and the achievement of human rights as well. Family size, progress of family and society are directly proportional to women's status in society. Future generations, families and societies would be able to get ripple effects and benefits from empowered women (Srivastava, 2009).The gender roles are not biologically established in culture; they are social constructs. These assigned roles are not permanent and changeable.

Empowering women is a debatable subject. It was the strong urge of Indian constitution and national leaders just after Independence that women should enjoy equal status with men in society. Although today, in all walks of the fields, we have seen women holding respectable roles. Yet, some prejudice and violence against women in society has not been fully abolished. Number of women are very less who have been able to determine their ability. Gender inequalities and prejudice are still observed today in India. There is such a paradoxical situation that she is often concerned as a goddess and other times only as a slave. In terms of human rights, constitutional and legal provisions, women now enjoy a special equality status with men in India. Yet Indian women have to come a long way in order to reach their current roles.

Gandhi held extremely radical views that the liberation and strengthening of women. Recovery of women was an imperative a part of his valuable application, in which women were agreed unusual concept. He had dynamic vision on the specific issues and issues identifying with ladies, which has frequently been reflected in his numerous compositions and talks. He raised his voice against women feticide, child murder, teenager marriage, widowhood, irrelevant conduct of ladies, abusive behavior at domestic towards ladies, victimization young women tyke, disavowal of training to ladies, settlement framework and so on i.e., all the touching troubles and issues figuring out with ladies of the modern global. Gandhi stated *"Womanhood is not confined to the kitchen simply, while the women is free of the servitude of kitchen that her actual soul might be determined."* In attitude of that as a preliminary circulate in the direction of it, he gave them a clarion call amid the possibility development to return. Human rights of women are violated in different ways. Gender related abuse and discrimination is a major challenge to the human rights nowadays (Bunch, 1990).Cook (2012) pointed out that how human rights are not applicable over women. It is a matter of serious concern that masculine gender is still considered superior and feminism inferior. Despite of providing human rights to both genders, reality of gender equality is quite different. Women are discriminated at every level throughout the world.

Hazarika (2011) wrote that women empowerment is a matter of debate. After independence, Indian government facilitate women with many legal provisions and many national level commitments to ensure equal rights of women. But still nothing happens. Gender biasedness is everywhere in society. Women empowerment is multidimensional in nature. It the process in which women can realize their existence and powers in all spheres of life such as social, political, economic, educational and psychological etc. Nayak & Mahanta, (2012); Mandal (2013). Tandon (2016) revealed that empowerment is holistic and strategic in nature. It is an ongoing process of acquiring varies degree of powers. Basheer, (2018) pointed out the role of education for raising the status of women and removal of gender discrimination. Javed & Chattu(2021) reported that violence against women and girls is a worldwide pandemic. Any kind of violence based on gender is the violation of human rights. In order to reshape our patriarchy society, COVID-19 recovery plan should be framed that will promote gender equality. Young generations should come forward to overcome this patriarchy-based gender discrimination.

In spite of having good development in all spheres of life, Indian women are still in miserable condition. It is very surprised to know that Gender inequality norms are accepted by women themselves and still prevailing in almost all societies of India. Even the birth and rearing of a girl child is considered as burden; wife beating is justified by women of India for one reason or the other. Although, in order to protect the due rights of women and to combat gender discrimination several provisions are made; fewer women mark their appearance in political powers and decision making.

It is disheartening for us that even today, empowered woman is an elusive reality and among the various concerns of developing countries, women empowerment as chief concern of 21st century. Their movements are restricted. They are not allowed to select many professions of their choice. (Barman, 2013).

Obstacles to women empowerment

Despite of having national and international agreements to affirm human rights of women, they are still facing gender inequality in accessing resources. Women are still poor and illiterate as compared to men. They are victim of domestic violence, physically and mentally harassed by society and even family members also.*"Dhol, ganwar, shudra, pashu aur naari- Ye sab tadankeadhikari"* this quote itself reflects the status of women's in India. There are various hindrances to women empowerment such as:

- **Household responsibility**: Women role are confined within the house hold boundaries. They are bounded by many household obligations such as rearing of childrens, cooking, cleaning etc. They have no time even to think about themselves.
- **Lack of education:**Education act as powerful tool for empowerment but large number of women are still deprived of school education. Poor school environment for girls influences their education. Lack of awareness about various educational programmes, weak planning, lack of trained staff and family responsibilities of women has proved to be major obstacles in women's progress and place in society.
- **Poor mobility:** girl mobility is restricted or limited to access learning resources, social and economic opportunities.
- **Lack of desire to succeed:** it is observed that majority of women have lack of desire to succeed.
- **Gender inequality in family:** gender discrimination found everywhere in society and even in families. People still prefer the birth of boy rather than a girl child.
- **Early marriage and dowry system**: in India child marriage &dowry system still exists which hinders their progress.
- **Domestic and social violence:** it is common observation nowadays that women are gang raped, murdered and humiliated almost every day. Women and childrens are victim of human trafficking.
- **Economic Inequalities:**In India, maintenance of families, childrens rearing & education, taking care of elder ones etc. responsibilities lie on women's shoulders. These mentioned responsibilities/works are unpaid. Despite of performing lots of work, women are still dependent on men for money. Henceforth, economic inequalities persist within families.
- **Political backwardness:** As per legal provisions and human rights, women have equal right to participate as candidate in political process of elections power but is still not guaranteed by social and legal institutions. Anti-domestic violence law is still in papers, not enforced. Henceforth, there is a need to re-address gender equality and women's empowerment at all levels of programming and policy making.
- **Health issues:**in developing countries, such as India, women are still more vulnerable to reproductive health problems than men. This is because of both physiological and social reasons. Researches indicates that majority of women are suffering from reproductive organ's cancer. A significant, but preventable, cause of death and disability for women in developing countries is reproductive health problems. Lack of information, medical facilities and services constitutes gender-based discrimination and a violation of women's rights to health and life.

Challenges

- Access to formal education
- Eradication of poverty
- Eradication of hindrances in economic and professional opportunities
- Eradication of political backwardness
- Ensuring Maternal healthcare and safety of women

Steps to uplift women' status

Women empowerment can be achieved through:

1. Adequate Legal provisions to promote gender equality
2. Change in perspective of social mobility of women
3. Encouraging women for self-employment

4. Awareness Programs for gender sensitization
5. Increase Educational Access to women
6. Promoting greater economic opportunities and assets for women
7. Promoting gender equality in education and healthcare services.
8. Promoting participation of women in decision-making and leadership roles
9. Awareness programs for women' personal safety
10. Breaking gender stereotyping and bring about changes inpsychological mindset of society towards women

CONCLUSION

A century ago, Mahatma Gandhi advocated the importance of women empowerment and given a vision and roadmap for the upliftment of the women' status the country. But in reality, status of women in country depicts completely an opposite picture of the empowerment of women. It is a matter of regret for Indians that even in 21st century women mobility is still restricted in household boundaries. They are not supposed to select profession of their interest. Society decides their profession that which profession is suitable for them such as nursing, teaching etc. Women are facing domestic violence within family and in the society these women are already suffering the effects of social evils like dowry system, prostitution etc. On one side empowered women and gender equality are considered as an asset for development and progress of nation; on the other hand, women are still facing violence & inequality in society. In nutshell, no legal provisions or Government can bring women empowerment unless women come with and help to self-empower themselves.

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RESEARCH ARTICLE

The Kinetics of Oxidation of Fe(III)-Citrate by PMS

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ABSTRACT

The kinetics of oxidation of Fe(III)-Citrate by PMS was studied in the pH range 3.0 to 6.0. Detailed analysis of the [PMS]-time profile shows that the reaction follow autocatalysis in this system studied. Alcohol quenching studies showed the absence of radical intermediates such as sulphate ion radicals and hydroxyl radicals and the reaction may proceed through molecular mechanism involving oxygen atom transfer. The oxidative decarboxylation of Fe(III)-Citrate gives acetone dicarboxylate (ADC) which is responsible for the autocatalytic effect.

Keywords: Citric acid, Acetone dicarboxylic acid, oxirane, Peroxy monosulphate,

INTRODUCTION

Citric acid (2-hydroxy propane-1,2,3-tricarboxylic acid), a weak organic acid, is an important intermediate in the tricarboxylic acid cycle, a metabolic pathway involved in the conversion of carbohydrates, fats and proteins to generate energy [1]. Its versatility as ligand for transition metal ions in chemical reactions of biological and analytical interest is widely known. Ultrasound-assisted procedure for the extraction of pectins from various fruit peels [2,3] and Industrial wastes [4] with citric acid as the extracting agent was reported and its an efficient and eco-friendly extraction method. Citric acid chemical extraction technology in combination with ultrasonication was used to remove heavy metals from industrial and municipal mixed sludge. The presence of Fe^{3+}, Al^{3+} and Ca^{2+} improved the extraction efficiency of Cu from the sludge significantly [5]. The biological and industrial importance of citric acid results in an increased attention on its oxidation and no reports are available on the reaction with peroxomonosulphate, inorganic peroxide.

Peroxomonosulphate ion (PMS), commercially available as OXONE® is a powerful oxidant with a reduction potential of +1.8 V [6]. PMS in the presence of transition metal ions generates radical intermediates of high oxidizing capacity. Anipsitakis and Dionysiou [7] studied the activation of PMS by various transition metal ions with special

reference to the catalytic efficiency and the nature of radical intermediates generated. The sulphate radical, with a redox potential of 2.5 V to 3.1 V [8], is a better oxidizing agent than its precursor PMS. Results on the oxidation of cobalt(II) malate complexes by PMS [9] shows that in the pH range 4.0 to 5.9 the probable mechanism may be a molecular (oxygen atom transfer) one.

The α-hydroxy carboxylates undergo oxidative decarboxylation to corresponding aldehydes or ketones [10]. It has been shown that the oxidative decarboxylation proceeds mainly through two-electron process if the carboxylate contains both alpha hydrogen and alpha hydroxyl groups [11,12]. Citric acid/citrate has no hydrogen at hydroxyl carbon but the earlier reports [11, 13-16] suggest that the oxidation leads to carbon dioxide elimination giving acetone dicarboxylic acid(ADC). Aliphatic ketones catalyze the decomposition of PMS [17,18] through a stable intermediate oxirane [19] which is also a powerful oxidizing agent [20-22]. Not only simple ketones, but alpha keto acids/esters such as pyruvates [23,24] are also reported to give oxirane type intermediates. These ketone catalyzed reactions were observed only at neutral or weakly alkaline medium. Preliminary experiments from our laboratory show ADC catalyze the decomposition of PMS in moderate acidic pH(3.0) also. Results from this laboratory on the oxidation of metal (II)-α-hydroxy carboxylates by PMS [9,25] show that the reactions follow auto catalysis. The intermediate between α-hydroxy carboxylate and its oxidative decarboxylation product aldehyde is responsible for autocatalysis. Similar to this, the product acetone dicarboxylic acid may catalyze the oxidation of metal citrates or it can enhance the decomposition of PMS. Therefore, to explore the kinetics of Citric acid we studied the oxidation of citric acid by PMS in the presence of Fe (III) pH range 3.0 – 6.0 and the results are discussed in this report.

MATERIALS AND METHODS

All the chemicals used were of highest purity commercially available and were used as received. The peroxide under the name OXONE®, monopersulphate compound was from Sigma-Aldrich GmbH (Germany). $NH_4Fe(SO_4)_2.12H_2O$ (GR, Merck, India) was the source of Fe(III) ions. Acetone dicarboxylic acid (3-Oxo glutaric acid) was 96% pure and from Sigma-Aldrich GmbH (Germany). This compound was recrystallized repeatedly from ethyl acetate to get a sharp melting point of 135°C[26] and was also estimated by alkalimetry. The peroxide solution was freshly prepared daily and standardized by iodometry. The pH of the reaction mixture was adjusted with citric acid-phosphate buffer. The pH values were adjusted to the predetermined values by adjusting the concentration of disodium hydrogen phosphate while keeping the citric acid concentration at a predetermined value, usually at 0.05 M. The rates of the reaction were calculated by following the concentration of unreacted PMS iodometrically at various times.

The stoichiometry of the reaction was determined at pH 4.8. A large excess of PMS (0.05 M) over citric acid (0.01 M) and the metal ion (0.002M to 0.0002 M) were allowed to stand for six to eight hours and the unreacted PMS was estimated. The unreacted oxidant concentration was very small in Fe(III) ions and this may be due to the self decomposition of PMS catalyzed by the metal ions. Therefore, the stoichiometry was determined with equal concentrations (0.01 M) of PMS and citric acid. The evolution of carbon dioxide from this mixture was confirmed with freshly prepared lime water. The oxidation product from citric acid gave 2,4-dinitro phenylhydrazone derivative which decomposed on heating. The formation of acetone dicarboxylic acid (3-ketoglutaric acid) was confirmed by the color test with sodium nitroprusside [27]. Acetone dicarboxylic acid (ADC) was converted into acetone by the addition of aniline [28] and acetone was estimated as 2,4-dinitro phenylhydrazone (M.pt 126-127°C, lit.128°C [29]). The quantitative estimation indicated that ~ 95-97% of the keto compound is produced per mole of PMS. Therefore the oxidation of citric acid can be represented as in Eqn.(1).

$$\text{Citric acid} + HSO_5^- \xrightarrow{Fe(III)} \text{Acetone dicarboxylic acid} + CO_2\uparrow \text{---(1)}$$

The stoichiometric determinations were also made in the presence of acetone dicarboxylic acid also. One interesting observations was that in the absence of metal ions, the evolution of oxygen gas was observed and was confirmed by

the color test with alkaline sodium dithionite activated with indigocarmine [30]. Also the evolution of carbon dioxide was inhibited and could not be detected with lime water. The change in the concentration of acetone dicarboxylic acid, estimated as acetone, was practically negligible. More over the turn over number, the number of moles of PMS decomposed per mole of ADC, was greater than six. These observations clearly show that in the absence of metal ions the decomposition of PMS is observed as in Eqn. (2).

$$\text{Citric acid} + \text{ADC} + HSO_5^- \longrightarrow \text{Citric acid} + \text{ADC} + O_2 \quad \text{---(2)}$$

However in the presence of metal ions, the reaction reverted to the oxidation of citric acid as confirmed by the formation of carbon dioxide, absence of oxygen gas evolution and an increase in ADC concentration equal to that of PMS concentration. Thus the reaction in the presence of metal ions can be represented by Eqn.(3).

$$\text{Citric acid} + \text{ADC} + HSO_5^- \xrightarrow{\text{Fe(III)}} 2\text{ADC} + CO_2 \quad \text{---(3)}$$

RESULTS AND DISCUSSION

At pH values 3.0 to 6.0 ($3.0 \leq pH < 6.0$), the reaction between citric acid and PMS was very slow and the change in the concentration of PMS, even after five to six hours, was negligible. However at pH ≥ 6.0 the reaction proceeded at a slow speed the conversion of [PMS] was ~ 25% at pH 6.0 and ~ 50% at pH 6.6 for six hours. In the presence of metal ions with the concentration range ~10^{-5} M to 10^{-3} M, the oxidation of citric acid/ citrate proceeded smoothly at a measurable rate even at pH 3.0**.** This clearly shows that metal ions catalyzed the oxidation of citric acid/citrate by PMS. The $[PMS]_t$ - time profile for Fe(III) ion catalyzed reaction at pH 3.0 is shown in **Fig.1A**. The reaction shows an induction period, usually 10-20 minutes after which the rate becomes fast. This feature is observed at all the pH values (3.0 to 6.0) employed in this study. Analysis of the results suggests that the metal ion catalyzed oxidation of citric acid can be explained by simple autocatalysis mechanism shown in reactions (4)&(5).

$$O + S \xrightarrow{k_1} C + P \qquad (4)$$

$$S + C + O \xrightarrow{k_2} 2C + P \qquad (5)$$

The oxidant PMS (O) reacts with the substrate (S) to yield the products C and P. One of the products (C) accelerates the reaction. The rate in terms of the decrease in [PMS] is given in equation (6).

$$rate = \frac{-[PMS]}{dt} = k_1[S][PMS]_t + k_2[C]_t[PMS]_t \quad (6)$$

If the catalyst C leakage is negligible, then $[C]_t$ can be replaced by the term $[C]_0 + [PMS]_0 - [PMS]_t$ where $[C]_0$ is the concentration of the catalyst at the start and ($[PMS]_0 - [PMS]_t$) represent the catalyst produced in the reaction. Since [S]>>[O] eqn.(6) can be simplified as in Eqn.(7) where $k_{1obs} = k_1.[S]$ and $k_{2obs} = k_2.[S]$.

$$rate = k_{1obs}[PMS]_t + k_{2obs}([C]_0 + [PMS]_0 - [PMS]_t)[PMS]_t \quad (7)$$

According to Eqn. (7) the plot rate/$[PMS]_t$ vs. $[PMS]_t$ should be a straight line with a negative slope and positive intercept. This is found to be true **(Fig.2)** and this confirms that all these Fe(III) ion catalyzed reaction follow autocatalysis.

The better values of k_{1obs} and k_{2obs} can be obtained from Eqn. (7), the integrated form of eqn. (6).

$$[PMS]_t = \frac{k_{1obs} + k_{2obs}([PMS]_0 + [C]_0)}{k_{2obs} + ((k_{1obs} + k_{2obs}[C]_0)/[PMS]_0)\exp(t \times (k_{1obs} + k_{2obs}([PMS]_0 + [C]_0)))} \quad (8)$$

The kinetic constants k_{1obs} and k_{2obs} can be obtained from Eqn. (8) by non-linear regression analysis. The k_{1obs} and k_{2obs} values calculated from Eqn. (7) and Eqn. (8) are agreeable. However, as a rule we have calculated the k_{1obs} and k_{2obs} from non-linear regression analysis of Eqn. (8) using the values from the derivative plots (Eqn.7) as the approximate initial inputs for non-linear regression. The product analysis (Eqn. (1)) shows that the oxidation product from citric acid is acetone dicarboxylic acid. This is in accordance with the earlier reports [11,13-16]. Therefore, one would expect that the catalyst 'C' in eqn.(4) may be acetone dicarboxylic acid(ADC). The proposed kinetic scheme (Eqns. (4) & (5)) can be verified by calculating kinetic constants k_{1obs} and k_{2obs} with the added acetone dicarboxylic acid (Fig.1B).

Careful analysis such as the plot of (rate/$[PMS]_t$) vs. $[PMS]_t$ for the curve (Fig. 2) shows that the reaction also follows autocatalysis. The kinetic constants for the Fe(III)-Citric acid –PMS system with no ADC at the start (Fig.1A) calculated by Eqn.(8) are : k_{1obs} =1.01±0.07 ×10^{-5} s^{-1} and k_{2obs} = 17.56±0.45 ×10^{-2} $M^{-1}s^{-1}$. The kinetic constants k_{1obs}, k_{2obs} and k_{obs} were calculated at different experimental conditions. The effect of sulphate ion concentration on the rate was studied and the concentration used was 0.05 M to 0.25 M. The results show that sulphate ion has no effect on the rate. However all the kinetics studies were carried out only in the presence of 0.05 M sulphate ion. The kinetic constants k_{1obs}, k_{2obs} and k_{obs} were found to be independent of citric acid concentrations in all the reactions.

$k_{2obs} = k_2[Fe(III)]$ (10)

The initial oxidation of Fe(III)-Citric acid by PMS is very slow and this may be the reason why the initial part of [PMS]-time profile is almost parallel to time axis(Fig.2A).Therefore k_{1obs} values of Fe(III)-citrate may be associated with high uncertainty. Moreover the k_{1obs} values differ from k_{2obs} by a factor of ~10^{-4}. The kinetic studies were usually carried out with $[PMS]_0 \approx 4.0\times10^{-3}$ M and hence $k_{1obs} \langle\langle k_{2obs}[PMS]_0$. The k_{1obs} values for Fe(III)-Citric acid system calculated from Eqn.(8) by non-linear regression may involve large fluctuation and this may be the reason that we could not get a correlation between k_{1obs} and [Fe(III)].

In ferric citrate oxidation k_{2obs} showed a linear correlation with [Fe(III)]. The plots k_{2obs} vs.[Fe(III)] were straight lines passing through (approximately) origin. The kinetic constants values were also calculated in the presence of aliphatic alcohols such as ethanol and tert.butyl alcohol so as to ascertain the formation of radical intermediates such as sulphate ion radical and or hydroxyl radical [7,8,31,32]. The concentration of alcohols used were ~100: 1 molar ratio of the alcohol versus the oxidant PMS. The alcohols had no effect on the rate constants and this suggest that the possibility of the reaction mechanism involving radical intermediates such as $SO_4^{\cdot-}$ and $OH^{\cdot}$ radicals can be neglected.

Peroxomonosulphate dissociates to give the dinegative anion as in Eqn.(11).

$$HSO_5^- \underset{\longleftarrow}{\overset{K_d}{\longrightarrow}} SO_5^{2-} + H^+ \qquad (11)$$

The pKa value of PMS is 9.4 at 25°C[33] and at the experimental pH values (3.0 to 6.0) the equilibrium will be shifted towards left, that is all the PMS will exist almost completely as HSO_5^-.

The Fe(III) ion catalyzed oxidation of Citric acid

Citric acid (H_3Cit) is an alpha hydroxy aliphatic acid and has four removable hydrogen atoms (as protons), three from carboxyl groups and one from the hydroxyl group. Therefore, citrate ion acts as a multidentate ligand towards

transition metals. The Fe(III)-citrate complex equilibriums in solution have been investigated extensively and the various complexes can be represented by the following equations.

$$Fe(III) + Cit^{3-} \underset{}{\overset{K_1}{\rightleftharpoons}} Fe(Cit) \quad \text{------(12)} \qquad Fe(III) + Cit^{3-} \overset{K_2}{\rightleftharpoons} Fe(H_{-1}Cit) + H^+ \quad \text{----(13)}$$

$$Fe(III) + H^+ + Cit^{3-} \overset{K_3}{\rightleftharpoons} Fe(HCit^{2-}) \quad \text{----(14)} \qquad 2Fe(III) + 2Cit^{3-} \overset{K_4}{\rightleftharpoons} Fe_2(H_{-1}Cit)_2 + 2H^+ \quad \text{----(15)}$$

Ferric citrate exists mainly (>90 %) as complexes of deprotonated hydroxyl group and almost all the metal ions exist in the complexed state even at the lowest pH (3.0) used in this study.

The quenching studies with aliphatic alcohols such as ethanol and tert-butanol show that the formation of radical intermediates such as $OH^{\cdot}$ and $SO_4^{\cdot-}$ [7,8,31,32] in the present investigation can be excluded. Therefore the oxidation of metal-citrate by PMS proceeds through two electron processes, probably oxygen atom transfer, and the product is ADC. The catalyst ((C) in Eqn.(2)) ADC enhances the rate of oxidation through an intermediate with PMS. The detailed kinetic scheme for the oxidation of metal citrate by PMS can be written as in the following equations.

$$\text{Fe(II)-Citrate} + HSO_5^- \xrightarrow{k_u} \underset{\text{(ADC)}}{\text{Acetone dicarboxylic acid}} + CO_2\uparrow + \text{Products} \quad \text{--(16)}$$

$$\text{ADC} + \text{Fe(III)-Citrate} \overset{K_7}{\rightleftharpoons} \text{Oxirane} \quad \text{---(17)}$$

$$\text{Oxirane} + HSO_5^- \xrightarrow{k_c} \text{2ADC+ Products} \quad \text{-----(18)}$$

The first step in the autocatalytic kinetic scheme shown in Eqns. (16) to (18), corresponds to the oxidation of Fe(III)-citrate by PMS to give an intermediate 'C' (Eqn. (2)). The stoichiometric determination shows that citric acid in the presence of metal ion is oxidized to acetone dicarboxylicacid (ADC). The oxidative decarboxylation of citric acid is reported [34,35] as early as in 1910. The enzymatic oxidative decarboxylation to ADC is also reported [36] to occur in nature. The oxidations of aliphatic poly carboxylic acids have been studied in detail [11,12]. The oxidation of aliphatic poly carboxylic acids with both alpha hydroxyl group and α-hydrogen usually proceeds through two electron processes. The oxidation mainly involves the oxidative decarboxylation giving an aldehyde or ketone with one carbon atom less than the parent compound and carbon dioxide [11,12]. Citrates have no α-hydrogen atom, but the elimination of carbon dioxide and hydrogen bonding with the acetone dicarboxylate product may enhance the oxidation of citric acid/citrate [15]. Therefore, Eqn. (16) corresponds to the oxidative decarboxylation of Ferric(III)-citrate to Acetone dicarboxylate (ADC).

Literature information suggest that aliphatic alcohols containing α-hydrogen, such as ethanol react at high and comparable rates with hydroxy and sulphate radicals [8,31,32]. Alcohols with no α -hydrogen such as *tert*-butyl alcohols are used as effective quenching agents for hydroxyl radicals but they are found to react much slower with sulphate radicals [8,31,32]. The difference in reactivity with aliphatic alcohols ethanol and *tert*-butanol has been used to gain an insight in to the nature of the radical intermediates produced/involved in the reaction with PMS. Burrows and co-workers [37-39] have showed that the alcohol quenching studies can extensively be used for radical identification in M(II)-PMS system. They used high concentrations of alcohols so that the molar ratio of alcohol versus the oxidant/metal ion corresponds to ~500:1. In the present investigation, the quenching studies were carried out with high concentration of alcohols, namely ethanol 0.00 M – 2.57 M and tert.butanol 0.00 M –1.58 M. Perusal of results in the presence and absence of ethanol and tert.butanol shows no remarkable changes in the k values. This implies the absence of radical intermediates and the oxidations of Fe(III)-citrate by PMS may proceed through the molecular mode. The first step is the formation of hydroperoxide intermediate (Fig.3). This reaction is similar to the reactions of alcohols with hydrogen peroxide [40]. Earlier works [33,4] on the decomposition of PMS suggest a transition state involving the nucleophilic attack of SO_5^{2-} ion on the oxygen atom of the HSO_5^- ion resulting an atom transfer to give a hydroperoxide type intermediate. Usually, the reaction with peroxide proceeds through nucleophilic substitution followed by oxygen atom transfer. The complexation with transition metal ions enhances the ionization of citrate hydroxyl group [42,43] thereby leading to a metal-oxide bonding. The hydroxyl group is completely ionized and the tetraionized citrate complex predominates in Fe(III) citrate as in Eqns. (13) and (15).The introduction of Fe(III)-ions results in the formation of tetra ionized citric acid, in the complexed state, even at pH 3.0.

This may be the reason why the metal ion catalysed oxidation is observed even at the acidic pH. Under the experimental conditions, namely [citric acid] >> [Fe(III)], the metal ion complexes mainly with the tetra ionized citric acid.

Therefore, in Fe(III)–citrate, the hydroxy group involves in the bonding with the metal ion and this may protect the hydroxy group from the interaction with the peroxide. The observed results are in accordance with this expectation. The autocatalysis reactions were reported in the oxidation of α-amino acids by PMS in acidic pH 4.0 to 5.2 [44,45]. The oxidative decarboxylation of α-amino acids gives aldehyde which then reacts with the amino group to give hemiaminals. The hemiaminals may be more reactive than the parent α–amino acids resulting to autocatalysis. Result from this laboratory on the oxidation of metal(II) alpha-hydroxy carboxylate by PMS [9,46] show that the reaction follow autocatalysis. Results from this laboratory [46] showed that the oxidation of Cu(II) and Ni(II)–tartarate by PMS follow autocatalysis mechanism. The mechanism involves the initial oxidation of metal(II)–tartarate to tartronic acid semialdehyde. The aldehyde interacts with the hydroxy group of the tartarate to give a hemiacetal which may be responsible for the autocatalysis. The careful analysis of the above autocatalysis reactions cited shows that these reactions have one common feature, namely the substrate undergoes oxidative decarboxylation and the intermediate product is an aldehyde. Then the aldehyde reacts with the parent compound to give more reactive hemiaminal/hemicaetals resulting autocatalysis. By analogy with M(II)–tartarate-PMS reaction, we can assume that some intermediate is formed between Fe(III)–citrate and acetone dicarboxyic acid (ADC) may be responsible for the autocatalysis.

Aliphatic ketones catalyze the decomposition of PMS [17,18] through a stable intermediate oxirane19 which is also a powerful oxidizing agent [20-22]. Not only the simple ketones, but also alpha keto acids/esters such as pyruvates [23,24] are also reported to give oxirane type intermediates. These ketone catalyzed reaction were observed only from our laboratory shows that the ADC catalyzes the decomposition of PMS in the moderate acidic pH(3.0) also. Therefore, oxirane may be the intermediate formed during the oxidation of Fe(III)-citrate. Experimental results show that the autocatalyzed oxidation proceeds only in the presence of metal ion, such as Fe(III) and the rate of the reaction is first order **(Eqn. (10))** in metal ion. This means that the complexation with metal ions somehow enhance the formation of intermediate (oxirane). The reaction mechanism, for the oxidation of Fe(III)–citrate is shown in Fig.3.

The rate equation for the kinetic scheme shown in Eqns. (16)-(18) is given in Eqn. (19).

$$\frac{-d[PMS]}{dt} = k_u \times [Fe(III)-citrate] \times [PMS]_t + k_c K_7 .[ADC] \times [Fe(III)-citrate] \times [PMS]_t \quad (19)$$

If there is no loss or leakage of the catalyst in the oxidation of the acetone dicarboxylic acid (ADC), the kinetic scheme suggests that the concentration of this catalyst can be equated to the amount of oxidant, [PMS], reacted. As discussed above, different Fe(III)-citrate complexes, such as Fe(III)-L, Fe(III)-H_{-1}L etc., exist at the experimental conditions. These complexes may react with different rate constant values. This is shown in the following equation.

$$k_u[\text{Fe(III)-Citrate}] = k_{u1}[\text{Fe(III)-Citrate1}] + k_{u2}[\text{Fe(III)-Citrate2}] + .. \quad --(20)$$

Where k_{u1} is the rate constant value for Fe(III)-citrate1 etc. Similarly, we can express $k_c K_7$ also. Simple calculation on the concentration of the complexes under the experimental conditions shows that the concentration of a given complex is constant at all pHs from 3.0 to 6.0. Therefore, we can approximate the concentration of a complex as proportional to [Fe(III)], that is [Fe(III)-citrate1]=C1.[Fe(III)] where C1 is a proportionality constant.

$$k_u .[Fe(III)-citrate] = C1.k_{u1}.[Fe(III)] + C2.k_{u2}.[Fe(III)] + ...$$

$$= [Fe(III)](C1.k_{u1} + C2.k_{u2}. + ...)$$

$$= [Fe(III)].\text{(Constant)} \quad (21)$$

From the limited data over the pH range (3.0 to 6.0) it can't be calculated the individual rate constant values. Therefore Eqn. (19) can be simply represented as in (22).

$$\frac{-d[PMS]}{dt} = k_u.[Fe(III)].[PMS]_t + k_c.K_7.[Fe(III)].([PMS]_0 - [PMS]_t).[PMS]_t \quad --(22)$$

Eqn. (22) is identical to Eqn. (3) if $k_1 = k_u.[Fe(III)]$ and $k_2 = k_c.K_7.[Fe(III)]$ and it will explain all the experimental observation. Therefore, Eqn. (22) explains all the experimental observations, namely the linear correlation between k_{2obs} vs. [Fe(III)]and independent of k_{1obs} with respect to [Fe(III)]. The results **(Table-1)** show that the rate constant for the autocatalyzed part does not changes with increase in pH.

Therefore, k2 can be expressed as in Eqn. (23).

$$k_2 = Kk_c[Fe(III)] \quad (23)$$

CONCLUSION

The kinetics of oxidation of Fe(III)-citrate by PMS were studied in the pH range 3.0 to 6.0. The oxidation of Fe(III)-citrate by peroxomonosulphate proceeds through autocatalysis. The oxidation of citrate results acetone dicarboxylic acid which may react with Fe(III)-citrate to form a oxirane type intermediate. This oxirane intermediate may react with PMS faster than malate and this may be the reason for autocatalysis.

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Table 1: Kinetic constants for the Fe(III)-Citrate oxidation

pH	$k_2(M^{-2}s^{-1})$	
Temp(°C)	**25.0**	**31.0**
3.0	71.22(±8.34)	102.78(±12.65)
4.0	70.59(±8.70)	123.33(±4.15)
4.8	53.69(±4.64)	85.17(±7.01)
6.0	56.64(±3.35)	95.63(±12.34)

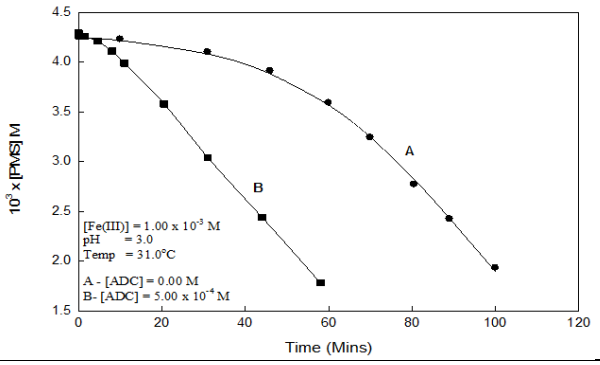

Fig 1: PMS-Time profile for Fe(III) ion catalyzed reaction.

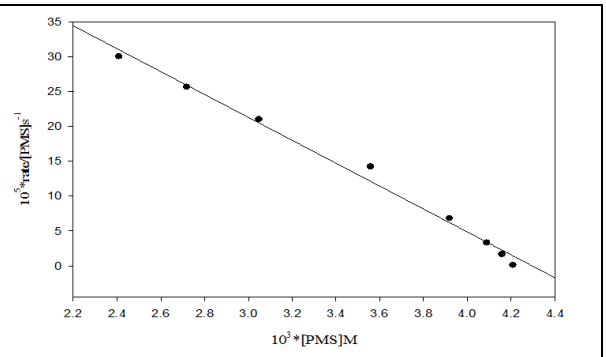

Fig 2: Plot of (rate/[PMS]) vs. [PMS] for Fe(III) ion catalyzed reaction at 31^{0}C and pH 3.

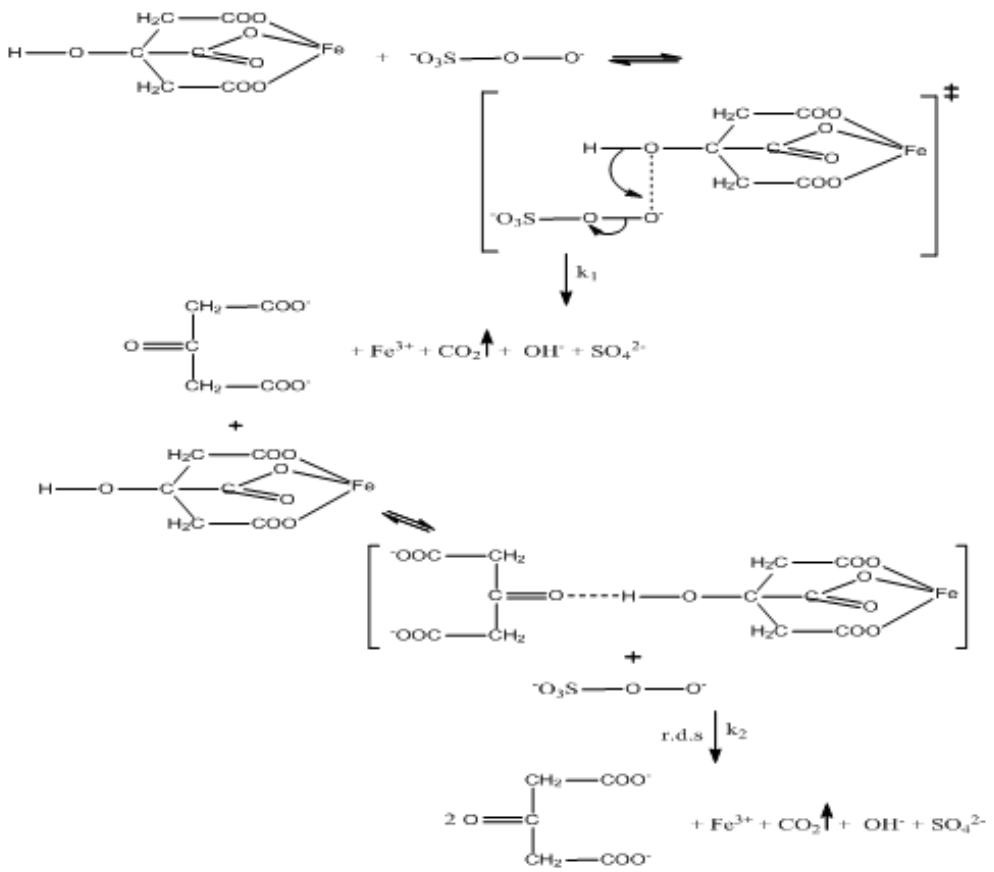

Fig: 3 Mechanistic scheme

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RESEARCH ARTICLE

Relative Link Chain Routing based Congestion Control using Adaptive Deterministic Packet Marking Algorithm for Improving Energy Efficiency in MANET

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ABSTRACT

By the communication medium Mobile Ad Hoc Network(MANET)becomes tremendous of self-configurable collection data transmissions that do not have any central internal infrastructure to control network services. Lack of congestion control as arrives problems with these networks. Due to the dynamic topology in MANET, a node cannot store data by passing it all. Mobile nodes varies on transmission accounting for a variety of challenges due to loss of transmission route packet drops leads network failure get poor congestion. Setting up and degrading energy consumption is a key attribute used by networks. To propose a Relative Link chain routing based congestion control (RLCR) using adaptive deterministic packet marking algorithm (DPMA) for improving energy efficiency in MANET. Send target information through multiple directions to simultaneously reduce node-to-end delay. In these protocols, traffic sent via the route affects the subsequent route, and the delay increases due to inadvertent use of the adjacent route. This is because competitions between adjacent nodes are repeated in order to obtain joint connections on adjacent paths. Referred algorithm attempts to identify different paths between source and target nodes and reduce congestion. Since the path is lost and received and optimized for energy consumption in all directions in communication routing performance as well as improved by this method.

Keywords: Adaptive packet transmission, Congestion Control, Link chain Routing, Node Discovery, Routing Delay, Traffic prediction.

INTRODUCTION

Mobile ad-hoc networks (MANET) include mobile independent mobile nodes that can be randomly placed on the network and can be left or joined. These nodes communicate and communicate with each other through wireless connections. Ad Hoc allows quick addition of new equipment. Each device in the network can move freely in any direction, resulting in a dynamic topology. Designing a network has many challenges and problems with its design, and the task is very difficult. Each node can also act as a router that sends packets from the target source. These nodes can be any personal devices such as laptops, mobile phones, and other mobile ad hoc network applications that range from small networks to very large dynamic networks. A mobile ad hoc network (MANET) (often without a fixed base station) and a fast-changing topology is an infrastructure. There is a significant shortcoming of wireless ad hoc networks that affect network capabilities in terms of congestion loss rate and bandwidth.

Time and effort can be lost through the use of congestion recovery mechanisms. MANET nodes do not have any centralized management engineering. Figure 1 shows the congestion traffic at communication delay tolerance Nodes and peer-to-peer connections are directly related to each other at any time, such as relocating the central access point node, which can be violent and unusual in topology, without involving MANET. The route between a raw node and a target manatee has multiple hops. MANET serves as a router to transmit data to each node. In order to expand and reduce the capability of congestion wireless ad hoc networks, various methods have been developed to address the source of congestion. Then, according to the transmission of congestion state, raw node retransmits or delays. The packets are uniformly distributed in the middle of each of its nodes engaged in broadcasting. The proposed protocol and the network lifecycle also serve as a hub for routing and congestion center transactions to improve network performance on other nodes. Choosing the right path in the route network provides the means. Routing protocols provide connectivity between routers and the packet from the target source through the appropriate route between the selected sender and the receiver. Designing a routing protocol is a very challenging task. Various cult protocols have been proposed so far.

Congestion Avoidance

In the network layer, when the message traffic is too crowded, it reduces the network response time. With the impact of increased congestion delay, performance decreases. If there is an increase in delay, it will make the situation worse. Wireless sensor network (WSN), computing savings, communication bandwidth, and most importantly, many sensor nodes are depleted of resources in terms of energy. One of the main challenges of these networks is the crisis triggered by factors from multiple knots, such as bandwidth, node buffer overflow, feedback in transmission media, propagation rate, and so on - to the nodes of a data transmission tank. Our concern is congestion avoidance, not congestion control. The difference between these two words is somewhat subtle. In short, the congestion avoidance program allows the network to operate in low latency and high-performance areas. These programs prevent the network from entering a crowded state in which packets are lost. We will discuss and discuss flow control and congestion control in the next section, and the rules for congestion avoidance. It has participated in a number of design challenges with Internet congestion control and resource allocation mechanisms, including the lining of networks with today's wireless wire. They continue to scale, twist, reach, and coordinate and expand the nest. It is very important to have a deeper understanding of how their resources are controlled with resources, and how the allocated resources can help them achieve congestion control and quality of service. Computer networks, such as those designed by the Internet, including micro-packet level control mechanisms, are formulaic and complex in terms of macro level after they understand the flow state.

Routing based Mobile computing

Mobile hosts like laptop, have powerful CPUs, big key memories, and routing on communication creates better compaction medium such as delay tolerance, on demand routing, distance routing etc. In at the same time, network connectivity as in form on MANET, including support also based on wireless network products Radio and infrared. If the static topographic collection of mobile nodes is evolving the instant network is called MANET Route warning. These networks this is due to the fact that it is very attractive for military installations that are capable of rapid deployment and reconstruction. Provides security and privacy attacks to hide routing, source and destination-related secure routing protocol. Active attackers can change, swap, or drop packets the slave network depends on its nature. So in much needs the congestion control determined by routing much taken packet transmission to improve the network and packet.

LITERATURE REVIEW

Mobile Edge computing (MEC) is an important factor in expanding vehicle use levels because it ensures low latency and high bandwidth requirements [1]. Whether it is a road hazard or damage to other vehicles, crashes include information on the vehicle and state information received, an anti-collision service, which is allocated to the receiving infrastructure much, will be processed and selectively notified of each vehicle. At MANET, personal wheels have a connection another via the remote platform [2]. MANET's node there is no centralized management engineering. However, get a flexible and low-cost mobile network with multiple hop data Transmission, however, remains a primary issue for traffic congestion Reduced network performance. Based on fuzzy logic algorithm cross-layer congestion detection and routing protocol. This protocol, whenever a network event is occurring, causes the event type to handle its identity accordingly.

One of the major causes of congestion is network inefficiency [3]. When traffic on the network increases beyond the specified capacity, congestion occurs. Traditional routing algorithms are not efficiently solved and cannot solve signal strength problems. Quality of service (QoS) is a quality requirement for data communications. Related to mobile ad-hoc network (MANET) real-time applications. Therefore, many routing protocols have been established for enhanced latency and energy efficiency, and there is a large optimization of the elaborated real-time system, which is the primary target of resource-constrained environmental resources. A new paradigm that has led to the creation of a large amount of computational workload and excessive networking using smart artificial intelligence algorithms in smart cities. Fog Computing, one of the mobile edge of computing formulas, installs some servers on the edge of mobile networks to solve these problems [4] [5]. One suitable solution for extending relationship battery life is offloading demanding large processing in conventional centralized cloud applications. However, the calculation of this option during the period of delivery plus the cloud and re-deletion application introduces a significant implementation delay to the cloud [6]. The Intelligent Manhole Cover Management System (IMCS) is one of the most important bases for smart cities to avoid frequent sewer crashes. There is a threat of personal protection, which is against the purpose of a smart city to seal the sewer, displace loss and damage [7]. Mobile cloud, like the emerging and future computing model of computing (MCC), can significantly increase the computing power of the SMD computing tasks by leveraging enriched SMD computing tasks for smart mobile devices (SMD) [8] [9]. Mobile Edge Computing (MEC) is a promising example of wireless enabled mobile devices that have recently come to improve data

Wireless sensor networks such as handle low-throughput networks and networking to noise ratio (population). The task of each computing wireless device (WDS) is to perform any part or full offload of the Mac server. Our goal is to get an online algorithm and the best fulfillment task is to change the wireless resource allocation over time [10]. Wireless channel conditions. Mobile cloud offloading computing is a promising solution to increase the computing power of mobile devices. In this article, consider personalized mobile devices, where personalized mobile devices can be offloaded by multiple access points or mobile cloud computing platform in order to reduce their computing

costs [11]. Mobile cloud computing provides the use of mobile internet cloud computing technology. With the advent of the 5G network era, mobile cloud computing offers lots of computing possibilities Parallel with various mobile terminals [12]. Computing Environment Mobile cloud-enabled resource-rich applications are computing and data cloud-free at the first load. However, landing on a remote cloud is always the best solution for long latency and energy consumption associated with intermittent wireless communication [13].

Mobile cloud computing or fog computing implies intensifying computing methods from mobile devices [14]. For example, in a cloud or intermediate cloud that saves resources, time and effort are on the mobile device [15] [16]. This article proposes a new no clouds or fog were available to resolve the situation. First, sensor network utilization queue-based network modeling, then techniques for making linear planning decisions. Various centralized and distributed algorithms are then presented thereby improving the overall efficiency of the system [17]. Stream processing system of multi-layer data, i.e. edge-stream, mobile computing edge (mech) to use the computing power of the entire network as a whole, such as the cloud top layer core (CC). Server on the middle layer, and on the edge device (ets) bottom layer [18]. Application sharing, which runs on location and remote areas play an important role in high performance mobile offloading systems. Best regional mobile devices mobile cloud computing (MCC) or mobile edge computing (MEC) will allow you to get many benefits [19]. Fixed partitioning solutions and speed assumptions system for unstable resources (network disconnection, bandwidth fluctuations, network latency, etc.), and service node (different speed mobile devices and Manet / edge servers, storage, etc.), due to wireless network with fixed bandwidth not suitable for unloading [20].

MATERIALS AND METHODS

Congestion sensors can affect various components of nodes that affect energy consumption and service quality. Therefore, one of the most important accounts for the avoidance, diagnosis and control of wireless sensor networks is to develop, and advanced technologies. In this paper, various methods of congestion control have been studied, and a new classification of concentrations based on the principle of congestion control has been proposed and uses different methods of detection and congestion control, as well as effective parameters. The issue of congestion control is more difficult to handle than connection-based protocols to address this proposed Relative Link chain routing based congestion control using adaptive deterministic packet marking algorithm for improving energy efficiency in MANET. Network resources are allocated to the network before the connection is established. Therefore, a simple way to control congestion is to prevent new connection attacks when congestion is detected. Calculate the packet spread rate of each node every step. If the data packet spread rate is too small, the node can continue to have a path where the data packet is dropped where the route is valid.Figure: 2 show the Relative Link chain routing based congestion control. A regional node evacuates the congestion can be effectively detected using the Continues links chain on determinist algorithm using a balanced message packet transition on route system.

Traffic prediction

Network traffic prediction is a very important on communication medium also structured to more complex and serious problem in network management and design. This is a model of a new algorithm in relative linking chain to verify the continuity of the route that performs in this article when modeling factors on traffic and updating the model's adaptive capabilities. To create a self-similar series for simulated network traffic and perform some experiments. This technique is used to infer the last interval of the average value of past observations n. This technique provides the same weight as all previous ideas. The number of past observations using Node (N) is called the order in MA (Multi-access).

Relative link routing Chain routing

The routing enhances the relay node transmission of packet, that the destination and intermediate node path are stored in the destination path routing table. This procedure is entered if a new path is required to be installed. Some

delays in this process are expected to be introduced, but the only successful retrieval is the response to the data packet target, which reduces waste to the network, using short pulses of the network, which can cost tons.

Algorithm 1

Input: Initialize the Mn as node Manet topology Mn1, Mn2…
Output: verified relative closest node
Step1 :If node Ling Nl→neighbor link equals{
Trans the closest node Cn
Cn→trans packet→Mn
Else
Reject node dynamic change resemble Cn;}
Step 2: Compute the information of Route R ant its ID Node
Step 3: Check **if** (Mn1 continuity Rn++)equalsthen
Packet transmission begins Cn→Mn++
Step 4: Compute the relative masseur between the Mn nodes
For equals Weightage on distance vector transmission Medium
Make continuity topology Link
Update Route Node Id Cn←Mn
Step 5: Compute relative weightage of transmission link Cn
Cn→Mn distance vector time equals
Remain same Mn
End for
Step 6Finalize closest Link As transmission Path Return
End if

This movement of the nodes in the manatee move freely without any obstructions from relative link. To track all mobile nodes, when they change their location, the mobile node on the network should send an update message. If the number of update messages is too large, a new path detection function must be installed to update the new location and intermediate node in a routing table. If any update message is received, we may assume that all routes are in the same location and that other information should not be taken in different primary senders and have the same detected paths. This application will reduce the overhead required by path discovery technology.

Regional node discovery

The Relative measure return the continues Link of route optimization to estimate how accurately a congestion can occur when an accident occurs. This type of congestion varies not in terms of traffic density; but it occurs because the road capacity is reduced to fill cars traveling with them. This reduction in efficiency is sudden, and the speed of the tooth and spread is very fast. The way it selects the appropriate data rate between links and allows new flows from the target source determines the storage delay. The route must meet the service delay requirements that are requested by the end-to-end packets must be less than the required delay.

Algorithm – Proposed algorithm

Step 1: Initialize the return traffic condition on route link TL
Step 2: By consideration traffic Ton Link L
Compute the node delay n route –Dy→LRP
Link on route probation on delay Path Dp
Step3: Compute the Coverage of distance on N nodes
Region Terms X and Y coordinated function remains
R →Dp(X,Y)
Step 4: compute For L = 1 to N Evaluate XL Link and region coverYL

Termscoverage XL and YL on region discovery
End For
Step 5: Using DX, find the distance to the node with the highest regularization (distance)
Step 6: Find the other connection node One: The coverage node on shortest Link is the direction of the Attain node= A1, A2 on region coverage on DPStep 7 :return the delay location Node resembles Mn

When the raw data communicates with the target node, it checks its routing table for effective routing to the target. If it looks, it sends the hop node parcel next to the target path. However, if the path is not a valid path and is not on the table, the source path detection function starts. During the route discovery process, the source generates a Route Request (RREQ) packet. In our simulations, we compared the efficiency of our proposed method with the conventional method of predicting traffic density in terms of an algorithm for traffic congestion forecasting in a fast-changing pheromone interaction model. Our method is more accurate than the traditional scheme. Traditional methods of predictive accuracy degradation, especially long-term inference, are used. However, we can maintain accurate predictions of our method.

Adaptive deterministic Packet transmission Protocols
Packet transmission on continues factor on relative link makes an contains communication and improve the network life time. Because of the transmitting mode and the intranet mode, we have added sleeping modes, which provide improved stability of the network, by listening; In order to maintain power over the network, nodes can run different modes of networking. In broadcast mode, the node is a node that can send other nodes via MS or Unicast. In the receiving mode, this node is a member of our Multicast Team or Unicast Broadcast Receiver.
Input: Initialize the route Link on regional Manet coverage
Output: optimized congestion occurrence:
Step 1: Compute the route Link RL on Mobile Node
Step 2 For Random node Rn varies each state{
Check Route Node variation delay tolerance
For(Mn→RL) each Link verifies packet flowReturn Packet on continues closest neighbor node}
Step 3: If progressive transition nodes refresh the delay on each link
Step 4 Determine the flow and delay →weightage Nw←delay Dw.Mn→delay free congestion if Yes
Step 5: Packet retransmission on close node on RL→L
End If; Return Mn End for

The node that is not contained in the multicast message means that it has enough energy to do so, in the manner of asking if it is ready to receive, but other nodes must transmit it. In a sleep mode device, there is a lack of node energyIt runs in sleep mode until the battery is charged. In some cases, node multicast messages can participate in sleep mode. In this case, after charging the battery, it can request unicast messages on the next node of the node. In our tree-based design, we need to know all the clusters with large residual nodes.

RESULTS AND DISCUSSION

In the real-time proposed routing-based approach, regional multi-factor approximation simulates the performance of various factors based on simulation. The simulation is performed using network simulator, and the results obtained are in this section. Table 1: Simulation Details .The information about the evaluation of proposed approach is presented in Table 1. Various factors are measured in the performance of different methods. The results of this review are presented in this section. The ratio of throughput introduced by different approach in different simulation time is presented in Table 2. RLCR-DPMA approach produced higher throughput performance at all the simulation period than other methods.

Output rate achieved by various methods is measured and compared with the results of other methods. The comparison results are presented in Fig. 3, and the debit RLCR-DPMA method significantly increases the output compared to the previous methods. Table 3 Performance on Packet Delivery Ratio The result on packet delivery ratio has been measured and compared in Table 3. The result MFQoS algorithm has achieved higher PDR than other methods. PDR performance is measured by various technologies and compared with the results of other methods. Comparison indicates that RLCR-DPMA algorithm achieves higher PDR than previous methods.Delay is a measure of the time at which the packet is sent between any source and destination. Total time is measured based on the number of groups taken and the number of groups taken. Delays generated by various methods can be measured and presented. Table 3 presents the algorithm RLCR-DPMA produced shorter delays than other methods. Figure 5 shows the packet delay ratio produced by various methods and RLCR-DPMAalgorithm has produced less latency value compare to other methods.The ratio of packets transmitted by different methods is measured based on the number of packets sent and received. The performance of the upper pocket transfer rate was measured and the results of other methods were compared.

CONCLUSION

Wireless ad hoc network provides computer and communication facilities, anywhere. Due to energy resources alone, energy use efficiency is an important issue for wireless ad hoc networks. If used correctly, the directional antenna provides further discounts on energy. Due to the rebuilt antenna, it offers a high compatible high energy savings. In this proposal RLCR-DPMA produce higher performance than similar methods, detailed comparisons have been made to measure the performance of Manet directional antennas. The proposed algorithm works well when using basic hardware directional reconstruction antennas. The proposed system offers greater energy savings realizes congestion avoids and improved network lifetime than existing protocols.

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Table 1: Simulation Details

Parameter	Value
Tool Used	Network Simulator
Number of Nodes	100
Transmission Range	100 meters
Simulation Area	1000×1000 meters
Simulation Time	5 minutes

Table 2 Performance on Throughput

Throughput Performance in %				
Time in Sec	**BEEDSR in %**	**TABR in %**	**MFQos in %**	**RLCR-DPMAC %**
30	13	18	39	51
60	26	36	47	73
120	42	51	68	82
240	68	74	84	91
300	82	93	97	98

Table 3 Performance on Packet Delivery Ratio

Packet Delivery ratio in %				
Time in Sec	**BEEDSR in %**	**TABR in %**	**MFQoS in %**	**RLCR-DPMA %**
30	19	26	35	46
60	36	47	58	61
120	49	63	74	76
240	58	79	87	88
300	73	87	94	96

Table 4 Packet delay comparative result

Latency in Seconds				
The rate of sending Packets/Sec	**BEEDSR in sec**	**TABR in sec**	**MFQoS in sec.**	**RLCR_DPMA %**
5	4.5	3.2	1.7	5
10	6.8	5.6	2.8	5.2
15	7.2	6.8	4.4	6.1
30	8.9	7.4	5.8	6.4
60	9.4	9.2	6.5	7.8

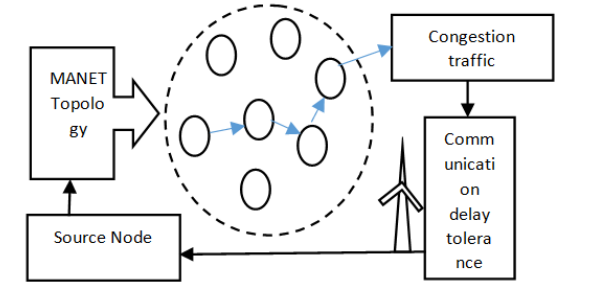

Figure 1: congestion traffic at communication delay tolerance.

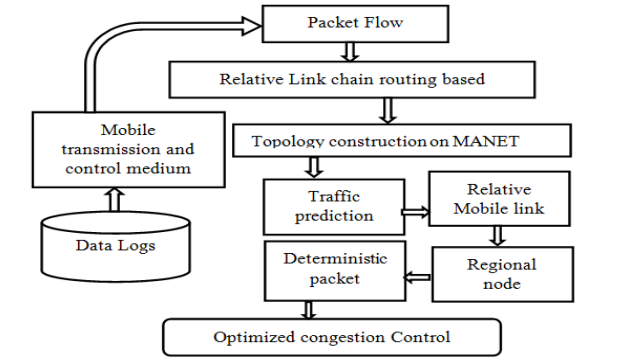

Figure: 2 Relative Link chain routing based congestion control

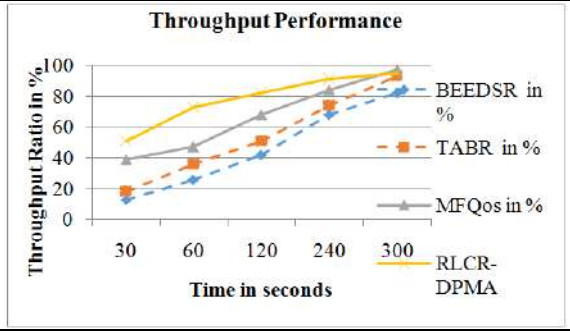

Figure 3 Performances on Throughput

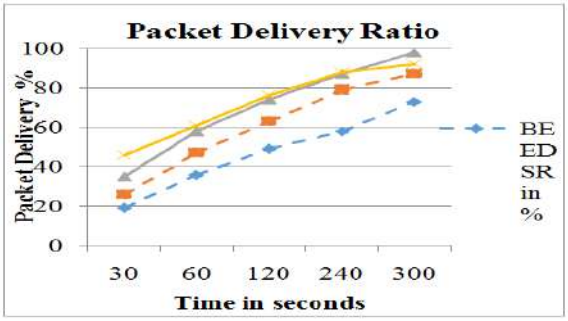

Figure 4: Performance on PDR

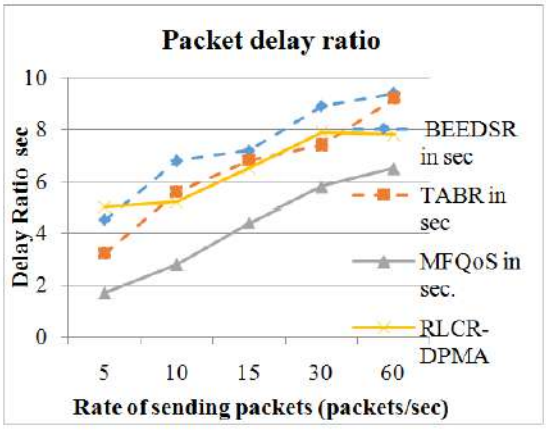

Figure 5 Packet delay Ratio of different methods

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RESEARCH ARTICLE

Influence of Weather Factors on Colony Growth of Indian Honey Bee, *Apis cerana indica* Fab. in Annamalainagar, Tamil Nadu, India

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ABSTRACT

The study was carried out to investigate the growth and development of Indian bee, *Apis cerana indica* colony in relation with the weather parameters during July 2019 to January 2020 in Annamalainagar. The sealed brood area, stored pollen area and adult bee population of the hive were observed in three selected colonies at apiary located in the Department of Entomology, Annamalai University, Annamalainagar. The results revealed that sealed brood area of *A. cerana indica* colonies were found higher during January 2020 (545cm^2) while the least sealed brood area was recorded during July 2019 (300.33 cm^2). It was gradually increased from July 2019 to January 2020. The pollen storage area reached its peak during January 2020 (163.67 cm^2) and found lowest during July 2019 and October 2019 with an area of 87.83 cm^2 and 90.83 cm^2 respectively. The adult bee population in the hive was observed high during January 2020 (5450numbers) but October 2019 was found with the lowest (3557numbers). The correlation study in relation with the colony growth parameters and weather factors revealed that the temperature and rainfall was negatively correlated while relative humidity was positively correlated. Influence of weather factors on colony growth parameters revealed that as the temperature decreased correspondingly sealed brood area, pollen storage area and adult bee population increased *i.e.* the weather factors were inversely proportional to colony growth parameters.

Keywords: *Apis cerana indica*, honey bee, pollen, sealed brood, weather factors

INTRODUCTION

Beekeeping is one of the important agriculture based industries in India. Honey bees interact with the cross-pollinated plants to produce byproducts like honey, bee wax, bee bread, propolis, bee venom and royal jelly

(Chaudhary, 2014). Among the cross-pollinated crops, 80% plants need honey bees as their potential pollinators and thus, honey bees maintain the biodiversity of the ecosystem (Sampson and Cane, 2000). The activity of the honeybee colony is influenced by many factors such as nectar and pollen flora (El-Kazafy *et al.,*2006). Growth of the bees depend on the availability of pollen and nectar. The flowers are the main sources for collecting pollen and nectar. The foraging of bees for nectar and pollen is continued throughout the year. It is highly influenced by the weather conditions and availability of flora in a particular locality.

The amount of pollen stored was regulated by both colony size and pollen amount had independent effects on brood rearing. Analyses of pollen grains collected from honey bee colonies provide relevant information for the bee visited plant sources of an area (Bilisik *et al.,* 2008). Number of pollen foragers, amount of pollen stored as beebread and brood in the colony differed significantly during different seasons. The growth and yield of the colony was identified by analysing the growth rate of the colony growth parameters like area of sealed brood, area of pollen storage, and adult bee population. The area of stored pollen, worker brood, population density and honey production were greater in strong colonies than the weak colonies (Taha and Al-Kahtani, 2013).The amount of brood present in a colony influence the foraging, egg production by the queen and adult population. In the absence of bee flora *i.e.* dearth period, the growth parameters of the colony like sealed brood, pollen storage, adult bee population and honey storage area were in reducing rate and the same was managed by using the pollen substitute for honey bees (Kumari and Kumar, 2019). The population of a bee hive fluctuates during the year according to the seasons and food resources. Successful beekeeping highly relies on the scientific management methods for their better improvement. Various factors influence the colony population and development of the honey bee among which, weather parameters and bee flora play a major role in determining the bee colony population. Hence, the present study was carried out to record the growth of colony parameters in relation with the weather parameters in Annamalainagar, Tamil Nadu.

MATERIALS AND METHODS

The colony population growth parameters include the area of sealed brood, area of pollen storage, and adult bee population were carried out during July 2019 to January 2020 from the selected three colonies at apiary established in the Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar. The data were recorded twice a month throughout the study period.

Area of sealed brood: Honey bees seal their brood with wax once the larvae turn into pupae. Thus, the sealed brood in a colony is an indication of future adult population. The area of sealed brood was recorded using transparent OHP sheet with grid markings of 4 cm^2 (Fig 1). Recorded data for each frame were summed for each colony and multiplied with four to get the total area of sealed brood (Hoffman *et al.,* 2008).

Pollen storage area: Bees normally store their pollen collected from flowers in the brood frames for future use. The pollen storage area in cm^2 on the face of the comb was observed with the help of a transparent OHP sheet marked with 4 cm^2 grid, with the help of a permanent OHP marker (Fig 2). The number of squares covering the pollen storage area on both sides of each brood comb was counted and added for all brood combs and the total was multiplied by four to get the total area of pollen storage a hive in cm^2 (Kishan and Srinivasan, 2016).

Adult bee population: Adult bee populations were made by estimating the number and area of comb covered with bees. The adult bee population was recorded by calculating the total number of frames entirely covered by honey bees and partially covered frames in the colony. The totally covered area with adult bees were summed up to get an approximate figure for each colony (Kishan and Srinivasan, 2016) (Fig 3).

Weather factors: The monthly weather parameters like maximum and minimum temperature (^{0}C), relative humidity (%), rainfall (mm) were obtained from meteorological observatory, Faculty of Agriculture, Annamalai University, Annamalainagar.

Statistical analysis: All the experiments were conducted using randomized block design (RBD) during the study period. The data were analyzed using the OPISTAT software and significance test was done by using Duncan's rule. Meteorological data were correlated with colony growth parameters (Sheoran *et al.,* 1998).

RESULTS AND DISCUSSION

The data recorded on the colonies of *A. cerana indica* during July 2019 to January 2020 are furnished in Table 1. The results revealed that the mean area of sealed brood in the hive was 545.00 cm^2 in January 2020, which was closely followed by December 2019 (449.33 cm^2). The area of the sealed brood found during January 2020 was highly significant than other months. The mean area of brood was declined during the month of November 2019 (359.67 cm^2) followed by September 2019 (334.67 cm^2) and August 2019 (311.83 cm^2). The least brood area was found during both months of July 2019 (300.33cm^2) and October 2019 (301.67 cm^2). The sealed brood area found during the months of July 2019, August 2019 and September 2019 were statistically on par with each other. The brood production was high in January 2020 due to the availability of natural pollen and favorable climatic conditions. The development of brood was recorded good during January 2020.

The highest mean pollen storage area was recorded during January (163.67 cm^2) 2020 followed by December (112.00 cm^2). The statistical analysis showed that pollen storage area in January month was highly significant than other months. Pollen storage area had shown that close reduction was found during November 2019 (100.17 cm^2) and September 2019 (97.17 cm^2). The pollen storage area in November 2019 and December 2019 were statistically on par with each other. The lowest pollen storage area was recorded during August 2019 (92.33 cm^2) followed by October 2019 (90.83 cm^2) and July 2019 (87.83 cm^2). The least average pollen storage area was found during the month, July 2019 (87.83 cm^2). The area of pollen storage was noticed in July 2019 and found significant than other months. The area of pollen stored in bee hives during August 2019 and October 2019 were found on par with each other (Table 1). The area of pollen storage was increased with the increase of pollen foragers. This was due to less availability of natural pollen and increased rainfall reduces the average development in the pollen storage area. The positive correlation was found within the brood area, stored pollen area and the adult bee population (Shawer *et al.* 2003).

The adult bee population was recorded and the colony strength in the bee hives are furnished in Table 1. The mean adult bee population of the honey bee was recorded high during the month of January 2020 (5450.00 numbers). Bee population was found statistically significant during January 2020 than other months. It was closely followed by December 2019 with an average of 4667.33 numbers. The lowest mean adult bee population (3557.00 numbers) was found during October 2019 and increased slightly in the months of September, August and July with an average of 4289.67, 3953.00 and 3608.33 numbers respectively. The hive population was found less during July 2019, which was on par with the population of bees in August 2019 and October 2019. The adult bee population was increased when the sealed brood area was started to increase. It was clear that the colony growth parameters had individual interlink between each parameters and also with the weather factors.

Colony growth parameters Vs weather factors

The weather factors during the study period is given in Table 1. Statistical analysis showed that the area of the sealed brood, amount of pollen storage area and adult bee populations were found significant during January 2020 than other months. Correlation between the population development of bees and weather factors showed their influence and relationship between them (Table 2). The maximum temperature showed negative correlation on the area of sealed brood covered and the coefficient of correlation was -0.853. The negative correlation was found between the maximum temperature and area of pollen storage covered was -0.766. The adult bee population had negative correlation with the maximum temperature in hive *i.e.* -0.722. The minimum temperature had shown negative correlation of -0.958, -0.895, -0.875 with the area of sealed brood, pollen stored area and the adult bee population of the hive respectively during the study period (Table 2). The correlation coefficient of sealed brood, pollen storage area and adult bee population with relative humidity was positively correlated with 0.676, 0.573 and 0.500 respectively. The correlation coefficient of sealed brood was negatively correlated with rainfall (-0.556) and pollen

storage area (-0.558) while rainfall and adult bee population of the hive was also negatively correlated (-0.608).The natural nutrition to the bees were obtained through pollen and feeding queen bees in the form of bee bread increased the egg laying of queen bee and sealed brood area. The area of sealed brood was declined during the maximum temperature, minimum temperature and heavy rainfall. Neupane and Thapa (2005) reported that the brood area was high during spring which fall in the month of January and declined during summer and rainy season. Collection of pollen was influenced by rainfall, maximum and minimum temperature. The current findings are in accordance with Kishan and Srinivasan (2016), who stated that the availability of pollen was less during rainy season and winter which reduced the pollen storage area of the colony. Reddy (1980) stated that foraging was influenced by the maximum temperature and rainfall. Similar reports were made by Mattu and Verma (1985) and Sharma (2010) who reported that unfavourable climatic condition had higher amount of rainfall, minimum temperature less than 20 ^{0}C had influenced the growth parameters like pollen storage and brood area. The adult bee population were negatively correlated with maximum temperature (-0.722), minimum temperature (-0.875) and rainfall (-0.608). The results are in accordance with the report of Mishra and Sharma (1998), they explored that the floral sources were less or even unavailable to the bees during June to September. This had influenced the pollen storage area which had direct role in the development of brood area and adult bee population.

CONCLUSION

Establishment and survival of apiary always depends on the availability of bee flora, weather factors and also management of natural enemies of honey bees like pest and diseases. From the results, it was noticed that the temperature decreased as a result, the enclosed brood space, pollen storage area, and adult bee population increased *i.e.* weather parameters were inversely proportional to the colony growth parameters. Conducive weather plays a major role in the development of bee colonies in apiary and the bee flora are the source for natural pollen and nectar, which is important in population establishment of colonies in a period of time. The inter-link between hive population and availability of natural flora are directly proportional to each other. A clear and detailed knowledge over the weather factors in a particular locality will bring the apiculture into a successful choice and a remunerative allied activity of farmers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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Table 1. Indian honey bee colony growth and weather parameters during July 2019- January 2020 at Annamalainagar

Months and year	Temperature (ºC)		Relative Humidity (%)	Rainfall (mm)	Mean area of sealed brood (cm²)	Mean area of pollen storage (cm²)	Mean adult bee population (Numbers)*
	Max.	Min.					
July, 2019	36.00	26.10	67.00	79.80	300.33 (17.32)^{d}	87.83 (9.36)^{e}	3608.33 (60.47)^{d}
August, 2019	35.20	25.50	65.00	113.10	311.83 (17.65)^{d}	92.33 (9.52)^{d}	3953.00 (62.03)^{d}
September, 2019	34.00	24.90	73.00	184.90	334.67 (18.28)^{c}	97.17 (9.80)^{c}	4289.67 (65.78)^{c}
October, 2019	31.90	24.60	82.00	207.40	301.67 (17.35)^{d}	90.83 (9.51)^{d}	3557.00 (59.60)^{d}
November, 2019	31.00	23.90	86.80	302.00	359.67 (18.96)^{b}	100.17 (9.96)^{b}	3890.50 (63.41)^{d}
December, 2019	29.20	22.50	87.20	67.10	449.33 (21.18)^{b}	112.00 (10.58)^{b}	4667.33 (68.64)^{b}
January, 2020	28.20	21.20	87.20	10.00	545.00 (23.34)^{a}	163.67 (12.65)^{a}	5450.00 (74.45)^{a}
SE.d					0.16	0.08	1.04
CD					0.42	0.17	2.10

In a column mean followed by a common letter are not significantly different by DMRT (P=0.05)
Values in Parentheses are $\sqrt{(X+0.5)}$ transformed values
* - Mean of three replications

Table 2: Correlation of colony growth parameters of *A. cerana indica* bees with weather factors at Annamalainagar

Weather parameters	Bee population development parameters		
	Area of sealed brood	Area of pollen storage	Adult bee population in hives
Max. Temperature	-0.853*	-0.766*	-0.722*
Min. Temperature	-0.958*	-0.895*	-0.875*
Relative Humidity	0.676*	0.573*	0.500*
Rainfall	-0.556*	-0.558*	-0.608*

*- Significant at P<0.05

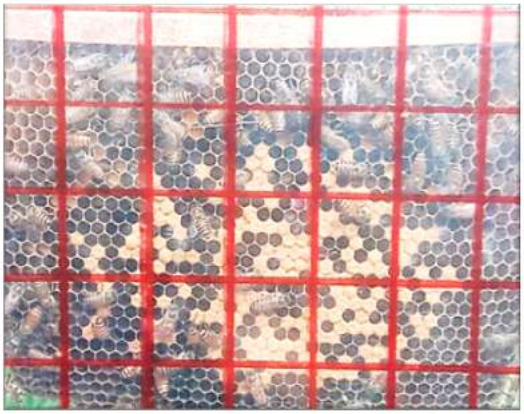

Fig 1. Assessment of sealed brood

Fig 2. Assessment of pollen storage area

Fig 3. Assessment of adult bee population

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RESEARCH ARTICLE

Design, Formulation, Optimization, Characterisation and Evaluation of Ramipril Loaded Seaweed Alginate – Natural Polysaccharides Composite Mucoadhesive Microspheres

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ABSTRACT

The aim of this study is to formulate and evaluate the microspheres of Ramipril (RAM) with the combination of Natural and synthetic polymers. Microspheres were prepared with the combination of HPMC, HEC, Xanthan Gum and Chitosan using sodium alginate by ionotropic gelation method. Microspheres were characterized by particle size analysis, percentage yield, and entrapment efficiency, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, *in vitro* release studies and release kinetics. The mean particle size, percentage yield were decreased significantly ($p < 0.05$) with decrease in drug-polymer ratio, SEM and FT-IR studies revealed that the microspheres were spherical; non-aggregated, and porous in nature and drug polymer is compatible. It was found that *in vitro* release was decreased significantly ($p < 0.05$) with decrease in drug-polymer ratio but increased

significantly ($p < 0.05$) with increase in polymer concentration. The analysis of dissolution kinetic data shows that it follows Higuchi model then zero order followed by first order. The optimized formulation was drug loaded in sodium alginate with HPMC as mucoadhesive polymer which may be useful for the delivery of Ramipril for continuous and prolonged drug release.

Keywords: Ramipril; Sodium alginate; Mucoadhesive; Microspheres; Ionotropic gelation.

INTRODUCTION

The gastro retentive drug delivery system (GRDDS) is of special interest in improving the bioavailability of drugs that are poorly soluble or unstable at higher pH of the intestinal or colonic environment1. In order to obtain local and sustained drug delivery in the stomach and proximal parts of the small intestine, it is desired to have prolonged gastric retention of the drug. This helps to have improved bioavailability and therapeutic efficacy which may also results in the reduction in dosing frequency of the dosage form.[1-3] the diminished efficacy of the administered dose may be observed due to intersubject variability and short time of gastric emptying which may results because of incomplete drug release from the drug delivery system above the absorption zone (stomach, upper part of small intestine).[4] moreover, it has been reported that drug delivery system is one of the commercial system which attributed to obtain the higher bioavailability than that of the non-floating system [1].

The GRDDS system is widely useful for the drugs which effectively act in the stomach and have absorption window in stomach [2, 7]. to formulate GRDDS the drug moiety should have good solubility at acidic pH and absorption window in upper GIT and short half-life. To overcome the disadvantages of conventional dosage forms, such as the intersubject variability of GI transit time, due to their all or none effect of the multiple unit dosage form systems are developed. Multiple unit dosage form have proven the lower possibility of dose dumping and reduced inter and intra subject variability of the drug absorption [8-10].

Ramipril inhibit angiotensin converting enzyme (ACE) which is identical to kininase ii catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex, thus inhibition of ace results in decreased plasma angiotensin ii, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The latter decrease may result in a small increase in serum potassium. Ramipril have dose proportional over the 2.5 - 20 mg dose range. The biological half life was 3-6 hours. The absolute bioavailabilities of Ramipril were 28 %, when 5mg of oral Ramipril was compared with the same dose of Ramipril given intravenously [11-12]. Ramipril degrades due to physical stress which influence the stability of Ramipril formulations are mechanical stress, compression, manufacturing processes, recipients, storage conditions, heat and moisture associated with formulating processes which can increase the rate the decomposition of Ramipril into degrading products. Formulations need special care when formulating into pharmaceutical preparations. [13-15]

Hence a Ramipril formulated as microspheres gives more stability to the drug from compression and other stress condition during formulation and storage conditions. Mucoadhesive microspheres of Ramipril, adheres to the GI mucosa, extend the drug release, maximize drug absorption and minimize local irritation of the drug which indicates microspheres for controlled extended drug delivery. The formulated microspheres are encapsulated in hard gelatin capsules and dispensed for extended drug release. Present work includes the development of mucoadhesive dosage form because it offers several advantages like improving patient compliance by decreasing dosing frequency, less inter and intra subject variability, gastric retention time is increased because of mucoadhesivity, reduced risk of local irritation, no risk of dose dumping. Beads can provide sustained release properties with more uniform distribution of drugs within gastrointestinal tract [16]. Hence the bioavailability of beads can be enhanced.

MATERIALS AND METHODS

Ramipril was a gift sample from Sun Pharmaceuticals Ltd, Mumbai, India. Sodium alginate, HPMC. HEC, Chitosan and Xanthan Gum was procured S.B. Fine chemicals Ltd, Mumbai. India. All other reagents and solvents used were of analytical grade.

Preparation of Microspheres

The beads were prepared by the ionotropic gelation technique [17]. Sodium alginate solution was prepared by dissolving sodium alginate in deionized water and heated at 60°C. Different concentrations of polymeric solutions (HPMC, HEC, Chitosan and xanthum gum) were used in combination with sodium alginate. The drug was dissolved uniformly in 50 ml of polymer solution below 40°C under continuous stirring. The stirring was continued until a uniform solution was obtained. The resultant homogeneous bubble free slurry dispersion was dropped through a 23 gauge syringe needle into 100 ml of 2% Calcium chloride solution which was kept under stirring to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. After 5 min of curing time, the formed beads were collected by filtration and dried at 40°C.

Evaluation parameters:

Thin layer chromatography of Ramipril

Thin layer chromatography (TLC) of Ramipril was performed to confirm the purity of Ramipril. A 100 mg of Ramipril in 5 ml methanol and this solution was spotted on silica gel based stationary phase [9]. The mobile phase consisted of a 30:3:1 mixture of toluene: tetrahydrofuran: glacial acetic acid. It was allowed to run on the stationary phase until it reached about 75% of the height of TLC plate. The plate was removed and allowed to dry completely and sample is visualized by iodine chamber method [10]

Beer-lambert plot of Ramipril

The wavelength of maximum absorbance (λ_{max}) of Ramipril was determined by scanning a Ramipril solution in pH 7.4 phosphate buffer between 200 and 400 nm using UV double beam scanning spectrophotometer (Schimadzu 1700 UV-Vis Double beam spectrophotometer). The λmax was found to be 272 nm. The absorbance of various concentrations of Ramipril in pH 7.4 phosphate buffer solution determined at 272 nm. The experiment was conducted in triplicate and a plot of absorbance versus Ramipril concentration was plotted. The prepared microspheres were evaluated for particle size, drug content, and entrapment efficiency, swelling index, in-vitro dissolution studies and stability studies.

Morphology

The morphology of spheres prepared was visually observed with naked eye under white light using a black background. The purpose of this examination was to observe shape, color, appearance and texture of the spheres. The spheres were also observed under optical microscope using a magnification of 3.2X in order to determine their surface morphology.

Determination of particle size and size distribution

Optical microscopy (Carl Zeiss standard binocular laboratory microscope, West Germany) was used to determine particle size and particle size distribution of Ramipril-loaded alginate spheres. A thin layer of the spheres was spread on a glass slide and the spheres were viewed using an optical microscope equipped with an eye piece containing a micrometer. The micrometer had a scale reading from 0 to 5 mm divided into 100 divisions, *i.e.*, each division measured 0.05 mm. For spheres that appeared to be oval or oblong, two dimensions (length and width) were counted, and the average of length and width was considered to be the particle size of the sphere in question. The particle size of 100 spheres was determined to calculate the average particle size of the batch of spheres. Every effort

was made to count each sphere once only. The diameters of 100 spheres were then treated mathematically to obtain mean particle size and size distribution.

Particle size analysis

The particle size of mucoadhesive microspheres was determined using optical microscopy method. Particle size of all the batches of the formulated beads in a sample was measured with an optical micrometer fitted with a calibrated eye piece. Calibration of the microscope was done prior to particle size measurement of the beads. Approximately 500 beads were counted for particle size using a calibrated optical microscope. All readings are average of three trials ± sd.

Scanning electron microscopy analysis (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35cf, JEOL, Japan) using gold sputter technique. The particles were vacuum dried, coated to 200 ao thicknesses with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.

Evaluation of swelling ratio

Swelling ratio was studied by measuring the percentage water uptake by the beads. About 80 mg of drug equivalent beads from all prepared placebo beads were accurately weighed and placed in 100 ml of 0.1N HCl (pH 1.2). Beads were removed from their respective swelling media after 16 hr and weighed after drying the surface water using filter paper. The water uptake was calculated as the ratio of the increase in weight of beads after swelling to the dry weight.

$$Swelling\ index = \frac{s - t}{t} x100$$

Where, s = weight of the beads after swelling t = initial weight of the beads.

Drug entrapment efficiency

Drug entrapment efficiency of Ramipril floating microspheres was performed by accurately weighing 80mg of drug equivalent floating microspheres and suspended in 100 ml of 0.1 n HCl and it was kept on a side for 24 hours. Then, it was stirred for 15 mins and filtered. After suitable dilution, Ramipril content in the filtrate was analyzed spectrop Hotometrically at 262 nm using Ultraviolet spectroscopy.

Estimation of Ramipril (drug content)

Equivalent weight of beads was weighed and dissolved in 5ml of water and methanol mixture in a standard flask. Shake for 30min and then make up with 0.1N HCl buffer and then centrifuge it. From that take 5ml of solution in 50 ml standard flask make up with 0.1N HCl. Generally, the drug content in any formulation should fall within the limit of 90 – 110%.

***In vitro* wash off test for mucoadhesion**

The time taken for detachment of beads from sheep stomach mucosa was measured in 0.1n hydrochloric acid (pH 1.2).this was evaluated by an in vitro adhesion testing method, known as wash off method. The mucoadhesive property of beads was compared with that of a non-adhesive material, ethylene vinyl acetate beads. A piece of sheep stomach mucosa (2×2 cm) was mounted onto glass slide (3×1 inch) with cyanoacrylate glue and one more glass slide was connected with a support. The beads (50 no) were counted and spread over the wet rinsed tissue specimen and immediately thereafter the support was hung on the arm of a USP tablet disintegrating test machine as shown in Photographs in figure 1. By operating the disintegration machine the tissue specimen was given a slow regular up and down moment. The slides move up and down in the test fluid at 37 ± 0.50 c. The number of beads adhering to the tissue was counted at 2-hour intervals up to 8 hours [18].

***In vitro* dissolution studies**

In vitro release studies of prepared floating micro beads were carried out using type I dissolution (basket) apparatus with 900ml of dissolution medium at 100 rpm. Dissolution was carried out for a total period of 16 hr using 0.1N HCl (pH 1.2) for 16 hrs for the rest of the period maintained at a temperature of 37±1°c. Sample was withdrawn at pre-determined time intervals replacing with an equal quantity of drug free dissolution fluid to maintain the sink conditions. The samples withdrawn were filtered through 0.45μ membrane filter, and concentration of drug in each sample was analyzed by UV spectrophotometer at 272 nm and cumulative percent drug release was calculated. The study was performed in triplicate.

Stability study

Stability studies were carried out at accelerated condition (25º c ±2º c at 60% RH ±5% RH), (30º c ±2º c at 65% RH ±5% RH) and (40˚ c ±2˚ c at 75% RH ±5% RH) for the optimized formulation F6. The beads were stored at (25º c ±2º c at 60% RH ±5% RH), (30º c ±2º c at 65% RH ±5% RH) and (40˚ c ±2˚ c at 75% RH ±5% RH) for accelerated temperature in closed high density polyethylene bottles for 3 months. The samples were withdrawn after predetermined period of 1 month, 2 month and 3 month. The samples were analyzed for its drug content and in-vitro drug release.

Kinetics of drug release

In order to understand the mechanism and various kinetics of drug release, the data obtained from the *in vitro* dissolution studies were analyzed with various kinetic equations like zero order (% release vs. Time), first order (log % retained vs. Time) Higuchi plot (% release Vs square root of time) and Korsmeyer's peppa K equation (log % release vs. Log Time). From these plots coefficient of correlation (r) values were calculated. [19]

RESULTS AND DISCUSSION

Drug-polymer Compatibility study

From the FTIR graphs it was observed that the presence of characteristic functional peaks of Ramipril, sodium alginate, and guar gum in the drug polymer physical mixture. It has also been evident that there was no major shifting and appearance of new characteristic peaks. Hence it was concluded that there was no interaction between the selected drug and polymers.

Formulation, evaluation and selection of best batches of placebo micro beads

From the table -1 it is evident that all the selected ratio of sodium alginate and guar gum were effective in producing microspheres of various sizes. But among various batches of micro beads made with guar gum as release modifier, the batch F6 was relatively better than other batches in terms of their physical characteristics. This batch was found to be the best based on its discrete nature, (fig-1) higher yield, lower particle size, lower drying rate and higher swelling ratio. Hence this batch was subjected to subsequent studies using the drug.

Estimation of drug loading efficiency of drug loaded microspheres

From the above table it is evident that all the twelve batches of microspheres show a satisfactory drug loading capacity. The percentage of drug loading ranges from 72 to 98%. But the drug loading capacity of F6 batch was better at a drug concentration of 500mg /500mg of polymers. As there was no much increase in drug entrapment after a concentration of 500mg/500mg of polymer the batch F6 was considered as the best batch. But all the drug loaded batches were subjected to *in vitro* analysis and *in vitro* wash off study.

Particle size determination of microspheres by scanning electron microscopy (SEM)

The morphological evaluation of the optimized formulation F6 was done by scanning electron microscopy. The study revealed that the microspheres were almost spherical in shape with rough outer surface, which subsequently enhances the drug release by channel formation.

***In vitro* drug release**

Fig-1 and 2 depicts the i*n vitro* release data of Ramipril loaded micro beads. It can be understood that disregard of drug loading, all the drug loaded batches from F1- F12 shows a cumulative percentage release of 92-100% with a sustained pattern for about of 16 hours. It is also clear that the selected release modifier is effective in retarding the release rate of drug at all selected concentrations. But among the batches the batch F6 , F9 and F12 has shown a linear drug release.

Release Kinetics

The dissolution data, obtained (table-7 and table 8) from all the drug loaded batches, a zero order graph was plotted with% drug release Vs time, first order plot Log % drug release Vs. time, a graph was plotted with % drug release Vs square root of time to fit the data to Higuchi's model and Graphs of log % of release Vs log time was plotted and the data was fit into Kosmeyer's- Pappas's model. Based on the 'R-value and 'n' (n > 1.0) value of the table it is evident that the selected batches of microspheres (F6) was found to exhibit a zero order release and shown a linear drug release in the first order, Highuchi and peppas model with r^2 0.949-0.994 [Tab-9].

The percentage of mucoadhesion of micro beads is shown in table 5 and 6. The results of the ex-vivo mucoadhesion study reveal that batches F6, F9 and F12 have good mucoadhesive character which could be due to the combined effect of both polymers. The increased viscosity of the polymer helps to increase adhesion with intestinal mucosa. Therefore, it could be assumed that the prepared microspheres will adhere to the intestinal mucosa for a prolonged period where they release the drug in a sustained manner before being eroded off

CONCLUSION

Sodium alginate microspheres of Ramipril prepared by Ionotropic gelation method with guar gum as release modifier were found to be a good carrier for the formulation of sustained release capsules or tablets in terms of good drug polymer compatibility, drug loading capacity, swelling behavior, percentage yield, *In vitro* release characteristics, and mucoadhesive strength Eventually it may improve the bioavailability of the selected drug with concurrent reduction in dose.

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Table 1. Formulation Composition of Mucoadhesive Ramipril Microspheres.

Formulation Code	Drug (mg)	Sodium Alginate (mg)	HPMC K100 (mg)	HEC (mg)	Xanthum gum (mg)	Chotisan (mg)	$CaCl_2$ (%)
F1	500	125	125				5%
F2	500	125		125			5%
F3	500	125			125		5%
F 4	500	125				125	5%
F 5	500	250	250				5%
F 6	500	250		250			5%
F 7	500	250			250		5%
F 8	500	250				250	5%
F 9	500	500	500				5%
F 10	500	500		500			5%
F 11	500	500			500		5%
F 12	500	500				500	5%

Table 2. Evaluation of Ramipril Microspheres of F1 to F12

Formulation Code	Entrapment Efficiency	Drug content (%)	Particle Size (μm)
F1	73.53±1.54	97.56±1.37	585.5±1.8
F2	83.23±1.84	98.52±1.45	577.84±2.08
F3	82.73±1.2	96.71±1.64	584.9±2.22
F4	81.46±0.58	98.31±1.33	572.4±2.39
F5	72.53±0.33	98.88±1.28	586.89±1.15
F6	**98.49±1.05**	**99.88±1.23**	**599.76±1.65**
F7	85.73±0.1	98.37±1.20	588.5±2.01
F8	86.97±0.43	98.19±1.46	588.33±1.64
F9	83.73±0.31	96.58±1.52	589.4±2.18
F10	84.32±1.54	97.56±1.80	590.8±1.29
F11	83.25±1.8	98.52±1.36	594.14±1.26
F12	86.97±1.2	96.71±1.28	592.77±2.2
	n=3 ± = Standard deviation		

n=3; ± = standard deviation

Table 3. Evaluation of swelling ratio of Ramiprilmicrospheres

Formulation code	1hr	2hr	4hr	6hr	8hr	10hr
F1	2.46±0.05	5.1±0.04	9.98±0.06	14.3±0.21	19.4±0.08	22.2±0.16
F2	4.44±0.11	6.9±0.18	11.8±0.22	16.4±0.17	20.6±0.16	22.5±0.09
F3	2.92±0.26	6.8±0.11	11.3±0.14	16.1±0.24	20.2±0.05	23.4±0.12
F4	2.55±0.14	5.8±0.16	10.1±0.06	15.0±0.15	19.7±0.01	22.8±0.15
F5	2.63±0.01	6.2±0.05	10.6±0.02	15.8±0.09	19.9±0.16	23.2±0.07
F6	**4.42±0.07**	**9.2±0.14**	**15.8±0.18**	**23.9±0.13**	**27.7±0.20**	**29.9±0.08**
F7	3.90±0.14	5.7±0.01	10.6±0.18	15.3±0.14	19.4±0.03	20.3±0.03
F8	3.77±0.04	7.2±0.16	12.1±0.07	18.1±0.22	22.6±0.28	25.2±0.10
F9	3.36±0.11	6.8±0.27	10.9±0.11	16.6±0.02	21.8±0.13	23.7±0.09
F10	3.40±0.26	7.0±0.33	11.5±0.19	17.6±0.09	22.1±0.11	24.9±0.05
F11	3.55±0.13	6.9±0.24	11.7±0.27	18.8±0.14	21.6±0.06	25.6±0.29
F12	3.32±0.03	4.53±0.08	9.8±0.05	14.1±0.19	18±0.16	18.2±0.08

n=3; ± = standard deviation

Table 4. Results of stability studies of optimized formulation F-6

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	25^{0}C/60%RH % Release	99.89	99.85	99.83	99.81	Not less than 85 %
F-6	30^{0}C/75%RH % Release	99.89	99.84	99.82	99.80	Not less than 85 %
F-6	40^{0}C/75%RH % Release	99.89	99.86	99.85	99.84	Not less than 85 %
F-6	25^{0}C/60%RH Assay Value	99.88	99.86	99.83	99.82	Not less than 90%.Not more than 110 %
F-6	30^{0}C/75%RH Assay Value	99.88	99.85	99.82	99.82	Not less than 90%.Not more than 110 %
F-6	40^{0}C/75%RH Assay Value	99.88	99.86	99.81	99.81	Not less than 90%Not more than 110 %

Table No 5: Percent Mucoadhesive Property of the microspheres of Ramipril in pH 1.2

Time (hrs)	Percent Mucoadhesive Property											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	34	40	46	30	76	**94**	54	66	96	44	34	83
1	6	18	28	8	68	**86**	56	88	54	34	78	74
2	-	-	10	16	-	**82**	13	14	42	44	78	40
3	-	-	-	-	44	**78**	15	-	82	12	-	62
4	-	-	-	-	-	**46**	10	32	60	38	-	26
5	-	-	-	-	-	**38**	-	-	20	22	-	16
6	-	-	-	-	-	**12**	-	-	18	-	-	4
7	-	-	-	-	-	**6**	-	-	11	-	-	-
8	-	-	-	-	-		-	-	-	-	-	-

Table No 6: Percent Mucoadhesive Property of the microspheres of Ramipril in pH 6.8

Time (hrs)	Percent Mucoadhesive Property											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F6	F11	F12
0	16	50	22	24	70	**78**	46	64	80	28	24	72
1	-	10	14	10	62	**78**	42	54	80	12	-	70
2	-	-	-	4	40	**76**	22	38	78	4	-	68
3	-	-	-	-	28	**74**	6	38	76	-	-	68
4	-	-	-	-	18	**74**	-	24	74	-	-	54
5	-	-	-	-	10	**58**	-	4	66	-	-	54
6	-	-	-	-	-	**70**	-	4	66	-	-	30
7	-	-	-	-	-	**58**	-	2	60	-	-	24
8	-	-	-	-	-	**54**	-	-	58	-	-	18

Table 7. Dissolution profile of formulations F1-F6

Time(hrs)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	**0**
1	32.6	28.6	25.1	23.8	22.8	**9.54**
2	50.4	48.4	46.9	45.8	44.3	**12.3**
3	65.6	62.3	60.6	57.6	56.8	**16.3**
4	78.8	73.21	68.4	65.4	67.9	**18.3**
5	84.6	81.7	78.1	76.4	78.6	**21.7**
6	90.8	89.1	85.6	88.7	89.4	**27.4**
7	94.2	92.19	90.4	93.14	94.4	**32.3**
8	98.3	95.7	94.1	97.5	99.77	**40.6**
9	-	98.4	99.4	99.8	-	**78.5**
10	-	-	-	-	-	**65.8**
11	-	-	-	-	-	**70.6**
12	-	-	-	-	-	**79.23**
13	-	-	-	-	-	**85.5**
14	-	-	-	-	-	**89.4**
15	-	-	-	-	-	**93.6**
16	-	-	-	-	-	

Table 8. Dissolution profile of formulations F7-F12

Time(hrs)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	20.6	37.12	27.84	40.81	14.95	19.84
2	31.40	46.91	37.80	54.37	20.04	28.37
3	38.31	60.69	47.69	61.89	27.11	36.96
4	47.0	68.48	58.43	70.94	28.84	41.39
5	51.72	74.18	68.46	81.63	31.58	44.61
6	55.13	76.67	76.67	90.46	36.79	52.43
7	59.00	85.44	79.76	95.42	39.41	53.91
8	62.54	89.17	83.59	97.28	41.62	58.14
9	57.38	95.42	89.87	100.44	49.54	67.82
10	75.36	99.86	95.44	-	60.81	71.67
11	80.35	-	98.65	-	75.33	76.65
12	87.92	-	-	-	89.38	80.33
13	98.84	-	-	-	99.14	91.25
14	-	-	-	-	-	99.24
15	-	-	-	-	-	-
16					-	-

n=3; ± = standard deviation

Table 9: Coefficient of correlation (R2) values of Ramipril mucoadhesive microspheres

Formulation	Zero order	First order	Higuchi's	Peppa's
F1	0.883	0.849	0.978	0.987
F2	0.856	0.83	0.973	0.989
F3	0.926	0.880	0.955	0.966
F4	0.913	0.904	0.964	0.994
F5	0.926	0.846	0.955	0.972
F6	**0.975**	**0.949**	**0.974**	**0.994**
F7	0.928	0.909	0.962	0.995
F8	0.937	0.920	0.942	0.981
F9	0.776	0.845	0.968	0.969
F10	0.854	0.897	0.964	0.994
F11	0.814	0.834	0.978	0.936
F12	0.904	0.856	0.967	0.993

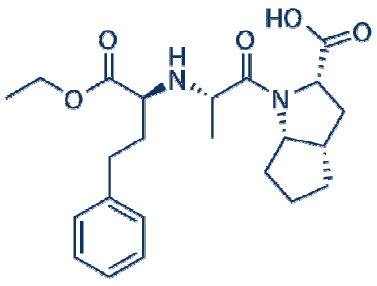

Figure 1. Structure of Ramipril

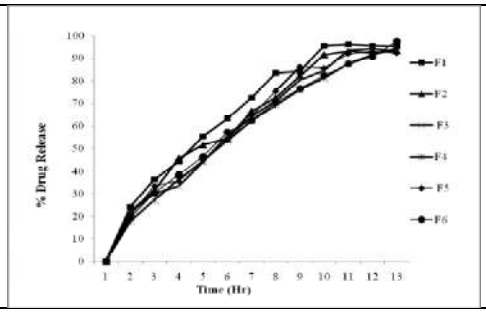

Figure 2. *In vitro* Drug Release Profile of Ramipril Microspheres F1 – F6

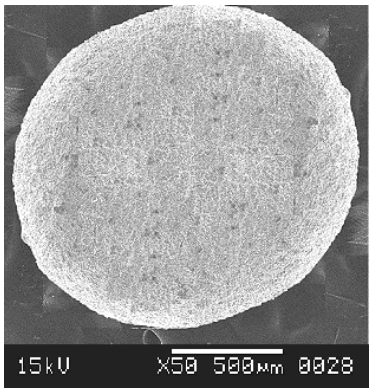

Figure 4. SEM PHotograpH of Optimised RamiprilMicrospheres

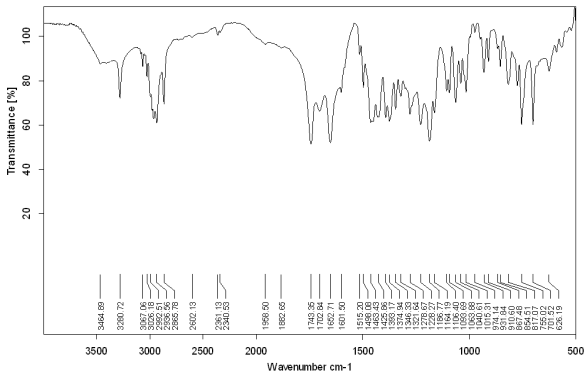

Figure 5. FTIR spectrum of Ramipril

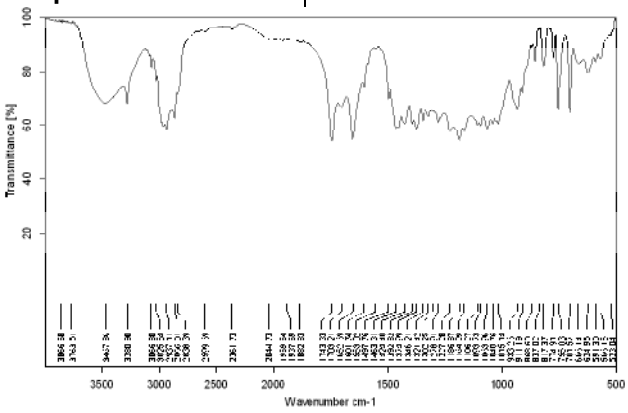

Figure 6. FTIR spectrum of optimised Ramipril formulation F6

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RESEARCH ARTICLE

Screening on Lipid Peroxidation and *In vivo* Antioxidant Activities of Various Extracts of *Cadaba farinosa* (Linn)

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ABSTRACT

Lipid peroxidation and in vivo cell reinforcement exercises of various concentrates of *Cadaba farinosa* (Linn) was examined. Lipid peroxidation (TBARS) and in vivo cell reinforcement movement was screened, for example, SOD, CAT, GPx, GST and Glutathione (GSH). AD treated rats were significantly increase the TBARS levels and decrease the enzymatic antioxidant level when contrasted and control group. Administration of methanolic concentrate of *Cadaba farinosa* in high fat eating regimen rats were indicated that the increased the levels of antioxidant enzymes, for example, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR) and level of non enzymatic antioxidant Glutathione (GSH) when contrasted and HFD rats (Group II). The methanolic concentrate of *Cadaba farinosa* in high fat diet rats were discovered lowered the concentration of TBARS when contrasted and HFD rats (Group II). In comparison of all the three extract treated group with standard group, the methanolic concentrate of *Cadaba farinosa* was showed significant ($p<0.001$) result than that of other groups. Taking into account the outcomes, we concluded that the methanolic concentrate of *Cadaba farinosa* is a significant source of antioxidant, which may be useful in keeping the advancement of different oxidative stresses.

Key words: *Cadaba farinosa ,* Atherogenic Diet, Lipid peroxidation, Antioxidant.

INTRODUCTION

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis, and cancer as well as in degenerative processes associated with aging (Halliwell B et al 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau JL et al 2002). Oxidative stress produced in the body due to the disruption in the balance between production of ROS and perturbation in detoxifying capability of the living system (De diego *et al* al 2009). Antioxidants reduce oxidative stress in the biological system by two processes Inhibiting the free radical production and Neutralization of free radicals ROS/RNS species via stimulation of CAT, GPx and SOD (Violi *et al* 2005). Our human system is equipped with ample number of scavenging enzymes such as CAT, SOD and GPx that play a vital role in quenching the free radicals and safeguarding the cell membrane from injury (Poongothai *et al* 2011).

Cadaba farinosa (family Capparidaceae) is generally known as "Indian cadaba" in ayurvedic traditional system. Quercetin, isoorientin, hydroxybenzoic acid,syringic acid, vanillic acid and 2-hydroxy-4-methoxy benzoic acid were isolated from *Cadaba farinosa*[Khare CP.,2006]. *Cadaba farinosa* was used for different diseases like anthelmintic, antisyphilitic, aperients, stimulant, antiscorbutic,antiphlogistic[Anonymous., 1986]. *Cadaba farinosa* was used rheumatic pain[Anonymous.,1986]. The flower buds are stimulant,antiscorbutic, purgative,antiphlogistic and anthelmintic especially for round worm[Nadkarni AK., 2002]. *Cadaba farinosa* was used as hepatoprotective activity[Umesh BT et al., 2010].*C. farinosa* was used for the treatment of wound healing[Habib A et al., 2004] and anticancer[Graham JG et al., 2000]. Still, no literature are available on the hypolipidemic activity.Hence, the objective of the present investigation to decide the hypolipidemic activities of different concentrates of aerial parts of *Cadaba farinosa* in experimental animals.

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents were purchased from Sigma, SD fine chemicals and Fisher.Fisher chemicals were of analytical grade .

Group & Identification of Plant

The aerial parts *Cadaba farinosa* (family Fabaceae) were gathered form senkottai, Tirunelveli District of Tamilnadu, India. Plant identification was made from Botanical investigation of India, Palayamkottai The *Cadaba farinosa* were desiccated under shadowy, segregate, crushed through grinder.[SivaKrishnan.S *et al* 2014].

Preparation of Concentrates

The pulverized materials were packed in muslin cloth and extracted with pet.ether, ethyl acetate and methanol as solvents respectively according to the increasing order of polarity [Shajiselvin CD et al 2010] through hot constant percolation method in Soxhlet equipment [Harborne JB, 1984]for twenty four hours. The concentrates were concentrated through rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired. [Satheesh Kumar.D *et al* 2010, Alagumanivasagam.G *et al* 2012].

Animals and treatment

Male Wister rats of 16-19 weeks age, weighing 150-175g were gotten from the Central Animal House, Sankaralingam Bhuvaneswari college of Pharmacy, Sivakasi, Tamilnadu, India .The creatures were kept in cages, 2 per confine, with 12:12 hr light and dim cycle at $25^0 \pm 2^0$C. The creatures were maintained on their separate diets and water *ad libitum.*

Animal Ethical Committee's clearance was approved by the Ethical Committee of Sankaralingam Bhuvaneswari college of Pharmacy, Anaikuttam, Sivakasi, Tamilnadu (SBCP/2020-21/CPCSEA/IAEC/1(2)/F16/1460/ 09-12-2020).

Experimental Design

Acute toxicity test

Albino Wistar rats were separated in six groups and each groups contain six animals (n = 6). Rats were fasted for four hours with free access to water only. The various concentrates of *Cadaba farinosa* suspended in normal saline: 0.5% CMC was administered orally at a dose of 5 mg/kg at first and mortality was noted for three days. The mortality was noted in 5/6 or 6/6 animals, and then the dose administered was measured as a toxic dose. However, the mortality was noted in less than 4 rats, out of 6 rats then the same dose was repeated again to confirm the toxic effect. If mortality was not noted, the procedure was repeated for higher doses i.e. 2000mg/kg.

Hypolipidemic activity

Rats were divided into following 6 groups of 6 rats each:

Group I : Standard chow pellet

Group II : Atherogenic Diet(AD)

Group III : AD plus treated with pet.ether concentrates of *Cadaba farinosa* (200mg/kg B.wt)

Group IV : AD plus treated with Ethyl acetate concentrates of *Cadaba farinosa* (200mg/kg B.wt)

Group V : AD plus treated with Methanol concentrates of *Cadaba farinosa* (200mg/kg B.wt)

Group VI : AD plus treated with Standard drug atorvastatin (1.2 mg/kg B.wt)

Animal diet

The compositions of the two diets were as follows (Kottai Muthu A *et al.,* 2005).

Normal diet

Wheat flour 22.5%, simmered bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt blend with starch 4% and vitamin and choline blend 0.5%.

High fat diet

Normal diet with coconut oil 9% and cholesterol 0.4%.

Experimental Design

Rats were partitioned into following six Groups of six rats each:

Group	Treatment	Dose
Group I	Standard chow diet	----
Group II	Atherogenic Diet(AD)	----
Group III	AD + PE concentrate of *Cadaba farinosa*	200 (mg/kg,p.o)
Group IV	AD + EA concentrate of *Cadaba farinosa*	200(mg/kg, p.o)
Group V	AD + ME concentrate of *Cadaba farinosa*	200(mg/kg,p.o)
Group VI	Atorvastatin	1.2(mg/kg, p.o)

Rat diet

The pieces of the twofold weight control plans were as underneath [Kottai Muthu et al 2007].

Ordinary eating regimen

Standard chow diet

Atherogenic diet
Standard chow diet with coconut oil 9% and cholesterol 0.4%.

Evaluation of *in vivo* cell reinforcement and lipid peroxidation
All the various concentrates of Cadaba farinosa just as atorvastatin were suspended in CMC independently and took care of to the individual rats by oral intubation. Toward the finish of 63 days all the creatures were yielded by cervical disengagement after overnight fasting. Liver, heart and aorta were freed from following fat, weighed precisely and utilized for the readiness of homogenate. Creatures were given enough consideration according to the Animal Ethical Committee's proposals (SBCP/2020-21/CPCSEA/IAEC/1(2)/F16/1460/ 09-12-2020).

Parts of the tissues were smeared, gauged and homogenized with methyl alcohol liquor. Folch et al.(1957) strategy was utilized to gathered the lipid concentrates. Nichans WH et al 1968 technique was used for assessment of TBARS level. Another bit of tissues were homogenized with buffer solution. The concentrate were utilized for the assessment of oxidative prevention agent exercises like Kakkar P et al., 1984 strategy was utilized for the assurance of Superoxide dismutase (SOD), Sinha AK et al., 1972 strategy was utilized for the assurance of Catalase (CAT), Rotruck et al 1973 procedure was used for assessment of Glutathione Peroxidase (GPx), Habig, WH et al., 1974 procedure was used for assessment of Glutathione – S-Transferase(GST) and Ellman GL et al 1959 technique was used for assessment of Glutathione (GSH) , Lowry OH 1951 et al strategy was utilized for the estimation of protein.

Statistical analysis
Results were verbalized as Mean±SEM and by applying One Way Analysis of Variance (ANOVA) measurable centrality was determined. $P<0.05$ was considered as huge (Dunnett's test).

RESULTS

The movement of different focuses of *Cadaba farinosa* on tissue TBARS in ordinary and AD treated rats were showed up in Table 1 and Fig 1. The AD treated Group of rats demonstrated expanded the degree of TBARS in liver, heart and aorta as 76.03±0.34, 83.29±0.35 and 65.04±0.18. Treatment of methanol concentrates of *Cadaba farinosa* indicated decrease level of TBARS in liver, heart and aorta as 30.82±0.50, 43.18±0.34 and 23.52±0.38. Treatment of methyl alcohol concentrates of *Cadaba farinosa* had critical decrease in the TBARS levels when contrasted with AD rats .This impact may because of quality of flavonoids in the *Cadaba farinosa* . The impact of different focuses of *Cadaba farinosa* on tissues SOD and CAT chemical levels in AD rats were introduced in Table 2. The AD treated rats indicated diminished exercises of SOD in liver (1.70±0.02),heart (0.73±0.01), aorta(1.45±0.02) when contrasted with control Group of rats. Treatment of PE concentrates of *Cadaba farinosa* demonstrated significantly SOD in liver (1.98±0.03),heart (0.93±0.01), aorta(1.70±0.02) Group III rats. Treatment of EA concentrates of *Cadaba farinosa* indicated significantly SOD in liver (2.16±0.03),heart (1.12±0.03), aorta(1.99±0.02) in Group IV rats. Treatment of methanol concentrates of *Cadaba farinosa* indicated extensively SOD in liver (3.39±0.02),heart (1.71±0.03), aorta(2.59 ±0.02) in Group V rats. Treatment of methanol concentrates of *Cadaba farinosa* having the convergence of tissue SOD, CAT and silymarin reestablished compound exercises to ordinary qualities.

The impact of different focuses of *Cadaba farinosa* on tissues SOD and CAT chemical levels in AD rats were introduced in Table 3. The AD treated rats indicated diminished exercises of CAT in liver (14.89±0.22),heart (30.52±0.22), aorta(21.08±0.34) when contrasted with control Group of rats. Treatment of PE concentrates of *Cadaba farinosa* demonstrated significantly CAT in liver (16.68±0.12),heart (32.61±0.34), aorta(22.84±0.22) Group III rats. Treatment of EA concentrates of *Cadaba farinose were* indicated significantly CAT in liver (19.04±0.14),heart (38.81±0.28), aorta(23.99±0.19) in Group IV rats. Treatment of methanol concentrates of *Cadaba farinosa* indicated extensively CAT in liver (25.20±0.28),heart (43.48±0.43), aorta(28.92±0.17) in Group V rats. Treatment of methyl alcohol concentrates of *Cadaba farinosa* having better tissue antioxidant activity when compared to standard drug .

The exercises of tissues glutathione peroxidase (GPx) in AD rats were appeared in Tables 4. Treatment with AD in Group II rats indicated extensively diminished action of tissues level of glutathione peroxidase (GPX) noted as 4.95±0.05, 6.98±0.06, 6.07±0.07 when contrasted with Group I rats tissues level of GPX chemical as 9.08±0.07, 16.15±0.08, 14.03±0.06. Treatment with EA extracts in Group IV showed increase in the activity of tissues level of glutathione peroxidase (GPX) enzyme activity as 6.17±0.03, 9.99±0.19, 7.24±0.05. Treatment with methyl alcohol extracts in Group V rats showed drastically increase in the activity of tissues level of glutathione peroxidase (GPX) enzyme activity as 8.07±0.06, 13.31±0.11, 12.77±0.06. Treatment with atorvastatin in Group VI rats showed enhanced activity of tissues level of glutathione peroxidase (GPX) enzyme activity as 8.69±0.07, 14.48±0.10, 13.59±0.14. Administration of methanol extract of *Cadaba farinosa* considerably (P<0.001) increased the tissues level of GPx enzyme activity.

The impact of tissues glutathione-S-transferese in AD rats were appeared in Tables 5. Treatment with AD in Group II rats indicated extensively diminished action of tissues level of GST compound as 9.76±0.14, 9.24±0.10, 7.05±0.04 when contrasted with Group I rats tissues level of 25.95±0.25, 20.73±0.19, 16.42±0.14. Treatment with EA extracts in Group IV showed increase in the activity of tissues level of GST enzyme activity as 14.74±0.18, 12.67±0.13, 8.29±0.11. Treatment with methyl alcohol extracts in Group V rats were showed drastically increase in the activity of tissues level of GST enzyme activity as 20.21±0.18, 17.10±0.11, 15.22±0.09. Treatment with atorvastatin in Group VI rats showed enhanced activity of tissues level of GST enzyme activity as 21.79±0.17, 18.15±0.80, 15.29±0.14. Administration of methanol extract of *Cadaba farinosa* considerably (P<0.001) increased the tissues level of GST enzyme activity.

As depicted in Table 6 and Fig. 2, the concentration of tissues glutathione (GSH) in control and AD treated rats. The level of tissues of glutathione (GSH) was incredibly decreased in AD rodent (Group II) as 1.60, 4.53, 2.39 when contrasted with control rats as 4.70, 8.02, 5.78. The Group III rats treated with PE extract of *Cadaba farinosa* along with AD showed slight increase in the tissues glutathione (GSH) as 1.85,4.85,2.74. The Group IV rats recurring EA extract of *Cadaba farinosa* along with AD showed increase in the tissues of glutathione (GSH) as 2.86, 5.22, 3.38 . The Group V rats recurring methanol extract of *C. asiatica* along with AD showed markedly increase in the aorta tissues of glutathione (GSH) 3.87, 7.17, 5,30. Atorvastatin managed in Group VI creatures recorded huge increment in the tissue of glutathione (GSH) as 4.17, 7.84, 5.69. Treatment of methanol concentrate of *Cadaba farinosa* significantly (P < 0.001) increment the tissues of glutathione (GSH) when contrasted and other two concentrates treated Groups.

As depicted in Table 7 and Fig. 2, the level of tissues Vitamin C in control and AD treated rats. The level of tissues of Vitamin C was incredibly decreased in AD rodent (Group II) as 0.43, 0.49,0.41 when contrasted with control rats as 0.92, 1.07, 0.90. The Group III rats treated with PE extract of *Cadaba farinosa* along with AD showed slight increase in the tissues Vitamin C as 0.44, 0.57, 0.49. The Group IV rats recurring EA extract of *Cadaba farinosa* along with AD showed increase in the tissues of Vitamin C as 0.49, 0.60,0.54. The Group V rats recurring methanol extract of *C. asiatica* along with AD showed markedly increase in the aorta tissues of Vitamin C 0.77, 0.88, 0.77. Atorvastatin managed in Group VI creatures recorded huge increment in the tissue of Vitamin C as 0.81, 0.91,0.79. Treatment of methanol concentrate of *Cadaba farinosa* significantly (P < 0.001) increment the tissues of Vitamin C when contrasted and other two concentrates treated Groups.

DISCUSSION

The raised degrees of TBARS were seen in tissues of rodents took care of with AD (bunch II). The AD is known to actuate oxidative pressure in the cells by delivering responsive oxygen species (ROS) (Khan SA et al., 2004). This outcomes in expanded lipid peroxidation prompting raised convergence of TBARS and formed dienes (Boccio et al., 1990). The huge decrease in the degree of TBARS in rodents directed with methanol concentrate of *Cadaba farinosa*. This impact might be expected to phytoconstituents present in the methanol concentrate of *Cadaba farinosa.*

The exercises of SOD and CAT in the tissue were essentially (P<0.001) brought down in rodents took care of with AD (bunch II). AD can cause the arrangement of harmful intermediates that can repress the movement of cell reinforcement proteins (Thampi et al., 1991). Supplementation of methanol concentrate of *Cadaba farinosa* alongside AD essentially expanded the exercises of SOD and CAT in tissues of rodents when contrasted with different concentrates treated gatherings.

The outcomes showed that the convergence of glutathione peroxidase (GPX), and glutathione-s transferase essentially diminished in tissues of rodents took care of with AD. Promotion diminished the proportion of oxidized glutathione/decreased glutathione in tissue (De La Cruz et al., 2000). Methanol concentrates of *Cadaba farinosa* alongside AD treated rodents were expanded the exercises of glutathione peroxidase and glutathione S-transferase in every one of the tissues. It very well may be because of help to spread of organic films observed to be related with expansion in the exercises of GPX. Glutathione peroxidase (GPX) engaged with the decrease of an assortment of hydroperoxides, for example, phospholipid hydroperoxides, unsaturated fat hydroperoxides.

GSH likewise works as free extreme forager in the maintenance of revolutionary caused organic harm (Meister, 1984). The decreased levels might be an endeavour by the tissue to balance the expanded arrangement of lipid peroxides that are dealt with by cell reinforcement catalysts, for example, Glutathione peroxidase which rummages H2O2 using GSH as substrate (Raja Shree et al., 1998). Expansion in glutathione level in methanol concentrate of *Cadaba farinosa* remove treated rodents with AD may be because of the increment in the movement of the chemical glutathione reductase which catalyzes the transformation of oxidized glutathione to decreased glutathione in liver.

CONCLUSION

The results gained from the pharmacological screening have incited the finishes that, AD-activated hyperlipidemia was associated with a rising in the oxidative tensions. Treatment of the methanol concentrate of *Cadaba farinosa* had amazingly cutting down oxidative nerves. The bio synthetic substances assessment might be liable for the lessening the TBARS and further develop the cell reinforcement exercises of methanol concentrate of *Cadaba farinosa*. Thusly it will in general be mishandled that methanolic concentrates *Cadaba farinosa* could fill in as trademark cell reinforcement action, which may be huge in keep away from free extremist-initiated infirmities.

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Conflict of interest statement

There are no conflicts of interest.

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Table 1. Impact of various concentrates of *Cadaba farinosa* on tissues TBARS in AD rats

Groups	TBARS (n mol of MDA formed/g tissue)		
	Liver	Heart	Aorta
Group I	23.62±0.24^{b*}	41.28±0.27^{b*}	17.93±0.23^{b**}
Group II	76.03±0.34^{a*}	83.29±0.35^{a*}	65.04±0.18^{a*}
Group III	71.06±0.23$^{a**, b**}$	79.20±0.29$^{a**, b*}$	57.79±0.22$^{a**, b*}$
Group IV	58.84±0.27$^{a**, b*}$	62.50±0.43$^{a**, b*}$	33.16±0.28$^{a*, b*}$
Group V	30.82±0.50$^{a*, b*}$	43.18±0.34$^{a*, b*}$	23.52±0.38$^{a*, b*}$
Group VI	28.85±0.26$^{a*, b*}$	41.80±0.23$^{a*, b*}$	20.36±0.31 $^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group
P values : *< 0.001, **< 0.05
a → Groups II, III, IV, V & VI compared with Group I.
b →Groups I, III, IV, V & VI compared with Group II.
I Group : Standard chow pellet. (Control)
II Group : Atherogenic Diet(AD).
III Group : AD + PE concentrate of *Cadaba farinosa* (200mg/kg B.wt)
IV Group : AD + EA concentrate of *Cadaba farinosa* (200mg/kg B.wt)
V Group : AD + ME concentrate of *Cadaba farinosa* (200mg/kg B.wt)
VI Group : AD+ Atorvastatin (1.2 mg/kg B.wt)

Table 2. Impact of various concentrates of *Cadaba farinosa* on tissues SOD in AD rats

Groups	**SOD (unit min/mg protein)**		
	Liver	**Heart**	**Aorta**
Group I	3.77±0.03^{b*}	1.93±0.02^{b*}	2.97±0.03^{b*}
Group II	1.70±0.02^{a*}	0.73±0.01^{a*}	1.45±0.02^{a*}

Group III	1.98±0.03$^{a**, b*}$	0.93±0.01$^{a**, b**}$	1.70±0.02$^{a**, b*}$
Group IV	2.16±0.03$^{a**, b*}$	1.12±0.03$^{a**, b*}$	1.99±0.02$^{a**, b**}$
Group V	3.39±0.02$^{a*, b*}$	1.71±0.03$^{a*, b*}$	2.59±0.02$^{a*, b*}$
Group VI	3.50±0.17$^{a*, b*}$	1.80±0.01$^{a*, b*}$	2.78±0.01$^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group
P values : * < 0.001, ** < 0.05
a → Groups II, III, IV, V & VI contrasted with Group I.
b → Groups I, III, IV, V & VI constrasted with Group II.
Group I-VI subtleties are comparable as in Table 1.

Table 3. Impact of various concentrates of *Cadaba farinosa* on tissues CAT in AD rats

Groups	CAT (μ moles of H_2O_2, consumed min/mg protein)		
	Liver	Heart	Aorta
Group I	30.63±0.46^{b*}	45.14±0.46^{b*}	32.48±0.23^{b*}
Group II	14.89±0.22^{a*}	30.52±0.22^{a*}	21.08±0.34^{a*}
Group III	16.68±0.12^{a**,b**}	32.61±0.34^{a**,b*}	22.84±0.22^{a**,b**}
Group IV	19.04±0.14^{a**,b*}	38.81±0.28^{a**,b*}	23.99±0.19^{a**,b*}
Group V	25.20±0.28^{a*,b*}	43.48±0.43^{a*,b*}	28.92±0.17^{a*,b*}
Group VI	29.02±0.21^{a*,b*}	46.74±0.24^{a*,b*}	31.23±0.18^{a*,b*}

Data be articulated as mean±SEM., *n* = six rats each Group
P values : * < 0.001, ** < 0.05
a → Groups II, III, IV, V & VI contrasted with Group I.
b → Groups I, III, IV, V & VI constrasted with Group II.
Group I-VI subtleties are comparable as in Table 1.

Table 4. Impact of various concentrates of *Cadaba farinosa* on tissues Glutathione Peroxidase in AD rats

Groups	GPx (mg of GSH consumed/min/mg protein)		
	Liver	Heart	Aorta
Group I	9.08±0.07^{b*}	16.15±0.08^{b*}	14.03±0.06^{b*}
Group II	4.95±0.05^{a*}	6.98±0.06^{a*}	6.07±0.07^{a*}
Group III	5.49±0.06$^{a**, b**}$	7.37±0.04$^{a*, b**}$	6.65±0.08$^{a**, b*}$
Group IV	6.17±0.03$^{a**, b*}$	9.99±0.19$^{a**, b*}$	7.24±0.05$^{a**, b*}$
Group V	8.07±0.06$^{a*, b*}$	13.31±0.11$^{a*, b*}$	12.77±0.06$^{a*, b*}$
Group VI	8.69±0.07$^{a*, b*}$	14.48±0.10$^{a*, b*}$	13.59±0.14 $^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group
P values : * < 0.001, ** < 0.05
a → Groups II, III, IV, V & VI contrasted with Group I.
b → Groups I, III, IV, V & VI constrasted with Group II.
Group I-VI subtleties are comparable as in Table 1.

Table 5. Impact of various concentrates of *Cadaba farinosa* on tissues Glutathione S-Transferase in AD rats

Group s	Glutathione – S – transferase (GST) (μ mole of CDNB – GSH – Conjugate to /min/mg protein)		
	Liver	Heart	Aorta
Group I	25.95±0.25^{b*}	20.73±0.19^{b*}	16.42±0.14^{b*}
Group II	9.76±0.14^{a*}	9.24±0.10^{a*}	7.05±0.04^{a*}
Group III	11.86±0.18$^{a*, b*}$	10.12±0.07$^{a*, b*}$	7.27±0.03$^{a*, b*}$
Group IV	14.74±0.18$^{a*, b*}$	12.67±0.13$^{a*, b*}$	8.29±0.11$^{a*, b*}$
Group V	20.21±0.18$^{a*, b*}$	17.10±0.11$^{a*, b*}$	15.22±0.09$^{a*, b*}$
Group VI	21.79±0.17$^{a*, b*}$	18.15±0.80$^{a*, b*}$	15.29±0.14$^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group
P values : * < 0.001, ** < 0.05
a → Groups II, III, IV, V & VI contrasted with Group I.
b → Groups I, III, IV, V & VI constrasted with Group II.
Group I-VI subtleties are comparable as in Table 1.

Table 6: Impact of various extracts of *Cadaba farinosa* on tissues Glutathione in AD rats

Groups	Glutathione (GSH) mg/dL		
	Liver	Heart	Aorta
Group I	4.70±0.06^{b**}	8.02±0.05^{b*}	5.78±0.04^{b*}
Group II	1.60±0.03^{a**}	4.53±0.02^{a*}	2.39±0.05^{a*}
Group III	1.85±0.04$^{a*, b**}$	4.85±0.04$^{a**, b**}$	2.74±0.04$^{a**, b**}$
Group IV	2.86±0.04$^{a**, b*}$	5.22±0.05$^{a**, b*}$	3.38±0.06$^{a**, b*}$
Group V	3.87±0.04 $^{a*, b*}$	7.17±0.03 $^{a*, b*}$	5.30±0.04$^{a*, b*}$
Group VI	4.17± 0.02 $^{a*, b*}$	7.84±0.08 $^{a*, b*}$	5.69±0.07$^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group
P values : * < 0.001, ** < 0.05
a → Groups II, III, IV, V & VI contrasted with Group I.
b → Groups I, III, IV, V & VI constrasted with Group II.
Group I-VI subtleties are comparable as in Table 1.

Table 6: Impact of various extracts of *Cadaba farinosa* on tissues Vitamin C in AD rats

Groups	Vitamin C μg/mg protein		
	Liver	**Heart**	**Aorta**
Group I	0.92±0.01^{b**}	1.07±0.01^{b*}	0.90±0.02^{b*}
Group II	0.43±0.01^{a**}	0.49±0.02^{a*}	0.41±0.01^{a*}
Group III	0.44±0.03$^{a*, b**}$	0.57±0.01$^{a**, b**}$	0.49±0.01$^{a**, b**}$
Group IV	0.49±0.01$^{a**, b*}$	0.60±0.01$^{a**, b*}$	0.54±0.01$^{a**, b*}$
Group V	0.77±0.01$^{a*, b*}$	0.88±0.02 $^{a*, b*}$	0.77±0.01$^{a*, b*}$
Group VI	0.81±0.01 $^{a*, b*}$	0.91±0.02$^{a*, b*}$	0.79±0.01 $^{a*, b*}$

Data be articulated as mean±SEM., *n* = six rats each Group

P values : * < 0.001, ** < 0.05

a → Groups II, III, IV, V & VI contrasted with Group I.

b → Groups I, III, IV, V & VI contrasted with Group II.

Group I-VI subtleties are comparable as in Table 1.

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REVIEW ARTICLE

Plant Derived Anticancer Agents Used In India - A Review

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ABSTRACT

Cancer is one of the life threatening diseases which affect the human beings very severely. There is a huge demand for newly developed methods and measures for the prevention of this disease. The advantages of using plant based drugs over synthetic drugs have increased and it makes the medicinal plants more important in the health care field. Secondary metabolites isolated from the medicinal herbs are capable of having anticancer properties due to its ability to inhibit DNA damage, cell cycle arrests, ability to inhibit angiogenesis of tumour cell angiogenesis, or sometimes their ability to induce apoptosis. In this present review, a small effort has been done to provide information about some important medicinal plants available in India which has anticancer activity against different cell lines.

Keywords: Cancer, cell lines, medicinal plants, cytotoxic, apoptosis, cell inhibition.

INTRODUCTION

Indian herbal medicines have had a record of protected utilization for the previous few hundreds of years. In latest years, the work on Indian herbal medicine has received momentum due to each treatment and preventive options. The growing popularity of herbs as medicines over the extra frequent allopathic system is chiefly a result of the threat of mortality and long term morbidity linked to surgeries and facet effects of allopathic medicines. Phyto medicines have been proven to benefit patients by means of imparting comfort from a plethora of ailments. It also offers the users the power to pick out the medications that they favour to use. Hence, herbs have been used due to the fact that a long time both directly or as dietary supplements. This is also due to the realization that natural products act in a way comparable to pharmaceuticals but pose no side effects. Natural anti-inflammatory compounds are observed in plenty in the natural plant life and have been already stated in green tea, the spices

turmeric and rosemary, feverfew, and many others[1]. In 2018, the global cancer burden increased to 18.1 million new cases and 9.6 million deaths. Among the different types of cancer, colorectal, liver, lung, prostate, and stomach cancers primarily harm men, while breast, cervix, colorectal, lung, and thyroid cancers primarily affect women. Cancer treatment has spawned a new field of study. There are both traditional and unconventional options. Modern techniques like chemotherapy, radiation therapy or surgery are used in treating cancers nowadays. These methods have some disadvantages also. The use of synthetic chemicals based treatment bears toxicities and side effects. As a result, new cancer preventive and treatment measures are required to keep the disease's death rate under control [2].

The search for anticancer agents from plant sources began in the early 1950s and is mainly with the discovery of vinca alkaloids and isolation of podophyllotoxins. The United States National Cancer Institute initiated a significant plant collection program in 1960, targeted generally in temperate regions, as a result of these discoveries. This led to the discovery of many novel compounds showing a variety of cytotoxic activities, which includes the taxanes and camptothecins. The improvement of these compounds into clinically active agents spanned a duration of some 30 years, from the early sixties to the nineties. This plant collection program was once terminated in 1982. The development of new screening technologies has led to the revival of collections of plants and different organisms in 1986, with a focal point on the tropical and sub-tropical areas of the world. It is interesting to notice that no new plant derived clinical anti-cancer agents have, as yet, reached the stage of general use, however a variety of agents are in preclinical development [3].

Herbal-based treatments have been determined to be one of the greatest options for treating and/or preventing cancer, according to researchers. This is due to the wide range of active chemicals found in plants, which function against a wide range of malignancies through a variety of ways. These chemicals can be isolated and utilised alone or in conjunction with other anticancer medications. These natural chemicals are discovered to be naturally available, cheaper, and easier to take orally than synthesised medications, and they have low or minimal adverse effects, as well as being rich in physiologically active chemotypes. Chemotherapy resistance is one of the most serious issues in cancer treatment, which is why researchers are working hard to prevent or lessen resistance by identifying new anticancer medicines as alternatives [4]. Traditionally, several medicinal plants all over the world especially in India are being used for the prevention and treatment of cancer. Cancer is a disease in which our body cells grow abnormally and finally results in death. It causes an imbalance in the body by destroying regular cells. In both developing and developed countries, it is one of the most serious health issues. It is extremely difficult to pinpoint the exact etiology of cancer. Tobacco usage, alcohol intake, environmental pollution, infectious agents, customary practises, and lifestyles are some of the most well-known causes of this disease.

One of the very important parameter in determining the severity of cancer is the different staging. According to the stage of cancer, patients can be recommended by drugs. There are four stages of cancer, each showing different properties and symptoms. These are tabulated as under:
STAGE 1:This is the first stage of cancer, and there are no symptoms. The tumour hasn't reached its full potential. A normal medical examination can aid in the detection of early-stage cancer. It would be easier to cure cancer if it was discovered at this stage.
STAGE 2:In this stage, the tumour is easily evident on scans. There are a few obvious signs and symptoms.
STAGE 3:The benign tumour has reached full maturity and is causing symptoms
STAGE 4:This is the final stage of cancer, and there is no cure at this point. The tumour has metastasized (spread) to other areas of the body. Cachexia (sudden considerable weight loss) has noticeable symptoms, as do spots on the skin in the case of skin cancer.
There is another way to classify cancer depending on its severity and spread, which is as follows:
T:This is the earliest stage of cancer, and the severity of it is determined by how far the tumour has migrated from its original location.
N:When a tumour extends from its original location to lymphatic nodes, it is classified as stage 'N' cancer.
M:This is the point at when the tumour has spread to other parts of the body and there is no way to cure it.

Dietary changes, cessation of cigarette use, efficient treatment of inflammatory illnesses, and the use of nutritional supplements that help immune functioning are all essential preventive approaches for most malignancies. Radiotherapy, chemotherapy, and chemically generated medications are among the current cancer treatments. Anti-metabolites, alkylating drugs, platinum analogues, and anti-tumor antibiotics are among the most regularly utilised cancer chemotherapeutic agents. Chemotherapy and radiotherapy, on the other hand, put patients under a lot of stress and harm their health. As a result, new anticancer medicines from nature, particularly plants, are currently being researched. As a result, new anticancer medicines from nature, particularly plants, are currently being researched. Plants have long served as a foundation for traditional medicinal systems, and they have provided humans with ongoing cures for thousands of years [5].

Anticancer Activity of Medicinal Plants

***Abrus precatorius* (Fabaceae)**.

It is native to India and is seen in all tropical countries. It is commonly called as Jequirity in English. The plant *Abrus precatorius* is widely used in many traditional systems of medicine including Ayurveda, Homeopathy, Folk, Tibetian, Sidha, and Unani. Its leaves are used as nerve tonic, applied on swellings and cuts and mouth ulcer. The leaves of the herb are used to cure fever, cough and cold. The roots are used to treat jaundice and haemoglobinuric bile. Paste of roots is used to cure abdominal pains, tumors and also for abortion. Root is chewed as a snake bite remedy. *A. precatorius* is traditionally used to treat tetanus, and to prevent rabies. Some importantphytoconstituents like alkaloid (abrin, choline, 5 cholinic acid, hypaphorine, abralin), steroids (abricin, abrectorinabridin), triterpene glycosides (abusosides A, B, and C), flavonoids (vitexin, liquirtiginin-7-mono-diglycosides, toxifolin-3-glucosides, 4,5,7-trihydroxy flavon) have been isolated from the leaves, roots and seeds of *A. precatorious* [6-8]. M. Lebri *et al* studied the in vitro anticancer effect of aqueous extract of *Abrus precatorius* leaves and the evaluation was done by the cellular cytotoxicity against the murinmastocytoma cell line (P815)by MTT assay. The studies showed the *in vitro* anticancer effectis adose dependant one [9]. The *in vitro* and *in vivo* antitumor properties by the induction of apoptosis were reported by a compound Abrusabrin isolated from the seeds of Abrus precatorius[10]. Bhutia SK *et al* reported the*in vivo* therapeutic effectiveness of abrin-derived peptide (ABP) fraction in Dalton's lymphoma (DL) mice model [11].Subba Reddy and Sirsi isolated a protein from the seeds of *Abrus precatorius* and conducted antitumor activity studies on Yoshida sarcoma (solid and ascites forms) in rats and a fibrosarcoma in mice. They found that the extract had a direct cytotoxic effect on the tumor cells [12]. Sivakumar and Alagesaboopathi performed the studies of crude extract and its fractions of red and white forms of *Abrus precatorius* on cancer cell line A 549 by MTT and SRB assays. They concluded that the methanol insoluble fraction of crude red forms were toxic to the cell in both assays[13].

***Acacia catechu* (Mimosacea):**

It is indigenous to India, other Asian countries, and East Africa. In recent years, numerous studies have examined various pharmacological properties of extracts prepared from heartwood, bark, leaves, seeds, and seed pods of Acacia species.Traditionally, *A. catechu* has been used as an antimicrobial, anti-inflammatory and antifungal, coagulant, vermifuge, antidiarrheal, and astringent, and has also been employed to heal wounds, treat obesity and diabetes, and maintain oral hygiene[14]. Acacia catechu contains mainly catechin, epicatechin, epicatechingallate, procatechinic acid, tannins, alkaloids quercetin and kaempferol, porifera sterol glucosides etc. Monga *etal.* in their three studies evaluated the aqueous, catechin-rich heartwood extract of *A. catechu* and was found to exhibit potent anticancer activity by the prevention of squamous cell, mammary, and liver cancers in a dose-dependent manner [15-17]. *Thangaveluet al* evaluated the cytotoxic activity of ethanolic extract of *Acacia catechu* seed against SCC-25 human oral squamous carcinoma cell line and was found to be cytotoxic at lower concentrations and induced apoptosis in human oral squamous carcinoma SCC-25 cells [18]. Nadumane*et al*have concluded the antiproliferative and apoptotic potentials of *Acaciacatechu*bark extract on HeLa, COLO- 205, and fibrosarcoma HT-1080 cell lines. They have concluded in their studies that the methanolic and hexane extracts of *Acacia catechu* have significant anticancer, cytotoxic effects on both the COLO-205 and the HeLa cell lines which are quite safe on human peripheral lymphocytes [19]. Ghate NB *et al*assessed theability to inhibit the proliferation of human breast adenocarcinoma

(MCF 7) cells by inducing apoptosis withthe methanolic extract of "Katha" which is the heartwood of *Acacia catechu*[20] .

***Allium sativum* (Liliaceae).**

Allium sativum is rich in various chemical compounds that are useful in prevention and treatment of different types of cancer. It contains a compound known as allicin which possess antioxidant and anticancer activities. Allicin can be penetrated very rapidly into different cells and can be completely metabolized in the liver. Experimental studies shown that garlic and its organic allylsulfur components are effective inhibitors of tumor growth [21]. Diallyltrisulphide, diallyldisulphide and s-allyl cysteine are the constituents with anticancer properties [22].The rapidly dividing cancer cells contain high concentration of sulphur enzymes. *Allium sativum*can react with these types of enzymes and thereby decreasethe concentration which can lead to the inhibition of tumour growth. The extract of *Allium sativum* enhances the activity of natural killer cells and macrophages. It can also increase the count of suppressor T cells which makes the lymphocytes more cytotoxic to cancerous cells [23]. The extract prevents the adhesion of circulating cancer cells thereby inhibiting metastasis. *Allium sativum*can protect DNA from the effects of carcinogens and increases the activity of detoxifying enzymes[24].Laboratory studies and experimental animals have demonstrated that the solid indication of organosulphur compounds in garlic might influence cancer cells by promoting the early mitotic arrest followed by apoptotic cell death devoid of disturbing healthy cells[25].Zhang *et al* studied the effect of Allicin, an organosulphur compound from the bulbs of Allium sativumcan induce apoptosis of gastric cancer cells by activating the p38 (mitogen-activated protein kinase pathway) MAPK signaling pathway, which further stimulates the hydroxylation of caspase-3 [26]. A recent study conducted by Li et al. showed that seven-year garlic supplementation was significantly associated with either a reduced risk of gastric cancer or a reduced risk of cancer-related death [27].

***Alstonia scholaris* (Apocynaceae)**

The medicinal herb *Alstonia scholaris*) has been used in folk and traditional systems in India to treat several diseases. The ripe fruits of plants are used for treating syphilis, insanity, and epilepsy. It is also used as a tonic, antiperiodic, and antihelminthic. The milky juice of *Alstonia scholaris* has been applied to treat ulcers. The most commonly used part is bark and is used in many herbal formulations. The phytochemical constituents of *Alstonia scholaris*have been widely investigated and alkaloids, iridoids, coumarins, flavonoids, leucoanthocyanines, reducing sugars, simple phenolics, steroids, saponins and tannins were documented as the chief chemical constituents [28]**.** SwafiyaJahan*et al* demonstrated the chemopreventive potential of *Alstonia scholaris* bark extract in DMBA-induced skin tumorigenesis in Swiss albino mice.Their study clearly gives the evidence that the extracts can be able to induce reversal of altered enzyme activities [29]. Jagetia and Baliga also studied the cytotoxic activity of *Alstonia scholaris*ethanolic bark extract in different cell lines in vitro as well as in vivo and was found to exhibit the cytotoxicity. The exact mechanism of action of the extract was not known, but the anticancer effect was may be due to induction of apoptosis, DNA damage, decrease in the antioxidant enzymes and total thiols, increase in the lipid peroxidation and immune modulation [30].

Andrographis paniculata (Acanthaceae)

In English it is commonly known as King of bitters and in Hindi as kalmegha. *Andrographis paniculata* is a medicinal plant traditionally used for the treatment of cold, fever, laryngitis and several infectious diseases ranging from malaria to dysentery and diarrhea in China, India and other South East Asian countries.*It is mainly seen in India and SriLanka. Traditionally the roots and leaves are used for the medicinal purposes. It contains mainly* flavonoids, stigmasterols and diterpenes [31]. Kumar *et al* demonstrated that the major constituent andrographolide, a diterpene showed anticancer and immunostimulatory activities. They have conducted in vivo study by hollow fiber assay method in immunocompetent Swiss albino mice andhas shown that andrographolide significantly inhibits the cancer cell proliferation without showing any signs of toxicity in mice even at high doses [32]. Due to this ability, plant is effective against various oncogenic and infectious agents. Siripong*et al* in their studies showed the cytotoxic effect against breast cancer cells (MCF-7), P388 lymphocytic cells and colon cancer cells (HCT-116). Andrographolide shows inhibition of growth in colon cancer cell line HT 29 and enhance growth and division of human peripheral

blood lymphocytes on mouse myeloid leukemia M1 cell lines [33]**.** Andrographolide exhibited both direct and indirect effects on cancer cells by inhibiting proliferation of cancer cells, cell-cycle arrests, or cell differentiation, enhancing body's own immune system against cancer cells; and inducting apoptosis and necrosis of cancer cells [34]. Geethangili*et al* showed the effective cytotoxic activity of ethanol extracts against human cancer cells including Jurkat (lymphocytic), PC-3 (prostate), HepG2 (hepatoma), and colon 205 (colonic) cancer cells [35].

***Amorphophallus campanulatus* (Araceae)**

It is commonly known as Elephant foot yam and is a tuber crop of South East Asian in origin. It is cultivated all over India and used as a food crop [36].Its rhizome is extensively used in many Ayurvedic medicines and also against liver infections, bronchial infections, abdominal pain, asthma, dysentery, spleen enlargement, elephantiasis diseases due to vitiated blood and sore swellings. The rhizome contains some important constituents like lupeol, sitosterol, stigmasterol, galactose, palmitic acid, betulinic acid, glucose, rhamnose and xylose[37]. It has been reported that the rhizome possess antibacterial, antifungal and cytotoxic activities [38]. Ansil*et al* evaluated the dose-dependent cytotoxic and apoptosis inducing effects of the methanolic extract and its subfractions of *Amorphophallus campanulatus* tuber. They have conducted the studies using colon cancer cell line, HCT-15. Antiproliferative effects of the sub fractions were studied by MTT assay. Apoptotic activity was assessed by 4′,6-diamidino-2-phenylindole, annexin V-fluorescein isothiocyanate and 5,5',6,6' tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide fluorescent staining. They have also evaluated the activity on human liver cancer cell line with the same subfractions using the same methods. The conclusion obtained from their studies was that the subfractions induce apoptosis by suppressing the cell growth [39-40].

***Annona reticulata* L. (Annonaceae)**

Annona reticulata is commonly known as custard apple or bullock's heart. The plant has traditionally used for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark has astringent activity and used as a tonic. The leaves are used in the treatment of helminthiasis [41]. Mondal*et al* have investigated the *invitro* cytotoxic activity of methanolic extract of leaves of *Annona reticulata* by caspase inhibitory assay and examined against human colorectal adenocarcinoma and human hepatocellular carcinoma. They have concluded that the extract is effective against colon and liver cancer and in the treatment of degenerative disorders [42]. Suresh *et al*have studied the ethanol and aqueous extracts of the roots of *Annona reticulata* for *invivo* and *invitro* anticancer activity by MTT colorimetric assay. Their observations proved that these extracts exhibit inhibitory activityaganist mice melanoma cells and human melanoma cells [43]. They have also investigated the major chemical constituents of the ethanolic extract of *Annona reticulata* roots and was found to present acetogenins, alkaloids, flavonoids, proteins, carbohydrates etc [44].

***Berberis aristata* (Berberidaceae)**

It is commonly known as Indian barberry or tree turmeric. The fruits are eaten by people as a dessert. Fruits are juicy and contain plenty of sugars and other useful nutrients that supplement their diet. Its stem, roots, and fruits are used in Ayurveda. Aqueous roots extract of this plant was found to have antimalarial activity in animal models. The plant as a whole is a good source of dye and tannin which is used for dyeing clothes and for tanning leather. Roots extract of *B. aristata* was found to have good dyeing properties on cotton [45]. Uniyal and Tewari hasconducted an ethnobotanical documentation studies on the traditional medical practices in remote villages of India and concluded that this plant is used by herbal healers to treat cancers, especially the oral cancers [46]. The root bark contains berberine, a quaternary ammonium salt of isoquinoline alkaloid.Pai *et al* studied the antineoplastic activity of the extracts of *Berberis aristata* in Ehrlich ascites carcinoma bearing mice in the advanced stage of tumorigenesis. Their study concluded that *Berberis aristata* has prominent antineoplastic activity [47].Mamata *et al* reported that the methanolic stem extract of *Berberis aristata* has anticancer activity in human breast cancer cell line (MCF-7) [48].

***Basella rubra* (Basellaceae)**

It is commonly known as Malabar spinach, Indian spinach, Ceylon spinach and Vine spinach. The various uses of this plant are mentioned in Charaka Samhita, and Sushruta Samhita. It is rich in calcium, iron, vitamin A and vitamin C. In Ayurveda, the plant has shown immense potential in androgenic, antiulcer, antioxidant, cytotoxic, antibacterial activity, anti-inflammatory, central nervous systemdepressant activity, nephro protective and wound healing properties[49].The plantcontains flavonoids, phenolic compounds, caffeicacid, chlorogenicacid, cinnamic acid, gomphrenin I, gomphrenin II, gomphrenin III etc.[50]. The phenolic, flavonoid, betalains rich ruit extract of *Basella rubra* was investigated for their antioxidant and anticancer activities against human cervical carcinoma (SiHa) cellsby Sandopu Sravan Kumar *et al* [51]. A bioactive compound isovitexin which is a C-glycosylflavone was isolated from the methanolic extract of the leaves of Basellarubra and was studied for the cytotoxicity against human colon cancer (HT-29) cells and the study was carried out by BhanupriyaKilaria *et al* [52].

***Cassia auriculata* (Cesalpinaceae)**

It is also known as Tanner's Cassia or Avartaki. It is extensively used in cancer treatment as well as a cure for rheumatism in traditional system of Indian medicine. Many activities like antipyretic, hepatoprotective, antidiabetic, antiperoxidative, antihyperglycemic and antimicrobial activities were reported in this plant. *Cassia auriculata* contains preliminary phytochemical constituents such as alkaloids, phenols, glycoside, flavonoids, tannin, saponins, protein, carbohydrate and anthraquinone derivatives which are responsible for the pharmacological activities. Muruganantham *et al* have studied the ethanolicflower extract for its anticancer activity against liver cancer HePG2 cell line by MTT assay [53]. Prasannaa*et al* conducted a study and concluded that the leaf extract of *Cassia auriculata* possess *in vitro* anticancer effect on human breast adenocarcinoma MCF-7 and human larynx carcinoma Hepd-2 cell lines. The extract inhibited the growth of both the cell lines and the inhibition is due to nuclear fragmentation and condensation which is an indication of apoptosis [54]. M. Irshad*et al* evaluated the anticancer activity of different extracts of *Cassia fistula* fruit against human cervical cancer (SiHa) and breast cancer (MCF-7) cell lines. The phytochemicals analysis has also performed and it revealed the presence of some important compounds like 2(3H)-furanone, rhein, thymol, oleic acid, inositol, palmitic acid, inositol and 2-pyrrolidone [55]. Padmalochana*et al* elucidated the antioxidant and anticancer activities of acetone and methanolicflower extract and the activity was examined by inhibition on liver cancer cell line [56].

***Cedrus deodara* (Pinaceae)**

Cedrus deodara commonly called as deodar is a species of cedar and is extensively used by the local people of the Himalayan region. The plant is used for the treatment of fever, diabetes, intestinal parasite and sinusitis and for many other ailments. The plant is rich in flavonoids and terpenes. Stem bark contain dihydroflavanol called deodarin, wood contain sesquiterpenehimachalol, needle oil include terpineol, linalool, limonene, anethole, eugenoletc [57]. Sudhir Kumar *et al* concluded in their studies regarding the cytotoxic effect of taxifolin rich fraction of *Cedrus deodara* bark extract and found to have efficient cytotoxic effect on human breast cancer cell lines MCF-7 [58]. Xiaofeng Shi reported that the total flavonoids extracted and purified from pine needles possess in vitro antitumour effect [59]. Bhagat *et al* has explored the anticancer potential of the essential oil obtained from the bark of *Cedrus deodara* and found that the essential oil inhibits the cell proliferation and induces apoptosis in colon cancer cells [60].

***Drosera indica* (Droseraceae)**

Drosera indica is commonly called dew plant, flycatcher and Indian sundew is an insectivorous plant as well as medicinal plant and consists of approximately 170 species in all over the world. It has been reported that these species have highly therapeutic properties like recovery of memory loss, overall body weakness, improvement of defective eyesight, and helps in fertilization in human and also curing of early aging etc. Phytochemical profiling of this species has revealed the presence of highly valuable phytochemicals like quercetin, hyperoside, isoquercetin, naphthoquinonesetc.Another study revealed that naphthoquinones and ramentaceone has potentialanti-cancer activity, to destroy the breast cancer cell in proper way [61]. Raju*et al* has conducted many studies regarding the

antitumor potentials of *Drosera indica*. In one study they evaluated the effectiveness of ethanolic and aqueous extract against Daltons lymphoma ascites induced tumour and concluded that the extracts has exhibited significant antitumor activity in DLA- bearing mice [62].

***Nigella sativa* (Ranunculaceae)**

*Nigella sativa*is commonly called Black cumin and is used as a spice in Indian cuisine. The seeds are used as carminative, aromatic, stimulant, diuretic, anthelmintic, galactagogueand and diaphoretic. Seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpenealcohols and the essential oil containsthymoquinone, p-cymene, pinene, dithymoquinone and thymohydroquinone. The essential oil also contains significant amounts of fatty acid ethyl esters. The fatty acids present in *Nigella sativa* was studied for antitumour activities against Ehrlich ascites carcinoma, Dalton's lymphoma ascites and Sarcoma 180 cells by Salomi et al. They have reported that the seeds possess 50% cytotoxicity against these cells [61]. Swamy and Tan have reported the in vitro cytotoxicity of the ethyl acetate fraction of *Nigella sativa* seeds against different cancer cell lines by using MTT assay [62]. Al-Sheddi *et al* has concluded in their investigation of anticancer activity of the seed extract and seed oil against lung cancer cell line that these reduce the cell viability and alter the morphology of A-549 cells significantly [63].

***Podophyllum hexandrum* (Berberidaceae):**

Podophyllum hexeandrum is commonly known as May apple and is found in Himalayanregion. It contains podophyllotoxin, kaempferol,quercetin, asiragalin, essential oil, podophyllin. *Podophyllum hexandrum* rhizomes, roots and fruits are used as anticancer agents and used in the treatment of ulcers, hepatic disorders, wounds, purgative and tuberculosis. It is currently being used as a lead compound for the semisynthesis of anticancer compounds like teniposide, etoposide and etopophos which are used in the treatment of lung and testicular cancers and certain leukemias [66]. Abad *et al* in their studies shown that podophyllotoxin prevent the assembly of tubulin into microtubules thus persuading apoptosis which confirms the antineoplastic property [67]. Chen *et al* showed in their preclinical studies that podophyllotoxin has prevented the polymerization of microtubule resulting in mitotic detention [68].

***Solanum nigrum*(Solanaceae)**

It is commonly known as black night shade and deadly night shade. It possess medicinal properties like antimicrobial, antioxidant, cytotoxic properties, antiulcerogenic and hepatoprotective activity. *Solanumnigrum* is a potential herbal alternative as anticancer agent due to the presence of diosgenin. Sanjay *et al* has tested the inhibitory effect of methanolic extract of *Solanumnigrum* fruits on HeLa cell line and concluded that the methanolic extract has significant cytotoxicity effect by SRB and MTT assays [69]. Li J *et al* have reported in their studies that the crude polysaccharides isolated from *Solanumnigrum* decreased the number of ascites tumor by invivo administration against U14 cervical cancer cell lines [70]. Ding X *et al* have investigated the antitumour activity with the isolated steroidal glycoalkaloids by different methods like MTT assay, flow cytometry, colorimetric assay and immunocytochemical method [71]. Lai Y *et al* in their recent studies found that the aqueous extract of *Solanum nigrum* markedly inhibited the cell viability of MCF 7 breast cancer cell line through apoptosis induction and cell cycle arrest [72].

***Tinospora cordifolia (*Menispermaceae)**

It is commonly known as Guduchi or Fish berry and is native to India. *Sharma et al has concluded in their reports that in Indian system of medicine, Tinospora cordifolia* is a very popular medicinal plant and is used in the treatment of fever, urinary problem, dysentery, skin diseases leprosy, diabetes, and many more diseases. Chemical constituents like alkaloids, terpenoids, lignans, steroidsetc are also reported by them [73]. In another study, Rumana*et al* investigated the in vitro cytotoxic activity of methanolic extract of stem of *Tinospora cordifolia* against human breast cancer cell line MDA-MB-231 and normal Vero epithelial cell line. Their reports have shown that the methanolic extract of has significant anticancer activity against MDA-MB-231 human breast cancer cell line[74].Ali H and Dixit S have extracted

and optimised an alkaloid namely palmatine from *Tinospora cordifolia* and studied the anticancer potential of this alkaloid in DMBA induced skin cancer model in mice [75].Jagetia and Raohave concluded in their studies that the dichloromethane extract of *Tinosporacordifolia* has got cytotoxic effect in the mice transplanted with Ehrlich ascites carcinoma. The extract exerted cytotoxic effect by reducing the GSH concentration and increase in LPx simultaneously [76].

***Vitex negundoi*(Lamiaceae)**

It is commonly known as five leave chase tree and nirgundi in India. This plant is a folk medicine in many countries including India. Mainly the leaves are used for the treatment of eye diseases, inflammation, leucoderma, toothache, spleen enlargement, skin-ulcers, gonorrhea, rheumatoid arthritis, and bronchitis. It also possess various biological activities like antimicrobial, antiseptic, anti-inflammatory, antitumor, sedative, insecticidal, insect repellant, and nematicidal, etc. Issa*et al* have explored the cytotoxic potential of the essential oil [77].Badgujar *et al* in their studies concluded that themethanolic extract of *Vitex negundo* leaves showed the presence of a pronounced amount of flavonoids and phenolic contents. Their studies have concluded that the leaves showed marked cytotoxic effect against HELA and KB human cancer cell lines[78].Xin *et al* have extracted the lignan compounds and found that they possesses a broad spectrum cytotoxic effect *via* arresting cancer cells at G2/M phase cell cycle and subsequently inducing apoptosis [79]. Nandu K and Patel R have evaluated the anticancer activity of crude leaf extract in experimental animals [80].

***Vitis vinifera (V*itaceae)**

It is commonly known as grape vine and is one of the most widely consumed fruits in the world and is rich in the antioxid antpolyphenols.Nirmala *et al* carried out a study to assess the antiproliferative and apoptotic effects of *Vitis vinifera* peel and seed extracts in an in vitro model using human epidermoid carcinoma A431 cell lines [81].Kaur *et al* isolated extract from the grape seeds and stems and have demonstrated the antitumour activity in human breast cancer cell lines (MCF-7 and MDA-MB-23), colon (HT29), renal (786-0 and Caki-1), thyroid (K1), hepatocellular carcinoma cell lines, oral squamous cell carcinoma and normal human fibroblasts [82].

***Withania somnifera(*Solanaceae)**

It is commonly called as Winter cherry or Ashwagandha and is a well-known medicinal plant in Ayurvedic and Unani system of medicine. The plant has been documented to exhibit medicinal benefits against several ailments, including neurodegenerative diseases, cancer, and chronic diseases. It contains with anolides, with aferins, anferine, isopellertierine and sitoindosise. Due to its medicinal properties, leaves and roots have been used in the Indian traditional system of medicine and marketed globally. Halder *et al* evaluated for the first time, the cytotoxic effect of *Withania* root extract on human malignant melanoma A375 cells. The crude extract was tested for cytotoxicity against A375 cells by MTT assay. The crude water extract of *Withania somnifera* has potent cytotoxic effect on human malignant melanoma A375 cells[83]. Maliyakkal N *et al* in their current study investigated the cytotoxic and apoptotic effects of the ethanolic extract of *Withania somnifera* on human breast cancer cells (MCF7 and MDA MB 231). MTT-based assays revealed dose-dependent cytotoxic effects [84].Withanolide A and With aferin A are the main constituent of this plant. With aferin A which is mostly present in the leaves and produces rapid apoptosis in the cancer cells. Cell signaling pathways by this plant formulation are mainly due to the presence of high content of WithferinA present in it [85].

***Ziziphus nummularia (*Rhamnaceae)**

It is commonly named as Wild jujube and is mainly seen in Iran, India, Iraq, Israel, Pakistan and Afghanistan. Stem, bark, roots seeds and flowers of this plant used for the medicinal purpose. The plant shows anti-tumour activity due to the presence of Betulinic acid and betulin (present in stem and bark), the major active constituents [86]. Betulinic acid shows cytotoxicity against various tumor cell lines and induces apoptosis by topoisomerase I inhibition, reactive oxygen species generation, angiogenesis inhibition and pro-growth transcriptional activator modulation [87].

CONCLUSIONS

Plants have long been a source of extremely successful conventional medications for the treatment of a variety of cancers. Even though the compounds isolated from the plants may not be suitable for use as drugs, they do provide leads for the development of new agents. The agents which are failed in earlier investigations are now generating renewed interest due to the technological development[1]. Among the huge number of medicinal plants only a few have been professionally investigated for their pharmacological activity. Many plant-derived agents with medicinal properties have yet to be discovered [88]. Due to the immune modulatory and antioxidant properties, medicinal plants have shown to have great anticancer potential. Bioactive compounds isolated from the medicinal herbs have shown to have great impact on cancer researches and therapies. Secondary metabolites isolated from the medicinal herbs are capable of having anticancer properties due to its ability to inhibit DNA damage, cell cycle arrests, ability to inhibit angiogenesis of tumour cell angiogenesis, or sometimes their ability to induce apoptosis [89]. In this review, some selected Indian medicinal plants were investigated for their biological activity and further research is needed to find out the effective anticancer plants from nature.

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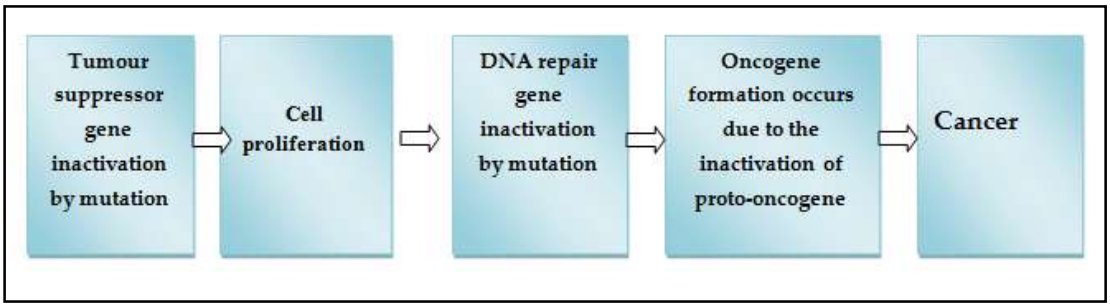

Figure 1.Development of cancer.

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RESEARCH ARTICLE

An M/G/1 Queue with Second Optional Service and Deterministic Repair Times

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ABSTRACT

We analyze the steady state behavior of an M/G/1 queue with Poisson arrivals subject to an optional service and random system breakdowns. Arriving customer must undergo first essential service and there is a second optional service. As soon as the first essential service of a customer is complete then with probability $r$the server may opt for second optional service or else with probability$1-r$, the server may opt to leave the system in which case another customer at the head of the queue is taken up for its first essential service. The service times follow arbitrary (general) service distributions. The system is prone to random breakdowns and just after a breakdown the server undergoes repair of a fixed duration. We obtain time dependent as well as steady state probability generating function for the number in the system. For steady state we obtain explicitly the mean number and the mean waiting time for the system and for the queue. Results for some special cases of interest are derived.

Keywords: Poisson arrivals, Probability generating function. Idle state. Deterministic repairs, supplementary variable technique.

INTRODUCTION

There is a natural interest in the study of queueing systems with server vacations or interruptions. In fact, queues and queueing networks occupy a prominent role in the performance analysis of wide range of systems in computer communications, logistics and manufacturing systems. Moreover, new results in queueing theory have often been inspired by new technological advances in computer, manufacturing, and communication networks. A classic

example is the celebrated Erlang loss model in the context of telephone networks. Important monographs on queueing theory for the performance prediction of computer networks and communications include Kleinrock (1976), Cohen (1982), Lavenberg (1988), Takagi (1991) and several other researchers.

Vacation queueing models have been of great interest due to its applicability in real situations. In fact, single server queueing models under vacation have been studied by numerous authors due to their wide applications in the analysis of processor schedules in computer and switching systems, the analysis of manufacturing system with machine breakdown etc. Several researchers have studied M/G/1 queue under various vacation policies during recent years due to its applications in real situations. Li et al. (2018)have studied an M/G/1 queue with vacations and multiple phases of operation. Analysis of a single server retrial queue with server vacation and two waiting buffers have been studied by Gao and Wang (2019). Saritha et al (2020) have studied an M/G/1 queue with vacation, two cases of repair facilities and server timeout. Vanitha (2020) has studied an M/G/1 queue with compulsory vacation and deterministic repairs. Kalita and Choudhury (2021) have studied a single server queue with random vacation policy. Several other researchers have done enormous work under various vacation policies of an M/G/1 queue in recent years.

In real life situations, a queueing system might suddenly breakdown and hence the server will not be able to continue providing service unless the system is repaired. Queueing models with service interruption have been studied extensively due to their widespread applications including production systems, transportation systems, complex modern communication systems and service systems. In recent years significant work has been done on queue with random breakdown by several authors. Rajadurai et al. (2018) have investigated a single server non-Markovian feedback retrial queue with breakdown, repair under multiple working vacations. Deepa and Kalidass (2018) have studied an M/M/1/N queue with working breakdown and two types of server vacations. Yang and Huang (2019) have analyzed an M/M/1 retrial queue with working vacations, in which the server is subject to starting failures. The proposed queueing model is described in terms of the quasi-birth-death (QBD) process.Gao et al (2020) have investigated an M/G/1 retrial with two types of breakdowns and delayed repairs. Agarwal et al. (2021) have studied an M/M/1 queueing model with working vacation and two types of server breakdown. In this paper the steady state probability distribution of the number of customers in single server Markovian queue is obtained by using matrix geometric approach with working vacation where server may breakdown in working vacation state as well as in busy state.

Study of an M/G/1 queue subject to various vacation policies or interruptions has been the subject of interest for several researchers during recent years. In fact, fascinating new results emerging from these kinds of queueing models have wide real time applications in day-to-day life. Not much works has been carried out on a single server queue subject to deterministic repairs. This gave way to study an M/G/1 queue with second optional service subject to random breakdowns and deterministic repair times. Such queueing models find several potential applications in the areas of production, computer and data communication systems. In the present work, we consider an M/G/1 queue with second optional service subject to random breakdowns and deterministic repair times of fixed length $d(> 0)$, using supplementary variable technique. Each customer must undergo first essential service and there is a second optional service. As soon as the essential service of a customer is complete, then with probability r he may opt for second optional service or else with probability $1 - r$ he may leave the system in which case the customer who is at the head of the queue will come to his first essential service. We assume that the breakdowns are random and time homogeneous which means that the service channel may fail not only while it is working but it may fail even when it is idle. Moreover, we assume that whenever service channel breaks down, it instantly undergoes a repair process and the repair times are deterministic of a constant (fixed) duration $d(> 0)$.

The rest of the paper is organized as follows. The mathematical description of our model is in section 2 and the equations governing the model are in section 3. The time dependent solution have been obtained in section 4 using supplementary variable technique and the corresponding steady state results have been derived explicitly in section

5. Mean number in the system and mean waiting time have been computed in section 6 and section 7 respectively. Some particular cases for this model are discussed in section 8.

ASSUMPTIONS UNDERLYING THE MODEL

The following assumptions describe the mathematical model

- Customers arrive at the system one by one in according to a Poisson stream with arrival rate $\lambda(> 0)$.
- There is a single server which provides the essential service to all arriving customers. Let $B_1(v)$ and $b_1(v)$ respectively be the distribution function and the density function of the essential service times and let $\mu_1(x)dx$ be the conditional probability density of completion of the essential service given that the elapsed time is x, so that

$$\mu_1(x) = \frac{b_1(x)}{1-B_1(x)}, \quad (1)$$

and therefore

$$b_1(v) = \mu_1(v)e^{-\int_0^\infty \mu_1(x)dx}. \quad (2)$$

- As soon as the first service of a customer is complete, then with probability r the server may opt for the second service, in which case the second service will immediately commence or else with probability $1-r$ the server may opt to leave the system, in which case another customer at the head of the queue (if any) is taken up for first essential service.
- The second service time is also assumed to follow general (arbitrary) distribution with distribution function $B_2(v)$ and the density function $b_2(v)$
- The service channel is subject to random breakdowns and the failure time distribution is exponential with mean $\frac{1}{\alpha}$. Consequently, the service channel may fail any time during the interval $(t, t+dt]$ with the probability αdt. Further we have assumed that the breakdowns are time homogeneous which implies that the service channel may fail any time even including the period of time when it is idle.
- We assume that whenever service channel breaks down, it instantly undergoes a repair process and the repair times are deterministic of a constant (fixed) duration $d(> 0)$.
- Various Stochastic processes involved in the system are independent of each other.

Definitions, Notations and the Time – Dependent Equations Governing the System

We define

$P_n^{(1)}(x,t)$: Probability that at time t, there are $n \geq 0$ customers in the queue excluding the one being provided the first essential service and the elapsed service time of this customer is x. Accordingly, $P_n^{(1)}(t) = \int_0^\infty P_n^{(1)}(x,t)dx$ denotes probability that at time t, there are n customers in the queue excluding one customer in the first essential service irrespective of the value of x.

$P_n^{(2)}(x,t)$:Probability that at time t, there are $n \geq 0$ customers in the queue excluding the one being provided the second optionalservice and the elapsed service time of this customer is x. Accordingly, $P_n^{(2)}(t) = \int_0^\infty P_n^{(2)}(x,t)dx$ denotes probability that at time t, there are n customers in the queue excluding one customer in the second optional service irrespective of the value of x.

$R_n(t)$: Probability that at time t, there are $n \geq 0$ customers in the queue and the server is under repair.

$Q(t)$: Probability that at time t, there is no customer in the system and the server is idle but available in the system.

Finally we assume that $k_r$is the parobability of r arrivals during a repair period of duration d so that

$$k_r = \frac{e^{-\lambda d}(\lambda d)^r}{r!}, r = 0,1,\ldots \quad (3)$$

The system has then the following set of differential – difference equations

$$\frac{\partial}{\partial x}P_n^{(1)}(x,t)+\frac{\partial}{\partial t}P_n^{(1)}(x,t)+(\lambda+\mu_1(x)+\alpha)P_n^{(1)}(x,t)=\lambda P_{n-1}^{(1)}(x,t), n=1,2,\ldots \quad (4)$$

$$\frac{\partial}{\partial x}P_0^{(1)}(x,t)+\frac{\partial}{\partial t}P_0^{(1)}(x,t)+(\lambda+\mu_1(x)+\alpha)P_0^{(1)}(x,t)=0, \quad (5)$$

$$\frac{\partial}{\partial x}P_n^{(2)}(x,t)+\frac{\partial}{\partial t}P_n^{(2)}(x,t)+(\lambda+\mu_2(x)+\alpha)P_n^{(2)}(x,t)=\lambda P_{n-1}^{(2)}(x,t), n=1,2,\ldots \quad (6)$$

$$\frac{\partial}{\partial x}P_0^{(2)}(x,t)+\frac{\partial}{\partial t}P_0^{(2)}(x,t)+(\lambda+\mu_2(x)+\alpha)P_0^{(2)}(x,t)=0, \quad (7)$$

$$\frac{d}{dt}R_0(t)=\alpha Q(t)+R_0(t)[-k_0-k_1-\cdots], \quad (8)$$

$$\frac{d}{dt}R_n(t)=\alpha\int_0^\infty P_{n-1}^{(1)}(x,t)dx+\alpha\int_0^\infty P_{n-1}^{(2)}(x,t)dx+R_n(t)[-k_0-k_1-\cdots], n=1,2,\ldots, \quad (9)$$

$$\frac{d}{dt}Q(t)=-(\lambda+\alpha)Q(t)+(1-r)\int_0^\infty P_0^{(1)}(x,t)\mu_1(x)dx+\int_0^\infty P_0^{(2)}(x,t)\mu_2(x)dx+\ V_0(t)K_0. \quad (10)$$

Equations (4) – (10) are to be solved subject to the following boundary conditions

$$P_0^{(1)}(0,t)=\lambda Q(t)+(1-r)\int_0^\infty P_1^{(1)}(x,t)\mu_1(x)dx+\int_0^\infty P_1^{(2)}(x,t)\mu_2(x)dx+R_0(t)k_1+R_1(t)k_0, \quad (11)$$

$$P_n^{(1)}(0,t)=(1-r)\int_0^\infty P_{n+1}^{(1)}(x,t)\mu_1(x)dx+\int_0^\infty P_{n+1}^{(2)}(x,t)\mu_2(x)dx+R_0(t)k_{n+1}+R_1(t)k_n$$
$$+\cdots+R_n(t)k_1+R_{n+1}(t)k_0, n=1,2,\ldots, \quad (12)$$

$$P_n^{(2)}(0,t)=r\int_0^\infty P_n^{(1)}(x,t)\mu_1(x)dx, n=0,1,\ldots, \quad (13)$$

We assume that initially there is no customer in the system and the server is idle so that the initial conditions are

$$Q(0)=1,\ P_n^{(1)}(0)=0,\ P_n^{(2)}(0)=0, R_n(0)=0=R_0(0), n\geq 0. \quad (14)$$

GENERATING FUNCTIONS OF THE QUEUELENGTH: THETIME DEPENDENT SOLUTION

We define the probability generating functions,

$$\left.\begin{aligned} P^{(1)}(x,z,t)=\sum_{n=0}^\infty z^n P_n^{(1)}(x,t), P^{(1)}(z,t)=\sum_{n=0}^\infty z^n P_n^{(1)}(t),\\ P^{(2)}(x,z,t)=\sum_{n=0}^\infty z^n P_n^{(2)}(x,t), P^{(2)}(z,t)=\sum_{n=0}^\infty z^n P_n^{(2)}(t),\\ R(z,t)=\sum_{n=0}^\infty z^n R(t). \end{aligned}\right\} \quad (15)$$

which are convergent inside the circle given by $|z|\leq 1$and define the Laplace transform of a function $f(t)$ as

$$\bar{f}(s)=\int_0^\infty e^{-st}f(t)dt, \Re(s)>0. \quad (16)$$

Taking the Laplace transforms of equations (4) to (13) and using (14), we obtain

$$\frac{\partial}{\partial x}\overline{P_n}^{(1)}(x,s) + (s+\lambda+\mu_1(x)+\alpha)\overline{P_n}^{(1)}(x,s) = \lambda\overline{P}_{n-1}^{(1)}(x,s), n = 1,2,\ldots, \quad (17)$$

$$\frac{\partial}{\partial x}\overline{P_0}^{(1)}(x,s) + (s+\lambda+\mu_1(x)+\alpha)\overline{P_0}^{(1)}(x,s) = 0, \quad (18)$$

$$\frac{\partial}{\partial x}\overline{P_n}^{(2)}(x,s) + (s+\lambda+\mu_2(x)+\alpha)\overline{P_n}^{(2)}(x,s) = \lambda\overline{P}_{n-1}^{(2)}(x,s), n = 1,2,\ldots, \quad (19)$$

$$\frac{\partial}{\partial x}\overline{P_0}^{(2)}(x,s) + (s+\lambda+\mu_2(x)+\alpha)\overline{P_0}^{(2)}(x,s) = 0, \quad (20)$$

$$s\overline{R}_0(s) = \alpha\overline{Q}(s) + \overline{R}_0(s)[-k_0 - k_1 - \cdots], \quad (21)$$

$$s\overline{R}_n(s) = \alpha\int_0^\infty \overline{P}_{n-1}^{(1)}(x,s)dx + \alpha\int_0^\infty \overline{P}_{n-1}^{(2)}(x,s)dx + \overline{R}_n(s)[-k_0 - k_1 - \cdots], n = 1,2,\ldots, \quad (22)$$

$$(s+\lambda+\alpha)Q(s) = 1 + (1-r)\int_0^\infty \overline{P_O}^{(1)}(x,s)\mu_1(x)dx + \int_0^\infty \overline{P}_0^{(2)}(x,s)\mu_2(x)dx + \overline{R}_0(s)k_0, \quad (23)$$

$$\overline{P}_0^{(1)}(0,s) = \lambda\overline{Q}(s) + (1-r)\int_0^\infty \overline{P}_1^{(1)}(x,s)\mu_1(x)dx + \int_0^\infty \overline{P}_1^{(2)}(x,s)\mu_2(x)dx + \overline{R}_0(s)k_1 + \overline{R}_1(s)k_0. \quad (24)$$

$$\overline{P_n}^{(1)}(0,s) = (1-r)\int_0^\infty \overline{P}_{n+1}^{(1)}(x,s)\mu_1(x)dx + \int_0^\infty \overline{P}_{n+1}^{(2)}(x,s)\mu_2(x)dx + \overline{R}_0(s)k_{n+1} + \overline{R}_1(s)(t)k_n + \cdots + \overline{R}_n(s)k_1 + \overline{R}_{n+1}(s)k_0, n = 1,2,\ldots, \quad (25)$$

$$\overline{P_n}^{(2)}(0,s) = r\int_0^\infty \overline{P_n}^{(1)}(x,s)\mu_1(x)dx, n = 0,1,\ldots, \quad (26)$$

Now multiplying equation (17) by z^n and summing over n from 1 $to\ \infty$, adding to equation (18) and using the generating function defined in equation (15), we get,

$$\frac{\partial}{\partial x}\overline{P}^{(1)}(x,z,s) + (s+\lambda-\lambda z+\mu_1(x)+\alpha)\overline{P}^{(1)}(x,z,s) = 0, \quad (27)$$

Performing similar operations on equations (19) to (22), we obtain

$$\frac{\partial}{\partial x}\overline{P}^{(2)}(x,z,s) + (s+\lambda-\lambda z+\mu_2(x)+\alpha)\overline{P}^{(2)}(x,z,s) = 0, \quad (28)$$

$$(s+1)\overline{R}(z,s) = \alpha\overline{Q}(s) + \alpha z\left\{\int_0^\infty \overline{P}^{(1)}(x,z,s)dx + \int_0^\infty \overline{P}^{(2)}(x,z,s)dx\right\}. \quad (29)$$

For the boundary conditions, we multiply both sides of equation (24) by z, multiply both sides of equation (25) by z^{n+1}, sum over n from 1 to ∞, add the two results and use equation (15) to get

$z\overline{P}^{(1)}(0,z,s) =$
$\lambda z\overline{Q}(s) + (1-r)\int_0^\infty \overline{P}^{(1)}(x,z,s)\mu_1(x)dx + \int_0^\infty \overline{P}^{(2)}(x,z,s)\mu_2(x)dx + \overline{R}(z,s)e^{-\lambda d(1-z)} - (1-r)\int_0^\infty \overline{P_0}^{(1)}(x,s)\mu_1(x)dx -$
$\int_0^\infty \overline{P_0}^{(2)}(x,s)\mu_2(x)dx - \overline{R}_0(s)k_0.$ (30)

Perform similar operation on equation (26), we have

$\overline{P}^{(2)}(0,z,s) = r\int_0^\infty \overline{P}^{(1)}(x,z,s)\mu_1(x)dx.$ (31)

Using equation (23), equation (30) become

$z\overline{P}^{(1)}(0,z,s) = (1-r)\int_0^\infty \overline{P}^{(1)}(x,z,s)\mu_1(x)dx + \int_0^\infty \overline{P}^{(2)}(x,z,s)\mu_2(x)dx + \overline{R}(z,s)e^{-\lambda d(1-z)} + \left[1 - s\overline{Q}(s)\right] - [-\lambda z + \lambda +$
$\alpha]\overline{Q}(s).$ (32)

Integrating equation (27) from 0 to x yields

$$\overline{P}^{(1)}(x,z,s) = \overline{P}^{(1)}(0,z,s)e^{-(s+\lambda-\lambda z+\alpha)x-\int_0^\infty \mu_1(t)dt}\ .$$ (33)

where$\overline{P}^{(1)}(0,z,s)$ is given by equation (32). Again integrating equation (33) by parts with respect to x yields

$$\overline{P}^{(1)}(z,s) = \overline{P}^{(1)}(0,z,s)\left[\frac{1-\overline{B_1}(s+\lambda-\lambda z+\alpha)}{s+\lambda-\lambda z+\alpha}\right],$$ (34)

where

$\overline{B_1}(s+\lambda-\lambda z+\alpha) = \int_0^\infty e^{-(s+\lambda-\lambda z+\alpha)X}\,\mathrm{dB_1(x)}$, (35)

is the Laplace - Steiltjes transform of the essential service time $b_1(x)$. Now multiplying both sides of equation of (33) by $\mu_1(x)$ and integrating over x, we obtain,

$\int_0^\infty \overline{P}^{(1)}(x,z,s)\mu_1(x)dx = \overline{P}^{(1)}(0,z,s)\overline{B_1}(s+\lambda-\lambda z+\alpha).$ (36)

Similarly, on integrating equation (28) from 0 to x, we get

$\overline{P}^{(2)}(x,z,s) = \overline{P}^{(2)}(0,z,s)e^{-(s+\lambda-\lambda z+\alpha)x-\int_0^\infty \mu_2(t)dt}$, (37)

Where $\overline{P}^{(2)}(0,z,s)$ is given by equation (31). Again integrating equation (37) by parts with respect to x yields

$$\overline{P}^{(2)}(z,s) = \overline{P}^{(2)}(0,z,s)\left[\frac{1-\overline{B_2}(s+\lambda-\lambda z+\alpha)}{s+\lambda-\lambda z+\alpha}\right],$$ (38)

where

$\overline{B_2}(s+\lambda-\lambda z+\alpha) = \int_0^\infty e^{-(s+\lambda-\lambda z+\alpha)x}\,\mathrm{dB_2(x)}$, (39)

is the Laplace - Steiltjes transform of second optional service time$b_2(x)$. We see that by virtue of equation (37), we have

$\int_0^\infty \overline{P}^{(2)}(x,z,s)\mu_2(x)dx = \overline{P}^{(2)}(0,z,s)\overline{B_2}(s+\lambda-\lambda z+\alpha).$ (40)

By using equation (36), equation (31) reduces to

$$\overline{P}^{(2)}(0,z,s) = r\overline{P}^{(1)}(0,z,s)\overline{B_1}(s+\lambda-\lambda z+\alpha). \quad (41)$$

Using equation (41), equation (40) become

$$\int_0^\infty \overline{P}^{(2)}(x,z,s)\mu_2(x)dx = r\overline{P}^{(1)}(0,z,s)\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha). \quad (42)$$

$$\overline{P}^{(1)}(0,z,s) = \left[\frac{\overline{R}(z,s)e^{-\lambda d(1-z)}+[1-s\overline{Q}(s)]-[-\lambda z+\lambda+\alpha]\overline{Q}(s)}{z-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)}\right]. \quad (43)$$

Substituting the value of $\overline{P}^{(1)}(0,z,s)$ into equation (34), we get

$$\overline{P}^{(1)}(z,s) = \left[\frac{\overline{R}(z,s)e^{-\lambda d(1-z)}+[1-s\overline{Q}(s)]-[-\lambda z+\lambda+\alpha]\overline{Q}(s)}{z-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)}\right]\left[\frac{1-\overline{B_1}(s+\lambda-\lambda z+\alpha)}{(s+\lambda-\lambda z+\alpha)}\right]. \quad (44)$$

Now using equations (41) and (43), equation (38) become

$$\overline{P}^{(2)}(z,s) = r\left[\frac{\overline{R}(z,s)e^{-\lambda d(1-z)}+[1-s\overline{Q}(s)]-[-\lambda z+\lambda+\alpha]\overline{Q}(s)}{z-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)}\right]$$
$$\overline{B_1}(s+\lambda-\lambda z+\alpha)\left[\frac{1-\overline{B_2}(s+\lambda-\lambda z+\alpha)}{(s+\lambda-\lambda z+\alpha)}\right]. \quad (45)$$

From equation (29),

$$\overline{R}(z,s) = \left[\frac{\alpha\overline{Q}(s)+\alpha z\{\overline{P}^{(1)}(z,s)+\overline{P}^{(2)}(z,s)\}}{(s+1)}\right]. \quad (46)$$

Let $\overline{W}(z,s) = \overline{P}^{(1)}(z,s) + \overline{P}^{(2)}(z,s)$ denote the probability generating function of the number in the queue irrespective of the type of service being provided. Then adding equations (44) and (45), we have

$$\overline{W}(z,s) = \left[\frac{\overline{R}(z,s)e^{-\lambda d(1-z)}+[1-s\overline{Q}(s)]-[-\lambda z+\lambda+\alpha]\overline{Q}(s)}{z-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)}\right]$$
$$\left[\frac{1-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)}{(s+\lambda-\lambda z+\alpha)}\right]. \quad (47)$$

Then substituting the value of $\overline{R}(z,s)$ from equation (46) into equation (47), we get

$$\overline{W}(z,s) = \frac{\overline{N}(z,s)}{\overline{D}(z,s)}, \quad (48)$$

Where

$$\overline{N}(z,s) = \{\alpha\overline{Q}(s)e^{-\lambda d(1-z)} - [-\lambda z+\lambda+\alpha](s+1) + [1-s\overline{Q}(s)](s+1)\}$$
$$\left\{\begin{matrix}1-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\\ \overline{B_2}(s+\lambda-\lambda z+\alpha)\end{matrix}\right\}, \quad (49)$$

$$\overline{D}(z,s) = (s+\lambda-\lambda z+\alpha)(s+1)$$
$$\left[z-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)-r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)\right] -$$
$$\alpha z e^{-\lambda d(1-z)}\left[\begin{matrix}1-\overline{B_1}(s+\lambda-\lambda z+\alpha)+r\overline{B_1}(s+\lambda-\lambda z+\alpha)\\ -r\overline{B_1}(s+\lambda-\lambda z+\alpha)\overline{B_2}(s+\lambda-\lambda z+\alpha)\end{matrix}\right]. \quad (50)$$

If we let $z = 1$ in equation (48), we can easily verify that

$$\overline{Q}(s) + \overline{R}(z,s) + \overline{W}(z,s) = \frac{1}{s}, \quad (51)$$

as it should be. Thus $\overline{R}(z,s), \overline{P}^{(1)}(z,s)$ and $\overline{P}^{(2)}(z,s)$ are completely determined from equations (46), (44) and (45) respectively.

STEADY STATE SOLUTIONS

In this section, we shall derive the steady state probability distribution for our queueing model. To define the steady state probabilities, we suppress the argument t wherever it appears in the time-dependent analysis. This can be obtained by applying the well-known Tauberian property,

$$\lim_{n\to 0} s\,\overline{f}(s) = \lim_{t\to\infty} f(t) \quad (52)$$

In order to determine $\overline{P}^{(1)}(z,s)$, $\overline{P}^{(2)}(z,s)$ and $\overline{R}(z,s)$ completely, we have yet to determine the unknown Q which appears in the numerators of the right sides of equations (44), (45) and (46) by using initial conditions (43) and (41). For that purpose, we shall use the normalizing condition

$$P^{(1)}(1) + P^{(2)}(1) + R(1) + Q = 1\ . \quad (53)$$

Thus multiplying both sides of equations (44), (45) and (46) by s, taking limit as $s \to 0$, applying property (52) and simplifying we have

$$P^{(1)}(z) = \left[\frac{R(z)e^{-\lambda d(1-z)} - [-\lambda z + \lambda + \alpha]Q}{z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)}\right]\left[\frac{1 - \overline{B_1}(\lambda - \lambda z + \alpha)}{(\lambda - \lambda z + \alpha)}\right], \quad (54)$$

$$P^{(2)}(z) = \left[\frac{R(z)e^{-\lambda d(1-z)} - [-\lambda z + \lambda + \alpha]Q}{z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)}\right]$$
$$\overline{B_1}(\lambda - \lambda z + \alpha)\left[\frac{1 - \overline{B_2}(\lambda - \lambda z + \alpha)}{(\lambda - \lambda z + \alpha)}\right], \quad (55)$$

and

$$R(z) = \alpha Q + \alpha z W(z), \quad (56)$$

Where

$$W(z) = P^{(1)}(z) + P^{(2)}(z) = \frac{N(z)}{D(z)}, \quad (57)$$

Where

$$N(z) = \{1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\}$$
$$\{\alpha e^{-\lambda d(1-z)} - [-\lambda z + \lambda + \alpha]\}Q, \quad (58)$$

$$D(z) = [-\lambda z + \lambda + \alpha]\{z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha) - \alpha z e^{-\lambda d(1-z)}\{1 -$$
$$\overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\}\}. \quad (59)$$

We see that for $z = 1$, $W(z)$ in equation (57) is indeterminate of the form $\frac{0}{0}$ form. Therefore we applyL'Hopital's rule on equations (57) and on simplifying we get

$$W(1) = \lim_{z\to 1} W(z) = \frac{[1-\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)\overline{B_2}(\alpha)][\lambda+\lambda\alpha d]Q}{\alpha[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]-[\lambda+\lambda\alpha d][1-\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]}. \quad (60)$$

Now using (60) in equation (56), we have

$$R(1) = \frac{\alpha^2 Q[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]}{\alpha[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]-[\lambda+\lambda\alpha d][1-\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]}. \quad (61)$$

Now since we must have $Q + W(1) + V(1) = 1$, we have

$$Q = \frac{\alpha[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]-[1-\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)\overline{B_2}(\alpha)][\lambda+\lambda\alpha d]}{\alpha(\alpha+1)[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]} \quad (62)$$

Which is the steady state probability that the server is idle but operative. Then we substitute the value of Q from (62) into (57) we have

$$W(z) = \frac{N_1(z)}{D_1(z)}$$

where

$$N_1(z) = \{1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\}$$
$$\frac{\alpha[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]-[1-\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]}{\alpha(\alpha+1)[\overline{B_1}(\alpha)-r\overline{B_1}(\alpha)+r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]}\{\alpha e^{-\lambda d(1-z)} - [-\lambda z + \lambda + \alpha]\}Q, \quad (63)$$

$$D_1(z) = [-\lambda z + \lambda + \alpha]\{z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\} - \alpha z e^{-\lambda d(1-z)}\{1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\} \quad (64)$$

Next using above equation in $R(z)$ and simplifying, we have

$$R(z) = \alpha Q\left\{\frac{(\lambda-\lambda z+\alpha)[\overline{B_1}(\lambda-\lambda z+\alpha)-r\overline{B_1}(\lambda-\lambda z+\alpha)+r\overline{B_1}(\lambda-\lambda z+\alpha)\overline{B_2}(\lambda-\lambda z+\alpha)][z-1]}{D(z)}\right\}, \quad (65)$$

where$D(z)$ is given by is given by equation (59) and Q is given by (62). Thus $W(z)$ and $R(z)$ have been completely and explicitly determined in above equations.

THE MEAN NUMBER IN THE SYSTEM

Let $P_q(z) = W(z) + R(z)$ denote the probability function of the queue length irrespective of whether the server is operative or in failed state. Then adding $W(z)$ and $R(z)$ and simplifying we have

$$P_q(z) = \frac{N_2(z)}{D_2(z)} \quad (66)$$

Where

$$N_2(z) = \alpha Q\left\{\frac{(\lambda - \lambda z + \alpha)(z-1)}{\left[\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\right]}\right\}$$
$$+\{1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\}$$
$$\{\alpha e^{-\lambda d(1-z)} - (\lambda - \lambda z + \alpha)\}Q, \quad (67)$$

$$D_2(z) = (\lambda - \lambda z + \alpha)\{z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\} - \alpha z e^{-\lambda d(1-z)}\{1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\} \quad (68)$$

and$Q$ is given by equation (62). Let L_q denote the mean number of customers in the queue under the steady state. Then

$L_q = \frac{d}{dz}P_q(z)$at $z = 1$.

Since this formula gives $\frac{0}{0}$ form. We use the following well known result in Queueing theory (see Kashyap and Chaudhry)

$$L_q = \lim_{z\to 1}\frac{d}{dz}P_q(z) = P_q'(1) = \lim_{z\to 1}\frac{D'(z)N''(z) - N'(z)D''(z)}{2\left(D'(z)\right)^2}$$

$$= \lim_{z\to 1}\frac{D'(1)N''(1) - N'(1)D''(1)}{2\left(D'(1)\right)^2} \quad (69)$$

where primes and double primes in equation (69) denote the first and second derivative at $z = 1$. Carrying out the derivatives at $z = 1$, we have

$$N'(1) = [\lambda + \alpha\lambda d]\{1 - \overline{B_1}(\alpha) + r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)\}$$
$$+\alpha^2\left[\overline{B_1}(\alpha) + r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)\right]. \quad (70)$$

$$N''(1) = \alpha(\lambda d)^2\{1 - \overline{B_1}(\alpha) + r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)\} + 2\lambda^2[1 + \alpha d]\left[\overline{B_1}'(\alpha) - r\overline{B_1}'(\alpha) + r\overline{B_1}'(\alpha)\overline{B_2}(\alpha) + r\overline{B_1}(\alpha)\overline{B_2}'(\alpha)\right]$$
$$-2\alpha^2\lambda[\overline{B_1}'(\alpha) - r\overline{B_1}'(\alpha) + r\overline{B_1}'(\alpha)\overline{B_2}(\alpha) + r\overline{B_1}(\alpha)\overline{B_2}'(\alpha)] - 2\alpha\lambda[\overline{B_1}(\alpha) + r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)], \quad (71)$$

$$D'(1) = [1 - \overline{B_1}(\alpha) + r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)][-\lambda - \alpha\lambda d] + \alpha[\overline{B_1}(\alpha) - r\overline{B_1}(\alpha) + r\overline{B_1}(\alpha)\overline{B_2}(\alpha)], \quad (72)$$

$$D''(1) = [1 + \alpha d]\left[-2\lambda - -2\lambda^2\overline{B_1}'(\alpha) + 2\lambda^2 r\overline{B_1}'(\alpha) - 2\lambda^2 r\overline{B_1}'(\alpha)\overline{B_2}(\alpha) - 2\lambda^2 r\overline{B_1}(\alpha)\overline{B_2}'(\alpha)\right] - \alpha\lambda^2 d^2[1 - \overline{B_1}(\alpha) +$$
$$r\overline{B_1}(\alpha) - r\overline{B_1}(\alpha)\overline{B_2}(\alpha)] - 2\alpha\lambda\overline{B_1}'(\alpha) + 2\alpha\lambda d[\overline{B_1}(\alpha) - r\overline{B_1}(\alpha) + r\overline{B_1}(\alpha)\overline{B_2}(\alpha)]. \quad (73)$$

Then if we substitute the values of $N'(1), N''(1), D'(1)$and $D''(1)$ from equations (70) to (73) into equation (69), we obtain L_q in closed form. Further let $P(z)$ denote the probability generating function of the number in the queue. Then from above equations and from (62) and simplifying we have

$$P(z) = Q + zP_q(z) = \frac{N_3(z)}{D_3(z)}, \quad (74)$$

where

$$N_3(z) = (\lambda - \lambda z + \alpha)\begin{bmatrix}\overline{B_1}(\lambda - \lambda z + \alpha) - r\overline{B_1}(\lambda - \lambda z + \alpha)\\ +r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\end{bmatrix}Q(1 + \alpha z), \quad (75)$$

$$D_3(z) = (\lambda - \lambda z + \alpha)\begin{bmatrix}z - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha)\\ -r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\end{bmatrix}$$
$$-\alpha z e^{-\lambda d(1-z)}\begin{bmatrix}1 - \overline{B_1}(\lambda - \lambda z + \alpha) + r\overline{B_1}(\lambda - \lambda z + \alpha)\\ -r\overline{B_1}(\lambda - \lambda z + \alpha)\overline{B_2}(\lambda - \lambda z + \alpha)\end{bmatrix} \quad (76)$$

and Q is given by equation (62). Moreover, we find the average system size L_s using little's formula. Thus we have

$L_s = L_q + \rho$ (77)

Where L_q has been found in equation (69) and ρ is obtained from equation (62) as

$\rho = 1 - Q$. (78)

The Mean Waiting Time

Let W_q and W denote the mean waiting time in the queue and the system respectively. Then using Little's formulas, we obtain,

$W_q = \frac{L_q}{\lambda}$, (79)

$W = \frac{L}{\lambda}$. (80)

Where L_q and L have been found in equations (69) and (77) respectively.

SPECIAL CASES

Case 1: Both services follow general distribution, assume $r = 1$, random breakdowns and deterministic repairs

In this case, we assume first essential service and second optional service follow general distribution and take $r = 1$ so that the model becomes M/G/1 queue with two stage heterogeneous service subject to random breakdowns and deterministic repairs.(Refer [16]).

Case 2: Both service times follow exponential distribution, assume $r = 1$, random breakdowns and deterministic repair

In this case, we assume first essential service and second optional service follow exponential distribution and assume $r = 1$, so that this model is a particular case of model discussed in 8.1.

Case 3: No optional service, random breakdowns and deterministic repairs

In this case, we assume that there is no second optional service, and the essential service is provided to all the arriving customers. Therefore, the results (62) to (65), (66) to (68) and (74) to (76) reduce to

$Q = \frac{\alpha\bar{B}_1(\alpha)-[1-\overline{B_1}(\alpha)][\lambda+\lambda\alpha d]}{\alpha(\alpha+1)\overline{B_1}(\alpha)}$, (81)

$W(z) = \frac{[1-\bar{B}_1(\lambda-\lambda z+\alpha)]\left[\alpha e^{-\lambda d(1-z)}-[-\lambda z+\lambda+\alpha]\right]Q}{[-\lambda z+\lambda+\alpha][z-\bar{B}_1(\lambda-\lambda z+\alpha)]-[1-\bar{B}_1(\lambda-\lambda z+\alpha)]\alpha z e^{-\lambda d(1-z)}}$, (82)

$R(z) = \frac{\alpha[-\lambda z+\lambda+\alpha][z-\bar{B}_1\lambda-\lambda z+\alpha)]Q}{[-\lambda z+\lambda+\alpha][z-\overline{B_1}(\lambda-\lambda z+\alpha)]-[1-\overline{B_1}(\lambda-\lambda z+\alpha)]\alpha z e^{-\lambda d(1-z)}}$, (83)

$P(z) = \frac{[-\lambda z+\lambda+\alpha]\overline{B_1}(\lambda-\lambda z+\alpha)[z-1](1+\alpha z)Q}{[-\lambda z+\lambda+\alpha][z-\bar{B}_1(\lambda-\lambda z+\alpha)]-[1-\bar{B}_1(\lambda-\lambda z+\alpha)]\alpha z e^{-\lambda d(1-z)}}$, (84)

$P_q(z) = \frac{P_q(Nr)}{D_q(Nr)}$,

Where

$P_q(Nr) = \left\{\begin{matrix}[1-\bar{B}_1(\lambda-\lambda z+\alpha)]\left[\alpha e^{-\lambda d(1-z)}-[-\lambda z+\lambda+\alpha]\right] \\ +\alpha[-\lambda z+\lambda+\alpha]\overline{B_1}(\lambda-\lambda z+\alpha)(z-1)\end{matrix}\right\}Q$, (85)

$P_q(Dr) = [-\lambda z+\lambda+\alpha][z-\bar{B}_1(\lambda-\lambda z+\alpha)]-[1-\bar{B}_1(\lambda-\lambda z+\alpha)]\alpha z e^{-\lambda d\setminus(1-z)}$, (86)

where Q in the right hand side of equations (82) to (85) is given by (81).

Case 4:Exponential essential service, no optional service, random breakdowns and deterministic repair times

In this case, we assume that there is no optional service, and the essential service follows exponential service time. Therefore, we have

$$\bar{B}(\alpha) = \int_0^{\infty} e^{-\alpha x}\, \mu e^{-\mu x} = \frac{\mu}{\alpha + \mu}$$

and similarly

$\bar{B}(\lambda - \lambda z + \alpha) = \frac{\mu}{\lambda - \lambda z + \alpha + \mu}$.

Use these substitutions into the results (62) to (65), (66) to (68) and (74) to (76), we obtain

$$Q = \frac{\mu - (\lambda + \lambda\alpha d)}{(\alpha+1)\mu}, \tag{87}$$

$$W(z) = \frac{\left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\left[\alpha e^{-\lambda d(1-z)} - [-\lambda z+\lambda+\alpha]\right]\left[\frac{\mu-(\lambda+\lambda\alpha d)}{(\alpha+1)\mu}\right]}{[-\lambda z+\lambda+\alpha]\left[\frac{(\lambda-\lambda z+\alpha+\mu)z-\mu}{(\lambda-\lambda z+\alpha+\mu)}\right] - \left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\alpha z e^{-\lambda d(1-z)}}, \tag{88}$$

$$R(z) = \frac{\left[\frac{\alpha\mu(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu)}\right][z-1]\left[\frac{\mu-(\lambda+\lambda\alpha d)}{(\alpha+1)\mu}\right]}{[-\lambda z+\lambda+\alpha]\left[\frac{(\lambda-\lambda z+\alpha+\mu)z-\mu}{(\lambda-\lambda z+\alpha+\mu)}\right] - \left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\alpha z e^{-\lambda d(1-z)}}, \tag{89}$$

$$P(z) = \frac{\frac{\mu(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu)}[z-1][(1+\alpha z))\frac{\mu-(\lambda+\lambda\alpha d)}{(\alpha+1)\mu}}{[-\lambda z+\lambda+\alpha]\left[\frac{(\lambda-\lambda z+\alpha+\mu)z-\mu}{(\lambda-\lambda z+\alpha+\mu)}\right] - \left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\alpha z e^{-\lambda d(1-z)}}, \tag{90}$$

$$P_q(z) = \frac{\left\{\left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\left[\alpha e^{-\lambda d(1-z)} - [-\lambda z+\lambda+\alpha]\right]\left[\frac{\alpha\mu(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right][z-1]\right\}Q}{[-\lambda z+\lambda+\alpha]\left[\frac{(\lambda-\lambda z+\alpha+\mu)z-\mu}{(\lambda-\lambda z+\alpha+\mu)}\right] - \left[\frac{(\lambda-\lambda z+\alpha)}{\lambda-\lambda z+\alpha+\mu}\right]\alpha z e^{-\lambda d(1-z)}}, \tag{91}$$

Case 5: Exponential essential service. no optional service, no random breakdowns

If there is no breakdown in the system, then take $\alpha = 0$ in all the main results so that we obtain $R(z) = 0$.

$$Q = \frac{\mu-\lambda}{\mu}, \tag{92}$$

$$P(z) = \frac{\bar{B}_1(\lambda-\lambda z)\left[1-\frac{\lambda}{\mu}\right][1-z]}{\bar{B}_1(\lambda-\lambda z)-2}, \tag{93}$$

$$P_q(z) = \frac{\left[\bar{B}_1(\lambda-\lambda z)-1\left[1-\frac{\lambda}{\mu}\right]\right]}{z-\bar{B}_1(\lambda-\lambda z)}. \tag{94}$$

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RESEARCH ARTICLE

Malathion Toxicity Induced Cytoskeleton Disruption: Implications on Cell Morphology and Behaviour of *Paramecium caudatum*

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ABSTRACT

The toxicity of organophosphate compounds has been widely tested on various groups of organisms. It is known to alter the functioning of many vital enzymes by disrupting the signaling pathways through phosphorylation. Malathion is one of the most used organophosphates in agricultural fields, was assessed in the present study using *Paramaecium caudatum*. We assessed the acute toxicity by subjecting the Paramaecia at 100, 150 and 200ppm. We calculated the LC_{50} value for 3 hrs of acute toxicity as 102.22±15.01 ppm. Several behavioural changes were reported during the first 30 mins, such as erratic swimming, jerky, and spinning movement. At the same time, cellular changes such as blebbing, blackening of cytoplasm and swelling of the cell were noticeable. Paramaecia were exposed to sub-lethal doses of 25, 50 and 100ppm to assess the chronic toxicity. Here cellular changes were assessed under quantitative and qualitative methodology. Under qualitative assessment, eight deformities such as decreased breadth, rod shape cells, narrow anterior, spindle shape cells, irregular shape, blebbing, blackening of cytoplasm and swelled cells were reported. The quantitative assessment involved comparing percent normal, percent deformed, and percent lysed cells in a concentration and time-dependent manner. These can be summarized as the entry of toxicants in the cell hampered cellular functioning and cytoskeletal system. Thus, the present study indicates the complete disruption of cytoskeletal functioning leading to noticeable changes in behaviour, morphology and functioning. The results also support the suitability of ciliates as an ideal single-cell eukaryotic test system for cytotoxic studies.

Keywords: Cytotoxicity, organophosphate, cytoskeleton, cellular deformity, *Paramecium*

INTRODUCTION

The use of chemical pesticides in agricultural fields has become common practice even though chemical pesticides have farfetched implications on the complete ecosystem. World Health Organization data has shown that only 2 percent of applied pesticide kills the target pest and remaining affects the non-target organisms. Aquatic systems are the sink of these toxicants as the agricultural runoffs containing pesticides enters directly into them. These toxicants then pass from zooplanktons to larger fishes while elevating the amount at every trophic level of the food chain leading to bioaccumulation, increasing the risk to organisms at higher trophic levels, where sometimes the higher organism is human. This problem has gained concern these days as the lethal effects of such toxicants can alter the tropic chain, further affecting the environmental balance[1][2].

In eco toxicology, test organisms that respond immediately and can be better correlated with higher organisms are selected. As the ciliates are unicellular, they serve as the simplest eukaryotic test system, which helps indirect investigations and better observations. Moreover, because ciliates are at the bottom of the food web, the effects of toxicants observed in these organisms would increase exponentially with each higher trophic level. We have selected *Paramaecium caudatum* (ciliate) in the present study due to its high sensitivity, large size, easy availability, and ubiquitous distribution. It is also possible to study the effects of toxicants on the large and genetically homogenous population as these have a short life cycle [3][4][5][6][7][8]. Moreover, it has already been reported by Gutierrez, J.C., *et al.* that *Paramaecium* shows high gene conservation along with better matches of coding sequences with humans [9].

Thus, the objective of the present study was to see the visible effects of sub lethal doses of organophosphate pesticide Malathion on behaviour and cellular functioning of ciliate: *Paramaecium*. Malathion was used in the present study because it is a commonly used pesticide known to contaminate almost all freshwater bodies through runoffs from agricultural fields [10].

MATERIALS AND METHODS

In the present study, commercially available Malathion was used. *Paramaecia*were cultured in the laboratory by hay infusion medium described by Nageshwara R A and Mohd Masood H in 2009 [11][12]. LC_{50} value and lethal concentration were calculated against the mortality curve for three hours duration. For acute toxicity studies, *Paramaecium caudatum* were exposed to various doses of 100, 150 and 200 ppm of toxicant and the cells were observed for the next 30 mins. However, for the chronic toxicity studies, *Paramecium caudatum* was exposed to sub-lethal doses of 25 ppm, 50ppm, and 100ppm Malathion for the next 96hrs to analyze the cell behaviour, viability and any deformity. After an interval of every 24hrs, a part of the culture was fixed using Carnoy's fixative and observed under the light microscope for any visible changes in *Paramecium* in all the Malathion concentrations. A culture devoid of toxicant was maintained as a control set and the treated organisms throughout the experiment.

RESULTS AND DISCUSSION

The LC_{50} value calculated for Malathion against mortality curve for 3 hrs exposure was 102.22 ±15.01 ppm. *Paramecia* exhibited a series of behavioural, morphological changes upon treatment with organophosphate pesticide, Malathion upon acute and chronic toxicity assessment. Quantitative analysis of treated cells has shown a significant decline in the percentage of normal cells. However, the deformed and lysed cell count increased. ANOVA was employed as the statistical tool to confirm that variation existed between the doses at various exposures. The F value for deformed and lysed cells was 9.147 and 7.989, respectively, much greater than the table value of 3.490 at 0.05 LS, confirming the

existence of variation. There was a significant decline in the percent deformed cells and increased percent lysed or dead cells with increased toxicant concentration and exposure.

Acute Toxicity

During acute toxicity assessment, both behavioral and cellular responses were observed.

Behavioural Implications

As a result of Malathion toxicity, cells showed high motility for the first few seconds with gradual difficulty in swimming and exhibited rocking movements. Nageswara R.A reported similar results in 2010 upon exposure to azadiractin[13]. After almost two minutes of Malathion addition, the spinning motion of cells was observed. Cell motility was further reduced as time progressed, and Paramecia started swimming with jerky movements followed by spinning around their body axes. This was found in accordance with the findings of Nageshwara in 2011[14]. Finally, cells were completely unable to swim, and no displacement of the cell was observed. Thus, by the end of 30 minutes of exposure, cells became completely motionless. These observations were in accordance with Nageswara R.A and Mohd Masood H. in 2008 in *Paramecium* under the influence of Delfin insecticide[15].

Cellular Implications

The functioning of a cell is greatly dependent upon the cell membrane and intracellular transport. The cytoskeleton is the basic framework of cells involved in maintaining cell shape, intracellular transport of molecules, movements of vacuoles, cell motility, exocytosis, and endocytosis. Collectively its involved in the intracellular motility of molecules and motility of the organism, such as in *Paramaecium*. Thus, any damage to the cytoskeleton would affect the cell adversely. Abou-Donia MB and Lapadula DM have already described in 1990 that organophosphates interact with CaM kinaseII (an endogenous phosphorylating enzyme of the cytoskeleton). Such interaction causes increased phosphorylating activity of CaM kinaseII, resulting in excessive phosphorylation of cytoskeletal proteins, including microtubules[16], causing the disassembly of microtubules and cytoskeleton. Such cytoskeletal disruption could be visibly observed by the formation of blebs (Plate No. 1c). A similar observation was reported in Hela cells when treated with a potent inhibitor of actin polymerization, cytochalasin D[17]. This is known to induce a significant disruption of F actin fibres, forming dense actin aggregates in cytoplasm and cytoplasmic blebs [17]. The increased contractile activity initiated by phosphorylated myosin causes intracellular pressure resulting in bleb formation [18] [19]. The intensity of blebbing (increase in bleb size or multiple blebbing) increased with increased concentration of Malathion. Increased contractile activity is often associated with abnormal cell motility.

Cell swelling (CS) (Plate No. 1 b) was the next cellular response observed, which is also reported by Venkateswara R *et al.* in 2007[20]. Blackening of cytoplasm (Plate No. 2 d, e and f) was observed and intensity of this blackening increased with an increase in the Malathion concentration (BC). This event could result from improper functioning of the cytoskeleton, causing the mixing of vacuolar contents leading to the blackening of the cytoplasm [13].A change in a cell shape, where the longitudinal axis length of a cell is decreased to form a rod-shaped cell, was observed and agreed with the findings of other scientists with similar work [21][11][12].

Chronic Toxicity

In chronic toxicity assessment, only cellular responses were observed. We performed qualitative and quantitative analyses to assess cellular responses.

Qualitative Analysis: The cellular deformities were observed for all concentrations and at all exposure periods. During qualitative, eight cellular deformities exclusive to chronic toxicity assessment were recorded. Narrowing of the anterior end(NA) (Plate No. 1 d), spindle-shaped deformity(SD) (Plate No.1 e), Irregular cell shape(IR) (Plate No.1 f),rod-shaped cells (RD) (Plate No.2 a), and decreased breadth (DB) (Plate No.2 b) were some of the significant cellular deformities. However, the remaining three deformities, like cell swelling, blebbing and blackening of cytoplasm, were in common with the deformities observed during acute toxicity assessment. Cell lysis (CL) (Plate

No.2 c) was the endpoint of cellular deformities. Narrowing of the anterior end(NA) and spindle-shaped deformity(SD) observed in the present case reported in similar studies by other scientists [15][13]. Similarly, Irregular cell shape(IR), rod-shaped cells (RD) and decreased breadth size (DB) observed in the present study were also observed by Nageswara R.A and Mohd Masood H in 2010[13].

Quantitative Analysis: The quantitative analysis involved observing the cell viability of 100 cells per concentration at each exposure time. It was observed that percent normal cells decrease (Figure No.1) while percent deformed and percent lysed cells increase with increased concentration and exposure time. However, on a close glance at the data, it was evident that the percent deformed cell decreased with the exposure time and concentration of toxicant (Figure No. 2). While the percent lysed cells increased with increased exposure time and concentration (Figure No. 3). Moreover, at lower concentrations of toxicants, the cells are more deformed, which shows that they are more tolerant and developed deformities instead of getting lysed and dying out due to the stress. Such results are supported by the fact that the generation time increases and regeneration capacity decrease with increased concentration and exposure time. Still, cells that survive this stress are more tolerant than other cells [20] [22].Thus, the cells develop tolerance when exposed to high concentrations for a prolonged time. The cytoskeletal system, an essential structural element of all cells, performs a key role in maintaining cell shape and motility [23]. Thus, it could be safe to conclude that the cytoskeleton disruption leads to distorted cell shapes and improper cell motility in the current study. Cytoskeleton being the basic framework of the cell, its disruption affects cells drastically that affects cellular morphology and alters behavioural patterns such as cell motility and feeding. Both of these activities involve cell membrane and cytoskeletal structures. Moreover, the formation of food vacuoles and the complete process of cyclosis is also cytoskeleton dependent. Thus, this approach of observing cellular and behavioural responses can be used in future studies to confirm cytoskeleton disruption in toxic conditions.

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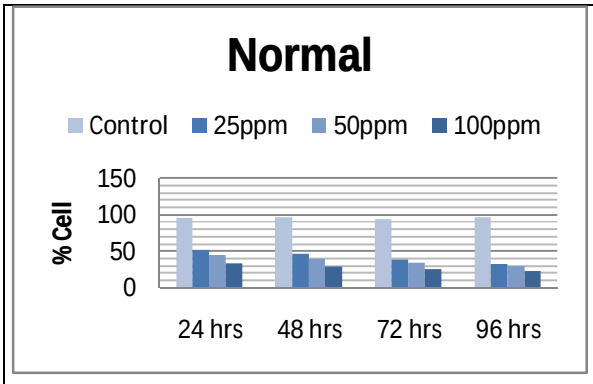

Figure 1 : Percent Normal cells at different concentration and exposures

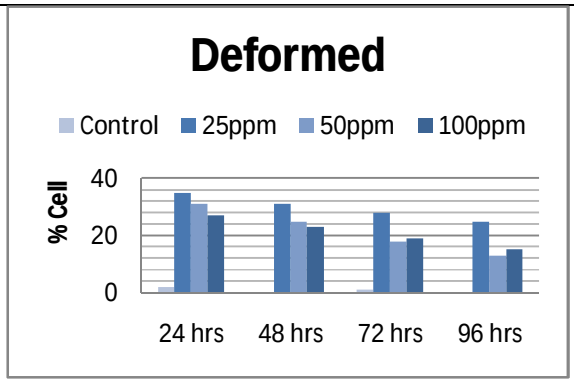

Figure 2: Percent Deformed cells at different concentration and exposures

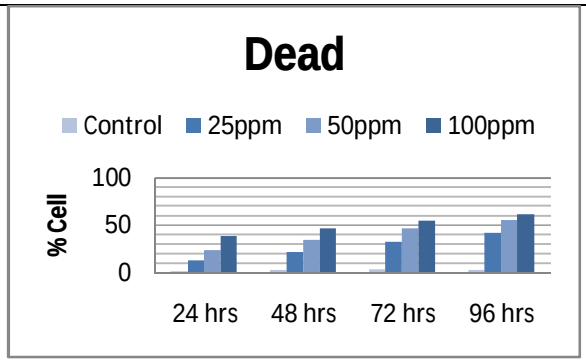

Figure 3 : Percent Lysed cells at different concentration and exposure

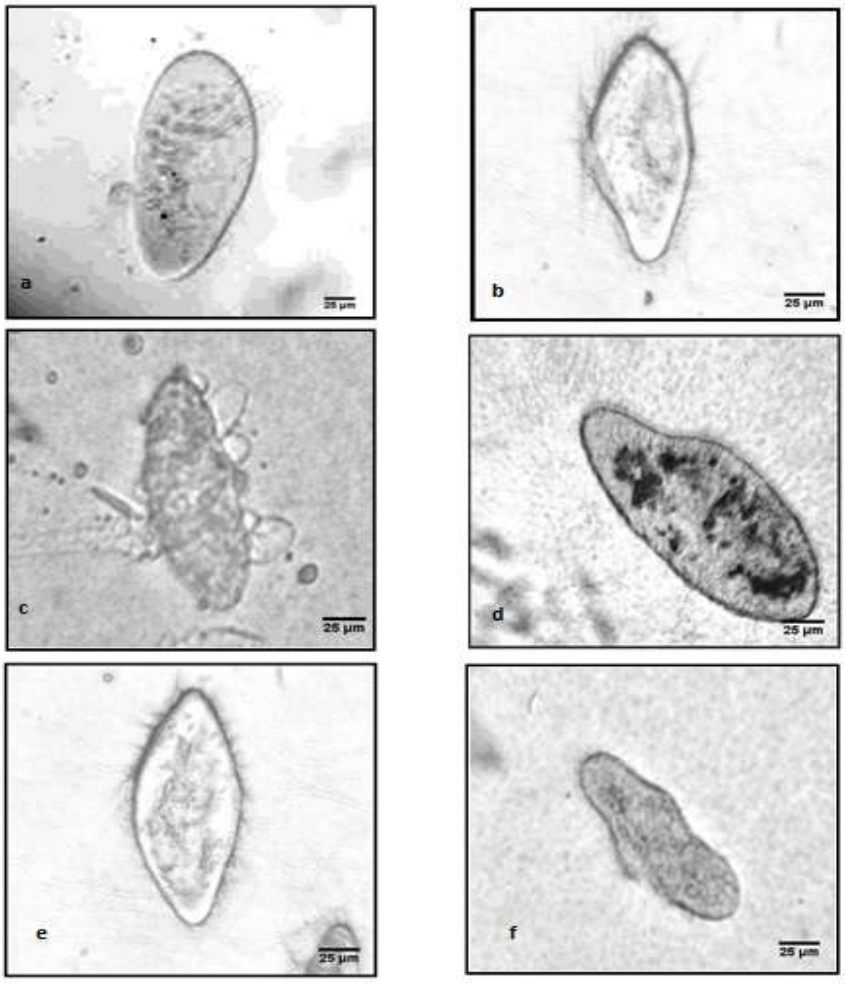

Plate No. 1: Morphological changes observed under the influence of Malathion; a: Control; b: Cell Swelling(CS); c: Multiple blebbing(MB); d: Narrowing of the anteriorend(NA); e: Spindle-shaped deformity (SD); f:Irregular cell shape (IRR).

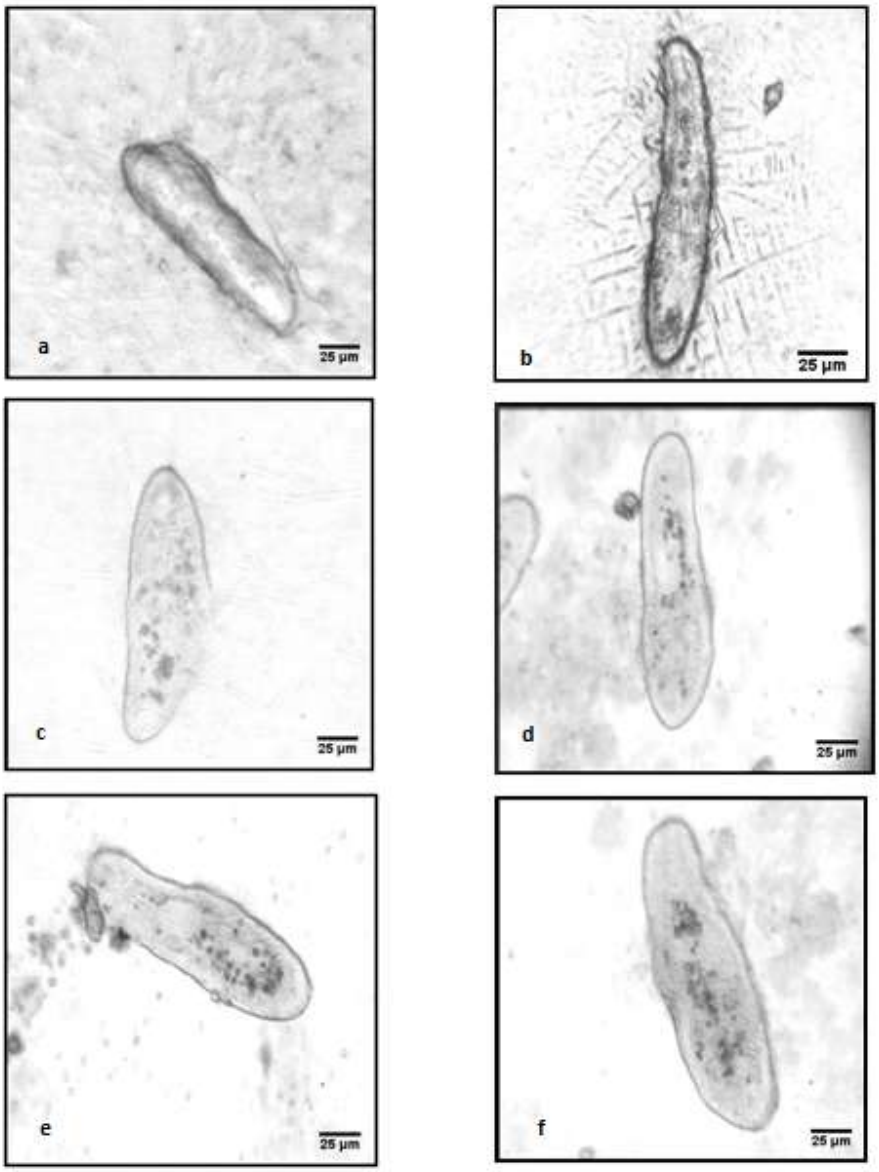

Plate No. 2: Morphological changes observed under the influence of Malathion; g: Rod-shaped cell (RD); h: Decrease in breadth size (DB);i: Cell lysis (CL); j: Blackening of cytoplasm(BC)at 25ppm; k: Blackening of cytoplasmat 50ppm;l: Blackening of cytoplasmat 100ppm.

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RESEARCH ARTICLE

India's Export Marketing Potentials of Palmyra Products

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ABSTRACT

The Palmyra products have been produced in all over the India from very long period. It is produced by the cottage industries, handy craft industries, small scale industries and Palmyra growers. The producers are selling their Palmyra products on retail, whole sale and export them to various countries. This report states about the overall export performance of Palmyra products. The development of Palmyra production may need helps to improve the country's economic growth. The study is based on empirical analysis and concludes that, from the agriculture report 5.10 crore Palmyra trees are there in Tamilnadu. Tamilnadu plays a major role in exporting Palmyra products. It may support state and central governments to Palmyra production, it will earning foreign money.

Keywords: Odiyal powder, Root powder, Jaggery, Candy, Industries, Palmyra fresh fruit

INTRODUCTION

Palmyra tree is economically useful and widely cultivated in all over the world. The Palmyra tree yields both edible and non-edible products. Africa is the native of Palmyra tree. The products of Palmyra tree is mostly produced and cultivated by the small scale industries and cottage industries. The government co-operative societies and co-operative federations are providing loan facilities, employment opportunities, welfare schemes, training programs for the rural artisans and awareness programs to the Palmyra product producers and enthusiast. The producers of Palmyra exporting their Palmyra based products to other countries.

Objectives of the Study

- To identify Palmyra products export marketing

- To find out Palmyra products export Growth
- To predict Palmyra products export in future period of 2021 to 2030.

METHODOLOGY OF THE STUDY

This study is based on only secondary data. Descriptive research design is used in this study. This exported Palmyra products were analyzed with using of following statistical tools.
(i) Growth rate
(ii) Forecast

Analysis of India's Top Ten Countries Export Growth and Forecast of Palmyra Products

Palmyra Wood Shaving Brush Handle

Naturally, stained brown shaving brush handle is made out of Palmyratree's wood. The products are produced by hand made industries. These industries are selling Palmyra wood shaving brush handle to the local markets and exporting to the various countries. This product export information's are given below

The Palmyra wood shaving brush handles have been exported from India for the past 10-years (2010-2011 to 2019-2020) it is exported to various countries like USA, UK, Canada, Germany, Hong Kong, Brazil, Spain, Singapore, Italy, Korea and others. India's total export of Palmyra wood shaving brush handle in the year of 2010-2011 was Rs.1425 lakhs. From this year its exporting performance is increasing level except 2019-2020. In the year of 2019-2020 its export is decrease as Rs.3428 lakhs. Here, needed special attention to improve its export.

Forecasting For the Next 10-Years Of Palmyra Wood Shaving Brush Handle Export

These are expected exports of Palmyra wood shaving brush handle from 2020-2021 to 2029-2030.

Palmyra Palm jaggery is made from the Palm syrup. The sugar made from the sap of the date palm, Palmyra palm and coconut tree. The Palmyra syrup is made by boiling raw Palm sap in large vessels. Palmyra jaggery is produced by cottage industries and small-scale industries. India exporting Palmyra jaggery and candy sugar to the various countries. The export performance of Palmyra jaggery and candy sugar from the year of 2010-2020 is as follows:

The Jaggery cube and candy sugar have been exported from India for the past 10-years (2010-2011 to 2019-2020) it is exported to various countries like Bangaladesh, Djibouti, Jordan, Iran, Somalia, Srilanka, Uarab EMIST, Sudan, Yemen republic and others. Total export of Plamyra Jaggery cube and candy sugar in the year of 2010-2011 was Rs.134785 lakhs. They are gradually increased export in every year. It is earning more foreign currency; we may improve more and more.

Forecasting For the Next 10-Years Of Jaggery Cube, Candy Sugar Export

The Palmyra fruits grow in cluster on tall Palm trees. The sugar palm fruits are about 4 to 7 inches in diameter and have black husk. Inside the Palmyra fruit sweet jelly seeds sockets appear which are translucent, pale white in colour are and have a mild sweet flavor. Palmyra fruit is season fruit, we cannot get them whenever we need so, that the producers introduced preserving Palmyra fruit method. Palmyra fresh and preserved fruit have been exported from India to the various countries. Its export performance is given below:

The Palmyra fresh and preserved fruit have been exported from India for the past 10-years (2010-2011 to 2019-2020),it is exporting to various countries like Bahrainis, Bangladesh, Kuwait, Malaysia, Nepal, Quatar, Saudi Arab, Singapore, U Arab EMIST, UK and others. Total export of Fresh and preserved Palmyra fruit in the year of 2010-2011 was Rs.15777 lakhs. These are gradually increased in every year. But 2010-11 and 2011-12 this both years export is constant.

Palmyra Root Powder

Palmyra root powder is used for making ayurvedic medicines and ayurvedic beauty cream products. The dried Palmyra root powder is stored in plastic bottles and plastic packets for the selling purpose. Its exporting performance is given below:

Forecasting For The Next 10-Years Of Fresh And Preserved Palmyra Fruit Export

The Palmyra root powders have been exported from India for the past 10-years (2010-2011 to 2019-2020) to various countries. It is exporting toUArab EMIST, USA, UK Kuwait, Newzealand, Malaysia, Hongkong, Thailand, Singapore and others. Total export of Palmyra root powder in the year of 2010-2011 was Rs.995 lakhs. These are gradually increased in every year except 2019-20

These are expected exports of Palmyra root powder from 2020-2021 to 2029-2030.

Palmyra Ayurvedic Beauty Products (Shaving Cream)

This product is produced from Palmyra root. This root powder is used for making beauty cream productsand it has used for medicinal purposes. It is produced in all over the India. This product is produced by small scale industries. Its export performance is given below:

The Palmyra ayurvedic beauty products have been exported from India For the past 10-years (2010-2011 to 2019-2020) to various countries like USA, U Arab EMIST, Singapore, Nepal, Bangladesh, Poland, Russia, Srilanka, Saudi Arab, Iran and others. Total export of Palmyra ayurvedic beauty product in the year of 2010-2011 was Rs.18587 lakhs. It is gradually increased in every year, but it is decreased in the year of 2017-18 hen compare base year.

Forecasting For the Next 10-Years Of Palmyra Ayurvedic Beauty Products Export Status

Palmyra Nut Shaker

This product is children's playing instrument made out of Palmyra wood. It is produced in all over the India. The Palmyra nut shaker is produced by handy craft industries. The detailed export information's are given below:

The Palmyra nut shaker have been exported from India for the past 10-years (2010-2011 to 2019-2020) it is exporting to various countries like, France, Germany, Canada, Nepal, Australia, Trinidad, South Africa, Srilanka USA, Japan, UK and others. Total export of Palmyra nut shaker in the year of 2010-2011 was Rs.271 lakhs. These are gradually increased in every year, except in the year of 2012-14. This export has some ups and downs.

Forecasting For the Next 10-Years Of Palmyra Nut Shaker Export Status

CONCLUSION

Palmyra products are exporting to various countries from all the states of India. All the states exporting performance were increasing trend. As per agriculture report 5.10 crore Palmyra trees are there in Tamilnadu. Tamilnadu plays a major role in exporting Palmyra products. It may support state and central governments to Palmyra production, it willbe earning foreign money. So, it is concluded that governments should provide special attention to this field.

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Table-1 Palmyara Wood Shaving Brush Handle (Hs – 96019090) Export Status From 2010-2011 to 2019-2020 values in Rs. Lakhs

S. No	Years	Country											
		USA	UK	CANADA	GERMANY	HONG KONG	BRAZIL	SPAIN	SINGAPORE	ITALY	KOREA	Total export	Growth%
1	**2010-2011**	399	251	204	139	138	81	75	54	45	39	**1425**	**100.00**
2	**2011-2012**	843	196	174	120	117	99	92	84	70	51	**1846**	**129.54**
3	**2012-2013**	1103	189	160	151	148	128	93	79	57	50	**2158**	**101.00**
4	**2013-2014**	1137	665	345	163	112	99	82	56	49	47	**2755**	**127.66**
5	**2014-2015**	1313	397	301	215	210	172	137	135	123	103	**3106**	**102.00**
6	**2015-2016**	1591	499	438	369	339	323	273	243	211	165	**4451**	**143.30**
7	**2016-2017**	1154	504	418	394	325	318	257	177	146	97	**3790**	**103.00**
8	**2017-2018**	1427	757	418	392	254	246	229	211	198	146	**4278**	**112.88**
9	**2018-2019**	1753	1154	632	412	270	200	198	182	139	127	**5067**	**104.00**
10	**2019-2020**	1743	458	309	216	192	171	111	88	78	62	**3428**	**67.65**

Source: DGFT/APEDA(Growth rate calculated in Excel)

Table-2 Source: (calculated in Ms- Excel)

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	5085	5422	5759	6096	6433	6770	7107	7444	7784	8119

Table-3 Jaggery cube, candy sugar (Hs-17019990) exporting status from 2010-2011 to 2019-2020 values in Rs.lakhs

S. No	Years	Country											
		BANGLADESH	DJIBOUTI	JORDAN	IRAN	SOMALIA	SRILANKA	UARABEMTS	SAUDI ARAB	SUDAN	YEMEN REPUBLC	Total export	Growth%
1	**2010-2011**	24291	8802	8050	6800	15185	39635	20972	0	0	11050	**134785**	**100.00**
2	**2011-2012**	43203	32219	0	10490	27890	100401	58956	18825	64296	47280	**403560**	**299.41**
3	**2012-2013**	0	14809	16166	50331	38039	45727	20358	95943	14511	0	**295884**	**101.00**

4	**2013-2014**	0	0	21197	17989	30728	43613	34600	103485	103485	11775	**366872**	**123.99**
5	**2014-2015**	6136	19416	10225	6745	70837	31994	49910	23053	125851	17958	**362125**	**102.00**
6	**2015-2016**	0	0	17960	18528	110597	46272	30342	26151	108180	299725	**657755**	**181.64**
7	**2016-2017**	0	70237	0	274351	102321	19217	35945	20358	102959	280406	**905794**	**103.00**
8	**2017-2018**	22541	17831	0	43203	59630	14105	57985	20063	128249	15356	**378963**	**41.84**
9	**2018-2019**	0	68822	32722	14171	91715	79986	33881	26813	195113	85894	**629117**	**104.00**
10	**2019-2020**	27818	67821	0	91397	142354	90706	22178	31775	206275	26733	**707057**	**112.39**

Source: DGFT/APEDA

Table 4

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	786096	8440988	895879	950771	1005663	1060555	1115446	1170338	1225230	1280122

Source: (calculated in Ms- Excel)

Table-5 Fresh and Preserved Palmyra Fruit (Hs – 08045020) Export Status from 2010-2011 to 2018-2019 values in Rs. Laksh

S. No	**Years**	**Country**											
		BAHARAINIS	**BANGLADESH**	**KUWAIT**	**MALAYSIA**	**NEPAL**	**QATAR**	**SAUDI ARAB**	**SINGAPORE**	**UARAB EMTS**	**UK**	**Total export**	**Growth%**
1	**2010-2011**	356	1859	391	163	210	200	618	207	10318	1455	**15777**	**100.00**
2	**2011-2012**	10318	1859	1455	618	391	356	210	207	200	163	**15777**	**100.00**
3	**2012-2013**	16287	3250	1200	887	840	776	610	578	420	300	**25148**	**101.00**
4	**2013-2014**	17231	4545	1219	824	655	562	504	411	404	323	**26678**	**106.08**
5	**2014-2015**	21498	1429	1238	811	695	688	606	588	505	473	**28531**	**102.00**
6	**2015-2016**	19199	3206	1733	1675	1298	1024	734	640	633	413	**30555**	**107.09**
7	**2016-2017**	24745	4957	2446	2147	1911	1604	1549	979	878	823	**42039**	**103.00**
8	**2017-2018**	18458	4801	2199	1981	1773	1631	1595	1588	908	819	**35753**	**85.05**
9	**2018-2019**	15336	6196	3004	2839	2442	1984	1564	14707	1357	1042	**50471**	**104.00**

Source: DGFT/APEDA

Table: 6 Forecasting for the next 10-years of fresh and preserved Palmyra fruit export

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	53717	57657	61596	65535	69475	73414	77354	81293	85232	89172

Source: (calculated in Ms- Excel)

Table 7. Palmyra root powder (Hs-11063090) export status from 2010-11 to 2019-20 in IndiaValues in Rs. Lacs

S. No	Years	Country											
		U ARAB EMTS	U S A	AUS TRALIA	U K	KUWAIT	NEW ZEALAND	MALAYSIA	HONG KONG	THAILAND	SINGAPORE	Total export	Growth%
1	**2010-2011**	447	258	88	78	27	24	23	19	16	15	**995**	**100.00**
2	**2011-2012**	1000	243	103	85	54	46	44	37	33	30	**1675**	**168.34**
3	**2012-2013**	608	343	236	151	113	113	76	59	43	27	**1769**	**101.00**
4	**2013-2014**	1396	1283	696	352	223	215	141	126	102	99	**4633**	**261.90**
5	**2014-2015**	2485	722	615	332	299	244	243	147	111	102	**5300**	**102.00**
6	**2015-2016**	2438	928	645	279	203	126	117	111	101	99	**5047**	**95.23**
7	**2016-2017**	1729	946	745	694	373	350	190	168	127	126	**5448**	**103.00**
8	**2017-2018**	2023	1444	1265	1248	474	355	279	203	186	124	**7601**	**139.52**
9	**2018-2019**	3545	1133	790	262	210	208	151	149	117	70	**6635**	**104.00**
10	**2019-2020**	767	314	124	93	82	24	24	24	23	21	**1496**	**22.55**

Source: DGFT/APEDA

Table- 8. Forecasting for the next 10-years of Palmyra root powder export status

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	9156	9958	10760	11561	12363	13165	13967	14769	15570	16372

Source: (calculated in Ms- Excel)

Table 9. Palmyra ayurvedic beauty products(Shaving cream Hs-33049990) export status from 2010-2011 to 2019-2020 in India Values in Rs. Lacs

S. No	Years	Country											
		U ARAB EMTS	NEPAL	BANGLADESH	POLAND	SINGAPORE	U S A	SAUDI ARAB	RUSSIA	SRI LANKA	IRAN	Total export	Growth%
1	**2010-2011**	10513	2396	1066	975	832	737	611	531	513	413	**18587**	**100.00**
2	**2011-2012**	7679	5279	2217	1155	1127	851	738	733	685	649	**21113**	**113.59**
3	**2012-2013**	6776	4585	2339	1496	1206	730	656	637	525	510	**19460**	**101.00**
4	**2013-2014**	7058	4681	3575	2102	1979	1628	1292	987	861	847	**25010**	**128.52**
5	**2014-2015**	6793	4158	3165	3018	1939	1255	1197	1122	1030	993	**24670**	**102.00**
6	**2015-2016**	6941	3816	3742	3107	2710	1892	1740	1414	1350	1288	**28000**	**113.50**
7	**2016-2017**	12834	6945	4108	2285	2253	1764	1705	1669	1572	1416	**36551**	**103.00**
8	**2017-2018**	8431	5028	3642	3132	2128	2073	1900	1694	1540	1404	**30972**	**84.74**
9	**2018-2019**	8403	6558	4530	3762	3102	1932	1927	1875	1360	1343	**34792**	**104.00**
10	**2019-2020**	7560	5573	4669	4090	3301	2663	2582	2581	1975	1755	**36749**	**105.62**

Source: DGFT/APEDA

Table-10. Forecasting for the next 10-years of Palmyra ayurvedic beauty products export status

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	39730	41922	44115	46308	48501	50694	52887	55079	57272	59465

Source: (calculated in Ms- Excel)

Table- 11. Palmyra nut shaker (Children's playing instrument Hs-92059090)

S. No	Years	Country											
		FRANCE	U S A	UK	GERMANY	CANADA	NEPAL	AUSTRALIA	TRINIDAD	SOUTH AFRICA	SRI LANKA	Total export	Growth%
1	**2010-2011**	97	58	29	26	24	11	10	6	5	5	**271**	**100.00**
2	**2011-2012**	243	188	56	33	32	28	13	12	11	7	**623**	**229.89**
3	**2012-2013**	93	66	39	23	19	18	8	7	7	6	**286**	**101.00**
4	**2013-2014**	73	56	36	28	28	19	18	7	7	6	**278**	**97.20**

5	**2014-2015**	94	53	30	25	12	11	10	10	7	7	**259**	**102.00**
6	**2015-2016**	117	105	18	16	11	9	9	8	7	7	**307**	**118.53**
7	**2016-2017**	133	133	47	23	21	17	16	15	8	7	**420**	**103.00**
8	**2017-2018**	168	105	44	30	18	16	15	15	11	6	**428**	**101.90**
9	**2018-2019**	130	109	43	26	25	18	16	15	12	11	**405**	**104.00**
10	**2019-2020**	134	131	46	26	23	16	12	8	8	7	**411**	**101.48**

Source: DGFT/APEDA

Table- 12. Forecasting for the next 10-years of Palmyra nut shaker export status

Year	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026	2026-2027	2027-2028	2028-2029	2029-2030
Forecast	389	393	397	401	405	410	414	418	422	426

Source: (calculated in Ms- Excel)

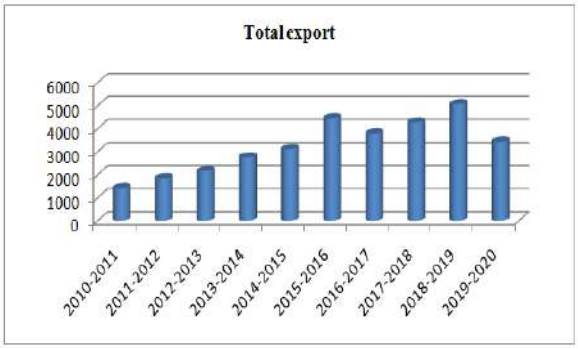

Fig.1 : Palmyra wood shaving brush handle

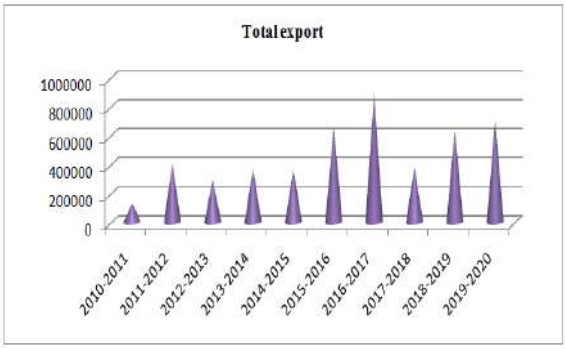

Fig.2 : These are expected exports of Palmyra jaggery cube, candy sugar from 2020-2021 to 2029-2030.

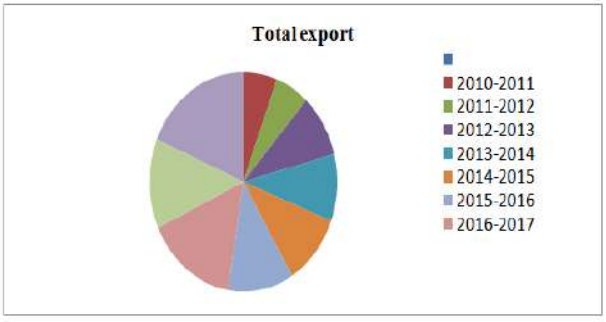

Fig.3 : These are expected exports of fresh and preserved Palmyra fruit from 2020-2021 to 2029-2030.

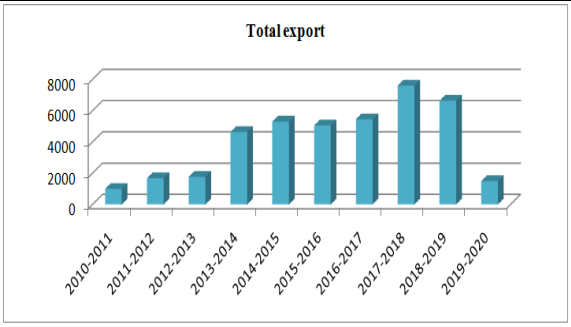

Fig. 4 These are expected exports of Palmyra root powder from 2020-2021 to 2029-2030.

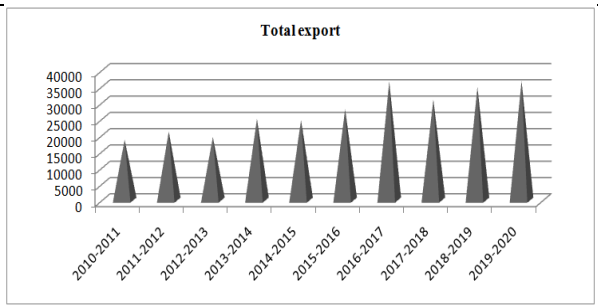

Fig.5. Palmyra ayurvedic beauty products export status

Fig.6 : Palmyra nut shakerexport status

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RESEARCH ARTICLE

Effectiveness of Mindfulness Based Stress Reduction Programme on Psychosocial Wellbeing of Elderly People

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ABSTRACT

Old age is a period when people need physical, emotional and psychological support. Stress is more common among old age people and it is higher in elderly living in old age home than living with family or alone. Stress affects wellbeing of elderly and leads to low-level psychosocial wellbeing and dispositional mindfulness. Mindfulness Based Stress Reduction is a very much effective relaxation programme to provide quick way to get rid of stress by improving psychosocial wellbeing and dispositional mindfulness among elderly people. The study was undertaken to evaluate the effectiveness of Mindfulness based Stress Reduction Programme on psychosocial well being of elderly people residing in selected old age homes, Salem. A quasi experimental design, pre and post test with control group and quantitative approach was used. Total 300 samples were selected by using multiphase sampling technique (Lottery method). Data collected by using structured interview schedule, Stress Scale, Psychosocial well being Scale and Mindful Attention Awareness Scale (MAAS). Mindfulness Based Stress Reduction Programme was administered for elderly people in experimental group. Techniques were demonstrated by investigator and instructed them to practice for 8 weeks after training session. Major findings of the study revealed that highest and more or less similar percentage (39% &44%) of elderly people in experimental and control group were in the age group of 60- 65 years, males (61% & 53%), Hindus, educated, employed (54%) and married in both experimental (62%) and control group

(67%). Highest percentage of them had two children in experimental group (49%), rich, nuclear family and from urban area, entered to old age home by family members (44% & 65%), staying more than five years at old age home, vegetarian and had the hobby of reading books and had no personal habits (60% & 51%), had hypertension (44% & 48%) and were staying at old age home due to the reason of children compulsion in experimental group (43%) whereas burden to the family (54%) in control group. Highly significant difference found in the overall and all the area scores of stress, psychosocial well being and dispositional mindfulness between pre and post test of elderly people in experimental group. Highly significant difference was found in the overall and all the area post test scores of stress, psychosocial well being and dispositional mindfulness between experimental and control group. Significant association was not found between pre and post test level of stress, psychosocial well being and dispositional mindfulness of elderly people in experimental and control group during and their selected demographic data. Highly significant positive correlation found between psychosocial wellbeing and dispositional mindfulness. Highly significant negative correlation found between Stress and psychosocial well being and Stress and dispositional mindfulness. Stress and lack of psychosocial wellbeing and dispositional mindfulness found in elderly people. Higher level of stress lower the psychosocial well being and dispositional mindfulness and lower level of stress lead to higher level of psychosocial well being and dispositional mindfulness among elderly. The study concluded that Mindfulness Based Stress Reduction Programme was a effective relaxation technique for elderly people in reducing their level of stress and improving higher level of psychosocial wellbeing and dispositional mindfulness.

Keywords: Mindfulness Based Stress Reduction Programme, Stress, Psychosocial Wellbeing, Dispositional Mindfulness, Elderly People

INTRODUCTION

India is fast developing country. Population of old age is large and increasing due to development of health care facilities. Oldage people are facing psychological physical, and health issues due to lo w economic status, urbanization, modernization. Globalizations made changes in lifestyle pattern which lead to disintegration of joint family system, and alternative living arrangements for elderly have come forward [1]. It is estimated 605 million people aged 60 years and above at globally. Improvements in health care facilities have brought longevity, which is considered as one of the greatest achievements of the 20th century. In India, 95 million people in the age of 60years at present, by the year 2025 nearly 80 million more will be increased. With improved life expectancy rate in our country, it has estimated that as many as 8 million people are currently above the age of 80 years. Economic compulsions of the children, changes in family values, neglect and abuse has caused elders to fall through the net of family care[2]. Aging is a consistent, natural and continuous irreversible changing process. In old age period, progressive generalized impairment of function leads to lack of coping response to stress. Inadequate family care surrounding leads to loneliness and depression[3]. Separation or death of spouse, deaths in the family and lack of social integration are common stressors, which may themselves cause physical and mental - ill health. Physical incapacity, the decline in the mental faculties and feeling of the generation gap, add to the problem[4].

Need for the Study

Ageing process is a chronic condition of human life cycle which is accompanied by conditions such as cardiovascular disease, hypertension, diabetes mellitus, cancer, and/or mental illness [5]. Globally, the population is ageing rapidly, and it is estimated that older people or elderly, defined as those aged 60 years and over, will constitute 22% of the total world population by 2025 (WHO, 2018) [6]. Mental health influences the health problems in older adults. It has estimated that more than 20% of older adults aged above 60 years suffered psychological and neurological problems

globally. Mental and neurological disorders is a cause for 6.6% of elderly with lack of daily living activities. The following reasons such as lack of independence, frailty, illness, separation, isolation, and simply due to their age, among other reasons, aged people are in more chances to get mental health issues and disorders [7].

Socio economic crisis and malpractice/ abuse of children mostly the reason for feeling of ignorance and lack of emotional support in elderly which often compel them to opt other places for living a problem free life (Akbar. et al, 2014)[8]. Changes in family relationships and psycho-social bonding and values often compel the elderly to live alone or to shift from their own homes to some institutions or old age homes [9]. Elderly are vulnerable to stress from various causes. The elderly in old age homes are a distinct population with inadequate family and social support lead to an increased signs of stress [10]. A lack of attention to signs of stress might lead to stress symptoms without appropriate coping responses, and subsequent reduction of health and well-being. An increased awareness of stress signs lead to higher dispositional mindfulness, might positively influence coping and buffer against the negative influence of stress. It can be revealed that higher degree of mindfulness enables increased clarity of awareness, intellectual and emotional well being[11]. Mindfulness is strongly related to well-being and perceived health. Dispositional mindfulness has negative influence of stress symptoms on psychological well-being. Use of mindfulness training as a way of improving psychological functioning among people experiencing stress[12]. Mindfulness meditation is a form of meditation that can improve psychological symptoms, such as anxiety and depression, and health-related quality of life (Relatively few clinical trials, however, have tested the benefits of mindfulness meditation on mind, body, health and wellness in aging. The therapeutic effects of mindfulness training continue to provide empirical support for the effectiveness of mindfulness-based interventions (MBIs) at reducing psychological distress and improving wellbeing[13]. MBSR program predicted a reduction in psychological distress and an increase in psychological wellbeing [14]. The above studies interpreted to keep pace with this continuing demographic shift, more information on morbidity rates in the elderly and particularly the size and burden of mental disorders is needed to optimize mental health care and to provide adequate services for elderly people. In the light of above facts and personal experiences, the researcher had felt that there is a need to assess the effectiveness of Mindfulness Based Stress Reduction Programme on Psychosocial wellbeing of Elderly people to promote mental health of the elderly people.

Statement of the Problem

A Study to assess the Effectiveness of Mindfulness Based Stress Reduction Programme on Psychosocial Wellbeing of Elderly People residing at old age homes, Salem, Tamil Nadu.

Objectives

1. To assess the stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group before administration of Mindfulness Based Stress Reduction Programme.
2. To assess the stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group after administration of Mindfulness Based Stress Reduction Programme.
3. To assess the effectiveness of Mindfulness Based Stress Reduction Programme on stress, psychosocial wellbeing and dispositional mindfulness of elderly people.
4. To compare the effectiveness of Mindfulness Based Stress Reduction Programme on level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people between experimental and control group.
5. To find out association between the pre and post test scores of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group and their selected demographic variables.
6. To find correlation between post test scores of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group.

METHODOLOGY ADOPTED

Research Approach and Design

Quantitative research approach and Quasi-experimental study, pre test and post test with control group was used to assess the effectiveness of Mindfulness Based Stress Reduction Programme on psychosocial wellbeing of elderly people

The schematic representation is:

Control group O_1 —— O_2

Experimental group O_1 X O_2

E = $O_2 - O_1$ (Experimental group)

E = Control group (O_2) – Experimental group (O_2)

The symbols used are:

O_1 : Pretest assessment of stress, psychosocial wellbeing and dispositional mindfulness of elderly people of control and experimental group

O_2 : Post test assessment of stress, psychosocial wellbeing and dispositional mindfulness of elderly people of control and experimental group

X : Administration of mindfulness based stress reduction programme on stress, psychosocial wellbeing and dispositional mindfulness of elderly people in the experimental group.

E : Effectiveness of mindfulness based stress reduction programme on stress, psychosocial wellbeing and dispositional mindfulness of elderly people between experimental and control group.

Setting of the Study

The study was undertaken in twenty-one old age homes in Salem district. Old age homes, which is authorized by Government of Tamil Nadu and administered by District Social Welfare Department, Salem District, were selected for my study. Two old age homes were selected for pilot study and one old age home for reliability. Remaining eighteen old age homes were divided equally as nine old age homes each in experimental and control group.

Variables under Study

Independent variable : Mindfulness Based Stress Reduction Programme.

Dependent variable : Stress, Psychosocial wellbeing and Dispositional mindfulness

Extraneous variables : age, gender, type of family, residential area, religion, education, previous occupation, marital status, total number of children, gender of children, economic status of children, mode of entry to old age home, family members visit to old age home, dietary pattern, hobby, personal habits, previous history of chronic illness and reason to shift to old age Home.

Population

The population for the present study were the elderly people above 60 years and staying in the old age homes, Salem district.

Sample

The samples for the present study were the elderly people above 60years and staying in the old age homes, Salem district & met inclusion criteria.

Sample Size

The sample size consisted of 300 elderly people in which 150 in experimental group & 150 in control group.

Sampling Technique

Multiphase random sampling techniques through lottery method was used for selecting sample as per availability and fulfillment of inclusion criteria

Inclusion Criteria

The elderly people who were,

1. 60 years and up to 80 years
2. able to do activity of daily living (ADL)
3. not attended any stress reduction programme in past 2 years
4. willing to participate in the study.
5. able to speak and understand Tamil

Exclusion Criteria

The elderly people who were,

- Critically ill
- Bed ridden
- Dumb and deaf
- Taking treatment for mental illness
- having disturbances in memory
- having recent history of chronic or recent back pain or injury.
- having recent history of High blood pressure and heart ailments
- having the complaint of Migraine.

Data Collection Instruments

Tools used in this study as follows.

Section - I : Semi structured interview schedule for demographic variables of elderly people.

Section - II : Stress Scale which is prepared by investigator to assess the level of stress of elderly people.

Section - III : Psychosocial Wellbeing scale which is prepared by investigator to assess the level of Psychosocial Wellbeing of elderly people.

Section - IV : Mindful Attention Awareness Scale (MAAS) is a standardized scale to assess the level of dispositional mindfulness of elderly people.

Data Collection Procedure

Mindfulness Based Stress Reduction Programme

The investigator obtained training in Mindfulness Based Stress Reduction techniques from authorized training institution & certified to do the intervention to elderly people.

Permission from the concerned authority

Obtained permission from District Social welfare officer, District social welfare department, Salem and Managing authorities of old age homes. Obtained informed oral consent from all the samples before data collection.

Pretest

- Brief introduction about the self were given to the elderly people following detailed explanations regarding the purpose of the study.
- The assessment was done by using rating scales to assess the level of stress, level of psychosocial wellbeing and dispositional mindfulness of elderly people in both experimental and control group.
- The total time taken for interview was averagely from 15 to 20 minutes.
- Elderly people answered all the items.

Intervention

- Immediately after the pre test mindfulness based stress reduction techniques were demonstrated by investigator for experimental group.
- Convenient time of elderly people was planned for the training period by investigator. Either 6.30 AM to 7.30 AM or 5.30 PM to 7.30 PM.
- Training was given for five days with the duration of 20 to 30 minutes per day as scheduled.
- Instructed the elderly people to practice continuously for eight weeks after the training period.
- In the period eight weeks for 15 to 20 minutes in the morning and night before the elderly going to bed.

- Elderly people' daily practice report was gathered by investigator from representative/incharge from each old age homes through in person or telephonic information.
- For the control group, No intervention were given and regular routine was followed.

Post Test

- Post test was done after eighth week of practice for both experimental group and control group in accordance with the plan of assessment.
- The assessment was done by using rating scales to assess the level of stress, level of psychosocial wellbeing and dispositional mindfulness of elderly people in both experimental and control group.
- The total time taken for interview was averagely from 15 to 20 minutes.

RESULTS AND FINDINGS

Percentage wise distribution of pre and post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group reveals that highest percentage of elderly people had high level of stress (61%) during pre test whereas highest percentage of them had moderate level of stress (58%) during post test, it might be due to that severe stress is reduced as moderate level of stress after intervention. Highest percentage of elderly people had moderate level of psychosocial wellbeing during pre test (56%) and post test (60%) none of them had low level of psychosocial(58%) during post test , it might be due to the effectiveness of intervention. Highest percentage of elderly people had moderate level of dispositional mindfulness (57%) during pre test whereas highest percentage of them had high level of dispositional mindfulness (69%) during post test, it might be due to that moderate level is improved as high level of dispositional mindfulness after intervention (Fig. No.1).

Percentage wise distribution of post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group reveals that highest percentage of elderly people had high level of stress (59%) in control group whereas highest percentage of them had moderate level of stress (58%) in experimental group, it might be due to that severe stress is reduced as moderate level of stress after intervention. Highest percentage of elderly people had low level of psychosocial wellbeing (52%) in control group whereas highest percentage of them had moderate psychosocial wellbeing (60%) and none of them had low level of psychosocial wellbeing in experimental group during post test. Highest percentage of elderly people had moderate level of dispositional mindfulness (52%) in control group whereas highest percentage of them had high level of dispositional mindfulness (69%) in experimental group, it might be due to that moderate level is improved as high level of dispositional mindfulness after intervention (Fig. No. 2).

Paired 't' test was calculated to analyze the significant difference in post test stress, psychosocial wellbeing and dispositional mindfulness scores of elderly people in experimental and control group revealed that there is a highly significant difference found in the overall and all the area scores of stress, psychosocial wellbeing and dispositional mindfulness between experimental and control group. Hence, it can be interpreted that research hypothesis was accepted and reveals that Mindfulness Based Stress Reduction Programme was effective among elderly people in reducing level of stress and to promote the psychosocial wellbeing (Table No. 1).

Association between the pre test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group during and their selected demographic data reveals that, all the selected variables were shown not significant association found between level of stress of elderly people in experimental group during pre test and their selected demographic data except economic status of children. There was no statistically significant association found between level of psychosocial wellbeing of elderly people in experimental group during pre test and their selected demographic data except residential area. There was no statistically significant association found between level of dispositional mindfulness of elderly people in experimental group during pre test and their selected demographic data except previous occupation. Hence, the alternative hypothesis is accepted. Therefore research hypothesis was rejected at 0.05 level of significance. Hence, it can be interpreted that mindfulness based stress reduction programme was effective in reducing the stress of elderly people in old age homes (Table No. 2).

'r'-value calculated to find out correlation between posttest level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group revealed that highly significant positive correlation found between psychosocial wellbeing and dispositional mindfulness. It reveals that when psychosocial wellbeing increases vice versa dispositional mindfulness also increasing. Highly significant negative correlation between Stress and psychosocial wellbeing and Stress and dispositional mindfulness. It reveals that when stress level increases psychosocial wellbeing and dispositional mindfulness decreasing whereas stress level decreased psychosocial wellbeing and dispositional mindfulness increasing. Hence, stated hypothesis accepted, it shows intervention was effective in elderly people in experimental group (Table No. 3).

DISCUSSION, CONCLUSION, IMPLICATIONS AND RECOMMENDATION

A quasi experimental design, pre and post test with control group and quantitative approach was used to evaluate the effectiveness of Mindfulness based Stress Reduction Programme on psychosocial wellbeing of elderly people. Total 300 samples were selected by using Probability multiphase sampling technique. The demographic data was collected using structured interview schedule, psychosocial wellbeing were assessed by Stress scale, Psychosocial wellbeing Scale and Mindful Attention Awareness Scale (MAAS; (Brown & Ryan, 2003).

The demographic variables reveals that highest and more or less similar percentage (39% &44%) of elderly people in experimental and control group were in the age group of 60- 65 years. Higher percentage of elderly people were males (61% & 53%), Hindus, educated, employed (54%) and married in both experimental (62%) and control group (67%). Highest percentage of them had two children in experimental group (49%), rich, nuclear family and from urban area. Highest percentage (44% & 65%) of them were entered to old age home by family members, staying more than five years at old age home, vegetarian and had the hobby of reading books. in both group. Highest percentage (60% & 51%) of elderly people had no personal habits, similar percentage (44% & 48%) of them had hypertension and were staying at old age home due to children compulsion in experimental group (43%) whereas burden to the family (54%) in control group.

Paired 't' test was calculated to analyze the significant difference in pre and post test stress, psychosocial wellbeing and dispositional mindfulness scores of elderly people in experimental group revealed that there is a highly significant difference found in the overall and all the area scores of stress, psychosocial wellbeing and dispositional mindfulness between pre and post test. Hence the stated hypothesis H_1 is accepted.

Paired 't' test was calculated to analyze the significant difference in post test stress, psychosocial wellbeing and dispositional mindfulness scores of elderly people in experimental and control group revealed that there is a highly significant difference found in the overall and all the area scores of stress, psychosocial wellbeing and dispositional mindfulness between experimental and control group. Hence, it can be interpreted that research hypothesis H_2 was accepted and reveals that Mindfulness Based Stress Reduction Programme was effective among elderly people in reducing level of stress and to promote the psychosocial wellbeing. The findings was supported by Laura A. Young, and Michael J. Baime, (2010) who conducted a study on Mindfulness-Based Stress Reduction: Effect on Emotional Distress in Older Adults and results revealed that overall emotional distress and all subscale mood measurements improved significantly following MBSR training. MBSR training resulted in >50% reduction in the number of older people reporting clinically significant depression and anxiety [15].

Association between the pre and post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group during and their selected demographic data reveals that, there was no significant association found between level of stress, level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group during and their selected demographic data.

'r'-value calculated to find out correlation posttest level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group revealed that highly significant positive correlation found between psychosocial wellbeing and dispositional mindfulness. Highly significant negative correlation between Stress and psychosocial wellbeing and Stress and dispositional mindfulness. Hence, stated hypothesis accepted , it shows intervention was effective in elderly people in experimental group. The findings was supported by

Srisailamaiah M, Suresh K, Srikanth Reddy V. (2016) who conducted a study on Depression and Psychological Well-Being among Living Institutionalized and Non- Institutionalized Elderly and the results revealed that there were (-0.68) negative correlations are seen between depression and psychological well-being [16].

CONCLUSION

A quantitative approach with quasi experimental design with control group was adopted to assess the effectiveness of Mindfulness Based Stress Reduction Programme on Psychosocial wellbeing of Elderly people in a selected old age homes, Salem, Tamilnadu**.** Major findings of the study reveals that reveals that highly significant difference found in the overall and all the area scores of stress, psychosocial wellbeing and dispositional mindfulness between experimental and control group. Hence, it can be interpreted that research hypothesis H_2 was accepted and reveals that Mindfulness Based Stress Reduction Programme was effective among elderly people in reducing level of stress and to promote the psychosocial wellbeing. Association between the pre and post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group during and their selected demographic data reveals that, there was no significant association found between level of stress, level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group during and their selected demographic data. 'r'-value calculated to find out correlation between posttest level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group revealed that highly significant positive correlation found between psychosocial wellbeing and dispositional mindfulness. Highly significant negative correlation between Stress and psychosocial wellbeing and Stress and dispositional mindfulness. Hence, stated hypothesis accepted , it shows that Mindfulness Based Stress Reduction Programme was effective among elderly people in reducing level of stress and to promote the psychosocial wellbeing.

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Table No. 1:Paired "t"-test to compare the post test scores of stress, psychosocial wellbeing and Dispositional mindfulness of elderly people in experimental and control group

Variables		Post test scores				Mean differ-ence	't'-value	Level of Signifi-cance
		Control		Experimental				
		Mean	SD	Mean	SD			
Stress	Physical	36.5	10.5	21.97	7.34	14.52	13.85	HS
	Psycho-logical	36.4	10.8	15.22	7.91	21.16	19.27	HS
	Social	33.3	9.44	19.54	6.51	13.72	14.65	HS
	Financial	14.2	3.43	9.64	2.71	4.59	12.85	HS
	Overall	**120.06**	**23.2**	**66.38**	**14.1**	**53.68**	**24.20**	HS
Psycho-social well-being	Physical	12.27	6.87	29.07	5.69	16.8	23.04	HS
	Psycho-logical	32.12	17.5	67.48	19.9	35.36	16.33	HS
	Social	19.63	7.33	31.3	7.85	11.67	13.31	HS
	Overall	**64.02**	**21.7**	**127.86**	**22.4**	**63.84**	**25.04**	HS
Disposi-tional mind-fulness	**Overall**	**36.4**	**14.0**	**70.34**	**14.7**	**33.9**	**20.4**	HS

P<0.001 level; HS - Highly significant

Table No. 2:Association between pre test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group and their selected demographic variables.

Demographic variables	Stress		Psychosocial wellbeing		Dispositional mindfulness	
	χ^2	Signifi-cance	χ^2	Signifi-cance	χ^2	Signifi-cance
Age in years	3.86	NS	2.37	NS	2.09	NS
Gender	1.19	NS	0.02	NS	1.12	NS
Religion	5.12	NS	0.204	NS	1.80	NS
Education	5.46	NS	3.75	NS	5.89	NS
Previous occupation	0.38	NS	0.044	NS	**5.20***	**S**
Marital status	2.67	NS	0.752	NS	1.74	NS
Number of children	2.23	NS	4.91	NS	6.81	NS
Economical status of	**8.39***	**S**	2.79	NS	2.39	NS

children						
Type of family	0.41	NS	2.61	NS	0.76	NS
Residential area	0.36	NS	**4.38***	**S**	0.01	NS
Mode of entry to old age home	0.22	NS	4.10	NS	4.92	NS
Duration of stay at old age home	2.78	NS	0.55	NS	5.27	NS
Family members visit to the old age home	0.048	NS	0.91	NS	1.17	NS
Dietary pattern	0.099	NS	0.55	NS	0.404	NS
Hobby	3.49	NS	2.69	NS	1.23	NS
Personal habits	3.82	NS	0.23	NS	2.005	NS
Chronic illness	0.686	NS	2.35	NS	0.09	NS
Reason for coming to old age home	0.686	NS	2.35	NS	0.09	NS

NS-Not significant, S-significant. *-P<0.05level - significant

Table No.3.:Correlation between posttest scores of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group

Variables	**'r'-value**	**p-value**
Stress and psychosocial wellbeing	-0.603	P<0.001*** HS
Stress and dispositional mindfulness	-0.477	P<0.001*** HS
Psychosocial wellbeing and dispositional mindfulness	0.386	P<0.001*** HS

***P<0.001 level HS-Highly Significant; NS-Not Significant.

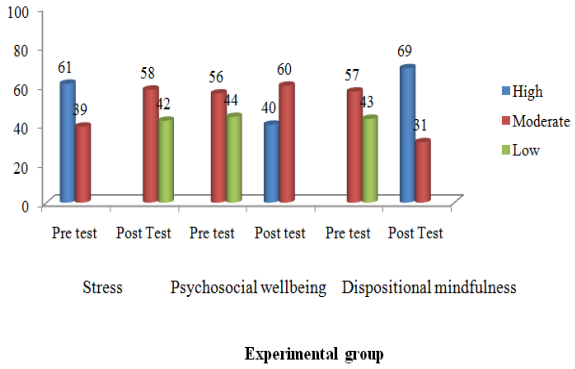

Fig. No. 1 : Bar diagram showing percentage wise distribution of pre and post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental group

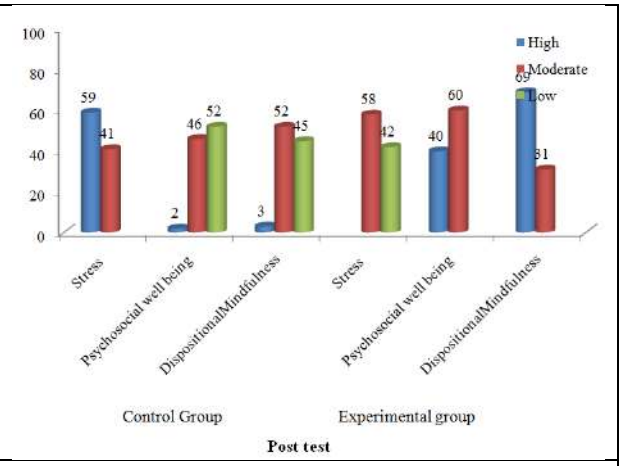

Fig. No. 2:Bar diagram showing percentage wise distribution of post test level of stress, psychosocial wellbeing and dispositional mindfulness of elderly people in experimental and control group

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RESEARCH ARTICLE

Copper Oxide Nanoparticles Synthesis using *Citrullus colocynthis* and its Antibacterial activity

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ABSTRACT

The current study describes a biologically based process as bio approach of CuO NPs using eco-friendly and non-toxic *Citrullus colocynthis* leaf extract. XRD, FTIR, and SEM with EDX were used to characterize the CuO nanoparticles. CuO is formed in a monoclinic structure, according to analytical techniques. This process uses a cost-effective, environmentally safe, and biocompatible reducing agent to make CuO nanoparticles. CuO's antibacterial activity was measured using the disc diffusion method against bacteria such as *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus.* CuO nanoparticles had strong antimicrobial activity against the bacteria strains that were examined.

Keywords: Nanoparticle Green synthesis, *Citrullus colocynthis,* XRD, Antibacterial Activity

INTRODUCTION

Copper oxide NPs have unique properties that have significant technological interest and also attract more attention. CuO with a small bandgap (Eg=1.2eV) is used in the fabrication of high-temperature superconductors [1-4], giant magneto resistance materials [8-9], gas sensors [5-7], and organic-inorganic nanostructure preparation [10]. Coatings, textiles, and plastics may contain antimicrobial, anti-fouling, antibiotic, and anti-fungal agents in their applications [11]. Cu and copper-based compounds with active biocidal properties are now widely used in pesticide formulations, and several health-related areas are being researched and/or introduced [12,13]. On the basis of the elemental and functional significance of CuO nonmaterials, well-defined CuO nanostructures with various

morphologies have been fabricated. Sonochemical[14], microwave irradiations [15], alkoxide-based path [16], sol-sel technique [17], one-step solid-state reaction method at room temperature [18], electrochemical method [19], precipitation-pyrolys [20], precursor thermal decomposition [21], or a combination of electro deposition and self-catalytic mechanism. Chemical synthesis methods cause the surface to absorb such toxic chemicals, which can have negative effects in medicinal applications. For material chemists, the synthesis of excellent nonmaterial with a composed state of agglomeration in practical processes for chemical purity, crystallinity, phase selectivity, and particle size homogeneity remains a challenge. Chemical synthesis methods often result in the presence of a toxic chemical on the surface, which can be dangerous in medicinal applications. The desire to develop an environmentally friendly solution arose as a consequence of the development of nanoparticles to better understand green chemistry and other biological processes. Green synthesis has many benefits in terms of environmental friendliness and accessibility for pharmaceutical and other biomedical applications because toxic chemicals are not used in the synthesis protocol [22,23,24,25]. Plant extracts and other biological methods are used in just a few studies on metal oxide nanoparticles, but there are many reports on metal nanoparticles. However, there have been few studies on the biological synthesis of CuO nanoparticles [26].

In this paper, we demonstrate that *Citrullus colocynthis* leaf extracts can be used to make green CuO nanoparticles, and we investigate its antibacterial activity against several bacterial pathogens *(Klebsiella pneumonia, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Staphylococcus aureus).*

EXPERIMENTAL WORK

plant collection

Chidambaram provided a fresh leaf from the plant, *Citrullus colocynthis,* which was disease-free. The Department of Botany at Annamalai University in Tamil Nadu, India, named and authenticated the leaves

Preparation of leaf extract

The fresh and healthy leaves of *Citrullus colocynthis* were collected and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles. When the leaves completely dried, they were chopped into fine pieces, each 20gm. The chopped leaves of *Citrullus colocynthis* were boiled with 100ml of double-distilled waste Carter r at 60^0C with constant stirring for up to 30mins. The obtained extract was filtered using Whatman NO.1 filter paper, and then brown extract was collected.

Synthesis of CuO NPs

In a typical reaction mixture, 1M Cupric Nitrate Trihydrate was dissolved in 50ml deionized water and magnetically stirred at room temperature for 10 minutes. Afterwards, *Citrullus colocynthis* aqueous leaf extract (5ml, 10ml, and 15ml) was added drop wise under stirring. As soon as the *Citrullus colocynthis* extract comes in contact with copper ions; it spontaneously changes the blue colour into green. The obtained green mixture began to change expected to the development of brown suspended CuO NPs, suggesting that water-soluble copper oxide nanoparticles had formed.

Characterization of CuO nanoparticles

CuO nanoparticles synthesized are crystalline in nature, with X-ray diffraction capping (XRD). FTIR spectroscopy was used to analyze peaks characteristic of the functional groups. SEM was used to examine the morphology structure of synthesised nanoparticles and to determine the particle size. The CuO can be identified by the EDX. CuO nanoparticles synthesized from *Citrullus colocynthis* plant leaf extract were used to further monitor antibacterial activity.

RESULTS AND DISCUSSION

X-ray diffraction

The nanoparticles are classified using X-ray diffraction phase detection and crystalline structure. The characteristic peaks seen in the XRD patterns confirmed the CuO NPs biosynthesized from *Citrullus colocynthis* leaf extracts (Fig 1). The peaks at 32.52^0, 35.49^0, 38.76^0, 48.62^0, 58.23^0, 61.67^0, and 66.22^0 correspond to (110), (022), (111), (202), (202), (113), and (311). Furthermore, the well-defined and sharp CuO NPs reflection XRD patterns (Fig.1) support the existence of CuO-NPs in a well-crystalline state. The well-crystalline presence of CuO-NPs in the well-defined and sharp CuONPs reflection is confirmed by typical XRD patterns. Figure 1 shows a standard XRD study of formed CuO NPs, which have a monoclinic single-phase structure close to that of monoclinic single-phase CuO NPs (JCPDS 89-5898). The XRD pattern showed no contamination peaks other than CuO-NPs, indicating high phase cleanliness. The diffraction peaks are becoming larger, suggesting that the crystal sizes are small. CuO NP crystallite size is measured using the Debye-Scherrer process [27]

$$D=K\lambda/\beta\cos\theta$$

Where Dare is the particle size in nanometers (nm), K is a constant of 0.94, λ is the x-ray wavelength ($1.5406A^0$), β is the full width at half maximum (FWHM) of the peak (in radians), and 2θ is the Bragg angle (degree). The crystallite size of the prepared sample is 27.77nm in 5ml, 28.80nm in 10ml, and 25.04nm in 15ml. It has been discovered that increasing the concentration of leaf extract causes crystallite size to decrease.

FT-IR analysis

The sample's FTIR spectrum is captured and shown in figure 4. The properties of the spectrum are as follows. Peaks of 551 cm^{-1} (Cu-O asymmetric stretching) and 883 cm^{-1} (Cu-O asymmetric stretching) indicate the presence of a metal oxide group in the sample. The existence of a hydroxide group in the sample is determined by the vibration peak 3454cm-1. This can be due to the water attached to the CuO nanoparticles' surface, which is also a reaction byproduct that can be removed by further heating. CuO is formed when the metal-oxygen bond is attended at 1390cm-1 (M-O rocking in-plane) and 1625cm-1 (M-O rocking in the plane). According to their FTIR spectrum, CuO NPs are surrounded by a variety of organic molecules

SEM with EDX

The SEM with a EDX analysis is done with SEM machine: JEOL Model JSM-6701LV. SEM with EDX at a magnification of 20000 with a voltage of 20kv. The SEM picture of the synthesized sample is shown in figure 3. It is seen that the CuO nanoparticles formed have an agglomeration structure with no distinct morphology. The EDX analysis carried out on the samples is depicted in figure 3, in which characteristic peaks corresponding to Cu and Oxygen are seen and there are traces of forerunner, which shows the cleanliness of the CuO NPs formed

Antibacterial activity

Antibacterial activity of CuO- sample *Citrullus colocynthis* against bacterial pathogens. The CuO *Citrullus colocynthis* was analyzed for antibacterial activity against selected bacterial pathogens on MHA agar plates. The CuO *Citrullus colocynthis* displayed greater antibacterial activity against all the selected pathogens and the zone of inhibition has varied significantly according to the concentration used. The maximum growth inhibitory activity was observed against *Staphylococcus aureus, Klebsiella pneumonia* followed by *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. However, the CuO *Citrullus colocynthis* exhibited lesser inhibitory activity against *Escherichia coli* when compared with other tested bacterial pathogens. The standard antibiotic Chloramphenicol recorded zone of inhibition ranged from 13 to 15 mm

CONCLUSION

CuO nanoparticles generated quickly by biosynthesis using *Citrullus colocynthis* leaf extract are inexpensive, non-toxic, and co-friendly, with a size of 25nm and a monoclinic structure, according to the findings. Biosynthesis CuO nanoparticles have stronger antibacterial activity than commercial medicines. As a result, the study results are expected to have far-reaching effects in the pharmaceutical and biomedical field

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Table 1. Antibacterial activity of CuO- sample *Citrullus colocynthis* against bacterial pathogens

S. No	Bacterial Pathogens	Zone of Inhibition (mg/µL)			
		50 µg	100 µg	Positive	Negative
1	*Pseudomonas aeruginosa*	08.48±0.24	11.62±0.45	14.12±0.26	-
2	*Staphylococcus aureus*	09.08±0.12	12.42±0.38	15.34±0.82	-
3	*Klebsiella pneumoniae*	-	10.16±0.18	13.16±0.54	-
4	*Escherichia coli*	-	11.62±0.37	13.64±0.38	-

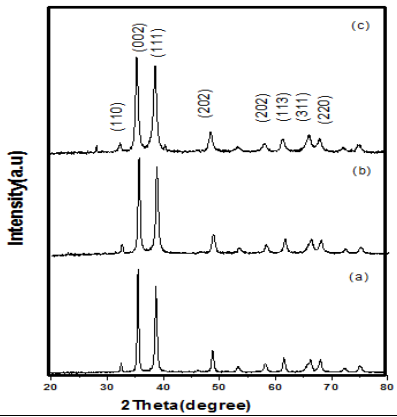


Fig.1.XRD patteren of CuO-NPs

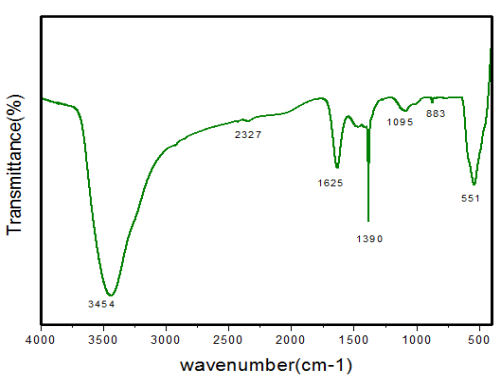
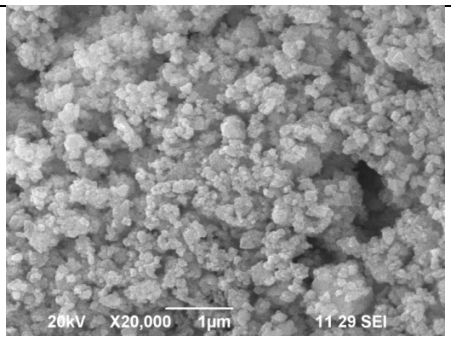


Figure 2: FTIR spectrum of CuO NPs



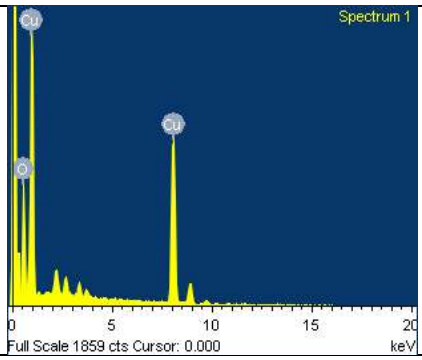
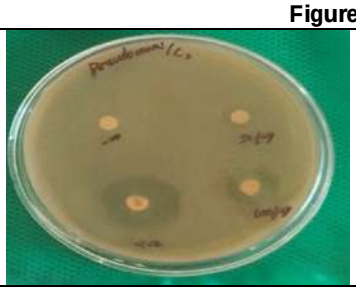


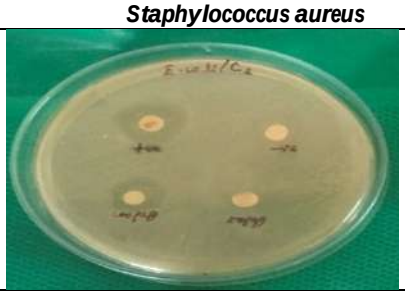
Figure 3: SEM with EDX of CuONPs



Pseudomonas aeruginosa

Staphylococcus aureus

Klebsiella pneumoniae



Escherichia coli

Figure 4: Antibacterial activity of CuO

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RESEARCH ARTICLE

Upshot of Polyherbal Formulation on Spatial Working Commemorative Performance in Diabetic Rats.

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ABSTRACT

Diabetes like conditions are key reasons for impaired memory functions, memory loss, amnesia and dementia rather further developed circumstances like Alzhemers disease and Schizophrenia is major problems. Nootropic agents such as piracetam, aniracetam, and choline esterase inhibitors like donepezil are being used for improving memory, mood, and behavior, but the resulting side effects associated with these agents have made their applicability limited.Our ancient knowledge is at its best which provide sound therapy for brain related elements. In this study we have evaluated Polyherbal formulation in streptozotocine induced diabetic rats for its effect on memory function. In result we found that the Polyherbal formulation in combination with Metformin successfully managed the diabetic memory loss complication. The meticulous mechanism of its action and major phytochemicals responsible can be discovered after thorough biochemical and phytochemical research which is in progress.

INTRODUCTION

Learning the various things and memorizing the information and developing the skills while remembering the specials deeds is called as memory function. Lower recall rates, confusion, panic poor memory and impaired learning capabilities are the common problems of today's fast food eating children's. Nothing less to elderly people stress and emotional burst are the conditions key reasons for impaired memory functions, memory loss, amnesia and dementia rather further developed circumstances like Alzheimer's disease and Schizophrenia is major problems [1]. In both the forms of diabetes mellitus (T1DM &T2DM) there is association with a bridged recital on manifold domains of cognitive function and with sign of abnormal structural and functional brain magnetic resonance imaging (MRI). Cognitive deficits may occur at the very initial stages of diabetes and are further aggravated by the metabolic syndrome [2]. As memory includes many interlaced brain roles, it results in different kinds of memories and almost any type of brain damage can result in one or other type of memory loss. Memory is often unstated as an informational dispensation system with clear and implied operational function that is made up of a sensory

processor, long-term memory and working memory (or short-term). This can be linked to various active neurons. The sensory processor permits information from the outside world to be distinguished in the form of physico-chemical stimuli and appeared to several stages of emphasis and intent. Working memory aids as an encoding and repossession mainframe. Information in the form of stimuli is encoded in accordance with explicit or implicit functions by the short-term memory processor. The working memory also retrieves information from previously stored material. Finally, the function of long-term memory is to store data through various categorical models or systems. Short-term memory is one of the types of memories which denote to a brain system that provides storage and operation of the information essential for multifaceted intellectual tasks like linguistic, knowledge, education and perceptive nature.[3]

Nootropic agents such as piracetam, aniracetam, and choline esterase inhibitors like donepezil are being used for improving memory, mood, and behavior, but the resulting side effects associated with these agents have made their applicability limited. As allopathic medicines are used as sole molecules and they directly act on molecular levels they show very high degree of side effects. Here our ancient knowledge is at its best which provide sound therapy for brain related elements. Bacopamonnieri, well known asbrahmi, water hyssop, thyme-leaved gratiola, and herb of grace, is a essential plant in traditional Ayurvedic medicine. It has many activities which are scientifically proven like Antianxiety, anti-inflammatory, boost brain function, also helps to reduce ADHD (Attention deficit hyperactivity disorder) is a neuro developmental disorder that is characterized by symptoms like hyperactivity, impulsivity, and inattentiveness. Bramhi is also useful in prevention of anxiety and stress [4]. An aphrodisiac herb named Akarkara (Anacyclus pyrethrum) is mentioned in ayurveda that is said to improve male vitality and virility in addition to being a brain tonic. Evidence is initial, but it seems to be a profertility agent and testosterone boosting herb with some neuroprotective effects. Akarkara is a pro-fertility and virility enhancing herb. The corebioactives in this plant are the alkylamides (similar to Spilanthes Acmella and Maca), and preliminary evidence seems to confirm its traditional claims of fertility and libido enhancement as well as its role as a 'brain tonic' (since it appears to have anti-amnesiac and anti-convulsive effects) [5].

Atibala or Indian mallow (Abutilon Indicum) which means “Very Powerful”. Plant hold simmunomodulatory, anti-inflammatory, antihyperlipidemic, anti-malarial, wound healing, diuretic, hepatoprotective, hypoglycemic, analgesic, antimicrobial, and anti-diarrheal properties. Tulsi Panchang Extract is an AyurvedicExclusive Medicine, with the blimey of Tulsi, which is known to warding off some of the most common illnesses. Struts immunity, fights bacterial & viral infections to combating and treating various hair and skin disorders. Valuable in heart disorder, sore throat, fever & common cold, cough, respiratory disorder, mouth infections, skin disorders, stress, constipation, immunodeficiency diseases, children's ailment [6]. It is well-known that taking Jatamansi (Nardostachysjatamansi) powder along with honey once or twice a day helps to improve memory functions. According to Ayurveda, roots of Nardostachysjatamansi have been clinically used for its activities like anti-ischemic, antioxidant, anticonvulsant, and neuroprotective. It acts as a brain tonic and helps to improve recollection of memory and brain functions also prevents cell injury due to its antioxidant properties. It also tranquilities brain cells and manages anxiety as well as insomnia. According to ancient Ayurveda, Jatamansi benefits in checking crinkles due to its oily nature (Snigdhaguna).[7] It also endorses healing of wounds due to its Ropan (healing) property. Vacha is a natural rejuvenator. It stimulates the brain cells, sharpens memory and recovers learning abilities in children. It has the potential of improving memory, cognition, intelligence, voice, and mental abilities. *Withania somnifera* (Family: Solanaceae), universally known as Ashwagandha consist of dynamic phytoconstituents chiefly with anolides, sitoindosides and alkaloids. Ashwagandha had been conventionally used for the treatment of multiple brain disorders from past ancient times in India [8]

Suvarnamakshika Bhasma and Moti Bhasmaare the safe and act as nervine tonic that is enriched with brain sharpening ingredients. Swarnamakshika is a compound of Copper, Iron and Sulphur having wide range of therapeutic efficacy. These bhasma protects the cells and increases the chemical related to learning and memory. Both reduce cortisol to minimize stress and anxiety levels by reducing blood pressure levels [9]. For diabetes there

are many drugs in the market which are capable of reducing blood glucose levels but they are unable to prevent diabetic complications. Here we are evaluating cognitive effect of Polyherbal formulation in diabetic cognition enhancement.

MATERIAL &METHODS

Apparatus

Redial 8 Arm- Maze (VJRM-01R) for Rat manufactured by V J Instruments was used.
Lane Width - 10cm
Arm Length Wall - 50cm
Height Rat -20cm
Thickness 5 mm
Specifications
1. Color black
2. Made of Aluminum material.

Drug

Test formulation was kindly received from Seveillar Clinical Supplies Services Pvt. Ltd.
Standard drugs were purchased from local shops.

Experimental Animals

Wistar rats weighing 160-200 gm were selected. Animals of either sex were housed under standard laboratory conditions of temperature 22±30C and relative humidity of 44-56% with free access to standard pellet diet and water ad libitum. Has received ethical approval by the Institutional Animal Ethical Committee & number is CPCSEA/IAEC/2020/021.

Acute toxicity

Acute oral toxicity studies were performed for Test formulation according to the OECD (Organization for Economic Co-operation and Development) guidelines. Male Rat (n = 6/each dose) were selected for acute toxicity study. The animals were fasted overnight with free access to water. Extract (suspended in 0.6% carboxy methyl cellulose) was administered orally at a dose of 5 mg/kg. The general behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for 3 days. If mortality observed in 4/6 or 6/6 animals, the dose administered was considered as toxic dose. However, if the mortality was observed in only one rat, then the dose was repeated with higher doses such as 100, 200, 500, 1000 and 2000 mg/kg. All doses were found to be safe at 2000 mg/kg, p.o. [10]

Streptozotocin induced diabetic cognitive dysfunction

100 overnight fasted rats were randomly divided into 10 groups (n=10). Group I served as normal control group which received 0.2 ml of 0.01 M citrate buffer (vehicle) with pH 4.5 orally. In groups II to IV diabetes was induced by a single intraperitoneal dose of freshly prepared Streptozocine (STZ) at the dose of 65 mg/kg dissolved in 0.01 M citrate buffer (pH 4.5). 48 hours after the administration of STZ, blood samples were collected from retro-orbital plexus of the overnight fasted rats for estimation of the blood glucose levels. The collected blood samples were centrifuged at 3000 RPM for 10 min, serum was separated and glucose levels were estimated immediately by using standard marketed kits. Rats which were found to have permanent diabetes mellitus [Fasting Blood Glucose (FBG) > 250 mg/dl] were considered as diabetic and used for the study. Rats who met the criteria being diabetic were divided into 03 groups (Groups II to IV) with 08 animals in each group while group I served as normal control group. Rats received the respective treatment as per mentioned in the Table for Eight weeks. [11,12]

Biochemical estimations

After successful On 7th, & 42ndday, one hour after the respective treatment, blood samples were collected from retro orbital plexus and blood glucose levels were estimated by using standard marketed kits (GOD- POD method). [13]

Radial arm maze task

After 35 days of treatment all the rats were trained for radial maze task performance by conducting daily training trial which consisted of two sessions wherein one food pellet was placed in a fixed arm and then in the variable arm to record the effect of test drugs on spatial reference and spatial working memory respectively. Rats maintained at 85% of their total diet were placed individually in the central hub and were allowed to choose the arm freely to get the food with upper cut off limit of 300 sec. The time taken by each rat to find the food along with number of reentries was considered to assess radial maze task performance. Rat was considered to be learned when he found the food with maximum one reentry for three consecutive days. The number of days required for making the rat learned and the latency to find the food along with number of initial correct entries (i.e. before first reentry) of learned rat was recorded as the effect of the drug on learning and memory process. One-hour interval was kept between the spatial reference and spatial working memory evaluation. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli [14].

Statistical analysis

Data obtained were subjected to one way ANOVA followed by the Dunnetstest to determine the level of significance at P<0.05 probability level. The results were expressed as Mean±SEM. The data was statistically analyzed using Prism version 6.01. Mean values were considered statistically significant when *p<0.05, **p<0.01 and ***p < 0.001. And when compared with Normal Control data represented as # as p<0.05, ## as p<0.01 and as ### p < 0.001

RESULT AND DISCUSSION

Diabetic patients are more prone to neuronal and Central Nervous System complications which are depicted by growing number of Alzheimer's disease in diabetics. To perform any activity it is necessity to perform oral toxicity study for safety of drugs. All doses were found to be safe at 2000 mg/kg, p.o. of Test formulation (T1). This study shows the safety of formulation. Data also suggests that formulation can be further studied with collateral models. As per Fig 01 Streptozotocine more prominently produced diabetic like condition in all the groups. On day 7 all the rats showed highly significant rise in hyperglycemia (## p<0.01). The results shows that metformin individually and in combination with Test Formulation reduced the blood glucose levels in highly significant manner (**p<0.01).

Spatial Memory – Short Term Memory

Spatial memory is short term memory which enables person to remember, manage and store evidences that is essential to wide-ranging complex cognitive errands. Spatial memory is a cognitive route that permits an individual to recollect diverse positions as well as spatial relations among the things. Here in this study comparison can be seen in diabetic control (DC), Metformin Treated (M) and Metformin + Test Formulation (T1) treated groups. As per Fig 02 there was statistically significant reduction in diabetic rats in the number of correct initial entries (## p<0.01).But Metformin Treated (M) and Metformin + Test Formulation (T1) treated groups were shown highly significant increased values (**p<0.01). As diabetic patients have short term memory loss our Diabetic control group (DC) showed the statistically increased latency to find the food (## p<0.01). Metformin Treated (M) and Metformin + Test Formulation (T1) treated rats showed highly significant reduction in finding of the food. (**p<0.01) which is well depicted in Fig. 03. As per the results shown in Fig 04 no of days required to train the rats were compared with normal control group and shown high rise in Diabetic control group. But treatment with Metformin (M) and Metformin + Test Formulation (T1) was effective in the reversal of effect of diabetes and showed highly significant reduction in No. of Days required to make rat learned.

Spatial reference memory is a system of long-term memory in lieu of the spatial, contextual, and factual facets of a task that leftovers constant among trials. Spatial reference memory delivers insight hooked on in what way the brain stores, retrieves, and encodes the information. Parahippocampal and Hippocampal cortices, widely accepted as the main brain areas sustaining this task, comprise several types of cell natures with distinct spatial firing patterns. [15]
In this context as per Fig 05 there was statistically significant reduction in No. of initial correct entries in diabetic rats (## $p<0.01$) but Metformin Treated (M) and Metformin + Test Formulation (T1) treated groups were shown highly significant increased values (**$p<0.01$). As diabetic patients haslong term memory loss too our Diabetic control group (DC) showed the statistically increased latency to find the food (## $p<0.01$). Metformin Treated (M) and Metformin + Test Formulation (T1) treated rats showed highly significant reduction in finding of the food (**$p<0.01$) which is well depicted in Fig. 06. As per the results shown in Fig 07 no of days required to train the rats were compared with normal control group and shown high rise in Diabetic control group. But treatment with Metformin (M) and Metformin + Test Formulation (T1) was effective in the reversal of effect of diabetes and showed highly significant reduction in No. of Days required to make rat learned.

As per the above results in this study, Metformin + Test Formulation (T1) significantly managing the Spatial reference as well as spatial working memory which supports the conventional claim of formulation being a tonic in dullness of intellect especially in spatial memory impairments. The variance in the significance of discrete parameters of sessions do not unavoidably narrate specific aspect of spatial reference or spatial working memory because of the dearth of appropriate animal model with single parameter to be chronicled which can exactly illustrate the human learning and memory processes. Hence significant improvement in most of the parameters is usually considered as effect of the drug. The existing results when assessed on this basis bare the simplification of learning and memory. In count the preliminary phytochemical investigation of Test Formulation (T1) showed the presence of flavonoids, alkaloids, steroids, carbohydrates, glycosides and saponins [17].

CONCLUSION

The oxidative stress, formation of free radicals and lack of oxygen are common causes for neurodegeneration and related cognitive impairments particularly in spatial learning and memory deficits. Thus, the current results documented significant upgrading in radial arm maze performance and thereby facilitation of spatial learning and memory. These outcomes sign posts the probable use of the Test Formulation (T1) as a part of therapy to improve meager learners and patients with impaired spatial memory functions. Furthermore, it can be employed as a barrier in contradiction of speedy age related deterioration in mental tasks observed with various neurological diseases. The meticulous mechanism of its action and major phytochemicals responsible can be discovered after thorough biochemical and phytochemical research which is in progress.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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Table 1 Treatment schedule for streptozotocin induced diabetic neuropathy and nephropathy

Group No	Treatment	Dose
I	Normal control (NC)	1% CMC (1 ml/kg)p.o.
II	Diabetic control (DC)	1% CMC (1 ml/kg)p.o.
III	Metformin (M)	100 mg/kgp.o.
IV	Metformin + Test Formulation (T1)	100 mg/kg + 200 mg/kg p.o.

Apparatus

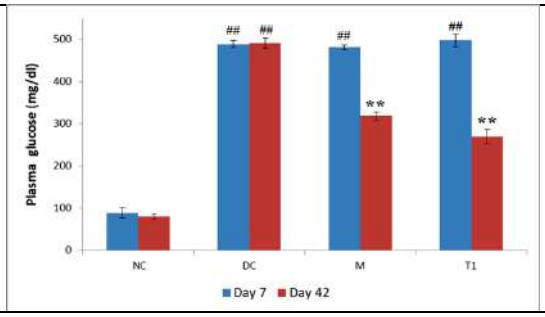

Fig 01: Effect of Drugs on Fasting Blood Glucose Levels

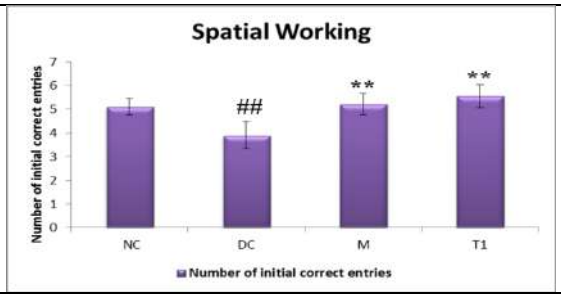

Fig 02: Spatial Working Memory- No. of initial correct entries.

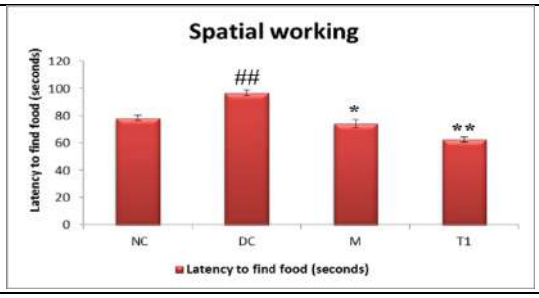

Fig 03: Spatial Working Memory- Latency to find Food.

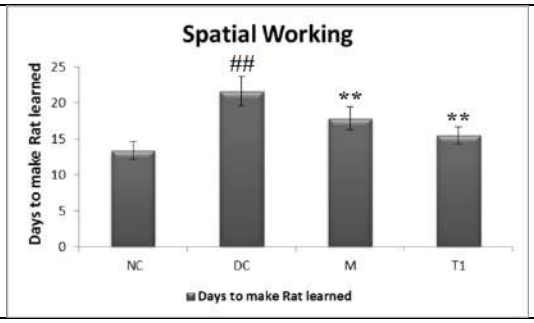

Fig 04: Spatial Working Memory- No. of Days required to make rat learned.

Spatial Reference Memory - Long Term Memory

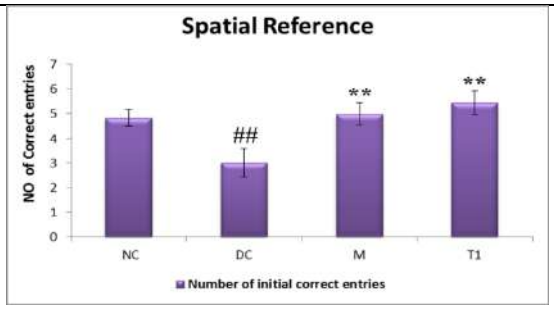

Fig 05: Spatial Reference Memory - No. of initial correct entries.

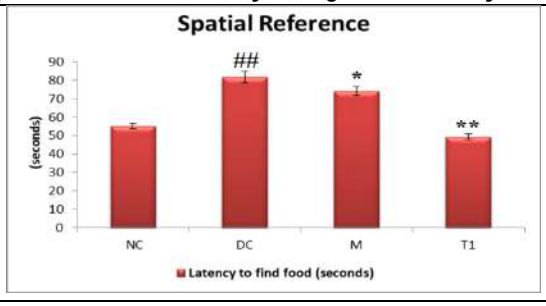

Fig 06: Spatial Reference Memory - Latency to find Food.

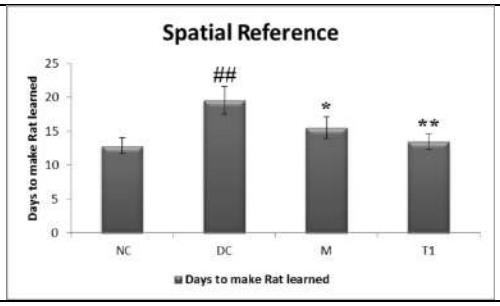

Fig 07: Spatial Reference Memory - No. of Days required to make rat learned.

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RESEARCH ARTICLE

Recent Updates on Antitubercular Discovery

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ABSTRACT

Tuberculosis (TB) is an infectious sickness caused by bacterium of the Mycobacterium genus, specifically through Mycobacterium tuberculosis (MTB). Tuberculosis (TB) is a major health trouble, with about one-third of the arena's population infected with Mycobacterium tuberculosis, 8 million human beings in the energetic disease country, and two million dying annually. Furthermore, the superiority of TB/HIV co-infection, and the emergence of multidrug-resistant tuberculosis (MDR-TB) and substantially drug-resistant tuberculosis (XDR-TB) have in addition aggravated the unfold of this disorder and as a consequence mortality by it. There is an urgent need for novel antitubercular marketers with advanced homes, consisting of decrease toxicity, shortened length of therapy, speedy bactericidal movement, and stronger hobby against MDR traces. The World Health Organization targets to notably lessen the number of instances in the coming years; however, the increased range of multidrug-resistant (MDR) and extremely drug-resistant (XDR) sorts of the bacterium and the lack of treatment for latent tuberculosis are demanding situations to be conquer. This evaluation pursuit to summarize the contemporary advances in and promote insights into anti-tubercular drug discovery.

Keywords: Tuberculosis, Mycobacterium tuberculosis, multidrug-resistant tuberculosis, extremely drug-resistant

INTRODUCTION

Tuberculosis (TB), an old ailment considered tied to the beyond, began again taking pictures the specialists' attention in the early Nineteen Nineties for specific motives, consisting of the HIV/AIDS pandemic and the arrival of Mycobacterium tuberculosis drug-resistant lines. In 2011, about 9 million human beings had been laid low with TB and 1.4 million died, more often than not in Asia (60% of instances) and Africa (24%). Multidrug-resistant TB (MDR-TB) is also a annoying public fitness trouble in Eastern Europe, in which the said occurrence of MDR-TB levels from 18 to 35% in new TB instances [1]. Generally, TB remedy is based on the aggregate of four drugs, rifampicin, isoniazid, ethambutol and pyrazinamide for two months, accompanied via rifampicin and isoniazid for four months [2]. Unfortunately, terrible patient compliance, as well as insufficient health care specialised structures, favored the choice of MDR-TB that calls for as a minimum 20 months of treatment with 2d line capsules like fluoroquinolones, amikacin, kanamycin and capreomycin, which are more poisonous and less efficient, with remedy quotes in the variety of 60–75% [3]. In 2014, the World Health Organization (WHO) estimated nine million new tuberculosis (TB) cases had passed off globally in 2013, 480000 of them being laid low with multidrug-resistant (MDR) Mycobacterium tuberculosis lines. MDR-TB is defined as resistance in vitro to as a minimum isoniazid and rifampicin, while drastically drug-resistant (XDR)-TB is immune to at the least one fluoroquinolone and one injectable 2d-line anti-TB drug similarly to isoniazid and rifampicin [4].

As MDR/XDR-TB scientific results are in large part suboptimal and their remedy very lengthy, poisonous and pricey, these difficult-to-treat instances are considered a serious hazard to TB manage and removal [5]. Fortunately, quite a number of recent capacity antitubercular candidate drugs with heterocyclic jewelry, which are maximum probably to be powerful in opposition to resistant traces, have entered scientific trials in current years [6]. This evaluation highlights current advances in the research of novel heterocyclic compounds, with specific attention on their antimycobacterial pastime, mechanisms of motion, toxicity, and structure-interest relationships. Over 480000 cases of multidrug-resistant (MDR) tuberculosis (TB) occur every year globally, 9% of them being stricken by substantially drug-resistant (XDR) lines of Mycobacterium tuberculosis. The remedy of MDR/XDR-TB is regrettably lengthy, poisonous and luxurious, and the achievement rate largely unsatisfactory (<20% amongst instances with resistance patterns past XDR) [7, 8].

Development of Antituberculous Drugs: Current Status and Future Prospects

Worldwide, tuberculosis (TB) remains the most frequent and essential infectious sickness causing morbidity and demise. One-0.33 of the sector's populace is inflamed with Mycobacterium tuberculosis (MTB), the etiologic agent of TB [9]. The World Health Organization estimates that approximately eight to 10 million new TB cases arise yearly worldwide and the incidence of TB is presently increasing. In this context, TB is inside the top three, with malaria and HIV being the main causes of death from a single infectious agent, and approximately million deaths are attributable to TB yearly [10]. In precise, pulmonary TB, the most commonplace form of TB, is a exceptionally contagious and existence-threatening infection. Moreover, stronger susceptibility to TB in HIV-infected populations is any other extreme fitness hassle at some point of the sector [11, 12]. In addition, multidrug-resistant TB (MDR-TB) has been increasing in occurrence in many areas, now not most effective in growing countries but industrialized countries as properly, all through the past decade. These conditions, especially the global resurgence of TB and the fast emergence of MDR-TB, underscore the significance of the improvement of recent antituberculous pills and new protocols for efficacious medical control of TB patients the usage of everyday antimycobacterial tablets [13]. Concerning the improvement of recent antituberculous tablets, the subsequent points are of particular significance. Development of medicine which display lasting antimycobacterial interest in vivo is proper, on account that they can be administered with long periods and consequently facilitate immediately located remedy and enhance patient compliance [14]. Development of novel antituberculosis compounds to fight MDR-TB is urgently wished. The eradication of slowly metabolizing and, if feasible, dormant populations of MTB organisms that purpose relapse, using new training of anti-TB pills may be very promising for prevention of TB occurrence, as it will markedly

reduce the prevalence of active TB from humans who are latently inflamed with MTB [15]. Unfortunately, no new tablets besides rifabutin and rifapentine have been marketed for TB within the US and other international locations during the forty years after launch of rifampicin. There are some of constraints that have deterred businesses from making an investment in new anti-TB drugs. The studies are expensive, slow and difficult, and calls for specialized centers for managing MTB [16]. There are few animal fashions that carefully mimic the human TB ailment. Development time of any anti-TB drug could be lengthy. In truth, medical trials will require the minimal six-month therapy, with a follow-up duration of three hundred and sixty five days or extra. In addition, it's miles hard to demonstrate apparent benefit of a new anti-TB retailers over pre-present drugs, in view that medical trials contain multidrug aggregate remedy the usage of fairly effective everyday anti-TB drugs [17]. Finally, there may be the perceived loss of business go back to companies engaged within the improvement of recent anti-TB pills, because over ninety five% of TB instances worldwide are in developing countries. In this symposium, we reviewed the following areas. Critical new data at the complete genome of MTB these days received and increasing know-how of diverse mycobacterial virulence genes are significantly promoting the identification of genes that code for brand new drug objectives [18].

The status of recent styles of compounds which can be being evolved as anti-TB drug. He additionally discussed the development of latest antimycobacterial pills consistent with new and ability pharmacological goals and the first-class scientific development plans for brand new-TB capsules when it comes to company method [19]. Using such findings for mycobacterial genomes, bioinformatics/genomics/proteomics-based totally drug design and drug improvement the use of quantitative structure-activity relationships can be possible within the close to destiny. The usefulness of chemical genomics in searching novel drug targets for development of latest antituberculous drugs [20]. The authors reviewed the history and present reputation of chemical genomics that is defined because the systemic look for a selective small molecular modulator for each feature of all gene products, recent studies of the authors on profiles of the interactions between various forms of human proteins and small molecule modulators the usage of the brand new technology devised with the aid of Reverse Proteomics Research Institute, and destiny potentialities of the development of new antituberculous drugs based totally on chemical genomics. 3. It seems also promising to broaden new forms of drug management structures the use of drug cars, which enable efficacious drug shipping to their target in vivo [21].

The usefulness of liposome- and polymer-primarily based technologies, which enable efficacious shipping of encapsulated tablets at required doses for prolonged intervals of time with simplest a unmarried shot without toxicity [22, 23], and also allow fantastically targeted transport of medication to their target in vivo. They indicated that the applications of drug shipping device the use of conventional anti-mycobacterial retailers are challenging to enhance the compliance of remedy and better medical final results. Immunoadjunctive therapy seems to be promising in enhancing outcome of clinical control of refractory mycobacterial infections, inclusive of MDR-TB and M. Avium complex infection [24-26].

The prevailing repute of immunotherapy of mycobacterial infections in mixture with antimycobacterial tablets. They indicated that the improvement of latest lessons of immunomodulators aside from cytokines (IL-2, IFN-gamma, GM-CSF, IL-12, and so on.) mainly those with no extreme aspect-consequences, are urgently needed [27]. Their review dealed with some promising immunoadjunctive retailers, specially ATP and its analogues, which potentiate macrophage antimycobacterial pastime via purinergic P2 receptors. The intention of this symposium is to deal with the future possibilities of the improvement of latest pills and drug regimens for anti-TB chemotherapy [28]. There are some of difficulties in drug-layout for the development of latest drug formulations with increased capacity for antimycobacterial consequences, wonderful pharmacokinetics, and tolerability [29]. It must be emphasised that the most pressing intention of chemotherapy of TB and MAC infections, in particular that related to HIV infection, is to increase tremendously active, low-cost pills which can be used now not simplest in industrialized nations but additionally in growing countries, since the incidences of AIDS-associated intractable TB and MAC infections are rapidly growing in the latter [30]. We strongly wish a incredible boost of fundametal and sensible research in

developing such styles of new anti-TB tablets inside the close to destiny. Prospects for non-medical or scientific improvement of recent antituberculous pills in relation to company strategy [31]

Tuberculosis (TB) remains one of the deadliest threats to public health. No new anti-TB capsules were introduced into the medical institution inside the past 40 years. Current non-clinical works with advanced era and Global Alliance for TB Drug Development, a non-earnings business enterprise installed in 2000, accelerate research and improvement of faster-acting anti-TB compounds [32]. We reviewed the status of recent varieties of compounds which are being developed as anti-TB drug, such as diarylquinoline (TMC 207), nitroimidazole (PA-824 and OPC-67683), and moxifloxacin (MFLX). We also mentioned the nice medical development plans for new-TB pills in relation to corporate approach. 2. Exploring novel drug goals through the chemical genomics technique and its viable utility to the improvement of anti-tuberculosis pills [33]. In advance to a totally massive scale national challenge in US began ultimate yr, Reverse Proteomics Research Institute Co., Ltd. (REPRORI) has developed the middle technology for chemical genomics. Here we describe the define of chemical genomics observe, in particular that of REPRORI, and talk approximately its feasible software to the development of anti-tuberculosis drugs [34].

Drugs for Tuberculosis

Effective treatment of tuberculosis (TB) is reliant on numerous bactericidal and sterilising drugs administered in combination for an adequate duration, to assure antimicrobial efficacy at the same time as preventing choice of drug-resistant mutants and obtain permanent therapy.1 Current remedy regimens are, however, unsatisfactory because of low efficacy, excessive toxicity, long duration and sizable health aid burden with treatment for multidrug-resistant TB (MDR-TB described as resistance to isoniazid and rifampicin); drug–drug interactions also are splendid as exemplified by rifampicin with protease inhibitors and different antiretrovirals (ARVs) [35]. Some combos include capsules that have been registered for symptoms apart from TB and are consequently repurposed and used 'off-label,' consisting of oxazolidinones, carbapenems, or clofazimine, for the treatment of noticeably-resistant TB instances. In May 2016, WHO issued guidance that human beings with TB immune to rifampicin, without or with resistance to different capsules, have to be handled with an MDR-TB remedy regimen. Despite latest advances, TB consisting of drug-resistant forms, continues to give some of challenges to physicians and country wide TB programmes. With 10.Four million infections in 2015 TB became one of the top 10 reasons of dying worldwide killing 1.Eight million people, rating above HIV/AIDS as the main cause of demise from an infectious disorder and one of the predominant international health issues. This is regardless of the reality that with a well timed analysis and correct treatment, most people of humans growing TB disorder can be cured. Global development depends on significant advances in TB prevention and care. Worldwide, the price of decline in TB occurrence remained at simplest 1.Five% from 2014 to 2015. This needs to boost up to a four–5% annual decline by way of 2020 to reach the primary milestones of the End TB Strategy. New TB capsules and regimens are urgently needed to improve treatment prices for people with drug-resistant TB (currently round 50% globally) and to shorten the remedy of each drug- inclined and drug-resistant TB (currently at the least six and as a minimum nine months respectively). For the first time in almost four many years, new TB pills, bedaquiline, and delamanid, have emerge as to be had. These are encouraged via the World Health Organisation (WHO) for the remedy of drug-resistant TB under certain conditions. These tablets have, however, been examined for efficacy as add-ons to the conventional (or longer) WHO-endorsed remedy routine for MDR-TB, their use in mixture and with repurposed capsules are nevertheless under take a look at, it is hoped that those new regimens will lead to improved treatment efficacy treatment length at the equal time enhancing safety [36].

Antitubercular *in vitro* Drug Discovery: Tools for Begin the Search

Antitubercular drug resistance rather than the invention and improvement of new antitubercular dealers The drug resistant TB (DR-TB) emergence and spread is a multifactorial hassle produced by using the usage of fitness mismanagement hobby; inadequate remedy courses, antibiotic misuse, inadequate socioeconomic conditions, presence of immunodeficiency issues and coffee affected person compliance [37]. In addition, coinfection TB-VIH complicates the present day treatment recurring due to the truth: lower compliance and boom drug interactions

producing poisonous side results [38]. The need for more effective and lots less toxic anti TB pills is in fact pressing, but the antibiotic drug discovery and development is a long and expensive technique with only a few compounds making it to the marketplace [39]. The modern anti-TB drugs have been advanced when you consider that Fifties until Nineteen Eighties which represented a omitted period in TB drug studies that contributed drastically to new annoying situations for enhancing treatments for DR-TB and save you LTBI [40]. Actually, the most important project for discover and expand a modern day technology of TB drugs is prevention of drug resistance, this is vital for cope with the patients underneath useless restoration regimens [39]. Because of this, all efforts among sponsors, TB drug researchers, regulators and funders must be directed to the improvement of recent and optimized portfolio of multidrug treatments. Antitubercular *in vitro* drug discovery program design In vitro experiments searching for to evaluate the interaction between the drug and the bacteria, which validates the choice of candidate compounds and the willpower of the goal drug concentrations for in addition trying out [41]. Is a truth that drug candidates fail in the level of clinical development, within the Tuberculosis Antimicrobial Acquisition and Coordinating Facility Program (TAACF) have been evaluated 88601 compounds and ultimately had been determined on five ability leads that could be an immoderate cost drug discovery program? An in vitro antitubercular drug screening technique should undergo in thoughts and integrate severa factors as whole mobile screening; unmarried enzyme goals, toxicity attempting out and the inclusion of in vitro pharmacological checks for optimize the choice of promissory new drugs and predicts their scientific behaviour.

Anti-TB Drug Targets

Despite the relative efficacy of contemporary treatment, the diverse antibiotics that represent first- and 2nd line pills for TB therapy goal handiest a small quantity of core metabolic processes such as Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) synthesis, cell wall synthesis, and strength metabolism pathways [41]. New training of drugs with additional drug goals which might be hard to overcome with the aid of mutation are urgently wanted [42]. Desirable new goals ought to be involved in crucial elements of bacterial boom, metabolism and viability whose inactivation might cause bacterial dying or an incapability to persist, for this reason remedy will be shortened and drug resistant strains could be removed or substantially reduced. Moreover, objectives worried within the pathogenesis of the disorder process need to additionally be considered for drug improvement. The discovery of the whole genome collection of TB bacteria helped to pick out numerous essential drug goals [43]. Various businesses have used this genomic information to become aware of and validate targets as the idea for development of new Anti-TB sellers. Besides, mycobacterial genetic tools, which includes transposon mutagenesis, gene knockout, and gene transfer, greatly facilitate goal identity.

Cell Wall Biosynthesis Related Targets

Cell wall biosynthesis is a particularly precise supply of molecular goals due to the fact the biosynthetic enzymes do not have homologues within the mammalian machine[44]. The cell wall of M. Tuberculosis may be very important for its survival within constrained conditions along with the ones inner of human macrophages. The biosynthesis of the cellular wall additives involves many crucial degrees and distinctive enzymes that are absent in mammals and can be attractive drug targets. Recently, the 2C-methyl-D-erytrol four-fosphate (MEP) pathway turned into found as a potential drug target since the give up product of the pathway leads to the formation of isoprenoids, which can be responsible for the synthesis of numerous cellular wall additives [45]. Peptidoglycan biosynthesis is any other source of potential drug objectives. For instance, alanine racemase and D-Ala-D-Ala-ligase catalyze the primary and 2nd dedicated steps in bacterial

Recent Advances in Selected Therapeutic Targets and Rational Drug Design

Peptidoglycan biosynthesis, and due to the fact those steps are essential for essential polymers, they may be top drug objectives. Both alanine racemase and D-Ala-D-Ala ligase are inhibited by means of D-cycloserine, a second line anti-TB drug [46]. Another appropriate drug goal is the pyridoxal 5'-phosphate containing enzyme Alr that catalyzes the racemization of L-Alanine into D-Alanine, a first-rate issue inside the biosynthesis of peptidoglycan [47]. Arabinogalactan biosynthesis, a singular arabionofuranosyl transferase that catalyzes the addition of the

primary key arabinofuranosylredisude of the galactan core, isn't sensitive to EMB, however is critical for viability. The ribosyltransferase that catalyzes the first devoted step in the synthesis of decaprenyl-phosphoryl-D-arabinose, the lipid donor of mycobacterial d-arabinofuranosyl residues, has additionally these days been characterised and proven critical for increase .

Mycolic Acid Biosynthesis Related Targets

Within the mycobacteria lipid metabolism, mycolic acids are essential structural additives of the mycobacterial cell wall [34]. The early degree of fatty acid biosynthesis, which generates the precursors of mycolic acids, is a wealthy source of antibacterial goals[22]. It is also the website online of movement of INH and ethionamide[42]. M. Tuberculosis has both styles of fatty acids synthase (FAS) structures found in nature, FAS-I and FAS-II. FAS I is the gadget liable for de novo synthesis of C16-C26 fatty acids and the FAS II system extends those fatty acids up to C56 chains to make precursors of mycolic acids, which can be critical for boom. Since enoil-ACP reductase (InhA) is the target of INH, it's miles reasonable to count on that each one steps inside the FAS-II pathway can be critical for the viability of M. Tuberculosis. Many of the person enzymes of the FAS-II gadget had been expressed, purified and characterised [48]

New Medical Tenet for the Treatment and Prevention of Drug-Resistant Tuberculosis

Treatment for MDR- and notably drug-resistant (XDR)-TB is lengthy and difficult. The availability of strong new and repurposed drug treatments lets in practitioners, for the first time, to pick options to injectable capsules, that have lengthy been taken into consideration an essential element of treatment regimens for DR-TB. These injectable tablets have well-known toxicities, which includes irreversible hearing loss [48].By prioritizing the use of orally administered medicines, the rule of thumb writing committee believes clinicians can spare sufferers a number of the most debilitating consequences of TB treatment, make remedy greater tolerable and enhance outcomes

Anti-Arthritis Drug Also Stops Tuberculosis Bacillus from Multiplying In Blood Stem Cells

Tuberculosis (TB) can also have an effect on any part of the frame, however the unfold of the sickness might begin in the bone marrow. Immunologists from KU Leuven and Brazil have shown that the TB bacillus hijacks the blood stem cells from the bone marrow to turn them into ideal host cells for multiplication. They also located that this mechanism may be stopped by using administering an anti-arthritis drug [49].

Hiding in the Bone Marrow

About a quarter of the arena populace is a provider of Koch's bacillus, that can motive tuberculosis (TB). Most folks that are inflamed have latent tuberculosis, that means that they do not come to be ill. However, this latent TB can turn into active tuberculosis when the immune device becomes weaker, as an instance inside the aged or in HIV sufferers. Worldwide, TB claims extra than 1.Five million lives each year. WHO categorisation of 2d-line antituberculosis pills encouraged for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis [50].

MDR-TB are strains resistant to the maximum strong first-line drugs, rifampicin and isoniazid. In 2012, 450000 people evolved MDR-TB in the world. It is predicted that approximately 9.6% of those cases have been considerably-drug-resistant (XDR-TB), displaying extra resistance to at the least one fluoroquinolone and one injectable drug (amikacin, kanamycin or capreomycin). For sufferers stricken by XDR-TB, the therapeutic efficacy is pretty constrained [36]. Recently, a few reviews have claimed about the emergence of ‘totally drug-resistant TB’ without a danger of therapy.7 However, there is a want to come to an settlement at the definition of these traces commonly in terms of severity.Therefore, new antitubercular capsules, as well as novel TB goals, are urgently required to overcome the problem of drug resistance and to ultimately eradicate TB [28]. Many molecules below scientific evaluation, which includes fluoroquinolones, were advanced to deal with other infectious sicknesses and have now been repurposed for TB treatment, at the same time as other drugs had been particularly found for TB remedy with hobby in opposition to MDR and XDR-TB strains [51]. In this mini-overview, we are able to consciousness simplest on some new capsules which might be in different levels of improvement as well as on a few ‘hot’ TB targets. Several comprehensive and up to date critiques on this topic are to be had. Bedaquiline, a diarylquinoline, turned into

accepted via the FDA (Food and Drug Administration) in December 2012 as part of the mixture remedy for the treatment of grownup patients tormented by MDR-TB and it is now in segment II of medical improvement (Figure 2). It may be considered the primary drug permitted by using FDA for TB within the remaining 40 years.33 The diarylquinoline came out from a phenotypic screening of chemical molecules against mycobacterial increase, while the corresponding target changed into identified thru complete-genome sequencing of M. Tuberculosis and M. Smegmatis spontaneous mutants proof against the compound. These mutants showed missense mutations in the atpE gene, encoding the c subunit of ATP synthase, as a consequence interfering with the energy manufacturing [52].

The mechanism of motion of bedaquiline is atypical and constitutes an alternative approach to modern-day antimycobacterial killing, by means of inhibiting ATP synthase; it is powerful in opposition to each replicating and dormant M. Tuberculosis traces. It is well-known that pulmonary lesions can incorporate both forms of populations, that are tough to eliminate with conventional antitubercular pills, as a consequence favoring the improvement of resistance [17]. It has been posted that the human mitochondrial ATP synthase is 20 000fold less sensitive to diarylquinoline than the mycobacterial one, consequently validating it as an crucial drug target, despite the truth that ATP synthase is tremendously conserved among Prokarya and Eukarya. Although bedaquiline has been related to an improved risk of inexplicable mortality and QT prolongation, it represents a first rate addition for the remedy of MDR- and XDR-TB strains, specifically in areas of the sector wherein TB is endemic [6].

Two new nitroimidazoles, PA-824 and OPC67683 (renamed delamanid), are in Phase II and Phase III scientific development, respectively. They are both pro-capsules whose activation depends on a F420-deazaflavin-based nitroreductase (Ddn) present in M. Tuberculosis. The energetic shape of PA-824 is the corresponding des-nitroimidazole molecule, which generates reactive nitrogen species like nitric oxide. The respiration poisoning via nitric oxide launch seems to be critical for its anaerobic interest [3]. PA-824 is lively against both replicating and dormant mycobacteria and preclinical and scientific investigations, suggesting that it is able to make contributions in the shortening of TB length remedy. The mechanism of motion of delamanid is to inhibit mycolic acid biosynthesis and has been connected to an growth in sputum-subculture conversion amongst patients suffering from MDR-TB. Moreover, it showed efficacy with suited toxicity as part of a MDR-TB regimen [5].

Rifapentine is a semisynthetic cyclopentylrifamycin derivative; it binds the β-subunit of RNA polymerase, just like the rifampicin. Rifapentine is extra powerful towards M. Tuberculosis, each in vitro and in vivo and the MIC degrees from 0.02 to zero.06 μg ml−1. As expected, there may be go-resistance between rifamycin and rifapentine [53]. In 1998, the USA FDA accepted rifapentine (10 mg kg−1) for oral management a couple of times weekly for each lively and latent TB remedy. Regarding the latent TB, the use of rifapentine plus isoniazid for three months (as soon as-weekly routine) is supported with the aid of top medical evidence. Unfortunately, the outcomes are quite unique for the treatment of lively TB, in which the rifapentine is authorized through the FDA at six hundred mg dose orally, twice weekly during the extensive section of TB remedy (2 months), then once weekly during the continuation section [54].

CONCLUSION

Tuberculosis remains the main infectious sickness global, no matter the provision of TB chemotherapy and the BCG vaccine. This is further demonstrated by the truth that half a year of remedy with multiple pills is wanted. Recent genetic and genomic tools in addition to excessive-throughput screening, and structure-based drug design techniques have allowed the invention of latest anti-TB capsules. These are more and more receiving greater attention, and a huge quantity of recent compounds or derivatives from current pills are below investigation. With this and a higher knowledge of the specific biology of TB, more targets might be confirmed, and with a bit of luck a pattern will emerge as a way to assist us attain the goals of more potent compounds that allow a couple of degrees and drug objectives to be addressed

MDR-TB is a significant mission for the manipulate of TB in lots of components of the arena and a threat to TB elimination. The beginning of this problem has been the sub-top-quality control, man or woman or programmatic, of sufferers with susceptible TB. Inadequate management of cases can be the origin of 50% of the brand new RR/MDR-TB instances. The different 50% is due to lively transmission of RR/MDR-TB strains in the community or healthcare settings. Therefore, to govern this epidemic, we will want to enhance the control of the prone TB cases in addition to find and remedy maximum of the RR/MDR- TB cases, to which exceptional treatment options should be confident. Fortunately, after nearly 4 a long time with practically the equal diagnostic equipment and armamentarium, there have been substantial advances in this field, with a focal point on the brand new global drug-resistant TB epidemic. Drug resistant TB treatment has advanced extensively over the last years, the brand new shorter MDR-TB regimens and increasing availability of recent or repurposed capsules like bedaquiline, delamanid, clofazimine and linezolid envisaged that extra sufferers can be able to be treated and more will continue to exist. If we are cautious, we will no longer repeat preceding mistakes with the new pills and the dream of developing a prevalent new regimen with these new capsules for all TB sufferers, susceptible and immune to all of the antique tablets may additionally grow to be a reality

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Table 1 : Classification of TB Drugs

Group A: fluoroquinolones
• Levofloxacin
• Moxifloxacin
• Gatifloxacin
Group B: second-line injectable agents
• Amikacin
• Capreomycin
• Kanamycin
• (Streptomycin)

Group C: other core second-line agents
• Ethionamide/prothionamide
• Cycloserine/terizidone
• Linezolid
• Clofazimine
Group D: add-on agents (not part of the core multidrug-resistant tuberculosis regimen)
D1
• Pyrazinamide
• Ethambutol
• High-dose isoniazid
D2
• Bedaquiline
• Delamanid
D3
• Para-aminosalicylic acid
• Imipenem plus cilastatin (requires clavulanate)
• Meropenem (requires clavulanate)
• Amoxicillin plus clavulanate
• (Thioacetazone)[a]

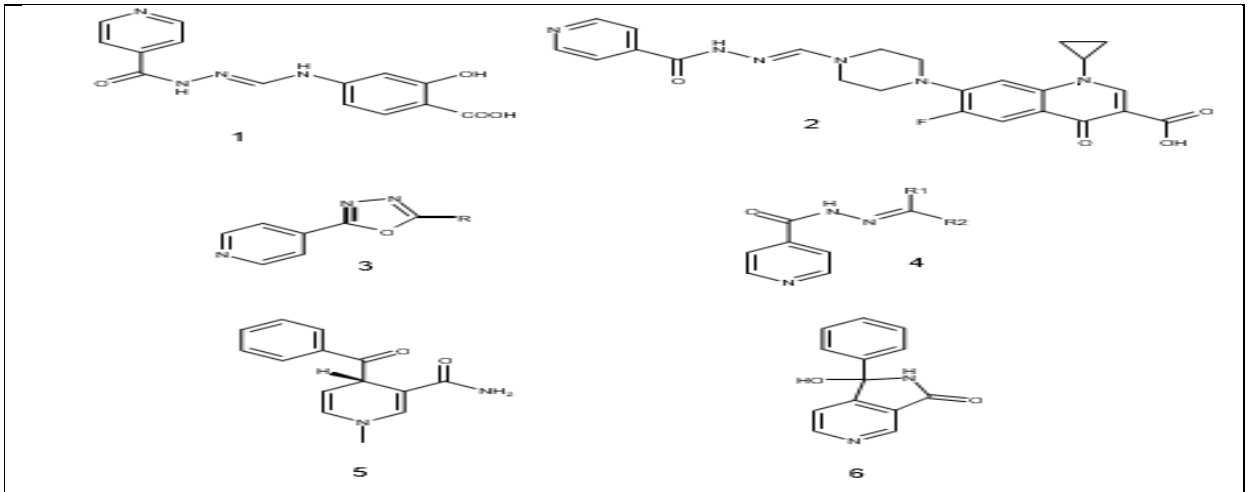

Fig.1 Isoniazidderivatives

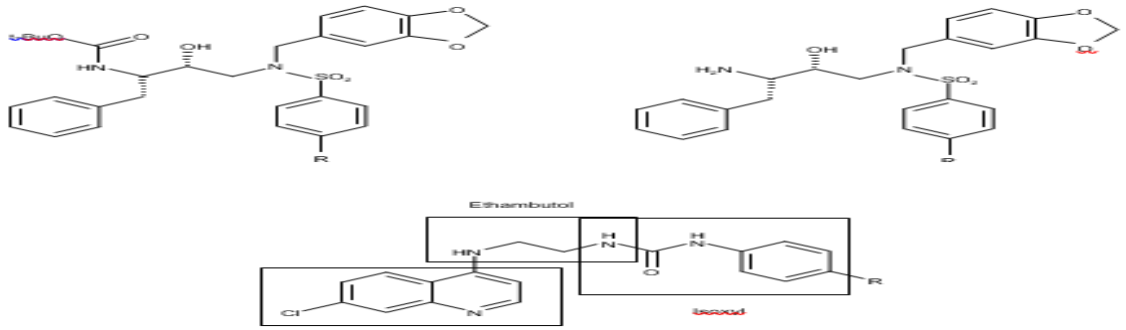

Fig.2 : Ethambutol derivatives

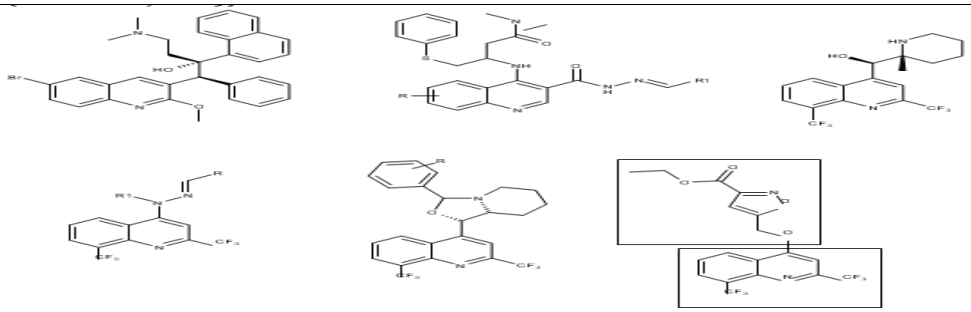

Fig.3 : Quinoline derivatives

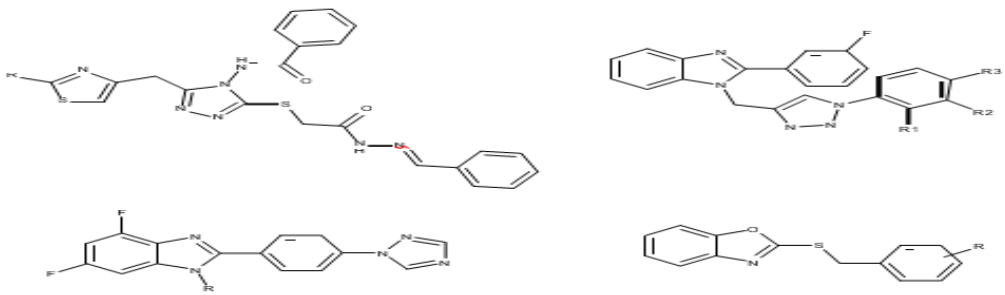

Fig.4 : Azolesderivatives

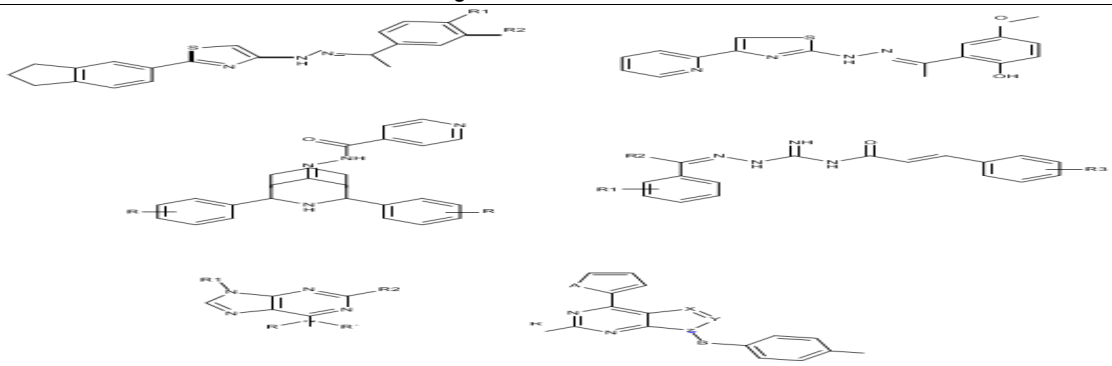

Fig.5 : Nitrogen heterocyclic derivatives

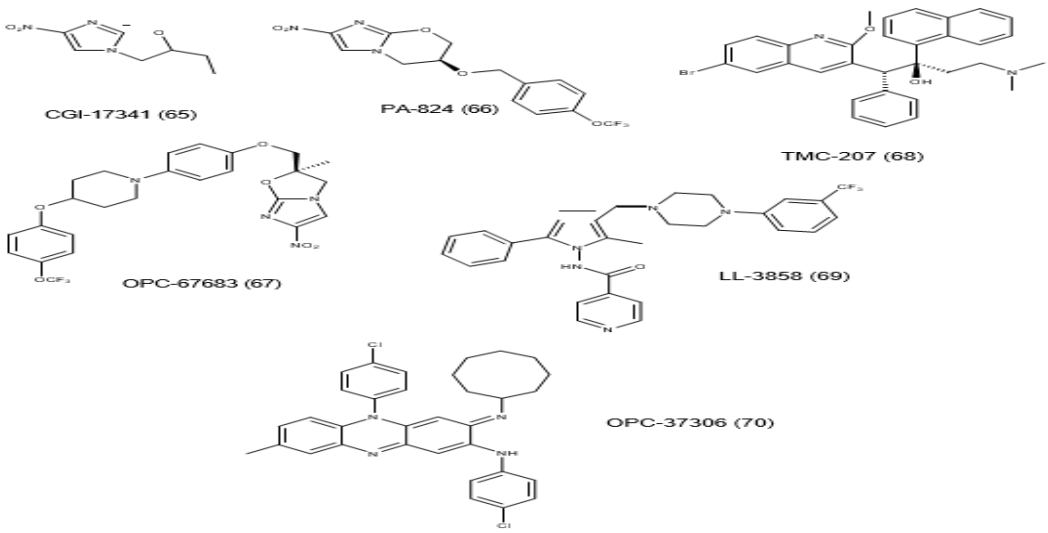

Fig.6 : Drugs in clinical trials

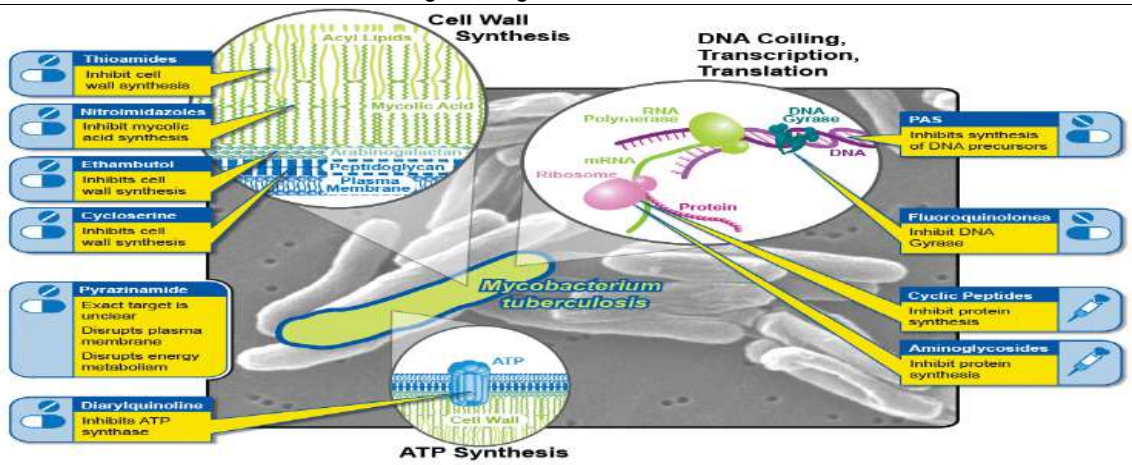

Figure 7: Mechanism of action of different groups of TB drugs

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RESEARCH ARTICLE

Immunopathological Properties of the Enigmatic COVID-19 and Therapeutic Interventions against It: an Update

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ABSTRACT

Coronavirus disease 2019 or COVID-19, the unprecedented global pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-COV-2 which has a long incubation period, higher transmission rate than other viruses, and it is transmitted through zoonotic modes where bats are found to be the main reservoir for these types of viruses. The innate and adaptive immune system plays a very crucial role in preventing viral infection. But, sometimes, the host's own immune system is dysregulated causing hyper inflammation and impaired pulmonary gas exchange, severe multi-organ damage and eventually death. In the present review, we have highlighted the "battle of triad", i.e., interaction among SARS-COV-2, healthy immune system and dysfunctional immune system. The association between dysfunctional immune system and disease severity resulting in hyper-inflammation, cytokine storm and penultimate death of COVID-19 patients may serve as a key factor for developing effective vaccines and other therapeutics against SARS-COV-2. Till now, a number of therapeutic strategies have been developed to combat COVID-19 and in the present review, we have summarized several potential therapeutic strategies such as immunomodulatory drugs, plasma therapy, anti-viral treatments and active immunizations via vaccine development that has been proved to be useful to prevent the severity of this disease. The present review may help to understand the overall perspective of host-pathogen interaction that may resolve the enigma in further therapeutic development to combat the devastating effect of this disease.

Key-words-SARS-COV-2; dysfunctional immune system; cytokine storm; ARDS; COVID-19.

INTRODUCTION

Severe acute respiratory syndrome corona virus 2 (SARS-COV-2), traced back to the city of Wuhan, China at the end of 2019, arises as an unrivalled enemy to the entire world in the form of an unprecedented health crisis. The causative pathogen of COVID-19 is a β-corona virus which has high sequence homology to the bat corona viruses (CoVs). It uses angiotensin-converting enzyme 2 (ACE2) receptor as the dominant mechanism of cell entry [1,2]. The zoonotic transmission mode of the virus led the researchers to speculate that the virus jumped from an animal reservoir to a human probably during the first week of November 2019. Although, the intermediate host of the virus is still to be identified, it is now well-known that bats are the main reservoirs for these types of viruses [3,4]. The causative virus, SARS-COV-2, is capable of human- to-human transmission and spread rapidly throughout the world. By the 11th of March 2020, the World Health Organization (WHO) declared the Corona virus disease 2019 (COVID-19) as a pandemic that had infected more than 118,319 people and killed more than 4292 in 113 countries around the world [5]. While the mortality rate in the case of SARS-COV-2 is not as much as SARS-COV-1 or Middle Eastern Respiratory Syndrome (MERS) COVs (MERS-COV) that caused a local outbreak of zoonotic epidemic during 2003 and 2012, respectively, it was revealed that the causative RNA virus of SARS-COV-2 is closely related to other two diseases [1,6,7]. According to WHO, as of the 2nd week of September 2021, more than 223, 022, 538 confirmed cases of COVID-19 and 4,602,882 deaths are reported from its fatal implications [5]. Although a plethora of novel findings on COVID-19 is published daily throughout the world, the pathogenesis of COVID-19 still remains to be fully elucidated for the development of better intervention strategies. The present review discusses the immunopathogenesis of SARS- COV-2 and its interaction with target cells, the immune defence mechanism to the virus, the contribution of dysfunctional immune responses to disease progression and some of the available therapeutical strategies. Moreover, the hallmarks of SARS-COV-2 are also discussed in the present review.

Immunopathogenesis

Viral Entry and Replication into the Host Cell

SARS-COV-2 only infects that host cells which express the cell surface receptor angiotensin-converting enzyme 2 (ACE2) and TMPRSS2. ACE2 is a transmembrane metallocarboxypeptidase, consisting of a C-terminal collectrin-like domain (CLD) and the N-terminal, which is expressed mostly in the nasal and bronchial epithelial cells along with intestinal epithelium [8,9]. In the case of SARS-COV and SARS-COV-2, the entry of the virus is accomplished by the viral RBD of spike protein (S1 subunit) which directly binds to the N terminal peptidase domain (PD) of the ACE2 receptor of the host cell and starts the infection event [10,11]. After the binding of RBD with its receptor, it triggers the clathrin-dependent and independent endocytosis which triggers a proteolytic cleavage event by endosomal proteases that cleave away the S1 subunit, exposing the fusion peptide region of the S2 subunit that insert into the host cell membrane [12,13]. After that, the HR1 and HR2 region of S2 subunits comes together, leading to membrane fusion and release of the viral genome into the host cytoplasm. The viral genome is then translated into polyproteins and structural proteins and begins to replicate after which the newly synthesized envelope glycoproteins are inserted into the membrane of the endoplasmic reticulum or Golgi, and the nucleocapsid is formed by the combination of genomic RNA and nucleocapsid protein [14]. Then, viral particles germinate into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Finally, the vesicles containing the virus particles fuse with the plasma membrane to release the virus that causes pyroptosis of the host cell-associated with vascular leakage in the airway epithelial cells and tissues. Pyroptosis, a highly inflammatory programmed cell death is commonly seen in the case of cytopathic viral infection as observed in the case of SARS-COV-2 infection [15,16].

Recognition and Antigen Presentation

Due to the genetic similarity between SARS-COV and SARS-COV-2, it is assumed that the immunological signaling mechanisms of both CoVs are similar. Initially, the virus is recognised by the antigen-presenting cells (APCs) as well as pattern recognition receptors (PRRs) including toll-like receptor (TLR), RIG-I like receptor (RLR), NOD-like receptor (NLR), C type lectin-like receptors (CLmin), and free molecule receptors in the cytoplasm, such as cGAS,

IFI16, STING, and DAI, when it enters into the airway epithelial cell [17]. The active PRR then detects the viral RNA and DNA oligomers that trigger the downstream signaling cascades by the secretion of various pro-inflammatory cytokines and chemokines which creates a local wave of inflammation. Among these, type I/III interferons (IFNs) are considered the most effective against viral infections [18]. There are also some other cytokines and chemokines, such as pro-inflammatory tumour necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1), IL-6, and IL-18, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1 that are also released into the blood of affected patients and induce the antiviral responses via Th1 cell in target cells and initiate the adaptive immune response [19]. Secretions of these cytokines and chemokines attract monocytes and T lymphocytes, but not neutrophils, from the blood into the infected site promoting further inflammation and establishing a pro-inflammatory feedback loop [20,21]. Lymphocyte infiltration from the blood into the airways may explain the lymphopenia and increased neutrophil-lymphocyte ratio seen in around 80% of patients with SARS- COV-2 infection [22,23]. In a healthy individual, the antigenic peptides are presented by APCs to the virus-specific cytotoxic T lymphocytes (CTLs) via major histocompatibility complex (MHC) or human leukocyte antigen (HLA) to the site of infection where they can eliminate the infected cells before it spreads. In the case of SARS-COV, the antigen presentation is mainly dependent on the MHC I molecules [24]. There is a strong correlation between the HLA polymorphisms and the SARS-COVs. Some of the HLA such as HLA-B*4601, HLA-B*0703, HLA-DR B1*1202 and HLA-Cw*0801 are more susceptible to SARS-COV [25,26], but other alleles of HLA such as HLA-DR0301, HLA-Cw1502 and HLA-A*0201 are not susceptible to SARS-COV, moreover, these alleles provide protection from SARS infection [27].

Generation of "Cytokine Storm Due To" Covid-19 Infection

Like other virus-induced pro-inflammatory responses, SARS-COV2 leads to the accumulation of an excessive amount of pro-inflammatory cytokines in the lung causing overwhelming inflammation called cytokine storm (CS), which can spread to all organs in case of severe infectious diseases. The released pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGF-β) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10) by the effector's cells cause a variety of diseases like infectious diseases, rheumatic diseases and induce tumour [28–30]. In the case of SARS-COV-2 and other coronavirus induced pneumonia like SARS and MARS, it is accompanied by rapid viral replication, a large number of inflammatory cell infiltration and CS, causing acute lung injury and eventually leading to multiple organ failure [31,32]. IL-6 seems to play a key role in response to cytokine storm as an elevated level of IL-6 has been measured in a non-survival and critically ill patient with COVID-19 than the survival group of patients [33,34]. The contradictory report is observed in several COVID -19 patients where an elevated level of IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A and TNF-α were measured but there was no significant difference of serum IL-6 level between severe and moderately stable patients [35,36]. Recent clinical studies revealed that severely infected COVID-19 patients with exaggerated and dys regulated immune response lead to massive production of pro-inflammatory cytokine levels that are responsible for plasma leakage, vascular hyperpermeability, and disseminated vascular coagulation, severe pulmonary deterioration and multiorgan failure [37] Significant increase was also observed in the number of CD14+ and CD16+ inflammatory monocytes in peripheral blood of severe patients that is responsible for inflammatory cytokines secretion contributing to the cytokine storm causing sepsis and ultimately death in 28% of fatal COVID-19 cases, mainly due to multiple organ failure, especially of the cardiac, hepatic and renal systems [38–40]. The clinical report shows that acute respiratory distress syndrome (ARDS) is the main symptom in severe COVID-19, characterized by difficulty in breathing, low level of oxygen saturation and susceptibility to some secondary bacterial infections [41]. ARDS, the common immunopathological event for SARS-COV-2, SARS-COV and MERS-COV infections caused by cytokine storm may lead directly to respiratory failure. In case of COVID-19 death in 70% of fatal cases is due to ADRS [21,39].

Involvement of T Cells and Their Responses

T cells play a very crucial role in eliminating SARS-COV-2 and also orchestrate other immune cell responses. CD8 T cells or killer T cells directly take part in viral infections by attacking and killing the virus-infected cells. It is well known that the CD4 T cells are able to promote the virus-specific antibody production via T cell-dependent B cell activation. The study revealed that about 80% of total infiltration in the pulmonary interstitium in SARS-COV-2

patients are accounted for by cytotoxic T cells [42]. T cells could survive in the MARS-COV infected lungs and completely destroy the infected cells which indicate that T cells have a crucial role in neutralizing the MARS-COV infection rather than B cells [43]. In case of dysregulation of T cell responses, the number of CD4 and CD8 T cells in the peripheral blood diminish, a condition known as lymphopenia, is observed in the case of severe and mild COVID-19 patients [44,45]. A correlation is found between lymphopenia and disease severity in COVID-19 patients who were admitted to ICU with a striking reduction of CD8 T cells causing greater disease severity and mortality [46,47]. Clinical studies of several systematic infected patients have unveiled an increased accumulation of CD4 and CD8 T cells, as well as follicular helper T cells (TFH) in the lung [48]. One mechanism that contributes to the reduction of T cells in the peripheral blood and its accumulation in the lungs suggests that T cells are attracted away from the blood and into the infected site to control the viral infection [21]. One mechanistic pathway shows the correlation between the pro-inflammatory cytokines and the accumulation of T cells into lungs from peripheral blood. It is observed in patients recovering from COVID-19 that the number of T cells in peripheral blood circulation is restored with a low level of pro-inflammatory cytokines [49,50].

Involvement of B Cells and Their Responses

The humoral immune response plays a crucial role in inhibiting cytopathic SARS-COV-2 infection and also induces the memory response that prevents reinfection. The SARS-COV-2 can initiate the humoral immune response that drives virus-specific cell proliferation, antibody isotype switching, virus-specific immunoglobulin production, cell maturation, and cell residence in systemic and mucosal sites. These first lines of defence initiated by the humoral immune response block the entry of viruses by neutralizing them and further prepare the body for passive protection [51]. It is revealed that B cell response in a SARS-COV-2 patient has been developed concomitantly along with follicular T helper cells approximately one week after the onset of symptoms [52]. SARS-COV-2 elicits a profound B cell response, as indicated by the rapid increase in the virus-specific IgM, IgA, and neutralizing IgG antibodies (nAbs) following infection. It is speculated that the initiation of the antibody-mediated responses against SARS-COV-2 infection occurs approximately 4–6 days after IgA and IgM peaks and more than 10 days for virus-specific IgG after exposure of virus in the most affected patient [53,54]. The virus-specific antibodies typically arise against the viral nucleocapsid protein and S protein within 4-8 days after the onset of symptoms [55,56], whereas the neutralizing antibody responses to the S protein are reported to be developed by the 2nd and 3rd week in most patients [57,58]. Furthermore, it is revealed that immunoglobulins possess receptor-binding domain (RBD) or hapten repeat (HR) domains that prevent the viral attachment and entry to the host cell, cease host-dependent viral replication and also induce the activation of natural killer cell and complement system [59,60]. In the case of SARS-COV infection, RBD is the primary target for neutralizing antibodies [61]. Similar to the SARS-COV, the high binding affinity of antibodies to the external S glycoprotein and internal N protein of SARS-COV-2 has been detected in most patients. Indeed, a case study of COVID-19 patients reveals the identification of RBD specific IgG memory cells in the blood [59,62,63]. The effects of B cell responses not only protect us from initial acute infection, but it also plays a very crucial role in eliciting serological memory that offers extended immunity against reinfection via recruiting memory B cells which can rapidly respond against reinfections providing long term protection through the generation of plasma cells with high affinity to the reinfection substances. A recent study on SARS-COV-2 infected rhesus macaques revealed that the macaques that had resolved the primary infection were resistant to reinjection 28 days later [64]. In addition, a case study of a single patient described the induction of a specific subtype of antibody secreting cells (ASCs), concomitant with an increase in circulating follicular T helper cells (Th) cells [52]. The serological investigations have unveiled the presence of antibody producing B cells and increased concentration of virus neutralizing antibodies in clinically recovered patients suffering from severely symptomatic COVID-19 [37].

Immunogenic Interaction of Host against Sars-Cov-2 Infection

The possible host immune strategies to give protection against the viral infection COVID-19 and the prospective way out of SARS-COV-2 to escape the protective host innate immunity have been graphically shown in Figure 1. SARS-COV-2 infects and enters into the airway epithelial cells by interacting with the surface receptors ACE2 and TMPRSS2. Upon entering the viral particle, TLR3/7 is activated in endosomes along with the cytosolic RNA sensors

RIG-I and MDA-5 which in turn activate the interferon regulatory factor 3/7 or IRF 3/7 and enhance the synthesis of pro-inflammatory cytokines in the nucleus. NFkβ, also activated by TLR3/7, regulate the cytokine gene expression to wipe out the viral infection. The released cytokine type 1 IFNs binds to its receptor (IFNAR) expressed on the infected host cells and also on the neighbouring cells through STAT transcription factors (STAT1 and STAT2). This pro-inflammatory cytokine expression can efficiently be inhibited by the evasion strategies employed by the novel coronavirus. The inhibitors TRAF3, TRAF6 and MAVS suppress the activated TLR3/7, RIG-I and MDA-5 to limit the action of IRF3/7 and NFkβ, thereby inhibiting the pro-inflammatory gene expression. SARS-COV-2 also limit the antiviral immune responses by suppressing the action of IFNAR [65]**.** Due to these evasion mechanisms, the antiviral response is not initiated and the active replication of the viral RNA continues that releases the viral particles. After the release of the virus, the host cell undergoes pyroptosis causing the release of host DNA, ATP, IL-1, nucleic acids which are recognized by the neighbouring epithelial cells, endothelial cells and alveolar macrophages. This recognition triggers the generation of IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1, pro-inflammatory cytokines and chemokines, causing the recruitment of the monocytes, macrophages and T cells to the site of infection and establish a pro-inflammatory positive feedback loop to promote further inflammatory responses. The activated CD8 T cells induce the apoptosis of SARS-COV-2 infected airway epithelial cells before the spreading of the virus. B cells are activated by CD4 helper T cells and release the neutralizing antibodies, blocking viral infection which is further recognised by the alveolar macrophages that clear them by phagocytosis [66]. The initial inflammation also recruits NK cells which clear viral infection via antibody dependent cell mediated cytotoxicity (ADCC). Chemo attractants released by alveolar epithelial cells, macrophages and stromal cells lead to the recruitment of monocytes into the lungs from the blood, differentiating monocyte into monocyte derived inflammatory macrophages with the help of CCL2 and CCL7 releasing from the infected cells, causing delayed type I interferon response by the infected host cell, activated NK cells and T cells through the production of TNF-α, granulocyte macrophage colony stimulating factor (GM CSF), and interferon- γ (IFNγ). It is assumed that type I interferons triggers the expression of ACE2 receptor on the surface that is used by the virus to enter into the monocyte derived inflammatory macrophages. This activates the NLRP3 inflammasome that leads to the secretion of mature IL-1β and/or IL-18, amplifying the activation of monocyte derived macrophages. It may also lead to the reduction of type I interferon production in the infected lungs. The engagement of Fcγ receptors (FcγRs) by anti-spike protein leads to a massive amount of cytokine production including CXCL10 and IL-1RA which contribute to the COVID-19 cytokine storm [67]. Activation of the complement system also occurs through different pathways which lead to the generation of C3a and C5a complement proteins that may contribute to the cytokine storm in severe COVID-19 patients [68].

Therapeutic Strategies for COVID-19

The therapeutic strategies against COVID-19 are developed to neutralize hyper-inflammation, antiviral activity for viral clearance, convalescent plasma therapy for passive immunization, antigen-specific active immunization via vaccine administration(Table 1).

CONCLUSION

The present review has presented a generalized overview of the immunopathogenesis and different perspectives of the immune defence mechanisms against SARS- CoV-2 infection, controlling the inflammatory responses in targeting the virus. In this study, we have demonstrated that the T cells play a significant role to protect the host from viral infection. The association of dysfunctional immune system to the disease severity resulting in hyper-inflammation, causing cytokine storm and death of COVID-19 patients that may serve as a key factor for developing effective vaccine and other therapeutics against SARS-COV-2. In this review, we have also summarized interventions of various available therapeutics controlling the viral load and inflammatory responses of COVID-19. Though there are so many shreds of evidence about the host pathogen interaction of SARS-COV-2, the rapid mutating nature of this novel virus caused a lack of information about the specific immune defensive strategy that provides better protection

against this infectious disease. The current consequences of COVID-19 at the population level are devastating. The unprecedented nature of SARS-COV-2 has demanded urgency to develop effective therapeutics by the scientific community to provide immune protection before the exposure of the virus.

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Table 1: Summarization of available therapeutic strategies against SARS-COV-2 infection

Time line	Type	Name	Consequences	Adverse Effects	Ref.
Passive Immunotherapy					
21st March 2020	Intravenous Immunoglobulins (IVIG)	Convalescent Antibody	Antigen specific dose dependent immune modulation, such as regulation of immune cells proliferation,	The transfusion (immunoglobulin)-related acute lung injury	[69,70]

			activation of cytokine network and neutralize autoantibodies. Anti-inflammatory activity via interactions between the Fc domain of the IgG and their receptors (FcγRs).	(TRALI), acute respiratory distress.	
27th March 2020	Plasma Therapy	Convalescent Sera	Enhanced production of the NAbs against SARS-CoV-2 virus.	Serum sickness and anaphylaxis associated with bronchospasm, transfusion related acute lung injury, remote possibility of SARS-CoV-2 reinfection antibody dependent enhancement (ADE)	[71]
Immunomodulatory Drugs					
14th April 2020	Anti-inflammatory	Lianhuaqingwen	Downregulation of the pro inflammatory cytokines and chemokines (TNF-α, IL-6, CXCL10 and MCP-1) expression	NA	[72]
	Anti-inflammatory	Thalidomide	The oxygen index improved rapidly. Downregulation of inflammatory cytokines expression (IL-6, IL-10, and IFN-γ) and recovered lymphocytes count.	NA	[73]
	Interleukin-6 Inhibitor	Tocilizumab	Reduction of the pro inflammatory cytokine IL-6 level	Dose dependent elevation in liver enzyme levels, tuberculosis, bowel perforation	[74]
	Interleukin (IL)-1 inhibitors	Anakinra	Reducing the expression of IL-1 cytokine level to control cytokine storm.	Not associated with any significant adverse effects in short-term use.	[75]
	Granulocyte-Macrophage Colony-Stimulating Factor Inhibitors	Gimsilumab, Lenzilumab, Namilumab, Ntilimab,	Inhibiting the intracellular signalling between GM-CSF and its cell surface	Risk of bacterial infection, acute kidney injury and elevated level of liver transaminases.	[76]

		Mavrilimumab	receptor to neutralize its biological function.		
Anti-Viral Therapy					
21st April 2020	Antimalarial drug	Chloroquine or Hydroxychloroquine	Blocking the transport and release of SARS-CoV-2 genome via inhibiting their fusion with endocytic vesicles.	May cause sudden cardiac death in patients who experienced severely prolonged QTc.	[77]
1st May 2020	RNA polymerase inhibitor	Remdesivir Favipiravir	Blocking viral replication via binding with the viral RNA-dependent RNA polymerase and premature termination of RNA transcription.	Elevated transaminase levels, hyperuricemia, teratogenicity and gastrointestinal symptoms.	[77]
Steroidal and Nonsteroidal Drugs					
22nd June 2020	Immunosuppression	Dexamethasone	Non associative to viral clearance in COVID-19 patient. It is a potent anti-inflammatory agent.	High risk of opportunistic fungal infections like mucormycosis, aspergillosis and latent infections like hepatitis B virus, herpesvirus infections, tuberculosis	[78]
	Anti-inflammatory	Indomethacin	Increasement in the oxygen saturation level and marked symptomatic relief.	No adverse effects.	[79]
Active Immunotherapy					
Dec 2020 to Jan 2020	Nucleic acid (DNA and RNA)-based vaccine	GX-19, INO-4800, LUNAR-COV19, BNT162, mRNA 1273, CVnCoV	NA	Still unknown	[80,81]
	Recombinant protein subunit vaccines	NVX-CoV2373, SCB-2019, COVAX-19, Plant-based adjutant covid-19 vaccine			
	Live-Attenuated/Whole Virus Vaccines	Janssen's AdVac®, adenoviral vector, PER.C6 technology, AZD 1222, Codegenix and dia, Covaxin,			

	Adenovirus vectored vaccine	Sputnik V, Covidshield,			
	adenovirus-based vaccine	AD5-nCoV			

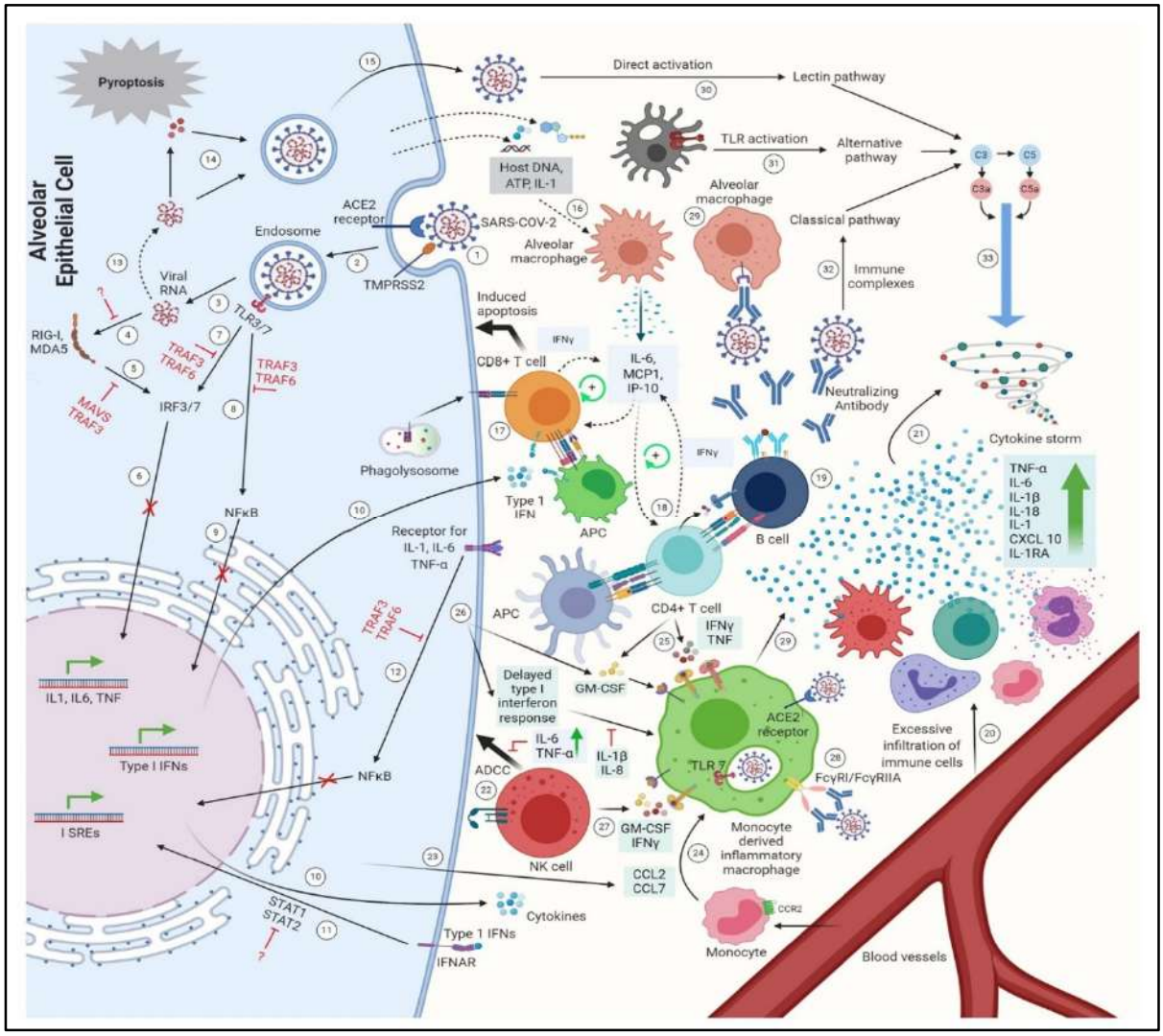

Figure 1: Possible immunogenic interaction between host and SARS-COV-2 (Figure illustrated with the help ofhttps://app.biorender.com/)

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RESEARCH ARTICLE

Effect of Yoga Practice in Hand Discomfort Caused by Mobile Phone Usage with its Correlation on Morphometry of Hand

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ABSTRACT

Cell phone usage has become part of our day to day's life. This study put forward some of the ill effects and potential problems that arise due to cell phone usage. The objective is to study the ill effects and various problems arising from the use of the cell phones for texting and to investigate the effects of hand-Anthropometry and gender difference on mobile phone texting. The study was done on 60 healthy volunteers of 18 to 40 years (30 male and 30 female) without any history of upper extremity musculoskeletal disorders from VMKV Medical College & Hospital, Salem. A structured questionnaire about cell phone usage was given to the participants. Anthropometric data was collected from each participant that includes age, gender, height, weight, handedness, hand length, palm width, finger breadth, and finger length along with other body hand discomfort information. The 3 simple yogasnas were demonstrated to the participants and a survey was taken regarding the relief of discomfort cause by cell phone typing. The data was analyzed by student t test to compare between the two anthropometry groups. Hand-size is significant in cell phone usage which includes the speed, special character selections and navigation. Smaller hand-sized participant more satisfied when compare to large hand- sized participants. Effect of hand-size and were gender was found to be significant in cell phone usage to improve speed and special character selections. The complications of pain and tenderness in hand and thumb were found to be more in participants with lengthier hand and thumb. The present study found

that texting is actually beneficial for improving the students writing skills. The present study helps to prevent the complications of prolonged cell phone usage and message texting using the treatment modalities through simple yoga practices.

Keywords: Cell phone Usage, Upper Extremity Discomfort, Hand-size variations, texting in mobile phone, Yogasnas.

INTRODUCTION

Technical advancement of smart phones has totally changed the pattern of its usage worldwide. The method of its usage may potentially cause musculoskeletal symptoms. Prolonged usage of smartphones results in musculoskeletal disorder over time. Repeated light exertions over longer periods associated with the usage of phone resulted in the risk of upper extremity musculoskeletal discomforts due to awkward postures. In cell phone use, the upper extremity performs light work continuously. The head and neck are stabilized for visual purposes [1]. Low-level static exertions for an extended durations results in musculoskeletal discomfort and signs of fatigue [2]. The mobile phones with preferred grip sizes are in correlation with length-strength muscle which result in maximizing strength and minimizing muscle activity. Muscle length is altered by moment arms which are controlled by postural demands. Muscles usually have a greater ability to generate active force when they are in resting length. Stretching or shortening the muscle length inhibits it to produce tension [3].

Recent smartphone designs come with increased size which forces the user to sub optimize length-strength principles. Standard cell phone requires power and precision grips to hold it, which depends on size of phone in correlation with hand anthropometry [4]. If length and strength of muscle properties are ignored during designing the smartphones, musculoskeletal discomfort and fatigue will result due to prolonged usage of phones. Most of the research publications so far have focused on the smartphone usage while driving and the exposure to its radiation. There is a gap in the literature regarding effect of cell phone usage, its biomechanics and upper extremity musculoskeletal symptoms with its treatment modalities. Thus the current study was designed to bring out a solution through treatment modalities for the above problem faced by many in the community [5].

Aim & Objectives

Aim

To study various effect of the cell phone usage on upper extremity discomfort and its treatment by yoga therapy

Primary Objective

To correlate the cell phone usage and participants anthropometry resulting in development of discomfort and muscle fatigue due to over usage.

Secondary Objective

To assess the effect of cell phone usage and message typing over long period in the upper limb musculoskeletal discomfort. To study the effect of simple yoga practices for upper extremity to reduce the musculoskeletal discomfort caused by prolonged cell phone usage.

MATERIALS AND METHODS

The randomized cross sectional study was done on 60 healthy volunteers of 18 to 40 years (30 male and 30 female) with no history of any upper extremity musculoskeletal disorders. Study participants were taken from Salem district of Tamil Nadu. The study was conducted in Central Research Laboratory of VMKV Medical College & Hospital,

Salem after getting Institutional Human Ethical Committee approval (Reference - VMKVMC&H/IEC/19/79). Informed consent was obtained from all the study participants. Participants were given the option of preferred side (dominant or non-dominant) to hold a phone. Anthropometric measurements were taken. A structured questionnaire about cell phone usage was given to the participants to give all the necessary basic information. Anthropometric data (Figure 1) was measured and collected from each participant that includes height, weight, hand breadth, hand length, thumb length, thumb circumference, index finger length, index finger circumference [6].

Mobile Phone Usage

Cell phone was selected based on the design parameters which are currently in use. The cell phone designs varied in length, depth, breadth, as well grip style. As per the survey cell phones typically had a grip area of 600 to 1,300 Sq.mm [7]. To minimize variability we had selected a cellular phone model that have a similar grip area. The subjective measures consist of a questionnaire regarding cell phone usage related discomfort [7].
During the task, the participants were asked to hold the phone as usual for texting [16].

Treatment modality - Yoga Therapy

The following 3 simple Yogasnas[8] were demonstrated to the participants by the yoga instructor and were trained them using recorded videos for practicing it daily. The asanas includes, Vajraasnas, Urdhva Baddha Anguliasana and Namaskarasana. The therapy was given initial with few sessions to make the participant feel comfortable without any problem in doing the yoga posture and later slowly full-fledged session of 1 hour practice for 10 days were continued at their own pace of time and a feedback was taken regarding the relief of discomfort caused by cell phone usage.

RESULTS AND OBSERVATION

The data's were collected and analyzed by student t test to compare between the two anthropometry groups (Male & Female) and the musculoskeletal discomforts were noted as per the participant's expression throughout the experiment. Table 1 shows the usage of cell phone for talking by the participants regularly with maximum of 1 to 2 hours by 50% of the study participants and a minimum of 30 minutes in 8.3% and 4 to 5 hrs.in 8.3% of the study participants. Cell phone texting as per the survey shows that 33.3% of the study participants spend 1 to 2 hrs.of their time in texting to the least of 5% study participants were texting for 4 to 5 hrs. (Table 2).

Around 58.3% of participants were using both the hands for texting and most frequently the right hand was used by 25% of study participants (Table 3). Right hand was used more frequently for holding the phone while talking and left hand was used while texting the message where as in 41.7% of people use both the hands (Table 4). Thumb was used for texting messages by most of the study participants (75%) whereas both thumb and index fingers were used by 16.7% of people for texting (Table 5). Text entry factors include alphabets, special characters, learn ability and speed. Most of the people often used the navigation (53.3%), alphabet texting (100%) and special characters (60%) while texting. Around 73.3% of people were so fast in typing the messages / texts (Table 6). The distance of mobile phone while texting were held at 30 cm by most of the study participants (45%)(Table 7).The present study showed that texting is actually beneficial for students writing skills as shown in the Table 8. The anthropometric data of the study participants were measured and analysed. The right and left side measurements were compared by student t test in both the sex and tabulated below with $P<0.05$as significant (Table 9). The upper extremity discomforts that were experienced by study participants on prolonged usage of cell phones were tabulated below (Table 10).

DISCUSSION

The usage of cell phone for talking by the participants regularly with maximum of 1 to 2 hours by 50% of the study participants and a minimum of 30 minutes in 8.3% . Study shows that there is a positive correlation between prolong cell phone use and neck pain.[9] Anne-Marie Chany et al., 2007 had done a study on the effect of phone design correlating with upper extremity discomfort and muscle fatigue. The results of this present study provide some insight into the effect of cell phone size with its discomfort and muscle fatigue [10]. Minkyung Lee et al., 2015 had done a study on the effects of smartphone use on upper extremity by comparing the muscle activity and pain threshold. The results showed that smart phone usage with one hand caused greater pain in hand due to increased muscle activity [11]. Junhyuk Park et al., 2015 the study shows that prolonged phone usage produces considerable stress on cervical spine and thus result in altering the cervical curve and pain threshold of muscles of the neck region. Smartphones also cause ill effects psychologically status, such as depression. Therefore, individuals should maintain appropriate posture and reduce the duration of cell phone usage [12]. Ewa Gustaffson et al., 2016 had done a study in which associations were found between mobile phone text messaging and musculoskeletal discomforts. The short-term effects were found to be at a lesser extent, long-term wer found to affect muscles in neck and upper extremities [13].

In the present study cell phone texting as per the survey shows that 33.3% of the study participants spend 1 to 2 hrs. in texting (Table 2). A significant positive correlation was found in-between the duration of mobile phone use and severity of neck pain [13]. Around 58.3% of participants were using both the hands for texting. Right hand was predominantly for holding the phone while talking and left hand was used while texting the message (Table 4). Aitthanatt et al., 2018 had done a systematic review which shows that the use of smartphones may lead to musculoskeletal changes which is associated with head–neck, shoulder–arm and hand–thumb areas.[14] In the present study the thumb was used for texting messages by most of the study participants (75%) whereas both thumb and index fingers were used by 16.7% of people for texting [14].

Mohammed et al., 2018 have measured the pain intensity using the McGill pain scale and was positively correlated with the shoulder and wrist range of motion. The findings have reported a positive correlation between pain intensity and upper extremity range of motion in smartphone users [15]. Abdulrahman et al., 2018 in their research reported the children's use of electronic devices is associated with neck pain. In the present study also there is association between long time cell phone usage and neck pain [16]. Al Hadidi et al.,2019 had studied the association of prolonged mobile phone use in correlation with neck pain in university students using a numeric rating scale for evaluation of neck pain. This study suggests an association between duration of mobile phone use and the duration of neck pain. The increased severity of neck pain is an increasing threat on the healthcare system [17].

The anthropometric data of the study participants were measured and analysed. The right and left side measurements were compared by student t test in both the sex and tabulated below with $P<0.05$as significant (Table 9).In the present study hand-size is significant in cell phone usage which includes the speed, special character selections and navigation. Smaller hand-sized participant more satisfied when compare to large hand- sized participants. Effect of hand-size and were gender was found to be significant in cell phone usage to improve speed and special character selections. The upper extremity discomforts that were experienced by study participants on prolonged usage of cell phones were tabulated below (Table 10). The complications of pain and tenderness in hand and thumb were found to be more in participants with lengthier hand and thumb.

A research survey also showed that 70% of texting had harmful effects on students writing skills. However, present study showed that texting is actually beneficial. The upper extremity discomfort were overcome by administering Yoga therapy by a trained yoga instructor which includes Vajra asnas, Urdhva Baddha Anguliasana, Namaskarasana were found to be useful and the survey of the participant after practice of yoga showed that 80% of them got relieved

from the discomfort of upper extremity especially hand discomfort. The study was planned to be extended further in the near future with a diverse population and also the muscle activity will be observed using EMG before and after yoga therapy in correlation of upper extremity discomfort.

CONCLUSION

The anthropometry of hand showed that females were found to be more satisfied with the speed and special character selections of text entry mechanism than males. Smaller hand- sized participants are more satisfied with the text entry speed, special character selections and navigation, encounters less pain and other complications when compared to participants with medium and large size hands and thumbs. The present study showed that texting is actually beneficial for improving students writing skills. The complications of pain and tenderness in upper extremity, hand and thumb were found to be more in participants with lengthier hand and thumb. The Preventive measures for upper extremity discomfort were done by administering Yoga therapy which includes Vajra asnas, Urdhva Baddha Anguliasana, Namaskarasana. Today texting is most widely used which is of around 72% of all mobile phone users worldwide. The study will also help to associate the knowledge and attitude regarding prevention of complications of prolonged cell phone usage and message texting. The treatment modalities through simple yoga practices will help the prolong cell phone usage community like businessman, medical representatives etc., to be relieved from upper extremity musculoskeletal discomforts.

Limitations

The study was planned to be extended further in the near future with a diverse population and also the muscle activity will be observed using EMG before and after yoga therapy in correlation of hand and upper extremity discomforts.

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Table 1 - Talking hours in Phone/Phone usage

Talking hours	10-15mins	30 mins	1-2 hrs	4-5 hrs	>5 hrs
Number of persons	10(16.7%)	5(8.3%)	30(50%)	5(8.3%)	10(16.7%)

Table 2 –Texting hours in phone

Message typing	10-15 mins	30 mins	1-2 hrs	4-5 hrs	>5 hrs
No. of Participants	12(20%)	15(25%)	20 (33.3%)	3(5%)	19(31.7%)

Table 3 - Hands used for texting

Hand used for texting	Right	Left	Both
No. of Participants	15(25%)	10(16.7%)	35(58.3%)

Table 4 - Hands used for holding phone

Hand used for holding	Right	Left	Both
No. of Participants	30 (50%)	5(8.3%)	25(41.7%)

Table 5 - Fingers used for texting

Fingers used for texting	Thumb	Index	Both
No. of Participants	45(75%)	5(8.3%)	10(16.7%)

Table 6 - Text Entry Factors

Navigation		Alphabet texting		Speed			Learnability		Special characters	
Often	***Rare***	***Easy***	***Hard***	***Fast***	***Medium***	***Slow***	***Easy***	***Hard***	***Often***	***Rare***
32	28	60	-	44	11	5	60	-	36	24
53.3%	46.7%	100%	-	73.3%	18.3%	8.3%	100%	-	60%	40%

Table 7 - Distance of mobile phone held while typing

Distance of mobile phone held while typing	**40 cm**	**30 cm**	**35 cm**	**15 cm**
No. of Participants	7(11.7%)	27(45%)	10(16.7%)	16(26.4%)

Table 8 - Texting & Student's writing Skills

Texting in correlation with Student's writing Skills	YES	NO
Have you ever found that text language has affected students writing skills?	19	41
Do you use text language with complete sentences and proper punctuations while texting?	17	43
Do you use text language with complete sentences and proper punctuations while emailing?	34	23
Do you think cell phone texting has negative effects on student's writing skills?	21	39

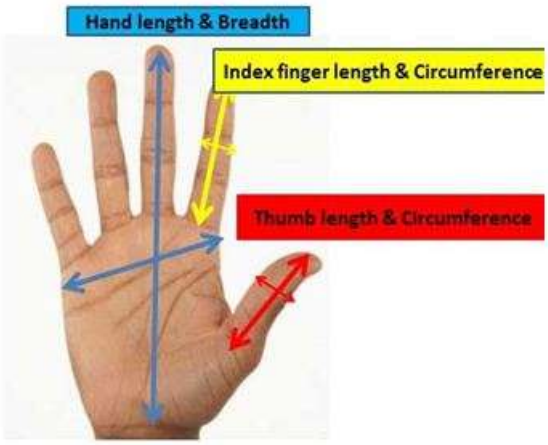
Table 9 - Hand & Finger measurements

Measurements (cm)	**Male**		**Female**	
	Right	**Left**	**Right**	**Left**
Hand length	19.2± 1.21	18.2 ±0.4*	17.5±0.3	16.7±1.4*
Hand breadth	7.3± 1.32	7.5± 0.67*	7.5±0.37	7.7±1.2 #
Thumb length	7.7± 1.21	6.2±0.76*	6.2±0.45	6.5±0.91*
Thumb circumference	7.3±1.25	7.5±0.73 #	6.7±0.92	6.5±1.4 #
Index finger length	8.3±1.67	8.1±0.68 #	7.2±0.94	7.1±1.3 #
Index finger circumference	6.9±1.54	6.4±0.84 #	5.9±0.78	5.4±1.3*

Table 10–Hand discomforts and others associated symptoms

Symptoms	No. of participants
Pain in fingers used for typing	11(18.3%)
Pain in hands used for holding the phone	16(26.7%)
Pain in hand used for texting	7(11.7%)
Pain in wrist	8(13.3%)
Neck and Shoulder pain while texting	14(23.3%)
Back pain while texting / talking for a prolonged time (more than 30 minutes)	8(13.3%)
Pain in elbow while talking phone	3(5%)
Head aches	16(26.7%)

Figure 1 –Anthropometry of Hand



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RESEARCH ARTICLE

Intuitionistic Fuzzy Contra Semi γ* Generalized Continuous Mappings

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ABSTRACT

Aim of this article consists of the introduction of intuitionistic fuzzy (IF) contra semi γ* generalized continuous mappings and their properties. Some characterizations of the above mappings are obtained using some new spaces

Keywords: "Intuitionistic fuzzy sets(IFS), Intuitionistic fuzzy topology(IFT), Intuitionistic fuzzy semi γ* generalized closed sets(IFSγ*GCS), intuitionistic fuzzy semi γ* generalized continuous mappings (IFSγ*GCM), intuitionistic fuzzy contra semi γ* generalized continuous mappings(IFCSγ*GCM)".

INTRODUCTION

Continuity is an important topic in the topological space. Zadeh [11], Atanassov [3], Coker [4] have introduced fuzzy sets, intuitionistic fuzzy sets (IFS) and intuitionistic fuzzy topological space(IFTS). Intuitionistic fuzzy semi γ* generalized closed set is the base definition which was introduced by Abinaya and Jayanthi [1]. Here we deals with intuitionistic fuzzy contra semi γ* generalized continuous mappings and its properties.

Preliminaries

"Definition 1: [3] An **intuitionistic fuzzy set** (IFS) A is an object having the form
$A = \{\langle x, \mu_A(x), \nu_A(x)\rangle : x \in X\}$
where the functions $\mu_A : X \to [0,1]$ and $\nu_A : X \to [0,1]$ denote the degree of membership (namely $\mu_A(x)$) and the degree of non-membership (namely $\nu_A(x)$) of each element $x \in X$ to the set A, respectively, and $0 \le \mu_A(x) + \nu_A(x) \le 1$ for each $x \in X$. Denote by IFS(X), the set of all intuitionistic fuzzy sets in X.
An intuitionistic fuzzy set A in X is simply denoted by $A = \langle x, \mu_A, \nu_A \rangle$ instead of denoting $A = \{\langle x, \mu_A(x), \nu_A(x)\rangle : x \in X\}$.

Definition 2: [3] Let A and B be two IFSs of the form
$A = \{\langle x, \mu_A(x), \nu_A(x)\rangle : x \in X\}$ and $B = \{\langle x, \mu_B(x), \nu_B(x)\rangle : x \in X\}$.
Then,
(a) $A \subseteq B$ if and only if $\mu_A(x) \leq \mu_B(x)$ and $\nu_A(x) \geq \nu_B(x)$ for all $x \in X$,
(b) $A = B$ if and only if $A \subseteq B$ and $A \supseteq B$,
(c) $A^c = \{\langle x, \nu_A(x), \mu_A(x)\rangle : x \in X\}$,
(d) $A \cup B = \{\langle x, \mu_A(x) \vee \mu_B(x), \nu_A(x) \wedge \nu_B(x)\rangle : x \in X\}$,
(e) $A \cap B = \{\langle x, \mu_A(x) \wedge \mu_B(x), \nu_A(x) \vee \nu_B(x)\rangle : x \in X\}$.
The intuitionistic fuzzy sets $0_{\sim} = \langle x, 0, 1\rangle$ and $1_{\sim} = \langle x, 1, 0\rangle$ are respectively the empty set and the whole set of X.

Definition 3: [4] An **intuitionistic fuzzy topology** (IFT) on X is a family τ of IFSs in X satisfying the following axioms:
(i) $0_{\sim}, 1_{\sim} \in \tau$,
(ii) $G_1 \cap G_2 \in \tau$ for any $G_1, G_2 \in \tau$,
(iii) $\cup G_i \in \tau$ for any family $\{G_i : i \in I\} \in \tau$.
In this case the pair (X, τ) is called an **intuitionistic fuzzy topological space** (IFTS) and any IFS in τ is known as an **intuitionistic fuzzy open set** (IFOS) in X. The complement A^c of an IFOS A in an IFTS (X, τ) is called an **intuitionistic fuzzy closed set** (IFCS) in X.

Definition 4: [10] Two IFSs A and B are said to be **q-coincident** ($A\ q\ B$) if and only if there exits an element $x \in X$ such that $\mu_A(x) > \nu_B(x)$ or $\nu_A(x) < \mu_B(x)$.

Definition 5: [10] Two IFSs A and B are said to be **not q-coincident** ($A\ \overline{q}\ B$) if and only if $A \subseteq B^c$.

Definition 6: [5] An **intuitionistic fuzzy point** (IFP), written as $p_{(\alpha,\beta)}$ is defined to be an IFS of X given by

$p_{(\alpha,\beta)}(x) =$

An intuitionistic fuzzy point $p_{(\alpha,\beta)}$ is said to belong to a set A if $\alpha \leq \mu_A$ and $\beta \geq \nu_A$.

Definition 7: [7] An IFS $A = \langle x, \mu_A, \nu_A \rangle$ in an IFTS (X, τ) is said to be an
i. **intuitionistic fuzzy γ closed set** (IFγCS) if $cl(int(A)) \cap int(cl(A)) \subseteq A$,
ii. **intuitionistic fuzzy γ open set** (IFγOS) if $A \subseteq cl(int(A)) \cup int(cl(A))$.

Definition 8: [6] An IFS A of an IFTS (X, τ) is said to be an **intuitionistic fuzzy** (IF) **continuous mapping** if $f^{-1}(V)$ is an IFCS in (X, τ) for every IFCS V of (Y, σ).

Definition 9: [9] An IFS A of an IFTS (X, τ) is said to be an **intuitionistic fuzzy γ^* generalized** (IFγ^*G) **continuous mapping** if $f^{-1}(V)$ is an IFγ^*GCS in (X, τ) for every IFCS V of (Y, σ).

Definition 10: [1] An IFS A of an IFTS (X, τ) is said to be an **intuitionistic fuzzy semi γ^* generalized closed set** (IF semi γ^*GCS) if $int(cl(A)) \cap cl(int(A)) \subseteq U$ whenever $A \subseteq U$ and U is an IFSOS in (X, τ).
The complement A^c of an IF semi γ^*GCS A in an IFTS (X, τ) is called an **intuitionistic fuzzy semi γ^* generalized open set** (IF semi γ^*GOS) in X.

Definition 11: [1] An IFTS (X, τ) is an **intuitionistic fuzzy semi γ^*_γ $T_{1/2}$ space** (IF semi γ^*_γ $T_{1/2}$ space) if every IF semi γ^*GCS is an IFγCS in X.

Definition 12: [1] An IFTS (X, τ) is an **intuitionistic fuzzy semi γ^*_c $T_{1/2}$ space** (IF semi γ^*_c $T_{1/2}$ space) if every IF semi γ^*GCS is an IFCS in X.

Definition 13: [2] A mapping $f: (X, \tau) \to (Y, \sigma)$ is called an **intuitionistic fuzzy semi γ^* generalized** (IF semi γ^*G) **continuous mapping** if $f^{-1}(A)$ is an IF semi γ^*GCS in X for every IFCS A of Y.

Definition 14: [8] Let f be mapping from an IFTS (X, τ) into an IFTS (Y, σ). Then f is said to be an
i. **intuitionistic fuzzy contra continuous mapping** (IF contra CM) if $f^{-1}(B) \in$ IFC(X) for each IFOS B in Y.
ii. **intuitionistic fuzzy contra α-continuous mapping** (IF contra αCM) if $f^{-1}(B) \in$ IFαC(X) for each IFOS B in Y.
iii. **intuitionistic fuzzy contra pre continuous mapping** (IF contra PCM) if $f^{-1}(B) \in$ IFPC(X) for each IFOS B in Y."

intuitionistic fuzzy contra semi γ^* generalized continuous mapping

Here we introduced the definition of IF contra semi γ^* generalized continuous mappings, examples & its properties.

Definition 15: A mapping d: P → Q is said to be an **intuitionistic fuzzy contra semi γ* generalized continuous mapping** (IFCSγ*GCM) if $d^{-1}(R)$ is an IFSγ*GCS in P ∀ IFOS R of Q.

Example 16: Let P = {m, n} and Q = {e, f}. Then ι = {0~, D_1, 1~} and C = {0~, D_2, 1~} are IFTs on P and Q respt, where D_1 = ⟨p, (0.5_m, 0.6_n), (0.5_m, 0.4_n)⟩ & D_2 = ⟨q, (0.2_e, 0.3_f), (0.8_e, 0.7_f)⟩. Define a mapping d: (P, ι) → (Q, C) by d(m) = e and d(n) = f.

The IFS D_2 = ⟨y, (0.2_e, 0.3_f), (0.8_e, 0.7_f)⟩ be IFOS(Q) and $d^{-1}(D_2)$ = ⟨p, (0.2_m, 0.3_n), (0.8_m, 0.7_n)⟩ is an IFSγ*GCS in (P, ι). Therefore d is an IFCSγ*GCM (P, ι).

Theorem 17: Every IF contra CM is an IFCSγ*GCM.

Proof: Let d: (P, ι) → (Q, C) be IF contra CM, R be an IFOS in Q. Then $d^{-1}(R)$ is an IFCS in P, by hypo's. Hence $d^{-1}(R)$ is an IFSγ*GCS in P. Thus d be an IFCSγ*GCM.

Example 18: Assume P = {m, n}, Q = {e, f}, D_1 = ⟨p, (0.5_m, 0.4_n), (0.5_m, 0.6_n)⟩, D_2 = ⟨p, (0.4_m, 0.3_n), (0.6_m, 0.7_n)⟩ and D_3 = ⟨q, (0.3_e, 0.4_f), (0.5_e, 0.6_f)⟩. Then ι = {0~, D_1, D_2, 1~} C = {0~, D_3, 1~} are IFT's on P and Q respt. Define a func d: (P, ι) → (Q, C) by d(m) = e and d(n) = f. Here d be an IFCSγ*GCM but not IF contra CM.

Theorem 19: Every IF contra SCM is an IFCSγ*GCM.

Proof: Consider d: (P, ι) → (Q, C) be IF contra SCM. Let R be an IFOS in Q. Then $d^{-1}(R)$ is an IFSCS in P, by hypo's. Then $d^{-1}(R)$ is an IFSγ*GCS in P. Hence d be IFCSγ*GCM.

Ex 20: Consider P = {m, n}, Q = {e, f}, D_1 = ⟨p, (0.5_m, 0.4_n), (0.5_m, 0.6_n)⟩, D_2 = ⟨p, (0.4_m, 0.3_n), (0.6_m, 0.7_n)⟩ and D_3 = ⟨q, (0.3_e, 0.4_f), (0.5_e, 0.6_f)⟩. Here ι = {0~, D_1, D_2, 1~} & C = {0~, D_3, 1~} are IFT's on P & Q respt. Here a func d: (P, ι) → (Q, C) by d(m) = e and d(n) = f. Then d be an IFCSγ*GCM but it does not satisfies IFcontra SCM.

Theorem 21: Every IF contra PCM is an IFCSγ*GCM.

Proof: Let d: (P, ι) → (Q, C) be an IF contra PCM, R be IFOS in Q. Now $d^{-1}(R)$ be IFPCS in P, by hypo's. Thus, $d^{-1}(R)$ is an IF semi γ*GCS in P. Hence d is an IFCSγ*GCM.

Example 22: Consider P = {m, n}, Q = {e, f}, G_1 = ⟨p, (0.5_m, 0.4_n), (0.5_m, 0.6_n) ⟩, G_2 = ⟨p, (0.4_m, 0.3_n), (0.6_m, 0.7_n)⟩ and D_3 = ⟨q, (0.5_e, 0.4_f), (0.5_e, 0.6_f)⟩. Then ι = {0~, D_1, D_2, 1~}, C = {0~, D_3, 1~} are IFTs on P and Q respt. A mapping d: (P, ι) → (Q, C) by d(m) = e and d(n) = f. Then d is an IFCSγ*GCM but not an IF contra PCM.

Theorem 23: Every IFCα CM is an IFCSγ*GCM.

Proof: Assume d: (P, ι) → (Q, C) be an IFCα CM, R be an IFOS in Q. Then $d^{-1}(R)$ is an IFαCS in P, $d^{-1}(R)$ is an IF semi γ*GCS in P, by hypo's. Hence d is an IFCSγ*GCM.

Example 24: Consider P = {m, n}, Q = {e, f}, D_1 = ⟨p, (0.5_m, 0.4_n), (0.5_m, 0.6_n)⟩ and D_2 = ⟨q, (0.2_e, 0.3_f), (0.8_e, 0.7_f)⟩. Here ι = {0~, D_1, 1~} C = {0~, D_2, 1~} are IFT's on P & Q respt. Define a func d: (P, ι) → (Q, C) by d(m) = e and d(n) = f. Then d is an IFCSγ*GCM but not an IFcontra αCM.

Theorem 25: Every IFcontra γ CM is an IFCSγ*GCM.

Proof: Assume d: (P, ι) → (Q, C) be an IF contra γ CM, R be an IFOS in Q. Then $d^{-1}(R)$ is an IFγCS in P, by hypo's. Thus $d^{-1}(R)$ is an IF semi γ*GCS in P. Hence d is an IF contra semi γ*GCM.

Example 26: Assume P = {m, n}, Q = {e, f}, D_1 = ⟨p, (0.5_m, 0.4_n), (0.5_m, 0.6_n) ⟩, D_2 = ⟨p, (0.4_m, 0.3_n), (0.6_m, 0.7_n)⟩ and D_3 = ⟨q, (0.5_e, 0.3_f), (0.5_e, 0.6_f)⟩. Then ι = {0~, D_1, D_2, 1~} and C = {0~, D_3, 1~} are IFTs on P and Q respt. Define a func d: (P, ι) → (Q, C) by d(m) = e and d(m) = f. Therefore d is an IFCSγ*GCM but it does not an IFcontra γCM.

Relation between various types of IF contra CM and IFCγ*GCM is mentioned in the below figure. From the figure 'cts' means continuous mapping. The reverse implications of the below diagram is false.

Theorem 27: A mapping d: (P, ι) → (Q, C) is an IFCSγ*GCM iff d^{-1}(J) every IFCS of Q is an IFSγ*GOS in P.

Proof: Necessity-: Assume J be IFCSet in Q. Which ⇒ J^c is an IFOSet in Q. Then $d^{-1}(J^c)$ is an IFSγ*GCS in P, by hypo's. Since $d^{-1}(J^c) = (d^{-1}(J))^c$, $d^{-1}(J)$ is an IFSγ*GOS in P.

Sufficiency: Consider J to be an IFOSet in Q ⇒ J^c is IFCSet in Q. By hypo's $d^{-1}(J^c)$ is an IF semi γ*GCS in P. We know that $d^{-1}(J^c) = (d^{-1}(J))^c$, $(d^{-1}(J))^c$ is an IF semi γ*GOS in P. Thus $d^{-1}(J)$ is an IF semi γ*GCS in P. Hence d is an IFCSγ*GCM.

Theorem 28: Let d: (P, ι) → (Q, C) be bijective function. Suppose that one of the flg statements hold good:

1) $d^{-1}(cl(E)) \subseteq int(\gamma cl(d^{-1}(E)))$ ∀ IFSet E in Q,
2) $cl(\gamma int(d^{-1}(E))) \subseteq d^{-1}(int(E))$ for each IFSet E in Q,
3) $d(cl(\gamma int(J))) \subseteq int(d(A))$ for each IFSet J in P,
4) $d(cl(J)) \subseteq int(d(J))$ for each IFγOS J in P.

Then d be an IFCSγ*GCM.

Proof: 1) ⇒ 2) is obvious by taking complement of 1).

2) ⇒ 3) Consider $J \subseteq P$. Put $L = d(J)$ in Q. This implies $A = d^{-1}(d(J)) = d^{-1}(L)$ in P. Now $cl(\gamma int(J)) = cl(\gamma int(d^{-1}(L))) \subseteq d^{-1}(int(L))$ by (ii). Therefore $d(Cl(\gamma int(J))) \subseteq d(d^{-1}(int(L))) = Int(L) = Int(t(J))$.

3) ⇒ 4) Let $J \subseteq P$ be an IFγOSet. We know $\gamma int(J) = J$. By hypothesis, $t(cl(\gamma int(J))) \subseteq int(d(J))$. Hence $d(Cl(J)) = t(Cl(\gamma tnt(J))) \subseteq int(t(J))$.

Suppose 4) holds. Consider J be IFOSet in Q. Now $d^{-1}(J)$ is IFSet in P & $\gamma int(d^{-1}(J))$ IFγOS in P. Hence by hypo's, $d(cl(\gamma int(d^{-1}(J)))) \subseteq int(d(d^{-1}(J))) = Int(J) = J$. Therefore $cl(\gamma int(d^{-1}(J))) = d^{-1}(d(cl(\gamma Int(d^{-1}(J))))) \subseteq d^{-1}(J)$. Thus $d^{-1}(J)$ is IFPCS in P & hence an IFSγ*GCS in P. Hence d be an IFCSγ*GCM.

Theorem 29: Consider d: (P, ι) → (Q, C) be a func. One of the flg to subsequent:

1) $t(\gamma Cl(L)) \subseteq int(d(J))$ *for each* IFSet J in P,
2) $\gamma Cl(t^{-1}(L)) \subseteq t^{-1}(int(L))$ ∀ IFSet L in Q,
3) $t^{-1}(cl(L)) \subseteq \gamma int(t^{-1}(L))$ ∀ IFSet L in Q.

Then t be an IF contra semi γ*GCM.

Proof:- 1) ⇒ 2) Consider L ⊆ Q. Now $d^{-1}(L)$ is an IFSet in P. By hypo's, $d(\gamma Cl(t^{-1}(L))) \subseteq int(d(d^{-1}(L))) \subseteq Int(L)$. Here $\gamma Cl(d^{-1}(L)) \subseteq d^{-1}(d(\gamma Cl(d^{-1}(L)))) \subseteq d^{-1}(int(L))$.

2) ⇒ 3) obvious from 2).

Suppose 3) holds. Assume J be an IFCSet in Q, $cl(J) = J$ & $d^{-1}(J)$ is an IFSet in P. Now $d^{-1}(E) = d^{-1}(cl(E)) \subseteq \gamma Int(d^{-1}(E)) \subseteq d^{-1}(E)$, by hypo's. This implies $d^{-1}(E)$ is an IFγOS in P and consequently an IFSγ*GOS in P. Accordingly d is an IFCSγ*GCM.

Theorem 30: Consider d: (P, ι) → (Q, C) be bijective func. Subsequently t is an IFCSγ*GCM if $cl(t(J)) \subseteq t(\gamma Int(J))$ for every IFSet J in P.

Proof: Assume L be an IFCSet in Q, cl(L) = L also $l^{-1}(J)$ is an IFSet of P. By hypo., $cl(l(l^{-1}(L))) \subseteq l(\gamma int(l^{-1}(L)))$. Since l is bijective, $l(l^{-1}(L)) = L$. Therefore L = cl(L) equal to $cl(l(l^{-1}(L))) \subseteq l(\gamma Int(l^{-1}(L)))$. Now $t^{-1}(J) \subseteq l^{-1}(l(\gamma int(l^{-1}(L)))) = \gamma int(l^{-1}(L)) \subseteq l^{-1}(L)$. Hence $l^{-1}(L)$ is an IFγOS in P and hence an IFSγ*GOS in P. Thus d is an IFCSγ*GCM.

Theorem 31: If d: (P, ι) → (Q, C) is an IFCSγ*GCM, where P is an IF semi γ^*_γ $T_{1/2}$ spaces, then the flg conditions are true:

[1] $\gamma cl(d^{-1}(T)) \subseteq d^{-1}(int(\gamma cl(T)))$ for all IFOSet T in Q,
[2] $d^{-1}(cl(\gamma int(T))) \subseteq \gamma int(d^{-1}(T))$ for all IFCSet T in Q.

Proof: [1] Consider T ⊆ Q be IFOSet. By hypo's $d^{-1}(T)$ is an IFSγ*GCS(P). We know that P is an IFsemi γ^*_γ $T_{1/2}$ spaces, $d^{-1}(T)$ be IFγCS(P). This implicit $\gamma cl(t^{-1}(T)) = t^{-1}(T) = t^{-1}(int(T)) \subseteq t^{-1}(int(\gamma cl(B)))$.

[2] Result is obvious from taking the complement of [1].

Theorem 32: Suppos d: (P, ι) → (Q, C) is an IFCSγ*GCM and k: (Q, C) → (L, Ψ) is an IFCM then $k \circ d$: (P, ι) → (L, Ψ) is an IFCSγ*GCM.

Proof: Consider R to be an IFOSet in L. Now $k^{-1}(R)$ is an IFOS of Q. We know that k is an IFCM, d is an IFCSγ*GCM, $d^{-1}(k^{-1}(V))$ is an IFSγ*GCS in P by hypo's. Therefore $k \circ d$ is an IFCSγ*GCM.

Theorem 33: Suppose d: (P, ι) → (Q, C) is an IFCSγ^*GCM and k: (Q, C) → (L, Ψ) is an IF contra CM then k o d: (P, ι) → (L, Ψ) is an IFSγ^*GCM.

Proof: Consider R to be an IFOSet in L. Now $k^{-1}(R)$ is an IFCS of Q, since k is an IFcontra CM, d → IFCSγ^*GCM, $d^{-1}(k^{-1}(R))$ is an IF semi γ^*GOS in P. Therefore k o d is an IFSγ^*GCM.

Theorem 34: For a mapping d: (P, ι) → (Q, C), where P is an IF semi γ^*_γ $T_{1/2}$ space, then below conditions are equal:

1. d be IFCSγ^*GCM,
2. For every IFCSet J in Q & for every IFP $h_{(\alpha,\beta)} \in P$, if $d(h_{(\alpha,\beta)})$ q J then $h_{(\alpha,\beta)}$q $\gamma Int(d^{-1}(J))$,
3. For every IFCSet in Q & for any IFPoint $h_{(\alpha,\beta)} \in P$, if $d(h_{(\alpha,\beta)})$ q J then there exist an IFSγ^*GOS E s.that $h_{(\alpha,\beta)}$ q E and $d(E) \subseteq J$.

Proof: 1) ⇒ 2) Assume d be an IFCSγ^*GCM, J ⊆ Q be IFCSet & $h_{(\alpha,\beta)} \in P$. Consider $h(p_{(\alpha,\beta)})$ q J, now $h_{(\alpha,\beta)}$ q $d^{-1}(J)$. By hypo's $d^{-1}(J)$ be IFSγ^*GOS in P, P is an IF semi γ^*_γ $T_{1/2}$ space, $d^{-1}(J)$ be IFγOS(P). Hence $\gamma Int(d^{-1}(J)) = d^{-1}(J)$. Thus $h_{(\alpha,\beta)}$ q $\gamma Int(d^{-1}(J)$

2) ⇒ 1) Consider J ⊆ Q be IFCSet, now $d^{-1}(J)$ be IFS(P). Assume $h_{(\alpha,\beta)} \in P$ & $d(h_{(\alpha,\beta)})$q J then $h_{(\alpha,\beta)}$ q $d^{-1}(J)$. By hypo's, which implies $h_{(\alpha,\beta)}$ q $\gamma Int(d^{-1}(J))$. i.e., $d^{-1}(J) \subseteq \gamma Int(d^{-1}(J))$. We know $\gamma int(d^{-1}(J)) \subseteq d^{-1}(J)$. Hence $\gamma int(d^{-1}(J)) = d^{-1}(J)$ & an IF semi γ^*GOS in P. This implies d is an IFCSγ^*GCM.

2) ⇒ 3) Assume J ⊆ Q be IFCSet, now $d^{-1}(J)$ is an IFSet(P). Consider $h_{(\alpha,\beta)} \in P$, $d(h_{(\alpha,\beta)})$ q J then $h_{(\alpha,\beta)}$ q $d^{-1}(J)$. By hypo's, this implies $h_{(\alpha,\beta)}$ q $\gamma int(d^{-1}(J))$. i.e., $d^{-1}(J) \subseteq \gamma int(d^{-1}(J))$. We know $\gamma int(d^{-1}(J)) \subseteq d^{-1}(J)$. Thus $\gamma int(d^{-1}(J)) = d^{-1}(J)$. Therefore $d^{-1}(J)$ be IFγOS in P and IFSγ^*GOS in P. Let $d^{-1}(J) = E$. Therefore $h_{(\alpha,\beta)}$ q E and $d(E) = d(d^{-1}(J)) \subseteq A$.

3) ⇒ 2) Assume J ⊆Q be IFCSet, now $d^{-1}(J)$ is an IFSet in P. Consider $h_{(\alpha,\beta)} \in P$, $d(h_{(\alpha,\beta)})$ q J then $h_{(\alpha,\beta)}$ q $d^{-1}(J)$. By hypo's there exist an IFSγ^*GOS E(P) s.that $h_{(\alpha,\beta)}$ q E & $d(E) \subseteq J$. Assume $E = d^{-1}(J)$, P is an IF semi γ^*_γ $T_{1/2}$ spaces, $d^{-1}(J)$ is an IFγOS in P & $\gamma Int(d^{-1}(A)) = d^{-1}(J)$. Hence $h_{(\alpha,\beta)}$ q $\gamma Int(d^{-1}(J))$.

Theorem 35: A funct d: (P, ι) → (Q, C) is an IFCSγ^*GCM iff $d^{-1}(\gamma Cl(B)) \subseteq \gamma Int(d^{-1}(cl(E)))$ ∀ IFS E in Q, where P → IF semi γ^*_γ $T_{1/2}$ space.

Proof:- Necessity:- Consider E ⊆ Q be an IFSet. Now cl(E) is an IFCS(Q). By hypo's, $t^{-1}(cl(E))$ be IFSγ^*GOS(P), P is an IF semi γ^*_γ $T_{1/2}$ spaces, $t^{-1}(cl(E))$ be IFγOS(P). Hence $t^{-1}(\gamma Cl(E)) \subseteq d^{-1}(cl(E)) = \gamma Int(d^{-1}(cl(E)))$.

Sufficiency: Consider E ⊆ Q be IFCSet, cl(E) = E. By hypo's, $t^{-1}(\gamma Cl(E)) \subseteq \gamma Int(t^{-1}(cl(E))) = \gamma Int(d^{-1}(E))$. We know $\gamma cl(E) = E$. Thus $d^{-1}(E) = d^{-1}(\gamma cl(E)) \subseteq \gamma int(d^{-1}(E)) \subseteq t^{-1}(E)$. This implies $t^{-1}(E)$ is an IFγOS in P and hence an IFSγ^*GOS in P. Thus d is an IFCSγ^*GCM **.**

Theorem 36: A mapping d: (P, ι) → (Q, C) is an IFCSγ^*GCM if $t^{-1}(\gamma Cl(E)) \subseteq Int(t^{-1}(E))$ forevery intuit., fuzzy set E in Q.

Proof: Assume E ⊆ Q be an IFCSet, cl(E) = E. We know every IFCSet be IFγCS, $\gamma cl(E) = E$. Now by hypothesis, $\varphi^{-1}(E) = \varphi^{-1}(\gamma cl(E)) \subseteq Int(\varphi^{-1}(E)) \subseteq \varphi^{-1}(E)$. Which implies $\varphi^{-1}(E) = Int(t^{-1}(E))$. Thus $t^{-1}(E)$ be IFOSet in P, t is an IF contra CM. By Theorem 17, d is an IFCSγ^*GCM.

Theorem 37: Let d: (P, ι) → (Q, C) and k: (Q, C) → (Z, δ) be mappings. Then the flg statements are equivalent if P is an IFS γ^*_c $T_{1/2}$ spaces:

1. k∘d : (P, ι) → (Z, δ) is an IFCSγ^*GCM
2. $Cl(int(Cl(k\circ d)^{-1}(E))) \subseteq (k\circ d)^{-1}(E)$ ∀ IFOS E in Z.

Proof: (i) ⇒ (ii) Consider E be IFOS(Z). Now $(k \circ d)^{-1}(E)$ be IFSγ^*GCS in P by hypothesis. Here P be IFsemi γ^*_c $T_{1/2}$ spaces, $(K\ o\ d)^{-1}(E)$ is an IFCS in P. Here $cl((K\ o\ d)^{-1}(E)) = ((k\circ d)^{-1}(E))$. Then $cl(int(cl(K\ o\ d)^{-1}(E))) \subseteq cl((K\ o\ d)^{-1}(E)) = (k\circ d)^{-1}(E)$.

(ii) ⇒ (i) Consider E be IFCSet of Z, E^c be IFOS in Z. By hypo's, $Cl(int(cl(k\circ d)^{-1}(E^c))) \subseteq (k \circ d)^{-1}(E^c)$. Hence $(k \circ d)^{-1}(E^c)$ be IFαCS in E., $(k\circ d)^{-1}(E^c)$ is an IFSγ^*GCS in P. As $(k\circ d)^{-1}(E^c) = ((k\circ d)^{-1}(E))^c$, $(k\circ d)^{-1}(E)$ is an IFSγ^*GOS in P. Hence k ∘d is an IFCSγ^*GCM.

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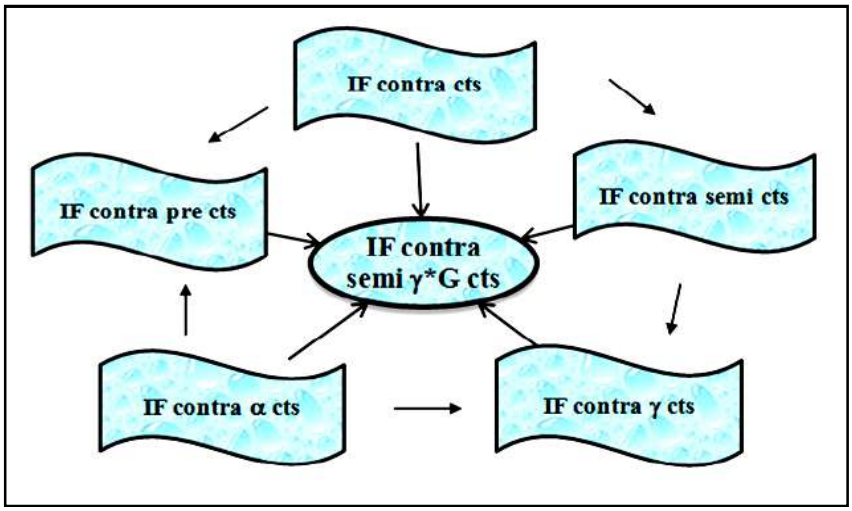

Fig.1 : Relation between various types of IF contra CM and IFCγ*GCM is mentioned

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RESEARCH ARTICLE

Therapeutic Applications of Benzopyrans and its Analogues–An Over View

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ABSTRACT

The naturally arising compounds comprising oxygen moiety is benzopyran. The adequate writing support and the way that benzopyran has shown pharmacological properties, such as, antitumor, against HIV, antimicrobials, mitigating and anticoagulants and so forth. Benzopyran is a flavonoid which happens generally in numerous characteristic innovations which show proof of assortment of organic and pharmacological activities. The benzopyran derivatives support their use as therapeutic agents for multiple diseases. Their structural character correlated to physicochemical properties seems to define the extent of the biological activity. Several benzopyran derivatives have *invivo/invitro* biological responses. Their clinical assessment will be basic to evaluate remedial utility. The compounds covering benzopyran moiety is well defined as lead compounds for proposal of new more favorable molecules. The current appraisal places of interest are to weigh up the fused benzopyran analogues as therapeutic agents.

Keywords: Benzopyran, lead compound, flavonoid, HIV, biological responses

INTRODUCTION

Benzopyran is a bicyclic heterocyclic system, constitutes a stable structure in medicinal chemistry. The structure of benzopyran ring exist in many in many natural products like α-tocotrienol, γ –tocotrienol, δ-tocotrienol, and tocopherol carrying phytyl chain on the pyran ring [1]. They exist in pigments in leaves and in numerous food sources such as in olive oil, red wine, fruits, and tree [2] and also in some naturally arising Warfarin [3], genistein [4],

nebivolol [5], hesperidin [6], and umbelliferone [7]. Due to its structural diversity it illustrates a profound impact on drug discovery. Currently, many experts are inspired in their search for new molecular entities with pharmacological activity by the natural products [8]. Among them, it was in concert to more and more consideration to benzopyran. In the early decades, benzopyran byproducts were main compounds with a widespread range of biological properties, including anti-inflammatory, antibacterial, antimicrobial, antiviral, antioxidant, antiplatelet, anticancer activities etc [9]

Chemistry of Benzopyran

Benzopyrans is also entitled as chromens, bicyclic heterocyclic systems covering benzene ring fused to a heterocyclic pyran ring (Figure 1), establish an esteemed structure in medicinal chemistry. Based on the current idea the six membered ring containing oxygen as a hetero atom is widely spread in variety of natural flavanoids owning variety of activity is been appraised.It is well-known that on the reduced form it has been classified into 2H chromene, 5H chromene, 7H chromene, 1H isochromene, 2H isochromene [10]. The instantaneous family members of benzopyrans or flavonoids comprise flavones, flavonols, isoflavones and flavanones (2, 3-dihydroxy derivative of flavones).

The uniqueness of the title benzopyran is because of its diverse functionalities. Benzopyrans are glowing famous by the terms chromones and flavonoids which show an important role in plants growth, progress and in protection against microorganisms and pests [11]. Benzopyrans, in human regimen, act as important antioxidants [12]. A large number of plant medicines comprise benzopyrans as antibacterial, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory action [13]. For example, Khelin has been perceived to possess coronary dilating action [14] however 7-hydroxy-8-dialkyl aminomethylflavones are observed to act as heart stimulants [15]. Benzopyrans have also been established to prevent a widespread of enzymes involved in oxidation system such as 5-lipoxygenase, cyclooxygenase, and monooxygenase or xanthine oxidase [16]. Benzopyrans comprising a highly conjugated aromatic system, display extreme and characteristic absorption spectra in ethanol. Complexation or chelation of benzopyrans with various reagents indicates about the shifting in the spectra, by this it gives more evidence about the structure of benzopyrans [17]. As much as the analytical presentations of benzopyrans are concerned, the flavonols such as morin, quercetin, galangin etc. have been used successfully. These chelating ligands need oxygen donor carbonyl and the hydroxyl groups in their structures which are liable for their analytical characteristics.The 3-hydroxyl group and carbonyl group with metal result in a chelating structure as shown in figure 2.

Biological Applications of Benzopyrans

Benzopyran Derivatives for Breast Cancer

Breast cancer is measured as the utmost universally diagnosed cancers and is the second foremost cause of cancer death among women in India. The mechanism of breast cancer evolution is related with cell proliferation and apoptotic cell death. Even though chemotherapy is extensively known as the mainstay of cancer therapy, unwanted side effects narrow the use of anticancer drugs inchemotherapeutics. Benzopyranpharmacophore has provided fertile ground for breast cancer research due to discovery of Ormeloxifen [18], B43-genistein [19] and KBU2046 [20]. Ormeloxifen a 3rd generation selective estrogen receptor modulator, 1-[2-[4-[(3*S*,4*S*)-7-methoxy-2,2-dimethyl-3-phenyl-chroman-4-yl]phenoxy]ethyl]pyrrolidine[21] available in trade name Novex-DS, centchroman and Sevista [22-23]used as a non-steroidal oral contraceptives and in the treatment of fibroadenoma [24]**.**

Genistein, 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one a naturally occurring isoflavone influences multiple biochemical functions in living cells. Many studies have reported a wide range of biological effects such as anticancer, antiangiogenic, antioxidant and anthelmintic activity. Research data confirms that Genistein increases the growth rate of some estrogen receptor expressing breast cancers.Presently over 20 clinical trials of Genistein analogues are carried, mainly for hormone-dependent ailments, including menopause symptoms, osteoporosis and cancer [25]. Many drugs based on isoflavones are currently being tested. A good example is B43- Genistein[26] and KBU2046.

Benzopyrans are selective estrogen receptor agonists (SERBAs), which bind the ER receptor subtypes and in opposite orientations.Based on drug design the unique phenomenon has been exploited via substitution at the 8th position of the benzopyran A-ring to disrupt binding to ERa, thus improving ERb subtype selectivity. X-ray co crystal structures with Era and Erb are supportive of this approach to improve selectivity in this structural class [27]

Benzopyran Byproducts for Anti-Inflammation

Inflammation is a shielding response to eradicate the initial cause of cell damage and plays a important role in the development of several diseases such as asthma, rheumatoid arthritis (RA), atherosclerosis, inflammatory bowel diseases, and various human cancers. In response to the stimuli, inflammatory cells produce a lot of pro-inflammatory mediators such as nitric oxide, ROS, prostaglandins, and cytokines. The structure revealed in figure 5 demonstrates that it has potential action against inflammation [28]. On justification of gastrointestinal protection, the classical non steroidal anti-inflammatory drugs (NSAIDs) were substituted by COX-2-selective inhibitors (such as coxibs) [29]. PGE2and PGI2areCOX-2-mediated metabolites which play an significant role in renin release, sodium excretion, the conservation of renal blood flow, and glomerular filtration rate [30]. In the clinical medicine, coxibs was changed in view of the side effects about cardiovascular risks of stroke and myocardial infarction, bromo substituent benzopyrans were the most effective and selective inhibitors of COX-2 shown in figure 6.

Today, arthritis disturbs many of the people all over the world. Arthritis was allocated into two kinds: osteoarthritis and RA. The pharmacological treatment for arthritis comprises analgesics, steroidal and NSAIDs. These drugs may possibly control inflammation and pain in arthritis rather than the cause. At the similar period, these drugs have side effects of gastrointestinal tract, while using classical NSAIDs [31] and additional side effects when using COX-2 inhibitors, the compounds mentioned in figure 7 plays a major role in the prevention and management of arthritis.

Benzopyrans Derivatives for Treating Alzheimer's Disease

AD is a chronic neurodegenerative progression taking place in the central nervous system and the furthermost common is that it affects adult dementia disturbing more than 44 million people worldwide. The disease catches severe behavioral abnormalities, loss of cognitive ability, and at last causing death. So far, the precise cause of AD remains vague, it has been stated that the pathogenesis of AD comprises inflammation, oxidative/nitrosative stress, deficiency of cholinergic transmission, tau protein accumulation, too much metal ions, and presence of β-amyloid (Aβ) plaques. There are manykinds of Aβpeptides. In all aggregates, Aβ1–42which cause's tough toxicity toward neuronal cells has a higher tendency to form fibrils than others in the brain [32].Rendering to the currentdata, the below mentioned Compound has the capacity of selectively inhibiting the MAO-A (IC50= 673 nM) in figure 8.

Benzopyrans for Antidiabetics

Diabetes mellitus is a metabolic disorder characterized by insulin deficiency or insulin resistance. This type of deficiency may result in secondary complications affecting nerves, kidney, eyes and heart [33]. The non-insulin dependent diabetes mellitus is of higher incidence when compared to insulin dependent diabetes mellitus.The hunt of innovative drug with new properties is still in progress but none of them are free of side effect or problem with long term efficacy so an unconventional move towards to avert as well as to treat may be a good suggestion in this regard [34]. Nowadays attentiveness has been focusing on flavonoids which are naturally occurring over 8000 flavonoids are revealed and is still growing. The primary nucleus for flavanoids is benzopyrone. The family members include flavones; flavones, anthocyanadin, and catechin possessing poly phenolic compounds which are used to control carbohydrate metabolism. Flavanoids are the widely occurring natural polyphenolic compounds which are having many beneficial effects [35]. The consumption of flavanoids rich food gives several improved effect. The current existing antidiabetic agents are not interfering in the modulation of the complications. Benzopyran-4-one is the simple moiety present in natural plants that contain flavonoids. A wide variety of flavonoids has been found to be antidiabetic and the newer drugs modulate diabetes related complications by acting on newer targets. Henceforth, an endeavor was made to review the prospects of modulating benzopyran-4-one scaffold for main optimization in antidiabetic drug discovery [36, 37].

The role of quercetin in streptozotocin induced diabetic rats when compared with normal rats shows decreased plasma glucose level but raised hepatic glucokinase activity [38]. Naturally the Flavanoids with Gamma benzopyran nucleus possess antidiabetic property [39] so it's a good lead molecule for synthetic derivatives (Figure 9). Yogendra Nayak [40-42] reported the antidiabetic activity of synthetic benzopyrone analogues by nicotinamide- streptozotocin induced diabetes in rats. Test compounds showed hypolipidemic activity along with the antidiabetic activity in diabetic rats. Compared with the below mentioned quercetinderivatives Figure 12 exhibited maximum efficacy and was often equal or superior to standard drug glibenclamide.

Benzopyran Analogues

An insilico antidiabetic acivity has been carried out using *Euphorbia thymifolia* inorder to evaluate the molecular interaction amongst few bioactive compounds of *E.thymifolia* relating to Target protein of Type 2DM (11-β hydroxyseroid dehydrogenase type 1 glutamine,fructose-6-phosphate aminotransferase,protein-tyrosine phosphatase 1B, Mono-ADP-ribosyltransferase siruin-6) for the compounds mentioned in Figure 14,15,16,17.The result showed high binding affinity with all four target proteins.The 2D Figure of pharmacophore features also showed good hydrophobic,hydrogen bond donor and acceptor interactions between carbonyl oxygen of the ligands.[43]

Quercetin a naturally occuring flavanoid, and finding its antidiabetic activity in the liver and skeletal muscle demonstrated it as a promising lead for synthetic analogues. A novel twenty two derivatives of quercetin were synthesised and drug likeness property has been observed.The compound were docked against human alpha amylase inhibition and the tested compound showed least to significant acivity with IC_{50} 12-125µM and spectral analysis was carried to characterize the synthesised compounds [44]. The synthetic derivatives of coumarine Figure 18 showed very good binding energy compared with vildagliptin and found that it has the same pharmacophore. The protein PDBID:3W2T was designated for docking and Molecular visualization was done by using PYMOL viewer.Ligplots were used to visualize the docking result [45]. The synthesized compound exhibited less activity compared with standard Vildagliptin and sitaglptin but the two compounds possessing cyclic secondary amines and aromatic amines showed good acivity at 10µM concentration.

Series of 3-[4-(phenylsulfonamido) benzoyl]-2H-1-benzopyran-2-one derivatives were synthesized and evaluated for α- glucosidase inhibitor activity. Most of the compounds showed significant inhibition and 7-hydroxy-6-methoxy substituted compounds motif of 3-[4-(phenylsulfonamido) benzoyl]-2H-1-benzopyran-2-one figure 19 manifested most potent activity [46]. The biological activity of *Tinospora sinensis* was invesigated for antioxidant and antidiabetic activity using methanolic exract at different concentration and showed good inhibitory action on *alpha glucosidase* and *alpha amylase* enzyme compared with standard drug acarbose The extract posses a very good amount of total phenolic,flavanoid,flavanol also showed good antioxidant activiy using DPPH,ABTS radical scavening ability[47].

The phytochemical constituents of *Melia dubica cav* possesing alkaloids, flavanoids, carbohydrates, steroids, tannins, saponins and glycosides showed excellent antioxidant activity and*invitro alpha amylase inhibition*at the concentration(IC_{50}-24.82µg/ml) may be due to the presence of flavanoid [48]. The novel cyclohexylehanoid , Megastigmane sesquiterpenoid sulphonic acid and several derivatives are obtained by the leaves of *Wedelia chinensis* .The structural identification was done by spectral characterisation.The methanolic exract of the below mentioned compound showed good inhibitory of *alpha glucosidase* and *alpha amylase* enzyme.Figure 20 showed moderate inhibitory activity [49].

The chrysin, diosmetin, apigenin, luteolin are isolated from plants and are used for the synthesis by using flavanoid as an initial material. The resulting derivative is evaluated for alpha glucosidase inhibitory activity. The α-glucosidase inhibitory activity was compared with standard agarbose (IC_{50} =563.601± 40.492µmol/L)and 1-deoxynojirmycin and hexyl diosmetin shows effective inhibitor when compared with luteolin derivatives due to the

replacement of OH group with long alkyl chains at position 3,4 and 7 of the flavanoid was the vital factor which shows the activity [50].

CONCLUSION

The natural benzopyran derivatives posess wide therapeutic potentials. Owing to the diversed activity a special attention has been focussed on this flavanoid posessing antidiabetic activity. The research added continuous study in this field for further investigation . Benzopyran is a distinct structure which has several modifications which was perceived in many biologically active natural products, and it also plays a chief role in binding with many biopolymers. The synthetic bioactive benzopyran derivatives have been extensively studied, and combinatorial library of benzopyran templates has been reported by Nicolaou and coworkers. As evident from several cited papers, the benzopyran scaffold is the building block of various chromans, coumarins, xanthones and flavonoids occurring in various natural plants and pharmaceutical products. The overall decision is that benzopyran being one of the privileged heterocycles has revealed a wide array of biological activities, chiefly against cancer and diabetic.

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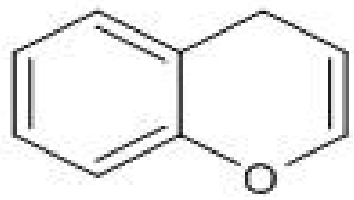

Figure 1: Structure of Benzopyran

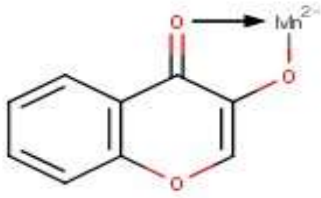

Figure 2: Chelation of Benzopyrans

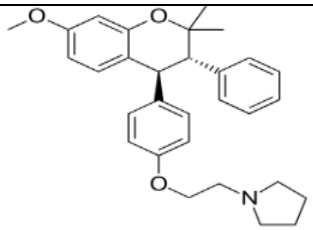

Figure 3: Ormeloxifen

Figure 4: Genistein

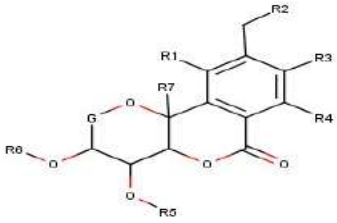

Figure 5: Benzopyrans derivatives for *anti-inflammation*

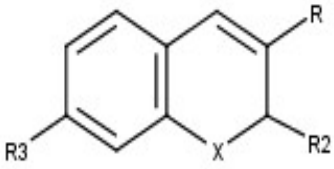

Figure 6: Benzopyrans derivatives for COX-2 inhibitors

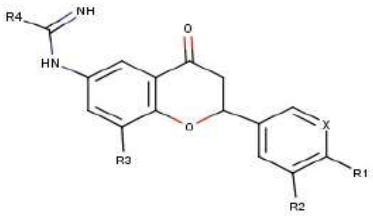

Figure 7: Benzopyrans derivatives for arthritis

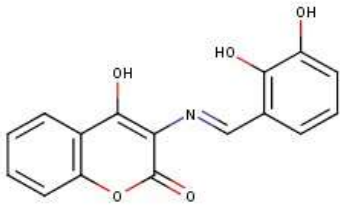

Figure 8: Benzopyrans derivatives for AD

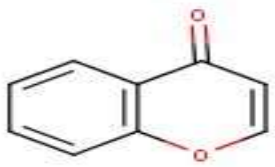

Figure 9: Benzopyran-4-one scaffold

Figure 10 : Benzopyran analogues

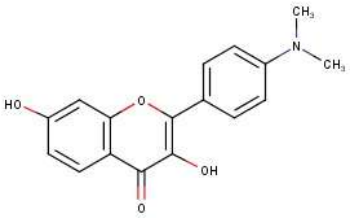

Figure 11: Benzopyran analogues

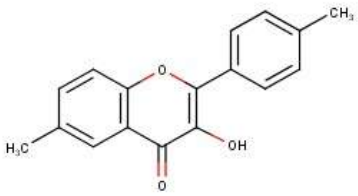

Figure 12 : Benzopyran analogues

Figure 13 : Benzopyran analogues

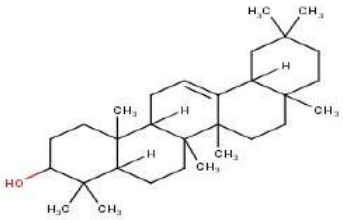

Figure 14: B-amyrin

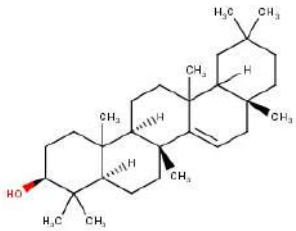

Figure 15: Taraxerol

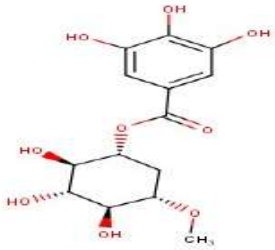

Figure 16 : 1-0-Ganoyl-beta-D-glucose

Figure 17: Quercetin 3-o-galactoside

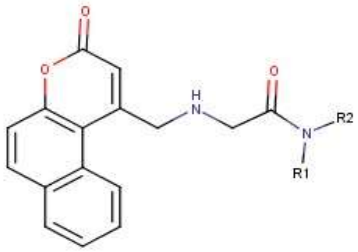

Figure 18: Coumarine derivative

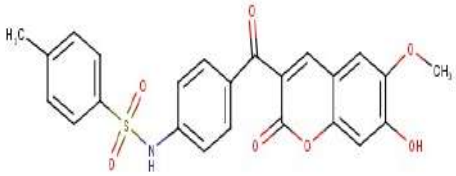

Figure 19: Benzopyran derivative

Figure 20: Benzopyran derivative

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RESEARCH ARTICLE

Mass Segmentation in Mammogram using a Hybrid Approach based on Weighted Coefficient Adaptive Local Fitting Model

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ABSTRACT

Breast cancer is the most recurrent invasive cancer among women, affecting one in every eight women. Segmentation of masses in a mammogram is a crucial task for early detection and treatment. Medical images are frequently obtained with intensity inhomogeneity, including distortion due to the complex atmosphere, image properties, and retrieval device limitations. Image inhomogeneity is common in real-world pictures and can cause significant problems during the segmentation process. Even though several researchers have focused on numerous methods based on active contour models for fine segmentation of regions of interest, segmentation in certain small areas of inhomogeneous sections remains difficult. The adaptive local fitting model, including weight coefficient, is used in this article which is a hybrid approach, considering region-based local and global energy functions. Using both local and global energy functions for each area helps the contour correctly map the correct object boundary. The proposed approach accurately determines the border in a dynamic inhomogeneous area and eliminates dependence on the original contour location with an accuracy of (91.77%). The suggested method's findings are compared to state-of-the-art techniques like LBF, LLBF, and ALF-AC using a mini MIAS and DDSM database using 110 (60+50) images respectively. It is observed by comparison that the proposed method produces better outcomes than the others.

Keywords: Active Contour, Breast Cancer, Intensity inhomogeneity, Image segmentation, mammogram

INTRODUCTION

One of the most common cancers in females is breast cancer. Behind skin cancer, it's also the most widespread cancer. Breast cancer affects concerning one in every eight people. The level of breast cancer at the time of diagnosis affects survival [1]. Breast cancer is responsible for about 5,08,000 female mortality, as per the World Health Organization [2]. As a result, starting at the age of 40, mammogram testing is necessary every two years, as recommended by the American Cancer Society to reduce the risk of cancer [3]. Mammography is among the most effective ways to early diagnose breast cancer. Early identification is the most significant factor in lowering mortality. Automatic image recognition is beginning to help with diagnosis. Computer-assisted diagnosis aims to help the radiologist in making decisions [4]. Mammography is by far the most powerful and generally recognized imaging technique for the analysis of breast cancer. Mammography is one of the gold standards for breast cancer detection, as per the American Cancer Society. Medical image processing and diagnosis treatment depend heavily on the segmentation of the region of interest (ROI) in image processing [5].

A mammogram classification as malignant or benign can be performed in three stages using the CAD procedure [6]. The tumor or mass is first removed from the background area, then relevant features are extracted, and finally, the cancerous mass is classified using a specific classification technique. Since the ground truth for the boundary of the mammogram image is not available, manual detection of a tumor by the radiologist is known to be a safe practice; but, due to manual mistakes, they may skip some minor cancerous regions. Furthermore, this procedure is time-consuming. Using automated segmentation methods for tumor detection is one of the effective practices. Because of the varying structure of the tumor and intensity inhomogeneity, developing automated methods for identifying a mass in a mammogram is challenging. The critical goal of image segmentation is to separate the relevant regions of interest to be processed [7]. A region of interest is a set of pixels identified by a border that can construct various shapes like polygons and irregular shapes. The segmentation method does not include details about the whole image but rather correlates just the ROI's data values [8].

Active contour is one of the segmentation techniques that use energy forces, including constraints, to distinguish the subjects of interest from the rest of the picture to be studied and analyzed further. The most common use of active contours in image recognition is to describe a smooth shape inside an image and create a sealed contour for just an area [9]. Snake models are used in active contour models. External energy, internal energy, and energy of the image under consideration are three categories of energy in the snake model, each of which conveys essential information about the brightness depicting the given image. The standard active snake model has several drawbacks, including noise sensitivity and false contour identification in high-complexity objects. The MS model is known for the Mumford-Shah model, and it is one of the suitable methods for image segmentation based on the region [10]. It is amongst the most widely observed mathematical form that can accomplish dual goals simultaneously using a piecewise smooth representation of an image. The MS model exhibits non-convex efficiency and non-regularity of the edge phrase, causing problems during the optimized design process. MS model is also having issues concerning the complexity in computation. Variational approaches are based on minimizing an energy function that includes a border and an image field. Similar image segmentation strategies have been implemented to focus on the variational process, like classical snakes centered on gradient [11], geodesic active contours [12].

Chan and Vese develop the Chan-vese method to remove the limitations of the MS model. CV model has two energy internal and external energy functions, and the intensity in this is considered the constants [13]. This model is helpful for suitable computation. As in the CV model intensities in ROI are considered to be constants; they do not help segment the inhomogeneous regions. The chan-vese method is used in [14] in association with the fuzzy contour for accurately extracting contour. For image segmentation, [15] uses a hybrid approach that combines a gravitational search algorithm and a chan-vese model. Image segmentation of inhomogeneous images is a drawback of the Chan vase model. One of several active contour-dependent local binary fitting models is proposed in [16] to address the

problem of intensity inhomogeneity. Inside a local window in which the present pixel is located in the middle, rather than the entire inner or outer region of interest, LBF considers the grey value to become fixed. Various authors have worked on the variation of the LBF model; in [17] Gaussian distance is used instead of using the square distance because it will help adjust the value with the discrepancy in the existing pixel. In [18] author has introduced a method beneath a forced energy minimization outline. They have applied two different steps to prevent contour from being stuck in the local minima, and the next is reducing the computational cost.

In this work, the main contribution involves is in the hybrid model proposed for mass segmentation in mammograms based on the adaptive local fitting model and weight coefficient, As per the analysis done it is clear that the use of local region and global region model can be done for restricting the boundary of the tumor correctly. Combining the effect of the local and global regions concerning the adaptive local fitting model parameterized with the weight coefficient will help segment the tiny region of inhomogeneous images. The remaining paper is planned as follows. Here, Section 2. Describes the related work while section 3. Describes the methodology relating to the novel segmentation approach, Experimental results represent the results obtained in sections 4 and section 5. Discussion is given representing comparative results. , finally, in section 6, the conclusion is shown.

RELATED WORK

Chan-Vese Method(CV method)

Chan-Vese Active Contours is among the approaches for segmenting mammograms without looking for edges. A level set function is used to describe the segmentation border automatically, allowing segmentation to accommodate topological changes more efficiently than explicit snake approaches. This will be useful in minimizing the energy function. The basic idea behind this is to split the image into two dissimilar parts. CV model overcomes the problems of the Mumford Shah model.

$$E_{CV}(C, C_1, C_2) = \mu.Length(C) + v.Area\big(in(C)\big) + \lambda_1 \int_{in(C)} (\mu_0(x,y) - c_1(C))^2 dxdy + \lambda_2 \int_{out(C)} (\mu_0(x,y) - c_2(C))^2 \text{dx dy} \quad (1)$$

Chan-vese model consists of internal energy and external energy. In CV model, the intensities in both of the regions are considered to be constants, but if work is done on the inhomogeneous image, then CV model will not work properly because of the assumption made for constant intensity.

Local Binary Fitting Model (LBF model):

Chan -Vese model is in demand for its effective use and simple calculation. But this will not deal properly with the inhomogeneous images. In [16] a method is proposed which deal with the intensity inhomogeneity. This model is a local binary fitting model which concentrates on the local information of the image, which will help them in handling inhomogeneous images. The energy function in LBF is given as

$$E^{LBF} = \int \sum_{i=1}^{N} \Big(K_i \int w(x-y) |I(y) - f_i(x)|^2 M_i\big(\varphi(y)\big) dy \Big) dx + \beta \int \frac{1}{2} (|\nabla\varphi(x) - 1)^2 \, dx + v \int \delta\big(\varphi(x)\big) |\nabla\varphi(x)| dx \quad (2)$$

The major thought of the LBF model lie down in $f_i(x)$which is presumed to be constant in the local region compared with the Chen-Vese model where it considers it constant in all regions in and out.

Local linear binary fitting model (LLBF)

A variant approach based on adjustment in the fitting term is proposed in [19] based on the definition of the LBF model. In the case of LLBF, we have $b(x)c_i$, and in the case of LBF, we have $f_i(x)$for approaching I(y). Since the

intensities in real images will differ drastically even in a small area, it's better to match the local intensities with a variable matrix rather than a fixed one.

$$E^{LLBF} = \int \sum_{i=1}^{N} (\int w(x-y)|I(y) - b(x)c_i|^2 M_i(\varphi(y))dy)dx + \beta \int \frac{1}{2}(|\nabla\varphi(x) - 1)^2 dx + v \int \delta(\varphi(x))|\nabla\varphi(x)|dx \quad (3)$$

METHODOLOGY

Image segmentation is a basic and difficult topic in computer vision, meaningfully divide an image so that objects can be identified and localized. In the field of medical imaging, this is necessary for future clinical analysis, diagnosis, and treatment. Planning of Treatment and tracking illness progression are two of the most important aspects of disease management. In biomedical imaging, high accuracy is essential [20, 21].In the case of inhomogeneous images the hypothesis of having the intensity of the outer and inner region of interest to be two different constants.

Adaptive Local Fitting model

In the case of inhomogeneous images, the hypothesis is of having constant terms for the inner and outer energy in the chan-vese model will not support hence local binary fitting is useful in minimizing the power of intensity of inhomogeneous images. As in the case of some tiny regions, pixel intensity differs. so it is confirmed that in LBF it will reject the local information. in the case of local linear binary fitting (LLBF) uses constant terms but still in some real-world images in tiny areas intensity will not be constant so LBF and LLBF will not be useful in segmentation of inhomogeneous images. In [22] Author has worked on an approach for inhomogeneous image segmentation using the adaptive local fitting method. In the case of equations 2) and 3) the inner terms of LBF and LLBF can be shown as:

$$\int w(x-y)\,|I(y) - f_i(x)|^2 dy \quad 4)$$

$$\int w(x-y)\,|I(y) - b(x)c_i|^2 dy \quad 5)$$

both of the equations are minimized for the constant values C but as it is not useful so $f_i(x)$ and $b(x)c_i$ are replaced with the following term.

$$\mu_i(x) + \lambda(x).\sigma(y) \quad 6)$$

Where $\mu_i(x)$ is considered to be mean intensity value $\sigma(y)$ is representing the deviation degree while $\lambda(x)$ is taken as a multiplier for adjusting the deviation. The adaptive local fitting can be represented as below

$$\boldsymbol{E^{ALF}} = \int \sum_{i=1}^{N} (K_i \int w^x(x-y)|I(y) - \mu_i(x) - \lambda(x).\sigma(y)|^2 \; X \; M_i(\varphi(y))dy)dx + \beta \int \frac{1}{2}(|\nabla\varphi(x) - 1)^2 dx + v \int \delta(\varphi(x))|\nabla\varphi(x)|dx \quad 7)$$

Weight coefficient Adaptive Local Fitting model (WC-ALFAC)

Based on the segmentation applications and image types, many methods have been suggested [23]. Over the last two decades, the active contour model (ACM) has become one of the most widely used unsupervised segmentation approaches. The ACM operating concept is based on two primary constraints: external and internal energy. It may be divided into two types: edge-based and region-based. In case, region-based methods are further divided into two different categories one is local region-based and the other is a global region-based method. Most of the authors have worked earlier on various approaches like chan-vese, LBF, LLBF, although these approaches work well for segmenting images, some were not good for inhomogeneous images and it is very dependent on the initial contour location. In the case of local image fitting (LIF) model have used the sliding window method for minimizing limitations in earlier methods but still in some images have intensity fluctuations [24], ACMs with several object

characteristics give greater segmentation accuracy than LR or GR alone [25]. To segment the image having intensity inhomogeneity the energy function can be represented as follows.

Proposed_HybridF = F_{hybr} + V. A(Φ) 8)

F_{hybr} is a hybrid energy function that includes both global and local energy Fitting models. and A is the area term that is utilized to accelerate the contour evolution process, and v is a positive coefficient that is used to penalize the area term. The hybrid function F_{hybr} can be represented as

$$F_{hybr} = \int \frac{w}{(1-w)}(I - I_b LFI)\,dx + \frac{w}{(1-w)}(I - I_{GFI})dx \quad 9)$$

In equation **9)** I_bLFI And I_{GFI} Represents the Local fitting and global fitting model. Here w is the scaling parameter. here w can be represented as w = avg (C_N). (1-C_N), here C_N represents how the pixel intensity is going to be changing for the local window of size N. Where CN = $\frac{I_{max} - I_{min}}{I_g}$, here max and min are intensity levels within the window.

It is considered that the global energy models which are built include homogenous intensity and due to which they are not able to capture the required object in the inhomogeneous images. Local energy fitting models, on the other hand, can handle inhomogeneous pictures but are inefficient in terms of time. As a result, both models have their own set of compromises.

level set function can be given as

$$\Phi_t = 0 = \begin{cases} -p, & x \in \Omega_0 \\ 0, & x \epsilon \sigma \Omega_0 \\ p, & x \epsilon\ \Omega - x \epsilon \Omega_0 \end{cases} \quad 10)$$

As shown in earlier work, the bias field is reliant on the initial location of the contour. For the same reason, the bias field is taken as follows to make it independent of the starting position. Where b_0 is the new bias field initialization, K the Gaussian kernel, and N0 denotes the average of picture intensities.

$$\boldsymbol{b_0 = K_\sigma\left(\frac{1}{N_0}\right)} \quad \textbf{11)}$$

$$\left(\frac{w}{1-w}\right)\boldsymbol{b(x)\delta(\varphi)(m1 + m2)(I(x) - I_{LIF}) - v\,\delta(\varphi) + \left(\frac{w}{1-w}\right)\delta(\varphi)\,(I(x) - I_{GFI})(a1 + a2)} \quad \textbf{12)}$$

Overall the minimization process and steps involved are shown as follows.

Algorithm: for the proposed hybrid method WC-ALFAC method

step1: Set up the initial value of , K_i, ε, β,λ, l the iteration number T and i=1
step 2: Update the level set function by Eq. 10);
step 3: Set the new bias field initialization with 11);
step 4: Apply Equation 7)
step 5: Apply Equation 12) to obtain the new value of $\boldsymbol{\varphi}$
if $i < T$, $i = i + 1$, return to step2; else output $\boldsymbol{\varphi}$.

RESULTS

In this section performance evaluation of the work is given based on the specified hybrid approach. To evaluate the results two databases are used one is MIAS [26] which is also called mini MIAS, and the second is DDSM [27].

Figure 1 represents the comparative results based on various state of art methods using some of the sample images taken from the MIAS database these are shown in the first column as (mdb028, mdb072, mdb115, mdb178, mdb256), in next columns segmented region is shown respect to the methods used like LBF [16], LLBF [19], ALFAC [22], and WC-ALFAC. Final column d) shows a segmented region of the proposed method i.e. WC-ALFAC. Results of the proposed work are also evaluated using some performance measurement parameters such as Precision, Specificity, Recall, and Accuracy. In the following table, they are represented. Table 1 is represented in the following chart. which represents that WC-ALFAC is best among other methods. The DDSM database is also used to assess the planned work. Images are captured in MLO and CC views. We utilized CC view images in this work. It includes images of all sorts of cancers, the proposed studies are compared to several approaches using DDSM images, and performance metrics parameters are used to assess the results. Figure 3 shows the comparative analysis for these images. In the case of DDSM ground truth is available. based on the actual output for the segmented region and ground truth we can compare the results based on some performance for quality of segmentation for that purpose we are using which are true positive rate, false-positive rate, precision, Jaccard similarity rate, and Dice coefficient rate. In measurement, of values, true positive rate, precision, Jaccard similarity rate, and Dice coefficient rate should have high values while false positive rate should be low.

DISCUSSION

To emphasize the effectiveness of the effort for segmentation, Figure 4. depict the relative results for input image (mdb271) which is taken from mini MIAS database, this image is of an ill-defined type after that various method like LBF [16], LLBF [19], ALFAC [22], WC-ALFAC have been applied for this image and the results are shown in figure 5. By these methods, it is clear that the segmented region extracted with the help of the proposed method is having a more accurate boundary of the tumor region found. To further examine the performance of the proposed method WC-ALFAC, the image is taken from the DDSM image dataset and is compared with all state of art methods; Figure 6 shows the output for all segmented regions with ground truth.

CONCLUSION

As per the literature survey done it is found that previous methods are not efficient in segmenting the inhomogeneous images in tiny regions. In this work, a novel hybrid approach is proposed based on an Adaptive local fitting model along with weights assigned to local and global region parts to adjust the two parts. In this, a new bias field was added for making the system independent of the initial contour position. The performance of the proposed model work is evaluated based on two databases MIAS and DDSM. Results are carried out based on recall, precision, specificity, f-score, and accuracy while segmentation performance is evaluated using Jaccard similarity rate, and Dice coefficient. Results show that the accuracy (91.77 %) of the proposed work is better than other state of art methods. Future work will be focused on increasing the efficiency of the proposed work.

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Conflict of Interest

The authors state that they have no conflict of interest.

Compliance with Ethical Standards

Ethical approval does not arise for this article as it does not contain any studies with human participants or animals performed by any of the authors. The authors declare that they have no conflict of interest.

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Table 1. Performance evaluation of the MIAS dataset.

Methods/ Parameters	RECALL	PRECISION	SPECIFICITY	F_SCORE	ACCURACY
WC-ALFAC (proposed)	0.7141	0.7664	0.942	0.6884	0.9177
ALF_AC	0.6454	0.6391	0.925	0.6319	0.8790
LLBF	0.6944	0.7054	0.9362	0.6822	0.8974
LBF	0.7141	0.7282	0.9401	0.7068	0.9036

Table 2 Values representing five different evaluation matrices for different methods

Method	True Positive (TP)	False-positive (FP)	Precision (P)	Jaccard similarity (JCS)	Dice Coefficient (DC)
Proposed Algorithm	0.89±0.09	0.19±0.07	0.84±0.06	0.74±0.02	0.81±0.01
ALF_AC	0.87±0.09	0.20±0.07	0.82±0.06	0.71±0.02	0.80±0.01
LLBF	0.90±0.09	0.49±0.30	0.68±0.08	0.63±0.05	0.77±0.03
LBF	0.85±0.08	0.27±0.04	0.75±0.03	0.65±0.06	0.78±0.04

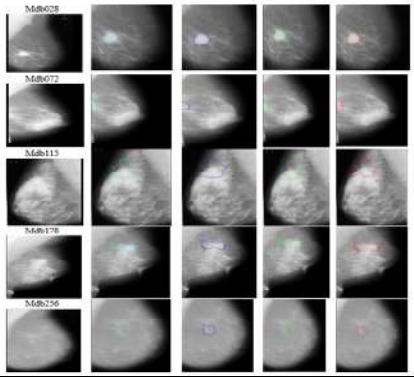

Figure. 1. Input Image (MIAS), segmented region obtain using a) LBF b) LLBF c) ALF-AC d) WC-ALFAC

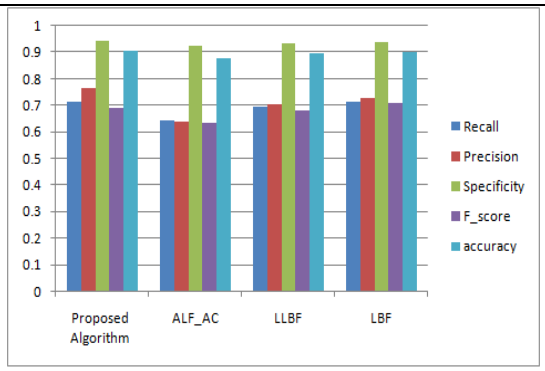

Figure 2: Chart representing comparative results

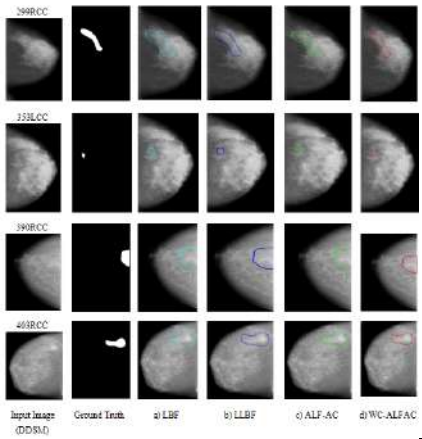

Figure. 3. Input Image (DDSM), Ground Truth and segmented region obtain using a) LBF b) LLBF c) ALF-AC d) WC-ALFAC

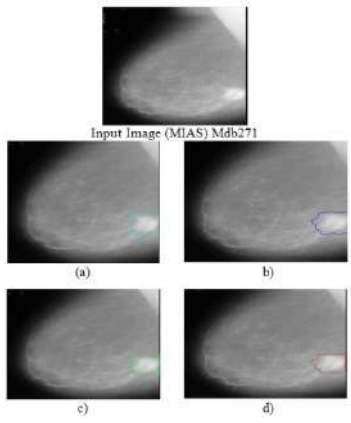

Figure. 4 results for [mdb271] image from MIAS database showing a) LBF b) LLBF c) ALF-AC d) WC-ALFAC

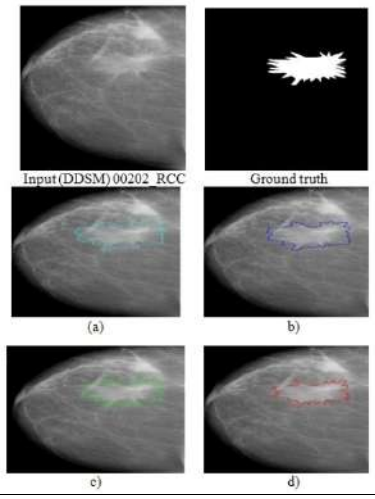

Figure. 5 results for [Input DDSM 00202_RCC] image from DDSM database showing input image, Ground truth, a) LBF b) LLBF c) ALF-AC d) WC-ALFAC

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RESEARCH ARTICLE

Induced Breeding of Fringed Lipped Peninsula Carp, *Labeo fimbriatus* at Bhadra Fish Seed Farm, Karnataka: A Case Study

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Shivamogga, Karnataka, India

ABSTRACT

The present study deals with the induced breeding of *Labeo fimbriatus* at Bhadra fish seed farm of Karnataka. Hypophysation is a scientific approach to the modem fish breeding procedure where riped brood fishes are stimulated to release the eggs by pituitary or any other synthetic hormone to breed in captive condition. In this study, Ovatide to be used for induced breeding of Kemmenu, *Labeo fimbriatus*. A total of 4 sets of female fishes were used for induced breeding with application of ovatide in August and September 2008 at Bhadra fish seed farm, Karnataka. The fertilization rate ranged from 88-93% and hatching rate varied from 75-88% respectively. A total of 1.45 to 7.36 lakh eggs were released. Number of spawns ranged from 1,00,000 to 5,00,000 with a survival rate of 68-77%.

Keywords: Induced breeding, Kemmenu, Ovatide hormone, Bhadra fish seed farm**.**

INTRODUCTION

Labeo fimbriatus is a cyprinid fish and it is the native from Pakistan, India, Nepal, Bangladesh and Myanmar. It is categorised as Least Concern as per the IUCN Red List of threatened species globally (Dahanukar,2011).. Fish reproduction is a periodic phenomenon and is managed with the aid of using environmental (exogenous) in addition to internal (endogenous) regulatory mechanism. The act of breeding happens below most desirable environmental situations which are beneficial to the survival of the younger ones. Environmental stimuli are detected with the aid of using sensory organs, relayed to brain-that triggers endogenous mechanism into action. Endogenous mechanism is mediated thru cascade of numerous neurotransmitters and hormones secreted with the

aid of using tissues of brain-hypothalamus-pituitary-gonadal axis. The secretions of above axis are regulated thru effective and bad remarks mechanisms related to particularly hormone receptors. The maximum vital reproductive neuro-hormones are hypothalamic gonadotropin-releasing hormones (GnRH) and gonadotropin-release-inhibiting factors (hormones) (GRIF or GnRIH) that alter secretions of pituitary gonadotropin hormones (GtH) which in turn, alter the synthesis of gonadal steroids chargeable for very last maturation of gametes. An suitable environmental stimulus can also additionally sign the advent of most desirable situations for the fry, triggering spawning i.e. spermiation and ovulation. Fish in captivity won't constantly reproduce on the maximum favorable time. In this situation, hormones play a important function within the reproductive processes. Hormone-precipitated spawning strategies impact this sequential mechanism at numerous steps, both with the aid of using selling or inhibiting the process. Induced duplicate in fish consists of fundamental techniques. The first is the manipulation of lifestyle surroundings to imitate vital traits of natural spawning surroundings of that unique fish. However, latest researches and business aquaculture practices recommend the emergence of traces of hormone induced spawning because the fine for a hit breeding as a minimum expense. These are i) injection or oral management of GnRH analog (LHRH analog) with dopamine antagonist, and ii) injection of purified gonadotropin (e.g. human chorionic gonadotropin-HCG) both or combined with not unusual place carp pituitary extract to enhance its potency. In spite of those, software of steroids, pheromones and prostaglandins have been additionally used, as a brand new rising and much less studied area and required greater studies earlier than its business software to gain captive spawning in majority of cultured fishes (Ram Singh and Akhilesh Kumar Gupta, 2011).

The approach of induced breeding offers very promising bring about fishery factor of view as it supply natural spawn of positive species of fishes below cultivation. Spawn amassed from herbal water isn't always natural as due to the fact a few unwanted wild species can also additionally include them in lifestyle pond. Sorting of natural seed is pretty not possible in the ones ranges. In later ranges it's far possible, however time consuming. It assures well timed to be had of natural seed, wherein as in nature the provision of seed is pretty unsure. It can fulfil any amount of call for in any time. Many carps take their complete adulthood in limited water however do now no longer breed. The approach is quite simple and does now no longer want an excessive amount of technical help or knowledge. It may be without problems learnt with the aid of using a layman with out lots training. The fee of expenditure may be very low than the herbal collections of spawns. This paper provides information on the hypophysation of cyprinid carp, *Labeo fimbriatus* and this study may support the fish farmers who are involved in aquaculture fisheries or hatchery management as well as to the researcher and students who need to understand the scientific method of fish breeding.

MATERIALS AND METHODS

Study Area

The present studied fish seed farm of Karnataka is positioned at 13º 41' N latitude and 75º 38' E longitude. Bhadra fish seed farm hatchery unit consisting of 04 parts, i.e., spawning pool, incubation pools, egg/spawn collection tank and overhead water storage tank. This hatchery unit is situated in Bhadravathi taluk of Shivamogga district of Karnataka for operation and seed production (Figure 2). Water circulation is given in the breeding pool with elbows fitted with carrying nipples. These are fitted in the same direction. A water inlet is also fitted in the sidewall of the pool bottom. All the water inlet pipes are interconnected with individual full-way valves to regulate the flow of water. A water shower is provided on the top margin of the pool for water showering. The pool has drainage outlets at the center and also at the lower part of the outer chamber of the pool. Matching to the tank size, the nylon inner hapa is used inside it to collect egg/spawn from spawning/ incubation pool, respectively. Generally, spawns were collected from the incubation pool on 4th day of fish spawning (Partha Chakrabarti et al.,2017). Ovatide is an native, cheaper and new hormonal formulation for induced breeding of fishes. It has low viscosity injectable solution and commercially available as spawning agents (Anita Jhajhria,2017).

The present minor carp breeding experiment was conducted in the Bhadra fish seed hatchery installed at BRP Village during August/September 2008. Normal hatchery operation practice was followed for production of fish seed (Mohapatra et al.,2005; 2007,2008). Selected brooders were then carefully transferred to the hatchery avoiding much handling and conditioned for one hour in the spawning pool prior to administration of inducing agent, Male and female brooders were injected with ovatide at the rate of 0.2-0.25 ml/kg and 0.3-0.4 ml/kg body weight, respectively, into the intra-peritoneal region of the fish in a single dose. After egg and milt release, the brood fishes were removed from the breeding/spawning pool with the help of a scoop net. Water circulation was maintained as per recommendation of Mohanty et al (2009). The flow rate of water during egg collection was maintained and the flow rate in the pool during operation was maintained.

Fertilization percentage for female was calculated as per Adebayo and Popoola (2008).

Fertilization = Number of fertilized eggs/ Total Number of eggs × 100

Hatchability percentage was determined according to Haniffa and Sridhar (2002).

Hatchability = Number of hatchlings / Total Number of fertilized egg × 100

Data Collection and Statistical Analysis

The data was collected from the Department of Fisheries at Bhadra fish seed farm of Karnataka and the data is compiled in the form of scientific paper. One-way ANOVA with post-hoc Tukey HSD with Scheffé, Bonferroni and Holm multiple comparison tests for fertilization, hatching, dose of the hormones for male and female fishes were carried out by using astatsa.com software.

RESULTS AND DISCUSSION

The results are appended in Table 1, 2-6. The fertilization rate ranged from 88-93% and hatching rate varied from 75-88% respectively. A total of 1.45 to 7.36 lakh eggs were released. Number of spawns ranged from 1,00,000 to 5,00,000 with a survival rate of 68-77%. The total length of the fishes ranged from 21-32 cm for males and 22-35cm for females. While, the total weight of fishes varied from 400-1000gm and 500-1250gm for male and females respectively. The dose of the ovatide ranged 0.20-0.25 ml/kg for males and 0.3-0.40 ml/kg for females. Table 3 depicts the physico-chemical characteristics of brooders pond water. The water temperature ranged from 21 to 29^{O}C. While ,pH of the brooders pond was alkaline in nature. The dissolved oxygen level varied from 4.70- 6.8 mg/l and CO_2 content ranged between 2.4 and 12.4 mg/l respectively. Total alkalinity fluctuated from 88 to 137 mg/l. However, the hardness values varied from 76 to 144.5 mg/l. BOD level ranged from 0.40 to 1.8 mg/l. Water parameters were within the suitable range for induced breeding of *Labeo fimbriatus.*

In *Anabas testudineus* (Bloch) the time taken for spawning response was found to be within a narrower range of 11.30 - 12 hours among group II fishes and 10 - 12 hours among group III fishes (Pius, 2010). Pius (2010) observed, a dosage of 1.0 ml kg^{-1} B.W. of ovaprim may be considered as a standard for induced breeding of *A.testudineus*. Kuldeep Kumar *el.al.,* (2010) conducted induced spawning experiments on *Anabas testudineus* during pre-monsoon and monsoon months . However, in spite of higher breeding response (80-100%) and egg production (295.7 - 374.2 g^{-1}) recorded during March to June, the higher larvae production (186 - 233.8 g^{-1}) could be obtained during May to July. Their study revealed that ovaprim @ 1.5 ml/kg body weight efficiently induced *Anabas testudineus* female for normal spawning.

Shankar Murthy *et al* (2013) have reviewed the induced breeding of Indian major, minor carps and cyprinid fishes of India by various hormonal analogues based on published literature. They reported the comparative efficiency of synthetic hormones viz., ovaprim , ovopel and ovatide used for the induced breeding of fishes revealed the breeding performance resulting in high fecundity and fertilization rate.

Strict enforcement of fishing rules to prevent fishing with explosives, poisoning, etc. Prevention of killing of brood fish and juveniles. Replenishment of stock by artificial propagation and fish farm should be constructed in close proximity of every dam, Few ponds should be held in reserve in each farm.

Tukey HSD Test

The p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.01 which strongly suggests that one or more pairs of treatments are significantly different.

CONCLUSION

Efforts have to be made to reproduce *Labeo fimbriatus* on a huge scale. Once the fish seed is to be had nation governments of India and fish farmers can use fish fingerlings for river ranching, raceway ponds and walking water subculture. Hatchlings ought to be grown to the fingerling length after which launched into reservoirs and downstream rivers. Thus if the recommended that remedial measures are applied in degrees the effective clean water fishes of India may be restored to its glory lots to the pride of anglers and scientists within side the country. Many species of fish will now no longer effortlessly reproduce beneath positive subculture conditions. Others will, however now no longer always whilst the farmer desires. In those cases, induction of spawning may be of incredible value. Methods range from species to species. However, as a minimum generalizations may be drawn. First, brooders are very liable to tough handling. Care must to continually be used to keep away from unfavourable those treasured animals. Second, a fish that doesn't have mature gametes will now no longer produce feasible eggs or sperm irrespective of how in many instances it's far injected with hormones. Ripeness is the end result of environmental elements running over a length of time, main to maturation of the gonads and manufacturing of feasible eggs. Many processes had been evolved for inducing fish to go through the closing steps of spawning. Farmers ought to very well studies the processes which have been evolved for his or her species of fish via experimentation, and pick out people who high-satisfactory in shape the circumstances. In addition, as soon as the fish have spawned, there are numerous strategies concerned in incubating and worrying for the eggs, and worrying for the hatched fry. These too have to be very well researched (Minnesota Sea Grant, 2008). The above findings of accumulated literature endorse the utility of artificial hormones for inducing ovulation and a success breeding and attaining high-satisfactory egg and larval manufacturing for a success fish cultivation in India.

Fish hatchery operators have to study on higher brood inventory control, hatchery control and nursery control to supply high-satisfactory fish seed. More emphasis have to to be laid on a couple of spawning of carps as a way to make certain the supply of seed over an extended period in a year for sustainable fish seed Production. The above findings of accumulated literature endorse the utility of artificial hormones for inducing ovulation and a success breeding and attaining high-satisfactory egg and larval production for a success minor carp cultivation in India.

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Table 1 : One-way ANOVA, Post-hoc Tukey HSD Test, Scheffé, Bonferroni and Holm multiple comparison for Fertilization, Hatching and Dose of the Ovatide hormone for *Labeo fimbriatus*

Treatment →	% of fertilization	% of hatching	Dose of hormone for males	Dose of hormone for females	Pooled Total
N	8	8	8	8	32
sum	708.0000	659.0000	1.8000	2.8000	1,371.6000
mean	88.5000	82.3750	0.2250	0.3500	42.8625
sum of squares	62,844.0000	54,415.0000	0.4100	1.0000	117,260.4100
sample variance	26.5714	18.5536	0.0007	0.0029	1,886.1356
sample std. dev.	5.1547	4.3074	0.0267	0.0535	43.4297
std. dev. of mean	1.8225	1.5229	0.0094	0.0189	7.6774

Table 2 : One-way ANOVA of independent treatments:

Source	Sum of squares SS	Degrees of freedom ν	Mean square MS	F statistic	p-value
Treatment	58,154.3050	3	19,384.7683	1,718.1814	1.1102e-16
Error	315.9000	28	11.2821		
Total	58,470.2050	31			

Table 3 : Tukey HSD Test

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	5.1577	0.0055865	** p<0.01
A vs C	74.3339	0.0010053	** p<0.01
A vs D	74.2286	0.0010053	** p<0.01
B vs C	69.1762	0.0010053	** p<0.01
B vs D	69.0710	0.0010053	** p<0.01
C vs D	0.1053	0.8999947	insignificant

Table 4 : Scheffe multiple comparison

Treatments pair	Scheffé T-statistic	Scheffé p-value	Scheffé inference
A vs B	3.6470	0.0113625	* p<0.05
A vs C	52.5620	1.1102e-16	** p<0.01
A vs D	52.4876	1.1102e-16	** p<0.01
B vs C	48.9150	1.1102e-16	** p<0.01
B vs D	48.8405	1.1102e-16	** p<0.01
C vs D	0.0744	0.9998876	insignificant

Table 5 : Bonferroni and Holm results: all pairs are simultaneously compared

Treatments pair	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni inference	Holm p-value	Holm inference
A vs B	3.6470	0.0064395	** p<0.01	0.0021465	** p<0.01
A vs C	52.5620	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
A vs D	52.4876	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
B vs C	48.9150	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
B vs D	48.8405	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
C vs D	0.0744	5.6471878	insignificant	0.9411980	insignificant

Table 6 : Bonferroni and Holm results: pairs relative to Fertilization simultaneously compared

Treatments pair	Bonferroni and HolmTT-statistic	Bonferroni p-value	Bonferroni inference	Holm p-value	Holm inference
A vs B	3.6470	0.0032198	** p<0.01	0.0010733	** p<0.01
A vs C	52.5620	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01
A vs D	52.4876	0.0000e+00	** p<0.01	0.0000e+00	** p<0.01

Table 7: Length-weight data *Labeo fimbratus* brooders in experimental ponds of Bhadra fish seed farm

Date	Total length (cm)		Total weight (gm)	
	Male	Female	Male	Female
25/08/2008	32	32	1000	1200
	27	33	750	1250
	25	33	750	1250
	28	--	750	--
27/08/2008	25	30	750	1000
	26	25	750	750
	27	27	750	750
	26	25	750	750
	21	--	500	--
	21	--	500	--
04/09/2008	32	30	1000	1000
	23	33	500	1250
	31	34	1000	1250
	32	33	1000	1250
	23	32	500	1000
	27	35	500	1250
	22	--	500	--
04/09/2009	23	25	500	750
	22	28	500	1000
	23	22	500	500
	22	24	500	750
	22	23	500	500
	22	--	500	--
	21	--	400	--
	22	--	500	--

Table 8: Experiments on some threatened fish species in India

Fish species	Hormone used	Sex ratio M:F	Dose ml/kg	Hatching %
Ompok pabo	Ovatide	2:1	3.0	70
Mystus vittatus	Ovatide	2:1	8.0	80
Clarias batrachus	Ovaprim	1:1	2.5 (♀)	50

(Source: Mijkherjee *et.al.*, (2002))

Table 9: Physico-chemical characteristics of Brooders pond water during 2008-09

Months	Water temperature (°C)	pH	DO	Free CO_2	Total alkalinity	Hardness	BOD
April	28	7.3	4.70	12.4	88.0	76.0	1.50
May	29	7.4	4.85	8.8	127.0	98.4	1.80
June	24	7.3	6.6	8.6	102.0	106.0	0.40
July	22	8.0	6.8	5.4	137.0	144.5	0.50
August	21	7.5	5.8	2.4	90.0	112	1.10
September	22	7.8	6.10	6.2	96.2	88.5	0.60

Table 10:Fecundity, fertilization and hatching percentage of *Labeo fimbrialus*

Date	Fecundity	% of fertilization	% of hatching	Water temperature (°C)	Air temperature (°C)	Dose of hormone ml/kg body weight	
						Male	female
25/08/2008		80-88	75-80	22.5-24	26-27	0.20-0.25	0.3-0.4
27/08/2008		85-90	80-85	22-24	26-27.5	0.20-0.25	0.3-0.40
04/09/2008		90-95	85-88	23-24	27-28.5	0.20-0.25	0.30-0.40
04/09/2009		85-95	80-86	23-24.5	27-29	0.2-0.25	0.30-0.40

Table. 11: Breeding performance & spawning response of *Labeo fimbriatus* to ovatide

Experiment date	Age of brooder (year)	Number of brooders		Weight of brooders		Fertilization rate (%)	Total No. of eggs (Lakhs)	Total No. of 3 day old spawn (Lakh)	Remarks
		♂	♀	♂	♀				
25/08/2008	♂1.5-2 ♀2-3	03	04	3.25	3.75	88	3.64	2.80	
27/08/2008	♂1.5-2.5 ♀1.5-2	06	02	4.0	2.50	90	1.45	1.0	
04/09/2008	♂1.5-2.0 ♀2-3	07	07	5.0	8.25	93	7.36	5.0	
04/09/2009	♂1.5 ♀1.5-2.0	08	05	4.0	3.5	-	-		a) No breeding since existing brooders were partially good for breeding. b) Environmental factors and hot weather

Table 12: Average weight of brooders before and after spawning

Date	Brooders	Range of average weight (gm) before spawning	Range of average weight (gm) after spawning	Number of spawn (Lakh)	% of survival of spawn
25/08/2008	Male (♂)	750-1000	748-995	2.80	77
	Female (♀)	1200-1250	1100-1205		
27/08/2008	♂	500-750	495-746	1.0	69
	♀	750-1000	700-950		
04/09/2008	♂	500-1000	498-980	5.0	68
	♀	1000-1250	948-1190		
04/09/2009	♂	400-500	394-490	--	--
	♀	500-1000	450-940		

Table 13: Spawning response of fishes to ovaprim, Wova F.H. and ovatide hormones

Species	Hormone dose	Fertilization rate (%)	Hatching rate (%)	References
Anabas testudineus	Ovaprim (1.0 ml)	96.37 ± 4.12	96.53 ± 4.88	Pius (2010)
Esomus danricus	Ovatide (1.0 ml)	85.0 (Dry)	--	Jyoti *et.al.* (2010)
Catla catla	Ovaprim (0.4-0.6)	94.20	92.05	More *et.al.* (2010)
Labeo rohita	Ovaprim (0.4-0.6)	94.06	91.36	More *et.al.* (2010)
Cirrhinus mrigala	Ovaprim (0.4-0.6)	92.89	88.34	More *et.al.* (2010)
Nandus nandus	Ovaprim (0.3 ml)	85 ± 3.2	57 ± 3.8	Sarkar *et.al.* (2009)
Nandus nandus	Wova- FH (0.3 ml)	80 ± 2.8	90 ± 4.0	Sarkar *et.al.* (2009)
Anabas testudineus	Ovaprim (2.0 ml)	73.11	92.06	Bhattacharyya and Home Choudhuri (2009)

Table 14:Comparative account on induced breeding by ovatide and ovaprim in different fish species in India

Species	Ovatide dose (m/kg^{-1} BW)	Latency period(hr)	Reference
Gibelien Catla	0.2-0.5	7.4	Thakur & Reddy (1997)
Cirrhinus mrigala	0.2-0.4	9.32	Thakur & Reddy (1997)
Ctenopharyngoden idella	0.2-0.65	10.5	Thakur & Reddy (1997)
Labeo calbasu	0.3-0.5	6.0	Thakur & Reddy (1997)
Heteropneustes fossilis	0.4	10.0	Marimutthu *et.al.* (2000)
Puntius javanicus	0.3-0.6	8.0	Thakur & Reddy (1997)
Channa striatus	0.4	24.0	Marimuttu & Haniffa (2007)
Esomus danricus	1.0	--	Jyoti *et.al.* (2010)
Channa punctatus	0.4	25-31.0	Marimutthu *et.al.* (2009)
Clarias batrachus	0.6-1.0	14-16	Yadav *et.al.* (2011)
	Ovaprim dose	**Latency**	

	(m/kg^{-1} BW)	**period (hr)**	
Anabas testudineus	1.5	20	Kuldeep Kumar *et.al.* (2010)
Anabas testudineus	1.0	10-12	Pius (2010)
Catla catla	0.2-0.6	10-12	More et.al., (2010)
Cirrhinus mrigala	0.2-0.6	10-12	More et.al., (2010)
Clarias batrachus	0.8-1.0	14-16	Yadav et.al., (2011)
Nandus nandus	0.3	7-9	Sarkar et.al., (2009)
Anabas testudineus	2 ml	10-12	Bhatta Charyya and Home Choudhuri (2009)

Figure 1: *Labeo fimbriatus* fish

Figure 2: Fish hatchery used for induced breeding of *Labeo fimbriatus*

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RESEARCH ARTICLE

Fern Biodiversity of Phalgam, District Anantnag, Kashmir, India

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ABSTRACT

The Present study was carried out in Phalgam, Anantnag district of Jammu and Kashmir, India during the year 2021. The region is located on the banks of Lidder River at an altitude of 7,200 feet, within the coordinates of 34.01°N and 75.19°E. The area is surrounded by lofty mountains which contain great variety of fern and their allies. Therefore, the current study was aimed to undertake deep systematic survey of different habitats of the area. A total of 25 species of ferns were collected from the area, out of which 17 species were identified of various families like *Pteridaceae, Aspleniaceae Cystopteridaceae and Dryopteridaceae* etc. A list of fern and their allies along with nomenclature, description, synonym, distribution and habitat have been provided here.

Key Words: Ferns, Habitat, Identification, Kashmir Valley, Phalgam

INTRODUCTION

Ferns are the second largest group of vascular plants, consist of about 10,000 dwelling species. Ferns attained remarkable levels of variety and abundance from the carboniferous period to the Jurassic period (3000 – 150 million years ago) (Skog, 2001). There are about 34 families, 144 genera and more than 1100 species of ferns with about 235 endemic species from India (Chandra Shubhash, 2000). Ferns and Fern allies are the natural group of vegetation in an essential division of plant kingdom known as the pteridophytes. They are distributed all over the world. Ferns develop vigorously in moist, shady, tropical and temperate forests. Natural environment comprises of biotic and abiotic components. Of the biotic components, plant life plays an important position on earth's surface, besides which other living things cannot survive. About 250, million years ago fern and fern allies have been the dominant plant groups on this planet. However, they are now changed via seed bearing plants, still grow luxuriantly in moist tropical and temperate forests (Dixit, 2000). There are about 13,600 species of ferns and their allies worldwide (Moran, 2006). India as a mega diversity country has a massive and rich diversity of Fern and their allies represented by 1157 species (Fraser-Jenkins et al, 2016). After the east Indo Himalayas, the Manipur-Khasi range, and south India, the west Himalayas are the fourth richest area for pteridophytes in India, with about 402 species accounting for 40%

of the country's pteridoflora (Kumari et al. 2010). The Kashmir Himalaya, a scenic south Asian region, is a unique biospheric unit located in the Himalayan biodiversity hotspot's northwestern extremity (Rodgers and Panwar 1988).

The abundant biodiversity that adorns Kashmir's beautiful scenery is one of the key aspects contributing to its worldwide recognition (Lawrence 1895). The valley harbours almost all groups of land plants due to the vast variety of edapho-climatic and physiographic heterogeneity and diverse habitats including lakes, springs, swamps, marshes, rivers, cultivated fields, orchards, subalpine and alpine meadows, mountain slopes and terraces, permanent glaciers, and so on; many species are distinct from those found elsewhere in the country and are endemic to the valley. However, only the Phanerogams have been fully recorded in the published literature on Kashmir's flora. In the past, cryptogams, particularly pteridophytes, received minimal attention in terms of survey and inventory (Dar et al. 2002). The valley has been occasionally explored in the past for pteridophyte diversity by Clarke (1880), Beddome (1883, 1992) and Hope (1899-1904). Some isolated fern collections have also been made in this region by the botanists of botanical survey of India, like R.B. Keshavanand, T.A. Rao, P.K. Hajra, U.C. Bhattacharyya, B.M. Wadhwa, M.V. Vishwanathan, etc. (Wani et al. 2012). As a result, the current authors have set out to investigate the pteridophytic wealth and potential of fern species in the Kashmir valley, and have restricted their study to district Anantnag, which possesses the majority of topographical features of the large expanse of valley.

MATERIALS AND METHODS

The research was carried out in the Phalgam, district Anantnag Kashmir. The region is situated between 34.01°N and 75.19°E on the banks of Lidder River at an altitude of 7,200 feet. Figures b and c depict the primary research sites. As specimens were collected, photographed and numbered, the detail notes were recorded in the notebook. The collected specimens were pressed and dried in the standard way and described and identified with the relevant literature (Beddome, and Fraser- Jenkins etc.). The identities of specimens were authenticated from Department of Botany, Annamalai University.

RESULTS AND DISCUSSION

Adiantum capillus-veneris L., Sp. Pl. 2: 1096(1753).

Synonym: *Adiantum formosum* R. Br.; *A. michelii* H.Christ; *A. modestum* Underw.; *A. remyanum* Esp. Bustos; *A. schaffneri* E. Fourn.; *A. wattii* Baker. Lithophytic medium-sized fern, rhizome long-creeping, stipe castaneous black. Stem short-creeping; scales golden brown, concolored, iridescent, margins entire or occasionally with single broad tooth near base. Leaves arching or pendent, closely spaced, 15--75 cm. Petiole 0.5--1.5 mm diam., glabrous. Blade lanceolate, pinnate, 10--45 × 4--15 cm, glabrous, gradually reduced distally; proximal pinnae 3(4)-pinnate; rachis straight, glabrous. Segment stalks 0.5--3.5 mm, dark color extending into segment base. Ultimate segments various, generally cuneate or fan-shaped, about as long as broad; base broadly to narrowly cuneate; margins shallowly to deeply lobed, incisions 0.5--7 mm, apex rounded to acute. Sori covered with false, yellow or yellowish brown, broadly reniform or orbicular-reniform indusia, infrequent, altitudinal range 1700-2800 m.

Distribution: China, Japan, Vietnam and are common in temperate and tropical climates across Africa, America, Asia, Europe, Oceania, and India (throughout India).
Habitat: Rainforests, shrub and woodlands, broadleaf and coniferous forests and desert cliff seeps and springs.

Pteridaceae
Pteris cretica L., Mant. Pl. 1: 130 (1767)

Synonym: *Pteris serraria* Sw.; *P. treacheriana* Baker; *P. trifoliata* Fee; *P. triphylla* M. Martens & Galeotti; *P. nervosa* thumb. *Pycnodoria cretica* (L.) Commonly occur in open rocky meadows having dimorphic fronds, sterile fronds are usually small and bend backwards, fertile fronds have longer and stronger stipes, lamina imparipinnate, herbaceous-sub-coriaceous, pinnae lanceolate, margin spinulose-serrate, sori continuous, sub marginal covered by false indusium of reflexed pinnae margin, common from 1800-3000 m altitude.

Distribution: Afghanistan, Australia, Africa, Europe, Iran, Bhutan, Burma, China, Nepal, Sri Lanka, Pakistan, the Philippines, Korea, Taiwan, Thailand, Japan, and India (Kashmir, Himachal Pradesh, Garhwal, Kumaun, West Bengal, Assam, Nagaland, Madhya Pradesh, Tamil Nadu, and others).
Habitat: Prefer to grow in vertical habitats, such as steep hills or even actual rock walls.

Aspleniaceae
Asplenium adiantum-nigrum L. Sp. Pl. 2: 1081 (1753).

Synonym: *Asplenium andrewsii* A. Nelson; *A. chihuahuense* J. G. Baker; *A. dubiosum* Davenport.
Lithophytic medium-sized fern with evergreen fronds that grows on rocky woodlands, hedge banks, shaded walls, and rocks, stipes clustered, lustrous, lamina 2-3 pinnate, dark green, glossy, thick, coriaceous, edges acutely dentate to serrate, and a distributional range of 1700-3000 m altitude. Plants 15-40 cm tall. Rhizome ascending, apex scaly; scales dark brown, narrowly triangular, up to 6 mm. Fronds caespitose; stipe 8-15(-20) cm, base conspicuously swollen, thickened, semiterete, abaxially shiny castaneous to blackish purple, with scattered brownish black hair like scales or subglabrous, adaxially sulcate; lamina triangular-ovate, 9-25 × 4-6(-10) cm, apex acute to acuminate, tripinnate; pinnae 8-13 pairs, alternate, stalked, basal pair largest, triangular, 4-6 × 1.8-2.4 cm, base truncate, bipinnate, apex acute to acuminate; pinnules 5-8 pairs, anadromous, basal pairs largest, base cuneate-truncate, shortly stalked or subsessile, pinnate, apex acute; ultimate pinnules 4-6 pairs, alternate, anadromous, basal pair largest, elliptic to oblong or linear, 5-7 × 2.5-3.5 mm, margin serrate, apex obtuse. Costa obvious, sulcate adaxially, veins obscure, anadromously branching, simple or 2-forked, not reaching margin. Sori 1-3(or 4) pairs per pinnule or segment, median on subtending veinlet, confluent at maturity, linear, 1-2.5(-5) mm; indusia white or brownish, linear, membranous, repand to entire, opening toward costa or costule. Spores brown to dark brown, perispore lophate (cristate-alate), with average exospore length 32-37 µm. Plants sexual allotetraploids: 2*n* = 144.

Distribution: Java, Nepal, Taiwan, Turkey, Pakistan, North, Africa, Afghanistan, Europe, Japan, Iran, North America, India (Kashmir, Himachal Pradesh, Garhwal, Kumaun).
Habitat: Evergreen perennial fern occurs on a wide range of well drained, usually basic substrates, in lightly shaded habitats.

Asplenium trichomanes L. Sp. Pl. 2: 1080 (1753).

Synonym: *Asplenium melanocaulon* Will. *A. trichomanoides* Houtt.; *A. minus* Bl.; *A. pusillum* Bl.; *A. densum* Brack.; *A. melaolepis* Col. *Chamaefilix trichomanes* (L.) Farw. *Trichomanes crenatm* Gilib.; *Pylilitis rotundifolia* Moench. Lithophytic fern. Plants 10-30 cm tall. Rhizome erect, short, scaly; scales narrowly triangular, 3(-4) × 0.5 mm, with opaque, red to dark brown central stripe and paler narrow clathrate borders, entire. Fronds caespitose; stipe shiny castaneous-brown, 2-8 cm, base scaly, upward subglabrous, abaxially semiterete but adaxially grooved, with brown, membranous and subentire narrow wings, texture papery, stipe and rachis usually persisting after shedding of pinnae; lamina linear, 10-25 × 0.9-1.6 cm, base slightly reduced, 1-pinnate, apex acute and 2-4 mm wide; pinnae 20-30 pairs, usually obliquely inserted, sessile, middle pinnae elliptic or ovate to orbicular, 2.5-7.5 × 2-4 mm, base nearly symmetrical, cuneate, margin crenate, apex obtuse; lower pinnae gradually reduced. Veins pinnate, costa obscure,

veins obliquely simple or up to 2-forked, basal acroscopic vein usually 2-forked. Sori 4-8 per pinna, oval to linear, 1-3.5 mm, usually on acroscopic vein; indusium white or brown after drying, oval to linear, membranous, free margin repand to entire, opening toward costa, persistent. Spores with lophate perispore, average exospore length 27-31 µm. Plants sexual diploid: 2*n* = 72.

Distribution: worldwide in all temperate zone, in tropics on high mountains, India (Kashmir, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Sikkim, Assam, Meghalaya, Arunachal Pradesh, Manipur, Rajasthan, Tamil Nadu).

Habitat: Prefers acidic rocks such as sandstone, basalt and granite, terrestrial habitats, forested bluffs, cliffs, talus slopes etc.

Cystopteridaceae

Cystopteris fragilis (L.) Bernh. Schrad. Neues J. Bot. 1: 26 (1805)

Synonym: *Aspidium dentatum* Sw.; *A. fragile* (L.) Sw.; *A. viridulum* Desv.; *A. dentatum* (Sw.) Gray; *A. fragile* (L.) Spreng.; *A. fumarioides* C. Presl; *Cyathea anthriscifolia* (Hoffm.) Roth*; C. cynapifolia* (Hoffm.) Roth; *C. fragilis* (L.) J. Sm.; *Cystea angustata* (Hoffm.) Sm.; *C. dentate* (Dicks.) Sm.; *C. fragilis* (L.) Sm.; *Cystopteris acuta* Fee.; *C. baenitzii* Dorfl.; *C. canariensis* C. Presl.; *C. dentate* (Sw.) Desv

Morphologically similar to *Cystopteris dickieana* except in spore ornamentation, spore exine is echinate not rugose or verrucose, common from 1800-2800 m altitude. The leaves are up to 30 or 40 centimeters long and are borne on fleshy petioles. Each leaf is divided into many pairs of leaflets, each of which is subdivided into lobed segments. The underside of the leaf has many rounded sori containing the sporangia.

Distribution: Afghanistan, China, Nepal, Russia, and the United States of America

Pakistan, Japan, Iran, Africa, Europe, North America, Pakistan, Taiwan, Korea, India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Nagaland.

Habitat: Worldwide, generally in moist, shady areas, cliffs deciduous woodlands etc.

Cystopteridaceae

Gymnocarpium dryopteris (L.) Newman, Phytologist 4: 371 (1851).

Synonym: *Aspidium dryopteris* (L.) Baumgarten; *Carpogymnia dryopteris* (L.), *Currania dryopteris* (L.) , *Dryopteris dryopteris* Britton; *D. pulchella* (Salisb.) Hayek; *D. linnaeana* (L.) Medium size fern growing on damp areas and among rock crevices near moisture in forests, rhizomes long creeping, black-brown, thickly scaly, apex densely scaly, stipe base purplish, above stramineous, lamina pentagonal-ovate, veins apparent abaxially, sori exindusiate, abundant from 2000-2900 m altitude. Stems 0.5--1.5 mm diam.; scales 1--4 mm. Fertile leaves usually 12--42 cm. Petiole 9--28 cm, with sparse glandular hairs distally; scales 2--6 mm. Blade broadly deltate, 2-pinnate-pinnatifid, 3--14 cm, lax and delicate, abaxial surface and rachis glabrous or with sparse glandular hairs, adaxial surface glabrous. Pinna apex entire, rounded. Proximal pinnae 2--12 cm, perpendicular to rachis, with basiscopic pinnules, perpendicular to costa; basal basiscopic pinnule usually sessile, pinnatifid or rarely pinnate-pinnatifid, if sessile then with basal basiscopic pinnulet often equaling or longer than adjacent pinnulet. Spores 34--39 µm. 2 n = 160.

Distribution: Europe, North America, Pakistan, India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim), Japan, China, Tibet, Nepal, Korea.

Habitat: Coniferous woodlands and on shale talus slopes, moist forests, thickets and rocky slopes etc.

Dryopteridaceae

Dryopteris barbigera (T. Moore ex Hook.) Kuntze, Revis. Gen. Pl. **2**: 812 (1891).

Synonym: *Aspidium barbigerum* (T. Moore ex Hook.) H. Christ; *Dryopteris falconeri* (Hook.) Kuntze; *Lastrea barbigera* (Hook.) T. Moore ex Bedd.; *L. falconeri* (Hook.) Bedd.; *Nephrodium barbigerum* Hook.; *N. falconeri* Hook. Terrestrial fern with densely scaly and fibrillose stipe, rachis, and lamina; scales reddish brown, lamina thickly herbaceous, pinnule lobes rounded with serrate and conspicuous acute teeth, rare from 2800-4700 m height. Plants 60-80 cm tall. Rhizome

caespitose, rhizome and stipe base densely clothed with ovate or oblong-lanceolate, ferruginous scales. Fronds caespitose; stipe 20-30 cm, up to 1 cm in diam., densely clothed with scales and brown, fibrillose scales; lamina ovate or oblong-lanceolate, bipinnate-pinnatifid, not narrowed to base, apex obtuse; pinnae more than 20 pairs, lanceolate, ca. 13 × 3 cm, obtuse, shortly stalked; pinnules 20 pairs, oblong, apex rounded; segments dentate, remote, reflexed when dried. Lamina: rachis, costa, and costule all densely clothed with brown fibrillose scales and narrowly lanceolate scales; veins distinct on both surfaces. Sori 1 on each pinnule, on each side of costule; indusia ferruginous, orbicular-reniform, often early deciduous.

Distribution: India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Assam, Sikkim, West Bengal), Bhutan, Pakistan, China, Tibet, Taiwan, Nepal.
Habitat: Suitable for light (sandy), medium (loanmy) and heavy (clay), soils.

Dryopteris filix-mas (L.) Schott, Gen. Fil. , sub pl. 9 1834.

Synonym: *Aspidium depastum* Schkuhr; *A. erosum* Schkuhr; *A. expansum* D. Dietr.; *A. filix-mas* (L.) Sw.; *A. mildeanum* Gopp.; *A. nemorale* (Salisb.) Gray; *A. opizii* Wierzb.; *A. umbilicatum* (Poir.) Desv.; *A. veselskii* Hazsl. ex Domin. Terrestrial fern with long-erect rhizome, stipe grooved, scaly and fibrillose, lamina pale to mid-green above, pinnae short petiolate, pinnules lobed, lobe ending in an acute tooth, sori crowded, top portion of the lamina fertile, scarce. The bipinnate leaves consist of 20–35 pinnae on each side of the rachis. The leaves taper at both ends, with the basal pinnae about half the length of the middle pinnae. The pinules are rather blunt and equally lobed all around. The stalks are covered with orange-brown scales. On the abaxial surface of the mature blade 5 to 6 sori develop in two rows.

Distribution: Africa, China, Argentina, Afghanistan, Russia, Greenland, Malaya, Peru, Mexico, Kazakhstan, Pakistan, Russia, Iran, Jamaica, Europe, North America, India (Kashmir, Uttarakhand).
Habitat: Favours damp shaded area in the understory of woodlands, but also shady places on hedge-banks, rocks and screes.

Dryopteris intermedia: (Muhl. Ex Wild.) A. Gray TSN: 17538

Synonyms: *Polypodium intermedium, Aspidium* americanum, Aspidium intermedium, Dryopteris austriaca var. intermedia, Dryopteris spinulosa var. concordiana, Dryopteris spinulosa var. intermedia, Thelypterisspinulosa var. Intermedia.
It is a perennial fern that reaches a height of 40 to 90 cm and a width of 60 to 90 cm. Its base is comprised of an underground rhizome from which the plant's fronds develop in a spiral pattern. Each frond is made up of a stipe with light brown scales at the base and short glandular hairs higher up. The leaves are bipinnately compound. The pinnules of the species are lobed and toothed.

Distribution: Eastern North America, Alabama, New Hampshire, Canada, India etc.
Habitat: Forests, woodlands, ravines, swamp edges and rocky slopes.

Polystichum setiferum: (Forssk.) Moore ex Woyn.

Synonyms: *Acropelta T. Nakai, Adenoderris J.Sm, Aetopteron Ehrh. Ex House, Hemesteum H. Lev, Hypopeltis Michx, Papuapteris C. Chr, Phanerophlebia C. Presl, Plecosorus Fee, Sorolepidium Christ*. Many ferns in this genus have stout, slowly creeping rootstocks that create a crown with a vase-like ring of evergreen fronds measuring 30-200 cm in length. The sori are spherical in shape and have a circular indusium. The stipes feature large scales with cilia that resemble hairs, but no genuine hairs.

Distribution: Southern and Western Europe, Ireland, Britain, Western France, Iberia, Turkey, Scotland, India etc.
Habitat: Evergreen fern, found in warm temperate and tropical mountainous regions in rocky habitats.

Deparia allantodioides (Bedd.)

Synonyms: *Asplenium thelypteroide*, *Athyrium allantodioides*, *Athyrium thelypteroides*, *Deparia sikkimensis*, *Lunathyrium allantodioides*, *Lunathyrium mackinnonii*, *Lunathyrium sikkimensis.*
Rhizome is short and scaly. Scales are brown, ovate, and acuminate, tufted fringes stipes are long, about 25 cm long, thick, 0.4 cm in diameter, brown, hairy, scaly at the base, sparsely scaly higher up; rachis is fibrillose and hairy. Pinnae 28 pairs, alternate, sessile, lanceolate, up to 13 cm long and 2 cm broad, apex acute, pinnae proximate, lower pinnae deflexed downwards, reduced sometimes up to auricles, texture herbaceous; lamina 2-pinnatifid, very large, ca. 85 cm long, 26 cm broad, lanceolate, apex acuminate, Costae and costules hairy; pinnae deeply lobed; lobes (pinnules), 18-22 pairs, 1.2 cm long and 0.4 cm wide, alternate, apex rounded, margin serrate-dentate, connected by a narrow wing, basal basiscopic pinnule outwards; costae and costules hairy. Simple veins. Sori indusiate, large, linear, oblique, up to 6 pairs per pinnule; indusia dark-brown, same shape as sori, margin fimbriate, persistent; indusia dark-brown, same shape as sori, margin fimbriate, persistent. Spores are dark-brown in color.

Distribution: **World**; Bhutan, China, Nepal, Tibet, Pakistan, **India**; Jammu & Kashmir, Himachal Pradesh, Dehradun, Garhwal, Sikkim, Arunachal Pradesh, West Bengal

Dryopteris juxtaposita H. Christ

Synonyms: *Aspidium filix-mas* var. *normale*; *Dryopteris odontoloma*; *Lastrea odontoloma*.; *Lastrea odontoloma*; *Nephrodium filixmas* var. *normale.* Rhizome erect-short, Scales are brown and scaly, rachis stramineous, almost glabrous; stipes stramineous, up to 20 cm long, scaly and fibrillose, base densely scaly. Lamina 2-pinnate, ovate-oblong, apex acuminate, up to 30 cm long and 15 cm wide, glabrous, subcoriaceous texture, upper surface blue-green, Pinnae 13 pairs, subopposite, short stalked, upper pinnae sessile, distant, lowest pairs more distant, lower surface whitish-green. Pinnules 11–13 pairs, oblong, basal pair largest, lower pinnules very briefly stalked, upper ones adnate, base truncate, apex rounded, margin entire in the pinnules of the distal part of the lamina and apex toothed, basal pinnules pinnatisect, lowest basiscopic pinnule of each pinnae curved, lobe Pinnate veins, 6-8 pairs per pinnule, forked, visible, Indusia brown, rounded-reniform, persistent, glabrous, deciduous; sori indusiate, 5-6 pairs per pinnule, small, round, mostly upper part of lamina fertile; indusia brown, rounded-reniform, persistent, glabrous, deciduous. Dark-brown spores, perinate, perine folded.

Distribution: **World**; Afghanistan, Burma, Bhutan, Japan, Nepal, Tibet, Vietnam, China, Nepal,Thailand. **India**; Jammu & Kashmir, Himachal Pradesh, Dehradun, Garhwal, Sikkim, West Bengal, Assam, Nagaland, Manipur, Meghalaya, Tamil Nadu.
Habitat: In forests along stream banks

Dryopteris ramosa (Hope) C.

Synonyms: *Nephrodium ramosum* Hope
Rhizome thick, long-erect, densely scaly, surrounded by stipe bases. Fronds large, tufted, deciduous, monomorphic. Scales pale-brown, long, lanceolate to ovate-lanceolate, apex acuminate; scales pale-brown, long, lanceolate to ovate-lanceolate, apex acuminate; higher up scales narrowly lanceolate and dark-brown. 3-pinnate, deltate or triangular-lanceolate, herbaceous, pale-green, ca. 50 cm long and 25 cm wide, base wide, Pinnules up to 20 pairs, alternate, shortly petiolate, distant, oblong-lanceolate, lowest pair of pinnules larges, 2.5 cm long and 0.7 cm broad, apex acute bearing prominent teeth, deeply lobed up to costa or pinnate; lobes (pinnulets) small, oblong-lanceolate, apex acute with fine acute teeth; pinnae ca. 18 pairs, alternate Simple or forked veins, up to 9 pairs per pinnule; sparsely scaly

costae and costules. Spores indusiate, large, close to costule, up to 7 pairs per pinnule, in one row on both sides of costule; indusia flat, thin, brown, reniform, persistent; indusia flat, thin, brown, reniform, persistent. Brown spores, perinate, ridged perine.

Distribution: **World**; Afghanistan, Bhutan, California, Caucasus, Tibet, Europe, Nepal, Pakistan, **India**: Jammu & Kashmir, Himachal Pradesh, Dehradun, Garhwal.
Habitat: Forest floor

Dryopteris stewartii Fraser-Jenkins.

Synonyms: *Dryopteris odontoloma* (Bedd) C. Chr. forma *brevifolia* Mehra and Khullar
Rhizome erect, stout, scaly. Fronds deciduous, monomorphic. Scales long, ovate-lanceolate, apex acuminate, blackish-brown to brown to pale-brown, glossy, sometimes bicolorous with blackish-brown central part and light-brown above, margins serrate, apex acuminate, stipe further up sparsely scaly; rachis stramineous, fibrillose, and scaly; rachis stramineous, fibrillose, and scally; Scales are few, scattered, and small, with a mid-to-dark brown colour and a lanceolate shape. Pinnae up to 20 pairs, shortly petiolate, alternate, triangular-lanceolate, widest at middle; pinnules 16 pairs, long, 2.6 cm long, 0.7 cm broad, alternate, basal shortly stalked, higher pinnate-pinnatifid, herbaceous, elongated triangular-lanceolate, up to 60 cm long and 25 cm broad, widest just above the base, lobes rectangular with rounded-truncate apices, bearing several acute teeth, 7-8 pairs per pinnule, crowded, basal pair of pinnae larger than above it, basiscopic pinnules more developed, oblong-lanceolate, apex acute with several teeth but ending in one, margin deeply lobed in lower pinnules of pinnae, above pinnules crenate; lobes rectangular with Costae and costule grooved, sparsely scaly; veins free, forked, 7-10 pairs per pinnule, pinnately divided, visible abaxially; veins forked, forked, 7-10 pairs per pinnule, pinnately divided, visible abaxially; veins forked, forked, 7-10 pairs per pinnule, pinnately divided, visible abaxially. Sori indusiate, round, 6-8 pairs per pinnule, one to per lobe, not crowded, in two rows, one on each side midway; indusia reniform, persistent, glabrous, flat or slightly curved at the edges, thin, margin entire; indusia reniform, persistent, glabrous, flat or slightly curved at the edges, thin; indusia reniform, persistent, glabrous, Brown spores, perinate, perine irregular.

Distribution: **World**; Afghanistan, Pakistan, Nepal, **India**; Jammu & Kashmir, Himachal Pradesh, Dehradun, Garhwal.
Habitat: Small stream banks

Dryopteris wallichiana (Spreng.)

Synonyms: *Aspidium donianum*; *Aspidium filix-mas*; *Aspidium filix-mas* var. *parallelogrammum*; *Aspidium paleaceum*; Aspidium paleaceum; *Aspidium parallelogrammum* Kunze; *Aspidium patentissimum*; *Aspidium Wallichianum* .
Rhizome erect, massive, bearing several fronds in whorled fashion, densely clothed with brown, lanceolate scales. Fronds are monomorphic, evergreen, and shuttlecock shaped. Scales at stipe base blackish mixed with pale ones, scales upwards along with rachis light-brown to paler, mixed with few blackish ones, scale base usually dark, scales lanceolate to narrowly lanceolate, margins with projections, apex acuminate; rachis densely scaly and fibrillose; Pinnae numerous, 30-38 pairs, alternate, green to deep-green, lanceolate to oblong-lanceolate, large, ca. 70 cm long and 22 cm broad, base narrowed, glabrous adaxially, scantly scaly on abaxial side, coriaceous in texture; lamina 1-pinnate-pinnatisect, green to deep-green, lanceolate to oblong-lanceolate, middle pinnae large, 11 cm long and 1.8 cm broad, broadest at base, apex acute, lanceolate, shortly petiolate, margin deeply lobed, sometimes pinnate, lower pinnae reduced; pinnules (lobes) up to 22 pairs, rectangular, obliquely spreading, roundly truncate to truncate at apex, apex toothed, basal pair of pinnules clearly separate from next to it, other pinnules are joined by a narrow wing, basal basiscopic pinnule with an auricle towards below. Costae and costules grooved above, groove continuous from rachis to costae, scaly and fibrillose. Veins free, forked; costae and costules grooved above, groove continuous from rachis to costae, scaly and fibrillose. Sori indusiate, round, 4-5 pairs per pinnule, in a single row on

either side of pinnule, medial, 2/3rd of frond is fertile; indusia dark-brown, reniform falling off at maturity, glabrous, entire; indusia dark-brown, reniform falling off at maturity, glabrous, entire; indusia dark-brown, reniform falling off at Brownish spores, perinate, granulose perine.

Distribution: **World**; World; Argentina, Bhutan, Borneo, Burma, China, Malaysia, Myanmar, Nepal, Jamaica, Cuba, Brazil, Tibet, Nepal, Taiwan, Japan, Mexico, Vietnam, Philippines, Java,**India**; Kashmir, Himachal Pradesh, Dehradun, Garhwal, Sikkim, Arunachal Pradesh, Meghalaya, Nilgiri hills.
Habitat: Forest floor

Polystichum piceopaleaceum Tagawa in Acta Phytotax.

Synonyms: *Aspidium angular*; *Polypodium setiferu* (Forssk.)*; Polystichum aculeatum* var. *fargesii*; *Polystichum bicolour*; *Polystichum doianum*.
Rhizome short-erect, densely scaly. Scales ovate-lanceolate, blackish to dark-brown, sometimes bicolorous with central region darkbrown surrounded by paler region, margin serrate, apex acuminate, fibrils numerous, brown; rachis scaly and fibrillose, scales ovate-lanceolate to linear-lanceolate, higher up sparsely scaly; rachis scaly and fibrillose, scales ovate, 2-pinnate, lanceolate, 35 cm long and 13 cm wide, slightly narrowed base, acuminate apex, subcoriaceous lamina, Pinnae up to 24 pairs, largest pinna ca. 7 cm long and 1.7 cm broad, alternate, petiolate, linear lanceolate, broadest at base, apex acuminate, lower 1-2 pairs deflexed downwards; scaly and fibrillose abaxially, glabrous adaxially, middle part widest, alternate, rhomboidal-ovate in shape, apex acute to rounded, auricled onacroscopic side, auricle rounded or toothed, margin spiny serrate, sometimes lobed, basal pair largest; costae and costules scaly. Veins free, forked, visible adaxially, 4-5 pairs per pinnule. Sori indusiate, terminal on veinlets, medial, 4-6 pairs per pinnule, in one row on either side of the mid-vein, lower 4 pairs of pinnae sterile; indusia small, fimbriate round, brownish, persistent. Spores brown, perinate, perine smooth.

Distribution: **World**; Afghanistan, Bhutan, Burma, China, Japan, Myanmar, Nepal, Pakistan, Sri Lanka, Taiwan. **India**; Jammu & Kashmir, Himachal Pradesh, Dehradun, Garhwal, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nilgiri hills, Nagaland.
Habitat: Forest Floor, Rocky meadows

Pteridium aquilinum (L.)

Synonyms: *Pteridium aquilinum* var. lanuginosum Henriq.; *Pteridium aquilinum* subsp. typicum R.M. Tryon; *Pteridium japonicum* Tardieu and C. Chr.; *Pteridium latiusculum* (Desv.).
Rhizome slender, long-creeping, woody, thick, 0.3-0.4 cm, hairy; hairs short brown. Stipes are 50 cm or longer, dark-brown at the base and lightening in the distal region, and hairy, hairs are glandular, colourless or brown-tinged. Pinnae ca. 15 pairs, the large lower pinnae opposite, upper pinnae often alternate, petiolate, lanceolate to deltate; pinnules 12-18 pairs, basiscopic pinnules larger than the acroscopic ones, lanceolate, margin lobed to the costae; lamina 3 pinnate, ovate to deltate, 40-70 cm long and 20-40 cm broad, herbaceous, ultimate segments oblong, ca. 10 pairs, 1cm long by 0.5 cm wide, apex rounded, margin crenate or lobed, often reflexed, Free, simple, or forked veins, rachis dark-brown and course, grooved, hairy, hairs as on stipe. Exindusiate sori, round, intramarginal, 2-6 pairs per pinnule, unprotected, but partially protected by reflexed teeth Exine uniformly spinulose, spores yellowish brown.

Distribution: **World**; World; Bangladesh, Burma, China, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Taiwan, Thailand, Vietnam. **India**; Jammu & Kashmir, Dehradun, Garhwal, Himachal Pradesh, Manipur, Sikkim, Arunachal Pradesh, Uttar Pradesh, South India.
Habitat: Commonly occurs in open, sunny slopes, forest floors and edges of forests

CONCLUSION

Botanical study of the flora of particular area is an essential aspect as it forms baseline information for the distribution of plant species or communities and their relation with physical environment. The paper gives a broad outlook about the exploration, investigation of ferns of Phalgam, Anantnag. The study will provide insight and act as a monumental resource in the field of identification of fern species in this region, allowing individuals to become more familiar with these fern species. Ferns and their allies are an important part of the ecosystem, with the majority of them living in forests and thus serving as good indicators of the severity of issues such as deforestation and habitat destruction.

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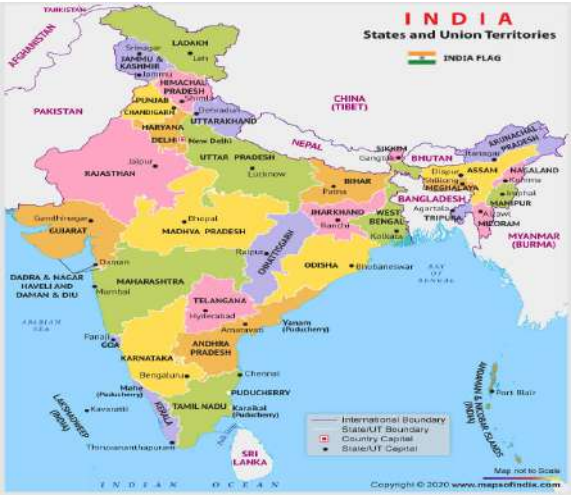

Fig. 1. India Map

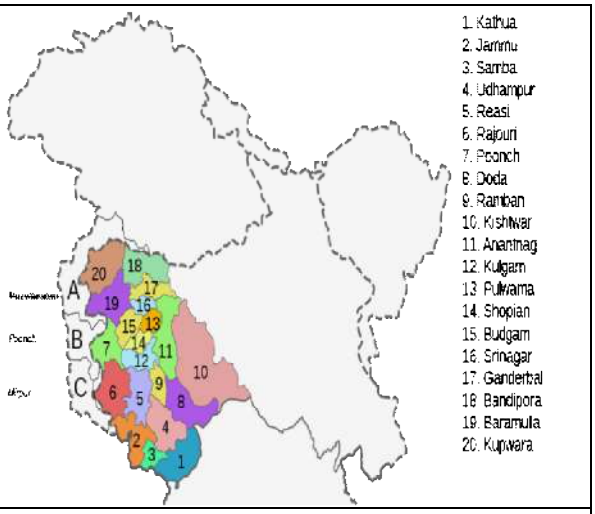

Fig. 2. Jammu And Kashmir Map

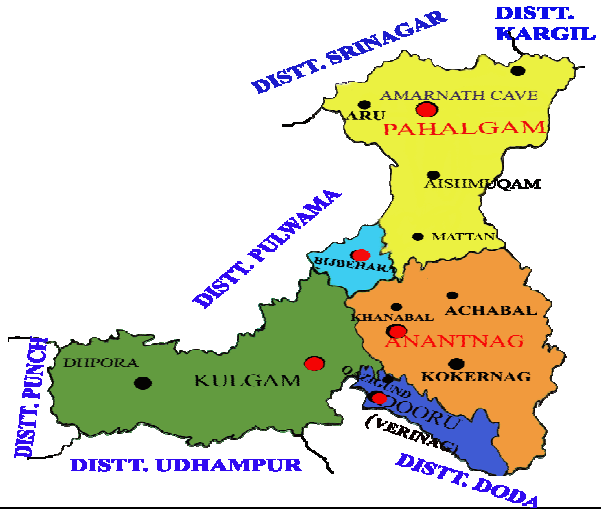

Fig. 3. Anantnag (Phalgam) Map

			
Adiantum capilus-veneris	***Pteris cretica L***	***Asplenium Adintum-nigrum L***	***Asplenium trichomanes***

			
Cystopteris fragillis	***Gymnocarpium Dryopteris L***	***Dryopteris barbigera***	***Dryopteris filix-mas***
			
Dryopteris intermedia	***Polystichum setiferum***	***Deparia allantodioides***	***Dryopteris juxtaposita***
			
Dryopteris ramosa	***Dryopteris stewarti***	***Dryopteris wallichiana***	***Polystichum piceopalacum***
			
Pteridium aquilinum			

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RESEARCH ARTICLE

Antimicrobial, Antioxidant Activity and Phytochemical Analysis of Lichen *Parmotrema perlatum*

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial, antioxidant and phytochemical analysis of lichen *Parmotrama perlatum.* It is commonly known as kalpasi, belongs to the family Parmeliaceae categorized under the genus Parmotrema and this lichen has been traditionally used as medicine. The preliminary phytochemical analysis of hexane extract of *Parmotrema perlatum* shows wide range of chemical compounds including alkaloids, flavanoids, resins, phenol and the Identification of bioactive compounds was done by thin layer chromatography. Antibacterial activity of lichen extract showed inhibitory activity on all the bacterial pathogens, the highest activity was against *Staphylococcus aureus* and *E.coli* with 23 and 22 mm zone of inhibition respectively. The strong antifungal activity was found against *Aspergillus flavus* with 19 mm zone of inhibition. Antioxidant properties of lichen extract was assayed using DPPH free radical scavenging method, Lichen showed significant antioxidant activity. Thus *Parmotrema perlatum* has lots of medicinal values against pathogens and antioxidant potential which could be used as therapeutic drugs.

Keywords: *Parmotrema perlatum*, antimicrobial activity, antioxidant activity, phytochemical analysis.

INTRODUCTION

Lichen is a combination of fungi (mycobiont) and cyanobacteria (photobiont) have symbiotic relationship. Lichen produce a varied range of secondary metabolites and also some of them are unique to lichen symbiosis including depsides, depsidones, dibenzofurans and pylvinic acid [1]. The genus *Parmotrema* is typically characterized by large foliose thalli with broad lobes, commonly with a broad marginal zone on the lower surface, pored epicortex, thick walled hyaline ellipsoid ascospores, filliform conidia and with or without marginal cilia [2]. Lichen occurs in some extreme environment on earth-artic tundra, hot desert, rocky coast, and toxic slag heaps. Lichens are widespread and may be long lived; however many are also vulnerable to environment disturbance and may be useful to scientist in assessing the effects of air pollution, ozone depletion, and metal contamination. Lichen has also been used in making

dyes and perfumes, as well as in traditional medicines. Recently much attention has been paid to several lichen species and their secondary metabolites as resource of natural antioxidants and antimicrobial, antiviral, antitumor activity [3, 4, 5]. Infection disease caused by pathogenic microorganisms is a major threat to public health. Emergence of multi drug resistance leads to unavailability of drug, so searching the new bioactive natural drug or antibiotic molecules is needed for therapeutics [6]. In our present study was used, four bacterial and three fungal pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas sp.,* and *Aspergillus niger*, *Aspergillus flavus*, *Penicillum sp.* The sensitivity of the pathogen against *P.perlatum* was examined by measure the zone of inhibition (ZOI) by disc diffusion method. Free radicals and other reactive oxygen species (ROS) are continuously produced in our body, as by-product of various essential processes like energy generation; phagocytosis and detoxification reaction, antioxidant can prevent the oxidation of lipids or other molecule by inhibiting the initiation or propagation of oxidative chain reaction. The antioxidant activity of lichen extract was checked by using DPPH assay.

MATERIALS AND METHODS

Sample collection

The lichen species was collected from the local super market, Tirupur, Tamil Nadu.

Preparation of lichen extracts

The collected lichen sample was dried and 100g of lichen was grounded using electric blender, made it into a fine powder form. From that powdered sample 30g] was weighed and extracted in 300ml of hexane with soxhlet extractor. [7]

Identification of Lichen Secondary Metabolites

Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out of *Parmotrema perlatum* extracts for the presence of phenolic glycosides, alkaloids, flavonoids, tannins, terpenoids, saponins, oils, sugars and gums [8].

Thin layer chromatography (TLC)

Hexane extract of lichen was subjected to TLC for identify the bioactive compounds. Extract was dissolved into a 1 mg/mL of hexane and spotted onto the TLC plate using a capillary tube. The TLC plate was kept in toluene/1,4-dioxane/acetic acid (180:45:5) mixture in TLC chamber for 1 h. The plate was then air-dried and sprayed with 10% H_2SO_4 solution. Visualize the colour and separate patterns of extract on TLC plate. [9,10]

Test Microorganisms

The bacterial pathogens were collected from the KMCH laboratory and isolated plant fungal pathogens were used in this study. The bacterial samples were serially diluted and cultured in nutrient agar, EMB agar and Manitoal salt agar and incubated the plates for 24 hrs at 37°c. The fungal samples were cultured in potato dextrose agar, and sabouraud dextrose agar in the dilution 10^{-2}, 10^{-3}, 10^{-4}, and incubated the plates for 2-3 days at 25°c.

Antimicrobial activity

The antimicrobial potential of the hexane extract obtained from the lichen *Parmotrema perlatum* were tested again the collected bacterial pathogens by disc diffusion method. Bacterial isolates were swabbed onto Muller-Hinton agar plates. Sterile paper discs soaked with different concentrations of. Lichens extracts (50 µg/ µl, and 100 µg/ µl) were impregnated on the MHA medium. The plates were incubated for 24 hr, and the zone of inhibition was measured around discs. Streptomycin (1mg/ml) used as positive control and DMSO as negative control. Antifungal activity of lichen extract was checked against selected fungal pathogens by disc diffusion method. Paper discs were laid on PDA plate after being soaked with 50 µg/ µl, and 100 µg/ µl concentrations of lichen extract and incubated the plated at room temperature for 2-3 days. Flucanozole used as positive control and DMSO as negative control. Antifungal activity was measured by zone inhibition around the disc [11]

Total Phenolic Content

Phenolic content is important constituents with redox properties, which is responsible for antitoxidant activity. Determination of total phenolic content present in the lichen extract was performed using the Folin-Ciocalteu method [12]. The extract was diluted in the concentration of 1mg/mL, and aliquots of 0.5mL were mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5 % sodium bicarbonate. The mixture was allowed for 15 min at the 45°C and absorbance was measured at 765nm against blank sample. Total phenolic content in the lichen extracts were expressed in gallic acid equivalents (mg GA/g extract).

Antioxidant activity

DPPH scavenging activity: The free radical scavenging activity of lichen extracts was measured using the DPPH (1,1-diphenyl-2- picryl-hydrazil[13,14]. DPPH (4mg) was dissolved in 100 mL methanol to obtain a concentration of 40 µg/ mL. Then serial dilutions were carried out with the stock solution (1mg/mL) of the lichen extract. The 2mL of stock solutions mixed with DPPH (2 mL) and allowed to stand for 30 min, and absorbance was measured at 517nm. As reference standards were used ascorbic acid and dissolved in methanol were used to make the stock solution with the concentration (1mg/ mL). Control sample was prepared with same volume and Methanol 95% was used as blank. The following formula was used to check the DPPH free radical scavenging activity.

% inhibition = [(Ac – As) / Ac] × 100

Statistical analysis: All data obtained from results were expressed as mean ± SD from a three replicates. Inhibition concentration IC_{50} (50%) was calculated by using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Identification of Lichen secondary metabolites

The qualitative analysis of phytochemicals in the *Parmotrema perlatum* extract was revealed and present some of the important phytochemicals are alkaloids, flavanoids, tannins and phenols.

Thin layer chromatography

The results showed the separation of bioactive compounds from in lichen hexane extract was identified based on Rf value. The data indicated that presence of important compounds atranorin at Rf value of 0.72 and stictic acid at Rf value of 0.32.

Antimicrobial activity

Antibacterial activity of *Paromtrema perlatum* hexane extract was tabulated (Table.1). Streptomycine was used as control. The standard shows inhibition against all tested organism The hexane extract of *Parmotrema perlatum* was tested against four bacterial pathogens *E.coli, Pseudomonas sp*., *staphylococcus aureus* and *Streptococcu pyogenes.* The results indicated that lichens extract has high efficacy against all the tested bacteria. The highest zone of inhibition was recorded against *Staphylococcus aureus* (23 mm) and *E.coli* (22 smm) in 100µg/ µl concentration**.** The lichen extract also showed good activity on *Streptococcus pyogenes* and *Pseudomonas* sp., with 19 mm and 18 mm zone of inhibition. Among various fungal pathogens the lichen extract showed highest inhibition against *Aspergillus flavus* (15mm) and *Penicllium* sp.,(14 mm) and least against *Aspergillus niger* with (12mm) Zone of inhibition (Table 2).

Antioxidant Activity by DPPH Assay

The total phenolic content of the *Parmotrema perlatum* extract was exhibited about 69.78 mg GAE/g (Table 3). The phenolic content may be the responsible for bioactivity of lichen. The results showed percentage of inhibition in DPPH radical scavenging activity of Lichen extracts and standard at different concentrations. The lichen extract recorded IC_{50} value of 0.393µg/ mL which is lowest IC_{50} compared with standard ascorbic acid (0.513µg/ mL) in 100 µg/ mL contration. Lichen *Parmotrea perlatum* hexane extract showed good antioxidant activity (Table 4).

CONCLUSION

The objective of this study was to investigate the antimicrobial and antioxidant activity and phytochemical analysis of *Parmotrema perlatum* hexane extract. Compared to the antifungal activity, Lichen extract showed the high antibacterial activity against various bacterial pathogens. The antioxidant activity was analyzed using DPPH radical scavenging assay method, the % of inhibition and IC5o value of lichen extract were identified. The results revealed that thelichen extract has good antioxidant activity when compared to standard. The phytochemicals and bioactive compounds present in the lichen extract might be responsible for bioactive potential. Finally the above study concludes that lichen *parmotrema perlatuum* has various bioactive compound and effective against bacterial and fungal pathogens and has good antioxidant potential, so it could be used as a good source of medicine.

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Table 1: Antibacterial activity of hexane extract of *P. perulatum* against bacterial pathogens by disc diffusion method

Test Bacteria	Zone of Inhibition (mm)		Standard (streptomycin), 1mg/ml
	50µg/ µl	100µg/ µl	
E.coli	17 ±0.23	22 ± 0.26	21 ± 0.20
Pseudomonas sp.	16 ± 2.14	18± 3.26	20 ± 2.11
Staphylococcus aureus	18 ± 0.54	23 ± 1.2	22 ± 1.0
Streptococcus pyogenus	17 ± 0.74	19± 0.82	19 ± 0.92

Table 2: Antifungal activity of lichen extract by disc diffusion method

S.No	Test Fungi	Zone of Inhibition (mm)		Standard (Fluconazole) 1 mg/ml
		50µg/ µl	100µg/ µl	
1.	*Aspergillus niger*	9±0.23	12 ±0.11	20 ±0.13
2.	*Aspergillus flavus*	10 ±0.45	15 ±0.33	19 ±0.34
3.	*Penicillium sp.,*	8 ±0.23	14 ±0.23	21 ±0.12

Table 3: Total phenolic content of lichen extract

S.No	Lichen Extracts concentration	TPC (mg GA/g)
1.	50µg/ µl	37.45±0.23
2.	100µg/ µl	69.78 ±0.34

Table 4: Antioxidant Activity of Lichen extract by DPPH Assay.

S.No	Concentration of Lichen Extract	DPPH % Inhibition	IC_{50} (µg/mL)
1.	50µg/ µl	62±0.56	0.534±0.12
2.	100µg/ µl	78±0.47	0.393±0.32
	Standard Ascorbic acid		
1.	100µg/ µl	74±0.43	0.513±0.27

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RESEARCH ARTICLE

A Miniaturized Biosorption-Desorption Process for Removal of Pb (II) Ions from Wastewater Using Biosorbent of *Tectona grandis* Leaves

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ABSTRACT

Heavy metal ion contamination is increasing by the day, degrading the quality and reliability of water on both the surface and beneath. This research focuses on the reduction of Pb (II) ions from wastewater employing Tectona grandis leaves as a biosorbent (TGLB). Variations in pH (2-7), contact time (20-120min), biosorbent dose (1-5g), temperature (298-338K), and initial metal ion concentration (10-50mg/l) were used to investigate the adsorption procedure. A Pb (II) ion solution with a purity of 10 mg/L resulted in 93.93% of maximal removal with TGLB at optimum pH 6 and temperature 298±1.5K for a biosorbent dose of 5gm and a 45minute contact period. The adsorption was spontaneous and exothermic, with TGLB yielding a maximum monolayer adsorption of 7.35 mg/g. The adsorption of Pb (II) ions by TGLB followed pseudo-second-order kinetics (R^2 > 0.998). Hydrochloric acid has a strong potential as an eluent for desorption of Pb (II) ions, according to the desorption experiments. The findings suggested that TGLB might be employed as an efficient and cost-effective biosorbent for the removal of Pb (II) ions. The spectroscopic assessment was done using Fourier transform infrared (FT-IR) analysis, whereas the morphological characterization was accomplished using Scanning electron microscope (SEM) images.

Keywords: Biosorption, isotherm, optimum, removal, wastewater.

INTRODUCTION

Water purity deterioration is a crucial impediment to long-term sustainability and a grave danger to the ecosystem. Extensive study on management of various water sources and pollution abatement has been motivated by global population increase, industrialization, and the quest of the latest technological innovations. Contaminants in untreated industrial wastes, including heavy metal ions, degrade the integrity of the water resources. Since they can

not be decomposed, they can accumulate to harmful amounts in a living body, this becomes a major cause of concern [1]. Lead (Pb) is one among the most noxious heavy metals and is extremely stable in its +2 oxidation state. The International Agency for Research on Cancer (IARC) classifies it as a group '2A' potential human carcinogen. The World Health Organization (WHO) recommends a maximum lead quantity of 0.05 mg/L in drinkable water, while the USEPA recommends a threshold of 15 g/L. Lead is prevalent in wastewater from a significant number of sectors, including pigments, dying, plumbing, glass, painting, pesticides, oil refining, batteries, metallurgical engineering industries, coal combustion, processing, and lead product production [2, 3]. Pb (II) can cause kidney and liver damage, neurological disorders, skin cancer, gastrointestinal bleeding, damage to red blood cells, anemia, hearing problems [4]. There are various conventional wastewater treatment procedures e.g., chemical precipitation, ion exchange, reverse osmosis, coagulation and flocculation, flotation, solvent extraction are the common methods of removing hazardous metals from water, but each of them has various disadvantages including a high operational and capital cost, a massive amount of secondary sludge generation, slow process, high energy needs, incomplete removal of the contaminants [4, 5]. With its easy accessibility and operation, ease of design, excellent efficacy, and minimal formation of hazardous by-products, biosorption is known as an effective approach. Using biological, agricultural, or commercial wastes or microorganisms as a sorbent of solid phase to remove pollutants such as heavy metals from adsorbate of the aqueous phase is referred to as biosorption. There are numerous trees and plants whose leaves, bark, fruits, fruit peels, and other parts have been utilized for formation of biosorbents for eliminating toxic heavy metal ions e.g., Mahogany fruit shell [2], *Mangifera indica* [3], pumpkin char [4], *Aspergillus niger* [5], *Rubus ellipticus* leaves [6], *Pyrus pashia* leaves [7], sesame husk [8], *Arthrobacter* sp. biomass [9], *Phytolacca americana* L. biomass [10], *Artemisia vulgarise* [11], *Dicliptera bupleuroides* leaves [12], meranti wood [13], *Jatropha curcas* seeds [14], *Cladophora* sp. [15], *Peganum harmala* seeds [16], pine needle biochar [17], coir pith biochar [18], date bead activated carbon [19], waste activated sludge of water treatment plants [20], Vermiculite [21], pomegranate peel [22], orange peel [23], activated sewage sludge biomass [24], moringa pods [25], brown algae *Cystoseira trinodis* [26], marine red algae *Callithamnion corymbosum sp* [27], fungus *Penicillium purpurogenum* [28], fungus *Mucor indicus* [29], jelly fungus *Auricularia polytricha* [30].

The present study aims to investigate the viability of the biosorbent prepared with *Tectona grandis* (teak) tree leaves which are abundant in Kumaun region of Uttarakhand, India for removal of Pb (II) ions from contaminated water. *Tectona grandis* is a tall tree that grows to a height of 30 meters and belongs to the *Lamiaceae* family and genus *Tectona.* The optimal values of pH, initial metal ion concentration, contact time, temperature, and biosorbent dosage were all determined. The Langmuir, Freundlich and Temkin isotherm models were used to explain the biosorption isotherm in this study along with the pseudo-first-order kinetic model and pseudo-second-order kinetic model to examine the biosorption kinetics of using TGLB.

MATERIALS AND METHODS

Preparation of biosorbent

Tectona grandis leaves were collected from the immediate vicinity of board office Colony, Ramnagar, Uttarakhand, India (29°23'37"N 79°07'08" E).To eliminate dust and soluble contaminants, these leaves were washed thoroughly with double distilled water separately. The leaves were then dried for 48 hours in a hot air oven (Popular Traders S.N.-1680) at 333 K. Then the air-dried leaves were crushed and ground in a domestic grinder to get the powdered form. The powdered biomass was soaked in 0.1N $HNO_3$for activation up to 24 hours at room temperature, then filtered and rinsed with double distilled water. The activated biomass was placed in a hot air oven for drying at 333K for 48 hours. Then it was passed through 63-micron sieves (240 BSS). Then, the sieved biomass of *Tectona grandis* leaves was stored in an airtight bottle for experimental use as *Tectona grandis* leaves biosorbent (TGLB).

Preparation of adsorbate

The stock solution of Pb (II) ions of 1000mg/L concentration was prepared by dissolving 1.598 g of $Pb(NO_3)_2$ in 1000mL of double-distilled water. This solution was kept in an airtight bottle to prevent the solvent water from evaporating. The pH of the working solutions was adjusted in the range of 2-7 using 0.1 N HCl and 0.1N NaOH. A digital pH meter (Model: Systonic 361) was used to monitor the pH value of the solution. A. R. grade reagents were used throughout the study.

Biosorbent surface characterization

FT-IR spectroscopic analysis

The occurrence of functional groups on TGLB surface before and after Pb (II) adsorption was measured using a Fourier transform infrared (FT-IR) spectrometer (Perkin Elmer, USA, Model: Spectrum 400) in the 400-4000cm^{-1} range.

Scanning electron microscopy (SEM)

The images of TGLB surface before and after adsorption of Pb (II) were captured and analyzed using a scanning electron microscope (SEM) (JEOL, Japan, Model: JSM 6100).

Biosorption experiments:

Batch experiments had been used to analyze the impact of numerous parameters on metal ion removal, like pH, contact time, biosorbent dose, metal ion concentration, and temperature. The optimum pH was determined prior to all and the rest of all experiments were performed on the same pH. By altering the parameter under study while keeping the others constant, the optimum condition of each parameter was identified. The experiments were conducted with 100mL of standard solution using conical flasks of 250mL. The initial metal ion concentration was 10mg/L for all batch experiments other than the experiments of varying metal ion concentrations. The solution was shaken for 45min at 298 ± 1.5 K temperature using a rotatory flask shaker (Popular S.No. 1685) fixed at 170rpm for other than the contact time experiments. The solution was then filtered with Whatman No. 42 filter paper and filtrate was digested with conc. HNO_3. The digested solution was analyzed by atomic absorption spectrophotometer (Optima 4300DV ICP, Perkin- Elmer, Boston, MA). A representative diagram of whole procedure is shown in Figure1.

The removal efficiency for each experiment was calculated using equation (1) as follows:

$$\text{Removal Efficiency } (\%) = \frac{C_i - C_e}{C_i} \times 100 \qquad (1)$$

Where C_i represents the starting metal ion (adsorbate) concentration (mg/L) and C_e denotes the metal ion concentration (mg/L) at equilibrium.

The quantity of adsorbed metal ions adsorbed per gram of biosorbent (Q_e) was evaluated by applying the preceding expression:

$$Q_e = \frac{(C_i - C_e)}{1000} \times \frac{V}{m} \qquad (2)$$

Where Q_e represents equilibrium adsorption potential of biosorbent (mg/g), V implies the volume of working solution (L), C_i and C_e denote the initial and equilibrium metal ion concentration (mg/L) and mass of biosorbent (g) is denoted by m.

Adsorption isotherm models

Langmuir adsorption model

Irrespective of the scale of coverage, in the Langmuir isotherm model, solutes are assumed to be adsorbed as a single layer upon a substratum of adsorbent, with the exact number of identical metal-binding sites and the same adsorption energy [31]. The Langmuir isotherm model implies:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{max}} + \frac{1}{K_L Q_{max}} \quad (3)$$

Where metal ion concentration at equilibration (mg/L) is denoted by C_e, while the quantity of metal ions adsorbed at equilibrium (mg/g) is Q_e. K_L is Langmuir's constant (L/mg) and Q_{max} is the maximal adsorption capacity (mg/g). Metal-ion-adsorbent-surface interactions are further computed using non-dimensional constant (separation factor) (R_L). R_L is expressed as follows:

$$R_L = \frac{1}{1 + K_L C_0} \quad (4)$$

Where C_o is the initial concentrate of adsorbate (mg/L) and K_L denotes the Langmuir constant (L/mg). This isotherm is favorable if $0 < R_L < 1$, unfavorable if $R_L > 1$, irreversible if $R_L=0$ and linear if $R_L=1$.

Freundlich adsorption model

This isotherm model consists of a pragmatic equation that proposes multilayer adsorption on a heterogeneous surface [32]. It is expressed mathematically as follows:

$$Q_e = K_F C_e^{1/n} \quad (5)$$

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \quad (6)$$

Where, K_F (Freundlich constant) is associated with the adsorption capacity of the biosorbent, whereas n is an experimental constant. If the calculated valuation of 1/n is between 0 and 1, then the adsorption mechanism is likely to be favorable.

Temkin adsorption model

This model was introduced on the premise that adsorption energy drops linearly with surface penetration owing to various interactions between adsorbent and adsorbate [33]. The linear expression is as below:

$$Q_e = b_T \ln A + b_T \ln C_e \quad (7)$$

Where b_T implies the adsorption heat constant ($b_T = RT/b$, where R=8.314J/mol/K, T is absolute temperature, and b is the adsorption heat in J/mol), while A represents the constant of steady-state binding (L/g).

Adsorption kinetics models

The dynamic adsorption process was described by pseudo-first-order and pseudo-second-order kinetic models for the time intervals of 20min, 40min, 60min, 80min, 100min, 120 min.

Pseudo-first-order kinetic model [34]

$$\ln(Q_e - Q_t) = \ln Q_e - K_1 t \quad (8)$$

Where Q_e is the concentration of metal ions adsorbed at the equilibrium condition in mg/L while Q_t is the metal ion concentration at any time t. K_1 indicates the rate constant of the pseudo-first-order kinetic model (min^{-1}).

Pseudo-second-order kinetic model [35]

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{1}{Q_e} t \quad (9)$$

Where K_2 implies the rate constant for the pseudo-second-order kinetic model (g/mg/min) and all other terms have the previously stated meanings.

Thermodynamic study of adsorption

The thermodynamic studies were conducted at temperatures 298K, 308K, 318K, 328K and 338K. Thermodynamic parameters i.e., a change in Gibbs free energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) were calculated with the following equations:

$$\Delta G° = -RT\ln K_c \quad (10)$$

$$K_c = \frac{Q_e}{C_e} \quad (11)$$

$$\ln K_c = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \quad (12)$$

Where, R implies the universal gas constant (8.314 J/mol/K), T represents the temperature (K), the concentration of adsorbate metal ions which are adsorbed (mg/L) is implied by Q_e, C_e represents the metal ion concentration (mg/L) at equilibrium and K_c is equilibrium constant.

Desorption studies

This research also investigated the desorption of Pb (II) ions from loaded TGLB. Eluents are examined using three distinct chemical reagents: 0.1M HCl, 0.1M NaOH, and double-distilled water. For the utmost efficient effluent, the biosorption-desorption procedure was repeated three times in succession. Desorption tests were performed out in 100 mL conical flasks with 50 mL of eluent solution and 1gm of loaded biosorbent. The mixture was agitated for 45 minutes at a stirring speed of 170 rpm and a temperature of 298±1.5°C. Filtration with Whatman 42 filter paper removed the biosorbent from the working solution. Atomic Absorption Spe was applied for determining the percentage of Pb (II) ions within the desorption solution.

The percentage of Pb (II) ions desorbed from the loaded material is calculated as follows:

$$\text{Desorption (\%)} = \frac{Q_{e,\text{desorption}}}{Q_{e,\text{adsorption}}} \times 100 \quad (13)$$

Where $Q_{e,adsorption}$ is the Pb (II) ions concentration loaded on the biosorbent before desorption calculated by equation (2).

Statistical analysis

The average and standard error of data from at least three different experiments are shown. The TGLB adsorption outcomes were compared to the controlled outcomes using a t-test ($P<0.05$) [36]. Using the online software OPSTAT, a single-factor assessment of research data was performed, with a significance level of 5% used to compare treatment means. Using statistical tools in Microsoft Excel 2007, the contextual relevance of the linear equations of Langmuir, Freundlich, Temkin isotherm models, pseudo-first-order, and pseudo-second-order models, as well as pseudo-first-order and pseudo-second-order models, was investigated using the correlation coefficient (R2) (version Office XP, Microsoft Corporation, USA).

RESULTS AND DISCUSSIONS

FT-IR characterization

The presence and shift of functional groups on TGLB before and after adsorption of Pb (II) ions was analyzed Fourier transform infrared (FT-IR) spectrometer (Perkin Elmer, USA, Model: Spectrum 400) within the range 400-4000cm^{-1}, which is shown in Figure 2. In Figure 2, the FT-IR spectrum of TGLB before the adsorption of Pb (II) ions indicates the peaks at 3433.652cm^{-1} (representing –OH stretching vibrations of phenols, alcohols, and carboxylic acid groups of cellulose and lignin present on biosorbent surface) [3, 10], 2921.477cm^{-1} (represents asymmetric $–CH_2$ and –CH stretching vibrations) [22], 1632.010cm^{-1} (attributed to stretching vibrations of C=C and C=O of carboxylic acids or their esters and N-H bending vibrations), 1059.917cm^{-1} (belongs to C-O stretching) [3, 10, 14] and 619.151cm^{-1} (indicates the presence of alkyl halides and aromatic amino acids (-NH_2 group)) [22]. After the sorption of Pb (II) ions on the TGLB surface, these peaks shifted to 3425.622cm-1, 2921.690cm-1, 1631.440cm-1, 1056.634cm-1, and 615.750cm-1. The signal at 1382.35 cm-1 corresponds to –CH3 symmetric bending, O-H in-plane bending, and ionic carboxylic symmetric groups [22], which vanished after Pb (II) ions sorption, emphasizing its importance in the biosorption. The emergence of distinct peaks implying respective active moieties and the switch in their frequencies after Pb (II)

ion sorption indicate that these functional groups participated in binding the Pb (II) ions on the biosorbent surface, and the sorption process may involve chemisorption as a mechanism [8].

SEM analysis

The surface morphology of the biosorbent surface was captured and analyzed by Scanning Electron Microscope (SEM) (JEOL, Japan, Model: JSM 6100). The SEM images of the TGLB and LCLB surface before and after adsorption of Pb (II) ions are shown in Figure 3. It is visible that before the biosorption process (some indicated by orange colored arrows) the TGLB surface had holes that were filled after the adsorption of Pb (II) ions. The visible presence of holes i.e., active sites is most probably due to the HNO_3 activation during the preparation of biosorbent [8].

Influence of various parameters on %removal efficiency of TGLB for removal of Pb (II) ions

The parameters under study affect the removal of metal ions in different ways the outcome of the study was analyzed by single-factor analysis as shown in Table 1.

Effect of pH of working solution

By varying the pH from 2 to 7, for a contact time of 45minutes, initial metal ion concentration of 10mg/L using 1g of biosorbent dose at 298±1.5K temperature and 170rpm of stirring speed; the effect of pH on the percent removal of Pb (II) ions by TGLB was investigated. The percent removal efficiency of Pb (II) increased from pH 2-6 (from 58.7% to 89.8%) and subsequently decreased from pH 6 to pH 7 (Figure 4 (a)). It is attributable to the relatively high number of H^+ ions at lower pH levels, which have superior mobility than heavy metal ions and attach to biosorbent active sites before metal ions. However, if pH is raised, the possibility of forming metal hydroxide complexes increases, reducing the quantity of free-moving metal ions [23]. As the optimum pH for both the biosorbents came out to be 6, all other batch experiments were performed at pH 6.

Effect of contact time

The effect of varying the contact time on % removal efficiency of TGLB was analyzed under optimum conditions of pH 5, 1gm of biosorbent dose, 10mg/L of initial metal ion concentration at 295±1.5K temperature and 170 rpm stirring speed. On increasing the contact time from 20min to 80min, there was a steep increase in the % removal (from 70.06% to 89.73%). The % removal increased very slowly from 80min to 100min of contact time and from100min to 120min, it was monotonous as shown in Figure 4(b). The process can be broken down into three different processes in this way: the first is very fast owing to a large number of unoccupied active sites that induce a rapid increase in Pb (II) ion adsorption on TGLB. The second step includes a gradual increase in percent removal, implying that the amount of vacant active sites reduced with increasing contact time, and the third step implies that the active sites on biosorbent surfaces became saturated and the biosorbent-biosorbate system reached equilibrium [14].

Effect of biosorbent dose

The percentage of adsorbed Pb (II) ions increased from 86.43% to 93.94 % using TGLB when the biosorbent dose was increased from 1gm to 5gm (Figure 4 (c)) for an initial metal ion concentration of 10mg/L with the solution of pH 5 for a contact time of 45min at 295±1.5K temperature and 170 rpm stirring speed. This initial rise is obvious because raising the biosorbent dose increases the number of functioning sites for metal ions to chelate with. However, the small changes in the later phase can be attributed to accretion in the number of active functioning sites while the metal ions concentration was fixed at 10mg/L, which worked as a concentration gradient [14]. Therefore, the % removal of Pb (II) ions augmented with the rising adsorbent dose while Q_e decreased (Figure 5 (a)). There can be one more possibility that increasing adsorbent dose leads to aggregation of biosorbent particles, consequently, the net surface area of biosorbent (available for adsorption) also decreases and so does Q_e [32].

Effect of initial metal ion concentration

The % removal decreased from 87.33% to 76.8% using TGLB on increasing the metal ion concentration from 10mg/L to 50mg/L (Figure 4 (d)). All the variable parameters were in their respective optimum values. As the concentration of metal ions increases, all active sites eventually fill, and there are no more vacant sites for the remaining metal ions to fill, resulting in a decrease in the overall removal percentage. However, to overcome the resistance to mass transfer of metal ions between aqueous and solid phases, a greater concentration gradient can be utilized as a driving force, increasing the chance of Pb (II) ions colliding with the active sites [8]. Thus, as the metal ion concentration rises, the effective collision of the metal ions and active metal binding sites available on the biosorbent surface rises, increasing Qe as demonstrated in Figure 5(b), and notably, the initial uptake of metal ions is rapid at small concentrations, indicating a rapid surface reaction [23].

Effect of temperature

In the case of physisorption, Vander Waal's forces are the vital forces of attraction between adsorbate and adsorbent, which may be easily dissipated by thermal treatment or lowering the pressure that leads to desorption. Chemisorption contains stronger chemical interactions involving electron sharing and exchange that are far more stable at inclined temperature values and low pressure [8]. Using TGLB as a biosorbent, the percentage removal initially increased from 87.2% to 89.16% on rising the temperature from 298K to 308K and then decreased to 62.8% on rising the temperature from 308K to 338K as depicted in Figure 6 (a), which again indicates the process to be physisorption [8, 15]. The ideal pH value for these experiments was 6, with a period of 45 minutes, a biosorbent dose of 1g, and an initial metal ion concentration of 10mg/L at a swirling speed of 170rpm.

Biosorption thermodynamics

The decline in % removal efficiency with the temperature rise suggests the biosorption should be exothermic. The negative values of $\Delta H°$ (-33.763 KJ/mol) also supported this fact (Figure 6 (b) and Table 2). If the standard enthalpy change of biosorption ranges between 2.1 and 20.9 KJ/mol, then the process corresponds to physisorption, whereas it is considered as chemisorption if enthalpy change ranges between 20.9 and 418 KJ/mol [9]. Therefore, the biosorption of Pb (II) ions on TGLB surface can be demonstrated as chemisorption. The negative $\Delta G°$ outcomes demonstrate that the sorption process occurred spontaneously in the case of both biosorbents (Table 2).

If $\Delta S° > 0$, the reaction proceeds with dissociative mechanism and it proceeds with associative mechanism if $\Delta S° < 0$. The negative output of $\Delta S°$ (-94.530 J/mol/K) demonstrated a diminution in standard entropy on the biosorbent surface after biosorption process implying the process to be associative [8], which can be accomplished by the emergence of activated compounds between the adsorbed metal ions and the adsorbent. Similar results were obtained in the case of biosorption of Cd (II) using meranti wood biosorbent and biosorption of Cu (II) ions using orange peel [13, 23].

Adsorption isotherms

The biosorption equilibrium data of Pb (II) adsorption on TGLB were investigated using three isotherm models: Langmuir isotherm, Freundlich isotherm and Temkin isotherm. These isotherms are useful for determining adsorbent surface properties and adsorbate molecule distribution across liquid and solid phases at equilibrium. Isotherm plots depicted experimental outcomes of Pb (II) ions adsorbed per unit mass (mg/g) of biosorbents versus equilibrium solution concentration for solutions of concentration ranging between 10 mg/L and 50 mg/L at constant temperature (298K) keeping the other conditions also constant.

Langmuir adsorption isotherm

The correlation coefficient (R^2) for Langmuir adsorption isotherm using TGLB was 0.759. To compute the isotherm constants Q_{max} and K_L, the slope and intercept of the plot C_e/Q_e vs C_e (Figure 7(a)) were used. For the initial metal ion concentrations of 10, 20, 30, 40, and 50 mg/L, R_L values were 0.548, 0.377, 0.288, 0.232 and 0.195 showing that the biosorption of Pb (II) on TGLB surface was favorable.

Freundlich adsorption isotherm

The solute concentration adsorbed on a particular amount of adsorbent does not remain constant at different metal ion concentrations. The Freundlich constant K_F was determined from the intercept and n was determined from the slope of the plot $\log Q_e$ versus $\log C_e$ (Figure 7(b)). The correlation coefficient for Freundlich isotherm using TGLB as a biosorbent was 0.980. The value of 1/n came out to be 0.665 (using TGLB) i.e., between 0 and 1, implying that the biosorption of Pb (II) ions on TGLB surface was favorable [7].

Temkin adsorption isotherm

The equilibrium binding constant A and adsorption heat bT for Pb (II) ions adsorption were determined using the plot Q_e against $\ln C_e$ (Figure 7(c)). Using TGLB, the coefficient of correlation was 0.887. The characteristics of the Langmuir, Freundlich, and Temkin adsorption isotherms for Pb (II) adsorption on TGLB are shown in Table 3. The Freundlich isotherm is the most adequately predicted over the concentration range investigated, based on computed values of all the variables of the isotherm models (Table 3). Table 4 presents a comparative assessment of the maximum adsorption capabilities of various biosorbents.

Adsorption kinetics

Pseudo-first-order rate equations are used to explain the kinetic process of adsorption in the liquid-solid phase, whereas pseudo-second order rate equations are used to differentiate between the two [3]. The data were investigated with pseudo-first-order and pseudo-second-order kinetic models as shown in Figures 8 (a) and 8 (b) and Table 5. Kinetic analysis was performed at predetermined time intervals ranging from 20, 40, 60, 80, 100, and 120 minutes. The relevance of a kinetic model is determined on two factors: 1) correlation coefficient value and 2) comparability of Q_e (experimental) and Q_e (calculated). The closer the correlation coefficient is to 1, the more relevant the model is, and the smaller the difference between Q_e (experimental) and Q_e (calculated), the more applicable the model is [14].

Pseudo-first-order kinetic model

The correlation coefficient of pseudo-first-order kinetics for Pb (II) ions adsorption on TGLB was 0.977. The Q_e (experimental) using TGLB was 9.21 mg/g and Q_e (calculated) was 5.078 mg/g.

Pseudo-second-order kinetic model

The Q_e (experimental) for pseudo-second-order kinetic model using TGLB was 9.21mg/g and Q_e (calculated) was 9.8 mg/g. Moreover, the correlation coefficient in the case of TGLB was 0.998. The findings (in Table 5) suggested that adsorption of Pb (II) ions on TGLB followed the pseudo-second-order kinetic model. This implies that the chemisorptions can be a rate-limiting step involving the sharing or transfer of electrons [5, 14]. From FT-IR spectra of the biosorbent, it is evident that there was an involvement of the functional groups in the binding of Pb (II) ions on TGLB surface, which lend credence to the use of chemisorption as a major mechanism [24]. The adsorption kinetics (applicability of pseudo-second-order kinetic model supported chemisorption) and adsorption thermodynamics ($\Delta H°$= -33.76KJ/mol) also support the same fact for TGLB. Except this, it is also notable that the spontaneity of the biosorption process decreased with increasing temperature, this trend implied the physisorption to be a major part of the mechanism. Therefore, these all results indicate that the biosorption of Pb (II) ions on TGLB followed both chemisorption and physisorption [8].

Desorption studies

From the desorption studies using 100mL of double-distilled water, 0.1M NaOH and 0.1M HCl as eluents, it was evident that both double-distilled water and NaOH have a low propensity of removing the adsorbed Pb (II) ions. The 0.1M hydrochloric acid (HCl) was the most appropriate eluent removing Pb (II) ions among all three eluents as depicted in Table 6.

The reusability of TGLB was further investigated by three consecutive cycles of adsorption-desorption processes using 100mL of 0.1M HCl as an eluent. The effect of the number of cycles on the Pb (II) ions desorption capacity was

shown in Figure 9. For the first cycle, the desorption efficiency was 93.21% for Pb (II) using TGLB as a biosorbent, while these desorption percentages dropped up to 88.69% for the second cycle and 80.43% for the third cycle. It may be deduced that TGLB can be utilized for up to 2-3 cycles with a minimal drop in desorbing capability between the first and last cycles. The reduced desorption percentage implies the possibility of structural change and swelling in biosorbent owing to the presence of 0.1M HCl as an eluent during consecutive cycles [34, 38].

CONCLUSION

TGLB was found to be a significant and cost-effective biosorbent for removing Pb (II) ions from wastewater in batch experiments conducted in this study. The optimum pH level was 6. The maximum monolayer adsorption efficiency utilizing TGLB was determined to be 7.35 mg/g, and the Freundlich isotherm was reported to be the best acceptable isotherm model for the biosorption of Pb (II) ions on TGLB surface. The pseudo-second-order kinetic model best explained the biosorption kinetics. The whole process was spontaneous, exothermic, and followed an associative mechanism that led to a reduction in disorderliness at the solute-solvent interface. Considering the findings of FT-IR spectroscopic analysis, change in % removal efficiency with increasing temperature and value of standard enthalpy change, the biosorption of Pb (II) ions on TGLB surface should have consisted of both physical as well as chemical interactions. TGLB can be recycled for three sessions of adsorption-desorption with minimal drop in their respective capacities. Using *Tectona grandis* leaves as biosorbent for decontaminating the water is cheaper, eco-friendly and can also serve as a good example of waste management.

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Table 1: Effect of various parameters on Pb (II) ions removal using TGLB

pH		Contact time		Biosorbent dose		Metal ion concentration		Temperature	
pH	**C_e (mg/L)**	**t (min)**	**C_e (mg/L)**	**Dose (g)**	**C_e (mg/L)**	**C_i (mg/L)**	**C_e (mg/L)**	**T(K)**	**C_e (mg/L)**
2	4.13±0.083	20	2.49±0.090	1	1.35±0.052	10	1.26±0.067	298	1.28±0.095
3	2.99±0.076	40	2.04±0.058	2	0.93±0.046	20	4.11±0.084	308	1.08±0.058
4	2.33±0.046	60	1.02±0.041	3	0.81±0.023	30	6.54±0.081	318	1.45±0.066
5	1.73±0.059	80	0.93±0.015	4	0.62±0.023	40	8.90±0.050	328	3.07±0.108
6	1.02±0.035	100	0.79±0.023	5	0.60±0.022	50	11.60±0.057	338	3.72±0.135
7	2.54±0.043	120	0.79±0.023	-	-	-	-	-	-
CD	0.193	0.153		0.115		0.221		0.308	
SE (m)	0.062	0.049		0.036		0.069		0.097	

±Standard Error, SE (m) Standard Error Mean, CD Critical Difference

Table 2: Thermodynamic parameters for adsorption of Pb (II) ions on TGLB

Metal ion	**ΔG°(KJ/mol)**					**ΔH°(KJ/mol)**	**ΔS°(J/mol/K)**
	298K	**308K**	**318K**	**328K**	**338K**		
Pb (II)	-4.753	-5.406	-4.691	-2.220	-1.471	-33.763	-94.530

Table 3: Langmuir, Freundlich and Temkin isotherm parameters for Pb (II) ions adsorption using TGLB

Metal ion	Langmuir isotherm parameters			Freundlich isotherm parameters			Temkin isotherm parameters		
	Q_{max} (mg/g)	K_L (L/mg)	R^2	K_F (mg/g)	1/n	R^2	B_T (mg/g)	A (L/g)	R^2
Pb (II)	7.35	0.0824	0.759	0.703	0.665	0.980	1.271	1.245	0.887

Table 4: A comparison between TGLB and other biosorbents for Pb (II) ions adsorption capacity

Biosorbent	Adsorption capacity	References
Mahogany fruit shell	322.58	[2]
Fungus *Penicillium purpurogenum*	252.8	[28]
Phytolacca americana leaves	13.19	[10]
Pyrus pashia leaves	5.73	[7]
Rubus ellipticus leaves	3.38	[6]
Dicliptera bupleuroides leaves	1.76	[12]
Artemisia vulgarise	0.86	[11]
Vermiculite	0.238	[21]
Tectona grandis	7.35	Present study

Table 5: The parameters of kinetic models for the adsorption of Pb (II) ions on TGLB

Metal ion	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	Q_e (mg/g)	K_1(/min)	R^2	Q_e (mg/g)	K_2 (g/mg/ min)	R^2
Pb (II)	5.078	0.045	0.924	9.8	0.0148	0.998

Table 6: Desorption % of TGLB for Pb (II) ions using different eluents

Metal ions	Eluents		
	Distilled water (H_2O)	NaOH	HCl
Pb (II)	5.58±0.69	2.52±0.35	95.67±0.65

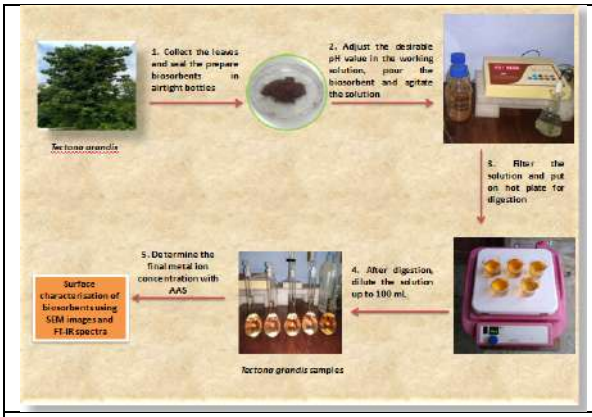

Figure 1: A representative diagram of biosorption process for removal of Pb (II) ions from wastewater using TGLB

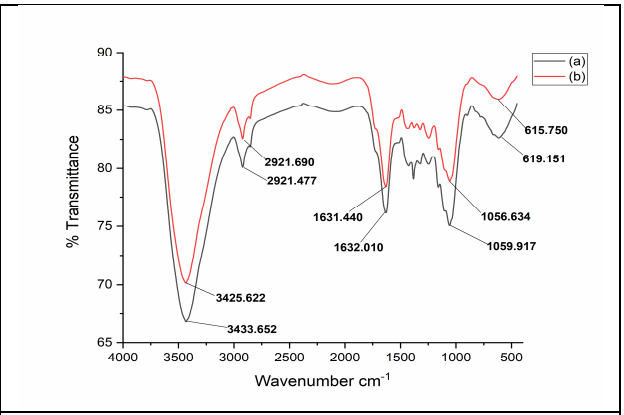

Figure 2: FT-IR spectra of TGLB (a) before and (b) after adsorption of Pb (II) ions

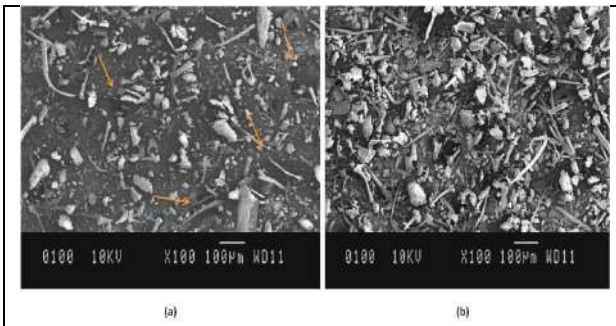

Figure 3: SEM images of TGLB surface (a) before and (b) after adsorption of Pb (II) ions

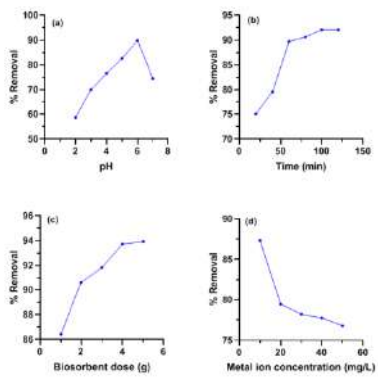

Figure 4: (a) Effect of pH, (b) effect of contact time, (c) effect of biosorbent dose and (d) effect of initial metal ion concentration respectively on the % removal efficiency of the TGLB

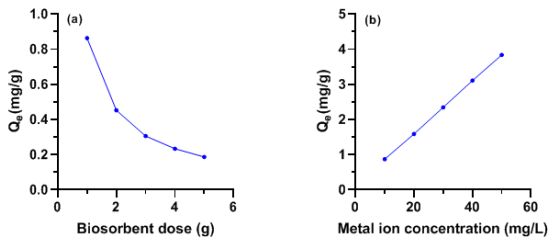

Figure 5: Effect of (a) biosorbent dose and (b) initial Pb (II) ions concentration on biosorbent adsorption capacity respectively

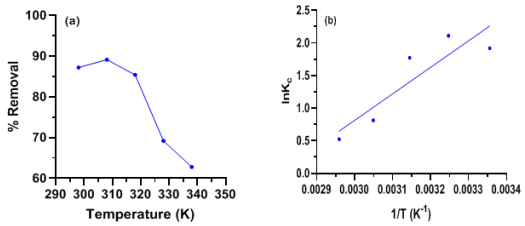

Figure 6: (a) Effect of temperature on % removal efficiency of Pb (II) ions using TGLB and (b) plot of lnKc versus 1/T for Pb (II) ions biosorption using TGLB respectively

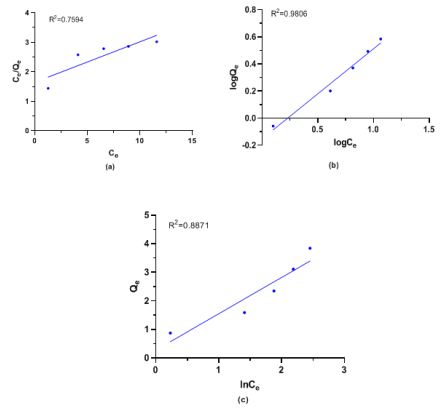

Figure 7: (a) Langmuir, (b) Freundlich and (c) Temkin adsorption isotherms respectively for adsorption of Pb (II) ions using TGLB

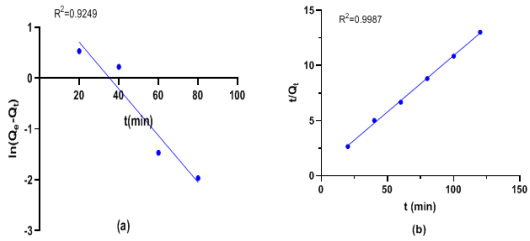

Figure 8: (a) Pseudo-first-order and (b) pseudo-second-order plots respectively for adsorption of Pb (II) ions using TGLB

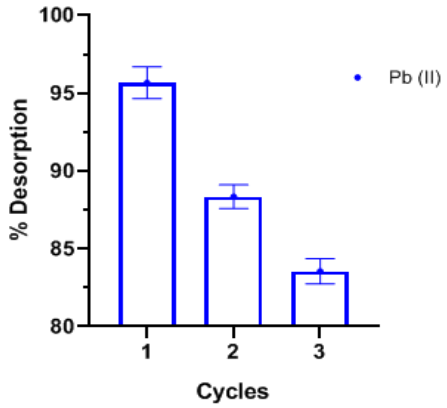

Figure 9: Desorption % of TGLB using 0.1M HCl to elute Pb (II) ions during different cycles

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REVIEW ARTICLE

Medical Errors in Clinical Care and Ethical Facets

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ABSTRACT

Medical or clinical errors and adverse events are common problems faced by health care professionals. Though ethics and the law recommend sincere and timely disclosure of error to be the standard practice, healthcare professional find it difficult to report errors. The underlying fear principle decrease the confidence in reporting. A confined and systematic system established in one's clinical practice and healthcare institute can minimize errors. This can lead to a decrease in the medicolegal liabilities of health care professionals. The concept of error disclosure should be included and imparted as a part of the healthcare education system. This article reviews the basic aspects of medical errors, its ethical, and practical implications.

Keywords: Medical error, apology law, error disclosure, medical negligence

INTRODUCTION

"*To err is human/ Errare humanum est*" is a common phrase that encapsulate the frailty of human nature [1]. Healthcare professionals as humans can be subjected to committing mistakes, but being associated with a noble profession, entails within it certain ethical or moral values. Clinical ethical practice not only revolves around the use of expert knowledge and skill obtained for the benefit of the patient or the society but also impregnates within it honesty, integrity and human ideals. The medicolegal issues that arise during practice or when treating a patient can be included under medical error, malpractice and negligence, informed consent, errors from irrational use of drugs or use of costly sophisticated technology and breach of confidentiality or privacy. In case of dental practice, this includes extraction of wrong teeth, administration of saline instead of local anaesthetic, administering or prescribing

wrong drug or dose, misplacement of biopsy samples, operator injury [2]. Medical error can be defined as the failure of a planned action to be completed as intended (an error of execution) or the use of a wrong plan to achieve an aim (an error of planning) [1,3,4] OR An act of omission or commission in planning or execution that contributes or could contribute to an unintended result [1]. These errors are usually considered to be "preventable adverse medical vents" [5]. Patients are harmed as a result of what is done to them – errors of commission - or as result of what is not done but should have been done to prevent an adverse outcome - errors of omission [6]. Medical malpractice usually occurs when a health care professional causes an injury to the patient due to a negligent act or omission and medical negligence occurs due to lack of action by the professional without intent. An error may or may not produce an adverse event. Adverse events are injuries that result from medical interventions that cause harm to the patient like death or any disability like facial paralysis.

Various Medical Error Reporting Systems (MERS) have been initiated to acquire more secure and higher quality patient care. A few examples of MERS designed for hospitals and health systems and accessible to health personnel include [7,8] the (i) Centers for Disease Control's National Nosocomial Infection Survey, (ii) U.S. Pharmacopeia MEDMARX - a national medication error-reporting program, (iii) the American Surgical Association's National Surgical Quality Improvement Program, (iv) Medical Event Reporting System for Transfusion Medicine, (v) Swiss Anesthesia Critical Incident Reporting System, (vi) Edinburgh Intensive Care Unit Critical Incident Reporting System, and (vii) Australian Incident Monitoring Study; which has led to documenting information on medical errors and numerous research publications highlighting the acute need for improved patient safety [7–9].MERS is underutilized by the healthcare professionals and less than 10% of medical errors are being reported as stated by Anderson and Abrahamson [10].

Few of the common reasons for healthcare professionals not reporting medical errorsare: (i) too busy routine and therefore too worn-out to report, (ii) wary of disciplinary or legal action or being perceived as being incompetent, (iii) unaware that they need to report, (iv) unaware on how to do the reporting, (v) demotivated by the lack of any form of immediate feedback and having the assumption that the institution or system does not take visible corrective actions to prevent recurrence of the errors [7,8,11,12].

Epidemiology

The Institute of Medicine (IOM- 1999), USA reported that 98,000 Americans die each year as a result of preventable medical errors, nearly half of the adverse events that occur in US patients are due to avoidable medical errors and cost 17 to 29 billion US dollars [13]. A Harvard study by Prof Jha (2013) showed that 5.2 million medical errors are happening in India annually and around 43 million. Studies done in Uttarakhand and Karnataka have documented medical error rate to be as high as 25.7% and 15.34%, respectively, in hospitalized patients [14].

Types of Medical Errors

Elder et al., [15] had derived a classification system of medical errors on three main categories of preventable adverse events related by primary care: diagnosis, treatment, and preventive services(Table I). Another classification that has been under consideration[16]is based on the prevention, treatment, diagnosis, system and communication errors (Table II) .

Potential harms of error disclosure

Coping with medical error is never easy and numerous factors prevent error disclosure: legal (the hazard of malpractice litigation), economic (effect on professional practice if the error is leaked to the public), psychological (erosion of self-esteem)[6], loss of referrals, credentials, licensure and patient reaction. Doctors fear, often justifiably, that media may use these incidents as fuel to fire a campaign against medical profession.

Ethics

The patient doctor relationship is fiduciary in nature relying on principles of autonomy, beneficence, nonmaleficence, justice & fidelity in all actions. The doctors must always act on the patient's interest. Ethics and law advocate that doctors have a moral commitment to disclose their errors to the patient in a timely and openly manner [17]. Failure or nondisclosure of errors to patients destabilizes the trust in healthcare [18] and this can be considered as a breach of professional ethics — a lapse in the commitment to act solely for the patient's interests. Failure to disclose can further harm the patients if they are injured and may require relevant information about what transpired during and after the treatment that led to the injury and this will further help them to consent properly to treatment for an injury caused by error [19]. To maintain autonomy and to give true informed consent, patient must be aware of relevant errors. Patients should be also be due informed about errors out of respect for them as humans. Thus, they have a right to know about critical incidents even if the errors do not produce any physical harm. If the error has resulted in increased cost to the patient, justice would dictate disclosure to ensure patient compensation. Fidelity demands truth telling at all times.

Disclosure of error, by contrast, is consistent with recent ethical advances in medicine toward more openness with patients and the involvement of patients in their care. This ethical rationale for disclosure, based on a strong notion of autonomy, goes beyond what the law might require one to do. Improved communication may play a major role in reducing the risk of litigation. Patients often appreciate candour and empathy that may also help to assuage the patient's apprehension and in turn may decrease the medico legal liability of the health care professionals [6]. An open and transparent approach may help to strengthen the doctor–patient relationship. Nondisclosure may be rationalized by concerns about increasing patient anxiety or confusing the patient with complicated information. It may also undermine efforts to improve the safety of clinical practice if the error is not reported to the appropriate authorities.

Potential harms and benefits of disclosure to patient

Disclosure can lead to anxiety, alarm, and discouragement, destroy faith and confidence. The American College of Physicians Ethics Manual states "society recognizes the therapeutic privilege which is an exemption from detailed disclosure when such disclosure has a high likelihood of causing serious and irreversible harm to the patient. However, on the balance, this privilege should be interpreted narrowly; invoking it too broadly can undermine the entire concept of informed consent [20]. On the contrary doctors can gain absolution for the mistake, decrease in likelihood of legal liability, improve and to take constructive changes in their practice.

How should doctors disclose medical errors?

Disclosures are complex and subtle discussions and should be tailored to the nature of the event, the clinical context, and the patient– provider relationship. Error disclosure should never be an apathetic/ cursory or a perfunctory process [21]. The nature of mistake, consequences and corrective action taken or to be taken has to be stated. Offering an apology for harming a patient should be considered to be one of the ethical responsibilities of doctors. Full and honest disclosure of errors is most consistent with the mutual respect and trust that patients expect from their doctors. If it has led to a major adverse impact, compensation offers be made with necessary supportive services. Financial amends should include all extra expenses incurred such as doctor's fees, error generated lab fees, hospital expenses and drug costs. Hospital risks management teams can adopt malpractice insurers to reduce the size of malpractice suits.

Measure for error disclosure/disclosure standards: Key Elements of Safe Practice for Disclosing Unanticipated Outcomes to Patients [21]

Content to be disclosed to the patient

- Provide facts about the event
- Presence of error or system failure, if known
- Results of event analysis to support informed decision making by the patient

- Express regret for unanticipated outcome
- Give formal apology if unanticipated outcome caused by error or system failure
- Feedback and results from the investigation team

Institutional requirements

- Integrate disclosure, patient-safety, and risk-management activities
- Establish disclosure support system
- Provide background disclosure education
- Ensure that disclosure coaching is available at all times
- Provide emotional support for health care workers, administrators, patients, and families
- Use performance-improvement tools to track and enhance disclosure

Medical error prevention and disclosure systems

Many organizations are adopting disclosure policies and educational programmes. These programs strongly encourage transparent communication with patients after unanticipated outcomes, and they supply some impressive tools for helping clinicians achieve this goal. Clinical auditing /clinical governance issue/ error proofing systems: The value of Six Sigma Quality has been documented in other fields and industries. Few factors that are required to be added into healthcare delivery system include: • Accountability • Raising the standard of health care organizations and professionals • Using standardized procedures, checklists and results • Data integration and integrity, documentation• Building public awareness [16]. In order to encourage open disclosure more specifically by physicians, a number of countries have enacted *disclosure laws* mandating disclosure of medical errors under specific circumstances.[22]Several countries have also enacted *apology laws*, *i.e.,* laws providing that an apology given after an adverse event cannot be used in ulterior legal proceedings (Australia - enacted such a law).[23]The actual effect of those laws on professional behavior is debatable. The Professional insurance coverage/ indemnity policies can be adopted which can to some extend help in the coverage of legal expenses.

CONCLUSION

Every healthcare professional holds the responsibility to communicate truthfully and the responsibility of error disclosure to patients that occur in their care. Many barriers imposed by health care systems, patients and the law confound the practice of disclosing errors to patients. Such barriers should be overcome by the hospitals or health care institutions. Policies and procedures should be developed to encourage and support the reporting and disclosure of errors. Such an error disclosure attitude should be inculcated into the professional training courses so as to avoid fear and to instigate the responsibility in the future generation doctors.

Conflict of Interest

The authors declares that there are no existing competing interests.

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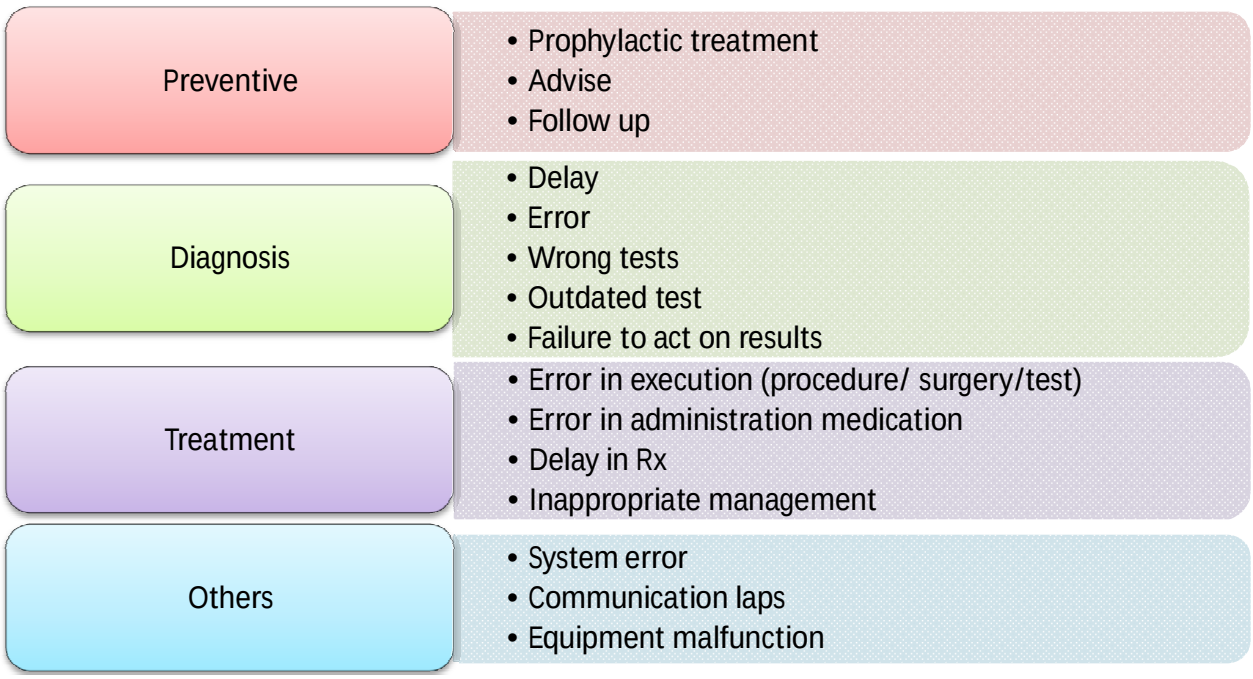
Table I: Types of medical errors [15]

Diagnosis	Related to misdiagnosis • Misdiagnosis ▪ Missed diagnosis ▪ Delayed diagnosis
	Related to prevention • Misdiagnosis ▪ Missed diagnosis ▪ Delayed diagnosis

Treatment	Drug ▪ Incorrect drug ▪ Incorrect dose ▪ Delayed administration ▪ Omitted administration
	Non-drug ▪ Inappropriate ▪ Delayed ▪ Omitted ▪ Procedural complication
Preventive services	▪ Inappropriate ▪ Delayed ▪ Omitted ▪ Procedural complication

Table II: Classification of errors [16]



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REVIEW ARTICLE

Review on Monoazaphenothiazine Analogues

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ABSTRACT

Azaphenothiazines are the modified structure of phenothiazines, and exhibit various biological importance. This interesting group of compound has diverse biological activities such as anticancer, antimicrobial, CNS activity, ant obesity, antioxidant, anti-inflammatory, antiemetic, cytotoxic activity etc., It is hoped that this review focused on the documentation of chemistry and biological activities of various azaphenothiazines scaffolds which will be helpful for new thoughts in the quest for rational designs of more active azaphenothiazine analogs.

Keywords: Azaphenothiazines, CNS activity, Anticancer, Anti-inflammatory, Antioxidant.

INTRODUCTION

Azaphenothiazine are obtained by structural modification of phenothiazine ring by introducing one or more nitrogen atom in the benzene ring. The research and developments related to azaphenothiazines based medicinal chemistry have become a promptly developing and increasing. Medicinal properties of azaphenothiazine includes neuroleptic, antipsychotic, schizophrenia, CNS depression, acute-mania, sedative, hypnotic, anticholinergic, anticonvulsant, antiviral, antimicrobial, antimalarial, antiparasitic, antihistaminic, antioxidant, analgesic, anti-inflammatory activity and antiarrhythmic activities[1]. Its wide biological activities are summarized in numerous comprehensive review papers and patents but the present review summarizes the recent details about the chemical classification, synthesis and biological importance of azaphenothiazines analogs.

General Concept of Azaphenothiazines

Azaphenothiazines are to be tricyclic, tetracyclic, pentacyclic and hexacyclic. About 50 tricyclic azaphenathiazine are obtained based on the position and number of nitrogen as well as nature of fused ring system [2]. Modification of phenothiazines by replacing azine rings such as pyridine, pyridazine, pyrimidine, pyrazine, or quinoline in the place of benzene ring gives azaphenothiazine like Pyridobenzothiazines, Pyridazino, Pyrazino, Pyrimidino, Tricyclazino, Pyridoquinothiazines, Pentacyclic etc. Depending upon the number and position of nitrogen in phenothiazinering about 50 types of azaphenthiazines are known with various heterocyclic rings[2].Based on the number of nitrogen atom tricyclic azaphenothiazinesare classified into monoazaphenothiazine. Diazaphenthiazine, Triazaphenothiazine and tetra azaphenthiazines.

Monoazaphenothiazine

Pyridobenzothiazines are monoazaphenothiazines and this category covers 1-Azaphenothiazine, 2-Azaphenothiazine, 3-Azaphenothiazine and 4-Azaphenothiazines (Figure: -1). It is a linear fusion of 1.4 thiazine ring with pyridine and benzene. The1-Azaphenothiazine (Pyrido [3,2, -b] benzo [1,4] thiazines) substituted with rothipendyl, isothipendyl, oxypendyl, cloxypendyl and pipazethate givesProthipendyl, Isothipendyl, Oxypendyl, Cloxypendyl, and Pipazethate, respectively [3]**.** Prothipendyl, 10-(3-dimethylaminopropyl)-1-azaphenothiazine, (Figure: -2) are used in the treatment of anxiety and agitation in psychotic syndromes, available under brand name Dominal and Tolnate[4]and used in Schizophrenia [5], treatment-resistant depression[6], acute mania[7], unspecfic sedation[8] and dementia[9]. Prothipentyl differs from Promazine by the replacement of a one carbon atom in phenothiazine ring. In recent times Prothipentyl showed antiviral action against a mosquito- transmitting alphavirus (CHIKV) including CHIK fever [10,11]. Isothipendyl, 10-(2-dimethylamino-2-methylethyl)-1-azaphenothiazine (Figure: 3) is a shorter alkyl chain between the amine and thiazine nitrogen atom, sold under the trade names Actapront and Alergis. It is a first generation H1 antagonist antihistaminic, anticholinergic and antipruritic (Reference). This Isothipendyl is exhibited some phototoxic properties with ultraviolet A(UVA) and it reduced the erythema response to UVB radiation [12].

Oxypendyl, 10-[3-(hydroxyethyl-4-piperazinyl) propyl]-1-azaphenothiazine (Figure: -4a) exhibits antiemetic activity and also called Pervetral[13]. Cloxypendyl, 3-chloro-10-[3-(hydroxyethyl-4-piperazinyl) propyl]-1-azaphenothiazine (Figure: -4b) are used as potent sedative and neuroleptic activities. This compounds derivatives as dimethylaminoethyl, dimethylaminopropyl, hydroxypiperazinylpropyl groups were less active. There are very good tolerance and favorable therapeutic range[14]. Pipazethate, 2-(2-piperidinylethoxy) ethyl 1-azaphenothiazine-10-carboxylate (Figure 4c) by schuler *et al.* first introduced phenothiazine as an antitussive drug, suppressing irritative and spasmodic cough by inhibiting theexcitability of the cough Centre and the peripheral neural receptors in the respiratory passage, but not depressing respiration[15].

Chemistry of Azaphenothiazines

N-unsubstitutedAzaphenothiazine can be synthesized by any one of the following ways

- Cyclization 2-phenylaminopyridine with sulphur
- Condensation of 2-amino benzene thiols and substituted pyridines
- Smiles rearangementofo-amiophenylpyridylsulphide containing a leaving group
- Phenyl pyridyl amine on thionation with sulphur or thionyl chloride

Synthesized 1-Azaphenothiazine (Figure: 5) using 2-chloropyridine and aniline as starting material via two steps including condensation and cyclization of formed 2-phenylaminopyridine with sulphur. Buchwald–Hartwig tandem amination protocol was achieved successfully by Egbujor and his co-workers for the synthesis of various aniline substituted derivatives of 3-chloro-1-azaphenothiazine (Figure: 6). The basic nucleus azaphenothiazine was prepared by the reaction of 2-aminothio phenol with 2,3,5 trichloropyridine[16]. Synthesize and antimicrobial activity of 1-azaphenothiazine derivatives by condensation of 2-amino benzene thiols and 2-chloro-3-5-dinitropyridines was carried out by Swathi *et al.* ., By condensation of the zinc salt of substituted 2-amino benzene thiols and 2-chloro-3,5-dinitropyridinesmesoionic compound viz substituted 1-hydroxy 2-chloro acetyl piperidino acetyl and piperizino

acetyl-4-nitro 6-thia-10b-aza-2azonia aceanthrylene hydroxide inner salts and substituted 1-hydroxy -4-nitro 6-thia-10b-aza 2a-azaniaaceanthrylene hydroxide inner salts were prepared and were screened anti-microbial activity[17]. Condensation of 2-aminobenzenethiol **4** with 2-chloropyridine in the presence of iodine gives directly 10H-azaphenothiazine [18] (Figure 7).

Most commonly reported synthesis for N-substituted azaphenothiazinesis from *o*-aminophenyl pyridyl sulphide either by ullmanncyclisationandsmiles rearrangement of it to o- mercaptophenylpyridylimines then to azaphenothiazine (Figure 8).

The smiles rearrangement of pyridyl phenyl sulphide depends upon on the nature of sulfides, steric hindrance, reaction conditions (basic acidic and even neutral and thermal) and presence of catalysts. Depending on the type of sulfide undergoing the rearrangement, phenyl azinylsulfides and diazinylsulfides, and a type of azine (pyridine, pyridazine, pyrimidine, pyrazine, 1,2,4-triazine, quinoxaline and benzoquinoxaline) various types of Azaphenthiazineslikemonoaza-, diaza-, triaza-,tetraazaphenothiazines and their benzo- and dibenzo derivatives were obtained.Yale and Sowinski in 1958 first carried out the cyclization of phenyl 2-pyridinyl sulfides[11]. 2-Acetylaminophenyl 3- nitro-2-pyridinyl sulphide on heating with ethanolic potassium hydroxide and acetone under nitrogen form an intermediate amine by smiles rearrangement and cyclizes to 10-acetyl-1-azaphenothiazine (Figure: 9) Refluxing 2,3,5- trichloropyridine and 2-aminobenzenethiol in presence of DMF gives an intermediate sulfide which rearranges to 3-chloro-10H-1- azaphenothiazine[16]. The halogeno derivatives of 10H –3-nitro-1-azaphenothiazines without any groups at the thiazine nitrogen atom and evaluated anti-bacterial activity. The phenoxy and methoxy groups are attached in the substituted 10H-3-nitro-1-azaphenothiazines (Figure: 11a) revealed better antimicrobial activity than other compounds[19].

The 10-(1-Methylpiperidnyl1-3-ethyl)-1-azaphenothiazines (Figure: 11b)shows high binding to the D2 dopamine and 5-HT2A serotonin receptors and also screened by anti-tubercularactivity [20]. Then the 1-Azaphenothiazines and their S-oxides established a mixed radical scavenging activity in both DPPH (1,1-diphenyl-2-picryl hydrazyl) and ABTS $^{+}$(2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assays and that revealed increase in sulfhydryl group (GSH)assay[21]. There are twenty-one 10-substituted 1-azaphenothiazines having hexyl linker ending with acyclic and cyclic amino groups, bromine atom or nitro group and halogen atom in position 7 were screened anticancer activity [22].

A new derivative of monoazaphenothiazines were produced under reaction of 2-aminothiophenol with 2,3,5-trichloro pyridine in antacid medium via Buchwald Hartwig coupling reaction. The new subsidiaries of monoazaphenothiazines such as 3-anilino-1-monoazaphenothiazine, 3-(4-nitroanilino)-1-monoazaphenothiazine, 3-(4-hydroxyanilino)-1-monoazaphenothiazine, 3-(3-nitroanilino)-1-monoazaphenothiazinewere synthesized reported for antibacterial activity [23]. The condensation reaction between 2-aminothiaphenol and 2,3,5 trichloro pyridine in aqueous basic medium to produce 3-chloro azaphenothiazine by applying Buchwald Hartwig Cross Coupling reaction, in good yield [24].

The structure activity relationship for the incorporation of a nitrogen atom into the phenothiazine framework result in increased potency and selectivity for HDAC6 as rationalized by molecular modelling and docking studies. The preparation of phenothiazines and their analogues containing a benhydroxamic acid moiety shows good zinc-binding group. This evaluated their ability by assessing their effects on various cancer cells[25]. The activity of a new synthesized azaphenothiazines as tricyclic 10-substituted dipyridothiazines, pentacyclic 6-substituted diquinothiazinesandhexacyclicdiquinothiazium salt was analysed for invitro cell lines. The azaphenothiazine was the first anticancer derivatives [26]. The presence of ethylene group in the aminoalkylazaphenothiazines act as a linker that found to be similar to the linkers in aminoalkylphenothiazines substituted by propylene and butylene.
The 2-Chloropyridine and aniline is used as starting material via condensation and cyclization with sulfur and the reaction temperature was maintained at 270°C. The product was confirmed by HPLC and 1-azaphenothiazine was

reported antihistamine and antipsychotic activity [27]. A new series of amino and aldehyde substituted azaphenothiazines are produced by reaction of 2-chloro-1-(10H-azaphenothiazin-10yl) ethenone with various amines and aryl aldehydes. The substituted aldehyde and amine are attached with some alkyl chain or heterocyclic moiety leads to potentially biological active compounds [28].

2-Azaphenothiazines

The 2-azaphenothiazine and 8-chloro -2-aza-phenothiazinewere synthesized by the ring closure of pyridylphenylsulphide like that of phenothiazine from diphenylsulphide [29]. A dimethylamino-2-methylpropyl group is attached to the 10th position of the nucleus obtained by 10-Aminoalkyl -2-azaphenothiazines. It should be generally less potent antipsychotics than their 1-aza analogs. However, their N-oxides had useful CNS depressant activity [30]. The 10H-2-azaphenothiazines are not reported in the literature about that antitumor activity. The amino alkyl group containing 2-azaphenothiazine are shows antipsychotic and sedative properties [31].

3-Azaphenothiazines

A 3-Azaphenothiazine (Figure: -11) is chemically Pyrido [3,4-b] benzo [1,4] thiazine reported for sedative, hypnotic, anticonvulsant and hypotensive activity [2]. The substituted 1-cyano -3-azaphenothiazin-3H (2)-ones (Figure:13) and their S-oxides and S-dioxides were verified by binding affinity to the benzodiazepine receptors and anticonvulsant activities. The binding nature is increased due to the presences of methyl group at the thiazine nitrogen atom. The methyl group are attached at 4th position diminished the affinity and the chlorine atom attached at position 7 and 8 does not alter the binding [32].

There are five 10-aminoalkyl 3-azaphenothiazines as the oxalate salts evaluated potential analgesic activity. The pyrrolidinyl propyl groups are attached at 10th position more active than those with ethyl chain [30]. Anopen and cyclic amine moiety containing 3-Azaphenothiazines namely 10H-3-aminoaklyl-3-azaphenothiazinium chlorides are potential hypotensive effects [33]. A new 3-Azaphenothiazines (Figure: 14) were prepared under reduction and nitration process. The condensation reaction between 4-chloro-3,5-dinitropyridine with o-amino thiophenol led to the formation of 1-nitro-3-azaphenothiazine. The corresponding oxazine analogue are not able to isolate the intermediate dinitropyridyl o-aminothiaphenol, to be appeared under spontaneous ring closure formationand that produce 1-nitro-3-azaphenothiazine further proceeds reduction process to be formed 1-amino -3-azaphenothiazine.

The 1-nitro-3-azaphenothiazine reacts with nitrous acid led to the formation of 3-azaphenothiazine 1,10-diazole (Figure: -15b) a typical reaction of an o-amino diphenylamine residue.That the 1-nitro-3-azaphenothiazine is concentrated with sulphuric acid undergo nitration process to givedinitrosulphoxide and a 1,7-dinitro-3-azaphenothiazine sulphoxide are produced whether nitration of the corresponding 1-nitro-3-azaphenothiazine or nitration of phenothiazine which lead to a 3,7-dinitrophenothiazine sulphoxide [34]. Reduction on 1,7 dinitroazaphenothiazine to be produced 1,7 diamino 3-azaphenothiazine (Figure: 15a). Among recent many years azaphenothiazines has been synthesized by smiles rearrangement of 2-acetamido phenyl 3-nitro 4-pyridyl sulfide in acetone solution by the addition of potassium hydroxide to giv 60% yeild of 3-azaphenothiazine. Then it has been alkylated with 3-dimethyl aminopropyl chloride using sodium amide in refluxing toluene to give 10(3-dimethylamino propyl)3-azaphenothiazine [31].

4-Azaphenothiazines

The 4-Azaphenothiazines containing skeleton is studies on their potential activity as allergic inhibitors. A new 4-azaphenothiazine were synthesized by 2-chloro pyridine reacts with o-amino thiophenol in the presences of biphenyl solvent at 125°C or by react with iodine in the presence of biphenyl solvent at 200°C[35]. An azaphenothiazines are originated the modifications of phenothiazine with azine rings. Such phenothiazine should not contain only a tricyclic ring system but also tetra and pentacyclic ones with additional nitrogen atom in aromatic rings with pyridine and quinoline rings. Among 150 compounds were screened for their potential biological activities, mostly in in vitro tests. The few selected compounds showing best suppressive activities were preferred for evaluation of their

potential suppressive in mouse *in vivo* models such as models of delayed type hypersensitivity (DTH) to ovalbumin (OVA) [36].

CONCLUSION

Azaphenothiazines are structurally modified phenothiazines, formed by substitution of one or both benzene rings with the azine rings such as: pyridine, pyridazine, pyrimidine, pyrazine, 1,2 4-triazine, quinoline,quinoxaline,benzoxazine and benzothiazine. The azine nitrogen atoms are concerned, they may form monoaza, diaza, triaza and tetraazaphenothiazines. But the structure diversity, azaphenothiazines required new methods of synthesis, different from those of the classical phenothiazines. Many of them NH-azaphenothiazines (having only hydrogen atom at the thiazine nitrogen atom) showed significant biological activities they produced valuable pharmacophoric scaffolds. The nature of the substituent R at the thiazine nitrogen atom and the substituent Z in the benzene or azine rings accustomed the biological activity. The authors hope that this review highlights the importance of the substituted azaphenothiazines in the search for lead compounds in the anti-depressant activity.

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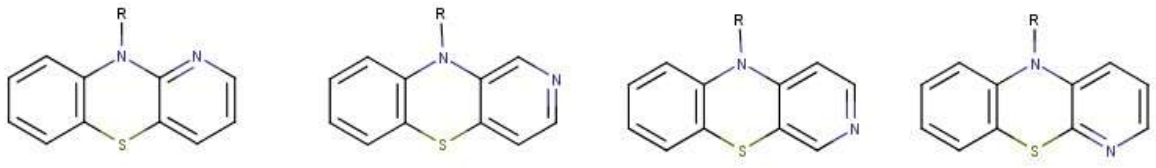

Figure -1 : Monoazaphenothiazine

Figure-2: Prothipendyl, 10-(3-dimethyl aminopropyl)-1-azaphenothiazine

Figure-3: 10-(2-dimethylamino-2-methylethyl)-1-azaphenothiazine

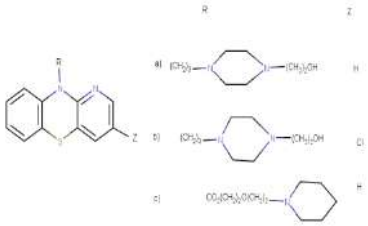

Figure-4: propyl]-1-azaphenothiazine,

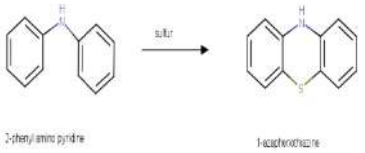

Figure: 5: Synthesized 1-Azaphenothiazine

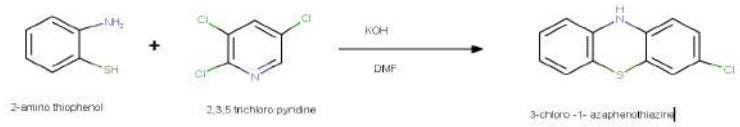

Figure: 6 3-chloro-1-azaphenothiazine

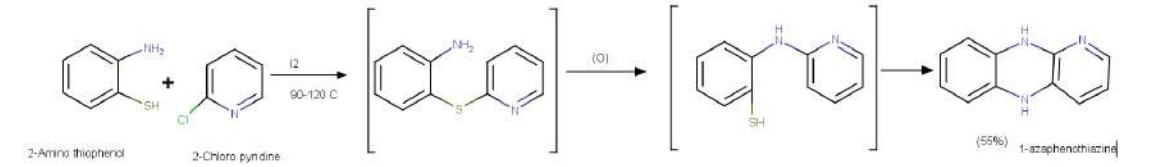

Figure-7 : 10H-azaphenothiazine

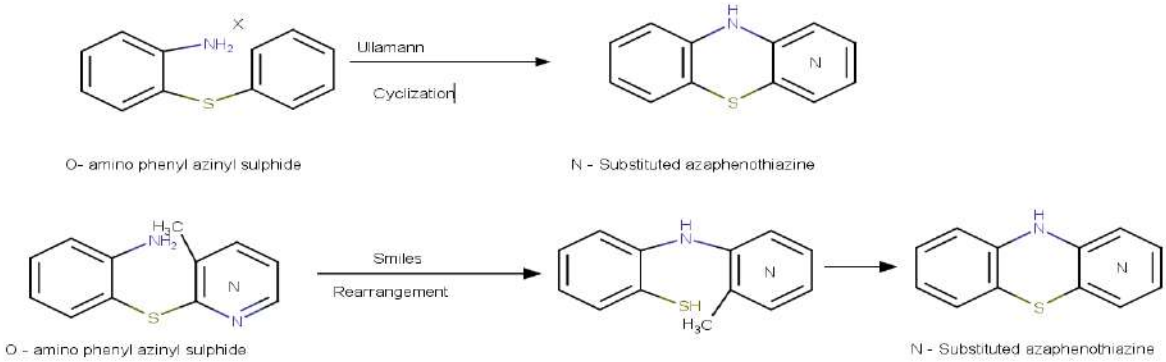

Figure- 8 - Synthesis of 1-Azaphenothiazines

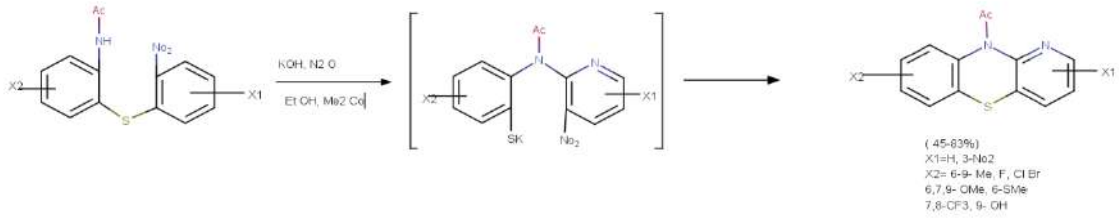

Figure-9 :10-acetyl-1-azaphenothiazine

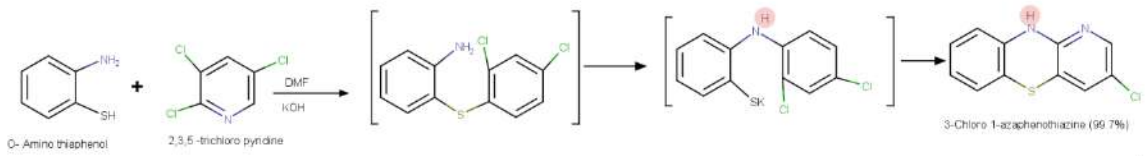

Figure-10 :3-chloro-10H-1- azaphenothiazine

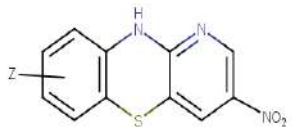

Figure-11a :substituted 10H-3-nitro-1-azaphenothiazines

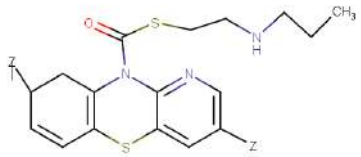

Figure – 11b 10-(1-Methylpiperidnyl1-3-ethyl)-1-azaphenothiazines

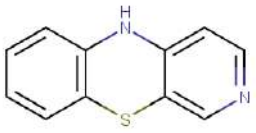

Figure – 12 3-Azaphenothiazines

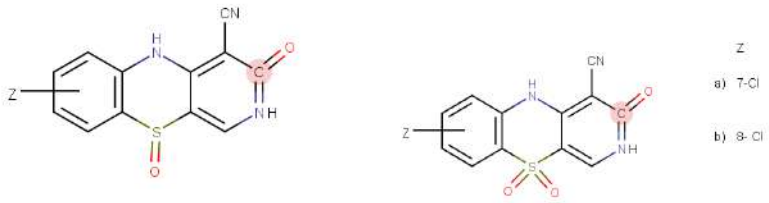

Figure – 13: The substituted 1-cyano -3-azaphenothiazin-3H (2)-ones

Figure -14 : new 3-Azaphenothiazines

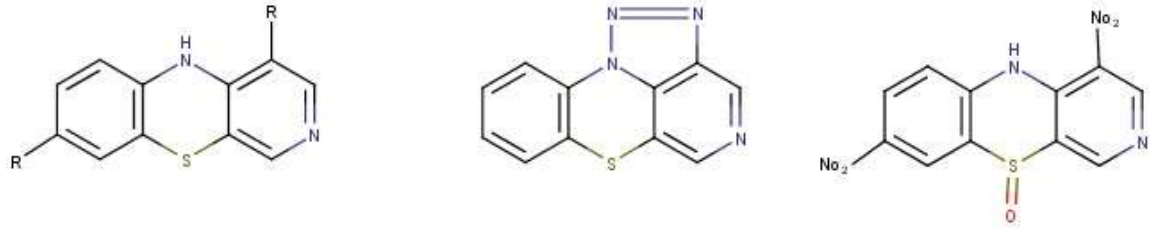

Figure 15a-c

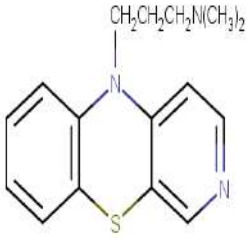

Figure 16

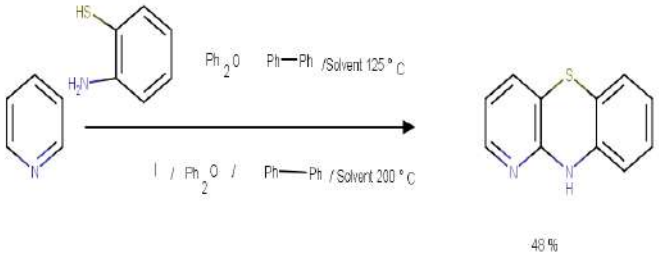

Figure -17 : 4-Azaphenothiazine

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RESEARCH ARTICLE

Challenges on *In-vivo* Culture and Recovery of Entomopathogenic Nematodes from *Galleria mellonella*

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ABSTRACT

In this paper, we have compiled all of the challenges encountered during the selection and culturing of the host, as well as the recovery of nematodes using the white trap process. *Galleria mellonella* is used as a host insect to culture EPN's via the white trap method. Galleria were cultivated in the lab using an artificial diet. During this time, great deal of discomfort was experienced including predator attacks on insects, larvae mortality due to a lack of food, fungal assault, and the content and quality of artificial diet has a detrimental impact on larval growth and reproduction. Also had trouble in detecting, collecting, and storing of EPN's as well. There were some suggestions given for recovery of entamopathogenic nematodes from *Galleria mellonella*. People may benefit from certain improvements and knowledge of EPN production. Management of obstacles will offer confidence to work more on EPN.

Keywords: *Galleria mellonella, Entamopathogenic nematodes, white trap method*

INTRODUCTION

Due to the negative impact of pesticides on environment, agriculture need for biological alternatives to control the pest in the field. Chemical pesticides are more restricted, costly and less effective. The discovery of EPN act as a good biological solution for the control of variety of insects pests in the agriculture. The EPN's *Heterorhabditis* and *Steinernema* carries symbiotic bacterium *Photorhabdus and Xenorhabdus* respectively. Injective juveniles of EPN's attack the host and release the bacteria. In the host blood bacteria multiplies and release the toxic substances with kill the insect within 24-48 hours by septicemia. EPN species are lethal and simple to use which can be mass produced either by *in-vivo* or *in-vitro* (solid or liquid). High virulence of nematodes is produced only by *in-vivo* method. This is low-cost technology method with low startup costs which involves the production of EPN's by using live insects, which are highly susceptible and easily available at a lower cost. The insects used under this method are larvae of the greater wax moth, *Galleria mellonella*, the rice moth, *Corcyra cephalonica*, or the meal worm, *Tenebrio molitor* which are reared in the laboratory. Generally, the last instar of *G.mellonella* is preferred , due to its high susceptibility, easy availability and high yield of IJs.

The greater wax moth*G.mellonella*is the most common host for mass-production EPN. The main advantages of the insect are their size and short lifecycle, easy rearing on artificial diets consisting of several ingredients, rearing at various temperatures (20-37°C), and high nematode yields [1]. Since the greater wax moth is a non-vertebrate model, using it as a mini-host has no ethical implications. The reasons for scientist choose the insects in immune studies are the following; (i) analysis of the host-pathogen interaction; (ii) understanding the innate immune mechanisms; (iii) testing the virulence factors of human pathogens; (iv) testing in vivo antimicrobial activity of new drugs; and (v) looking for bioactive molecules, for example, with antimicrobial, antiviral and anticancer activity and for use as biopesticides [2].

METHODS

Materials

- Containers for insects
- Forceps
- Artificial Food (Refer Table 1)
- Petri plates
- Watch Glass
- Double Distilled Water
- Storage Containers

Collection of *G.mellonella*

The worms were collected from the beekeeping site of our college. The bee colonies were fully fed by worms all over. With the help of forceps larvae and the pupa were carefully transferred into the clean plastic containers and maintained for the culturing.

Culture medium for *G.mellonella*

Artificial diet was prepared by adding the ingredients of different flour and mixed slowly with honey and glycerin. Diet should be mashed sparely without lumps well. The diet should not be too moist or without moisture. If too moist the diet will thicken so worms cannot penetrate inside. The diet was constantly stirred thoroughly and prepared diet was kept in a plastic container and used after 4 or 5 days.

Rearing of *G.mellonella*in containers

Fill 5 to 8 cm of well cleaned plastic containers with artificial diet food. Transfer the well-developed larvae into the container. These larvae form the cocoon and turn into pupa. The turned pupa should be kept in a separate container without food. Because, at this time they do not feed any food. If the cocoon not transferred into the separate container, they will come out as an adult moth and it is very difficult to transfer them because they are likely to fly out. Cover all the plastic containers tightly with cloth and wrap the opening with thread. The female insect that comes out from the cocoon will start laying eggs in the edges of the container. Once the eggs are laid, they die. In a week or two the tiny larva will come out of the eggs. Remove all the dead insects in the container and place the tiny larvae into clean container. Afterwards feed them with fresh culture media. It can take five to six weeks for tiny larvae to turn into mature larvae. Until then monitor them frequently and place the food they need in the container.

Soil collection for EPN's

Soil samples were collected randomly with a hand shovel from forest, orchards and field crop. Soil samples collected at a depth of at least 15cm. 5 random samples were collected within an area and 3 subsamples per sample were also collected. Each sample should be 250gm. samples were placed in polythene bags to avoid dehydration and carefully transported in laboratory. Samples are labeled with the water proof markers (Figure 2). The label should contain information of the site location, temperature, habitat description and vegetation. The hand shovel was sterilized with ethanol (70%) after each sampling.

Nematode isolation from soil sample

Remove all the debris, rocks and leaves from the soil samples. Moisten the soil with water. So, the nematode in the soil can easily move above. Add 200-250 gm of moist soil into the plastic container with lid. Add 5 to 10 worms in the bottle and cover with lid. Turn the bottle over and place it in a dark room. Every 2 to 3 days remove the dead larva and replace it with healthy for additional nematode infection. Rinse the cadaver with distilled water.

Nematode recovery

Nematode Recovery from Infected Cadavers- Modified White Trap [3]

Inside a larger dish (100 mm) place the watch glass. Set one single circular filter paper (Whatman #1) on the top of the watch glass. Place cadavers on the filter paper and make sure cadavers do not touch each other to avoid any contamination. Fill the outer (larger) Petri dish with 20 ml of sterile distilled water. The filter paper should touch the water to avoid the larva from desiccation. Cover the large Petri dish and its contents with the lid. Label dishes accordingly. Add the following information: Nematode name (species/isolate), infection date (date infection was set) and trap date (this is the date the trap is set up). Keep trap at RT until emergence of infective juvenile stages (IJs) occurs. This process can take between 10 - 25 days depending on the nematode species and/or strain considered. Harvest water with IJs by removing the larger dish of the trap and pouring water with nematodes into beaker. Allow nematodes to decant to the bottom of the beaker. This process may take a few minutes. Pour water carefully, making sure nematodes remain in the bottom of the beaker. Rinse nematodes by adding more water and allowing nematodes to decant. This step can be repeated 2 - 3 times until water is clean. Place nematode suspension in a tissue culture flask (250 ml). Keep concentration of the suspension to 1,000 - 3,000 nematodes/ml. Store flasks with nematode suspension in a cold room or in incubator between 10 - 20°C.

NOTE: check stored flasks periodically as shelf life of EPN is variable. Usually, *Steinernematids* can be stored for 6 - 12 months without the need of subculturing, whereas *Heterorhabditids* may require more periodic check-ups.

RESULTS

The following results were observed during the investigation.

Insect dead by deprived food

Usually, the larvae had more amount of food. The low content of food or overcrowding of larva in the container will lead to the death of the larva (Fig.4). The insufficient food will make the larva die. But the insects do not feed any food.

Attack by pest and predators

Rearing larva may attack by the pest and predators. Larva mainly attacked by the common red ants (*Solenopsis geminate* Fab.) and Spider (*Theridion sp*.). Also due to the sweet aroma of the diet food will attract the natural enemies. Figure .5 shows the attack of insect by red ants. Ants were fully eaten the insect body and wings were left over.

Fungal attack on diet food

Monitoring is an important note for culturing *G.mellonella*. The survival, development, fecundity, longevity and hatchability of the insects were extensively affected by the quality of the food. The food may damage by fungus and molds. (Fig.6)

Identification of EPN infected larva

The incubated larvae start to die after 24 hours and the pigmentation were developed after 72 hours. The characteristic brick red color is associated with the infestation of *Heterorhabditis* and yellow to brown in the case of *Steinernema* nematodes (Fig.7) EPN infected larvae will not develop any putrefying odour. Larvae shows no shrinkage on the skin and the muscles were completely charged into watery.

Fungal attack on larva

During the nematode recovery larva may affected by the fungal growth. The white mycelium will start to appear on the 4th day of infection and larva develop putrefying smell (Fig.8).

DISCUSSION

G.mellonella detection and collection are vital for nematode recovery. The larva serves as a nutritional source for the nematode's nourishment, reproduction, and development. Since galleria is a beekeeping pest, we can get it from beekeepers, ho honey manufacturers, or wax storage facilities. *In-vivo* culture of *G.mellonella* requires a special diet which provide vital nutrients such carbohydrates, lipids, proteins, vitamins, and minerals for normal metabolism. Many studies have attempted to optimize *G.mellonella* mass rearing by taking into account the cost and availability of diet ingredients, as well as the insect's ability to adapt to diets without seriously affecting its development [4-6]. Nessa Banville et al., reported that, *G.mellonella* larvae deprived of food with seven days showed decreased density of haemocytes, antimicrobial peptides (e.g., lipocalin) and immune properties (e.g., apolipophorin and arylphorin). It results in lower cellular and immune responses and increase the susceptibility to infection in larva with deprived food [7]. In this experiment artificial diet was followed by the composition recommended by PDBC [8]. The prepared diet should be soft this makes the larvae could easily move through the diet, which was reflected positively on its feeding and ultimately increase the weight of pupa.

Sweet aroma in the artificial diet food will attract the pest and predators. *Solenopsis geminata* (Hymenoptera: Formicidae) a smaller common red ant was feed on the larvae of greater wax moth. In the raising area, similar issues were noticed. It's kept in check by spreading ant powder around it. Among the different natural enemies recorded on Greater wax moth the hymenopteran larval parasitoid, *Apanteles galleriae wilkinson* was considered as important species. Hymenopteran parasitoid (*Antrocephalus galleriae Subbarao*), two species of ants (*Solenopsis geminata Fab.* and *Camponotus compressus Fab.*), one species of ear wig (*Euborelliastali Dohrn*) and Spider (*Theridion sp*.), one fungal (*Beauveria bassiana Bals*) and viral (*Densonucleosis* virus) pathogens were recorded [9].

To avoid food contamination, dead larvae and insects must be removed often from the rearing containers. Low terbinafine concentrations, could be used as an antifungal agent in insect-rearing diets. By incubating larvae in an artificial diet containing terbinafine at concentrations of 0.001, 0.01, 0.1, and 1 g, they demonstrated that the highest concentration of terbinafine (1 g) fully inhibits egg laying capacity. At all terbinafine concentrations, *G.mellonella* survival rates were drastically reduced at all stages of development. Females on a control diet produced 82.9 18.1 eggs, whereas females on a 0.1 g terbinafine diet produced 51.4 9.6 eggs [10]. In the insect baiting approach, the complete hard particle must be removed from the soil. Similar larvae were too delicate in nature, and instead of being bait by nematodes, the particles could harm the skin. The EPN-infected larvae are usually empty, with no signs of skin shrinking and a completely fluid fleshy internal muscle by the mutualistic bacteria present in EPN's which transform the host into a nutrient soup that the nematodes feed on [11].

Isolated entomopathogenic nematodes were confirmed using their characteristic qualities observed through the modified White trap method. The dead cadaver turned reddish in colour after being infested by entomopathogenic nematodes for 48 hours. The characteristic brick red colour is associated with *Heterorhabditis* infestation and yellow to brown with *Steinernema* nematode infestation. The body of the deceased cadaver was undamaged and released no putrefying odour. The mutualistic bacteria associated with this nematode encode several antibiotic compounds that prevent secondary microbes from attacking dead cadavers, which is the primary reason for the absence of putrefying odour from a dead cadaver. *G.mellonella* gut bacteria influence the cause of pathologies caused by bacteria, fungi, and parasitoids. It is extremely vulnerable to the ectoparasitoid fungal infection *Habrobracon hebetor* and the trophical fungal infection *Beauveria bassiana*. Oral administration of predominant bacteria (*Enterococcus faecalis, Enterobacter sp., and Serratia marcescens*) may prevent bacterial decomposition of envenomated larvae and promote fungal killing [12].

CONCLUSIONS

We have compiled all of the challenges encountered during *G.mellonella* selection and culturing, as well as nematode recovery using the white trap technique, in this paper.

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Table 1 Ingredients for artificial diet used for rearing of *G.mellonella*in the laboratory. (PDBC, 2007)

Ingredients	Quantity
Wheat flour	100g
Wheat bran	100g
Milk powder	100g
Maize powder	200g
Dried yeast	50g
Honey	175ml
Glycerin	175ml

Table 2. Question and tips for recovery of entamopathogenic nematodes from *Galleria mellonella*

Question	Solution and tips
Is it possible to recover EPN using ordinary water?	To avoid contamination, it is necessary to use distilled water.
Is it necessary to keep the petriplates sealed?	If not, other flies will lay eggs in the water and infect the nematodes.
How can we identify EPN?	The size of newly emerged EPNs is consistent.
How can we know if a larva is infected with EPN?	Infected larva will emit a fruity odour (No putrefying smell)
How do we avoid galleria's predators?	Against predators, use the insecticidal powder. However, stop using the powder during the egg hatching process to protect the newly hatched larva.

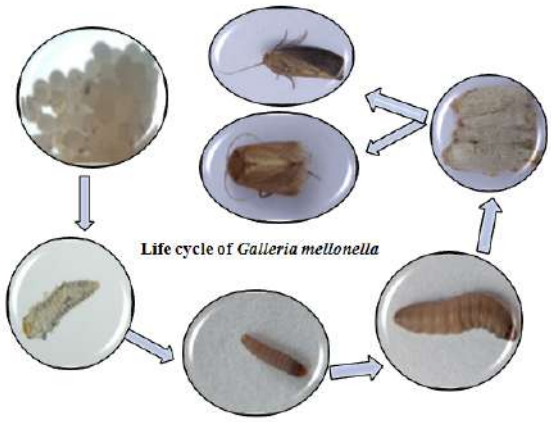

Fig. 1 Life cycle of *G.mellonella*. A- Tiny eggs B- newly emerged larva C-One week old larva D- Five-week-old larva E- pupa with cocoon F-Male G- Female

Fig.2 Soil sample collection for nematode infection

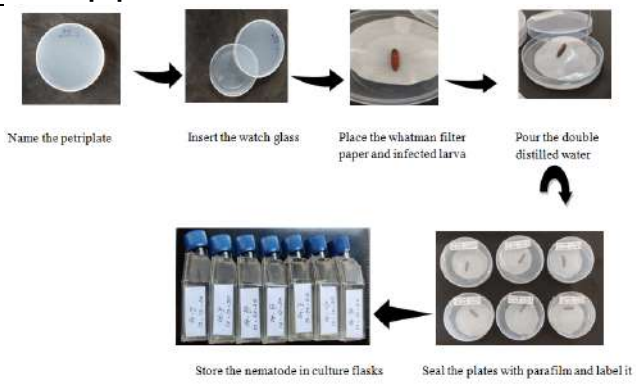

Fig.3. Steps involved in white trap method

Fig.4. Larva died by deprived food

Fig.5 Insects attacked by red ants a) Ants have eaten all of the body parts. b) Ants have taken over an insect container.

Fig.6 Diet food damaged by fungal and molds

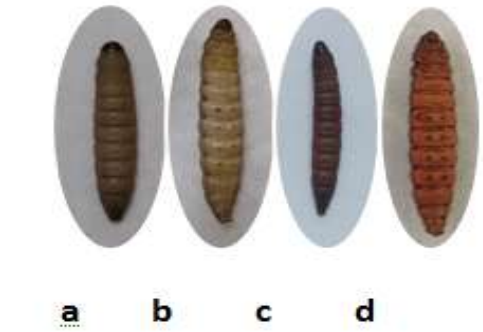

Fig.7 Color changes in EPN infected *G. mellonella*
a, b *Steinernema* infected larva
c,d *Heterorhabditis* infected larva

Fig.8Fungal attack in *G. mellonella* larva during white trap process
a) Initial stage of fungal attack b) Larva fully covered by fungus.

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RESEARCH ARTICLE

Investigation of Acute Toxicity and the Effect of Fenaxoprop-P-Ethyl Herbicide on the Behavior and Respiratory Dysfunction of the Common Carp (*Cyprinus carpio* L.,)

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ABSTRACT

Static renewal bioassay experiment was conducted to determine the acute toxicity (LC_{50}) of commercial grade herbicide Fenoxaprop-P-Ethyl (FPE) to common carp, *Cyprinus carpio*. The acute toxicity of Fenoxaprop-P-Ethyl to carp fingerlings exposed to 96 hrs was found to be 300µg/L. The one-fifth of LC_{50} for lethal studies i.e 75 µg/L for 1,2,3 and 4 days and for sub lethal studies 1/8th of LC_{50} i.e. 37.5 µg/L for 1,15,30 and 45 days, the data obtained were statistically evaluated by the use of the EPA and Finney's Probity Analysis Method. During our study period behavioral changes such as whirling cork movement was found to be frequent with altered opercula movement, Fin movement, Dyspigmentation, Mucus secretion. Variations in oxygen consumption were observed in both lethal and sub lethal concentration of Fenoxaprop-P-Ethyl respectively. There considerable variation in respiratory rates may be a consequence of impaired oxidative metabolism which leads to impairments in fish respiration physiology and behavioral responses even under recovery tenures may be due to slow release of sequestered FEP from the storage tissue in the sub lethal concentration, found under stress, but that was not fatal.

Keywords: Herbicide Fenoxaprop-P-Ethyl (FPE), Fresh water fish *C. carpio,* Fenoxaprop acid, Toxicity, Behavioral changes, Pesticide residue, Oxygen consumption

INTRODUCTION

The contamination of waterways and streams with compound impurities has got quite possibly the most basic natural issues of the century. A consequence of the toxins' vehicle from modern and rural territories into the climate and their compound diligence, numerous freshwater environments are confronted with spatially or transiently disturbing elevated levels of xenobiotics synthetics (S.R. Marigoudar *et al*., 2009)**.** The new advancement of

biomarkers dependent on the investigation of the reaction of life forms to poisons has given essential apparatuses to the usage of projects for pollution observing (KORAMI *et al.,* 2000). One of the significant elements defiling the normal living space is rural pesticides. These specialists utilized against bother, unwanted spices and agrarian illnesses were found to effect sly affect the climate. Among the herbicides utilized in Turkey with possible harmfulness against people are phenoxy mixtures, for example, 2,4-D [(2,4-dichlorophenoxy)acetic acid]; 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid]; MCPA [(4-chloro2-methylphenoxy)acetic acid] and their individual esters (Vural, 1996).Among these 2,4-D is the most generally utilized herbicide (Industry Task Force Research Data). There are various investigations done on the harmfulness of 2,4-D.According to **US** Environmental Protection Agency (1988) and Stevens and Sumner (1991), LC_{50} esteems range 2,4-D between 1 to 100 mg/l for Cutthroat trout relying on the plan utilized and Green sunfish presented to a centralization of 110 mg/l showed no unusual conduct. Gomez et al. (1998) explored the intense 2,4-D harming in *tench* and Holcombe et al.(1995) inspected the drawn out impacts of nine synthetics on Japanese medaka.2,4-D has no dirt ingenuity except for was recognized in groundwater supplies in the US and Canada (National Research Council, Canada, 1978).

Alexander et al. (1985) discovered the LC50 estimations of 2,4-D as 35 mg/l for *Daphnia magna* and 25 mg/l for *Pimephales promelas* (Cyprinidae). Rodrıguez *et al.(*1994) announced that ovaries of grown-up *Chasmagnathus granulata* crabs were discovered to be exceptionally delicate to exposures of pesticides. Barnekow *et al*.(2001) researched the impact of 2,4-D on bluegill sunfish named with 14C and estimated the radioactive buildup following a multi day exposures. They tracked down that complete radioactive buildup was 0.41 ppm right off the bat and 0.60 ppm on the third day. Sarıkaya *et al***.** discovered the LC_{50} estimation of 2,4-D on tench (tinca L., 1758) as 48 mg/l. There are concentrates in writing concerning the gathering of 2,4-D, its subordinates, and other agrarian synthetic substances in tissues (Koziollek *et al.,* 1996). Sarıkaya *et al.* (2002) decided the LC_{50} estimation of 2,4-D on tinca as 41.76 mg/l. The choice whether a certain xenobiotic is risky for the amphibians and the food cycle, must be made after the (a) mammalian intense harmfulness (b) microorganisms intense poisonousness (c) fish intense harmfulness and (d) natural separation tests have been completed in detail (Ardalı, 1990).The actuality that expanding utilization of defiling synthetic compounds in many industrialized pieces of the world makes the advancement of ecotoxicity estimation strategies a flat out need (Brando *et al*., 1992) The initial step is the intense toxicity test on green growth, fish and so on to show the expected dangers of these synthetic compounds (OECD, 1993).Although the underlying aquatic toxicity tests were conveyed by the utilization of microorganisms, spineless creatures like *Cladocera* and *Rotifera* and different gatherings, and they can not the slightest bit supplant the real test performed on fish. What is significant is the toxicity in fish which is the last chain in the food cycle (Castano *et al*., 1996).

Respiration is quite possibly the main physiological boundaries on which a considerable essential capacities like development and multiplication of fishes depend (Holden 1973), which thusly has an immediate bearing on the profitability of freshwater environments regarding fish. The freshwater air breathing fishes of tropicals possess waters of low O_2 substance and experience hypoxic water in summer and normoxic water during winter and stormy season. As per the variances in the physico-compound attributes of the encompassing waters, the air breathing fishes are furnished with dual mode gas trade hardware, utilizing breath utilizing profoundly vascularised air breathing organs and branchial integument trade of gases with water. Nowadays' pesticides are utilized unpredictably, which influence amphibian climate including fishes. One of the early indications of intense pesticide harming is the adjustment or disappointment of respiratory digestion (Holden 1973). Changes in oxygen take-up of fishes because of pesticide openness are fluctuating in various fishes presented to an assortment of pesticides (Karuppiah 1996). The impact of pesticides on oxygen utilization has been broadly concentrated in various water breathing fishes Mount 1962. Notwithstanding, these examiners assessed just the adjustments in oceanic breath despite the fact that the air breathing fishes like, *Mystus vittatus* (Gopalakrishna Reddy and Gomathy 1977**)** and *Channa punctatus* (Sambasiva Rao *et al*. 1984) were utilized in their examinations. An audit of writing shows that the impacts of pesticides on the extent of oxygen take-up from water and air via air breathing fishes were concentrated by a couple of laborers (Bakthavathasalam 1980, Natarajan 1981, Ganapathyraman 1987 and Karuppiah 1996), hence, the current work has been embraced in an air breathing fish, Channa gachua to propel our data in such manner.

Regardless of the way that these assessments have wind up being useful in depicting responses to sublethal exposures and have direct repercussions for strategy for action, the understandings of the ecological vitality of these different respiratory responses stay inconvenient. The changes in the respiratory activity of fish and aquatic small yellow animals have been used by a couple of trained professionals. Exposures to sub lethal obsessions are represented to augment respiratory activity, achieving extended Ventilation and from this time forward extended take-up of the toxic substance. Oxygen Consumption has been represented to stimulate Oxygen usage at sub lethal Concentrations and frustrate the oxygen take-up at lethal Concentrations. By virtue of produced pyrethroids a steady and reformist abatement in Ventilatory model and these by decline in oxygen Consumption is seen (Veeraiah and Durga Prasad 2001). At any rate there have been uncommon cases for this declaration and it is difficult to summarize (Murty 1986). The supreme oxygen Consumption is one of the pointers of the general flourishing of the fish. It may be moreover important to review the Physiological State of an animal aides in surveying the helplessness and obstacle plausibility and besides significant to Correlate the direct of the existence structure which Ultimately fill in as pointer of valuable aggravation of people. Hence the differential oxygen Consumption can be used as bio-marker to evaluate the key mischief caused on the animal which could either augmentation (or) decrease the oxygen take-up. In this way an undertaking has been had to analyze the effect of sub-lethal and lethal centralizations of cypermethrin 10% EC on oxygen use for 12 hours at two hour range to the Indian huge carp *Cirrhinus mrigala* (Hamilton).

Presence of pesticide in streams and lakes is to a great extent because of the overflow from farming fields and outfall from pesticide fabricating processing plants (Asuman Karadeniz *et.al*., 2015). Pesticides are not exceptionally specific yet are by and large harmful to numerous non-target creatures. Aquatic environment is additionally dirtied by pesticides and it prompts numerous adjustments in life form physiology (Bakthavathsalam, R. 1980). The impact of endosulfan on oxygen utilization of new water fish *Lipidocephalicthys thermalis* was concentrated by a few researchers **(**Anandkumar, S. 1988, Sethuraj *et al*., 1992). Fenoxaprop-P-ethyl has a place with the aryloxyphenoxypropionate (APP) herbicide class and it can restrain the movement of acetyl-CoA carboxylase (ACCase), which is a key catalyst that catalyzes the initial step of unsaturated fat biosynthesis. (Konishi *et al*., 1996). Substance weed control is a superior enhancement to ordinary techniques and structures a vital piece of the advanced yield creation. In this manner, utilization of herbicides is one of the alternatives left with the ranchers to dispose of harvest weed rivalry at early development phase of yield. The regular weed the executives practice is pre-development utilization of specific herbicides like pendimethalin, oxyfluorfen and oxadiazon followed by one hand weeding or utilization of quizalofop-ethyl as post-rise (Kalhapure *et al*. 2014, Sinare *et al*. 2014). In present examination, the herbicide considered for the investigation FEP has likewise injurious impact however the setting of this is to be concentrated as for *C.carpio* from this locale. Subsequently the examination is undertaken. The *C.carpio* is consumable fish from numerous areas of India. Consequently the impact of FEP will be assessed with deference Respiratory dysfunction and Behavioral to changes in the fresh-water fish *C.carpio*.

MATERIALS AND METHOD

Experimental animal

Healthy and active *Cyprinus carpio* fingerlings were procured from the State Fisheries Department, Dharwad, India. The test was performed in the Department of Zoology, Karnatak University, Dharwad. Fishes were maintained in large aerated cement tanks (6 X 3 feet) which treated with 1% KCl solution prior to the introduction of animal into the tank. Fishes were fed with balanced nutritious food pellets (Nova, Aquatic P. Feed) and allowed 12-14 hours of photoperiod during 14 days of acclimatization period at 24 ºC. The tank water was renewed daily and the Physico-chemical parameters of water were examined according to the guidelines of APHA.

Experimental toxicant

Fenoxaprop-P-Ethyl (6.9 % w/v EC) was procured from retail dealer in Dharwad, Karnataka, India, under the trade name Hyban, supplied by Hyderabad Chemical Supplies Limited, Hyderabad, India. The expiry date of the test

substance was confirmed prior to initiation of the exposure. Required quantity of Fenoxaprop-P-Ethyl for acute toxicity and sublethal studies (1/8th concentration) was drawn from freshly prepared stock solution which was prepared from the parent solution using variable micropipette.

Acute toxicity test

Semi-static renewal assay was performed for acute toxicity test which involve daily renewal of test solution and water **(**OECD 2019**)**.Range finding test was conducted to find out the upper and lower bound of the test substance for the fish. This step was employed to minimize unnecessary killing of animals. Healthy fingerlings of *C.carpio* weighing 4-6 ± 0.3 gm with 7±0.5 cm length was selected for the test. The fishes (10 each) were transferred to clean, fungus free glass aquaria and were not fed 24 hours prior to the initiation of experiment. All the test concentrations of commercial grade Fenoxaprop-P-Ethyl were introduced into the test aquaria which had triplicates of each concentration simultaneously except one control group. The test aquaria were kept under observation and the dead fishes during 24h, 48h, 72h and 96h experimental period were lifted off and recorded at every 24h. The recorded mortalities of each concentration calculated for mean value and this value was used to calculate LC_{50} of Fenoxaprop-P-Ethyl. The LC_{50} was calculated using mortalities of the fish and by performing probit analysis.

Behavioral studies

Healthy *C.carpio* (n=10) were introduced into a glass aquarium and were exposed to the sublethal concentration of Fenoxaprop-P-ethyl. One eighth of LC_{50} was selected and exposed for 10, 20 and 30 days. The fishes were frequently observed for the behavioral changes during each experimental period. Behavioral changes were recorded for further interpretation of the effect of toxicant on *C. carpio*.

Oxygen consumption

Fishes predominantly rely on dissolved oxygen in the living environment. Any disruption or variation of oxygen content in the water could cause hypoxia or anoxia in the fishes. Oxygen consumption is one of the parameters to study the stress level of the fish. In present study the respiration rate of *Cyprinus carpio* was measured in lethal (1, 2, 3 and 4days) and sublethal (1, 15, 30 and 45days) concentrations of Fenoxaprop-P-ethyl. The experimental set up was made in such a way that there should be minimum 75% oxygen at the end of experiment in order to minimize the effect of low oxygen level and metabolite accumulation. The whole experimental arrangement was made by following the method of (Rabia Sarikaya, Mehmet Yilmaz, 2003**)** as described by (Karuppiah, D. 1996**).** No mortality was observed during oxygen consumption tests and the measured oxygen was expressed in mg/L/g/h. The temperature and pH of the test water were 24±2ºC and 7.1±0.2 respectively.

Ethical statement

All the experiments performed in the present study abide by the guidelines of the Institutional Animal Ethics Committee (IAEC). The experimental animals used in the study were handled with care according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

RESULTS

Acute toxicity

The experimental fishes exposed to different concentrations of commercial grade Fenoxaprop-P-ethyl (6.9% EC). The water was tested for the Physico-chemical parameters to ensure optimum experimental conditions (Table 1). Toxicity of Fenoxaprop-P-ethyl to *C. carpio* exposed for 96 h exhibited 100% mortalities at 500 mg/L, no mortalities were observed at 100 mg/L and 50% mortalities were observed at 300 mg/L (Table 2).Mortality was gradually increased from 200 mg/L to 500 mg/L concentration of FEP. The LC_{50} values were calculated by probit analysis method (Finney D J., 1971).Table 3 shows 95% confidence limits calculations for the obtained concentrations. Figure 1 & 2 shows the

graph plotted percent mortality against concentration and percent mortality converted to probit against log concentration respectively. A straight line was obtained in Figure 1 & 2 indicating the LC_{50}value of 300 mg/L concentration of the test toxicant FEP. Sigmoid curve was obtained in the graph plotted percent mortality against log concentration (Figure 3). The values obtained from the experiment was manifested the LC_{50} of 300 mg/L and the upper and lower bound was shown in table 3.

Behavioral studies

Behavioral studies are one of the clinical observations which help to understand the overall response of the animal towards its environment. All the behavioral observations made in the study are followed OECD guideline 203 standards. In the present study the control fishes exhibited sensitive quick responses to the disturbances in the environment. The experimental fishes exposed to sublethal concentration of Fenoxaprop-P-ethyl showed various behavioral changes. The behaviors such as schooling, fright response, opercular beat, fin beat and buccal movement are normal in control fishes.10days exposed fishes were witnessed with medium schooling behavior, fright response, upward movement, burst swimming, opercular beat, fin beat and high intensity of buccal movements. 20day and 30day exposed fishes showed gradual increase of opercular beat, fin beat, mucus secretion and dyspigmentation of the body (Table 4). The fishes in 30th day of exposure more pronounced with mucus secretion on the body.

Oxygen consumption

The mean values of measured oxygen consumption in the fish *C. carpio* exposed to lethal and sublethal concentrations of Fenoxaprop-P-Ethyl were tabulated in Table 5.The fishes exposed to lethal concentrations for 1, 2, 3, 4 days exhibited decrease in oxygen consumption with 0.6815, 0.6175, 0.5365, 0.5131 mg/L/g/h respectively. At sub lethal concentration the fishes exhibited increase in oxygen consumption right from day 1, 15, 30 to day 45 with 0.7015, 0.7345, 0.7665, 0.8075 mg/L/g/h respectively. The percentage change in oxygen consumption in *C. carpio* is given in Figure 4.The results are significantly different from the control which exhibited oxygen consumption of 0.8983 mg/L/g/h. In lethal concentration the oxygen consumption drastically decreased and in sublethal concentration gradually increased.

DISCUSSION

Evaluation of toxicological impacts on aquatic life is one of the basic and important factors in order to introduce an eco-friendly herbicide. Fishes being the last chain of feeding cycle in aquatic eco-system highly impacts on the health of other aquatic organisms through food chain (Mehmet Yilmaz *et al*. 2003**)**. Technical grade Fenoxaprop-P-ethyl was found to have acute toxicity of 96 h LC_{50} = 0.39 mg/L to *Oncorhynchus mykiss* and LC_{50}=0.19 mg/L for *Lepomismacrochirus* Asuman Karadeniz *et al*., 2015**.** Present investigation assessed behavioral changes in *C. carpio* exposed to sublethal concentrations of Fenoxaprop-P-Ethyl. The fish exhibited erratic movement and hyperexcitability when exposed to the toxicant for the first time. However, these changes normalized in subsequent exposure days and seems to be a part of adaptive system in the fish. During the exposure period the control group maintained good co-ordination but the exposed carps failed to cope up with schooling and fright response behavior. Lateral swimming was found slowly evident from 10thto 30th day exposed fishes which is the main indicator of co-ordination between the muscles and nervous system Zabin SB *et al.,* 2018 .Some of the fishes shown sinking phenomenon, dashing movements, backward swimming and upward swimming which could be attributed to the loss of balance due to the impaired neurotransmission or disturbance in the body physiology. The behavioral changes in the toxicant media might be attributed to the impairment in the nervous system or in various effector sites Miguel Betancourt *et al*., 2006. The behavioral avoidance or the excretion of toxic substances from the body as early as possible are the possible ways of sustaining environmental intoxication by the toxic chemicals Connell D *et al.,* 1999.Our findings were in accordance with Jing lin *et al.,* 2007 .

In the study period the exposed carps manifested with increased buccal movement at 10th day exposure and observed medium buccal movement at 20th and 30th day of exposure. The opercular beat also increased till 30th day

that might be due the inability of the gills to supply enough oxygen in normal beat. The damaged gills could be one of the reasons for the increased opercular beat as the fish physiology demands more oxygen at sudden stress conditions. Increased mucus secretion is one of the major observations in the experimental fish compared to control fish. The experimental fish secreted excess mucus that made a barrier between the fish and environment to avoid the chances of exposure of body surfaces to the toxicant. Similar observations were made in the study of effect of 2,4-D herbicide on the behavior of *C. carpio* which was carried out by Mehmet Yilmaz *et al.* 2003 and in the experiment offish *Labeo rohita* exposed to malathion Vineetkumar K. Patil *et al*., 2008 **.**Even it could also be aiding in oxygen diffusion in the fish Jing lin *et al*., 2007 . Dyspigmentation of the exposed fishes was observed gradually from10th to 30thday which was coinciding the results of Ramesh Halappa *et al*., 2009, who worked on the effect of chlorpyrifos in *C. carpio.* Behavior irregularities in C. carpio (L., 1758) exposed to different convergences of 2,4-D are the uneasiness, unexpected jerks, loss of equilibrium, swimming topsy-turvy(up-side down) or vertical way, respiratory challenges, exorbitant mucosal emission, easing up in shading, accumulating at the surface for breathing, and hitting to the side dividers of the aquaria.Ferrando et al., in their examination on the impacts of eight chose organochloride pesticides, for example, endosulphane, diazinone, phenyltrithian and methylparathion on eels, decided their 96-h LC50 esteems and detailed conduct changes in fish. They likewise noticed tension, messes in swimming example, loss of equilibrium, unreasonable bodily fluid emission and easing up in shading (Ferrando et al., 1991). Albeit the methods of capacity of these insect poisons are especially not quite the same as Fenaxoprop-P-Ethyl, social changes noticed are like our own.

The boundless utilization of chlorophenoxyacetic corrosive as a herbicide and a development controller in farming, forestry, and cultivating has expanded the harm brought about by these poisonous mixtures on climate and human wellbeing **(**Duygu, 1979).The rural synthetic compounds contaminate all abiotic media especially water and soil. The pollution of underground waters and other water sources by rural synthetics represents an expected danger to amphibian living beings and fish. Toxic intensifies impact fish, which are the last chain of the taking care of cycle in oceanic eco-framework, and cause different creatures, which feed on fish, to be exposed to a similar harmful effect .The herbicide FEP utilized in this examination is exceptionally tocxic to fish and has extremely unfriendly consequences for the two people and animals. It likewise aggregates in tissues and causes intense harming (World Health Organization, 1984).In dislike of these tiring realities the investigation identified with the impacts of FEP on creatures are profoundly limited. It is evident that the quantity of studies coordinated to the examination of intense and persistent poisonousness of different herbicides is to be increased. The results acquired in this investigation obviously uncover the way that it is important to control the utilization of certain herbicides usually utilized in agriculture. The present experiment also focused on the effect of Fenoxaprop-P-ethyl on oxygen consumption of *C. carpio* under lethal and sublethal exposures. At lethal concentration oxygen consumption was decreased gradually that could be due to damage of epithelium of gills by the toxicant Saroja, K. 1959 .The fish at lethal concentrations experiences serious respiratory stress because of toxicant absorption through the gills Finney DJ 1971.However, the decreasing trend in oxygen consumption could also be a result of altered energy metabolism R.A. Maniyar 2011.It seems to be the fishes attempted to adapt to the surrounding toxic medium by slowing down the respiration process. However, in sub lethal concentration the oxygen consumption increased with respect to exposure period. The increase in oxygen consumption might be attributed to expellation of toxic substance via rapid metabolism Connell D *et al*., 1999. In long term exposure the fish might be trying to develop resistance against toxic substances via physical and physiological strategies.

CONCLUSION

The literature regarding the effect of herbicide on aquatic organisms is limited; however our study is one of the first attempts to evaluate the effects of Fenoxaprop-P-ethyl herbicide on *C. carpio*. The results are indicating the toxic effect of the herbicide on the fish behavior and the LC_{50} of 300mg/L makes it even more toxic to the sensitive fish *Cyprinus carpio*. Oxygen is the most important gas in the living world, the slightest variation in water could be fatal to fishes. The present experiment explains the possible effects of herbicide on oxygen consumption of the fish *C. carpio.*

Further studies are required to assess the possible detrimental effect of Fenoxaprop-P-ethyl on the aquatic organisms. This study offers the base data for future research on fresh water carps.

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CONFLICT OF INTEREST

Author claims no conflict of interest.

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Table 1: Physico-chemical parameters of the test water

SL. No.	Parameter	Obtained values
1.	Temperature	24±2ºC,
2.	pH	7.1±0.2 at 24ºC,
3.	Dissolved oxygen	9.6±0.8 mg/L
4.	Carbon dioxide	6.3±0.4 mg/L
5.	Total hardness	23.4±3.4 mg
6.	$CaCO_3$/L,	nil
7.	Phosphate	0.39±0.002 µg/L,
8.	Salinity	nil
9.	Specific gravity	1.001
10.	Conductivity	less than 10 µS/cm

Table 2: Acute toxicity (96 h LC_{50}) and 95% confidence limits of Fenoxaprop-P-Ethyl to *Cyprinus carpio*

Toxicant	96 h LC_{50} (mg/L)	95% confidence limits	
		Upper limit	Lower limit
Fenoxaprop-P-Ethyl	300	100	500

Table 3: Experimental mortalities of *Cyprinus carpio* exposed to different concentration of commercial grade Fenoxaprop-P-ethyl

Sl. No.	Concentration of Pesticide mg/L	Log concentration of Pesticide	No. of fishes exposed	No. of fish alive	No. of fish dead	Percent Kill (%)	Probit Kill
1.	100	2	10	10	0	Nil	0
2.	150	2.176	10	9	1	10	3.72
3.	200	2.301	10	8	2	20	4.16
4.	250	2.397	10	6	4	40	4.75
5.	300	2.477	10	5	5	50	5.00
6.	350	2.544	10	4	6	60	5.25
7.	400	2.602	10	3	7	70	5.52
8.	450	2.653	10	1	9	90	6.28
9.	500	2.698	10	0	10	100	0

Table 4: Behavioral changes in *C. carpio* exposed to different concentrations of Fenoxaprop-P-ethyl

Sl. No.	Observed behavior	Control	Exposure days		
			10 Days	20 Days	30 Days
1.	Lateral swimming	-	+	+ +	+
2.	Sinking phenomenon	-	+	-	+
3.	Schooling behavior	+++	+ +	+	-
4.	Fright response	+++	+ +	+	-
5.	Backward swim	-	+	+	+
6.	Dashing movement	-	+	+ +	+
7.	Upward swim	-	+ +	+	-
8.	Whirling cork movement	-	+	+ + +	++
9.	Buccal movement	+	+ + +	+ +	+ +
10.	Burst swimming	-	+ +	+ +	+
11.	Opercular beat	+	+ +	+ +	+ + +
12.	Fin beat	+	+ +	+ + +	+ + +
13.	Mucus secretion	-	+	+ +	+ + +
14.	Dyspigmentation	-	+	+ +	+ + +

Fishes exhibiting various behavior, indicates (+) as low, (++) as medium and (+++) as high intensity of behavior upon exposure to Fenoxaprop-P-ethyl.

Table 5: Oxygen Consumption (mg/L/g/h) of the fish *Cyprinus carpio* on exposure to the Lethal and Sublethal concentration of Fenoxaprop-P-Ethyl

Estimations	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	15	30	45
Oxygen Consumption (Mean)	0.8983^{A}	0.6815^{F}	0.6175^{G}	0.5365^{H}	0.5131^{I}	0.7015^{E}	0.7345^{D}	0.7665^{C}	0.8075^{B}
±SD	0.0003	0.0003	0.0003	0.0003	0.0038	0.0003	0.0004	0.0004	0.0003
% Change	----	-24.13%	-31.27%	-40.28%	-42.88%	-21.92%	-18.23%	-14.68%	-10.11%

Values are mean ± SD (n=6) for oxygen consumption in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test.

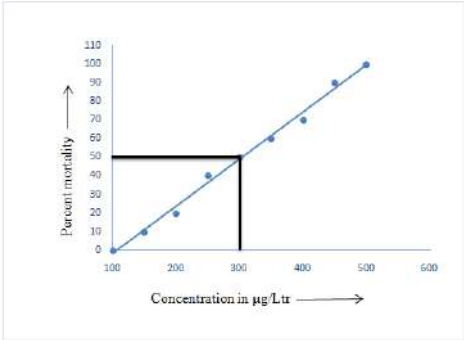

Figure 1: Percent mortality of *Cyprinus carpio* exposed to different concentrations of Fenoxaprop-P-ethyl

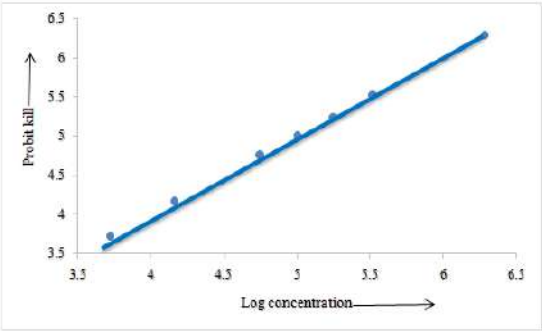

Figure 2: Probit kill of *Cyprinus carpio* exposed to different concentrations of Fenoxaprop-P-ethyl

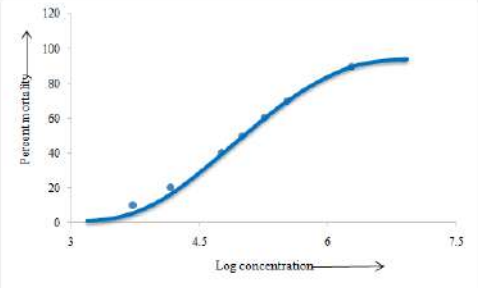

Figure 3: Percent mortality against log concentration of *Cyprinus carpio* exposed to different concentrations of Fenoxaprop-P-ethyl

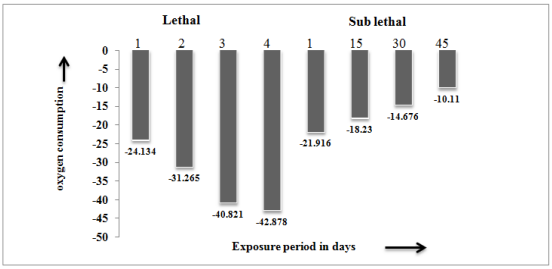

Figure 4: Oxygen consumption (mg of oxygen consumed/ gram wet weight of fish/h) of *C. carpio* following exposure to lethal and sublethal concentration of Fenaxoprop -P-Ethyl

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RESEARCH ARTICLE

Personalization of Medicine in Botanical Aspect

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ABSTRACT

Personalized or precision is a new paradigm that holds promise for individual diagnosis, treatment and care. Personalized evidence based herbal medicine aims to use stored health data to prevent future illness. Precision herbal medicine is difficult for patients with their genes, lifestyle, behaviours and environmental factors. Traditional medicine uses symptoms to diagnose disease, and drugs to treat these symptoms. Herbal remedies which are the form of herbal medicine in which the medicinal constituents are derived from herbal plants that aims in treatment of patient illness. The personalized herbal medicine was a roadmap for innovations of biomarkers. The tailoring of medicinal plant treatment includes Gastrointestinal, Urinary tract Gynaecology, Cough and cold etc. the precision herbal medicine also reports the covid cases in Africa and also some cases of malaria, HIV, tuberculosis, bleeding disorders etc.

Keywords: Medicine, herbs, Ayurveda, Disease, Precision, Treatment, Patient, Herbal Remedies, therapeutic, Health care.

INTRODUCTION [1-5]

Today, there are many forms of complementary medication, including herbalism, naturopathy, Ayurveda and conventional Chinese medicine. Natural products are reviewed for the protection and efficacy by the FDA if consumers or clinical professionals document hassle with them. The traditional system of drugs are the primary health care that includes spiritual, spiritual and natural remedy. The national Institutes of fitness (NIH) tactics a precision medicinal drug has a revolution for the disease prevention and treatment in character differences in life-style, biology and surroundings. The molecular biology is primary key to get the personalized medicinal drug to the

floor. Screening of genetic variations is ought to in expertise the underlying mechanisms of drug responses and to discover more focused and precise therapeutic techniques focussing at the individual variations.

BOTANICAL MEDICINE IN PERSONALIZATION [6-9]

Numerous corporations proposing natural extras which creates formulas in pill or capsule form to bring herbs without the need for patients to swallow a liquid tincture. Sometimes this is most suitable choice for patients, prescribing an herbal tincture permits the doctor to combine herbs at the time of the visit to treat the patient's precise condition. This is cost effective which removes the need to buy pre-formulated supplements, letting doctors to target specific protocols using the most useful herbs for that condition. The doctor can modify the treatment by altering the tinctures ingredients or proportions allowing for a truly personalized remedy. Botanical tincture may have side-effects and can interact with other herbs or medications.

AYURVEDA AND PERSONALIZED MEDICINE [10-15]

Ayurveda recognizes distinct patterns of individual variation and accordingly classifies individual into groups. The three dynamic patho-physiological objects Doshas.

1. Vata doshas (Nervous and Musculoskeletal functions)
2. Pitta doshas (Digestion, metabolism, hormonal actions, immune surveillance)
3. Kapha doshas (Homeostasis, biological strength and stability)

Patwardhan a first hypothesized genetic basis for prakriti and its protagonist in personalized administration of illnesses. Then provided experimental support for this thought by viewing relationship between certain Human Leucocyte Antigen (HLA) alleles and the individual prakriti types which is linked to autoimmune disease. Ayurveda has broad diagnostic system that senses all expressions of the primary disease and permits purpose of disease subtype and harshness.

For example,

a. Rogavastha indication classifies rheumatoid arthritis on the basis of presence or absence of ama (a pro-inflammatory substance produced by impaired metabolism)
b. Gavastha relates to prameha (metabolic syndrome and diabetes mellitus)

DEVELOPMENT OF BOTANICAL DRUGS FROM HERBAL MEDICINE [16-18]

Botanical medicines, advanced from herbal drug treatments, can potentially overcome the client suspicions and fulfil the promise of herbal drug treatments. An explanation of terminology is vital. A botanical drug, as defined through the FDA in its Draft guidance, is a botanical product organized from a botanical drug substance, and meant for use as a drug. A conventional FDA-authorised drug has a single well-characterized active aspect. Through contrast, a botanical drug, by using definition, comes in varieties of extracts which are composed of multiple chemical constituents. The concept of botanical drugs locations herbal medicinal drug into the lively FDA drug approval method. As a result, the development of botanical capsules will demand the mixture of the merits of advanced western technology and the empirical-primarily based, centuries-antique natural remedy way of life.

The modern crisis in new drug discovery highlights the issue of the traditional view of medicine as silver bullets. The conventional perception that a single chemical entity drug, which treats a single ailment, rests on a critical premise that human diseases have a uniform, underlying genetic foundation across patient populations. Generally, a silver bullet drug is a NCE drug with a single active chemical ingredient. While there have been blockbuster silver bullets like Amgen's EPO and Eli Lilly's Prozac, the hope for brand new blockbuster pills has diminished. Recent advances in genomics vindicate this pessimism. It appears that various genetic changes frequently underlie a single disorder, a phenomenon termed polymorphism. as a consequence, exclusive affected person populations may also require unique pills tailored to their non-public desires. The polymorphic nature of sicknesses indicates an individualized method in drug layout is more likely to be successful, making it even more difficult to broaden blockbuster drugs.

PRIMARY HEALTH CARE REMEDIES IN PERSONALIZED MEDICINE

Common cold treatment by trigatu powder [19-22]

Trigatu powder consists of fine powder of three pungent drugs. They are,

1. Shunthi (dry ginger) (Gingiberaceae)
2. Maricha (black pepper) (Piperaceae)
3. Pippali (long pepper) (Piperaceae)in equal proportion.

Common cold or coryza is a common respiratory problem caused by a variety of viral infections; commonest among them is the influenza virus and its variants. Individuals show considerable variation in susceptibility and environmental exposure is a contributory factor. The word "cold" is a catch-all term that describes symptoms such as sneezing, wet nose, running nose, scratchy throat, heaviness in head, body ache, headache, indigestion, nausea, stomach ache, vomiting, diarrhoea and fever. Frequent attacks of cold are a reflection of a temporary dip in immunity that is most often caused by insufficient rest; too many rich foods, stress and exposure to cold and dampness can also precipitate its occurrence. Frequent colds can make the individual vulnerable to secondary lower respiratory tract infections. Early and consistent attention, along with adequate time for convalescence, keeps all colds from becoming chronic.

Therapeutic properties

Anti-inflammatory, expectorant, analgesic, carminative, digestive.

Acidity and gastritis treatment by amalaki powder [23-25]

Amalaki- *Phyllanthus emblica* (Linn.)(Phyllanthaceae), Heartburn is because of excessive secretion of gastric acid or its reflux to the food pipe alongside delayed gastric emptying and fermentation of food. Gastric acidity and infection of the stomach is referred to as gastritis, which produces a common symptom of burning sensation within the middle part of the higher half of the stomach, at the same time as acid reflux causes throat and heartburn and behind schedule gastric emptying with fermentation results in gaseous distension of stomach and belching. These abnormalities cause symptoms like nausea, lack of appetite, indigestion and mild to moderate higher abdominal pain and distress.

Therapeutic properties

Antioxidant, anti-emetic, anti-ulcer, Anti-inflammatory, antacid, immuno modulator, rejuvenator and a rich source of vitamin C.

Conjunctivitis treatment by daruharidra decoction [26-28]

Daruharidra-*Indian berberry* (Berberidaceae), Vision is generally normal but a slight blurring may occur if excess secretions form a film over the cornea. Conjunctivitis may begin in one eye but often spreads to involve both eyes. Conjunctivitis is most commonly due to viral and sometimes bacterial infections. But it can also result from allergic reactions or from chemical irritants, air pollution, smoke, shampoos, dirt, swimming pool chlorine or noxious fumes. Rarely, underlying chronic inflammatory conditions can also cause a persistent conjunctivitis. The infectious form of conjunctivitis is very common in children and is highly contagious. Traditionally, home remedies have been successfully used for soothing inflamed eyes with uncomplicated symptoms, minor infections, or allergies.

Therapeutic properties

Anti-inflammatory, anti-diarrhoeal, anti-microbial, anti-pyretic, and anti-trachoma activity.

HERBAL REMEDIES IN INDIVIDUAL TREATMENT [29]]

Herbal treatments are a form of conventional medicine. They consist of herbs or compounds that come from herbs. They also can include fungi or algae.

Herbal remedies for coughs and sore throats [30, 31]

A number of OTC (over the counter) products for coughs comprise compounds that come from plant life. Cough lozenges frequently incorporate menthol to soothe a sore throat.

For example,

a) Sage
b) Liquorice root
c) Eucalyptus oil

Herbal remedies for stress and anxiety[32,33]

There are many historic treatments for pressure and anxiety. a few herbal merchandise that can help reduce pressure and anxiety

For example,

a) Essential oils
b) Lemon balm
c) German chamomile

Herbal remedies for migraine [34,35]

Migraine is a neurological situation that normally reasons painful headaches, in addition to other signs and symptoms such as nausea and light sensitivity. There are also a few natural treatments people use for migraine.

For example,

a) Ginger
b) Butterbur
c) Feverfew
d) Other peppermint oil may help

Herbal remedies for allergies [36]

Allergies are the condition in which immune system reacts abnormally to a foreign substances. There are most famous remedy are available for this treatment.

a) Garlic
b) Butterbur
c) Stinging nettle
d) Curcumin

PERSONALIZED HERBAL MEDICINE [37, 38]

The personalized herbal medicine was a roadmap for convergence of herbal and precision medicine biomarker innovations. The precision medicine faces clinical reality and develop a companion diagnostics for drugs as well as herbal medicines. Asymmetry in biomarker innovation strategy needs urgent attention from a wide range of innovation actors worldwide including,

a) Scientists
b) Government
c) Research funders
d) Community leaders
e) Policymakers and scientists
f) Civil society organizations
g) Herbal, pharmaceutical and insurance industries.

Africa, with the aid of distinctive feature of its substantial revel in advert publicity in herbal medication and a pregnant lifestyles sciences innovation environment, ought to play a recreation changing position the birth of

biomarker- informed personalised natural medicinal drug within the close to future. Precision/personalized herbal medicines is both well timed and essential for present day therapeutics.

Medicinal plant treatment tailoring in an individual with precision medicine [39-40]

Drugs are tested on a larger population of people through phase studies and the average response is reported in a sort of evidence- based medicine. The genomics, proteomics and metabolomics are the technology that can enable researchers to further understand the causative mechanisms or biomarkers. Furthermore, they might be used to assess optimized drug efficacy and safety. The most common indications for traditional medicine includes,

a) Gastrointestinal
b) Wound healing
c) Urinary tract
d) Gynaecology
e) Cough and cold
f) Pain and inflammation
g) Mental stress and mood disorders

The conventional use of these natural drug treatments mostly consists of a customised technique and emphasis on prevention instead of a single disease focused remedy with traditional mono factor drugs due to the fact these drug treatments have a couple of aspect with more than one objectives in the body.

Herbal decoctosome- a novel form of medicine[41,43]

Historically, herbal remedy is consumed by drinking decoctions produced by means of boiling herbs with water. The warmth stable is the useful element of the decoction. Small RNA (sRNAs) have been stated as a new class of useful components in decoctions. The heat stable decoctosomes (ELNs) exosome like nanoparticles which referred to as botanosomes from decoction have healing efficacy. A medical decoctosome mimic is known as bencaosome. Decoctosomes is constructed from,

a) Lipids
b) SRNAs.
c) Proteins
d) Chemical substances

This examine demonstrates that herbal decoctosome represents a combinatory treatment in precision medicine but additionally provide powerful oral delivery route for nucleic acid remedy.

PRECISION HERBAL MEDICINE IN COVID CASE OF AFRICA [44-45]

The financial problems skilled through most African nations including Zimbabwe supposed that what most governments ought to do became to impose lockdowns and quarantine, with minimum or no contact tracing because of lack of sources and insufficient essential governance. With a lack of funding in fitness infrastructure and studies and improvement, maximum African countries couldn't supply the tons-wanted ventilators and PPEs because the standard resources of those materials in the global village, the industrialized countries, are busy stocking for their personal unmet desires. The dearth of a well-functioning cutting-edge fitness care structures, each private and public, is augmented through the truth that a large percentage (nearly 80%) of the African population inclusive of Zimbabwe uses traditional natural remedy for its immediately health want.

As a new disorder, COVID-19 has no regarded treatment or vaccine. In the developed global, pills for different associated diseases are in clinical trials for COVID-19. With new findings suggesting thatCOVID-19 is a systemic disorder, impacting the respiratory machine and past in some people, we need new molecular targets for therapeutics innovation more than ever. There have been latest debates concerning the herbal practise in Madagascar, Artemisia Africa that is often used at some point of Africa to alleviate respiratory disorder signs, a

number of which might be shared with COVID-19. It's far on this and similar contexts of African natural medicine that omics technologies offer possibilities to discover the mode of motion of natural medicines and, therefore, can usefully make contributions to the pool of novel molecular goals for therapeutics innovation for COVID-19.

Given the systemic nature of COVID-19, we can want new remedies towards this new disorder over the times, months, years, and decades beforehand. African herbal medicinal drug which includes Artemisia Africa and the related know-how in Africa warrant consideration in scientific trials and mechanistic omics studies now and in the close to destiny, in a great deal the equal manner other healing applicants are presently being evaluated.

The implications of pharmacogenomics by using medicinal plants on African population's health transition[46-48]

The general public in Africa receive herbal medicines as generally secure without an extreme detrimental consequences. Patients regularly concurrently are trying to find treatment from each conventional and conventional health structures for the equal situation. The encountered diseases includes,

a) Malaria
b) HIV/AIDS
c) High blood pressure
d) Tuberculosis and
e) Bleeding issues

Each traditional and conventional drug entities are metabolized by way of the identical enzyme device within the human body, ensuing in each pharmacokinetics and pharmacodynamics interactions, whose properties continue to be unknown.

ADVANTAGES OF PERSONALIZED HERBAL MEDICINE

1. Delivering better treatments to patients. Delivering benefits to healthcare systems and society. More efficient development of new herbal medicines[49]
2. Selection of optimal therapy and reduce trial-and-error. Increase patient adherence to treatment (Inherited forms of hypercholesterolemia) [50]
3. The benefits to the healthcare system and society are evident from improvements in patient management and in terms of offsetting costs through reduced use of ineffective treatment, reduced cost of chronic conditions and reduced hospital stays [51]
4. Shift the emphasis in medicine from reaction to prevention (Breast Cancer) Prescribing (Herceptin and Colon Cancer). Help avoid adverse drug reactions (Warfarin and Clopidogril)[52]
5. Reduced healthcare costs (Warfarin, Heart diseases and Cancer). Better Quality of life (Heart transplant follow-up) [53]

DISADVANTAGES OF PERSONALIZED HERBAL MEDICINE

1. Infrastructure requirements. Environmental population-level prevention measures are shown to be more cost-effective and equitable than efforts directed to individuals [54]
2. Limitations in Applying Genetics for Primordial, Primary, and Secondary Prevention of Chronic Diseases [55]
3. The drawbacks could also result from factors that impact precision treatment variability such as gender, weight, ethnicity, and renal and hepatic function [56]
4. Designing choices in ways that affect decision making is termed "choice architecture." In workplace environments, designs that reduce obstacles toward healthful choices may be promising for promoting beneficial eating behaviour [57]
5. Predicting risk based on genetic variants is that traits for chronic diseases are complex [58]

CONCLUSION

It is without a doubt that the principle behind personalized herbal medicine holds a great potential for translational prescription by improving diagnostic and therapeutic approaches for patient care. Theherbal genomic science will undoubtedly change the future of medicine. Nowadays the people prefers traditional system of medicine because of lesser side effects. The national policy to ensure arrangement of personalized herbal medicine should work hand in hand with existing health strategic plans. Eg, (National cancer plans) and the resources funding needs to be aligned to aspiration. The traditional system of medicine, ayurvedic medicine, botanical drugs, personalized herbal medicine, herbal decoction, primary herbal remedies plays a vital role in the aspects ofprecision medicine. The individualized treatment with herbal constituents and therapeutic treatment aims in bringing the patients wellness and health care.

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RESEARCH ARTICLE

Lessons for India on Demographic Dividend: Experiences of China, South Korea, and Brazil.

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ABSTRACT

The phenomenon of demographic dividend signals transition of a country characterized by minimal use of technology, low level of education, and low economic growth having high birth and death rates to an industrialized nation with advanced technology, higher literacy level, and income growth having low birth rates and low death rates. The existing scientific literature confirms that now developed nations were able to successfully exploit their demographic dividend and translate it into sustained economic growth and improved standard of living. The birth rates and death rates are affiliated to and correlate with accompanying stages of manufacturing growth. The objective of this study is to review the experience of three countries in exploiting their demographic dividend and map out the lessons that India can implement to benefit from this window of opportunity. The countries selected for examination are the Republic of Korea, Brazil, and China. The nations selected had varied success in unlocking the demographic dividend. South Korea along with other Asian tiger economies has successfully utilized both first and second demographic dividend. With sustained investment in health and education along with increasing women's participation in the labor force and utilizing increased saving rates for capital accumulation, it was successful in leveraging its demographic dividend for economic development. China too greatly benefitted from its first demographic dividend becoming the 'factory of the world'. Comprehensive planning and its effective implementation along with an export-oriented growth strategy led to accelerated economic growth. With an aging population and the demographic effects of the one-child policy, China's ability to capitalize on the second demographic dividend in the future is not certain. Brazil on the other has failed to take advantage of its favorable demographic transition. With misplaced priorities and the absence of determined policy action to manage its demographic transition, Brazil has left itself vulnerable to demographic 'disaster' instead. The paper concludes that demographic dividend is not a guaranteed event for a country. To successfully benefit from demographic dividend a country needs conducive policy planning and investment in the development and utilization of the country's

human capital. India also needs to correct the problem of 'missing women' in its labor force. It needs to empower local public administration to ensure efficient public services and fostering local opportunities. Also, India should have the foresight to formulate a comprehensive economic and social strategy to ensure a smooth demographic transition from a young country to a middle-aged one. A country's success with demographic dividend ultimately needs integrated demographic, political, economic and, social policies altered a country's requirements.

Key Words: Demographic dividend, Demographic transition, Economic growth, Dependency ratio, Brazil, South Korea, China, India , Human capital, Female labor.

INTRODUCTION

As per United Nations Population Fund, the demographic dividend is a period of economic growth that a country experiences due to a large section of the population being of working age. This change in age structure gives rise to a large labor force. Increased labor supply accelerates production activity. Increased production leads to higher per capita income and an improved standard of living (Pool,2007).

The demographic transition which can lead to demographic dividend occurs in four stages. The first stage is associated with high birth rates and death rates, commonly present in pre-industrialized nations. In the first stage, population increase is gradual and partly aided by the surplus availability of food. Population growth rate increases in the second stage due to higher fertility and lower mortality rates. Improvement in nutrition and sanitation decreases the frequency of disease outbreaks and extends life span. The birth rates and death rates fall in the second stage due to improved social status and literacy rate in females, increasing per capita income, higher wage rates, rapid urbanization, societal shifts, and access to contraception. Growth independent population (ages less than 15 and more than 64) is slower than growth in the labor force. With fewer younger people to feed, resources become available for investment in economic activities. Resources freed up are utilized by the working-age population (ages between 15 and 64) to increase economic activity. Stage Four indicates low birth rates and death rates. Population born during the second stage enters the working-age cohort. Birth rates fall below the replacement rate. During the fourth stage size of the population shrinks. Death rates either remain low or there is a slight increase due to increment in lifestyle diseases linked with little exercise, high diet, and high obesity. The third and fourth stages are the phase of demographic dividend (Bloom et al.,2003).

Demographic dividends last for an average of fifty years or more (Lee et al.,2006). Eventually working population ages and fertility decreases the growth rate of the labor force. The age distribution again shifts in favor of less productive age groups(ages between 1 to 14 years and 64 and above population). The proportion of dependent population increases while working-age population share diminishes. Given everything else is equal, growth in per capita income slows down and more resources would need to be devoted to dependent age groups. Demographic dividend eventually turns negative(Ross,2004). The burden of financing long-term health care and social security would fall on the economically active population. The limited resources a country might have would be used up by the dependent age groups (Cotlear, 2011).

A country can unlock a second demographic dividend utilizing advantages accrued during the first dividend. The first demographic dividend was a period of high per capita income and a lower fertility rate, which ensured fewer children to spend resources on. This translates to increased savings of the now-retired population. Nudged by a conducive policy environment, accumulated savings can be invested in capital formation that would raise the income levels of the nation. Unlike the first demographic dividend, this virtuous cycle of investment and higher returns can last indefinitely. It is important to note that the dividends are not automatic and can only be ensured by

effective policy planning and implementation. The second dividend will transform transitory advances of a country into sustainable economic development (Bloom et al.,2007). Presently India is in the third stage of demographic transition. India's dividend window began in 2018 and is predicted to last till 2055 (Balachander,2020). With a population of 1.33 billion, India lags behind Japan and other countries in European Union by almost 40 years. Most of India's large population base resides in its 1.6 lac villages. The young population has an opportunity to prosper and yield significant demographic dividends for the country(Ravishankar, 2021).

This paper aims to review the experiences of countries with varied success with demographic dividends and map out the lessons India can learn from those experiences. I have selected the Republic of Korea, Brazil, and China as the three countries for review. The three countries performed differently in exploiting the demographic dividend. The Republic of Korea like fellow other three industrialized Asian tiger economies in east Asia have sustained their pace of growth having unlocked its second demographic dividend. China starting with similar economic conditions as India during the 1950s has raced ahead and achieved remarkable growth in a short duration of time, unprecedented in history. With an aging population and the demographic effects of the one-child policy, China's ability to capitalize on the second demographic dividend in the future is not certain(Golley,2010). Brazil, the biggest South American economy, a developing country just like India, has missed out on taking advantage of its demographic window and is caught in the middle-income trap like its fellow Latin American nations. (Turra,2005)

Review of Literature

It is important to note that in research on population growth and its impact on development, not every literary work supported the dividend theory. It's an age-old debate of Marx vs. Malthus(Desai, 2010). The demographic dividend is termed as an optimistic theory as population growth increases human and intellectual capital and furnishes expanding markets promoting economic growth. Pessimistic and neutralist theories as their names suggest believing in detrimental impact by reducing capital per worker and reducing productivity and independence of population growth to the development of the nation(Pool, 2007).

The demographic dividend is delivered through various mechanisms .1) Labor Supply- Generation born during the period of high fertility enters the workforce. Women with fewer children are free to seek profitable employment. Surplus and cheap workforce also attract foreign investment. 2) Savings-Working adults tend to save more as they earn more. Low levels of fertility ensure a smaller young population (0 to 14 years of age) needs financial support. Personal income savings is one of the sources of investment in economic activities that boost growth. 3) Human capital-Income earning households can focus on providing better health through better nutrition, access to safe water, sanitation, and education for limited young members of families especially the erstwhile neglected girl child in developing countries which translates into a more productive future working population (Bloom et al., 2000). Wongboonsin and Phiromswad in their 2017 paper titled " Searching for empirical linkages between demographic shape and monetary growth", proved that developed and developing countries' experience with the third stage of demographic transition is different. For developed countries, an increase in the share of middle-aged workers has a positive effect on economic growth through institutions, investment, and education channels. On the other hand, an increase in the share of the senior population dampens economic growth through institutions and investment channels. For developing countries, an increase in the share of young workers dampens economic growth through investment, financial market development, and trade channels.

Faster growth of the working population relative to consumers, which is the first demographic dividend, directly increases economic input, while the second demographic dividend – increased saving and investment with wealth accumulating – causes physical and human capital input to rise massively (Ha et al, 2016). The dividend is not automatic and some countries had gained more than others capitalizing on demographic advantage through policies related to health, fertility, financial and labor markets, and education in place before demographic transition allowing maximum gains from the phenomenon (Ross,2004). Misra in her 2017 paper, found a positive relationship between GDP growth rate and demographic dividend. The study relied on data from BRICS countries and European

Union. Golley and Tyler in their 2012 paper titled,'Demographic dividends, dependencies, and economic growth in China and India' concluded that while China gained from its demographic transition, India was yet to enter the stage of its demographic dividend. This research reviewing different countries' degrees of success with demographic transition in the context of applicable policy lessons for India will add to literature suggesting policy actions for India to leverage its demographic transition for economic growth.

RESEARCH METHOOLOGY

Descriptive analysis of experience of countries with demographic dividend and lessons India can learn from it. Tabular and graphical representation is employed to add to the theoretical analysis.

Objectives of the Study

The basic objectives of the study are as follows

1. The primary objective of this study was to review and compare the performance of Republic of Korea, China and Brazil in achieving effectively exploiting their respective demographic transitions.
2. The second objective is to map out the policy lessons India can learn from the demographic transition of the three countries.

Data Collection

The study is based on secondary data which was collected from various secondary sources: Asian Development Bank Report, World Bank data bank, United States census bureau international database, online newspapers newspaper articles, magazines and journals.

Limitations of the Study

The study relies on secondary sources for its evaluation. Depending upon dependency ratios measured in the age range as an indicator of demographic dividend in this study has its limitations. Firstly it is incorrect to assume that after a certain age people just quit being economically active. The absence of a considerable number of females in the active working population and increasing length of professional training for young adults means not everyone in the working-age population should be automatically counted as economically productive and participating in the labor market

Analysis and Interpretation of the Study

South Korea

South Korea, Hong Kong, Taiwan, Singapore grew rapidly since the 1960s tripling their per capita income between 1965 to 1990. This impressive performance is sometimes referred to as 'East Asia's economic miracle'. This unrivaled growth was because of many factors including trade and industrial policies, technological progress, savings, and capital accumulation, governance, education and health spending, geography and culture, initial income levels, and demographic factors. Simultaneously growth rate of the working population was ten times faster than the dependent population during this time. The increase in the labor force helped South Korea reap demographic dividend (Ross, 2004). South Korea has favorable demographic characteristics in the form of high life expectancy and low fertility. To maximize the demographic dividend the government invested heavily in education at higher education, health services, and family planning (Bloom et al., 2000). Policy initiative for investment in healthcare and education, managing dependent population and promoting gender equity along increase in labor supply, population concentration and increased life expectancy proved critical for East Asia's economic growth(Bloom et al.,2000)

Ha et al.(2016) reviewed the literature for demographic dividend role in East Asian economic growth and the important role family planning policies played in their growth story. Mason and Kinugasa (2008) claimed that

demographic change accounted for about 92 percent of the increase in East Asian saving rates for 1965–1995. Deaton and Paxson (1997) elaborated on the effects of economic and population growth on increasing national savings and rising inequality. Lee et al. (2003) showed that East Asia's demographic transition multiplied saving price via a vast degree, implying that the 2d demographic dividend additionally performed a wonderful position in Asia's convergence. Asian tigers' growth miracle was only possible because policy-induced demographic characteristics and economic variables interacted in a mutually reinforcing manner. The baby boom post-world war could easily have turned into a demographic disaster if policies were not formed and enforced anticipating the demographic dividend in these countries. Surplus from the first demographic dividend was reinvested in the economies to ensure permanent growth with the second demographic dividend.

South Korea invested in a more egalitarian education system doing away with the previous Japanese multi-tiered education system. Presently its education system is ranked amongst the world's best ("From London", 6 August 2021). McNicoll,(2006) credits the role of South Korean public administration and the local opportunities it successfully fosters. Ensuring effective local institutions not only promotes demographic bonuses but also plays an important role in unlocking the second demographic dividend. Investing in health and education, skilling the labor force, utilizing national savings, and export-oriented economic planning and implementation were the factors behind South Korea's success story.

Brazil Demographic Dividend

Brazil on the other has failed to take advantage of its favorable demographic transition. With misplaced priorities and the absence of determined policy action to manage its demographic transition, Brazil has left itself vulnerable to demographic 'disaster' instead. Developing nations tend to miss upon the demographic transition phase that accompanies structural-demographic transition. It is due to adopting misplaced domestic policies or completely ignoring policy formulation in required sectors. But Brazil's case is peculiar. The country has a low literacy rate and a vast social security net (Turra et al., 2005).In 2012 the public pension systems transferred about 13 percent of the GDP to the elderly in Brazil, a significant amount for a country where there are ten over-65s for every hundred 15 to 64 year-olds ("Geronto-Genoristy",2017). As of 2018, Brazil spent more on pensions than the education, health, and social development budget combined(Jasper,2018).

Brazilian pension payments are one of the most generous in the world. It replaces 75% of the average income. In a developing country like Brazil, one might expect that a pay-as-you-go scheme would generate surpluses that would be further get invested in infrastructure, social, economic, and education. Higher pension does not translate into higher investments due to the lack of a conducive local investment climate. The Brazilian economy is in deficit, where investment is about 20% of Gross Domestic Product, out of which hardly 3% comes from the Brazilian government.

Even after accounting for the difference in income and demographic profile, Brazil spends twice of OECD average on each pensioner. And only two-third towards the education of each child. Brazil's spending on children is less than its spending on older age groups. It has resulted in a unique development where fewer elderly are below the poverty line, but a third of children are. It is not to say that there has been no improvement in literacy rates. Public Education faces a lack of resources as there's more emphasis on spending on the aged population (Turra and Quiroz, 2005). Low literacy levels in the younger population, increasing tax evasion and extensive social security benefits offset any gains the increase in the share of the working-age population accrues to the country. Lowering social security support ratios (the ratio of social security taxpayers to beneficiaries) signals the fiscal burden for the future working-age populace, thereby decreasing the ability of workers to save for the future and endangering any possibility of a second demographic dividend. Brazil's policymakers have formulated policies contrasting the country's actual requirements. They have ignored the importance of demographic transition for the economic growth of the country. Brazilian policymakers provide new forms of benefits without needed contribution (for example, the addition of rural workers in 1988), and without the approval of reforms for encouraging tax compliance; they have lower down

the benefits of the demographic transition and further increased the financial issues from the aging populace. The scenario might be different if there had been an appropriate institutional structure and sound policy framework (Berganza et. al.,,2020).

China's Demographic Dividend

China's economy grew at an unprecedented rate 1980s onwards. Utilizing its large working population in an export-oriented economic framework made it one of the world's fastest-growing economies in the world. Economic reforms in the seventies and open-door policy encouraged the entry of foreign companies into China which made it the 'factory of the world' (Fang, 2018). While liberalization policies were crucial for it to happen, Elhorst et al., in their 2011 paper titled, "Demographic transition and economic growth in China, India, and Pakistan. *Economic Systems"* estimates that demographic dynamics explain 46% of growth in per capita GDP in China between 1961 to 2003. A large section of the population saw improved living standards and higher per capita income. Chinese government's mandatory one-child policy led to a non-organic decline in fertility rates which helped save resources for industry-driven growth (Wei, 2010). The policy led to a substantial decline in dependent population and thus the forgone consumption was instead saved and contributed to capital deepening in the country. China invested in education and health (Wang et. al., 2007). The mortality rate started early declined in socialistic structure and public health system governed by the government of China. Reforms, population control policies, and investment in health and education helped China gain demographic dividend(Fang et. al., 2018).

The forced demographic transition comes with a cost. China saw a sharp decrease in the supply of its cheap labor that affects its export competitiveness. China entered the fourth stage of the demographic transition in 2014, with a population of 1.45 billion that constitutes 18.74% of the world population. The peak of the demographic dividend has passed in China (Fang et al.,2018). The fourth stage of the demographic transition will have low birth and death rates. People born during the second stage of demographic transition have started moving into older dependent age cohorts and depend on the current smaller working population to support it. Fall in birth rates below replacement level will lead to a shrinking population (Yang et.al.,2014). Death rates might remain low, or they will increase marginally. Prevalence of lifestyle diseases brought on by a low amount of physical activity and high obesity(Fang et.al., 2013). An aging population and the demographic effects of the one-child policy might turn China's demographic dividend into a demographic 'drag' in the future.

The Chinese government is taking steps to arrest this decline. It has phased out the one-child policy and is investing heavily in education and skilling its labor force. It is difficult to determine whether China will be able to achieve the second demographic dividend (Q et.al., 2021).

India's Demographic Dividend

India is in second stage of its demographic transition with high birth rate and low death rate. India's number of working-age people (people aged between 15 and 64) has grown faster than the dependent population since 2018. According to United Nations Population Fund study the increase in the labor force is going to last till 2055 or for 37 years (Bhattacharya,2021). Dependency ratio started decreasing in India in 1970s with baby boomer generation moving in working age group . An estimated 40 to 50 percent of increase in per capita income in India can be attributed to demographic dividend (Malwade et al., 2011).With dependency ratio predicted to decrease to 50 in 2050 from 79 in 1970s, India is likely to enjoy significant demographic dividend (Ghosh et al., 2011).

To assume that a demographic bonus is imminent would be incorrect. India has a long way to go before fulfilling its potential to be a global economic power. Several challenges remain like underwhelming employment prospects for the labor force, low allocation of funds for the health and education sector leading to poor human capital which results in lower productivity of low skilled labour, lack of efficient services supply by local public administration, lower female participation in the labor force etc.(Bhagat, 2014).

The education enrollment rate in India is rising. Subsidized public education and various other policies like mid-day meal scheme, scholarships are few ways through which the government is attempting to make education more inclusive and accessible(Misra, 2015). Many states have achieved universal primary enrollment. The problem of education persists mainly due to the quality of education and lack of or poor quality educational infrastructure in higher education. These problems have become more widespread in rural areas of the country Education in India faces the challenge of quality of education and lack of or poor infrastructure and its upkeep (Kaptan,2016). According to the 2020-21 Economic Survey report India allocated 2.8% of GDP during 2014-19 which is expected to increase between 3-3.5% in 2019-21. The allocation falls short of 6% of GDP recommendation by experts. Low levels of investment along with the quality of education across all levels of education will present a challenge for India in its quest to maximize demographic dividend.

India has a poor record in the health sector. Government makes policy efforts to provide affordable health services for all but the Covid-19 pandemic exposed the gaps in our medical infrastructure and lack of it in rural areas. A healthy individual ensures higher productivity with lower absenteeism rates and higher savings through less disease burden expenditure. India accounts for 16.5 percent of the world's population yet contributes to a fifth of the world's share of diseases. HIV/AIDS and TB, and drug-resistant malaria diseases are estimated to increase in the future. Infant mortality rate, maternal mortality, and reproductive health improvements will require increased and upgraded facilities. Gender disparities in terms of nutritional intake have become a characteristic feature of females in India. The lower nutritional status of expecting mothers and adolescent girls requires urgent intervention. Health services and infrastructure needs immediate attention if India wants to gain any social or economic benefit from demographic "bonus" (Kaptan,2016).

Job creation lags behind the growth in the labor force leading to the problem of unemployment in India. Unemployment levels for the young are truly alarming, accounting for nearly 20 percent of young men in age-group 15-19 years and 30 percent of women in age-group 20-24 years (Ghosh et al., 2006). India's success story also suffers from a shortage of females in the labor force. Female labor force participation in India is lower than female participation in other parts of the world, even after accounting for poverty and poor opportunities. As of 2005, the number of women absent from the labor market was equivalent to the total population in Brazil. The lower participation rates are due to social factors where women either possess appropriate skills but are discouraged from gaining employment or are excluded from access to capabilities necessary for gainful employment. Lower literacy rates and limited opportunities for skill development for young women persist, which impacts employment opportunities (Ghosh et al.,2006). In the absence of rapid social change, cultural factors will dominate economic compulsions and aspirations (Malwade., 2011). India has an opportunity to benefit from its demography dividend if priorities of economic development are clear and precise and proper policies are formed and implemented. Indias' 'window of opportunity' is still open for the next few decades. In the coming decades (Mody et al., 2011) would be able to add approximately 2 percent points annually to India's per capita GDP growth(Mody et al., 2011). If not managed demographic transition might become a demographic disaster. With limited resources spread over a large number of people who are not economically productive. India has entered the latter half of the third stage of demographic transition in 2013 with a population base of 1.23 billion, and behind forty years in comparison to European Union, Japan, etc. (Ravishankar,2021).

CONCLUSION AND POLICY RECOMMENDATION

Twenty-first century India with a population size of more than 1.3 billion people is facing a multitude of challenges like working with deficient social infrastructure, creating more gainful employment opportunities, managing macro-economic shocks, and mitigating and adapting to climate change. But India also has a big potential advantage: its young demographic populace which if nurtured right will yield a significant demographic dividend.

India can learn some useful and relevant policy lessons from the Republic of Korea, China, and Brazil's experience with demographic dividend. Every country is unique and no one framework will work for everyone but some common themes emerge. One, either of two demographic dividends are not automatic. Appropriate policy planning and its implementation are necessary for a country to leverage its demographic dividend into sustainable economic growth. Second, instead of a top-down approach to creating opportunities to nurture and develop critical human capital effective local public administration would be instrumental. Policies and programmes should be stimulating for the economy and not mandatory for people to comply as it will only result in temporarily artificial gains(UNFPA). Third, if a country wants to leverage its demographic dividend for sustainable economic development it cannot afford to divert its limited resources towards multiple unproductive avenues. India possesses vast entrepreneurship potential. The country needs to expedite the movement of labor into productive employment. To facilitate the capture of demographic 'bonus' a country must create an economic structure where allocation and implementation of property rights along with enforcement of contracts and rule of law are followed. Fourth, India also faces the challenge of 'missing women' workers in the labor market due to various socio-economic reasons which needs to be challenged. In contrast to high and increasing female labor force participation in the developed countries, India has one of the world's lowest female labor force participation rates. Developed countries have managed to do so by implementing gender-sensitive programmes and policies like increased parental leave and subsidized childcare. Skill development of the existing working population is also equally important. Fifth, at present India's expenditure in education and healthcare, is less than adequate. India's HDI index at 0.645 in 2019 is lower than the three countries in reference(UNDP, Human Development Report, 2020). India needs to develop its human capital by investing in quality education and healthcare infrastructure while also building a conducive economic structure for human capital to work and later invest in. Sixth, the quality of institutions and public accountability is needed to realize the benefits of demographic dividends. Seventh, as was the case with China and South Korea, greater integration into the global market is likely to bring more opportunities for employment and growth. Sixth, the quality of institutions and public accountability is also important to realize the benefits of demographic dividend. Seventh, as was the case with China and South Korea, greater integration into the global market is likely to bring more opportunities for employment and growth.

It is now time for India to initiate the next generation of economic reforms that ensures efficient public services focusing on neglected social needs like nutrition and health services, primary and secondary schooling, quality enhancement of tertiary education, water supply, and sanitation, and urban development. A country's success with demographic dividend ultimately needs integrated demographic, political, economic and, social policies in tune with a country's requirements.

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Table 1: Economic growth and demographic transition

Country	Period of first demographic dividend	Average annual growth rate of GDP per capita	Increase in life expectancy over the period	Change in Total fertility rate over the period
Republic of Korea	1960-1990	6.9	17	-4.3
China	1970-2000	4.9	9	-4.1
Brazil	1970-2020	1.8	17	-3.27

Sources: McNicoll,(2006) and Worldbank database,*Indicators*, various years.

Table2:Dependency Ratio in India

Year	Dependency Ratio	Child Dependency Ration	Old Age Dependency Ratio
1950	73	67	6
1955	74	68	6
1960	76	70	6
1965	78	72	6
1970	79	72	7
1975	77	71	7
1980	74	67	7
1985	72	65	7
1990	69	62	7
1995	68	60	8
2000	64	56	8
2005	60	51	8
2025	48	36	12
2050	50	27	22

Source: Population Division of Department of Economic and Social Affairs of United Nations Secretariat, World Population Prospects

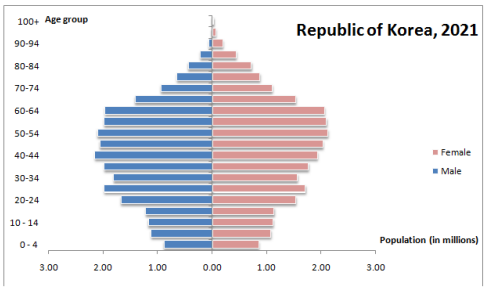

Figure 1: Population pyramid of South Korea (2021). Source: United States Census Bureau, International Database.

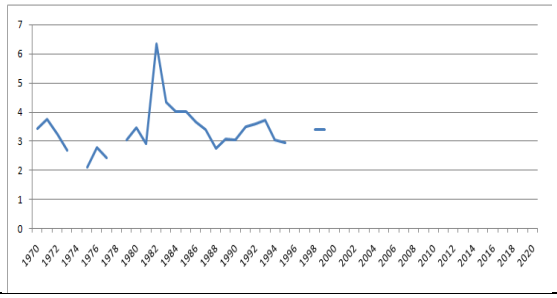

Figure 2: Republic of Korea expenditure on education (% of GDP).Source: World bank. *Indicators*,various years

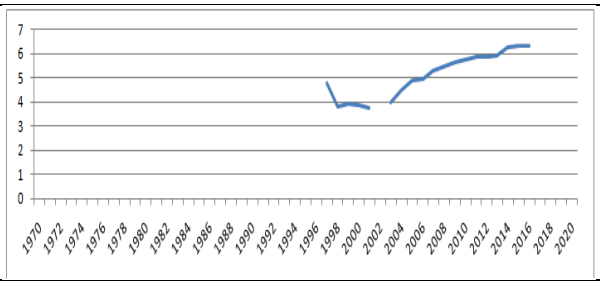

Figure 3 :Brazil total yearly expenditure on education as percentage of GDP. Source: World bank, *Indicators*, various years (Brazil)

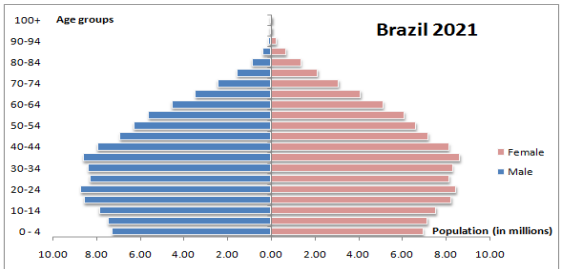

Figure 4: Population pyramid for Brazil,2021. Source: United States Census Bureau, International Database.

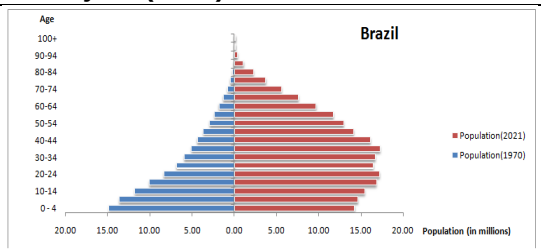

Figure 5: Brazil population pyramid in 1970 and 2021. Source: United States Census Bureau, International Database.

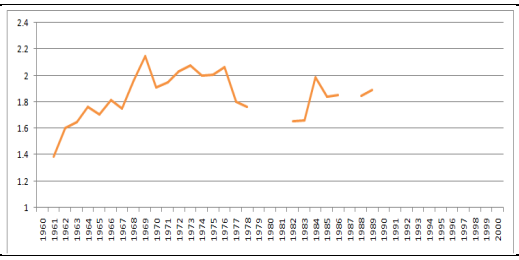

Figure 6: China total yearly expenditure on education as percentage of GDP. Source: World bank, *Indicators*, various years

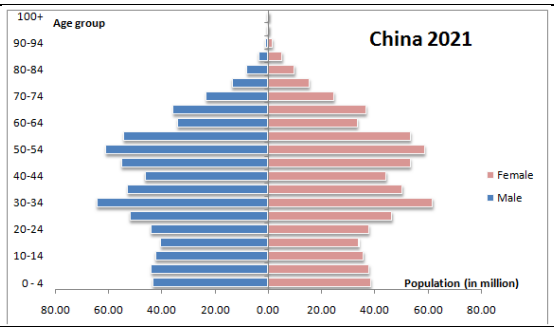

Figure 7: China population pyramid,2021. Source: United States Census Bureau, International Database

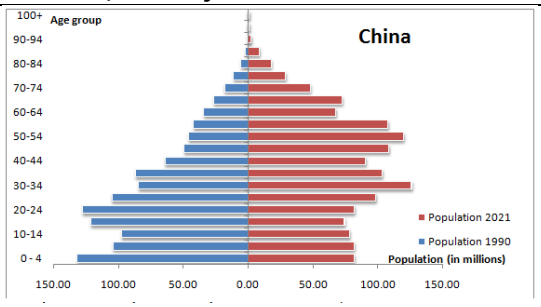

Figure 8: China population pyramid in 1990 and 2021. Source: United States Census Bureau, International Database.

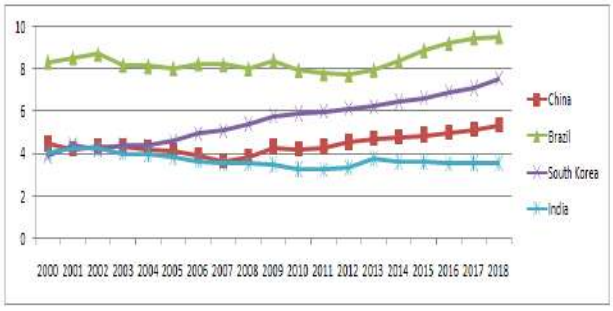

Figure 9 : Annual health expenditure as percentage of GDP. Source: World bank, *Indicators*, various years

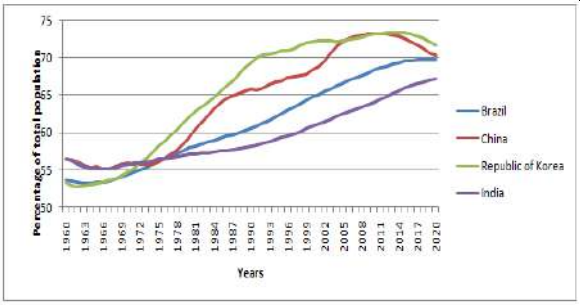

Figure 10: Working ages 15 to 64 as a percentage of total population. Source: World Bank, *Key Indicators*, various years

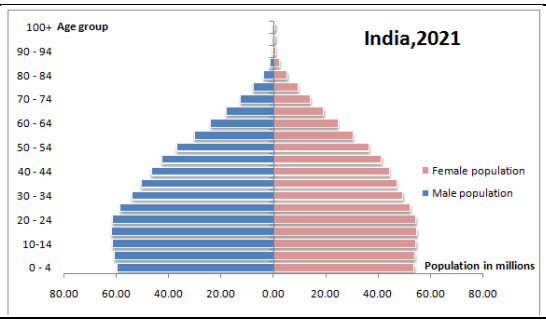

Figure 11 :India population pyramid,2021. Source: United States Census Bureau, International Database.

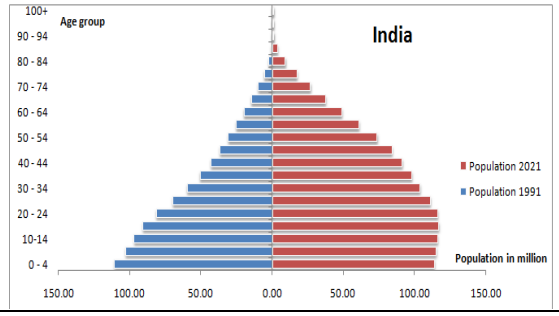

Figure 12 : India's population pyramid in 1991 and 2021. Source: United States Census Bureau, International Database.

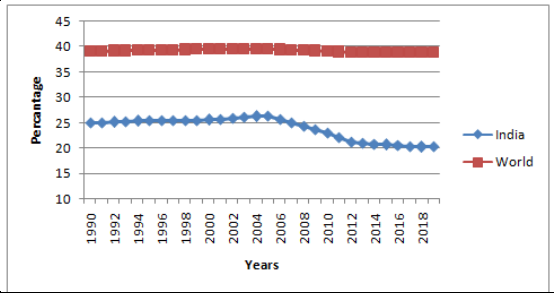

Figure 13: Labor force, female (Percentage of total labor force).Source: World bank. *Key Indicators*, various years.

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RESEARCH ARTICLE

Preference of Brinjal (*Solanum melongena* L.) Cultivars by Hadda Beetle, *Henosepilachna vigintioctopunctata* Fab. (Coccinellidae: Coleoptera) in Field Condition

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ABSTRACT

A field experiment was conducted to screen ten different brinjal cultivars towards feeding preference by hadda beetle, *Henosepilachna vigintioctopunctata* (Coccinellidae: Coleoptera) at Department of Entomology, Annamalai University, Tamil Nadu. The population of various stages of *H. vigintioctopunctata* and their leaf damage on the brinjal varieties were recorded. The results revealed that, among the varieties of brinjal evaluated against all the stages of *H. vigintioctopunctata*, green round variety was most preferred and found susceptible with a number of eggs (43.25), grubs (8.75), pupa (3.25) adult (5.00) and the leaf damage of 66.66% than other varieties whereas, brinjal 925 was resistant with the number of eggs (7.50), grubs (1.00), pupa (0.50), adult (1.00) and the lowest (4.00%) leaf damage. The varieties namely CO 1, PKM 1 and PLR 1 were found resistant against *H. vigintioctopunctata* with the average leaf damage of 8.33 %, 16.66 % and 24.99 % respectively but varieties like purple long, MDU 1 and Annamalai were found to be moderately resistant with the leaf damage of 33.33 %, 41.66 % and 49.99 % respectively. The cultivar brinjal 925 was found resistant against *H. vigintioctopunctata* and it can be incorporated in Integrated Pest Management modules so as to reduce the incidence of hadda beetles and their damage.

Keywords: Brinjal, Hadda beetle, Preference, Resistance, Susceptible, Varieties.

INTRODUCTION

India is a second leading vegetable producing country in the world, which grows variety of vegetables due to availability of different agro ecological zones. In south India, Tamil Nadu is a leading producer of vegetable crops and it occupies 0.24 million hectares area with an average production of 6.39 million tonnes [1]. Brinjal (*Solanum melongena* L.) is an important vegetable crop grown in India [2]. It is the 3rd most important vegetable crop in production after potato and tomato. There are about 2,000 species of brinjal cultivated in tropics and subtropics. Brinjal popularly cultivated in countries like Central, South and South East Asia, Central America and some parts of Africa [3]. Hadda beetle, *Henosepilachna vigintioctopunctata* Fab. is a key pest of solanaceous crops and found all over the world [4]. It is the only pest in the whole Coccinellidae family of order Coleoptera. Being polyphagous in nature, both adults and grubs feed voraciously by scrapping the chlorophyll of the leaves (in between the veins) resulting in skeletonization of the lamina which seems lace like appearance, later dries up and fall from the plants. In severe cases, even the calyx of the fruits may also be infested. The most damaging and voracious stages are 3rd and 4th instars and the damage may go up to 80% under favourable environment [5]. Use of resistant varieties is considered as a major tool in bio intensive pest management strategy. Attraction, feeding and oviposition of the pests will be coupled with morphological, physical and biochemical characteristics of plants and fruits. Although host plant resistance alone or in combination with other methods is environmentally safe and compatible with IPM, however this approach is realistic only when resistant varieties of crops subsist and recognized. Number of pesticide applications can be minimized even a moderate level of resistance [2]. Hence, the present study was undertaken to screen selected brinjal cultivars against *H. vigintioctopunctata* in field condition.

MATERIALS AND METHODS

The present study was conducted in the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu during 2018-19. Ten brinjal varieties namely Annamalai (AU 1), Green round, Super usha, CO 1, Purple long, PLR 1, PKM 1, MDU 1, KKM 1 and Brinjal 925 were selected and screened against *H. vigintioctopunctata*. Three weeks old seedlings were transplanted with a spacing of 60 x 60 cm. All the cultivation practices were used in the field except plant protection measures as per horticultural crop production guide of Government of Tamil Nadu. Ten rows of brinjal cultivars were planted and each row consisting of ten plants of brinjal cultivars. Four plants were randomly selected and maintained as replicates. Plants were tagged and observed for the incidence of *H. vigintioctopunctata*. Observations were taken from 15 days after transplanting upto harvest. Number of egg batches laid, grubs, pupae and adults of *H. vigintioctopunctata* were recorded from three leaves (top, middle and bottom leaves) of four randomly selected plants. Mean incidence was worked out and the percent leaf damage was also calculated. Based on percent leaf damage grading (Resistant (R) 0 - 25 %, Moderately Resistant (MR) > 25 - 50 %, Susceptible (S) > 50 - 100 %) was also done according to [2] and [5].

RESULTS AND DISCUSSION

The results exhibited that among the ten varieties screened against *H. vigintioctopunctata*, green round variety was highly preferred by all the stages of beetle which exhibited maximum number of eggs (43.25), grubs (8.75), pupae (3.25) and adults (5.00) followed by super usha (39.25), (7.50), (3.00), (4.75), KKM 1 (35.50), (6.25), (2.75), (4.25), Annamalai (AU 1) 28.75, 5.25, 2.50, 3.75 eggs, grubs, pupae and adults respectively, whereas, Brinjal 925 was least preferred which retarded the eggs (7.50), grubs (1.00), pupae (0.50) and adults (1.00) and statistically significant than other varieties tested (Table 1). Less infestation of *H. vigintioctopunctata* in certain cultivars could be due to the biochemical compounds present on the leaves, which could repel insects or affect host selection, indicating the existence of a possible chemical resistance factor in the variety. On the other hand, physical factors such as leaf area, pubescence and lamina thickness were also taken into consideration regarding host selection and might play a role in imparting resistance to *H. vigintioctopunctata* [5]. Nine brinjal cultivars against *H. vigintioctopunctata* were evaluated by [6] and reported that Pusa purple long was most preferred by these beetles. Similarly, six commercial cultivars

were screened against *H. vigintioctopunctata* by [7] and reported that the variety, Pusa Purple long had highly preferred by test insects which are diverged from present findings. A screening study was conducted by [5] on different cultivars of brinjal against various stages of *H. vigintioctopunctata* and they were concluded that at all the stages (egg, grub and adult) of Round F1 Hybrid was highly preferred while Blue Pearl cultivar was least preferred by *H. vigintioctopunctata*.

The percent damage done by both grubs and adults on leaf was worked out to find the presence of resistance in brinjal varieties. Results revealed that among the varieties evaluated, Brinjal 925 was resistant to hadda beetle which exposed a 4.00 percent leaf damage followed by CO 1 (8.33%), PKM 1 (16.66%) and PLR 1 (24.99%). However, the brinjal varieties manifested moderate resistance were Purple long, MDU 1 and Annamalai (AU 1) with leaf damage of 33.33 percent, 41.66 percent and 49.99 percent respectively whereas KKM 1 was moderately resistant (49.99%) (Table 2). The brinjal varieties which showed susceptibility were Super usha and Green round with the percent leaf damage of 58.32 and 66.66 respectively. Based on the present study, resistant cultivars were segregated against *H. vigintioctopunctata* based on per cent damage. The scale ranged as resistant, moderately resistant and susceptible. Among the brinjal cultivars, Pusa Purple Long was most susceptible than other cultivars reported by [7] and it had conflict with present study because Pusa Purple Long was observed as moderately resistant to *H. vigintioctopunctata*. The population and percent leaf damage was maximum in green round variety and minimum in Brinjal 925.

CONCLUSION

Based on the findings of the current study, it is concluded that minimum population of eggs, grubs, pupae and adult with minimal damage were recorded in Brinjal 925 cultivar. Hence, it was found that brinjal 925 was recommended as a promising cultivar for resistance to *H. vigintioctopunctata* and can also be incorporated in IPM practices in order to reduce yield loss in brinjal.

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Table 1. Varietal preference of various stages of *H. vigintioctopunctata*

Sl. No	Treatments (Cultivars)	Mean Numbers*/three leaves			
		Eggs	Grubs	Pupae	Adults
1.	Green round	43.25 (6.64)^{e}	8.75 (3.12)^{c}	3.25 (2.05)^{b}	5.00 (2.44)^{b}
2.	Annamalai (AU 1)	28.75 (5.44)^{d}	5.25 (2.49)^{b}	2.50 (1.86) ^{a}	3.75 (2.17)^{b}
3.	Super usha	39.25 (6.34)^{e}	7.50 (2.91)^{b}	3.00 (2.00)^{b}	4.75 (2.39)^{b}
4.	Brinjal 925	7.50 (2.14)^{a}	1.00 (1.41)^{a}	0.50 (1.20)^{a}	1.00 (1.28)^{a}
5.	PLR 1	19.50 (4.09)^{c}	2.75 (1.93)^{a}	1.25 (1.47)^{a}	2.25 (1.79)^{a}
6.	MDU 1	26.50 (5.20)^{d}	4.00 (2.23)^{b}	2.25 (1.79)^{a}	3.50 (2.11)^{b}
7.	Purple long	22.25 (4.80)^{c}	3.50 (2.11)^{b}	1.75 (1.65)^{a}	2.75 (1.92)^{a}
8.	CO 1	10.25 (3.05)^{b}	2.25 (1.79)^{a}	0.75 (1.31)^{a}	1.25 (1.47)^{a}
9.	PKM 1	11.25 (3.24)^{b}	2.50 (1.86)^{a}	1.00 (1.39)^{a}	1.50 (1.57)^{a}
10.	KKM1	35.50 (6.03)^{e}	6.25 (2.68)^{b}	2.75 (1.93)^{a}	4.25 (2.28)^{b}
	SE(d)	0.89	0.09	0.13	0.15
	C.D.	1.84	0.19	0.28	0.31

*Values are mean of four replications

Values in parentheses are square root transformed values

Values followed by alphabets shows significance among the treatments according to DMRT

Table 2. Varietal preference of *H. vigintioctopunctata* based on percent leaf damage

Sl. No	Treatments (Cultivars)	Mean percent leaf damage*	Grade
1.	Green round	66.66 (54.71)	S
2.	Super usha	58.32 (49.84)	S
3.	Annamalai (AU 1)	49.99 (44.97)	MR
4.	Brinjal 925	4.00 (5.92)	R
5.	PLR 1	24.99 (26.43)	R
6.	MDU 1	41.66 (40.14)	MR
7.	Purple long	33.33 (35.24)	MR
8.	CO 1	8.33 (8.81)	R

9.	PKM 1	16.66 (17.62)	R
10.	KKM 1	49.99 (44.97)	MR
	SE(d)	8.71	
	C.D.	17.97	

*Values are mean of four replications
*Values in parentheses are arc sine transformed
Grade: Resistant (R) 0 - 25 %
Moderately Resistant (MR) > 25 - 50 %
Susceptible (S) > 50 - 100 %

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RESEARCH ARTICLE

HPTLC Fingerprinting of Ethanol Extract of Leaves of *Abrus precatorius Linn.*

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ABSTRACT

Separation and identification of the constituents of ethanol extract of *Abrus precatorius Linn.* leaves was carried out using HPTLC analysis. The extraction of the plant was carried out using Soxhlet apparatus with absolute ethanol. The chromatographic separation of the compounds was performed on precoated silicagel $60F_{254}$ plates with the mixture of toluene: ethyl acetate: formic acid: methanol at a ratio of 3:6:1.6:0.4 v/v as mobile phase. The densitometric determination of the constituents was carried out at the absorbance of 254 nm using standard markers. The study concluded that the HPTLC fingerprinting analysis exhibited that the presence of flavonoids in the ethanol extract of *Abrus prectorius.*

Keywords: *Abrus precatorius*, HPTLC fingerprinting, Flavonoids, Densitogram profile

INTRODUCTION

Recently herbal preparations, nutraceuticals, dietary supplements are receiving more attention due to their effectiveness against various diseases. Unfortunately, there is an inadequate analytical validation to assure the quantity and quality of active principles in the preparation (Nicoletti., 2011). Hence, the scientists across the world are focussing their attention to isolate and validate the active ingredients of the herbal preparation. In that aspect the present study was carried out to assess the active ingredients of *Abrus precatorius. Abrus precatorius L.* (Fabaceae) is a climbing shrub, commonly distributed throughout the tropical and subtropical regions of the world (Morton., 1982). In India it is found in hedges and bushes. *A.precatorius* leaves were used as laxative, expectorant and aphrodisiac in ayurveda (Raamachandran., 2008). In addition to these, it is used to treat stomatitis, conjunctivitis, alopecia and leukaemia (Anbu *et al.,* 2011). The roots and leaves are astringent, anthelmintic as well as alexiteric and also useful to

treat the conditions of cough, pharyngodynia and pectoralgia. The leaves are sweet in taste, and the infusion of the leaves and roots are used for coughs, colds, and colic (Chakravarthy., 1969). Though it has lot of medicinal benefits, it is claimed as poisonous plant due to the toxic effect of the seeds (Davis., 1978). A separation technique "Thin Layer Chromatography"(TLC) was developed by Stahl (Stahl., 1965) is the most suitable method to analyse the known or unknown samples obtained from natural/synthetic sources. High Performance Thin Layer Chromatography (HPTLC) is a sophisticated version of TLC and it is an ideal tool to identify the unknown as well as reference samples in parallel on the same plate with the advantage such as reliable, flexible and rapid method to analyse many samples at the same time under the same chromatographic conditions (Nicoletti., 2011). A fingerprint represents the mixture of organic substances in a single chromatographic track, which is used to identify the components of the plant material (Nicoletti., 2011) with respect to number, sequence, position, and colour of separated zones.

MATERIALS AND METHODS

Chemicals and Reagents

Quercetin, rutin and gallic acid were procured from Sigma Chemical Company Inc., USA. The solvents such as methanol, toluene, ethyl acetate and formic acid were procured from Merck, Mumbai, India. Aluminium sheets backed silica gel 60 F_{254} plates (0.2 mm) for HPTLC was purchased from E-Merck, Darmstadt, Germany. A Camag HPTLC system comprising of Linomat 5 applicator and Camag TLC scanner 3 were used for the analysis.

Preparation of Test and Standard Samples

500 mg of ethanol extract of leaves of *A.precatorius* was dissolved in 5 ml of methanol and sonicated for 10 minutes so as to extract broad spectrum of compounds. After sonication, the test sample was centrifuged for 10 min. at 24°C and cooled. The supernatant was used for HPTLC analysis. Standard marker compounds were dissolved in methanol and prepared the solution containing 1 mg/1 ml of reference sample.

HPTLC Analysis

Sample Application

The sample solution was applied on aluminium backed silica gel coated 60 F_{254} plates (10 × 10 cm with 0.2 mm thickness) with a band width of 6 mm using CAMAG Linomat V applicator. Application position at 10 mm from the bottom of the plate. In total 6 bands inclusive of three samples and three standards were applied. The volume of the test samples was 2, 4, 8 µl and the standard/reference sample was 5 µl were applied on the silica gel plate.

Development of Chromatogram

Plates were dried at ambient temperature for 5 minutes and kept in a twin-trough chamber containing mobile phase consisting of toluene: ethyl acetate: formic acid: methanol at a ratio of 3:6:1.6:0.4. The saturation time was 20 minutes. Developing/migration distance was 80 mm and the developed layers were dried in an oven at 60°C for 5 minutes.

Detection

In order to carry out the densitometric analysis, the developed plate was fixed in photo documentation chamber Camag TLC scanner 3 coupled with win CATS software and the absorbance was carried out at 254 nm. The source of the light was deuterium as well as tungsten lamps and the scanning speed was 20 mm/s. TLC plates were air dried and densitometric scanning was performed at 254 nm. The Rf value of each compound and the peak area of each band was recorded.

RESULTS AND DISCUSSION

HPTLC determination of ethanol extract of *A. precatorius* leaves

The objective of this study was to identify the bioactive compound present in ethanol leaves extract of *Abrus precatorius*. The Rf values of the constituents were compared with the standard marker compounds such as quercetin, rutin and gallic acid. The above-mentioned flavonoids/standard marker have been among the active principles of some plant extracts. Hence, the Rf values of the test sample were compared with the standard marker compounds which is in agreement with the standard/marker compounds (Table 1). Densitogram profile of the active compounds observed at 254 nm for both the test sample as well as standard sample (Figure.1).The HPTLC profile of ethanol extract of *A.precatorius* leaves recorded under UV at 254 and 366 nm are given in figures 2A and B. The densitographic profile of ethanol extract of *A.precatorius* leaves at 254 nm for the volume of 2, 4 and 8µl are given as figures 3A, B and C respectively. The densitographic profile of standard samples of Quercetin, Rutin and Gallic acid are given in figures 4A, B and C. From the study, it was observed that the ethanol extract of *Abrus precatorius* contains flavonoids, quercetin and rutin.

Many literatures demonstrated that HPTLC is an important tool for the qualitative, semi quantitative and quantitative phytochemical analysis of herbal drugs and formulation (Kshirsagar *et al.,* 2008). Arivukkarasu and Rajasekaran *et al.,* 2018 developed a chromatogram using the mobile phase of toluene: ethyl acetate: formic acid: methanol at the ratio of 3:6:1.6:0.4 to find out the presence of secondary metabolites in commercial herbal formulation using the standard markers. The remedial properties of the plants are mainly due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins and glycosides (Lalnundanga *et al.,* 2012). Flavonoids are major components found in plants, possess medicinal properties used in traditional medicine all over the world. Flavonoids are also the phenolic compounds naturally exist in plants and constitute C6-C3-C6 carbon structure (Groot *et al.,* 1998). Besides plant growth and development, flavonoid's plays a vital role in pathogen resistance and UV light protection (Vvedenskaya and Vorsa., 2004). In addition to these, flavonoids are effective against human diseases due to its antioxidant nature (Verbeek *et al.,* 2004). It was reported that flavonoids possess anti-inflammatory, antioxidant, anticancer, antidiabetic and antiviral properties (Patel *et al.,* 2011).

Table.1. HPTLC fingerprint analysis of ethanol extract of *A.precatorius* leaves and the corresponding Rf values of test samples as well as reference samples.

Figure.1. Densitogram profile of ethanol extract of *A.precatorius* leaves at 254nm. Track 1-3 represents test samples and 4-6 belongs to reference samples.

Figure.2A and B: Chromatographic profile of ethanol extract of *A.precatorius* leaves under UV at 254 and 366nm respectively.

Figure.3A, B and C: Densitogram profile of 2, 4 and 8µl of ethanol extract of *A.precatorius* leaves at 254nm.

Figure.4. Densitogram of standard samples of 4A. Quercetin, 4B. Rutin, 4C. Gallic acid

CONCLUSION

HPTLC is a sensitive, reliable and rapid analytical technique used for the separation as well as identification of phytochemicals. In the present study HPTLC was used to find out the phytoconstituents present in the ethanol extract of the leaves of *Abrus precatorius Linn*. From the chromatographic fingerprint profile, it was found that the presence of flavonoids as compared to marker compounds such as rutin, quercetin and gallic acid. The study concludes that the presence of flavonoids may be responsible for the biological activity of the ethanol extract of *Abrusprecatorius* Linn. Hence, the flavonoids will be isolated for further studies and will be tested for biological activity.

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Table.1. HPTLC fingerprint analysis of ethanol extract of *A.precatorius* leaves and the corresponding Rf values of test samples as well as reference samples.

Track number	Amount of sample (µl)	Number of peaks	R_f value of the samples	Name of the marker present in the sample
1	2	8	0.06, 0.10, **0.19**, 0.29, 0.44, 0.64, 0.72, 0.88	Rutin
2	4	7	0.06, **0.19**, 0.39, 0.60, 0.67, 0.76, 0.87	Rutin
3	8	7	0.06, 0.20, 0.32, 0.58, 0.66, 0.75, **0.85**	Quercetin
4	5	2	**0.17, 0.83**	Rutin and Quercetin
5	5	1	**0.16**	Rutin
6	5	1	**0.77**	Gallic acid

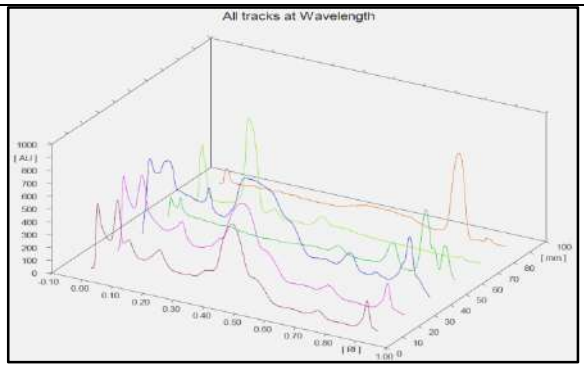


Figure.1. Densitogram profile of ethanol extract of *A.precatorius* leaves at 254nm. Track 1-3 represents test samples and 4-6 belongs to reference samples.

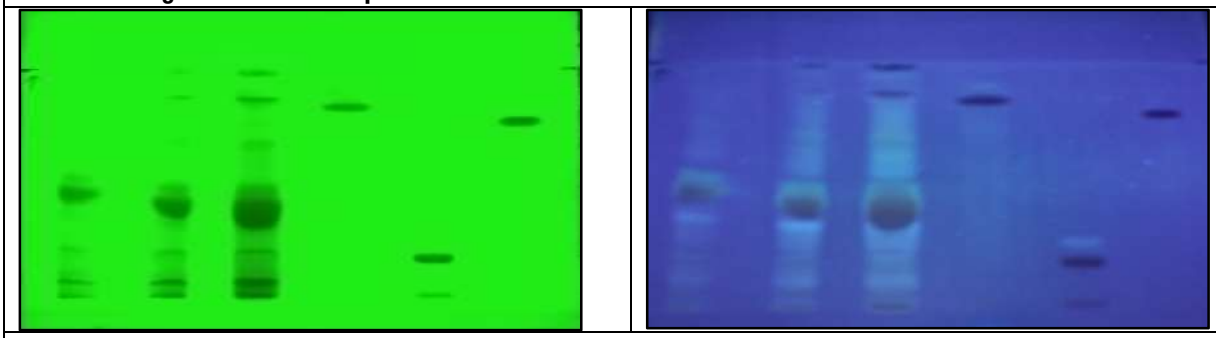
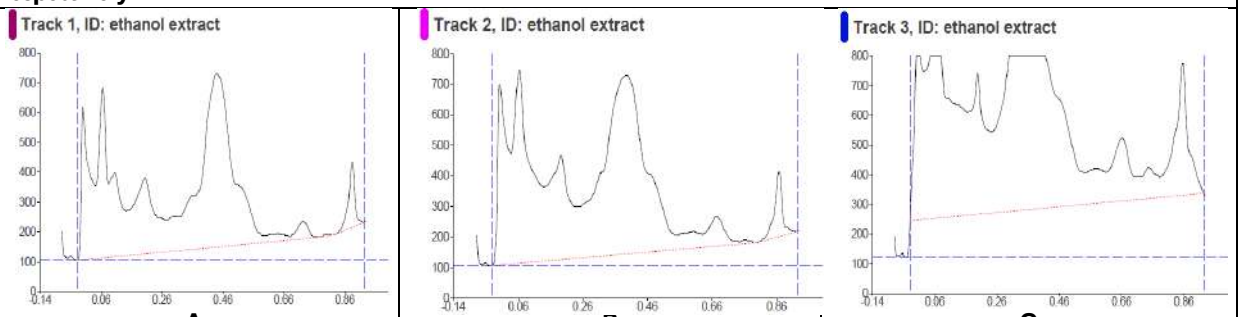
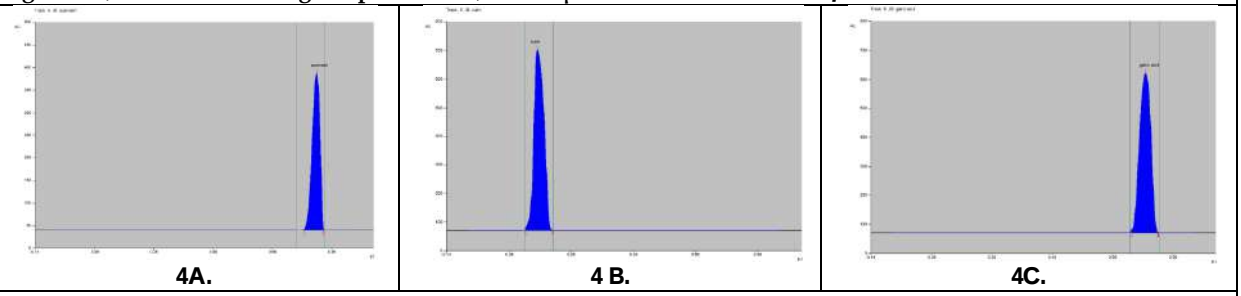


Figure.2A and B: Chromatographic profile of ethanol extract of *A.precatorius* leaves under UV at 254 and 366nm respectively.



A B C

Figure.3A, B and C: Densitogram profile of 2, 4 and 8µl of ethanol extract of *A.precatorius* leaves at 254nm



4A. 4 B. 4C.

Figure.4. Densitogram of standard samples of 4A. Quercetin, 4B. Rutin, 4C. Gallic acid

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REVIEW ARTICLE

An Overview on Cubosomes in Pharmaceutical Drug Delivery Systems

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ABSTRACT

Cubosomes are self-assembled, nanostructured, thermodynamically stable, square and rounded particles with cubic lattices visible. These are considered as versatile systems because of their properties and hence administrable in different ways such as orally, percutaneously, and parenterally. Self-assembled cubosomes act as active drug delivery systems; highly accepted, has got importance after innovation and nomination, they enclose a structure similar to "Honeycomb" through bicontinuous domains of water and lipid. Inside the surfactant, it is assembled into bilayers and wrapped into a three dimension, periodic and minimal surface, forming a strongly packed structure. They have very simple method of preparation; whereas biodegradability of lipids have the capability of encapsulating hydrophobic, hydrophilic and amphiphilic substances meanwhile targeted and controlled release of bioactive agent. Cubosomes are nanoparticles whose size ranges from 10-50nm in diameter. Each dot corresponds to the presence of pore containing aqueous phase cubic phases in lipid water system in X-ray scattering technique. and also covers some methods of preparation of cubosomes along with there characterization methods like Cryo-Transmission Electron Microscopy (CryoTEM), Small-Angle X-ray Scattering (SAX).

Keywords: Nanoparticles; Suspension; Liquid crystal; hydrophilic;hydrophobic.

INTRODUCTION[1-6]

The term "Cubosomes" were coined by Larsson that reflects the cubic molecular crystallography and similarity to liposomes[1-2].Cubosomes are defined as discrete, sub-micron, nano-structured particles of bicontinuous cubic liquid crystalline phase.3Cubosomes are useful over other drug delivery system due to their bioavailability improvement of poorly soluble drugs and enhances skin permeation, by this it reduces the cost of therapy[4-

5].cubosomes are composed of polymers, lipids and surfactants with polar and non polar components hence said as amphiphilic[6].

Structure of Cubosomes[7-11]

Cubosomes consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. Self assembled cubosomes as active drug delivery systems are receiving much more attention and interest after the firstdiscovery[7-8].Cubosomes are nanoparticles, more accurately nanostructure particles of liquid crystalline phases with cubic crystallographic symmetry formed by the self-assembly of amphiphilic or surfactant like molecules[9].Cubosomes are nanoparticles whose size ranges from 10–500 nm in diameter and appear like dots, which are likely to be spherical[10]. Cubosomes are amphiphilic carrier system which has a capability to encapsulate both hydrophilic and lipophilic drugs. The hydrophilic drug is encapsulated inside the vesicles whereas the lipophilic drug is partitioned between the hydrophilic domains[11].

Classification[12-15]

Cubosomes can be classified under two broad criteria as follows:

Based On Surface

Three structure of cubosomes have been proposed.

(i)(D-surface) (Diamond surface),

(ii)(G-surface) (Gyroid surface), and

(iii)(P-surface) (Primitive surface), in terms of nodal surfaces.

The structure generally maintains the efficacy; stability of actives such as vitamins and proteins[12-13].

i.Psurface:It is evalutated as follows:

$F(x,y,z)=\cos x+\cos y+\cos z$

.G surface: It is evalutated as follows:

$F(x,y,z)=\sin x \cos y+\sin y \cos z+\cos x \sin z$

iii.D surface: It is evalutated as follows

$F(x,y,z)=\cos [x-y]\cos z+\sin[x+y]\sin z$

Based on Physical State

Liquid Cubosome Precursors[14]:

The hydrotrope dilution process is found to produce more stable and smaller cubosomes. Nucleation process allows the formation of particles whose growth is seen under crystallization and precipitation processes. Monoolein is properly dissolved in a hydrotrope, such as ethanol, that prevents it from liquid crystalline formation. Thus, dilution of this mixture spontaneously “crystallizes” or precipitates the cubosomes. Quid precursor process is allowed for easier scale up of cubosome preparations and avoids the bulk solids handling and potentially damaging high energy processes.Drug releases slowly when compared powder.

Powdered Cubosome Precursor[15]:

Powdered cubosome precursors are composed of dehydrated surfactant coated with polymer. Such powders offer advantages to liquid phase hydrotropic cubosome precursors. Cubosomes with a mean particle size of 600 nm are formed by the hydration of the precursor powders, as confirmed by light scattering and cryo-TEM. Cubosomes which are made up with the use of lipids are waxy and sticky solids. Coating of the waxy lipid on water-soluble noncohesive starch generally prevents from agglomeration and controls the size of the particle. An excellent process for his purpose is spray drying. Powdered cubosomes precursor using freeze drying more expensive process than spray drying.

Manufacturing Techniques of Cubosome[16-22]

Cubosomes can be manufactured by two distinct methods[16]:

Top down Technique

Bottom up Technique
Heat treatment
Spray drying

Top Down Technique

The bulk cubic phase is first produced and then dispersed by high energy processing into cubosome nanoparticles. Bulk cubic phase resembles a clear rigid gel formed by water-swollen cross-linked polymer chains, but cubic phases differ in that they are a single thermodynamic phase and display periodic liquid crystalline structure[17].Less energy input is needed at high concentration to prevent the formation of liquid crystals. Dispersing inverse micellar phase droplets leads to the formation of cubosomes in water at 80 and allow them to slowly cool and droplets get crystallizes to cubosomes[18].

Bottom Up Technique

It is more recently developed technique of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale. The formation of cubosomes by dispersion of inverse micellar phase droplets in water at 80°C, then by slow cooling to allow the droplets to gradually crystallize into cubosomes.Dispersion of the nanoparticles produced in the cubosomes formation by several techniques[19].

1. Sonication
2. High pressure homogenization
3. Spontaneous emulsification
4. Spray drying
5. Sonication and high-pressure.

Heat Treatment

In this case, heat treatment can be regarded as a good approach. Note that in the strictest sense, heat treatment is not an integrated process for the manufacture of Cubosomes because it only promotes the transformation from non-cubic vesicles to well-ordered cubic particles. The dispersed particles, therefore, can be produced by a simple processing scheme comprising a homogenization and heat-treatment step. From the reported studies, heat treatment could cause a decrease in the small particle size fraction that corresponded to vesicles and form more cubic phases with narrow particle distribution and good colloidal stability. Taking the whole process of preparation into account, it is obvious that the transition takes place during the procedure of heat treatment. The reason for transition could be speculated as an elevated temperature giving rise to a reduction in solubility and stability. When the temperature was below cloud point, the surfactant had a high solubility and thus the particles could exist stably and the phenomenon of fusion was hardly observed. Once reaching cloud point, the solubility of surfactant decreased notably and a notable fast fusion among vesicles would occur[20].The lipids used to make Cubosomes are waxy, sticky Solids, rendering them unable to form small discrete particles. It is found that a water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. Spray drying is an excellent process to produce these particles. Spray drying produces encapsulated particles from an emulsion of liquid droplets or a dispersion of solid particles in a concentrated aqueous polymer solution[21].

The continuous and dispersed phases are sprayed through a nozzle to create suspension droplets that are contacted with a heated, dry air stream flowing in the opposite direction. Excess water immediately evaporates, leaving dry powder particles composed of the dispersed phase encapsulated by a shell of the formerly dissolved polymer. Spray-drying processes are easily scaled up and are already widely employed for manufacturing consumer products like detergents and foods. Further, the process provides an easy route to preload active into the Cubosomes prior to drying. Finally, the polymer coating on the powder imparts surface properties to the hydrated Cubosomes that can be tailored by proper selection of the encapsulating polymer. The liquid feed to the spray-dryer can be tailored to adjust the resultant powder properties. The production of starch coated Cubosomes powder precursors requires high shear treatment of monoolein in aqueous starch solution to form a coarse Cubosomes dispersion that is then pumped through a nozzle and dried. The initial composition pumped into the spray-drier is 60% w/w water, 30% starch, and

10% monoolein. Drying removes almost all water present and gravimetric tests of the powder generally indicate a final composition of about 4% w/w water, 72% starch, and 24%monoolein in the product powders.
The emulsion of both phases has low viscosity and is easily spray dried. The type of encapsulating starch also affects powder quality. Drying occurs as the dispersion is sprayed into droplets and moisture rapidly evaporates by convective heating. The Cubosomes in the dispersion form the nucleus of many of the sprayed droplets, surrounded by aqueous starch solution. As drying proceeds, the starch remains and forms a coating on the cubic gel particle, thereby encapsulating it. Because the cubic phase itself contains 40% (w/w) water, some drying must also occur at the core of the particles. Low molecular weight starches (84,000 MW) produce superior powders when compared to those made using high (335,000 MW) molecular weight starches. Provide a more comprehensive listing of feasible polymers and other materials for use as polymeric coatings to encapsulate Cubosomes[22].

Evaluation of Cubosomes[23,24]

Visual Inspection

The cubosomes are visually assessed for optical appearance (e.g colour, turbidity, homogeneity, presence of macroscopic particles).

Shape of the Cubosome

Transmission electron microscopy can be used to view the shape of the cubosomes.

Particle Size Distribution

Particle size distributions of cubosomes are mainly determined by dynamic laser light scattering using Zeta sizer (Photon correlation spectroscopy). The sample diluted with a suitable solvent is adjusted to light scattering intensity of about 300 Hz and measured at 25°C in triplicate.20 The data can be collected and generally shown by using average volume weight size. The zeta potential and polydispersity index can also be recorded.

Zeta Potential

The magnitude of zeta potential indicates the degree of electronic repulsion between adjust, similarly charge particle. Zeta potential is key indicator of the stability of formulation.

Entrapment Efficiency

The entrapment efficiency of cubosomes can be determined using ultra filtration techniques. In the later technique, unentrapped drug concentration is determined, which is subtracted from the total drug added. The amount of drug is analyzed by using spectrophotometer.

Measurement of Drug Release

Drug release from cubosomes can be done by pressure ultrafiltration method[23]. Using an Amicon pressure ultrafiltration cell fitted with a Millipore membrane at ambient temperature (22±2) °C. 5.7. Stability studies The physical stability can be studied by investigation of organoleptic and morphological aspects as a function of time. Particle size distribution and drug content can be assessed at different time intervals can also be used to evaluate the possible variations by time[24].

Characterization[25,26,27]

Cryo-Transmission Electron Microscopy (CryoTEM)

Cryo-Transmission electron microscopy (Cryo-TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultrathin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor. Cryo-TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small wavelength of electrons[25].

Small-Angle X-ray Scattering (SAXS).

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of Xrays (wavelength 0.1. - 0.2 nm) by a sample which has in homogeneities in the nm-range, is recorded at very low angles (typically 0.1 - 10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data. SAXS is capable of delivering

structural information of macromolecules between 5 and 25nm, of repeat distances in partially ordered systems of up to 150 nm[26].

Particle Size Distribution (PSD).

The particle size distribution of the dispersions was determined using photon correlation spectroscopy. Measurements were performed at 250C using a refractive index (RI) of Cubosomes at intervals of 100 s. Samples were diluted with water to adjust the signal level. The average particle size (z-average) and poly dispersity index were determined[27].

Advantages

1. They have ability to encapsulate both hydrophilic and hydrophobic also amphiphilic drugs[28].
2. Simple preparation method[29].
3. Biodegradability of lipids[30].
4. Biocompatible and non-toxic[31].
5. High drug payloads[32].
6. To targeting and controlled release of bioactive agent[33].
7. Improves efficacy and decreases risk of drug misuse and misdirection[33].
8. Cubosomes increases convenience and compliance[33].
9. Low cost of raw materials[34].
10. For longer time they are thermodynamically stable[34].

DISADVANTAGES:

1. High energy process[34].
2. Expensive[35].
3. Difficult to scale up[35].
4. Because of their high viscosity large scale production is sometimes difficult[36].
5. Low entrapment of water soluble drugs[36].
6. Large scale production is difficult for sometimes because of high viscosity[36].
7. Some big drugs cannot penetrate inside the channels and drugs can ruin the lattice structure of continuous liquid crystalline phase[37].

Application [38-40]

Topical Drug Delivery Systems:

Cubic phases have bioadhesive property, so that they can conveniently use in topical and mucosal depositions and delivery of diverse drugs.

Oral Drug Delivery System:

Cubosomes deal with the varied challenges in oral delivery of numerous promising compounds including poor absorption, poor aqueous solubility, and huge molecular size.

Melanoma (Cancer) Therapy:

Recently few anticancer drugs have been successfully encapsulated in cubosomes and characterized physicochemically. The unique structure of this promising nanocarrier suggests its application in melanoma therapy.

Topical Drug Delivery System:

Cubic phases are more bioadhesive in nature, so that they can conveniently use in topical and mucosal depositions and delivery of different drugs. Topical delivery systems are based on the exploitation of unique properties of liquid crystal (LC) and liquid crystal nanoparticle technologies (LCNT).

Intravenous Drug Delivery System:

Lipid nanoparticles comprising interior liquid crystal structures of curved lipid membranes are used to solubilize encapsulate and deliver medications to disease areas within the body.

Brain Targeting:
The delivery of drugs to brain for the treatment of CNS diseases is blocked by the BBB. This barrier poses a considerable challenge for the administration of both small and large drug molecules.

In Cancer Cell Targeting:
Many cancer drugs have been successfully encapsulated in cubosomes.The cellular uptake of this anticancer drug was increased by formulating into cubosomes.

Increasing The Corneal Permeability:
Because of low corneal permeability and bioavailability, ocular drug delivery faces different challenges. For glaucoma treatment a cubosome drug delivery was constructed for Timolol Maleate (TM) in a study with the help of glycerol monooleate and Poloxamer 407.

Vehicles for Biologically Active Substance.
Cubic phases were produced at 25 ºC in water monooleinalcohol mixtures. Ethanol was found to be more efficient than propanol and butanol. In the composition range of 49 to 56 wt% water, 31 to 40 wt% monooleine and 10 to 13% wt ethanol we identified a new transparent, low-viscosity (flowing) phase that we called OL. No structures were found by bright field light microscopy and polarized light microscopy, indicating that OL is an isotropic phase. CryoTEM showed large domains of this ordered phase, which by Fast Fourier Transformation was identified as a cubic phase.

Control Release of Solubilized Substance[40].
Cubic phase is more applicable for control release because of its small pore size (5-10nm), ability to solublisehydrophilic,nhydrophobic, amphiphilic. The molecules and its biodegradability by simple enzymes.

CONCLUSION

Recognizing the desirable properties of cubosomes, it has been proposed as a novel carrier for drug delivery system. The relatively recent discovery of cubosomes as a broad level of investigation become financially attractive will continue to narrow knowledge of cubosome formation and performance the precursor forms further scope in technological field. Moreover, the literature review also specify cubosomal utility as a control relief drug carrier.

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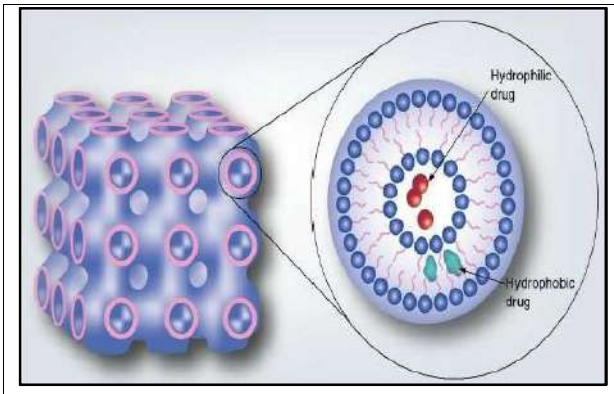

Fig.1 Structure of cubosomes

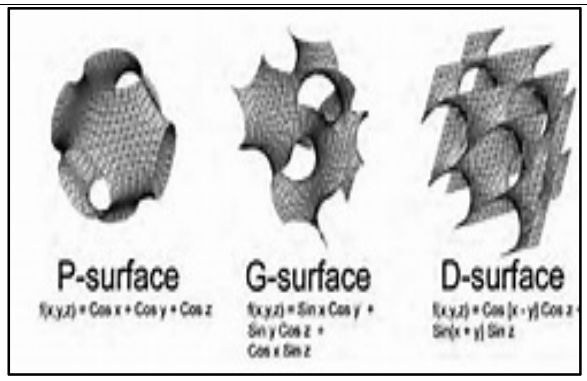

Fig.2 : Based on Surface

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RESEARCH ARTICLE

Experience and Expectation of Post Natal Mothers Regarding Nursing Care during Child Birth Process

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ABSTRACT

A descriptive survey with cross sectional survey approach was under taken to assess the experience and expectations of post natal mothers regarding nursing care during child birth process in JK hospital, Bhopal. Fifty post natal mothers were selected by purposive sampling technique and data were collected by using Rating scale.

Keywords: survey, hospital, technique, Nursing.

INTRODUCTION

Demographic findings revealed that 48% of the mothers were between the age group 20 to 25 years, 68% were Hindus and 50% had one child. Mothers who had primary education and higher secondary education were 38% each. Forty two percentage of the mothers belonged to the income group of above Rs. 2000 and 42% them were housewives whereas, majority (60%) of them were from nuclear family.

Finding shows that all most all (94%) the mothers were satisfied with nursing care to some extent whereas, mothers with satisfaction and not satisfaction were 4% and 2%, respectively. Most of the mothers (80%) were satisfied to some extent for the items "Cared like a family member", "Got strength due to motivation (70%)", and "Supported by nurse during labour pain (76%)".

Most (72%) of the mothers were satisfied with the nursing care for the items," Intimated sex of the child" whereas, most of them had no satisfaction for the items, " Informed to family members about the mothers condition (86%)"and "Family members not allowed in the labour room (84%)".

To assess the experience and expectation of post natal mothers related to nursing care during child birth process.
Childbirth experience is consistently described as a significant event of powerful psychological importance in a woman's life. Childbirth can be a development task or a time of crisis for women with the potential for either personal growth or negative outcomes(S.Swarna, 2006).

Worldwide extrapolated incidence of child birth is 14.86% (Dawn C.S, 2014). In India 18.5 birth / 1000 populations. In Tamil nadu the incidence rate of operative delivery is 85%, and normal delivery is 15% (Diana, 2015). Waldenstr, HM, U & Hildingsson (2014) revealed that 70% of the women had negative childbirth experience regarding nursing care. Mother wants the nurses to be calm, considerate, compassionate, concerned and friendly (Mackey & Lock, 1989).

One of the aims of ante natal services is to reduce worry, anxiety and alleviate fear of pregnant women related to child birth. This promotes, maintains and protects the physical as well as mental health of pregnant women.

METHODOLOGY

Design
Descriptive design with cross sectional survey approach.

Setting of the study
Jk hospital,Bhopal

Sampling technique
Purposive sampling technique.

Sample size
50 post natal mothers

Data collection procedure
The data were collected by using Rating scale, which had 14 items with 28 maximum scores.

FINDINGS AND DISCUSSION

Forty eight percentages of the mothers were between the age group 20 to 25 years, 68% were Hindus, 50% had one child and 38% each had primary education and higher secondary education. All most all (94%) the mothers were satisfied with nursing care to some extent whereas, mothers with satisfaction and not satisfaction were 4% and 2%, respectively. Most of the mothers (80%) were satisfied to some extent for the items "Cared like a family member". Most (72%) of the mothers were satisfied with the nursing care for the items," Intimated sex of the child". All most all the mothers expected that their mother should accompany along with them during the process of labour, and they need adequate information regarding the process of labour and they need adequate information regarding the process of labour and the condition of the baby.

CONCLUSION

Almost all the mothers were satisfied with the nursing care to some extent.

Recommendations

Same study can be conducted by using large sample to generalize the findings.

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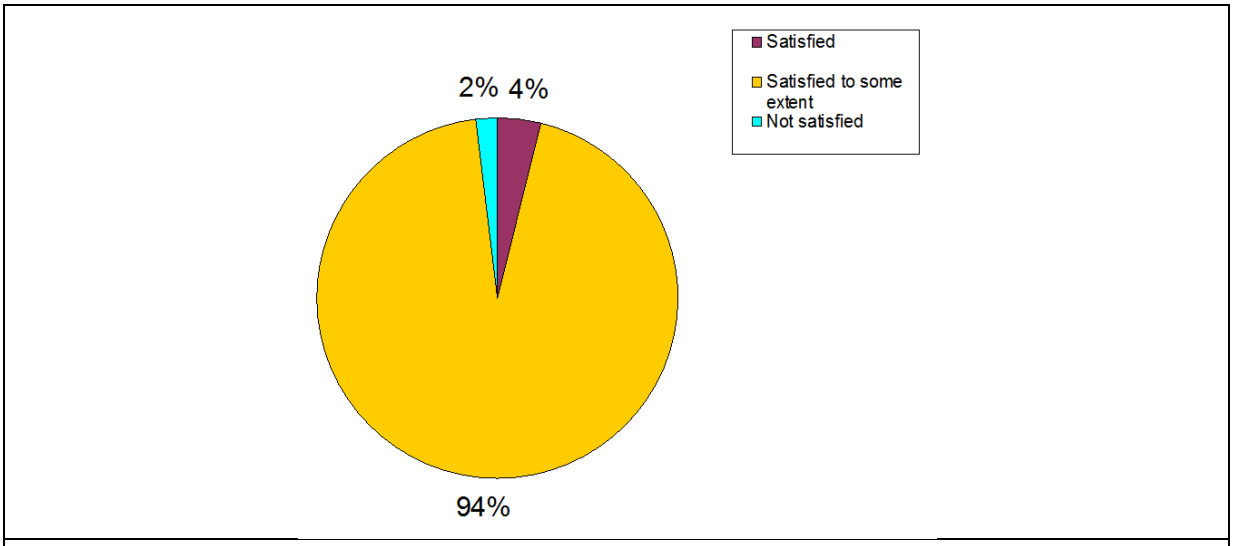

Figure 1: Pie diagram showing percentage wise distribution

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RESEARCH ARTICLE

Effectiveness of Structured Teaching Module on Knowledge of Adolescents Regarding Health Hazards on Use of Mobile Phones

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ABSTRACT

A quasi-experimental study with pre and post test without control group and quantitative approach was under taken from 27-12-2016 to 10-1-2017 data were collected from 60 adolescents by purposive sampling technique through structure interview schedule. The demographic characteristics reveal that highest (56.8%) of them were within the age bracket of 18-19 years. Highest (56%) of them were males and highest (77%) of them were from geographic region. Highest (48.3%) of them was within the income group of above Rs.15000 and highest (51%) of them were using smart phone, whereas (48%) of them were received information from the relations, friends. Knowledge score regarding peril of using portable shows that highest (64.9%) of them had adequate knowledge in post test. Area wise comparison of mean, SD and mean percentage of pre and post-test knowledge score shows that in post test the very best mean score (5.17± 0.63) which is 73.9% of total score was obtained the realm of "health hazards of portable use" and shows 49.5% effectiveness which was (24.4%) during pre test. However, the bottom mean score (4.07± 0.77) which is 67.8% was obtained within the area of "basic information of mobile phone" and therefore the effectiveness was 32.5%. Further the effectiveness various from 67.8% to 73.9%. Overall post test mean score was 22.3± 2.60 which is 74.3% of the overall mean score whereas during the pre test the mean score was 16.03±4.42 and also the effectiveness was 21.1%. Highly significant association was found between knowledge score in post test.

Keywords: Health Hazards, Knowledge, Mobile Phone, Adolescents, structure teaching module.

INTRODUCTION

Mobile phone could be a small, portable communication device that allows people to form phone calls whenever where they're. Signal transmission is that the very basic concept for mobile. The convenience of mobile is allowing people to speak with each other without the limitation of regions and time. Itinerant could be a device providing two-way communication. The technology influencing on portable started back within the mid twentieth century. The very first mobile telephony service was in Sweden. (Rithees.K, 2013). the full world is gripped by the mobile craze. Whether it's a student, housewife, shopkeeper, rickshaw driver, and milkman, professional, rich or poor, almost everyone carries a cellular phone in his/her hand. A itinerant could be a must have item for several a median teenager. many folks spend over six hours every day on their phones in talking, texting or playing games. The extensive use of cellular phone is making us addict of this small device. similar to every medicine has its side effects, cell phones even have some drawbacks. The increased usage of portable has increased the magnitude of potential health risks among its users.(Joseva.s,2011).

The adolescents and kids are more attracted for the mobile phones; they're more addicted and crazy for these mobile phones. The tissues of children's are tender and that they are likely to more affect by use of mobile phones. Children below 16 years should be discouraged from using mobile phones. The adolescents between 14-18 teens 96% of them have a minimum of one mobile and 22% of them own multiple mobile phones. all of them use mobile all the day a 3rd makes call over 6 minutes long, half is poorly informed about their potential health risks associated with electromagnetic pollution. They perceive its noxious but only 23% holds it aloof from the body, very small percent uses hand free kit.(Mukesh.L,2016). Tamilnadu Government has planned to ban the utilization (sale!) of mobile phones for kids up to the age of 16 years. the explanation for this ban is its adverse health effects. This comes almost 2 years after mobile phones were banned in Schools & Colleges (in Tamilnadu). The health minister said parents should discourage use of mobile phones by their under-age children reception also, which might cause cancer, brain disorders and adverse impact on system anervosum. a politician government notification on the ban are issued soon. The itinerant dealers are going to be informed to not sell handsets to anyone but 16 years. (Muthu Krishnan. V,2014).

The mobile telecommunication has the source of frequency radiation that produces energy; heat up the tissues .During use, mobile phones usually kept near the ear, which is incredibly close to the brain. it's suspected that continuous use of transportable for extended time may damage some brain tissues. Mobile phones are more injurious to people's health as compared to smoking. Mobile phones usage and brain cancer are linked to every other. Using mobile phones for over 10 years could double up one's risk of getting tumour. (LaxmiGowda.V, 2015). A study conducted in 1994, 16 million Americans subscribed to telephone services. Today, quite 110 million Americans are subscribers. Some experts predict that worldwide subscribership will reach 1.2 billion people by 2005. The incidence of brain cancer has increased 25% since 1973, consistent with the National Cancer Institute. Each year, 185,000 Americans are going to be diagnosed with a primary or metastatic neoplasm, per the National tumor Foundation.(Jackven.F,2014).

MATERIALS AND METHODS

Objectives

- To assesses the present knowledge of adolescents regarding health hazards on use of mobile phones.
- To evaluate the effect of structured teaching program imparted to adolescents regarding health hazards on use of mobile phones.
- To be told association between the amount of information among adolescents regarding health hazards on use of mobile phones with selected demographic variables.

Research design and approach : A quasi experimental design and quantitative approach was wont to evaluate the effectiveness of structured teaching module on knowledge of adolescents regarding health hazards on use of mobile phones in selected colleges, Salem.

Setting of the study: The study was conducted in Vinayaka Missions KirupananthaVariyar Engineering College which is 2 km removed from Vinayaka Mission's Annapoorana College of Nursing, Salem.

Population: The Population of the current study comprises 60 adolescents from selected college, Salem in Madras.

Sample size : Sample size of this study consists of 60 adolescents from selected college, Salem.

Sampling technique : Non probability purposive sampling technique was used to pick the samples of this study.

Inclusive criteria

- The adolescents who were
- between the cohort of 18-23 years.
- both male and feminine.
- willing to participate within the study.
- available during the info collection period.

Development of the tool

The subsequent tool was used for this study

- Structure Interview Schedule
- Structure Teaching Module regarding health hazards of using mobile

Description of the tool

The instrument used for this study was consists of two parts.

Section - A

The structured interview schedule was encompass demographic data like age, gender,, monthly income of family, sort of mobile, previous source of knowledge

Section - B

It consists of multiple choice knowledge item regarding health hazards of using portable. It includes basic information on movable, advantages, and downsides of portable, health hazards, and precaution

Validity

The content validity of the tool was established in consulting with experts from various fields like Community Health Nursing, Medical Surgical Nursing and Statistician. Their suggestion and opinion was incorporated in modification of the tool.

Reliability

Reliability of the tool was tested by implementing the structure interview schedule on 5 adolescents from Vinayaka Missions Annapoorana College of Nursing, Salem to check its feasibility. Test and retest method was wont to be told the reliability of the tool (r=0.8).

Data collection procedure

Ethical consideration

Before data collectionWritten permission was obtained from the principal Vinayaka Missions KirupananthaVariyar Engineering College Salem.Informed oral consent was obtained from the participants.

Planned data analysis

The collected data was organized, tabulated and analyzed by using descriptive statistician i.e percentage, mean, and variance, and inferential statistics like chi square test, and't' test. the information was presented within the type of tables and figures

RESULT AND DISCUSSION

Demographic characteristics of the adolescents

- Highest (56.8%) percentages of them were within the people of 18-19 years and 23.4% of them were within the people of 20-21 years.
- Highest (56%) percentages of them were males and 44% of them were females.
- Highest (77%) percentages of them were from populated area and 33% of them were from country.
- Highest (48.3%) percentage of them was within the income group of above Rs.15000 and also the lowest percentages (18.3%) of them were within the income group of below Rs.1000.
- Highest (51%) percentage of them were using smart phone and 38.8% of them were using basic movable with internet facilities. Further 10.2 % of them were using basic itinerant.
- Highest (48%) percentage of the adolescents were received information from the relations, friends and relatives and therefore the lowest percentage (18%) of them were received information from caregiver.

Paired 't' value of pre and post test knowledge score of adolescents regarding health hazards on use of mobile phones.

Paired 't' test was calculated to research the difference in pre and post test knowledge score on different aspects of health hazards of using mobile

Basic information of thetransportable, Advantages of the transportable, Disadvantages of the movable, Health hazards of transportable, Precautions for hazards of mobile phone shows that highly significant difference between the world wise score value of pre and post-test. Hence the stated null hypothesis rejected and statistical hypothesis was accepted ($P < 0.01$). Thus the difference observed in mean score value of pre and post-test where true difference

Association between the post-test knowledge score and demographic variables of adolescents.

Chi-square was calculated to seek out the association between the post test knowledge score and therefore the demographic variables of adolescents. There was highly significant association between knowledge score of adolescents in post test in comparison with age in year, Gender, area of residence, family monthly income, variety of transportable used, previous source of data. Hence H2 was accepted.

DISCUSSION

Area wise comparison of mean, SD and mean percentage of pre and post-test knowledge score adolescents regarding health hazards of using mobile shows that in post test the very best mean score (5.17± 0.63) which is 73.9% of total score was obtained the world of "health hazards of mobile use" and shows 49.5% effectiveness which was (24.4%) during pre test. However, all-time low mean score (4.07± 0.77) which is 67.8% was obtained within the area of "basic information of mobile phone" and therefore the effectiveness was 32.5%. Further the effectiveness various from 67.8% to 73.9%the general posttest mean score was 22.3± 2.60 which is 74.3% of the entire mean score whereas during

the pre test the mean score was 16.03±4.42 and therefore the effectiveness was 21.1%. It reveals that STM was effective on various areas of health hazards of mobileuse

ACKNOWELDEMENT

It gives us immense pleasure to thank our Principal **Dr. (Mrs) V. Selvanayaki, Ph.D (N),** Vinayaka Mission's Annapoorana College of Nursing, Salem for giving us permission to conduct this study with her guidance and valuable suggestion which is an vital support for this study.

I extend my deep sense of thanks to our guide **Dr.S.MalathiPh.D (N)** HOD**,** , Department of Community Health Nursing, Vinayaka Mission's Annapoorana College of Nursing, Salem for her source of inspiration, guidance, personal interest, encouragement and patience for making us to complete our study successfully.

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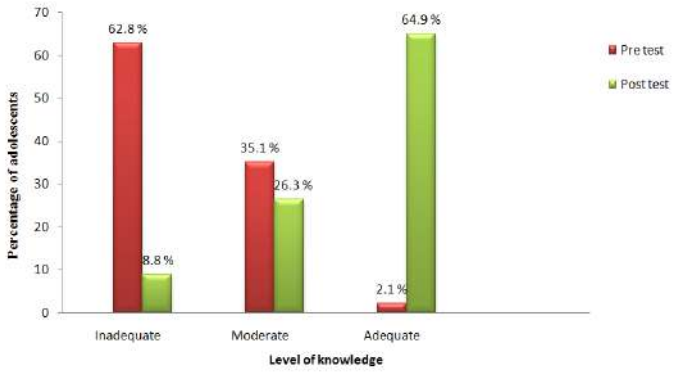

Fig.1. Assessment of the level of pre and post-test knowledge of adolescents regarding health hazards of using mobile phone.

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RESEARCH ARTICLE

Probing the Selective Flavonoids in *Tridax procumbens* L for Allergic Diseases by *In-silico* Docking Studies

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ABSTRACT

Allergens have the ability to causes allergic response while entering into the body. This allergic response leads to severe allergic disorders like asthma, allergic rhinitis, atopic dermatitis etc, by immune system's hypersensitivity. The primary immune cell lineages, receptors and chemical mediators like histamine receptor, IgE receptors, IL-5, IL-4, STAT-6 are the principle player in the allergic response pathway. In the present study aimed to investigate the anti- allergic activity of ethanolic extract *Tridax procumbens* L by *in-silico* docking studies. The flavonoids are Kaempferol, Catachin, Quercertin, Ellagic acid, Luteolin and Mycertin were selected and docked with molecular protein of selected receptors which leads to allergic reaction pathway by using autodock vina 2.0 software. Ligand and the target molecules were retrieved from RCSB PDB and pubchem respectively. The current work to understand and predict that *Tridax procumbens* L extract used in the treatment of allergic condition by *in-silico* docking studies. The molecular docking studies divulge or discuss that these phytochemical compounds have good binding energy and inhibition constant on allergic mediators. Among 30 docking studies 5ligands with 2 protein having good binding energy and best fit confirmation in the binding site of respective protein. Overall result outcomes that ethanolic extract of *Tridax procumbens* L arbitrate its anti-allergic activity on multi targeting allergic mediators. This molecular docking analysis may leads to the future development of novel drugs.

Keywords: Allergic response, *In-silico* docking, Kaempferol, Catchin, Quercertin, Ellagic Acid, Luteolin, and *Tridax procumbens L.*

INTRODUCTION

Allergies, often known as allergic illnesses, are a group of diseases caused by the immune system's hypersensitivity to normally harmless environmental contaminants [6]. These diseases include hay fever, food allergies, atopic dermatitis, allergic asthma, and anaphylaxis [2]. Red eyes, an itchy rash, sneezing, a runny nose, shortness of breath, and swelling are all possible symptoms in allergic condition [1]. Food in tolerances and food poisoning are separate conditions [4, 5]. The allergen can bind to the IgE molecules on the surface of mast cells or basophils if they have been subjected to the allergen over subsequently. When more than one IgE-receptor complex interfaces with the same allergenic molecule and activates the hypersensitive cell, cross-linking of the IgE and Fc receptors occurs [4,6]. Degranulation is a process in which activated mast cells and basophils release histamine and other inflammatory chemical mediators (cytokines, interleukins, leukotrienes, and prostaglandins) from their granules into the surrounding tissue, tends to result in vasodilation, mucous secretion, nerve stimulation, and smooth muscle contraction. This results in rhinorrhea, itchiness, dyspnea, and anaphylaxis leads to allergic disorders. Several drugs are used in the treatment of allergic diseases such as antihistamines, glucocorticoids, epinephrine (adrenaline), mast cell stabilisers, and anti-leukotriene drugs [7]. Anti-cholinergics, decongestants, and other drugs that are considered to inhibit eosinophil chemo taxis, anaphylaxis, asthma, and other allergic disorders. Our aim to target this chemical mediators to inhibitory activity by Ethanolic Extract of *Tridax procumbens* L [7].

Plant Profile

Tridax procumbens L, popularly called "coat buttons," could also be a perennial plant native to Central and South America that belongs to the Asteraceae family [8,9]. Since times of yore, this species has been employed in Ayurveda in India [10]. Different substances like oils, teas and skin poultices, among others, are manufactured using this species [11] .T. procumbens has diverse pharmacological properties including but not limited to immunomodulatory, anti-oxidant, anti-hepatotoxic, analgesic, anti-diabetic, anti-inflammatory, antifungal, and antimicrobial action. [9,12-14]The plant's defensive mechanisms, secondary metabolites like flavonoids, alkaloids, tannins, carotenoids, and saponins, are possibly responsible for the species diversity. On review of the plant *Tridax procumbens* L containing more quantity of flavonoids and its known flavonoids having anti-allergic property by natural. The aim of this research work is to ellucidate the new anti-allergic drug from a natural source to treat allergic diseases like asthma anaphylaxis and some inflammatory diseases.

MATERIALS AND METHODS

Collection and Authentication

The aerial parts of the *T. procumbens* L plant were collected from in and around of MTPG&RIHS college campus and its identity was confirmed by Mr.Iyyappan, Department of Botany, French Research Institute, Puducherry. After authentication the aerial parts of the plant was washed and shade dried for 2 weeks and its dried, transformed into a coarse powder by using an electric mixer [11,12].

Preparation of Plant Ethanolic Extract

The coarse powder (100g) of *Tridax procumbens* L were extracted with 500 ml of ethanol (95%) for 24 hour at 64°C by Soxhlet apparatus and it is filtered using Whatmann filter paper no.41(110 mm) to get the filtrate. The resultant solution were dried using vacuum evaporator to get concentrate and its stored in 4^0C for later usage [13].

Phytochemical Screening of the Ethanolic Extract of *Tridax procumbens* L (EETP)

Standard test procedures were accustomed conduct a preliminary phytochemical screening of Ethanolic Extract of *Tridax procumbens* L and quantitative test for the presence of phenols, tannins, flavonoids, alkaloids, terpenoids, steroids, and saponins[16]. These phytochemicals were identified by characteristics colour change using standard procedures [17].

Test for Phenol: The formation of intense colour on the addition of 0.5 ml of fecl3 (w/v) solution into 2 ml of test solution indicated the presence of phenol [22].

NaOH Test for Flavonoids: 2-3 ml of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of the intense yellow colour that didn't become colourless on the addition of a few drops of dilute HCl indicated the absence of flavonoids [23].

Shinoda Test for Flavonoids: 2-3 ml of extract and few fragments of magnesium metal were added into a test tube, followed by drop wise addition of concentrated HCl. Formation of magenta colour indicated the presence of flavonoids [23].

Lead Acetate Test for Tannins: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicated the presence of tannins [24].

Foam Test for Saponin: The extract was diluted into 20 ml of distilled water and shaken in a graduated cylinder for 15 min. A 1cm layer of foam indicated the presence of saponins [23].

Libermann-Burchard's Test for Steroid: Acetic anhydride (2 ml) was added to 0.5 g of ethanolic extract of each sample with 2 ml sulphuric acid. A colour change from violet to blue or green in some samples was an indication of the presence of steroids [24]

Molecular Docking Studies

Docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex. It is very useful and important method to the investigation of compound structure potentially without giving more exertion and investment in research work. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes [18,19].

Bioinformatics tools used in docking studies

RCSB PDB Database: RCSB PDB also provides tools for understanding collections of PDB structures, which in turn enables exploration of proteins from different organisms illuminating evolution at atomic and molecular levels[20].the data bank gives the structural information of the small molecule. From this database the protein were downloaded in pdb file format.

Pubchem Database: Pubchem is a public repository for biological activities of small molecules [21]. The ligand structure and identity number can be noted and the 3D conformer file was downloaded in 3D sdf file format.

Autodock Vina 2.0: Molecular docking technique has two molecules that gives a virtually screen on a database of a compounds and help to predict the strongest binders based on their docking score. ADT 1.5.6 software [21] is used by us to investigate the activity in terms of binding affinity (Kcal/mol) and inhibition constant (µmole).

Docking Process

Ligand Selection and Preparation: From the literature [15] review it reveals that *Tridax procumbens* L plant had 23 flavonoids. From this 5 flavonoids was selected based on the high quantity such as Kaempferol (17.593%), Catachin (7.875%), Quercertin (4.418%), Ellagic Acid (1.81%), Luteolin (1.707%) and Mycertin (1.40%).Retrieval of ligand from drug bank database called pubchem here the structure of the ligand namely Kaempferol, Catachin,

Quercertin, Ellagic Acid, Luteolinand Mycertin were downloaded in 3D sdf format and it was converted into PDB file format with the help of online smiles translator (NCI/ CADD-NIH) database.

Protein Preparation

The selected target proteins are H_1receptor code: (1jqe), fc receptor (4j6p), IL-4 (2b8u), IL-5(106z), stat-6(10j5) were downloaded as pdb file format by using RCSB- protein data bank. These protein were selected accordingly with X-crystallographic method having resolution less than 2 A^o with *E.coli* as an expression system for docking. From this the heteroatom were removed and the active sites were predicted with the help of pdb sum. In pdb sum database the proteins active sites were in liplot and their respective amino acids were noted.

Preparing pdbqt Format for Ligand and Target

By using autodock 4.2 version software, the 2 pdbqt files, gpf and dpf files of each protein are prepared using autodock tool software downloaded from MGL tools [14].

Grid Parameter Procedure

Grid parameters were generated by using altering the dimension of X,Y, and Z to 60. In genetics algorithms, the number of runs was made to 25 to get the desired docking conformation. After running the autogrid and autodock, the analysis procedure were carried out to obtain the docking score, inhibition constant and ligand efficiency valve based on their interaction between the protein and ligand molecule with help of conformation procedure and root mean square deviation (RMSD) table in dlg file [10-13].

RESULT

The phytochemical screening for chemicals constituents which in EETP by using standard protocols, and this extracts sample results in presence of phenols, tannins, flavonoids, phenol, steroids, and saponins which was shown in Table.1.

Pharmacological Screening using *In-silico* Docking Method

Higher negative binding score and greater inhibition constant represents the good and best binding property with the target and the ligand molecule. In the present study, 30 docking studies were carried out. The docking result of 6 ligand namely as lig.1, lig.2, lig.3, lig.4, lig.5 and lig.6 with respectively Kaempferol, Catachin, Quercertin, Ellagic acid, Luteolin and Mycertin with 5 protein, they were H1receptor, fc receptor, IL-4, IL-5, stat-6 were given in the Table-2-6. respectively. The docked image of protein and ligand molecule Figure: 1-5 were shown with hydrogen bond having bond distance, by this information we can confers rigidity of ligand to the protein structures and specificity to the intermolecular interaction. The chimera images of protein-ligand complex were shown below in Figure: 6-10, for highly extensible interactive analysis of proteins and ligands with their hydrogen bonds.

DISCUSSION

From the tables 2, 3, 4, 5, 6, the docking study exhibits good binding energy and inhibition constant, when 5 proteins docked with 6 ligand. Among 30 docking studies the macromolecule STAT-6 and IL-4 docked with this 6 ligands havingbest binding energy and inhibition constant whereas other macromolecules like histamine receptor, fc receptor and IL-6 results in highest binding energy ranges from 9-7 kcal/mole, but it has less inhibition constant ranges between 0-3 µmole. It denotes that more inhibition constant indicates the potent of the ligand when docked with protein, inhibition constant is the concentration required to produce half maximum inhibition. Hence, the *in-silico* analysis exhibits that ligand 5, 2 and 1 docked with STAT-6 and IL-4 showed good binding energy of -6.69 kcal/mole, -6.02 kcal/mole, -5.94 kcal/mole, -6.22 kcal/mole, -6.21 kcal/mole and inhibition constant of 12.56 µm, 38.92 µm, 44.15 µm, 27.41µm and 27.87µm respectively.

Ligand 5 (Ellagic acid) ligand 2 (Quercertin) and ligand 1 (Kaempferol) docked with IL-4: When IL-4 macromolecule docked with lig.5, 2, 1, the binding energy was found to be-6.69 kcal/mole, -6.02 kcal/mole, -5.94 kcal/mole respectively and the inhibition constant of 12.56 µm, 38.92 µm, 44.15 µm respectively. IL-4 is the pro-inflammatory mediator during allergic reaction. IL-4 has pivotal role in the development of immediate allergic reaction. It also contributes to airway obstruction in asthma by through the induction of mucin gene expression. IL-4 also promotes eosinophilic inflammation and increases the expression of eotaxin and other inflammatory cytokines from fibroblasts that may contributes to chronic diseases.

Ligand 3(mycertin) and ligand 1(kaempferol) docked with STAT-6: STAT-6 protein results in good binding energy of -6.22 kcal/mole, -6.21 kcal/mole and inhibition constant of27.41µm, 27.87µm respectively.STAT-6 is the member of STAT family. It is inhibitor protein and activated by IL-13 and IL-4. STAT-6 plays a vital role in intestinal inflammation and pathologic manifestation of asthma, anaphylaxis and atopic dermatitis includes pruritus, scratching and chronic eclapsing lesion.

CONCLUSION

Therefore, the multi-targetting of allergic mediators by molecular docking analysis arbitrate the anti- allergic activity of EETP. Hence our research work can further to do the *in-vitro* and *in-vivo* studies to establish the anti-allergic activity of EETP.

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Table 1: Preliminary Phytochemical Screening of EETP

S. No.	Test for	Name of the Test	Result
1.	Phenols	Phenol Test	+
2.	Flavonoids	NaOH Test	+
		Shinoda Test	+
3.	Tannins	Lead acetate Test	+
4.	Saponins	Foam Test	+
5.	Steroids	Libermann Burchard's Test	+

(**+→** indicates the presence of the compound)

Table 2: Docking result of H1 receptor docked with the 6 ligands

(Lig.1-Kaempferol Lig.2- Quercetin Lig.3-Myricetin Lig.4- Luetolin Lig.5-ellagic acid and Lig.6- Catechin)

S.No	Ligand	Protein Name	Binding Energy K Cal/Mol	Inhibition Constant (µm)	No. of Hydrogen
1.	Lig.1	H 1 receptor (1UQE)	-8.54	0.5551	1jqe:A:TRP115:HN 1jqe:B:TYR215:HH
2.	Lig.2		-9.27	0.1608	1jqe:A:LYS99:HZ2 1jqe:A:TRP115:HN
3.	Lig.3		-9.47	0.1140	1jqe:A:LYS99:HZ2 1jqe:A:TRP115:HN 1jqe:B:TYR215:HH
4.	Lig.4		-8.77	0.3744	1jqe:A:LYS99:HZ 1jqe:B:SER176:HG
5.	Lig.5		-9.19	0.1833	1jqe:A:PHE113:HN
6.	Lig.6		-8.46	0.6332	1jqe:A:LYS99:HZ2 1jqe:B:GLY175:HN 1jqe:B:SER176:HG

Table 3: Docking Result of IgE receptor docked with the 6 ligands

S.no	Ligand	Protein name	Binding energy K cal/mol	Inhibition constant (µm)	No. of Hydrogen
1.	Lig.1	IgE receptor (FceRI) (4J6P)	-8.43	0.667	4j6p:A:TYR189:HH 4j6p:C:ARG224:HH11 4j6p:C:ARG224:HH21 4j6p:A:ARG224:HH11
2.	Lig.2		-8.56	0.531	4j6p:A:TYR189:HH 4j6p:C:ARG224:HH21 4j6p:D:ARG224:HH11
3.	Lig.3		-8.96	0.271	4j6p:D:ARG188:HE 4j6p:D:ARG188:HH21
4.	Lig.4		-8.50	0.587	4j6p:D:ARG188:HE 4j6p:D:ARG188:HH21
5.	Lig.5		-8.83	0.334	4j6p:C:ARG188:HH21 4j6p:D:ARG188:HH21
6.	Lig.6		-9.06	0.229	4j6p:A:ARG188:HH11 4j6p:D:ARG224:HH11

Table 4: Docking result of IL-4 docked with the 6 ligands

S.no	Ligand	Protein name	Binding energy K cal/mol	Inhibition constant (µm)	No. of Hydrogen
1.	Lig.1	Interleukin [IL-4] (2B8U)	-5.94	44.15	2b8u:A:ARG85:HE
2.	Lig.2		-6.02	38.92	2b8u:A:ARG85:HE
3.	Lig.3		-6.33	23.03	2b8u:A:ARG53:HE 2b8u:A:ARG85:HE
4.	Lig.4		-6.00	39.66	2b8u:A:ARG85:HE
5.	Lig.5		-6.69	12.56	2b8u:A:ARG85:HE 2b8u:A:ARG85:HH22 2b8u:A:ARG88:HE 2b8u:A:ARG88:HH21
6.	Lig.6		-6.71	12.09	2b8u:A:ARG85:HH22

Table 5: Docking result of IL-5 docked with the 6 ligands

S.no	Ligand	Protein name	Binding energy K cal/mol	Inhibition constant (µm)	No. of Hydrogen
1.	Lig.1	Interleukin IL-5 (10BZ)	-8.04	1.28	1obz:A:ARG193:HE 1obz:A:ARG193:HH21 1obz:A:ARG197:HH21 1obz:B:PHE195:HN
2.	Lig.2		-8.02	1.31	1obz:A:ARG193:HE 1obz:A:ARG197:HH21 1obz:B:PHE195:HN 1obz:B:ARG197:HH12
3.	Lig.3		-8.07	1.21	1obz:A:ARG193:HE 1obz:A:ARG197:HH21 1obz:B:PHE195:HN 1obz:B:ARG197:HH12

4.	Lig.4		-8.10	1.16	1obz:A:ARG193:HE 1obz:B:PHE195:HN 1obz:B:GLU196:HE22 1obz:B:ARG197:HH12
5.	Lig.5		-7.51	3.1	1obz:B:GLN139:HE22
6.	Lig.6		-8.07	1.22	1obz:B:GLN139:HE22

Table 6: Docking result of STAT-6 docked with the 6 ligands

S.No	Ligand	Protein name	Binding energy K cal/mol	Inhibition constant (µm)	No. of hydrogen
1.	Lig.1	STAT-6 receptor (1JQE)	-6.22	27.41	10j5:B:LEU795:O 10j5:B:GLY270:O
2.	Lig.2		-6.21	27.87	10j5:A:GLN266:HE21 10j5:B:LEU795:HN2
3.	Lig.3		-6.59	14.76	10j5:A:GLN266:HE21 10j5:B:LEU795:HN2
4.	Lig.4		-6.92	8.52	10j5:B:LEU795:O
5.	Lig.5		-6.51	17.17	10j5:A:ARG293:HH11
6.	Lig.6		-6.51	16.92	10j5:A:ARG293:HH11 10j5:GLU99:HN

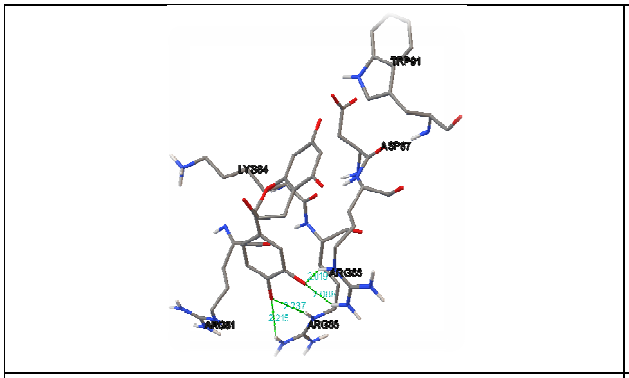

Fig 1: Ellagicacid(lig.1) docked with IL-4

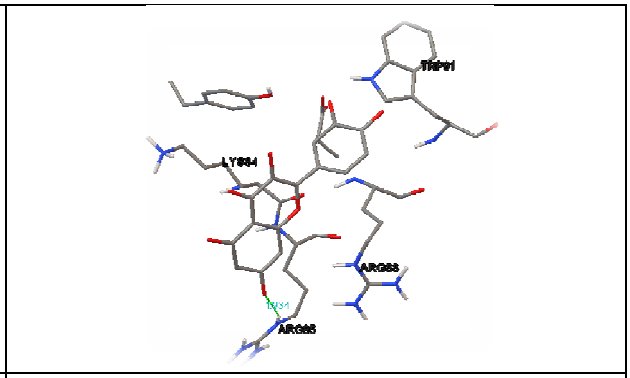

Fig 2: Qucertin (lig:2) docked with IL-4

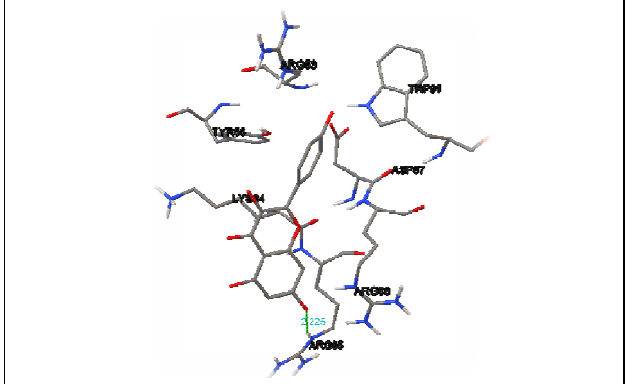

Fig 3: kaemperol(lig.1) with IL-5

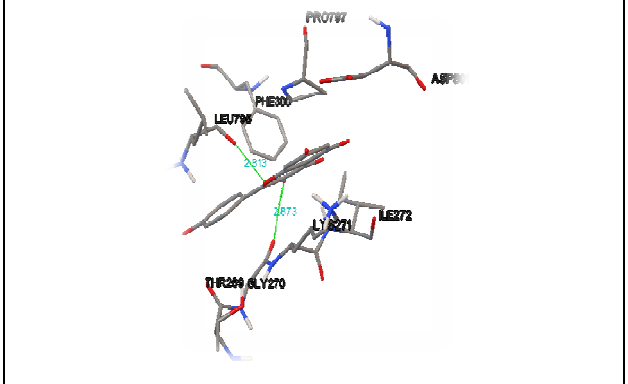

Fig 4: Kaempferol (lig.1) with STAT-6

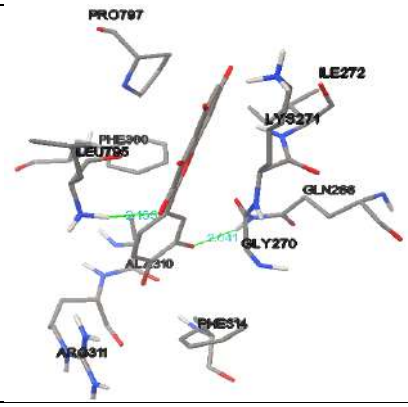

Fig 5: Qucertin (lig.2) with STAT-6

Docking image of proteins with their respective ligands

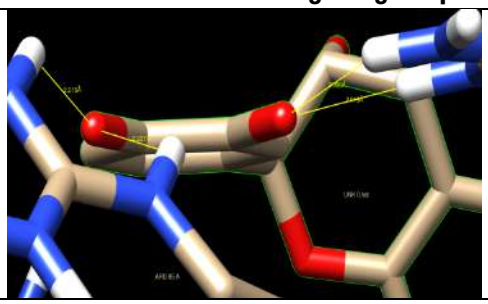

Fig 6: Chimera image of ellagicacid(lig.1) docked with IL-4

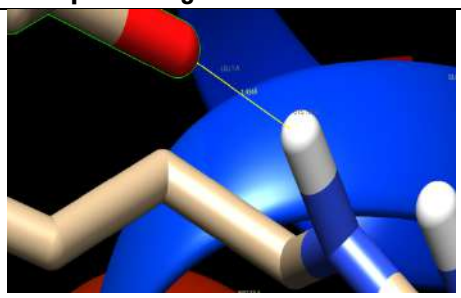

Fig 7: Chimer image of qucertin(lig.2) docked with IL-4

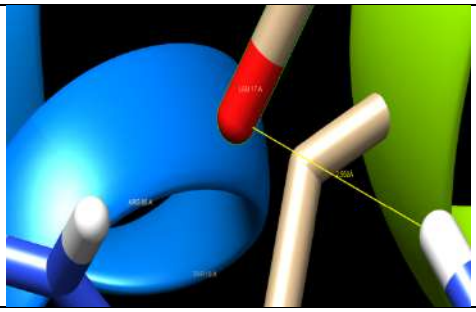

Fig 8: Chimer image of kaempferol with IL-4

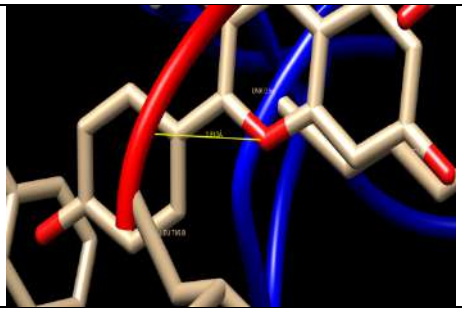

Fig 9: chimera image of kaempferol (lig 1)with STAT-6

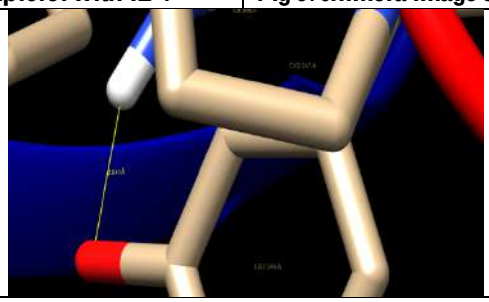

Fig 10: chimera image of Qucertin with STAT-6

Chimera image of the docked ligands with their proteins

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RESEARCH ARTICLE

Smart Multi- Channel Seed Counter for Six Row Peanut and Chickpea Planter

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ABSTRACT

The smart seed counters were developed for multicrop planter. The laboratory experiments indicated that the electronic seed counters developed for peanut (R-8808, GPDB-4 and KRG-1) and chickpea (Annigeri-1 and KGB-1 varieties) with accuracy of 97.7 and 99.3 percent with reference to actual (hand counted). Seeds sensing, the seed flow in seed tube by which the operator could observe functioning of the planter. A rotor speed of 0.212 ms^{-1} and forward speed of 3.5 kmh^{-1} and the cell area of each cell on rotor of 172.30mm^2 (R-8808), 139.32mm^2 (GPBD-4), and 124.37 mm^2 (KRG-1) for peanut verities and 99.88 mm^2 (Annigeri-1) 126.65 mm^2 (KGB-1) for chickpea verities were found to be optimum. Based on these optimised values, electronic counters for tractor drawn six row multi crop inclined plate planter was developed. The field performance of seed counters among six rows recorded an accuracy in the range of 97.5 to 98.8% for Peanut crop and 94.63 % to 96.69% for chickpea.

Keywords: electronic seed counters, peanut, chickpea

INTRODUCTION

India ranks second in peanut production after china and Karnataka ranks third in area and fifth in production in India with an average productivity of 713.1 kg per hectare (anon., 2005) [1]. The productivity not yet reached the potential, which ascribed to low plant population caused by improper plant spacing. Chickpea occupies a unique position in the world agriculture due to its high protein content. It is reported that the lower yield of crop is due to low plant stand owing to improper placement of seed. The cost of seed contributes 40 percent of total production cost. Electronic metering unit was developed at Indian Institute of Technology Kaharagpur for Inclined plate planter and tested laboratory the optimum condition was reported as at metering speed of 50 rpm [2] An electronic seed

metering mechanism was developed for Cowpea seeds tested in the institute found that a seed spacing of 16.2 cm was recorded against a standard of 15 cm [3] An inclined plate planter for multi crop planter with electronic seed counter was designed and developed at VTU, Research center Raichur. The power for metering mechanism was taken from ground wheel and the seed discharged from planter seed box was recorded the results indicated that the electronic counter had accuracy of 98% [4]A sensor for flow of seeds in seed tube was developed at IIT Kharagpur for Maize, Wheat and Mustard seeds. In laboratory testing, the sensing of seeds was found to be satisfactory [5]. The study conducted IIT Kharagpur reported that ground wheel which powers the seed metering mechanism faced problems like slippage of wheel due to sticking of wet soil to lugs of ground wheel in field testing [6]. The precision seed metering mechanism leads to recommended seed space which directly affects the saving of seeds and to get improved environmental condition for better seed germination [7]. According to statistics, the output of precision seeding increases by 10 per cent - 30 per cent compared with that of the conventional drill [8] The use of seed drill/planter with electronic counter for counting seeds is uncommon in India so far, the planting system adopt bulk or mass seeding kg ha^{-1} to determine the plant stand, which is not a precise method for achieving require plant population at optimum spacing, the counting o seeds failing per unit area is very much essential. Hence, this requires the incorporation of precise seed counting device in multi crop inclined plate planter.

MATERIALS AND METHODS

Electronic Seed Counter

An electronic seed counter was developed based on the principle of opto-electronics. The number of seeds dropped from the metering mechanism were sensed and counted by the sensor mounted in the seed tube (Reheman and singh 2003) [4]. The electronic seed counter consist of a power source , light emitting diode (LED), sensor (photo transistor), amplifer NAND gate and counter cum multiplexer cum driver. The specification of components of electronic seed counter is presented in table 3.4. The data seed of components used in electronic seed counter circuit is presented in Appendix 1. A 220V, 5A A.C. is converted to 5V, 100mA D.C. using an AC adaptor and connected to LED and counter circuit

A block diagram of regulated power supply and block diagram of different components of electronic seed counter as shown in Figure 1. The green light emitting diode (GB-333GD), which as visible radiant energy with peak wave length of 564ns was used in circuit. A photo transistor of 2N5777 is mounted exactly opposite to LED in seed tube of inclined plate metering mechanism and it forms an opto-couplar with a LED. The fine beam of light from LED is focused on a photo transistor (2N5777).When seed passed through seed tube, the light falling on phototransistor is interrupted and generates as pulse. The generated pulse is applied to the counter through digital switch i.e. NAND gate, which act as a clock pulse.

The circuit diagram of a electronic seed counter is shown in Figure 2 and Figure 3. The output of the phototransistor was fed to a high speed switching transistor (BC109C) which acts as an amplifier. The clock pulse generated in NAND gate is proportional to number seed which obstructs the light falling on phototransistor. The IC CD4093BC is selected as a NAND gate. It consists of four Schmitt trigger circuit, out of which only one gate has been used for making connection to the counter and used in inversion mode. The signal generated by phototransistor is not sufficient to drive the NAND gate and hence a performs as a single stage amplifier. The signals produced in photo transistor due to obstruction of light are amplified and feed to clock input of clock input of counter through NAND gate. The signal conditioning circuit consist of resistors R_1 to R_7 each of 330 ohms, R_8 to R_{14} 1K ohms , R_{15} of 100K ohms, R_{16} of 220K ohms and R_{17} of 22K ohms were used to minimize the current drawn by each segment which increases the life of the counter. The output of the decoders was connected to the respective segments of common anode of LED display through resistors. The circuit is assembled on a PCB (printed circuit board). The IC_2 (CD 4093) is used for inversion of voltage with buffer and it provides isolation between sensor and counter circuit.

The output of CD 4093 was fed to a counter come multiplexer cum driver (MM74C925) for counting and displaying the counts digitally. The display unit consist of seven segment displays a seven rectangular LED units (1, 2, 3,4,5,6 and 7).Each LED is the part of the character being displayed the rest switch S_1 is used for resetting or clearing the previous count and sensor is ready for counting the seeds. A multi channel electronic seed counter is using such six units embedded in single PCB so that it can be used to count the seeds for six rows of prototype multi-crop inclined plate planter.

CALIBRATION OF ELECTRONIC SEED COUNTER

The performance of electronic seed counter was evaluated for counting of three varieties of pea nut seeds viz., R-8808, GPDB-4 and KRG-1 and two varieties of chick pea seeds viz., Annigeri-1 and KGB-1. Discharged from metering mechanism using the test rig under laboratory condition with manual counting of seeds as controlled. The electronic seed counter was calibrated using the procedure suggested by malvino, 1990(67). The seed rotary designed for a particular variety of test crop (pea nut/chick pea) was fitted in the seed reservoir. The seed samples of three Kg were graded and filled in the hopper. Seed counter was set for zero reading using reset switch. The seed metering mechanism was driven by an electronic motor through variable speed gare box to obtain the required rotor speed (20, 30, 40 and 50). The calibration of electronic seed counter was carried out at stabilized speed. The rotor was operated at selected speed for 3000 cells at each trial and results were reported for 15 cycles dividing the data by 10. The seed rotor was operated at selected speed for 15 cycles and the number of seeds dropped from metering mechanism was counted by the electronic seed counter. The counter was reset to zero. The observations were recorded for different speeds. The actual number of seeds dropped from metering mechanism was counted manually. The trails were replicated six times and the data was analyzed using T-test. The correlation between electronic seed counting with actual seed drop from the metering mechanism was developed.

WORKING OF INCLINED PLATE METERING MECHANISM WITH ELECTRONIC COUNTER

The seed metering mechanism is driven by a single phase 0.735 kilo watt, 1440 rpm electric motor through variable speed gear box for testing of unit under laboratory condition, which imparts rotary motion to feed shaft. The feed shaft drives the seed rotor of the inclined plate metering device through a drive plate assembly. The seed rotor has cells around its periphery that moves through a seed reservoir, where the seeds are picked up by the cells and dropped in a delivery chute through a opening provided in the square plate (discharge point). As the seed passes from the delivery chute to the furrow opener through seed tube, it will be counted with a help of electronic seed counter mounted in the seed tube.

RESULTS AND DISCUSSION

An electronic seed counter was developed to count the seeds dropped from metering mechanism precisely and the details of component layout with a circuit diagram, construction and calibration procedure are explained above. The electronic seed counter was calibrated under laboratory condition for counting the number of seeds (pea nut and chik pea). Delivered from the metering mechanism at different rotar speeds. The performance of electronic seed counter for counting seeds of test crop is presented in Table.2. The mean number of seeds dropped (electronic count) from the metering mechanism at different rotar speeds for selected pea nut varieties varied from 294.70 to 297.01, 293.43 to 296.19, 290.36 to 295.21 for R-8808, GPDB-4 and KRG-1, varieties respectively, were as chik pea varieties the readings were in the range of 289.59 to 293.43 and 287.88 to 290.37 for Annigeri-1 and KGP-1 respectively. Theoretical number of seeds to be dropped from the seed rotor in one cycle (15 rotations of seed rotor with 20 cells.) are 300, however the variation was absorbed in the actual number of seeds counted by the counter and manual labour.

The comparative performance of electronic seed counter and manual counting for peanut and chickpea selected varieties shown in Figure 4 . The reduction in number of seeds counted by electronic counter is mainly due to the seeds passing through the counter in quick succession (2 - 3 seeds). While the counter has sensed one seed instead of more than one. The actual number of seeds dropped was also lesser than theoretical number(300), particularly at

higher rotor speeds. The percent cell fill of seed rotating metering mechanism for peanut and chickpea were found to be 99.36 and 98.42% respectively. The difference in the percent cell fill for the two crops may be due to the difference in the shape and size of the seed. This may be due to the less time available for the seed to fill in the cell area at higher speed. This is in agreement with a findings of Wangura and Hudspeth [9].

The analysis showed non-significant effect on the variance of seed counted by the electronic seed counter at the different rotor speeds (Table3). All the calculated values from "t" test were found lesser than the table values and that shows that there was no significant relation between rotor speed and electronic mean seed count. The percent of manually counted peanut and chickpea seeds that were counted by electronic seed counter was very high ranging from 98.32 to 98.70 percent. The more precise of counting of seeds was obtained due to the stopper provided inside the delivery chute of the metering mechanism which allowed the individual seed to pass through the counter. The developed electronic seed counter was found to be more accurate compared to developed by [5] with an accuracy of 82 to 90 percent for muster , wheat and maize.). The variation in the number of seeds counted by the counter and the actual number of seeds dropped was not significant. The calibration results are found to be superior than the results obtained from obtained from Rehman and Singh.[5] For all the rotor speeds at a particular cycle. The number of cumulative seeds counted and dropped was also found to be non-significant irrespective of rotor speed. The field performance of seed counters among six rows recorded an accuracy in the range of 97.5 to 98.8% for Peanut crop and 94.63 % to 96.69% for chickpea.

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Table 1: Components of Electronic Seed Counter

S no	Components	Model no	Qty	Specification
1	L1	GB-333GD	1	Green LED
2	S1	2N5777	1	Fast Response photo transistor
3	Q5	BC109C	1	Silicon planar epitaxial transistor
4	IC2	CD4093BC	1	Quad 2 – input NAND sehmitt trigger
5	IC3	MM74C925	1	Counter cum multiplexer cum driver
6	Q1-Q4	BC547B	4	Small signal transistors (NpN)
7	D1-D4	LTS543	4	Common anode 7 – segment display
1	R1-R7	330 ohms	7	10 %, watt carbon film resistor
2	R8-R14	1 K ohms	7	10 %, watt carbon film resistor
3	R15	100 K ohms	1	10 %, watt carbon film resistor
4	R16	220 K ohms	1	10 %, watt carbon film resistor
5	R17	22 k Ohms	1	10 %, watt carbon film resistor

Table 2: Performance of electronic seed counter for counting seeds of test crop

Crop/ variety	Rotar speed rpm	Mean number of seeds (electronic count)	Mean number of seeds (manual count)	Percent of manually counted seeds counted electronically, %	Percent fill of cells, %
peanut					
R-8808	20	296.51	298.50	99.33	99.50
	30	297.01	299.40	99.2	99.80
	40	296.98	299.25	99.24	99.75
	50	294.70	297.48	99.06	99.16
GPDB-4	20	296.19	298.89	99.09	99.63
	30	293.43	297.30	98.69	99.10
	40	293.72	298.21	98.49	99.40
	50	294.71	297.90	98.92	99.30
KRG-1	20	292.8	298.50	98.09	99.50
	30	290.36	297.36	97.64	99.12
	40	292.47	297.03	98.46	99.01
	50	295.21	297.30	99.29	99.10
			Average	98.79	99.36
chickpea					
Annigeri-1	20	289.59	296.70	97.60	98.90
	30	291.75	296.10	98.53	98.70
	40	289.88	294.15	98.54	98.05
	50	294.3	294.90	99.79	98.30
KGB-1	20	287.88	295.80	97.32	98.60
	30	289.19	294.30	98.26	98.10
	40	288.05	294.60	97.77	98.70
	50	290.37	294.03	98.75	98.01
			Average	98.32	98.42

Table 3: Analysis of variance for electronic seed counter

Crop	variety	Calculated value	Table value		Calculated value	Calculated value
		F-value	F-value	correl	t value	t-value
peanut	R-8808	0.080495	3.238872	0.64611	1.19718	4.302653
	GPBD	0.129354	3.238872	0.4302	0.67395	4.302653
	KRG-1	0.276804	3.238872	0.607	1.08019	4.302653
Chickpea	Annigeri-1	0.210133	3.238872	0.729622	1.508871	4.302653
	KGB-1	0.067534	3.238872	0.707285	1.414925	4.302653

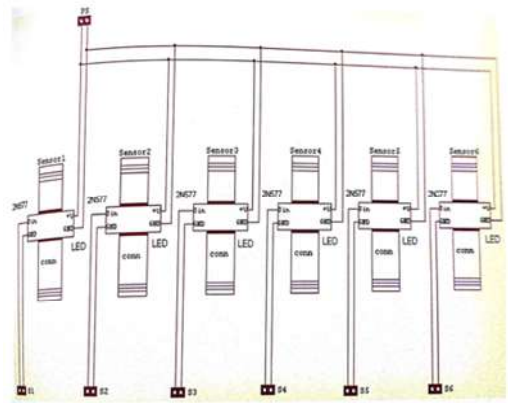

Figure 1: Circuit diagram of seeds sensors mounted in seed tubes

Figure 2: Multicrop inclined plate with electronic seed counter (Front View)

Figure 3: : Multicrop inclined plate with electronic seed counter (Rear View)

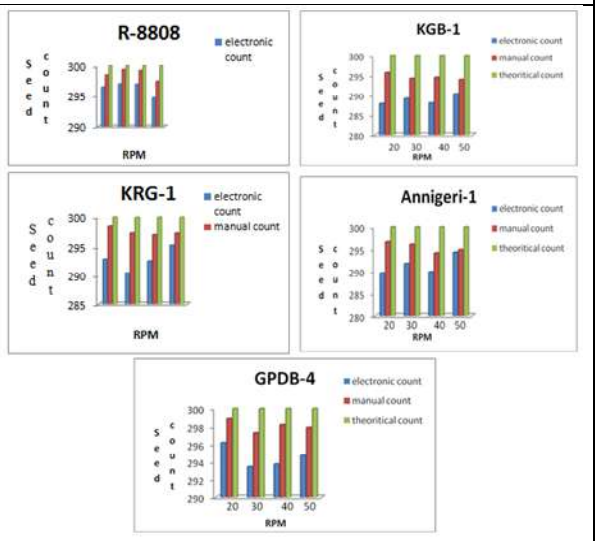

Figure 4: The comparative performance of electronic seed counter and manual counting for peanut and chickpea selected varieties

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REVIEW ARTICLE

An Overview on Herbal Based Nano Formulations

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ABSTRACT

Herbal medicines are widely used roundthe world since earlierperiod. The advancement of phytochemical and phytopharmacological sciences has enabled elucidation of the composition and biological activities of several medicinal plant products. The effectiveness ofthe many species of medicinal plants depends on the provision of active compounds. Many whole herbal preparations, herbal extracts and isolated phytocontituents are subjected to pharmacological and clinical research. This has highlighted issues like poor bioavailability, stability and distribution of herbal medicines when administered in traditional dosage forms and paved the way for research into the incorporation of herbal medicines into Nano formulations. Nanotechnology is one in all the key novel drug delivery methods under investigation, with Nano formulations thought to possess a good kind of benefits as compared with conventional preparations of plant constituents, which include enhanced permeability, solubility, bioavailability, therapeutic activity, stability, improved distribution within tissues and sustained delivery. Nanotechnology is that the study of extremely small structures which covers the varied area of matters at dimensions which are approximately between 1 to 100 nanometres. It identifies the assorted Nano formulation approaches that are developed and successfully used as a way to reinforce the topical delivery of natural bioactives, including Nano- emulsions, liposomes, phytosomes, microspheres, and transferosomes. This review will provide a short discussion of the main Nano pharmaceutical formulations moreover becausethe impact of nanotechnology on herbal drugs.

Keywords: Herbal medicine, Nano formulation, nanomedicine, nanotechnology, drug delivery.

INTRODUCTION

Nano formulation is an emerging trend that has already yielded some interesting results in the development of novel phytochemical delivery systems. Conventional phytochemicals used as drugs are highly water-soluble with low absorption properties due to their inability to cross lipid membranes in addition to having high molecular weights. In contrast, more than 40% of new chemical entities are poorly water soluble drugs having slow drug absorption [1]. Both results in low bioavailability and inefficacy in drug delivery, hence reduces their therapeutic value. A major reason behind poor absorption of conventional drugs is the challenge associated with finding the appropriate perfect formulation to account for physiochemical properties of the drug, and the type of target site and disease [2]. From ages, human diseases are treated by herbal medicines in almost every infection. Plants are natural source of treatment and are used from ages for food and medicine. Indeed, natural medicines have grabbed the attention again instead of fighting in the number of infections [3]. About 80% of the world population is now using herbal drugs for primary health care basically in developing countries. But, these herbal therapies have some restrictions, due to stability issues and poor lipid solubility [4]. Mostly in traditional drugs, only a finite amount of administered amount gain access to the targeted site and most of the dose get dispersed throughout the body depending on physiochemical and biochemical characteristics as a result giving low therapeutic effect. As there is more than one active ingredient in herbal formulations, stability profiles of the herbal medicines should be determined. Supply of active constituents has great significance in the efficacy of most of the species of plants having medicinal importance [5]. Herbal medicinal extracts containing biologically active components, like tannins, flavonoids, and terpenoids, are tremendously miscible in water, but cannot cross the lipid membranes of the cells so have less absorption, also have extremely large molecular size, causing loss of bioavailability and effectiveness. Because of these drawbacks some extracts are not use in practice. To conquer these problems, innovative systems of drug delivery has been developed for phytomedicines [6]. These herbal innovative systems of drug delivery include vesicular delivery systems such as liposomes, ethosomes, phytosomes, transferosomes, and particulate delivery systems such as micropellets, microspheres, nanoparticles, and micro and nano emulsions. For the enhancement of stability, bioavailability and depletion of toxicity, many natural drugs have been assimilated into these drug delivery systems [7].

In recent years, interests in the development of Nano-formulated drugs are prominent to enhance the therapeutic value of medicinal drugs. These drugs in the Nano-form possess unique features such as high surface to mass ratio and quantum-size effects such as electron confinement with absorption and drug carrier ability. These features play a crucial role to overcome the challenge of low therapeutic absorption associated with phytochemicals as drugs and new chemical entities. Various Nano-formulations have been employed in drug delivery research to improve targeted drug delivery, bioavailability, solubility, drug retention time, besides minimizing their side effects, including risks of toxicity [8]. Nano-formulations generally vary in size from 10-100 nm, and the drug is dissolved, entrapped, encapsulated or attached to the drug carrier. There are some major properties that are needed to be considered while formulating Nano-drugs. The formulation must facilitate the drug to reach site of action from the site of administration and should protect them from the detrimental effects of environmental factors such as pH, enzyme attack, and potential biochemical degradation. Moreover, the formulation should release the payload in its active form in or around the target site and to facilitate administration of lower doses to achieve high drug effect [9].

NANO-FORMULATIONS IN DRUG DELIVERY

Various Nano-formulations such as dendrimers, polymers, liposomes, Nano-emulsions and micelles are used in the pharmaceutical industry for drug delivery.

- Dendrimers are synthetic, hyper branched polymeric macromolecules with a well-defined core, backbone and multivalent periphery to form a globular tree like structures. These dendrimers has the capability to carry various drugs by using covalent conjugations or encapsulation within the cores [10]

- Polymeric nanoparticles such as Nano spheres and Nano capsules are also used as delivery carriers for drugs. Polymeric nanoparticles enhance the water solubility of drugs,yet capable in controlling the drug release rate [11].
- Liposomes are lipid bilayer composed of either synthetic or natural phospholipids in an aqueous phase that encapsulates drugs in a closed spherical vesicle. They are highly stable and hence are useful as delivery carrier for drugs such as steroids, vaccines and genetic materials [12].
- Nano-emulsions has droplet sized emulsions of 20-200nm which makes them highly stable against sedimentation. These emulsions possess interesting size depended properties such as optical transparency and high shelf stability against gravity driven creaming of particles for enhancing the performance of drug in pharmaceutical industry [13].
- Micelles are 10-100nm sized amphiphilic molecules which have core-shell architecture. The inner hydrophobic core and outer hydrophilic corona makes them an eminent carrier of drugs by enabling prolonged circulation in biological system [14].

STRATEGIES OF NANOTECHNOLOGY AS NOVEL DRUG DELIVERY SYSTEM

Nano-sized delivery system was selected because of the following reasons:

- They appear to be able to deliver high concentrations of drugs to disease sites because of their unique size and high loading capacities.
- Deliver the drug in the small particle size that enhances the entire surface area of the drugs allocating quicker dissolution in the blood.
- The concentration seems to persist at the sites for the longer periods.
- Shows EPR (enhanced permeation and retention) effect, i.e., enhanced permeation through the barriers because of the small size and retention due to poor lymphatic drainage such in tumor.
- Exhibits passive targeting to the disease site of action without the addition of any particular ligand moiety.
- Decrease in the side effects.
- Decrease in the dose of the drug formulation [15-16]

TECHNIQUES

The techniques commonly used for the formulation are:

High-pressure homogenization method

In this method, the lipid is pushed with high pressure (100 to 2 000 bar) through a very high shear stress, which results in disruption of particles down to the sub-micrometre or nano-meter range. High-pressure homogenization method is a very reliable and powerful technique for the large-scale production of nanostructured lipid carriers, lipid drug conjugate, SLNs, and parenteral emulsions [17].

Complex coacervation method

This is a spontaneous phase separation process of two liquid phases in colloidal systems, which results by the interaction of two oppositely charged polyelectrolytes upon mixing in an aqueous solution[18].

Co-precipitation method

This method is a modification of the complex coacervation method for the preparation of nanoscale core-shell particles. This method has been reported to provide good dispersion stability to poorly water-soluble drugs [19].

Salting-out method

This method is based on the phenomenon that the solubility of a non-electrolyte in water is decreased upon addition of an electrolyte [20].

Nanoprecipitation method or solvent displacement method

This method is based on interfacial deposition of a polymer after displacement of a semi polar solvent miscible with water from a lipophilic solution, thereby resulting in a decrease in the interfacial tension between the two phases, which increases the surface area with a subsequent formation of small droplets of organic solvent even without any mechanical stirring [21].

Solvent emulsification–diffusion method

The method involves preparation of an o/w emulsion using oil phase containing polymer and oil in an organic solvent, which is emulsified with the aqueous phase, containing stabilizer, in high shear mixer, followed by addition of water to induce the diffusion of organic solvent, thus resulting in formation of nanoparticles [22].

Supercritical fluid methods

This method can be used to prepare submicrometer-sized and nano-sized formulations. A supercritical fluid (SCFs) can either be a liquid or gas and used above its thermodynamic critical point of temperature and pressure. The most commonly used SCFs are carbon dioxide and water [23].

Self-assembly methods

Self-assembly is the physical process wherein pre-existing disordered components, atoms, or molecules organize themselves into regulated nanoscale structures by physical or chemical reactions without any contribution from any external source [24].

TECHNIQUES FOR PREPARATION OF NANOPARTICLES

Preparation and compression of drugs in polymeric nanoparticles

Polymeric nanoparticles are prepared by using numerous approaches depending upon the needs of its usage and a kind of drugs to be compacted. A large number of bioactive substances are being encapsulated by these nanoparticles and are broadly used to originate the nanomedicines. Bio decomposable polymeric nanoparticles are revealed as promising drug delivery system and extremely preferred. These types of nanoparticles offer precise or constant discharge characteristic, subcellular size and biocompatibility with tissue and cells. Besides these, they are unchanging in blood, non-thrombogenic, non-virulent, non-inflammatory, not susceptible to elicit an immune response, not causing activation of neutrophils, bio decomposable, don't have any action on reticuloendothelial system and appropriate to numerous particles such as medicines, proteins, nucleic acids or peptides [25].

Herbal Nano tablet

The developing global populations with have no means to get clean water for drinking are now using herbal nanoparticles in the form of tablets for water purification. Brahmi (Bacopamonniera) extract is used to make these herbal nano tablets on a small porcelain disk packed with silver or copper nanoparticles that is positioned in a water vessel. Inside the water vessel it can frequently sanitize water for about six months. For precise and target specific delivery of drugs, nano tablets comprising of herbal medicines are used. Bahamas layered nano herbal tablets are reported for the anticancer effects. For making of energy drinks, nano coffee energy tablets are used effortlessly. The active constituents used in these drinks are vitamin C, Vitamin B6 B12 B5, niacin, Guarana seed extract, Folate, Chromium, caffeine (from herbal sources), glucuronolactone [26].

HERBAL MEDICINES USING NANOFORMULATIONS

Nano herbal drugs are synthesized from active phytocomponents or systemized extracts. Effectiveness and bioavailability of the delivered drug increases by using nanotechnology. Nano herbal formulations also reduce the side effects and verilency of the administered drugs [26].

Artemisiaannuanano capsules

Artemissiaannua is a single stemmed annual herb of family Asteraceae. Artemisinin is the active principle of Artemissiaannua. It has the potent antimalarial action. Due to its poor pharmacokinetic properties and short half-life, its clinical application is restricted [28]. The Nano-coated artemisinin have been developed to overcome the problems associated with artemisinin. These Nano capsules dispersed well in aqueous solutions and hydrophilicity of artemisinin crystals were also improved after encapsulation. Resolved this issue. Compression in these small particles causing the fat soluble curcumin to be absorbed better and also reveal the steady discharge into blood stream, increasing and exceeding bioavailability. Many experiments in vitro and on animals have proposed that curcumin have antioxidant, antitumour, antiarthritic and anti-inflammatory activity. Pharmacokinetic researches in vivo showed that curcumin entangled nanoparticles reveal about 09 fold rises in oral bioavailability in comparison of curcumin used with piperine as absorption stimulator [29].

Berberinenano medicine

Berberine, a naturally occurring isoquinoline alkaloid present in the roots, rhizome and stem bark of number of medicinal plants such as Berberis vulgaris L.(Family- Berberidaceae) , Hydrastis Canadensis L.(Ranunculaceae), Phellodendronamurense (Rutaceae), Coptischinensis (Ranunculaceae) and Tinosporacordifolia (Menispermaceae). Berberine has tremendous potential to cure many physiological disorders. Moreover, the anti-tumor properties of berberine have been achieved on human malignant brain tumor, oesophageal cancer, human leukemic and colon cancer cell lines. Berberine loaded nanoparticles are successfully prepared using single emulsion, multiple emulsion and ionic gelation methods for sustained drug release [30].

Centellaasiatica nanoparticles

A small herbaceous creeping plant, Centellaasiatica. (Family- Apiaceous) has wide range of pharmacological applications such as anxiolytic, anti- anxiety, and antioxidant. It is also used for the treatment of leprosy, wounds, cancer, fever, allergy and syphilis. Centellaasiatica extract (CAE) possesses high potential biological activities; its clinical usage is limited to some extent due to its physical instability. CAE (powder extract) shows high hygroscopicity. Therefore, the development of nanocrystal in which the extract is entrapped inside could protect it from external moisture. Nano encapsulation of CAE provided physical stability compared to its extract alone [31]

Murvananoparticles

Murva is a controversial drug, obtained from many medicinal plants such as Marsdeniatenacissima (Roxb) Moon (Asclepiadaceae), Clematis triloba (Ranunculaceae), Sansevieriazeylanica (Roxb) (Agavaceae), Helicteresisora Linn. (Sterculiaceae), Chonemorphamacrophylla (Apocyanaceae), Bauhinia tomntosa Linn. (Caesalpinaceae) and Maeruaoblongifolia (Capparaceae). It has the potential to treat diseases like anaemia, fever, diabetes, stomach disorders, typhoid, urinary infection and cough. Due to its low aqueous solubility and poor bioavailability, its use is limited in clinical application. Thus to increase its solubility and bioavailability, it is formulated as nanocrystals [32].

Danshen (Tanshinone IIA nanoparticles)

Danshen, a dried roots of Salvia miltiorrhiza L (Family- Lamiaceae), are widely used as medicines for promoting circulation and improving blood stasis. Danshen is extensively used for the treatment of coronary heart disease, cerebrovascular diseases and hyperlipidaemia. Slow pharmacological action is the major drawback of this herbal drug. Nano-coated Salvia miltiorrhiza exhibits stronger antioxidant property and the release was much faster than the traditionally powered samples. Phospholipids complex loaded nanocrystal also enhanced oral bioavailability [33].

Curcuminnanophyto medicine

Curcumin is a constituent of the turmeric (Curcuma longa), revealed many benefits regarding the treatment of various diseases. Many researches and experiments have showed the pharmacokinetics, protection, and effectiveness of this constituent in case of ailments in human body. Curcumin is poorly soluble in water and highly soluble organic molecule which reducing its bioavailability. The metabolism of curcumin is so fast which causes further reduction in its bioavailability [34].

Bhasma nanoparticles

Bhasma are exclusive preparation, dried with herbal juice or decoction and extensively suggested for curing of a many chronic disorders. Bhasma is defined as an ash attained after incineration; there is an extensive process of purification for the initial substance and then this substance will undergo reaction phase which includes amalgamation of certain other minerals and/or herbal extract. Bhasma has many significant effects on health such as sustaining optimal alkalinity for optimal health; reduce the destructive effects of acids that cause ailment. Bhasma breakdowns the heavy metals in the body and never produce detrimental metabolites as it does not metabolized [35]. Bhasma is the oldest form of nanotechnology. Bhasma is used from many years but extremely modern nanomedicine synthesized from metal after many systematic techniques to raw materials to convert them into active form. All these processes involve frequent incineration and crushing with certain herbal juice and other definite drug. Its basic properties changed due to its tiny size. The size of the particle is 56 nm when assessed by different tools and methods such as AFM (atomic force microscope) and scaning electron microscope [36].

Nanoparticles of Aloe Vera extract

Many creams or gels extensively use the aloe Vera extract in its formulations for the care of skin. The creams or lotion preparations use for dermatitis, dryness, psoriasis, scaling, flaking, eczema, sunscreen and antiaging contain aloe Vera extracts mostly. The present researches on Aloe vera extract in Japan suggested that it is not able to cross the stratum cornium. There is an obstacle for aloe vera extract to penetrate the skin, as aloe vera is hydrophilic compound and stratum cornium consists of high protein cells and intracellular lipid domain which act as impermeable barrier for it. For resolving this issue dose of the extract to skin increases but it causes inflammation. This study explored liposome comprising Aloe vera from soybean lecithin that increases the penetrating power of extract. In vitro, human skin fibroblast and epidermal keratinocytes used for revealing the penetration power of prepared Aloe vera comprising liposome. They have diameter <200 nm. The research suggested that the rate of proliferation is dramatically higher after using liposome comprising Aloe vera than without encapsulation. Moreover, the synthesis of collagenase also enhanced by 23% with liposomal aloe vera extract in comparison to 4% without encapsulation extracts [37].

Celastrol nanoparticles

Celastrol, tripterine is obtained from the root extract of Trypterygiumwilfordii Hook F reveals significant importance for the cure of autoimmune syndromes. The family of Trypterygiumwilfordii Hook F is Celastraceae. It is also called as the ‘Thunder of God Vine’ and from many years used in ancient chinese remedies for the cure of rheumatoid arthritis. Further, therapeutic uses of celastrol confines to its lesser water solubility. Celastrol nanoparticles are prepared and their antitumor activity was tested in vitro which reveals that celastrol nanoparticles remarkably prevent the propagation of human retinoblastoma cells depending upon dose and time. They cause apoptosis in targeted cells and prevent the growth of retinoblastoma in a xenograft mouse model which proves it an significant substitute for the cure of retinoblastoma [38].

Genistein nanoparticles

Genistein, isoflavone was extracted from dried foodstuffs is a chief active constituent of soyabean, scoparius and other leguminous plants. It is a phytoestrogen and antioxidant. It also showed the potential to reduce the chances of osteoporosis, heart disorders, breast and uterine carcinomas. However, it has lesser bioavailability and water solubility which reduces its practical usages. Hence there was a need to synthesize the genistein nanoparticles to

enhance its water solubility and bioavailability. Genistein nanoparticles were prepared by using nano-precipitation technique. Research revealed that genistein comprising nanoparticles have greater (241.8%) comparative bioavailability than the genistein alone [39].

Cuscuta nanoparticles

Cuscuta reflexa is the plant of family Convolvulaceae, a parasitic plant; generally recognized as dodder plant, amarbel, akashabela. Conventionally it is termed as miracle plant. Flavanoids and lignins are the chief components of this plant. It possesses anti-cancerous, antiaging and immune-stimulatory properties. Due to its lower water solubility its oral administration is restricted [40].

Quercetin nanoparticles

Quercetin, a flavonoid expelled from air dried plant part (primarily from bark and leaf) of Spohora japonica L. belongs to family Fabaceae. Quercetin has greater antioxidant activity than eminent antioxidants like ascorbyl and trolox . Besides this, it exhibits anticancerous and antiviral properties. Dispite the large number of pharmacological characteristics, its applications in biomedical field is restricted due to its lesser water solubility and stability which leads to poor bioavailability. Quercitincomibines with superparamagnetic iron oxide nanoparticle (SPION) are proved to increase the bioavailability of quercetin. The study was suggested that these nanoparticles bind to specific proteins causing prevention of neural cell apoptosis and increases learning and memory. Hence, SPIONs might enhance the bioavailability of quercetin and increase learning and memory [41].

Paclitaxel nanoparticles

Paclitaxel is one of the active constituent of the plant Taxusbrevifolia Nutt. obtained from its bark. This plant belongs to the family taxaceae has anticancerous properties. Its greater lattice energy consequences in inadequate water solubility (0.7-30 µg/ml-1) restricted its effectiveness. Accordingly integration of paclitaxel into nanoparticles improved its anti-tumoral activity. Paclitaxel laden nanoparticles were synthesized by nano-precipitation method and by sequential simplex optimization method. Paclitaxel nanoparticles improve drug stability, maintain the steady drug discharge and increase bioavailability [42].

PROBLEMS ENCOUNTERED IN PHYTOMEDICINE PRODUCT DEVELOPMENT

Mostly, phytomedicines are called as secondary metabolites and these metabolites are being chemically isolated and identified. The production of active constituents of phytomedince represents a lot of challenges. These secondary metabolites which are present in plant are very low and their active constituents vary depending on a number of factors, such as botanical species, used chemo-types, the anatomical part of the plant used (seed, flower, root, leaf, and so on) and also storage, sun, humidity, type of ground, time of harvest and geographic area. Phytomedicine screening from the plant is another challenge, and even though the high throughput methods are normally employed in the screening of drugs in pharmaceutical field, it is not suitable for the phytomedicine as crude extracts contain numerous drug compounds. Moreover, some active constituents present in the plant gave false information when screening by high throughput techniques. In addition to this, identification, isolation of active constituents and fractionation process also takes weeks or even months and active ingredient supply is also another challenge, which needs several hundreds of grams for preclinical development depending upon the utility. Many phytomedicine and extracts of plant despite of their surprising potential in-vitro finding, exhibit least or no significant in-vivo activity due to their poor solubility, poor lipid solubility and improper size result in poor absorption and bioavailability. Another problem is their structural instability in biological milieu, premature drug loss through rapid clearance and biotransformation and some plant extracts are destroyed in gastric juice during gastric emptying when administered orally [43]

VARIOUS NANOTECHNOLOGIES APPROACHES FOR ENHANCING THE BIOACTIVITY OF PHYTOMEDICINE

Reducing the size of the phytomedicine into nanophytomedicine

Most of the phytomedicine formulation is administered orally because of patient convenience and manufacturing advantages compared with other dosage forms. For the orally administered drug, there are two critical slower rate-determining steps (RDSs) in the absorption. First is the rate of dissolution and the rate of drug permeation through the membrane is another step. Dissolution is the RDS for hydrophobic in poorly aqueous soluble drugs and absorption of such drugs is said to be dissolution rate limited. If the drug is hydrophilic with high aqueous solubility, then dissolution will be rapid and RDS in the absorption of such drugs will be the rate of permeation through the bio-membrane. That is said to be permeation rate limited or transmembrane rate limited. A well formulated nanophytomedicine prepared through various routes of synthesis, by virtue of their size, enhances the dissolution, absorption and bioavailability of drugs while reduces in the dose. Cuscutachinensis (*C. chinensis*) is a Chinese drug containing flavonoids and lignins as active medicament, which is poor aqueous solubility and poor absorption upon oral administration. The nano sized *C. chinensis* were prepared by a nanosuspension method for hepatoprotective and antioxidant effects after oral administration. The 25 and 50 mg/kg oral doses showed similar activity as that of 125 and 250 mg/kg ethanolic extract of *C. chinensis*, fivefold reduction in dose was observed with nano sized *C. chinensis* [44].

Radix salvia nanoparticles prepared by spray drying method for coronary heart disease, angina pectoris and myocardial infarction, nanosized Radix salvia showing improved bioavailability. The reduction of the size of the phytomedicine improves aqueous solubility. Generally water soluble drugs whose size smaller than the diameter of the pore of the biomembrane can penetrate easily. The driving force is constituted by the hydrostatic pressure or the osmotic difference across the biomembrane due to which bulk flow of water along with small solid molecules occur through such aqueous channels [45].

Modification of surface properties

Surface modification can be achieved by surface coating with hydrophilic, stabilizing, mucoadhesive polymers/surfactants or by the production of biodegradable copolymers with hydrophilic segments. These modifications change the zeta potential of nanoparticles, hydrophobicity, stability, mucoadhesive properties and protein adsorption on their surface. Surface properties of particles and size have a significant impact on particle uptake. The membrane passing and permeation can be increased by using the surface modification. Intestinal transit results in reducing the phytomedicine residence time and limits the bioavailability of herbal drugs. One possible way to increase the bioavailability of phytomedicine which has low mucosal absorptive properties and to increase its residence time at mucosal or epithelial level is by incorporating phytomedicine in micro or nanoparticles. When a suspension of micro or nanophytomedicine is administered orally, they diffuse into the gastrointestinal medium and encounter the mucus at which they could adhere. One of the big impediments of oral administration of phytomedicine is their lack of stability in the gastrointestinal tract. The surface-modified micro or nanophytomedicine can be used as an efficient strategy to circumvent this problem. The poly (lactic-co-glycolic) acid (PLGA) microspheres with chitosan, and PEGylated PLGA-based nanoparticles were used to modify the properties of formulations [46].

Attaching the polymers with phytomedicine

A variety of polymers are being utilized in these and other biomedical applications; for example, polyvinyl chloride used in the manufacture of cardiac catheters, surgical tapes, artificial hearts, blood pumps and artificial limbs. By modifying polymer surfaces one may achieve a number of desirable properties ranging from blood clotting prevention to controllable drug release, and other applications, while maintaining useful bulk polymer properties. Each particle is a matrix of the drug dispersed in the polymer and drug is released as a first order process. The polymers used for the fabrication of the microspheres are biodegradable or non- biodegradable. Various polymers have been used for the fabrication of these microparticulate carriers such as albumin, gelatin, modified starch,

polypropylene, dextran, polylactic acid and poly lactide-co-glycolide etc. The drug release is controlled by the dissolution and degradation of the matrix [47].

Nano carriers for phytomedicine

The main goals in designing nanoparticles as a delivery system is to manage particle size, surface properties and release of bioactive agents in order to accomplish the site-specific action of the drug at the therapeutically optimal rate and dose regimen. There are certain advantages of using nanoparticles in phytomedicine drug delivery system include,

(1) Particle size and surface characteristics of the nanoparticles can be modified easily for both passive and active target;

(2) Controlled release and degradation can be manipulated easily with matrix constituents, so drug can be incorporated into the system free from chemical reaction and with high loading of drug;

(3) Surface properties of the nanoparticles helps to achieve site specific delivery by attaching target ligands to the surface of the particles [48 – 50].

ADVANTAGES

- Improves solubility thus improved efficacy
- Feasability of variable routes of administration.
- Reduced dosing frequency.
- Biodegradable, non-toxic.
- Feasibility of incorporation of both hydrophilic and hydrophobic drugs.
- Improved safety.
- Improves selectivity of herbal drugs [51-53]

DISADVANTAGES

- Reduced ability to adjust the dose
- Productivity is more difficult
- Highly sophisticated technology
- Difficult to maintain stability of dosage form.
- Requires skill to manufacture
- High cost [54-56]

APPLICATION

- Widely used in case of cancer therapy.
- Used in intracellular targeting.
- Used for prolonged systemic circulation
- As a vaccine adjuvant.
- Used in DNA delivery.
- In case of ocular delivery.
- It is used in case of oligonucleotide delivery.
- Enzyme immunoassay [57-60].

CONCULSION

The main aim of this review is to provide information about the different synthesis methods, drug delivery, and different type of herbamedicinethat are available for Nano-formulations. It was observed that different types of preparation methods for each Nano-formulation are available and choosing the suitable method helps in effective usage of these Nano-formulations for drug delivery applications. On the other hand, synthesis methods are

responsible for altering the size and shape of Nano-formulations, thereby helps in altering and enhancing its properties for effective drug delivery. Efficacy of many traditional herbal drugs is limited due to their bulky size that limits their bioavailability and bioactivity. Novel drug delivery systems have tremendous potential in improving their bioavailability, pharmacokinetic profile, blood brain barrier and sustained delivery. This will enhance the efficacy of conventional drugs and provide several health benefits to the people. It is equally important to ensure the safety and simultaneous studies should be carried out on their potential toxicity.

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RESEARCH ARTICLE

Drug Master File: A Regulatory View

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ABSTRACT

The Drug Master File (DMF) is a technical document that is sent to the regulatory authority by either the active pharmaceutical ingredient (API) manufacturer or the holder of DMF, by an approved person appointed to protect confidential information or intellectual property, which the manufacturer of API does not want to disclose to the manufacturer of drugs products. The DMF includes production information, intermediate information, methodology, impurity profiling, safety information, and effectiveness information that which are supposed to be sent to the agencies in support of the drug substance. In order to file a DMF, the registration requirements for an active pharmaceutical ingredient (API) vary by country. As it is normal to use the format of the ICH-CTD for the International Council for the Harmonization of Technical Requirements for Human Use Pharmaceutical, country data is required to be included when submitting to a specific country. The key objective of the study is to summarise the intended requirements that must be considered for filing a DMF in the emerging nations (Singapore, South Arabia, Brazil, and South Korea), compare it with those in the regulated nation (US and EU), and to provide a snap regarding the specific strict requirements of the emerging regions.

Keywords: Drug master file, Regulated market, emerging market, Regulatory requirements, Drug substance, Variations.

INTRODUCTION

In recent years, the pharmaceutical industry's growth in established nations such as the United States (US) and European Union (EU) has stagnated, forcing numerous multinational companies to seek economic boom in emerging nations. The emerging nation's financial sector is evolving due to rapid economic development and industrialization. The major growth reasons in developing countries include greater government engagement to upgrade healthcare systems, a rise in consumer financial yields and wealth, and the increased rate of medical conditions like diabetes and cancer. As established markets stagnate, developing nations become increasingly important as a source of revenue growth for global pharmaceutical businesses [1]. There's a lot of promise in emerging markets, but their criteria aren't harmonized yet. So, they run into a lot of issues when it comes to registration. Due to the language and communication limitations, it is difficult to determine the authority's exact conditions. There is no clear public domain access to these emerging markets' legislation because their economies are continuously changing over the past few years. The regional criteria given in this study are real data-based and gathered from multiple submissions to all of these marketplaces, which may be utilized as a reference point in the future. With global plans and business prospects, Brazil, Saudi Arabia, Singapore, and South Korea have been chosen. A regulated market, such as the US or the EU, is used to compare emerging market DMF filing requirements and to demonstrate the strict standards set by emerging authorities [1].

In medicinal product manufacturing, an active pharmaceutical ingredient (API) is any material or mixture of substances that become the active ingredient of the drug product when used in production. These drugs are proposed to have pharmacological effects or have other important effects in the diagnosis, cure, mitigation, or prevention of the disorder. APIs should be manufactured by existing legislation about their uses for a high degree of protection, reliability, and consistency achievement [2]. APIs must be manufactured for pharmaceutical drugs following cGMP (current good manufacturing practices) and sold per GDP (good distribution practices). GMPs were implemented and monitored for the manufacture of drugs a long time ago (around 1970). The ICH (International Harmonization Committee) was established a few years ago (1990) to provide requirements that are applicable in many parts of the world, including the US and the EU. The ICHQ7A guideline, which is focused on the production of pharmaceuticals, complies with GMP. Simultaneously, complying with state and municipal legislation must be considered. The national health authorities' review would, in particular, confirm that the substance meets the requirements and the regulatory dossier filed by the manufacturer with the health authorities [2].

The prescription substance maker must apply a registration dossier to the Health Authorities to acquire a marketing permit. This dossier tends to follow the 'ICH M4 – CTD' format (common technical document), which is divided into five modules. Module 3 is concerned with the dossier's content section which is devoted to the drug substance (Section 3.2.S) and the drug product (Section 3.2.P). To summarise, Module 3.2.S is focused on API information and includes all details relating to the nature of the active material (manufacture, characterization, and regulation of drug compounds, container closure system, and stability data). The quality section of the registration dossier can have a different name and composition depending on the country [2]. The API manufacturer's quality details could be deemed proprietary. As a result, the API provider creates and submits a registration dossier to the relevant health authorities in the various nations of concern. The 3.2.S portion of this registration dossier follows the CTD format. In certain overseas nations, the regulatory dossier is required in order to market APIs as such (Brazil for example). After the certificate has been issued, the authorized dossiers must be restored on a regular basis and revised if there are any improvements or modifications in the API production method [1].

The revisions are introduced and the information of the revision dossier is based on locally accepted change classification criteria. (Minor, major, and so on...) When a product has a monograph in a National Pharmacopoeias, the manufacturer is expected to market the API in conjunction with the monograph [2]. A DMF is a document compiled by a drug manufacturing company and sent to the relevant national regulatory authority in the specified

country strictly at the manufacturer's discretion. There is no legal obligation to file a DMF. The book, on the other hand, gives the regulatory authority sensitive, comprehensive details about the equipment, procedures, or articles used in the manufacture, distribution, packaging, and storage of one or more human drug products [3]. When two or more companies collaborate on the development or manufacture of a prescription substance, a DMF is typically filed. It allows a firm to protect its intellectual property while still satisfying the legal requirements for processing information in an open and transparent manner [3].

The DMF usually covers the Chemistry, Manufacturing, and Controls (CMC) of a drug product's portion, such as the drug contents, excipients, or storage conditions. It is a file that contains all of the information of an API or a completed drug dosage type. In EU, an Active Substance Master File (ASMF) is the new title and it was formerly known as a European Drug Master File (EDMF). In US it is named asUS-Drug Master File (US-DMF) [3]. A DMF may provide drug product information as well as non-CMC information (e.g., equipment, toxicological). For every human drug product, a DMF offers accurate and complete facts on chemistry, manufacturing, durability and purity as well as information on packaging and cGMP compliance. When a major or vital defect is found, DMF is suspended or stopped. Until the holder provides a reason that is approved by the regulatory body, the termination is limited to two years. If this time span expires without being fulfilled, the DMF will be revoked. The holder must order the restoration, and it is not subject to revocation for two years. It tends to the interruption of importation for imported API's or suspension of API's.

DISCUSSION

USDMFs

A US- DMF is a classified comprehensive data filing to the Food and Drug Administration (FDA) about machineries, articles or procedures used in the production, refining, packaging, and storage of one or more human products. It is not mandated by the state of laws or FDA legislations to submit a DMF. A DMF is sent as per the holder's interest. The data in the DMF can be utilized to assist an Investigational New Drug Application (IND), a New Drug Application (NDA), an Abbreviated New Drug Application (ANDA), another DMF, an Export Application, or additions and revisions to any of these applications. An IND, NDA, ANDA, or Export Application are NOT alternatives for a DMF. It hasn't been accepted or denied. Only in the context of the evaluation of an IND, NDA, ANDA, or Export Application a DMF examined [4].

For the submissions mentioned below, there are 4 different categories of DMF.

- Type II - Drug substance, drug substance intermediate, and material used in their preparation, or drug product
- Type III - Packaging material
- Type IV - Excipient, colorant, flavour, essence, or material used in their preparation
- Type V - FDA accepted reference information (4)

CEPs / COS

"CEP" is an acronym for 'Certification of Suitability of European Pharmacopoeia Monographs'. COS (Certificate of Suitability) is an acronym that stands for the same thing but is not the standard acronym. The CEP's purpose is to rise that the purity of a drug manufactured by a specific producer is adequately regulated by the related European Pharmacopoeia monograph(s). Suppliers of raw materials may demonstrate their appropriateness to their pharmaceutical industry clients and/or regulatory authority by proving that their product has been awarded a CEP.

In order to comply with the most recent version of the Standard Operating Procedure (SOP), the application dossiers must be structured in CTD (Common Technical Document).

When it comes time to submit a marketing application dossier A copy of the CEP can be used to replace the documentation related to the drug substance or excipient that was included in the CTD,rules Governing Medicinal Products in the EU (NTA, Vol. 2 B-CTD module).

Legal Status

To apply for certification, the manufacturer or supplier of the product should follow the official process established by the EU, defined in Resolution AP-CSP (07) 1 and Directives 2001/83/EC, 2001/82/EC, and 2003/63/EC as amended of the EU.

- the monograph's suitability for controlling the microbiological quality and chemical purity of the substance; or
- the general monograph's reduction of TSE risk; or
- Assessment of the monograph's adequacy for the regulation of herbal medicines and herbal medicine preparations.

To receive a CEP, following documents submitted in electronic format and must be sent to the EDQM's Certification of Substances Department (DCEP).
It includes:

- a completed submission form including the invoicing information
- a single copy regards the Quality Overall Summary (QOS) according to CTD format, ideally written in one of the Europe's official languages or in English.

Upon receipt of the application, the EDQM reviews it and sends issues to the applicants. A CEP is issued to applicants who successfully complete the EDQM's review process. The validity of CEP is for 5 years from date of issue following renewal of same. To ensure the official timeline is met, the EDQM carries out a strict procedure for the assessment of CE applications. (5)

ASMFs / EDMFs

The EDMF, also identified as the Active Substance Master File (ASMF), is a request made to European Competent Authorities and/or EMEA for pharmaceutical products Marketing Authorization Application [MAA] or Marketing Authorization Variation [MAV]. The ASMF / EDMF must be in CTD format. EDMF is physically divided into two areas, one is Applicant Part (AP) and another is Restricted Part (RP).Data by the EDMF holder deems non-secret to the applicant / MA holder is provided in an AP, whereas confidential data is provided in an RP. (6)
A 'table of contents (TOC)' and a separate description of QOS for the AP and RP should be included in the EDMF, additionally to the AP and RP.The recent edition of the concerned SOP must be pursued for details on ASMF / EDMF content and submission procedures.(7)

Regional DMFs

For submissions outside the US and EU, there is a section called Regional DMFs.It is created based on market requirements for each country specifically.
The nations following regional DMF submission includes:

1) Turkey
2) Brazil
3) Korea
4) South Africa
5) Australia
6) Syria

The 'Regional DMF' should be prepared and submitted in accordance with the regional guidelines of the various nations. (3)

CONCLUSION

In current findings, API approval requirements for emerging countries are more stringent than those for regulated. In all countries DMF holders are submits DMF amendments and MAH is for a drug product undergoes a major change as Post approval changes. In contrast to developed markets, developing markets lack uniform norms and are less transparent. Uncertainty about DMF requirements in emerging countries like Saudi Arabia and South Korea hinders applicants in providing the relevant additional information. All use CTD submissions, still they differ in regional information, some technical data (test batches, stability data, etc.), and order layout for the data arrangement.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Table 1: Comparative review of the DMF filing requirements in US, EU, Brazil, Saudi Arabia, Singapore and South Korea [8,6,9,10,11,12,13,14,15]

Drug Master File Requirements	Regulated Markets		Emerging Markets			
	United States	**European Union**	**Brazil**	**Saudi Arabia**	**Singapore**	**South Korea**
Health Authority	Food and Drug Administration (FDA)	European Medicines Agency (EMA)	Brazilian Health Surveillance Agency (ANVISA)	Saudi Food and Drug Authority (SFDA)	Health Science Authority (HSA)	Ministry of Food and Drug Safety (MFDS)
For API	Drug Master File	Drug Master File	Active Pharmaceutical Ingredient Dossier (DIFA)	Drug Master File	Drug Master File	Drug Master File
Types of DMF	**Type II:** Drug substance, Drug substance intermediate , and materials used in their preparation or drug product **Type III:** Packaging Materials **Type IV:** Excipient,	NA	NA	**Type I:** Drug Substance or intermediate in the manufacture of a drug substance. This can include (API) or biological Ingredients. **Type II:** Container closure systems or components	NA	NA

	Colorant, Flavour, essence, or material used in their preparation **Type V:** FDA-accepted reference information			**Type III:** Excipients, colorants, flavours, and other additives. (2009) No types available (2014) amended guidelines		
CTD Modules	Module 1, 2, and 3 are submitted	Only Module 3 is submitted	Module 1, 2, and 3 are submitted	Module 1, 2, and 3 are submitted	Only Module 3 is submitted	Only Module 3 is submitted Once approved agent as to submit module 2
Applicant (Open) part (AP)/ Restricted (closed) Part (RP)	There is no distinction between AP and RP. The authority receives a consolidated DMF. The customer is only given AP. The customer receives RPupon request.	The AP is submitted by MAH as part of MAA The DMF holder submits the RP directly to the agency	The customer receives the AP, which is then sent to ANVISA. To get a DMF number, the RP is transmitted directly to ANVISA as a supporting document. DMF information is automatically sent to ANVISA if the API producer and formulator are the same entity.	Both AP and RP are submitted by DMF holder to the authority The customer receives the AP, which is then sent to SFDA.	Both AP and RP are submitted by DMF holder to the authority The customer receives the AP The DMF holder submits the RP directly to the agency	The agent receives the AP and forwards it to the appropriate authority. The applicant submits the RP to the authority directly.
Forms for registration	FDA Form 3938 to be implemente d in late 2021	NA	NA	NA	NA	Form 16 and Form 17
Filing format (eCTD/PDF)	eCTD	eCTD	PDF/CD	PDF/CD	PDF/CD	PDF/CD
Review Timeline	Complete the assessment within 60	Depends on type of marketing authorizatio	Initial queries will take up to two or three months.	DMFs are processed in the order in which they	Depends on type of application of drug	Queries need to be answered within one month after being

	days of receiving the required fees. The scientific review will take 6-8 months.	n procedure to which the DMF is linked	According to the questions raised, clearance takes more than a year For **Priority drug substance**approx. 120 days	were received. Within 10 days of assigning a DMF number to a full package, a letter of acknowledgment is issued to the DMF owner or agent, confirming that the DMF has been established.	product to which the DMF is linked	submitted. Clearance takes 6-18 months, depending on how long it takes to answer the queries.
Agent / Authorized Person Requirement	Not mandatory but recommended to have a agent	Not required	NA	Authorized DMF agent can be appointed for the correspondent for reply to the SFDA by notification to authority for appointment	Authorized agent is appointed to act as point of contact to the agency concerning the Drug Substance filed	Authorized person agent is required
Language	English	English or French	Portuguese	Arabic or English	English	Korean and English The RP filed in English language is directly sent to the authorities.
Pharmacopoeia	United states Pharmacopeia (USP)	European Pharmacopoeia (EP)	Depending on the applicant's submission, European Pharmacopoeia (EP) / United States Pharmacopoeia (USP)	USP/EP based on consumer requirements,	USP/EP, Dependent on consumer requirements,	Dependent on consumer needs, USP/EP
Module 1	1. Cover letters 2. Letter of authorizations (LOAs) 3.	1. Cover Letter 2. TSE/BSE certificate 3.Declarations	1. Letter of suitability (CADIFA) If required 2. Cover letters 3. Query letters	1.Cover Letter 2.DMF submission form 3.Letter of Access	1. Cover letter 2. DMF application and Amendmen	Open Part of the DMF applicant shall be forwarded to the agent and filed with the required

	Declarations 4. GMP certificate 5. Form 3794 6. Label with storage conditions and expiry date/retest date	4.Letter of Access (LOA) 5.GMP Certificate	4. GMP certificate 5. Application forms customer-specific 6.TFVS Payment receipt	4.GMP Information 5.Agent Authorization	t Form 3. Fee Form 4. GMP Information 5. Letter of Access 6. Agent Authorization	applications or forms by the agent. The company will provide **cover letters** before sending RP to the agency
Life cycle Management (Amendments/ Annual reports)	**Major changes** – tell and do; With Amendments **Minor changes** – Annual reports/ Annual updates. Also provided along with amendments	**Major variation** Type II (Prior approval) **Minor variations** Type IA (Do and Tell) Type IA (IN) Type IB. (tell and do) Annual Updates	**Major Changes** (Prior approval before 180 days) **Minor Changes** (prior approval before 120 days) **Immediate Notification** **Annual Notification** (Within 12 Months)	**Major Changes** (Prior approval) **Minor Changes** (Do and tell procedure) Annual reports *If no changes were made within the last 5 years, a letter indicating that the DMF remains current	**Minor Variations MIV-1** (Prior consent is required from agency) **MIV -2** (Notification / Do and tell)	**Major modifications –** tell and do; Annual updates should be presented by December or January
Submission Pathway or Forwarding address	Electronic Submission gateway (ESG)	Common European Submission Portal (CESP)	Electronic Petitioning system **or** À AgênciaNacional de VigilânciaSanitária (Anvisa) COIFA SIA Trecho 5, Área especial 57, Lote 200 CEP: 71205-050 Brasília – DF, Brasil	Saudi Food and Drugs Authority 4904 northern ring branch rd - HittinDist Unit number: 1 Riyadh 13513 – 7148, Saudi Arabia	Therapeutic Products Branch Health Sciences Authority 11 Biopolis Way Level 11, Helios Singapore 138667	Ministry of Food and Drugs Safety 187, Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Republic of Korea [28159] Tel: +82-43-719-1564 (for English) | 1577-1255 (for Korean)
Validity	**There is no validity** **The DMF is**					

	reported to be operational on the basis of the manufacturer's annual updates.					

Table 2: Comparison of Contents of DMF(8,6,9,10,11,12,13,14,15)

Drug substance 3.2.S (ICH-CTD)		**Regulated Markets**		**Emerging Markets**			
		United States	**European Union**	**Brazil**	**Saudi Arabia**	**Singapore**	**South Korea**
3.2.S.1 General information	**S.1.1 Nomenclature**	-Generic name -International Non-proprietary name (INN) -Chemical Abstract service (CAS) Register Number -Company or lab Code -US Pharmacopoeia monograph name	-European Pharmacopoeia monograph name -INN -another chemical name	-Brazilian Non-proprietary Name (DCB) - INN -compendial name - CAS registry number	Non-proprietary name (INN) -Chemical Abstract service (CAS) Register Number -Company or lab Code -US/EU Pharmacopoeia name	Non-proprietary name (INN) -Chemical Abstract service (CAS) Register Number -Company or lab Code -US/EU Pharmacopoeia name	
	S.1.2 Structure	-Structural formula -Molecular formula -Relative molecular formula -Therapeutic Class	-Structural formula -Molecular formula -Molecular structure - Therapeutic Class	-Structural formula, (stereochemical configuration) -Molecular formula - Relative molecular mass	-Molecular structure -Molecular formula -Therapeutic class -Molecular weight	-Structural formula, (stereochemical configuration) - Molecular formula - Relative molecular mass	
	S.1.3 General properties	-Description -Solubility - pH -pKa value by HPLC	A list of physicochemical properties and other relevant	-Solubility -PKa - polymorphism -	-Chirality -dissociation constant -Physical description	-Description -Specific optical rotation - Melting range Hygroscopicity	Solubility in ether

		-Partition coefficient	properties should be provided.	isomerism - log P -permeability and hygroscopicity.	-Thermo gravity analysis	- Polymorphism - Isomerism	
3.2.S.2 Manufacture	**S.2.1 Manufacturer**	- **Details of holder of DMF**: Name, designation, Telephone Number, fax No, contact person's name, Contactperson's email address **-Details of manufacturing and testing Facilities along with their function** Name, Address, Telephone No, Fax No, DSUN and FEI No, contact person's name and email address**-Regulatory Agent Details** Name, Address, Telephone No, Fax No, contact person's name with an email address	The name and address of each manufacturer, as well as their involvement in the production process from the introduction of raw material(s) to the finished substance, should be provided.	name, address, and responsibilities of the units that perform intermediate and API production processes Agent details are not vital			Agent details are not essential

	S.2.2 Description of manufacturing process and controls	-Codes of Starting materials, Intermediates and final drug substance -Synthetic Scheme -Process flow diagram -Detailed description of manufacturing stages -BPR's* -Reprocessing and Recovery procedure. (If applicable)	-Detailed description of the manufacturing process -Flow diagram (Structural formula for SM* & intermediates) -batch size -Reworking, recovery, blending of production batches (if applicable)	Synthetic scheme -Information on non-isolated intermediates -Batch size - Process parameters, -Identification of critical steps and process controls -Scale of manufacture and yield ranges			Data on ingredients, solvents and reagents used in manufacturing process-synthesis, extraction and grinding -documentation on test procedures involved
	S.2.3 Control of materials	S.2.3.1 Specification and test procedure for all SMs S.2.3.2 Specification and test procedure for all SMs S.2.3.3 Critical material attribute; COA's for SM's and RM's	-Specification & description of analytical methods -control and absence of carryover of potential impurities -Source and justification for selection of SM	- KSM justification	list of RM (SM, solvents, reagents, catalyst) used -Specification & analytical method Specification of recovered solvents (if applicable)		
	S.2.4 Control of critical steps & Intermediates	-Specification and test procedures for in-process stage -Critical process parameters -specifications and test	-specification & description on impurities found in isolated intermediates				

		procedures for intermediates					
	S.2.5 Process Validation	-3 batches of validation data -Stage wise yield -API batch data - Intermediate quality data	PPQ protocol and reports	-Validation studies, protocols and reports - validation of API from KSM prior to marketing			Protocols and reports
	S.2.6 Manufacturing process development	Contains a summary of manufacturing process development	Development & scale-up information; - manufacturing process controls -In process controls - Specifications & analytical procedures	NA			
3.2.S.3 Characterization	**S.3.1 Elucidation of structure and other Characteristics**	-NMR study [^{1}HNMR &^{13}CNMR], Mass, UV or IR spectra -Elemental analysis -Assessment of polymorphism, Isomerism - Qualitative analysis report	-Suitable Identification test as described in the Eu Pharmacopoeia	-explaining certain characteristics and other polymorphs associated to the API - Refractive Index & Chirality - Reference standard Structure elucidation - Possible isomers structural, geometric, optical data			-Although it is a pharmacopoeia product, structural elucidation (SE) for the working standard is essential (commercial batch) -Polymorphs and other associated characteristics

	S.3.2 Impurities	Stage-by-stage impurity source, removal or control of unknown impurities, and residual solvents should be given in the path of synthesis of starting material, intermediate, and API. -Validation data & Chromatograms	-detailed impurity discussion provided -Analytical methods & minimum validation data along with suitability - LOD/LOQ's	-Validation of critical parameters of analytical procedures used in carry-over studies -justification for the absence of tests for potential impurities that are not controlled			-All chromatograms, including batch analysis, and carryover studies -With the exception of class III solvents, skip lot test for genotoxic impurities and solvents. is necessary
3.2.S.4 control of Drug Substance	**S.4.1 Specification**	-	-	-Particle size distribution			-solubility in ether
	S.4.2 Analytical Procedures	The standard testing procedure (STP's) and specifications should be provided	Instead of the Ph. Eur. method, applicants should propose a quantitative technique to	Description on analytical procedure used for release & stability studies must be provided.			Data for solubility

			regulate the associated substances that are expected to be present in the substances				
	S.4.3 Validation of analytical procedures	Method validation protocol and report along with typical chromatograms	In the event of an in-house technique, it should be verified and cross-validated using the Eur. Ph monograph. - 3 batches are required for comparison with appropriate impurity spiking for substance which are very pure	Validation should be done in accordance to Article 43 of RDC 166/2017			
	S.4.4 Batch analysis	3 batches COA's is required	The results of 3 batches of complete tests should be provided.	3 batches COA's is required			DMF contains the raw data for one batch from three batches of CoA's.
	S.4.5 Justification for specification		Additional limits /deviations should be justified if applicable				
	3.2.S.5 Reference Standard	- CoA for primary reference standard (RS) and working standard (WS) - Data on drug substance qualification	IR spectrum and CoA for RS or in house standards	RS CoA's and validation data must be provided			

	3.2.S.5 Reference Standard	- CoA for the primary reference standard (RS) and the working standard (WS) -Qualification data on drug substance (RS)	IR spectrum and CoA for RS or in house standards	RS CoA's and validation data must be provided			
3.2.S.6 Container closure system		-Brief description of container closure system and type of material used. - Statements of certification for contact materials used in food and drugs. - Manufacturer, specifications, and representative CoA for primary and secondary packaging materials	Description, specification, and identification (IR spectra) of proposed materials -suitability with respect to choose of materials, protection from light, moisture & compatibility -Stability data	Materials safety data sheet (MFDS)			To make sure about the safe ty, specify the packing system and reasons for container selection in detail Materials safety data sheet (MFDS)
3.2.S.7 Stability	**S.7.1 Stability summary and**	-Stability acceptance criteria -Info on test	Stability data and storage condition justification	Forced degradation studies (NLT 3.6			Proposed storage conditions and retest

	Conclusion	performed and their limits - Retest period -data from forced degradation studies (1.3Million lux hours)	-observed trends to support the proposed retest period	million lux hours) One batch is required. 2 more batches considered if initial study is non conclusive			period or shelf life shall be provided Frequency of analysis
	S.7.2 Post approval stability protocol and stability commitmen t	Stability Protocol and stability commitment is provided	If the retest period is based on pilot scale batches- the manufacturer must submit the additional stability data	According to RDC318/201 9, a post-approval stability protocol and stability pledge should be given.			
3.2.S.7 Stability	**S.7.3 Stability Data**	Stability data of 3 process validation batches for -long term condition -Accelerated stability studies	-Stability data sheets -Zone II (30°C ± 2°C/35% RH ± 5% RH) long term stability studies - Chromatogra ms of each stability stations. - Photo stability studies	Zone IV b 30ºC±2 ºC /75%RH± 5% RH; API stability study. Intermediate stability studies are not required The stability results should be provided, according to RDC 318/2019.			-Long-term preservation and worst-case evaluation results -Accelerated test data -Intermediate stability is not required - Forced degradation studies are not required

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RESEARCH ARTICLE

Effect of Hydraulic Loading Rate

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ABSTRACT

The present study is an experimental research using a laboratory model on Fixed Bed Fixed Film (FBFF) reactor for evaluating its treatment performance of treating sugar effluent stream, as representative stream of highly biodegradable industrial effluent stream. The active anaerobic microorganisms attached with the surface of the filling media, offers low fill media ratio that will essentially biodegrade the Chemical Oxygen Demand (COD) under endogenous phase, resulting in COD removal at more than 78% under varies Hydraulic Loading Rates (HLR). The loadings are 0.002, 0.005, 0.007, 0.010, and 0.012 m^3/m^2/day. The experimental model is designed to have plastic models that have a higher net surface area for attached growth biomass. The model has an effective volume of 24.36 litres with plastic modules filled for 41% of the reactor volume with a total surface area of 5 m^2.The model was fitted with a peristaltic pump that can load influent at 0.5 to 2.5 lit/ hr. which correspond to hydraulic retention time (HRT) of 9.6 to 48 hrs.

Keywords: FBFF, COD, HLR, HRT, Peristaltic Pump and Plastic Modules.

INTRODUCTION

Now a day's water is more necessity parameter in our life. Environmental and water pollution has become an issue of serious international concern in recent years. So the waste water treatment technology is very important for prevent the environment, reduce the water pollution, increase the sources of water and also increase the renewable sources. Generally the waste water is divided into two classifications one is sewage waste water and another one is industrial waste water. Anaerobic treatment process is lower energy requirements, less costly, which is required small amount of space, simple operations and finally they have produce less amount of sludge as compared to the

aerobic processes. The COD removal up to 85% through anaerobic process is making it as the most effective and economic option for providing treatment to any industrial liquid streams.

Sources of Sugarcane Waste Water

The Sugar cane is collected, cleaned and washed before it is crushed to yield the juice from it. The juice is evaporated for the removal or evaporation of water to get sugar crystals. The sugar cane is first washed with fresh water and then it is shredded using shredding machine in the Mill house and sent for crushing to extract the juice from the sugar cane. About 93% of the juice is extracted and the fibrous residue will be left as Bagasse. The extracted juice is screened for removing the floating impurities. After screening, milk of lime is added to increase the pH from 7.6 to 7.8 in order to prevent from corrosion and to aid clarification by coagulating the colloidal impurities along with the addition of a coagulant aid. The mixture is pre heated using high-pressure steam and allowed to settle. The clarified juice is bleached and sent for evaporation, where the juice is reclaimed. The residue result in the filters is called Pressmud. The concentrated juice after evaporation is fed into a Multiple Effect Evaporator, where the sugar is concentrated. The concentrated syrup is known as massecuite which will be passed into a crystallizer. The crystal and syrup is separated in the Centrifuge systems. The spent liquor is collected as molasses and the sugar crystals are collected and dried as Sugar.

METHODOLOGY

The model of FBFF was constructed based on an empirical design approach for 24.36 litres of effective volume. The dimentions of the experiment is designed to match the size of peristaltic pump and proposed influent COD ranges which were decided in the particular characterization of the respective waste stream. The experiment was started with domestic waste water followed by real time waste water of Sugarcane and after the process stabilization; the reactor was continued run for simulated synthetic waste stream.

FBFF Reactor - Laboratory Model

The experimental setup consists of FBFFR model which is made out for flexi glasses. The cylindrical portion of the reactor is 1.05 m height and 0.2 m diameter. The top of the reactor hermitically sealed to avoid any air fit-up. The reactor is packed with polypropylene microbial support media for 41% v/v. The reactor is fed from the inlet (sugarcane wastewater) tank by means of a peristaltic pump of Miclin's make (model pp-15). The wastewater is pumped to move upward from the bottom passing through packed media. Two ports are provided one for desludge at bottom and another one for sampling at top. The diagram of the model is presented in figure.1 respectively. The design of reactor is construct on the basis of Flow Rate, Influent COD, Hydraulic Retention Time, Volumetric Loading Rate, Hydraulic Loading Rate, Reactor VSS and Organic Loading Rate. The physical dimensions and process in parameters for the experimental model of the FBFFR model as represented in the Table 1. The model was run the Sugarcane wastewater which was started from 94th day, from actual date of reactor commissioning by batch mode with domestic wastewater. The model was fed with real time effluent of sugarcane wastewater effluent, slowly in mixed state with sewage in stages of 20%,40%,60%,80% and 100% of real time effluent was made to pump in two weeks time. Then, synthetic effluent was mixed and replaced the real time effluent in the next two weeks in stages of 20%, 40%, 60%, 80% and 100%. The observations on model was started with % of COD removal as the treated wastewater started comes out as clear, colourless liquid and the biogas generation was observed for a maximum of 0.29 m^3 per kg COD removed.

RESULT AND DISCUSSION

The reactor was operated five different flow rates viz., 0.5, 1.0, 1.5, 2.0, and 2.5lit/hr. That corresponds to HRT of 48, 24, 16, 12 and 9.6 hrs. That corresponds to hydraulic loading rates (HLR) of 0.002, 0.005, 0.007, 0.010 and 0.012 m^3/m^2/day respectively.

Considering the evaluation of the reactor model in respect of the % of COD removal, the experimental model results were accounted for HLR. The appropriate graphs were presented in Fig 2 to Fig 6.Considering the evaluation of the reactor model in respect of HLR, the experimental results were accounted for bio-gas generation m^3/kg of COD removed. The appropriate graphs were presented in Fig 7 to Fig 11. The maximum % of COD removal was observed at 85.16% for an operating HLR of 0.002 m^3/m^2.day and HRT of 48 hrs. The minimum COD removal efficiency was observed at 65.59% for an operating HLR of 0.012 m^3/m^2.day and HRT of 9.6 hrs.The maximum bio-gas generation was observed for 0.309 m^3/kg of COD removed that correspondent HLR 0.002 m^3/m^2.day and the % of COD removal was observed 75.85 %.The minimum bio-gas generation was observed for 0.206 m^3/kg of COD removed that correspondent HLR 0.012 m^3/m^2.day and the % of COD removal was observed 70.15 %.

CONCLUSION

The FBFF reactor is establish to treat sugarcane effluent for a maximum COD removal efficiency of 85.16 % with 0.309 m^3/kg COD removed. Therefore, it can be used for removing up to 85 % COD in sugarcane effluent.

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Table 1 Physical Dimensions with Process Parameters of Experimental Model: FBFFR

Specifications	**Fixed Bed Fixed Film Reactor (FBFFR)**
Total volume of the reactor, litre.	32
Effective volume of the reactor, litre.	24.36
Total height of the reactor, m	1.24
Effective height of the reactor, m	0.87
Effective diameter of the reactor, m	0.19
Height of the microbial support fill media, m	0.36
Diameter of the influent and effluent pipes, mm	8
Peristaltic pump (Miclin's make)	PP-15 model
Mesh or bearing plate hole size mm,	2.5
Operating Parameters	
Influent flow rate, litre/hr.	0.5, 1.0, 1.5, 2.0 and 2.5.
Influent COD, mg/lit	1972, 3022, 4022, 4974, 6024
For synthetic sugarcane effluent, mg/lit	
Volumetric Loading Rate, kg.COD/ m^3.day	0.960 - 15.550
Organic Loading Rate, kg COD/ m^2.day	0.005- 0.075
Hydraulic Loading Rate, m^3/ m^2/day	0.002 - 0.012

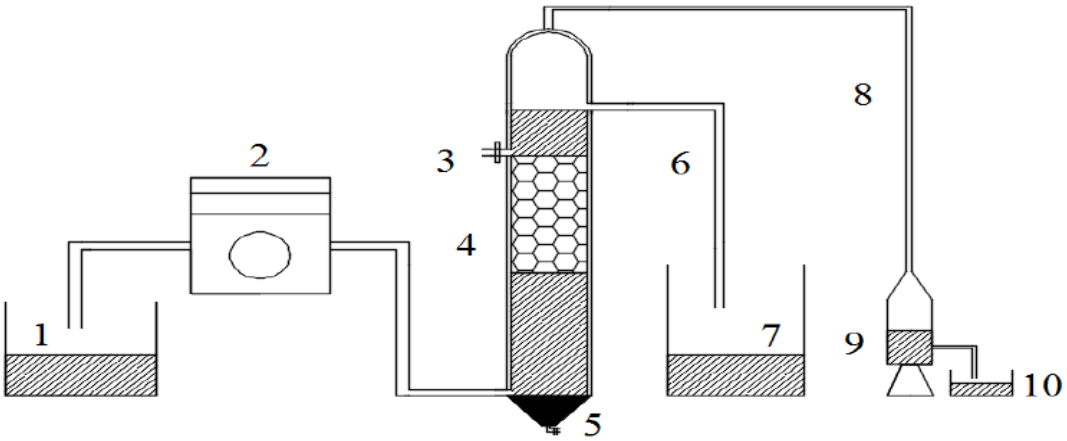

Figure 1: The Diagram of Experimental Setup.

1. **Influent tank**
2. **Peristaltic pump**
3. **Sample port**
4. **Microbial support media**
5. **Desludge pipe**
6. **Treated effluent pipe**
7. **Treated effluent**
8. **Gas pipe**
9. **Water displacement reactor**
10. **Displaced water collection jar**

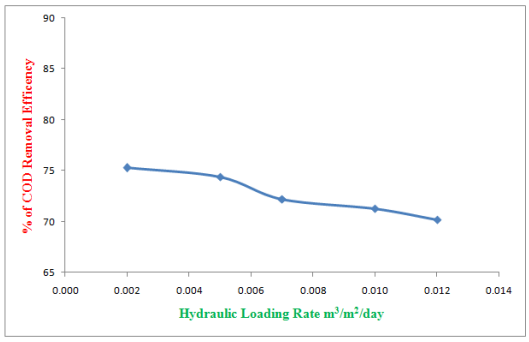

Fig.2 Hydraulic Loading Rate Vs% of COD removal.

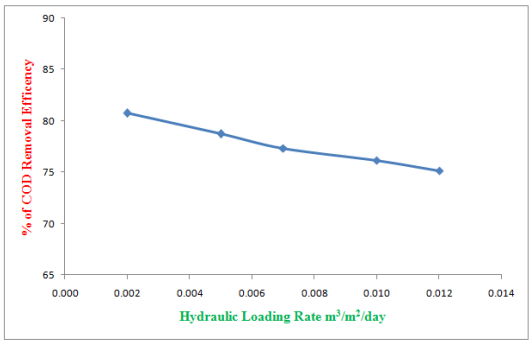

Fig.3 Hydraulic Loading Rate Vs% of COD removal.

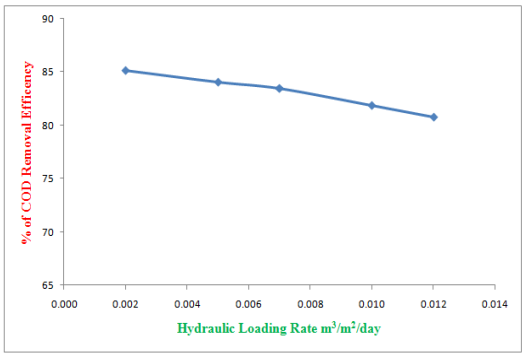

Fig.4 Hydraulic Loading Rate Vs% of COD removal.

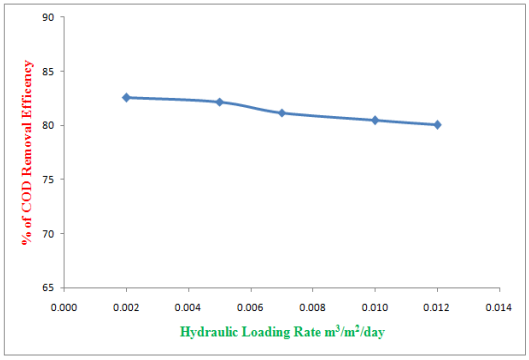

Fig.5 Hydraulic Loading Rate Vs% of COD removal.

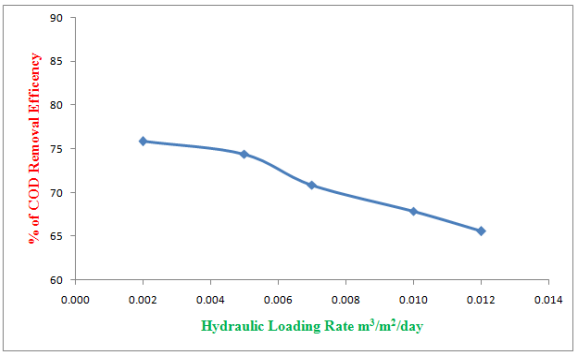

Fig.6 Hydraulic Loading Rate Vs% of COD removal.

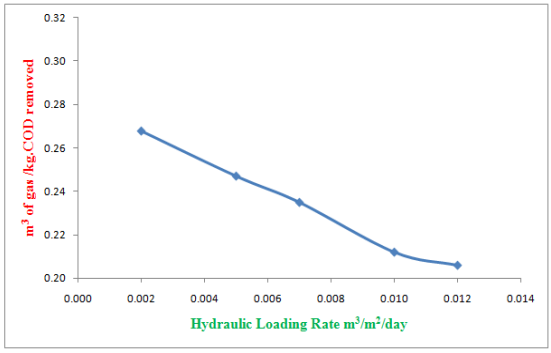

Fig .7 Hydraulic Loading Rate Vs m³ of gas / kg.COD removed

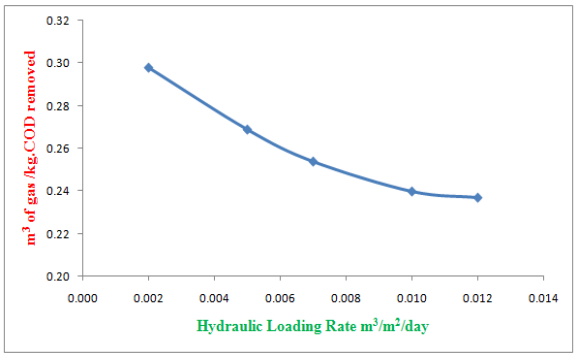

Fig.8 Hydraulic Loading Rate Vs m³ of gas / kg.COD removed.

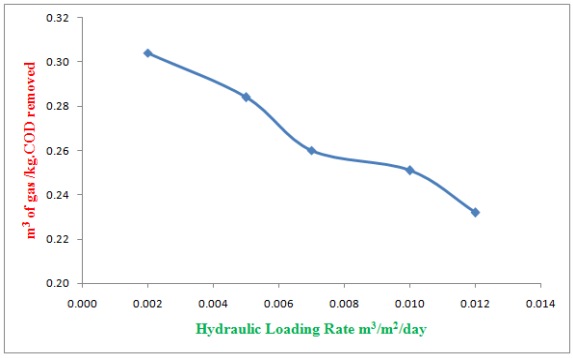

Fig. 9 Hydraulic Loading Rate Vs m³ of gas / kg.COD removed.

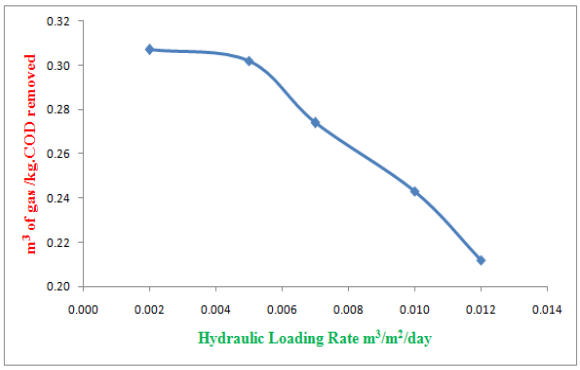

Fig. 10 Hydraulic Loading Rate Vs m³ of gas / kg.COD removed.

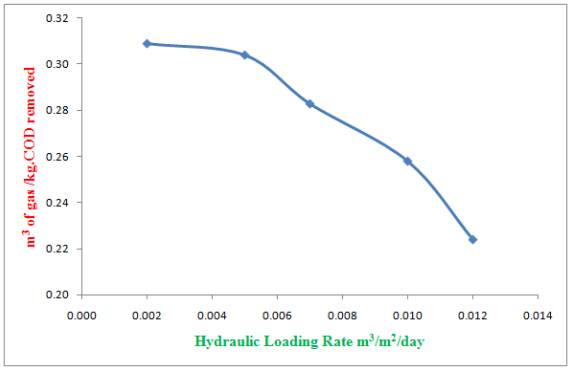

Fig. 11 Hydraulic Loading Rate Vs m³ of gas / kg.COD removed