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RESEARCH ARTICLE

Evaluation of *In-vitro* Rheumatoid Arthritic Activity of Polyherbal Ethanolic Extract Containing Formulations for Selected Potential Indian Herbs

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ABSTRACT

Arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response.[3] One fifth of the world's elderly suffer with arthritis .In the anti-arthritic activity the production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. The mechanism of denaturation probably involves electrostatic hydrogen, hydrophobic and disulphide bonding. Anti denaturation study which includes the albumin denaturation is performed by using Bovine serum albumin (BSA). When BSA is heated it undergoes denaturation and express antigens associated with type 3 hypersensitivity reactions and that is related to diseases such as serum sickness, glomerulonephritis, rheumatic arthritis, and lupus erythromotosus.

Keywords: Inflammatory, Arthritis, Medical response, Lysis

INTRODUCTION

Inflammation is a normal protective response to tissue injury which involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [1]. It is characterized by redness, swelling, pain, stiffness of joint and loss of joint function. Inflammation is associated with membrane alterations, increase in vascular permeability and protein denaturation [2]. Arthritis is a chronic, inflammatory, systemic

autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response [3]. One fifth of the world's elderly suffer with arthritis [4]. The current treatment of arthritis includes minimization of this associated pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using anti-rheumatic drugs [5,6]. Due to adverse reactions of the NSAIDs and disease modifying anti-rheumatic drugs, the arthritic patients tend to search for other treatments that are effective and less toxic. Therefore, complementary and alternative medicines are commonly preferred by such patients [7].

Polygonumglabrum: the tribes of Chhattisgarh use the root paste as a medicine for snake bite [20]. In some areas the root stock is used for the treatment of jaundice and piles [8]. The leaves are used as an antimalarial agent in Sudan [9]. In south India *Polygonumglabrum* leaves are used for the treatment of dysentery. [10] A decoction of the leaves and seeds are used as cardio tonic, astringent and anthelmintic [11]. The whole plant decoction is used as a remedy for colic pain, pneumonia and the boiled paste is applied in cuts and wounds [12]. Apart from medicinal use, the whole plant is powdered and used as bait for fishing. Peels from stem are used for treating rheumatism [13]. *Ochnaobtusata DC*. (Family Ochnaceae).is a small tree up to 8 m tall. The family is characterized by the presence of secondary metabolites like flavonoids, terpenoids [14]. and it is extensively used in Indian traditional medicine for the treatment of epilepsy, menstrual complaints, lumbago, asthma, ulcers, and as an antidote to snake bites [15]. Several studies conducted on *Ochna* species revealed the presence of glycosides, saponins, steroids, flavones and fatty acids [16]. The leaves and roots of *O. obtusata* are used for ulcer, asthma and bronchitis and also possess anti-ulcer genic activity [17].

Canthium dicoccum Ethanolic extract of whole plant of *Canthium dicoccum* for anti-inflammatory activity in Wister albino rats in various models of anti-inflammatory activity viz. Carrageenan induced paw edema, Formalin induced paw edema, fresh egg white induced paw edema and cotton pellet induced granuloma model. Results showed the extract with anti-inflammatory activity and suggests a potential alternative to NSAIDS like diclofenac [18]. ethanolic extract of *Canthium dicoccum* for anti-diabetic in an alloxan induced diabetic rat model. Results showed a significant drop in fasting blood sugar in a dose-dependent manner, with an effect on the beta-cell population in the pancreas. The extract showed almost equipotent anti-diabetic activity compared to standard drug Glibenclamide [19]. Ethanolic extract for anti-arthritic activity in albino rats. Results showed significant anti-arthritic activity against Egg-albumin induced arthritis model [20]. In the recent studies of the author ethanolic extract of the above plants and the polyherbal formulations with different fractions of ethanolic extract showed good anti-oxidant activity. The present study is deigned to evaluate the ant arthtric activity for the different polyherbal formulations

MATERIALS AND METHODS

Plant source and authentication

Polygonum glabrum, Ochnaobtusata DC, and Canthium dicoccum was collected from Tirumala Hills, Tirupati, and Chittoor district of Andhra Pradesh, near Seshachalam and Tirumala Hills. The plant specimen was verified to be of the correct species by Dr. MadhavaSetty, a botanist from the Department of Botany, S. V. University, and Tirupati.

Chemicals and reagents

All the chemical are used analytical grade and the Egg Albumin, Bovine Serum And Diclofenac sodium obtained from sigma Aldrich

Preparation of poly herbal extract

Aerial parts of *Polygonum glabrum, Canthium dicoccum, Ochnaobtusata* were collected and dried. Then the material was blended to form a fine powder and extracted Ethanol using Soxhlet apparatus for 6 hrs at 50ºC and water by maceration the solvent was completely removed by rotary evaporator (Rotavapor® R-210, BUCHI Corporation) and respective extracts preserved for various investigations.

Preparation of ethanol extract of four selected polyherbal formulations using different portions. The above extract used for the preparation of five different poly herbal formulations with varying proportions and working formula given in the table.1

Table 1. Five different poly herbal

S.NO	FORMULATIONS	PGEE	CDEE	OOEE
1	FORMULATION 1	1	1	1
2	FORMULATION 2	2	1	1
3	FORMULATION 3	1	2	1
4	FORMULATION 4	1	1	2

Preliminary phytochemical studies:[28-30]

Previously various preliminary phytochemical tests were performed using standard procedures and the above formulations showed the presence of mainly carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids and saponins which majorly responsible for the desired activity .

Evaluation of in-vitro anti-arthritis of polyherbal formulations

Inhibition of protein denaturation method using bovine serum [31 -34]

Preparation of the standard solution: The standard solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5 % w/v aqueous solution) and 0.05 ml of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml). Preparation of the test solution: The test solutions (0.5 ml) were prepared using 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml). Preparation of the test control solution: This solution (0.5 ml) was prepared using of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.

Experimental procedure

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

Inhibition of protein denaturation method using egg albumin [35-38]

Preparation of the standard solution: The standard solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml). Preparation of the test solution: The test solutions 5 ml were prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of different concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml). Preparation of the test control solution: This solution prepared using 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of distilled water.

Experimental procedure

All above solutions were incubated at 37 ± 2°C in incubator for 15 min and then heated at 70°C for 5 min. After cooling their absorbance were measured at 660 nm by using vehicle as a blank. The percentage inhibition of protein denaturation was calculated by using the following formula

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

HRBC membrane stabilization method [39-45]

The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and was mixed with equal volume of sterilized alsevers solution. The blood solution was centrifuged in a centrifugation machine at 3,000 rpm for 15 min and the upper layer was carefully removed with a syringe or sterile pipette. The packed cells remained at the bottom were separated and washed with isosaline solution and a 10% v/v suspension was made with isosaline. Human red blood cells suspension was used for the study. Preparation of the standard solution: The standard solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of Diclofenac sodium solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of distilled water Preparation of the test solution: The test solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline 0.5 mL of extract solution in various concentrations (10, 50, 100, 200, 400, 800 and 1000 µg/ml) and 2 ml of distilled water. Preparation of test control: The test control solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, and 0.5 mL of 10%w/ v human red blood cells in isotonic saline, 2.5 mL of distilled water

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Experimental procedure

All the assay mixtures were incubated at 37ºC for 30 min and centrifuged at the rate of 3,000 rpm. The supernatant liquid was poured out and the hemoglobin content was estimated by UV spectrophotometer at 560 nm. The percentage of human red blood cell membrane stabilization or protection against hypotonicity induced hemolysis was calculated by using the following formula.

Percentage protection = 100- [(optical density sample/optical density control) × 100]

RESULTS

Table no .2 Inhibition of protein denaturation method using bovine serum

Conc µg/ml	Standard solution	F1	F2	F3	F4
10	51.92±0.4	37.12±0.4	44.82±0.24	34.22±0.12	39.36±0.34
50	57.81±0.5	43.24±0.6	51.91±0.3	42.91±0.24	46.18±0.4
100	63.14±0.9	49.42±0.6	54.64±0.6	45.82±0.19	69.26±0.8
200	79.12±1.5	57.12±2.5	71.24±1.3	53.64±0.9	61.92±1.4
400	85.46±0.8	65.46±0.4	78.36±0.6	67.74±0.25	64.82±0.6
800	92.16±0.9	74.24±0.2	84.29±0.9	72.92±0.16	76.19±0.8
1000	94.28±1.0	81.26±0.5	89.16±0.6	79.29±0.24	82.28±0.28

Table no .3 Inhibition of protein denaturation method using egg albumin

Conc µg/ml	Standard solution	F1	F2	F3	F4
10	52.32±0.8	46.12±0.25	49.42±0.24	42.12±0.5	46.89±0.5
50	58.24±0.6	48.24±0.62	53.91±0.3	44.24±0.2	49.12±0.12
100	65.04±1.2	51.42±0.24	57.64±0.6	48.42±0.4	52.22±0.4
200	73.24±1.4	56.12±2.5	69.24±1.3	51.12±1.5	58.25±2.15
400	76.08±1.18	63.46±0.4	74.36±0.6	59.46±1.4	64.14±0.41
800	79.16±1.5	72.24±0.2	76.29±0.9	68.24±1.2	73.43±0.12
1000	86.14±1.2	79.26±0.5	83.16±0.6	74.26±0.5	81.12±0.15

Table no .4 HRBC membrane stabilization methods

Conc µg/ml	Standard solution	F1	F2	F3	F4
10	56.92±0.4	41.56±0.46	44.56±1.2	39.56±0.4	39.96±0.14
50	62.81±0.5	49.21±0.42	51.21±1.5	45.21±0.6	50.21±1.3
100	89.14±0.9	82.13±1.3	84.13±0.9	82.13±0.25	81.13±1.6
200	91.59±1.5	85.14±0.2	89.14±1.4	78.14±1.2	84.14±1.2
400	94.26±0.8	89.46±1.4	92.46±0.4	84.46±0.6	86.46±0.9
800	96.59±0.9	90.49±1.2	94.49±0.5	89.49±1.3	92.49±0.5
1000	98.98±1.0	92.98±0.25	96.98±0.26	93.98±0.4	93.98±0.6

DISCUSSION

The Ethanolic extracts of *Polygonum glabrum, Canthiumdicoccum, Ochnaobtusata,* are formulated into four ormulation with different portion as given in the above formula and evaluated for *in -vitro* anti-arthritis activity with concentrations varying from 10 µg/ml to 800 µg/ml.by the *in –vitro* Inhibition of protein denaturation method using bovine serum, Inhibition of protein denaturation method using egg albumin and HRBC membrane stabilization method comparing with standard drug as Diclofenac sodium.in the five formulation F2 and F4 having the significantly more inhibition of protein denature and membrane stabilization which are concentration dependent.

CONCLUSION

The above result gives a conclusion that the polyherbal formulations with the different concentration have the anti-arthritis activity and the F2 and F4 having significantly more potential. Further investigation is required to use the two formulations in the treatment of Rheumatoid Arthritis

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Conflict of interest

No conflict of interest

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RESEARCH ARTICLE

Fear and Misconceptions towards Suturing Procedure as a Hindrance to Healthcare Seeking Behaviour - A Questionnaire Survey

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ABSTRACT

The standard of oral health of a population greatly depends on elements such as diet, socioeconomic status, educational qualification and oral hygiene practices. Factors that might account for the less frequent utilization of oral health care by the general population are fear and negative attitudes toward dentists and dental care. Dental fears may be centered on a variety of facets of the dental experience including fear of pain, fear of needles or injections, fear of dental drills, fear due to past negative experiences, and fear of unsanitary practices or infections. The study aimed to evaluate the prevalence of misconceptions and fear towards suturing procedure and its influence on health seeking behaviour of the patients. A questionnaire descriptive “cross sectional study” that involved 200 patients as its participants was conducted among the outpatients in the age of 18-80 years attending the OPD of department of oral and maxillofacial surgery in our institution at Chennai. The percentage of people who believed in the myths were higher among the lower class and lower middle class of socioeconomic category. The findings of this study show that females have more belief in the myths and misconceptions than the males. Also, previous experience with extraction procedure is found to act as a factor in disrupting belief in these widespread misconceptions. The study population has significant belief in one or more misconceptions about suturing procedure and more than half the population has significant fear towards suturing procedure and suture needle which can significantly affect their health care seeking behaviour.

Keywords: Needle, Fear, Suture, Dental extraction, Health, Community, Phobia, Misconceptions.

INTRODUCTION

Favorable health behaviour refers to people's beliefs and actions aimed at avoiding harm, optimizing health and well-being, and preventing diseases. Poor oral health care behaviour is associated with oral health impairments and reduced oral health related quality of life [1]. The standard of oral health of a population greatly depends on elements such as diet, socioeconomic status, educational qualification and oral hygiene practices [2]. Indian population is composed of people from different cultural backgrounds and there is a significant influence of the myths on the health seeking behaviour of these people [3]. Factors that might account for the less frequent utilization of oral health care by the general population are fear and negative attitudes toward dentists and dental care. Dental fear research has been critiqued recently because most studies quantitatively assess fear with single item or a small number of possible dental fears. Consequently, they fail to examine the complexities of dental fear (e.g., types or sources of fear), leading some to argue that more research is needed on the specific types of situations that provoke dental fears [4]. Dental fears may be centered on a variety of facets of the dental experience including fear of pain, fear of needles or injections, fear of dental drills, fear due to past negative experiences, and fear of unsanitary practices or infections [5].

The term that is used in practice to describe an anticipatory fear of needle insertion is 'Needle phobia'. Needle phobia is a condition that has become an increasingly important issue in medicine because of the modern reliance on injections and needles for various health care procedures [6]. Needle phobia is not confined to children, is not an emotion-driven or transient phenomenon, and is not a rare condition. Clinicians need to be aware of needle phobia because it is a common condition and because needle-phobic persons tend to avoid medical treatment, which can lead to serious health problems as well as social and legal problems [7]. In addition to these fears being a response to physiological stimuli, it is also reinforced in the minds of the population by various myths and misconceptions regarding the usage of needles or the surgical procedure itself. Scientifically, myths are regarded as an extensive and unquestioned false perspective, that emerges from false traditional beliefs and non-scientific knowledge. These myths eventually get deep rooted in the minds of the future generations over a period of time and lead them towards a wrong protocol making it difficult for the dentist to provide satisfying treatment [8]. This kind of myths and misconceptions are also prevalent due to falsely exaggerated and manipulated information publicized by those who personally had a previous negative dental experience [9].

Understanding the prevalence of such myths and misconceptions becomes essential even to a health care provider to direct the society towards proper health care. The purpose of this study is to assess the prevalence of myths and misconceptions about suturing procedure and fear of suture needles among the population, the analysis of which provides an indirect measure of the knowledge, attitude and health seeking behaviour of the community.

MATERIALS AND METHODS

A questionnaire descriptive "cross sectional study" was conducted to assess the prevalence of myths and misconceptions about suturing procedure after dental extractions among the outpatients reporting to the department of oral and maxillofacial surgery of a dental college and hospital in Chennai. The study was conducted among the outpatients in the age of 18-80 years attending the OPD of department of oral and maxillofacial surgery in our institution at Chennai. The study involved 200 patients as participants. The patients who voluntarily agreed to participate were included in the study. Patients who refused to participate in the study and people who could not comprehend the questions of the study despite the assistance were excluded from the study. The identity of the patient who participated was maintained confidentially.

Collection of Data

A questionnaire was developed to assess the prevalence of fear and misconceptions among the outpatients of the department of oral and maxillofacial surgery. The questionnaire was developed in English language. All the questions were given alternative options to facilitate the participants to make quick choices, and participants were asked to tick the most appropriate answer from the given list of answers. Before finalizing the questionnaire, the questions were pretested in a pilot study on 20 patients, to assess their ability to interpret it. The questionnaire appeared to be easily understood and was finalized with no modification. Permission was obtained from the institutional ethical committee to distribute the questionnaire to the patients. The objective of the study was explained to all the patients who participated in the study and also verbal consent was obtained from all them. The completed questionnaire was collected back in 10-15 min by the investigator and checked for completeness. Any incomplete forms were asked to be completed. For the patients who did not understand the language a volunteer helped to translate all the questions with the choices of response and filled the form on behalf of the patient with the patient's choice of responses. The same volunteer translated the questions to every patient who had difficulty in understanding the language.

The questionnaire was divided into two parts. The first part of the questionnaire contained personal data of the patient such as name, age, gender, educational qualification, previous extraction history and B.G.Prasad's scale [10] was used to assess the socio-economic status. This scale divides the population into 5 categories based on their per capita income and the second part of the questionnaire contained 10 close ended questions about myths and misconceptions pertaining to suturing procedure and related fear about sutures after dental extractions. To statistically analyse the awareness among the study population regarding the dental myths, the responses to the questions were recorded as correct or wrong and each of the correct answers was given a score of 0 and the wrong answer was given a score of 1. Individuals receiving a total score of <5 was considered High attitude and those with total score ≥ 5 was considered Low attitude. Chi Square test was done to find their statistical significance. Level of significance was set as ≤ 0.05.

RESULT

Demographic data: (Table 1)

The majority of the respondents belonged to the age group 20-35 years (37%), followed by 35.5% of respondents from the age group 36-50 years. Only 3.5% of the respondents were from less than 20 years of age. Among the 200 patients surveyed 47% were males and 53% were females. Majority of the participants (45.5%) were from the middle-class category of socio-economic status.

Responses for questions regarding myth and misconceptions about dental extractions: (Table 2)

About 88(44%) of the participants believed that suturing the extraction socket is more painful than the extraction of the tooth itself, and 98(49%) participants stood against it. About 135(67.5%) participants felt that they got scared when they were told by their dentist that they might need sutures, while 60(30%) participants were not. Nearly 134(67%) participants thought more number of sutures indicated some complication in the surgery, while 59(29.5%) participants were against it. Almost 118(59%) participants believed that suturing the extraction site indicated some complication with the extraction while 67(33.5%) participants were against it. About 45 (22.5%) participants felt the return visit for removal of sutures is unwanted while 126 (63%) participants were ok with it. On the whole 59 (29.5%) participants believed that removal of sutures is a painful procedure and 121 (60.5%) participants stood against it. About 47(23.5%) participants thought that removal of suture requires administration of local anaesthetic through injections while 128 (64%) participants were against it. Nearly 71 (35.5%) participants believed that normal food

consumption is affected by placement of sutures while 105 (52.5%) participants were against it. Almost 102 (51%) participants believed that mouth opening is affected by placement of sutures while 78 (39%) participants were against it. On the whole 59(29.5%) participants avoided dental treatments due to the fear of sutures being placed while 138 (69%) participants disagreed with it. Majority of the patients had a significant fear towards suturing procedure and needle. Almost all the participants believed in one or more misconceptions pertaining to suturing procedure. Based on the scores given to the correct and wrong answers given by the participants as responses to the myths and misconceptions regarding dental extraction, the awareness of the participants was estimated.

Based on Gender

Among the participants of the survey 52.1% of males and 74.5% of females had a low attitude and fear about suturing, which showed females had more fear towards the suturing procedures and they had more belief on the false misconceptions. This was statistically significant ($P< 0.05$).(Figure 1)

Based on Socioeconomic Status

Among the participants of the survey 92.3% of the upper-class category, 84% of the upper middle-class category, 48.3% of Middle-class category, 14.3% of the Lower middle class and 3.4% of the Lower-class category had a high attitude. This showed that prevalence of fear and misconceptions about suturing after dental extractions were high among lower class category followed by lower middle-class category people. This was statistically significant ($P< 0.05$).(Figure 2)

Based on Previous Extraction History

Among the participants of the survey 87.2% of people who had previous experience of dental extraction had no fear towards suturing and presented with a high attitude while only 18.3% of people with no previous extraction experience showed a high attitude.(Figure 3)

DISCUSSION

Education provides an individual the means of empowerment and freedom to promote creative thinking and imagination. This may also help in propelling an individual positively towards general as well as oral health. Myths can be prevalent in a population due to a variety of reasons like poor education, socioeconomic status, cultural beliefs and social misconceptions [11]. India being a developing country, is struggling to provide the necessary oral health needs to its population as the majority of Indian population resides in rural areas [12]. India has a low budget to meet the oral health treatment needs, a high disease burden and a low literacy rate of its general population. All these act as some predisposing factors that direct the general population to poor oral healthcare, false treatment needs assumptions, and false beliefs [13]. Results of this study illustrate lack of knowledge and awareness about dental health on part of the general community. Prevalence of a large number of myths and misconceptions has adversely affected the community dental health and has inflicted fear in the minds of people towards health care procedures in one way or the other. Literacy level of people and their socioeconomic status plays a very important role in development of the health sector of a country. In this study it was noted that the percentage of people who believed in the myths were higher among the lower class and lower middle class of socioeconomic category. The findings of this study show that females have more belief in the myths and misconceptions than the males. Also, previous experience with extraction procedure is found to act as a factor in disrupting belief in these widespread misconceptions.

This study is similar to the results of study done by Sudarssan et al [11] which also concluded that the most prevalent myth among dental extraction was pertaining to the suturing procedure. Another study done by Siegel et al [5] also tried to evaluate various fears that act as a barrier to dental health seeking behaviour which also similarly reported fear of the needles being a significant contributing factor, but different from our study it only investigated the needle as a part of the injection and not in the form of suture needle. Several other studies have also reported fear of patients towards needles as a significant factor for a negative health seeking behaviour which is very similar to the outcome of our current study [14-20]. The result of the current study shows that more than half of the participants 59% believed that suturing the extraction socket is an indication of some complication or mishap during the procedure and nearly 67% of the participants had a misconception that a greater number of sutures implies a complication with the surgical procedure. These were the most prevalent misconceptions of the current study. The reason behind this might be the south Indian movies and media which are trying to portray suturing procedures and the number of sutures placed, to indicate severe injury and extensive trauma. People are misguided by this portrayal and are assuming that suturing itself is a serious surgical procedure that indicates complications.

LIMITATION

The results of this study cannot be applied to a larger population since this study was done in an urban setting. Furthermore, qualitative and quantitative research regarding the prevalence of fear and misconceptions about suturing procedures on a larger sample and for a longer period of time in different regions and different populations is necessary to validate the results of this study.

CONCLUSION

The study population has significant lack of awareness and exhibit belief in one or more misconceptions about suturing procedure and more than half the population has significant fear towards suturing procedure and the needle which can significantly affect their health care seeking behaviour.

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Table 1: Demographic data of the study population

Demographics	Frequency (n (%))
AGE (YEARS)	
Below 20	7 (3.5)
20-35	74 (37)
36-50	71 (35.5)
51-65	38 (19)
66-80	10 (5)
GENDER	
Male	94 (47)
Female	106 (53)
EDUCATION QUALIFICATION	
Illiterate	8 (4)
Below 10th Grade Education	14 (7)
10th Grade Education	14 (7)
12th Grade Education	29 (14.5)
Graduate	63 (31.5)
Post Graduate	42 (21)
Diploma/Professional Training	30 (15)
SOCIO-ECONOMIC STATUS	
Upper Class	13 (6.5)
Upper Middle Class	25 (12.5)

Middle Class	91 (45.5)
Lower Middle Class	42 (21)
Lower Class	29 (14.5)
PREVIOUS EXTRACTION HISTORY	
Yes	47 (23.5)
No	153 (76.5)

Table 2: Responses of participants regarding misconceptions and fear pertaining to suturing procedure.

Questions	Agree	Neither agree nor disagree	Disagree
Suturing the extraction socket is painful than extraction of the tooth	88	14	98
The very moment the dentist tells you that you might need sutures, you get scared	135	5	60
More the number of sutures, more complicated the surgery is	134	7	59
Suturing implies that there is some complication with your surgical procedure	118	15	67
The return visit for removal of the sutures makes you uncomfortable	45	29	126
Removal of sutures is a painful procedure	59	20	121
Removal of sutures requires Local anaesthesia through injection	47	25	128
Your food consumption is affected on placement of sutures	71	24	105
Suturing the third molar socket will affect your mouth opening	102	20	78
You avoid dental treatments due to fear of sutures being placed	59	3	138

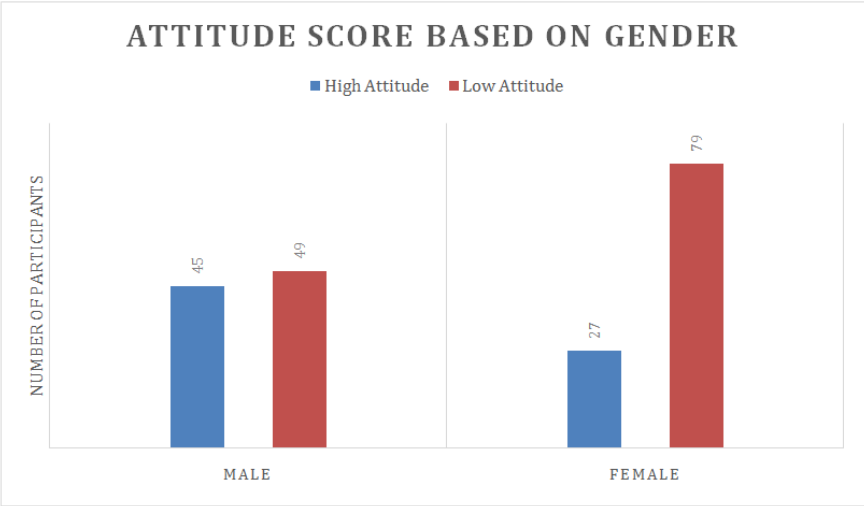

Figure 1: Attitude of male and female on Fear and Misconceptions about Suturing Procedure

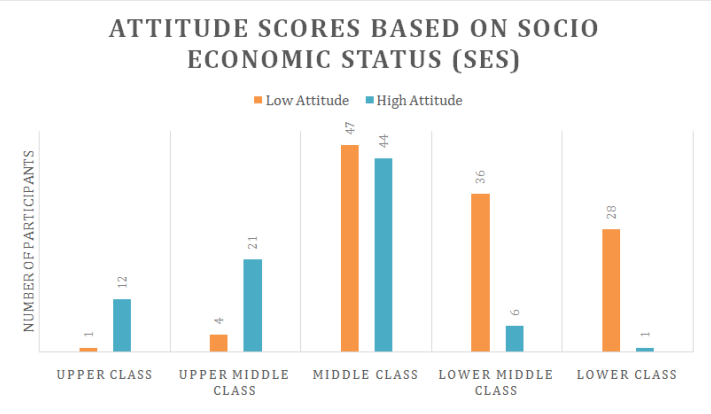

Figure 2: Association of Attitude scores with SES of the study population

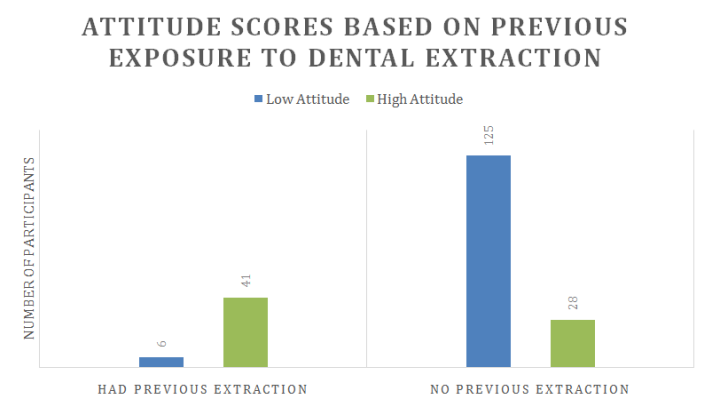

Figure 3: Association of Attitude scores with Previous Extraction Experience

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REVIEW ARTICLE

Articular Disc of Temporomandibular Joint Repair -Where are We Today? -Review of Literature

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ABSTRACT

Temporomandibular joint is a joint interface between the condylar surface of mandible and glenoid fossa of temporal bone ,with articular disc in between as a fibrocartilaginous structure that distributes the occlusal load ,maintaining harmony between the bony structures. TMJ begins to develop by the 10th week of intrauterine life from two separate mesenchymal condensations ,one for the temporal bone component and one for the condyle. MRI is the most reliable tool for evaluation of the TMJ soft tissues, the disc-condyle relationship, and for determination of disc displacement. Ultrasonography (US) may be considered for assessing TMJ disorders for their non-invasiveness, inexpensiveness, and ability in evaluating the integrity and correlation of the hard and soft tissues of the TMJ where patients needle alternatives for MRI like claustrophobia or with pacemakers (21). Fluoroscopic observation of the injection may reveal the presence of adhesions, perforations , discontinuities and accumulation of joint fluid in the capsule.A multidisciplinary team approach to TMD management, especially in which surgery is involved. Current advances in TMJ surgery have been overshadowed by the universal perception among practitioners that nonsurgical methods over surgery still entails unacceptable risks

Keywords: Temporomandibular, Ultrasonography, condyle, perforations, surgery.

INTRODUCTION

Temporomandibular joint is a joint interface between the condylar surface of mandible and glenoid fossa of temporal bone ,with articular disc in between as a fibrocartilaginous structure that distributes the occlusal load ,maintaining harmony between the bony structures .as proved any discal pathology will be the series of preceding events leading to degenerative changes that can involve the entire TMJ. The various conditions involving the articular disc are internal derangement (disc displacement), disc thinning, and perforation (1). the unique features of Temporomandibular joint are (1) The articulating surfaces are covered by fibroelastic tissue unlike any other joints by hyaline cartilage. (2) the condylar cartilage is considered a growth center that significantly contributes to the overall growth of the mandible (3) the TMJ is diarthrodial joint and mainly influenced by dental occlusion, and (4) it has an intact articular disc that is movable during all joint movements and functions as a shock absorber.
Discal thinning is an early sign of TMJ dysfunction.Temporomandibular joint dysfunction causes pain and limits movement of the jaw and is considered to be the one leading cause of altered quality of life in developing countries.Thus, successful treatment of early TMJ disorders continues to be an enigma.moreover , the complex and unique anatomy of this joint provides the challenge and demands for new surgical strategies irrespective of the severity of the pathology .

Temporomandibular joint embryology and Anatomy

TMJ has an evolutionary history being primary joint, as in mammals, integrated into the middle ear (2) .TMJ begins to develop by the 10th week of intrauterine life from two separate mesenchymal condensations ,one for the temporal bone component and one for the condyle. Superior to the condylar mesenchymal blastema, a band of mesenchymal cells develop will eventually differentiate into the disc .In the center of the condyle, cartilage develops which becomes the secondary cartilage contributing to the subchondral bone formation. The developing disc is highly cellular and vascular in nature. It continues with the developing lateral pterygoid muscle anteriorly and by a ligament with the superior end of the Meckel's cartilage posteriorly, where it develops into the malleus of the middle ear.by the 14th week of gestation ,all the components of the mature temporomandibular joint is evident (2) ,(3).

The disc is an avascular non-innervated fibrocartilage,biconcave in shape with a 2 mm thick anterior band and a 2.7 mm thick posterior band separated by a 1.0-mm thickness intermediate zone.It consists of three parts - anterior band, intermediate zone, and posterior band .The disc ligaments consist of the anterior and posterior bilaminar zones or ligaments, the lateral and medial collateral ligaments, and the discomalleolar ligament. All these ligaments are vascular, innervated, and fibroelastic in nature. The anterior ligament has a superior stratum inserting to the ascending slope of the articular eminence and an inferior stratum that inserts inferiorly at the anterior aspect of the condyle. The anterior ligament is supported by the superior and inferior heads of the lateral pterygoid muscle. The posterior ligament or the bilaminar zone consists of a highly elastic superior stratum inserting to petrotympanic fissure and an inferior stratum that also contains elastic fibers that inserts on the posterior aspect of the condyle below. The posterior bilaminar zone stretches considerably while jaw opening while allowing the disc to slip over the condyle at all ranges of motion.

The medial and lateral condylodiscal or collateral ligaments are collagenous which anchors the disc to the lateral and medial poles of the condyle, respectively. They are vascular and highly innervated. The most medial portion of the disc is connected to the Pinto's ligament. The extracellular matrix of disc is made up of fibers of type I and II collagen with a peculiar distribution being fibers of the anterior and posterior bands are transversely (mediolaterally) and intermediate band are mainly sagittally oriented (4), (5).

The retrodiscal tissue or bilaminar zone is the dorsal area of TMJ and made up of superior layer of elastic , collagen fibers and fat .(6),(7). In the open-mouth position, the vascular area fills with blood, rapidly increasing in volume(8).

The intermediate zone is the most resistant to load (9) (10) .It allows an optimal load distribution in the less resistant zones by ensuring a steady deformation of the whole structure and preventing the concentration of excessive loads in any single area. it has been hypothesized that the superior band of the lateral pterygoid muscle may pull the disk forwards and being the main risk factor for disc displacement (11)(12)(13).although, the biological possibility is debatable,since most fibers of the superior band of the lateral pterygoid muscle are attached to the condylar neck and very few fibers are attached directly to the posterior band of the disk(14)(15) .Moreover, the superior band of the lateral pterygoid does not seem to be active during jaw opening; it advances passively in response to the activation of the inferior band(16).

The possible mechanism of posterior shift of the disc is condylar traction. In both opening and closing phases, the condylar excursion is wider and more rapid .The disk also during the closing phase is posteriorly held back as due to its attachment . the masseter and the lateral pterygoid muscles, which are more active during the closing phase and, due to the characteristics of their insertions hold back the disk (17). When the condyle reaches its position under the disk, it drags the disk backwards passively mainly because of its concave shape of the disk (15).as said when disc loses its normal biconcave shape ,it cannot be dragged and an important pathogenic factor for displacement during the translation phase of the closing movement (18).

Age changes

Since the disc is made up of fibrocartilage with no regenerative capacity, perforation can be the terminal phase. Discal thinning and perforation may be part of the normal aging process and not pathologic, usually around 5th to 6th decade. The shape of the condyle may be categorized into five basic types: Flattened, convex, angled, rounded, and concave. As age advances, there is a progressive decrease in cellularity and an increase in collagen fibers in the disc(19).The disc becomes thinner and shows hyalinization and chondroid changes.

Diagnosis

MRI is the most reliable tool for evaluation of the TMJ soft tissues, the disc-condyle relationship, and for determination of disc displacement. the articular disk, or meniscus, in terms of its morphologic features and its location relative to the condyle in both closed- and open-mouth positions can be demarcated in the MRI .the most common method of assessing the disc in the imaging ,the most superior surface of the condyle as a reference point for the posterior band of the disk. A posterior band of the disk located anterior to the 12 o'clock position correlates to anterior disk displacement (20).Typically, the anterior band and the intermediate zone appears as hypointense (open mouth view) and the posterior band is slightly hyperintense(closed mouth view). MRI may assist the clinician in determining the degree of degeneration of the disc and aiding in management .

Ultrasonography

Ultrasonography (US) may be considered for assessing TMJ disorders for their non-invasiveness, inexpensiveness, and ability in evaluating the integrity and correlation of the hard and soft tissues of the TMJ where patients needle alternatives for MRI like claustrophobia or with pacemakers (21). ultrasonography images of the articular disc are variable although similarly to all major ligaments,it consists of dense fibrous tissue, however its visual aspect in,as it was reported to be hyperechoic,hypoechoic,isoechoic, and hypoechoic to isoechoic[77], probably for the presence of different structural, morphological and positional abnormalities in the patients examined. By ultrasonography it is also conceivable to identify sites of inflammation by detecting the presence of articular effusion.

Arthrography

Fluoroscopic observation of the injection may reveal the presence of adhesions, perforations , discontinuities and accumulation of joint fluid in the capsule. An arthrogram can clearly distinguish the synovial changes of an inflammatory arthritis from an internal derangement resulting from meniscal dysfunction and remains an indispensable tool when compared with static images obtained with CT and MRI (22),(23).

Articular Disorders
Internal derangement disorder

The direct contact between condylar surface and articular fossa when the disc is displaced from its normal position can cause further degenerative changes. When the displaced disc returns to its original position during closing of the jaw ,is called as reciprocal click (anterior disc displacement with reduction) usually as an initial symptom of the disc displacement of temporomandibular joint (24).The anterior disc displacement has different degrees of severity. Staging classifications has been represented for the TMJ related disc displacements (25) ,(26).The stages ranged from the early stage included slight displacement with clicking, and no pain or dysfunction to the last stage included degenerative changes to the disc with possible perforation, flattening of bones, pain, and restricted motion (27) (28). If the displaced disc does not return to the normal position during the mouth opening movement, this time it is defined as disc displacement without reduction.Thus, clinically limited mouth opening can be seen in the patients with disc displacement without reduction (29).The disc displacement frequently precedes the onset of TMJ osteoarthritis in susceptible individuals (30).

Osteoarthritis

Etiological factors of TMJ arthritis are mostly degenerative joint disease such as rheumatoid arthritis ,metabolic (gout), immunologic (ankylosing spondylitis, lupus).hence ,arthritis of TMJ has a multifactorial pathogenesis including biomechanical, biochemical, inflammatory, and immunologic traits (31). Inflammation by chronic mechanical or stress induced can modify the viscosity of synovial fluid which alters its ability to nourish the articular cartilage. As a result, cartilage metabolism changes. TMJ arthritis is classified as primary (no known predisposing factors) or secondary (associated with known abnormalities or injuries). Patients with TMJ arthritis usually complain of tremendous pain with functional morbidity . Crepitation may present as an indication of softening or perforated articular cartilage called chondromalacia (softening of the articular cartilage) of the TMJ (32)). Patients may have referred pain to head and neck regions.This condition progresses to the later stages, and crepitation may be developed secondary to bone exposure. Pain and adhesion formation result in limitation of mouth opening.

Articular repair
Non -invasive therapy

The noninvasive treatments include drugs, occlusal orthodontics, physical therapy, or acupuncture. The used drugs are analgesics, NSAIDs, anxiolytics, muscle relaxants, and opioids, all administered systematically .the occlusal splints have also been used for management of pain in TMD. Various techniques like exercises, neuromuscular stabilization, electrotherapy and transcutaneous electrical nerve stimulation (TENS), low-intensity ultrasound, and low-level laser therapy have been demonstrated efficient in managing TMD disorders with muscular origin.

Minimally - invasive therapy

The management of internal derangement of TMJ include conservative approaches and surgical approach if the former fails.The conservative treatment indicated are occlusal appliance therapy (33),and minimally invasive treatment such as Arthrocentesis of the upper joint space with or without intra articular medications such as Corticosteroids(34),Sodium hyaluronate(35),Platelet rich plasma(36) which has shown promising results to enhance the lubrication .

Surgical repair

TMJ Surgical Classification by Dimitroulis, 2013 (37), gave a classification for stages of TMJ disorders and their appropriate surgical management. According to classification the surgical repositioning of disc or discectomy or to perform an arthrotomy to restoring joint tissues or replace TMJ with autogenous or alloplastic material was indicated for moderate to severe changes in TMJ disc like non -reducing ,deformed and non -salvageable discs .

A.disc-repositioning using mini anchors (Mitek Mini Anchor)

The goal of disc-repositioning procedures is to relocate the disc to the normal condyle-disc-fossa relationship, helping to facilitate movement of the condyle previously blocked by the displaced disc. The reported clinical outcomes for TMJ disc repositioning surgery vary and are often unpredictable (38),(39).Traditional disc repositioning by suturing inflamed and often degenerated ligaments result in disc instability and scarring (40). Therefore, new surgical techniques have been developed for TMJ disc repositioning (41) ,(42). Mitek anchors were originally used for rotator cuff repair, medial and lateral collateral ligament repair, bicep tendon reattachment, and other muscle, ligament, and tendon repair orthopedic surgery procedures (43) .until Annandale in 1887 used it for surgical repositioning of the displaced temporomandibular articular disc (44). Disc repositioning became an acceptable surgical technique as in 1978 wilkes described detailed anatomy of TMJ using arthrography (45). As most the surgeons were not able to produce similar results,they developed modified techniques of disc repositioning (41),(46),(47). Some authors have proposed arthroscopic suturing techniques to reposition the disc (48),(49).

The Mitek mini anchor is cylindrical, measuring 1.8 mm in diameter and 5.0 mm in length. The body of the anchor is composed of titanium alloy (titanium 90%, aluminum 6%, vanadium 4%), and its arcs are composed of a nickel–titanium alloy (Nitinol), utilizing super elastic shape memory properties. An eyelet in the posterior aspect of the anchor allows placement of sutures that can function as artificial ligaments. The Mitek anchor technique uses a bone anchor that is placed into the lateral aspect of the posterior head of the condyle and the anchor will subsequently osseointegrate. Mitek anchor disc repositioning has a high success rate: 1. Disc repositioning at the onset of displacement within 4 years of displacement provides the greatest predictability of outcome. 2. Adolescent internal condylar resorption patients who are treated within the first 4 years of disease onset. Therefore, the use of mini-anchors (Mitek) for better stabilization of the disc in a more physiologic position with success rates reported between 82% to 91%.35-37 .

B.Disc Repositioning and Arthroplasty

Several authors have advocated combining a disc repositioning procedure with an arthroplasty of the condyle or the articular eminence (50),(51). Arthroplasty basically a reduces condylar/ eminence of about 2-4 mm which allows the amount of posterolateral repositioning required less and therefore permits an easier repositioning of an atrophic disc.Disc repositioning is then performed through the plication or excision technique. Intermaxillary fixation or training elastics can be used for 1 to 3 weeks to allow muscular adaptation and dental compensations.

C.Disc Repair and Discectomy

Small perforations of the disc (1 to 3 mm) can be treated with primary closure by Anterolateral release of the discal attachments to promote mobilization of the disc posteriorly. The atrophic displaced disc needs to be fully repositioned posteriorly such that the margins of the perforation should be excised and the posterior attachment on the posterior edge of the disc approximated to the tympanic portion of the retrodiscal tissue. The margins of the perforation are oversewn in a straight-line fashion with a nonresorbable material. The repaired retrodiscal tissue is intended to maintain the shape of the articular surface and to prevent ankylosis. Larger perforations will require more extensive repair usually with grating If the disc cannot be adequately repaired, then discectomy should be considered .

Disc Replacements

Despite the long-term reported success of TMJ discectomy, there is a perception among some surgeons that disc replacement is ideal to help prevent or reduce the significant osseous remodeling, intra-articular adhesions, and recurrent pain that can be seen following discectomy alone. After discectomy an interpositional material is considered to decrease joint noises by dissipating loading forces on the osseous surfaces. However, the effectiveness of interpositional materials and grafts are far superior to the short-term studies following discectomy and grafts and disc preservation procedures (52). The popularity of alloplastic disc replacement materials decreased considerably after the experience with Silastic and Teflon-Proplast (53),(54).The Vitallium alloy (cobalt, chromium, molybdenum) fossa-eminence prosthesis following discectomy and has been reported in the literature with moderate success (55).on a long haul the the condylar head from articulation against a metallic prosthesis causes degenerative change ,such that the surgeons have opted for autogenous grafts .few autogenous grafts are ,

Temporalis muscle/fascia flap.

This flap remains the most popular interpositional material primarily due to its close proximity, ease of use, and potential stability as a pedicled flap,less cosmetic and functional morbidity. The disadvantages include flap necrosis and fibrosis with the potential for decreased postoperative interincisal opening. Feinberg and Larsen described a technique utilizing a composite flap consisting of temporalis muscle, temporalis fascia, and the underlying pericranium,from the coronoid process and passed anteriorly around the zygomatic arch and then sutured to the retrodiscal tissues (56).Studies have reported that the muscle maintains its viability despite some fibrosis with high success rate (57).

Auricular cartilage

Autogenous cartilage has been described as a TMJ disc replacement by several authors (58). The quality and thickness of the aural cartilage is obviously variable in different patients, and caution is needed to avoid an iatrogenic tear during the harvesting process. Hall and Link described the procedure for harvesting the auricular cartilage and used as an replacement for disc . However, several studies have shown their tendency to fragment, proliferate, and result in condylar lysis or fibrous ankylosis with progressive restriction of mouth opening (59) .

Dermal graft

Georgiade originally reported the use of a dermal graft for the surgical correction of TMJ dysfunction followed by limited studies on the use of the graft for disc repair and disc replacement. Dimitroulis reported 35 joints in 29 patients that demonstrated dermis grafts were effective in reducing joint sounds, but conceded that dermis grafts were difficult to anchor and failed to prevent regressive remodeling of the condyle (60). The graft may be harvested

from the buttock, upper lateral thigh, groin, or the inner aspect of the upper extremity (61). In some cases, anchoring is difficult, and the graft is simply inserted as space filler.

Dermis fat graft

Dimitroulis published the first description of the abdominal dermis-fat graft as a disc replacement as its ability to be easily sculptured to fit precisely into any size cavity (62).Several studies by Dimitroulis and colleagues have proved that abdominal dermis-fat is a promising graft material is an ideal interpositional graft following discectomy (63).the graft promotes smooth joint function but fails to prevent further degeneration of the joint (64).

Tissue engineered disc replacements

The rapid advances in tissue engineering will provide alternatives for TMJ disc replacement (65). Tissue engineered articular discs are at the nascent phase of development, but the real challenge will come when researchers try to anchor the newly developed disc to the surrounding articular structures without compromising the normal function of the joint.

Temporomandibular Joint Derived Synovial Stem Cells in Repairing TMJ Disc Perforation

Another important aspect of cartilage tissue engineering is the design of three-dimensional scaffold which may maintain the initial shape of cell/scaffold construct and promote tissue regeneration.macroporous sponge-like chitosan was used as scaffold in hyaline cartilage repair (66),(67)). However, the intrinsic properties of fibrin gel such as poor mechanical strength and fast degradation make it unsuitable to be used independently. Several synthetic materials had been used in TMJ disc engineering, including polylactide (PLA), polyglycolic acid (PGA), and their copolymers (PLGA) (68). Natural chitosan is polysaccharide material which has been used extensively in the field of cartilage regeneration for its good biocompatibility and similarity to GAG cartilage (69).So improving cell seeding and distribution is essential for the limited amount of joint derived cells. In summary, fibrin gel improved the synthesis of fibrocartilage ECM by TMJ-SDSCs. This pilot study demonstrated that the regenerative ability of TMJ-SDSCs seeded fibrin/chitosan constructs could be applied for repairing TMJ disc perforation.

Tissue engineering toward temporomandibular joint disc regeneration

tissue-engineered implants produced from allogeneic, passaged costal chondrocytes can be applied to treat TMJ disc thinning (70). Using an innovative surgical approach in minipigs, reproducible defects modeling early-stage TMJ disc disease were treated with tissue-engineered implants. Healing was more robust in the implant-treated discs compared with untreated defects. Repair tissue formed in response to treatment exhibited a greater Young's modulus than that of the repair tissue formed in untreated discs. These results demonstrate a strategy capable of producing healing of TMJ disc thinning, paving the way toward new clinical applications of tissue-engineered solutions for patients with TMJ disc pathologies.

CONCLUSION

Although surgery is often considered as an option of last resort, there are instances in which surgery is the definitive and sometimes the only treatment option.In a fundamental sense, surgery is used to repair damaged tissue or remove tissue that cannot be salvaged. The TMJ surgery is benefited only when appropriate case selection,preceded by an accurate diagnosis. In these situations, the risks of surgery would far outweigh the benefits.A multidisciplinary team approach to TMD management, especially in which surgery is involved. Current advances in TMJ surgery have

been overshadowed by the universal perception among practitioners that nonsurgical methods over surgery still entails unacceptable risks. The future of discal repair in TMD diseases should be explored in free tissue engineering and their long term results especially with terminally damaged discs.

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RESEARCH ARTICLE

Synthesis of Sodium Salt of Partially Carboxymethylated Psyllium-g-4-Vinyl Pyridine and Study of its Swelling Behavior and Metal Ion Sorption

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ABSTRACT

Grafting of 4-vinyl pyridine (4VP) onto sodium salt of partially carboxymethylated psyllium (Na-PCMPsy) (DS = 0.13) has been carried out using bromate/thiourea redox pair initiator system under an inert atmosphere. The grafting parameters i.e. percentage grafting (%G) and grafting efficiency (%GE) increase with increase in concentration of Na-PCMPsy (DS = 0.13) from 0.1 to 0.3 g dm^{-3}. The grafting parameters increase on increasing the concentration of 4-vinyl pyridine from 10 x 10^{-2} to 30 x 10^{-2} mol dm^{-3}, BrO_3^- from 2 x 10^{-3} to 6 x 10^{-3} mol dm^{-3} and thiourea (TU) from 1.0 x 10^{-3} to 3 x 10^{-3} mol dm^{-3}. The optimized temperature and time for grafting of 4VP onto Na-PCMPsy are found to be 308.15 K and 2 h., respectively. The swelling ration and metal ion sorption have also been studied. The graft copolymer has been characterized by FTIR and thermal analysis.

Keywords: Sodium Salt of Partially Carboxymethylated Psyllium, Graft copolymer, swelling behavior, metal ion sorption, thermal analysis.

INTRODUCTION

Psyllium is a natural polysaccharide obtained from plantago ovata which is composed of neutral arabinoxylan (arabinose 22.6%, xylose 74.6%) having a straight chain of xylose with arabinose branches at 2 or 3 positions randomly attached [1]. Psyllium is highly viscous in an aqueous medium and it is a natural biodegradable polymer which decomposes before its melts, in order to overcome this difficulty, the carboxymethylated derivative of it [2]. Modification of natural polymers by graft copolymerization is the most promising technique as it functionalizes these biopolymers to their potential, imparting desirable properties onto them. In recent years, much attention has been paid on chemical modification of these natural macromolecules through grafting [3-6].

Due to immense potential of psyllium, in the present studies, it has been chosen as a backbone. In recent years, it is widely used as a flocculation behavior [7], in the textile industry [8], tannary effluent [9], drug delivery [10], hydrogel [11]. Psylllium, like other polysaccharides it is easily biodegradable which could be improved by grafting vinyl monomers onto it. Poly(4-vinyl pyridine) is an important class of polymer, which exhibit interesting properties due to presence of nitrogen atom in the pyridine ring. Therefore, in the present work decided to prepare graft copolymer of Na-PCMPsy and 4-vinyl pyridine so that to develop a product with high swelling capacity and could be used for metal-ion sorption.

EXPERIMENTAL

Materials

Psyllium was supplied by Gujarat Sat–Isabgol Factory, Unjha (Gujarat/India). Na-PCMPsy was prepared from Psyllium and purification as well as the measurement of the degree of substitution was followed as discussed earlier [2]. The DS was found to be 0.13. 4-vinyl pyridine (4VP) (E. Merck) was distilled out at atmospheric pressure and the middle fraction was collected. Potassium bromate, thiourea, and sulphuric acid were purchased from Merck. All the solutions were prepared in triple distilled water.

Synthesis of grafting

For each experiment known quantity of Na-PCMPsy added into the glass vessel containing triple distilled water with a stirrer. A calculated amount of 4-vinyl pyridine, thiourea and sulphuric acid solutions were added into the reactor and continuous flow of nitrogen gas has been passed. A known amount of potassium bromate solution was added to initiate the reaction and maintained the reaction temperature. After the desired time period the reaction was stopped and the grafted sample was precipitated by pouring the reaction mixture into pure methanol, where the graft copolymer precipitated out. The final product has been separated, dried and weighed.

Characterizations

FTIR analysis

IR spectra of Na-PCMPsy (DS = 0.13), Na-PCMPsy-g-4VP were taken in KBr pellet using Nicolet Impact 400 D Fourier Transform Infra-Red Spectrophotometer.

TGA analysis

Thermogravimetric Analysis (TGA) The thermal behavior of Na-PCMPsy (DS = 0.13), Na-PCMPsy-g-4VP has been examined in an inert atmosphere at a heating rate of 10^0C/min. with the help of the Mettler Toledo Star SW 7.01 thermogravimetric analyzer.

Swelling test

Swelling ratio (S_R) = (W_s - W_d)/W_d

Where W_s are weights of swollen polymers and W_d weight of dried polymers.

Percent swelling (P_s) = Swelling ratio (S_R) x 100

Metal ion sorption

The batch equilibrium method was carried out using the graft copolymer of Na-PCMPsy and 4VP as the sorbents. Sorption of the metal ions concentrations was determined by using the Perkin Elmer bulk scientific (AAS). Sorption was carried out by stirring 0.5 g graft copolymer kept for 24 h in 100 mL aqueous media containing a known concentration of Fe^{2+}, Pb^{2+}, $Cu^{2+,}$ and Cr^{6+} metal ions. The strength of sorbed metal ion has been determined by AAS after filtration. The results of sorption behavior of different graft copolymers have been determined in terms of different parameters like percentage uptake (P_u) and retention capacity (Q_r) [13].

$$\text{Percent metal ion uptake (Pu)Percent metal ion uptake (Pu)} = \frac{\text{Amount of metal ion sorbed}}{\text{Amount of metal ion in feed}}$$

$$\frac{\text{Amount of metal ion sorbed}}{\text{Amount of metal ion in feed}} \times 100$$

$$\text{Retention capacity } (QR) = \frac{\text{Amount of metal ion in polymer (mequiv.)}}{\text{Wt.of dry polymer (g)}}$$

$$\text{Retention capacity } (QR) = \frac{\text{Amount of metal ion in polymer (mequiv.)}}{\text{Wt.of dry polymer (g)}} \times 100$$

RESULTS AND DISCUSSION

Grafting parameters

The graft copolymer has been characterized by following grafting parameters [14].

$$\text{Grafting ratio (\%G)} = \frac{\text{Weight of grafted polymerWeight of grafted polymer}}{\text{Weight of substrate}\qquad\text{Weight of substrate}} \times 100$$

$$\text{Grafting efficiency (\%GE)} = \frac{\text{Weight of grafted polymer}}{\text{Weight of polymer formed}}\ \frac{\text{Weight of grafted polymer}}{\text{Weight of polymer formed}} \times 100$$

Determination of optimum grafting conditions

The effects of different concentrations of backbone, potassium bromate, thiourea, hydrogen ion,4-vinyl pyridine (4VP) along with temperature and time on grafting parameters have been studied.

Effect of bromate ion concentration

The effect of bromate ion concentration on grafting reaction has been studied and results are shown in Fig. 1. It was observed that the percentage of grafting (%G) and grafting efficiency (%GE) increases on increasing the concentration of bromate ion from 2 x 10^{-3} to 6 x 10^{-3}, but beyond this concentration range grafting parameters decrease. The increase of these grafting parameters up to this concentration is due to more availability of bromate ion and isothiocarbamide free radicals which attack on Na-PCMPsy molecule generating more free radicals site on to which monomer addition takes place. Beyond this concentration, the decrease of grafting parameters due to less availability of concentration of free radicals.

Effect on 4-vinyl pyridine(4VP) concentration

It can be observed from Fig. 2 the grafting parameters increase on increasing the concentration of 4-vinyl pyridine from 10 x 10^{-2} to 25 x 10^{-2} mol dm^{-3} but on further increasing the concentration of 4VP the grafting parameters decrease. The increase in grafting parameters could be attributed to the greater availability of monomer molecules at chain propagating site. The decrease in grafting parameters could be interpreted in terms of an increase in viscosity of the medium due to solubility of poly(4-vinyl pyridine).

Effect of backbone concentration (Na-PCMPsy)

The influence of the varying amount of Na-PCMPsy on %G and %GE is shown in Fig. 3. It can be observed from this table that, the value of %G and %GE is found to be increased with increase in Na-PCMPsy concentration and the maximum value is reached at 0.3 g dm^{-3} of Na-PCMPsy concentration in the case of grafting of 4VP. Beyond this optimum concentration of Na-PCMPsy, %G and %GE values are found to be decreased with further increase in Na-PCMPsy concentration. The results can be explained on the basis of the fact that the greater the amount of Na-PCMPsy for a given amount of monomer, the greater is the amount of complex formed during the course of reaction and consequently, the higher is the number of active sites generated on the polymeric substrate during the subsequent decomposition of the complex. As a result of this, the grafting yields are found to be increased with the

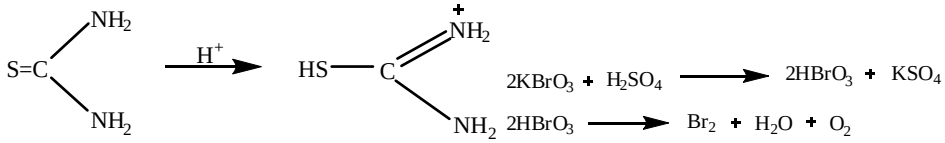
increase in the backbone concentration. The observed decrease in %G and %GE with a further increase in the amount of Na-PCMPsy indicates the formation of homopolymer (4-PVP).

Effect of thiourea concentration

From Fig. 4 it shows that the increase in grafting parameters and decrease in homopolymer formation from the concentration of thiourea 1.0 x 10^{-3} to 3.0 x 10^{-3} mol dm^{-3} beyond this grafting parameters decrease. The increment in grafting parameters within this range is due to availability of more free radicals and beyond this range protonation of thiourea and bromate ions are decreases. Therefore, a smaller number of free radicals are produced and hence grafting parameters decrease.

Effect of hydrogen ion concentration

From the Fig. 5 it has been observed that the grafting parameters increase up to 3 x 10^{-3} mol dm^{-3} and after this concentration homopolymer concentration increases. It could be explained that increase in concentration of hydrogen ion the rate of formation of protonated thiourea species increases which reacts with bromate giving rise to primary free radicals and these radicals react with Na-PCMPsy, giving rise to free radicals which were responsible for increasing the grafting parameter. But, beyond 3 x 10^{-3} mol dm^{-3} concentration excess hydrogen ion react with bromate ion to form $HBrO_3$ and hence percentage of grafting and grafting efficiency decreases.



Effect of time period

It is cleared from Fig. 6 the grafting parameters increasing upto 3 h due to propagation of grafting chain takes place for more availability of grafting sites and after that grafting parameters decrease because of the depletion in monomer and initiator concentrations and shortage of the available grafting sites.

Effect of temperature

The results obtained for grafting parameters at various temperatures have been evaluated in Fig. 7. It has been observed that as the temperature is increased from 298.15 K to 308.15 K, grafting parameters shown increment. It is concluded that as the temperature increase, rate of production of free radicals increases and therefore grafting parameters increase but beyond 308.15 K the decrement of grafting parameter was observed, which might be due to the destruction of the free radicals at a higher temperature.

Evidence of grafting

FT-IR spectroscopy

The O-H stretching absorption vibration for Na-PCMPsy forms a broad band around 3500-3200 cm^{-1}, which is due to the intermolecular hydrogen bonding, overlapped with the N-H stretching band that is supposed to occur in the same range. Primary amines have two bands in this region. The N-H bending vibration appears in the range of 1641cm^{-1} as sharp band. The spectrum of the grafted copolymer shows difference corresponding to the appearance of a band at 2129.95cm^{-1}, which is attributed to the poly 4-vinylpyridine chains. One characteristic band for CH bending of the aromatic ring of the vinylpyridine moiety that appears around 758 cm^{-1} is very indicative of the presence of the polyvinylpyridine polymer in the graft. Fig. 10 shows the primary thermograms obtained at a scan rate of 10^0C/min for Na-PCMPsy and Na-PCMPsy-g-4VP in an inert atmosphere. In the case of Na-PCMPsy shows a single step of degradation. The decomposition starts at 190^0C and proceeds at a faster rate upto 320^0C and at this temperature the sample loses 48% of its original weight. However, beyond this temperature, degradation proceeds at a slow rate upto 500^0C compared to the degradation proceeded in the earlier temperature range. This temperature range involves

about 14% weight loss. With a further increase in temperature, the degradation is found to occur at a relatively very slow rate upto 790^{0}C.The temperature at which the maximum rate of weight loss occurs is at 3000C. The overall degradation leaves about 24.5% residue.

Na-PCMPsy-g-4VP involves two steps of degradation. The first step of degradation begins at 110^{0}C and proceeds slowly upto 300^{0}C involving about 27% weight loss with a maximum rate of weight loss at 255^{0}C. The second decomposition step comprises of 300-790^{0}C involving about 32% weight loss with a maximum rate of weight loss at 465^{0}C. The sample leaves about 40% residue weight. The temperature characteristic values as well as the value of the integral procedural decomposition temperature (IPDT) of Na-PCMPsy and Na-PCMPsy-g-PVP are tabulated in Table 1. The examination of IPDT values indicates that the overall thermal stability graft copolymer increases as compared to Na-PCMPsy, this may be due to increase in ring formation at higher temperatures may be responsible for this greater stability.

Swelling behavior

Table 2 shows that the swelling behavior of grafted polymer, it indicates swelling ratio and percentage of swelling increase with increase in percentage of grafting which dependent on monomer concentration. Due to hydrophilic nature of monomer, it increases the water retention capacity of graft copolymer. Carboxymethyl group also increases the swellability of polysaccharides [6]. Hydrophilic nature of monomer and carboxymethyl group of substrates, both factors are responsible for good swelling capacity of graft copolymer. The amount of sorbed metal ion has been determined at constant pH and results have been shown in Table 10. For this study four metal ions have been chosen, that are Fe^{2+}, $Pb^{2+,}$ Cu^{2+} and Cr^{6+}. Results show that Cr^{6+} was least uptakable and Fe^{2+} was most uptakable in comparision to other three metal ions of them, which have been used. The value of percent uptake (P_u) and retention capacity (Q_r) increase as the percent grafting ratio increased. Which might be due to availability of additional functional groups of poly-pendent chain of monomer, fuctional group incorporated by grafting and its ability to interact with metal ion play important role in the determination of selectivity and quantum of metal ion uptake.

CONCLUSION

4-vinyl pyridine grafted onto Sodium salt of partially carboxymethylated Psyllium by redox pair system i.e. bromate/thiourea. The spectroscopic data confirm that the grafting has been take place at hydroxyl group of backbone (Na-PCMPsy). The thermogravimetric data shows that the graft copolymer is more stable than backbone (Na-PCMPsy). The graft copolymer shows good swelling ratio towards water as well as it can be used for the removal, separation and enrichment of hazardous metal ions in aqueous solutions and can play an important role for remediation of industrial wastewater.

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Table 1: Thermal analysis of Na-PCMPsy and Na-PCMPsy-g-PVP samples.

Sample	T_i ^{0}C (IDT)	T_f ^{0}C (FDT)	T_{max} (^{0}C)		T_{10} (^{0}C)	T_{50} (^{0}C)	IPDT (^{0}C)
			Step 1	Step 2			
Na-PCMPsy	190	790	300	--	260	330	538
Na-PCMPsy-g-PVP	110	790	255	465	230	590	599.6

Table: 2 Swelling behavior

Sample	[4VP] x 10^2 mol dm^{-3}	% Grafting (%G)	Swelling ratio (Sr)	Percent swelling (Ps)
1	10	217.37	5.2	525
2	15	258.00	6.2	620
3	20	306.38	7.4	740
4	25	349.99	8.8	880
5	30	353.49	9.2	920

[4VP] = 25 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K, time = 2 h, where 1,2,3,4 and 5 = graft copolymer.

Table 3 Extent of heavy metal sorption by the graft copolymer

Sample	[4VP] x 10^2 mol dm^{-3}	% Grafting (%G)	Percent uptake (P_u)				Retention capacity(ppm/g)			
			Fe^{2+}	Pb^{2+}	Cu^{2+}	Cr^{6+}	Fe^{2+}	Pb^{2+}	Cu^{2+}	Cr^{6+}
1	10	217.37	34.44	12.37	7.80	1.25	17.22	6.185	3.9	0.62
2	15	258.00	37.86	15.57	11.18	1.53	18.93	7.78	5.54	0.76
3	20	306.38	40.63	17.11	12.96	1.62	20.31	8.55	6.48	0.81
4	25	349.99	43.40	21.23	13.42	1.73	21.7	10.61	6.71	0.86
5	30	353.49	44.11	23.86	14.57	1.78	22.05	12.93	7.28	0.89

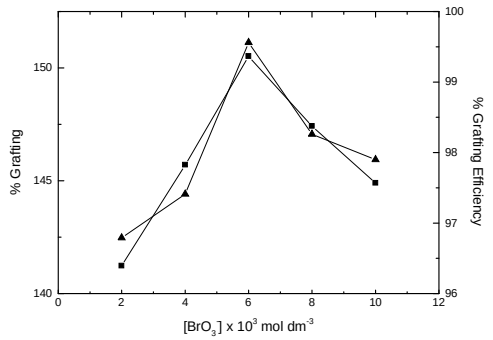

Fig. 1. Effect of bromate ion concentration: [4VP] = 30 x 10^2 mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K, time = 2 h.

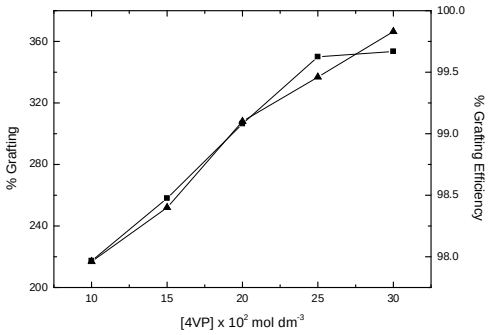

Fig. 2. Effect of 4-vinyl pyridine (4VP) concentration: [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K, time = 2 h.

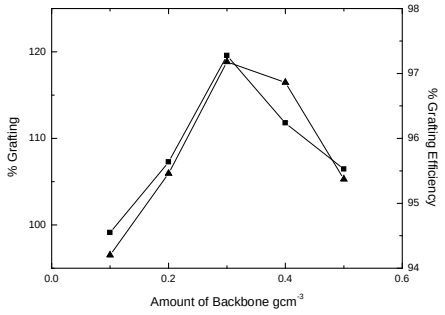

Fig. 3. Effect of backbone concentration: (Na-PCMPsy) [4VP] = 30 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K, time = 2 h.

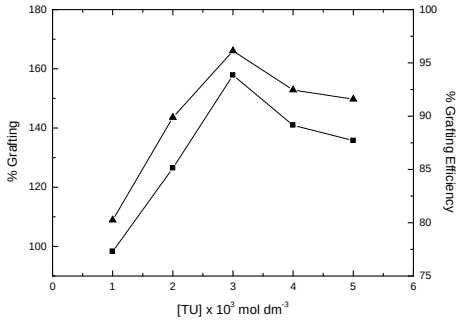

Fig. 4. Effect of thiourea (TU) concentration: [4VP] = 30 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K, time = 2 h.

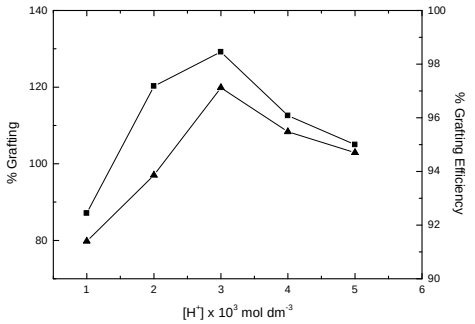

Fig. 5 Effect of hydrogen ion concentration: [4VP] = 30 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, temp. = 308.15 K, time = 2 h.

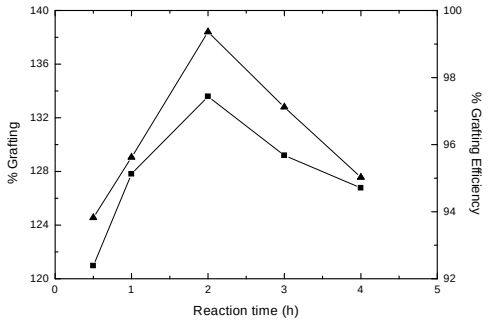

Fig. 6 Effect of time period: [4VP] = 30 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, temp. = 308.15 K.

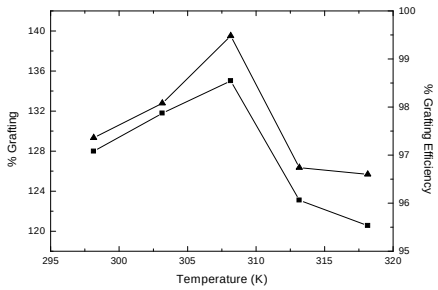

Fig. 7 Effect of temperature:[4VP] = 30 x 10^2 mol dm^{-3}, [BrO_3^-] = 6 x 10^{-3} mol dm^{-3}, [TU] = 3.0 x 10^{-3} mol dm^{-3}, [Na-PCMPsy] = 0.3 g dm^{-3}, [H^+] = 3 x 10^{-3} mol dm^{-3}, time = 2 h.

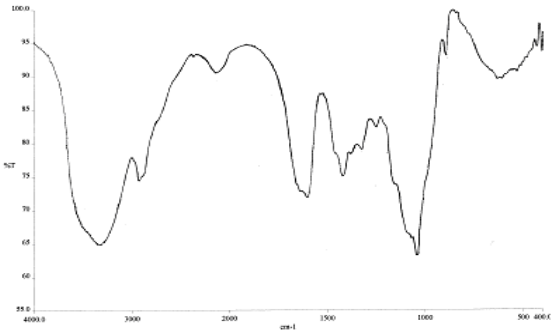

Fig. 8 IR spectra of Na-PCMPsy

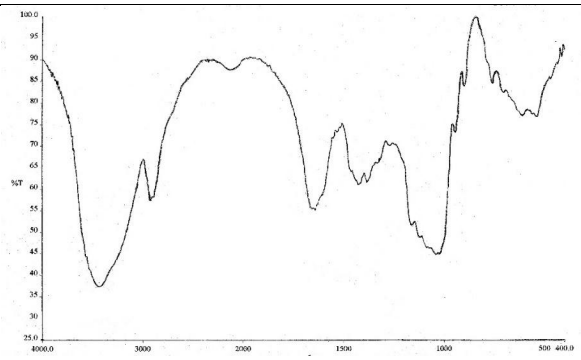

Fig. 9 IR spectra of Na-PCMPsy-g- 4VP

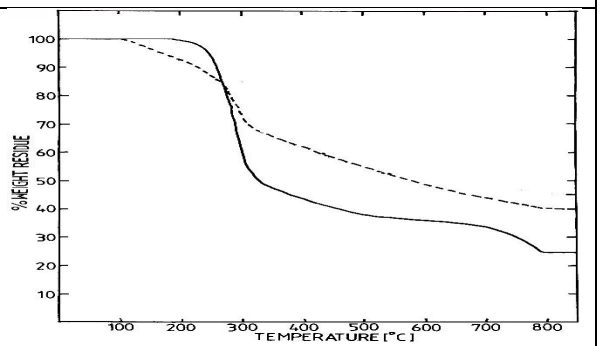

Fig. 10. TG thermogram for (——)Na-PCMPsy and (- - - - -) Na-PCMPsy-g-PVP at 10^0C/min

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RESEARCH ARTICLE

Annatto (*Bixa orellana*) δ-Tocotrienol on Nicotinic 4-Cell Murine Embryos: A Preliminary Study on the Changes in PI3K/Akt-Cyclin D1 Gene Expressions

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ABSTRACT

This preliminary study aimed to determine the effect of annatto-derived delta-tocotrienol (δ-TCT) in comparison to alpha-tocopherol (α-TOC) on the regulation of the PI3K/Akt-cyclin D1 pathway genes expressions in 4-cell embryos following nicotine treatment on female balb/c mice. Nicotine, a reproductive toxicant was used to induce damages. A total of 48 mice aged between 6–8 weeks old and weighed between 23–25 g were randomly divided into eight groups (Grp.1–Grp.8) (n = 6) and treated for 7 consecutive days with the assigned treatments (nicotine or/and annatto δ-TCT/soy α-TOC). On the 8th day, the females were hormonally superovulated and left for mating before sacrificed at 46 h post-coitum. Fifty 4-cell embryos were collected from each group and processed for gene expression analysis using Affymetrix QuantiGene Plex2.0 assay. Results showed that there were changes in the expressions of PI3K/Akt-Cyclin D1 pathway genes that may dysregulate its functions. Present findings suggested that maternal supplementations with annatto δ-TCT could potentially exerted anti-proliferative effect on the nicotinic embryonic cells through the upregulation of several important genes.To our knowledge, this is the first attempt in studying the benefits of annatto δ-TCT on murine preimplantation 4-cell embryos.

Keywords: annatto,*Bixa orellana*, murine pre-implantation embryos, nicotine, reproductive health, vitamin E, tocopherols, tocotrienols

INTRODUCTION

Tocotrienols (TCTs) and tocopherols (TOCs) are the two major constituents of vitamin E, and both are present in four different homologs, i.e. α-, β-, γ- and δ-TCT/TOC [1]. Vitamin E, a lipid-soluble vitamin [2,3], has been extensively studied and is a well-known antioxidant [4-6]. Health benefits of vitamin E have been widely reported including its benefits as an anti-proliferative [7], anti-survival [8], pro-apoptotic [9], anti-angiogenic [10], and anti-inflammatory [11] agent. Despite the numerous reported benefits of vitamin E, its effects on reproductive health including the preimplantation embryonic development remain largely unknown. Our recent studies have indicated the potential benefits of vitamin E [12-17], and these were in line with a few previously reported studies [18-22]. However, the details on the mechanisms of actions by vitamin E on reproductive health remain considerably lacking. The sources of vitamin E include various foods and plants, and among the most popular sources are palm oil and rice bran. Most of the vitamin E sources contain all the α-, β-, γ- and δ-TCT, and α-TOC homologs. However, this is exceptional for vitamin E deriving from the annatto (*Bixa orellana*) plant seeds, in which it contains 100% δ- and γ-TCT with 0% α- and β-TCT as well as 0% TOCs [23]. This is highly valuable, since α-TOC has been reported to interfere with the benefits of TCTs [24,25].

Embryonic development in human and animals are regulated by a network of signalling pathways. Amongst the pathways, phosphatidylinositol-3-kinase (PI3K)/Akt-Cyclin D1 pathway has been reported to play important roles in the governance of the pre-implantation embryonic development [16, 26–37]. Nicotine, a reproductive toxicant, has been extensively reported for its adverse effects in humans [38,39] and animals [40–44] including the effects on the major reproductive physiologic functions [45,46]. In addition, it was also reported that the nicotinic activation of the PI3K/Akt pathway contributed to the growth of several cancer types [47,48]. Taking together the adverse effects of nicotine and the protective effects of vitamin E, this study was conducted to determine theeffect of supplementation with annatto δ-TCT on the PI3K/Akt-Cyclin D1 gene expressionsin nicotinic murine 4-cell embryos.

MATERIAL AND METHODS

Ethics Approval

The ethical approval to conduct this study was obtained from the university's Committee on Animal Research and Ethics (CARE) and Animal Care and Use Committee (ACUC-7/13).

Animal Treatments

This study involved the use of a total of forty-eight (48) males and 48 females balb/c mice aged between 6–8 weeks old and weighed between 20–25g (Chenur Supplier, Selangor, Malaysia). Acclimatization was done for one week. Animals were kept at a controlled temperature and humidity setting at 24◦C, with 12-h light/dark cycle. Vitamin E-free pellets (Gold Coin Holdings, Kuala Lumpur, Malaysia) and water was provided *ad libitum*. Study samples of annatto (*Bixa orellana*)-derived δ-TCT in both forms (mixed = 90% delta and 10% gamma homologs; pure = > 98% delta homolog) and soy bean-derived alpha-tocopherol (α-TOC) were provided by American River Nutrition Inc. (ARN), Hadley, MA, United States of America (USA). Samples preparation involved mixing the samples extract with tocopherol-stripped corn oil (vehicle).

Experimental animals were randomly divided into eight groups (Grp.1 – Grp.8) and six mice were placed in each group (n=6). Grp.1 served as control and given 0.1 mL tocopherol-stripped corn oil (vehicle) (Acros, Belgium) through oral gavage, Grp.2 was given 3 mg/kg/day of nicotine through subcutaneous (s.c.) injection, Grp.3 received concurrent treatment with 3 mg/kg/day nicotine (s. c. injection) and 60 mg/kg/day of mixed δ-TCT (oral gavage), Grp.4 was concurrently given with 3 mg/kg/day nicotine (s. c. injection) and 60 mg/kg/day of pure δ-TCT (>98% purity) (oral gavage), Grp.5 received concurrent treatment of 3 mg/kg/day nicotine (s. c. injection) and 60 mg/kg/day of α-TOC (oral gavage), Grp.6 was given 60 mg/ kg/day of mixed δ-TCT alone (oral gavage), Grp.7 received 60

mg/kg/day of pure δ-TCT alone (oral gavage) and Grp.8 received 60 mg/kg/day α-TOC alone (oral gavage). Treatments were given between 10:00–11:00 a.m. daily for seven (7) consecutive days.

Superovulation

Following completion of the treatments, all females were super ovulated through subcutaneous injection (s.c.) with 5 IU of pregnant mare's serum gonadotropin (PMSG) (Sigma Aldrich, St. Louis, MO, USA). Injections took place between 10:00 a.m. to 11:00 a.m. on Day 8 and all females were left for 48 h. After 48 h, the females were injected (s.c.) with 5 IU of human chorionic gonadotropin (hCG) (Sigma Aldrich, St. Louis, MO, USA) (10:00 a.m. to 11:00 a.m. on Day 10) and immediately subjected for mating with fertile males at the ratio of 1:1. PMSG functions to mimic the oocyte maturation effect of the endogenous follicle-stimulating hormone (FSH) [49] and hCG functions to mimic the induction of ovulation effect of luteinizing hormone (LH), and to improve the maintenance of the corpus luteum [50].

Mating

All super ovulated females were arranged for mating in a cage for 48 h immediately after the hCG injections. Presence of a vaginal plug was taken as an indication of mating, and following this, the females were sacrificed by cervical dislocation between 8:00 and 9:00 a.m. on Day 12 (46 h post-coitum).

Embryo Collections and Culture

Fallopian tubes were retrieved immediately after euthanization and M2 medium was flushed into the Fallopian tubes (Sigma Aldrich, St. Louis, MO, USA) for embryo collection. This was done under a dissecting microscope (Leica Zoom 2000, Tokyo, Japan). Retrieved embryos were graded according to the quality of the embryos [51], and only embryos with good quality were selected for in vitro culture in 100 µL M16 culture medium (Sigma Aldrich, St. Louis, MO, USA). The culture media was overlaid with mineral oil (Sigma Aldrich, St. Louis, MO, USA) and prepared overnight prior to use for homogenization. Cultured embryos were incubated under 5% CO_2 at 37°C until they developed into 4-cell embryos. The standard protocol of embryo handling as described by [50] was used in this study.

Gene Expression Analysis

The list of the PI3K/Akt-Cyclin D1 genes is as listed in Table 1. QuantiGene Plex (QGP) assay (Affymetrix, Santa Clara, CA, USA) was used in the gene expression analysis. This analysis was conducted at i-DNA Biotechnology (M) Sdn. Bhd., Kuala Lumpur, Malaysia. The advantages of the QGP assay include direct quantification of mRNA from the embryonic cell lysate through the sequence-specific probe-gene hybridization and without the necessity of RNA extraction. These helped to reduce the errors that might be possibly introduced should the RNA extraction and amplification procedures were carried out [52–56].

Following *in vitro* culture, a number of fifty 4-cell embryos (n = 50) from each group (Grp.1–Grp.8) were collected and used in the gene expression analysis. The QGP procedures were followed as described in the manufacturer's protocol. At the end of the procedures, the relative fold-change (FC) values were obtained by normalization of the median fluorescence intensity (MFI) values (raw data) against the reference gene and control values (Reference gene = *hypoxanthine–guanine phosphoribosyl transferase 1* (*Hprt1*)).

Statistical Analysis

Statistical analysis was conducted using SPSS 24.0. Data were shown as mean ± S.E.M, and $p<0.05$ was considered as statistically significant.

RESULTS

Table 2 shows the data on the fold change values of the studied genes in 4-cell embryos. Four-cell stage embryos in Grp.2 showed that all of the PI3K/Akt-Cyclin D1 genes were either down-regulated or non-significantly changed (Table 2) following treatment with 3 mg/kg bw/day of nicotine. Intervention with δ-TCT in Grp.3 and Grp.4 resulted in a significant down-regulation of the *pik3cb* gene at 0.33-fold in Grp.3, and a significant up-regulation at 3-fold in Grp.4 ($p<0.05$). These were same for the *PTEN* gene expression where a significant down-regulation at 0.21-fold in Grp.3, and a significant up-regulation at 15.15-fold in Grp.4 were observed ($p<0.05$). Besides, down-regulations were also shown in *pdpk1* gene expression at 0.2-fold and *akt1* gene expression at 0.61-fold in Grp.3. The *GSK3β* gene expression in this group was significantly up-regulated at 1.82-fold ($p<0.05$). In contrast to the result of Grp.3, data from Grp.4 showed that the *akt1* and *GSK3β* genes expressions were significantly up-regulated at 15.15-fold and 27.27-fold respectively ($p<0.05$). In addition, data in Grp.3 showed the significant up-regulation ($p<0.05$) of *ccnd1* at 1.82-fold and *cdkn1a* at 11.5-fold, whereas the expressions of *atm*, *ccne1*, *cdk2*, *cdk6* and *cdkn1b* genes were shown to be down-regulated. Meanwhile in Grp.4, the gene expressions of *ccnd1*, *ccne1*, *cdk2*, *cdk6* and *cdkn1a* were significantly up-regulated ($p<0.05$), whereas the expressions of the other genes were either down-regulated or non-significantly changed.

Concurrent supplementation with 3 mg/kg bw/day of nicotine and 60 mg/kg bw/day of α-TOC in Grp.5 resulted in significant up-regulation of *PTEN* at 3.79-fold and *GSK3β* at 11.36-fold ($p<0.05$). Effects on the other genes were shown by a non-significant up-regulation of *ccne1* gene at 1.09-fold, as well as a significant up-regulation in *cdkn1a* gene expression at 4.72-fold ($p<0.05$). Expressions of the other genes were either down-regulated or non-significantly changed. Different expression patterns were observed in the studied genes in groups supplemented with mixed and pure δ-TCT alone in Grp.6 and Grp.7. In Grp.6, the *pdpk1* and *akt1* gene expressions were significantly down-regulated at 0.33-fold ($p<0.05$). The *pik3ca*, *PTEN* and *GSK3β* genes were non-significantly changed. The expression of *ccnd1* and *cdk6* were significantly up-regulated at 2-fold and 1.67-fold respectively ($p<0.05$). Meanwhile, the expressions of the other genes were either down-regulated or non-significantly changed. Treatment with pure δ-TCT alone (Grp.7) resulted in a significant up-regulation of *ccnd1* gene expression at 1.62-fold ($p<0.05$), whereas the other genes were either down-regulated or non-significantly changed. Supplementation with α-TOC alone (Grp.8) resulted in significant up-regulation of *ccnd1* at 1.15-fold increase ($p<0.05$), while the expressions of the other genes were either down-regulated or non-significantly changed (Table 2).

DISCUSSION

Present study is a preliminary study to determine the effect of annatto-derived delta-tocotrienol (δ-TCT) in comparison to alpha-tocopherol (α-TOC) on the regulation of the PI3K/Akt-cyclin D1 gene expressions in nicotinic 4-cell embryos. From the results, nicotine can be obviously seen to be exerting its damaging effect onthe expressions of PI3K/Akt-Cyclin D1genes (Table 2).Meanwhile, intervention with mixed δ-TCT (contains 90% δ-TCT and 10% γ-TCT homologs) (Grp.3) and pure δ-TCT (contains >98% of δ-TCT homolog) (Grp.4) showed significant changes in the expressions of the major genes including *PTEN*, *pdpk1*, *akt1*,*GSK3β*and *cdkn1a*that could potentially affect their normal functioning in the cells. In comparison of both Grp.3 and Grp.4, the suppressive effect of annatto δ-TCT (pure) in Grp.4 was more obvious with more genes were upregulated including *pik3cb*, *PTEN*, *Akt1*,*GSK3β*, *ccnd1*, *ccne1*, *cdk2*, *cdk6*and*cdkn1a*.Whereas, concurrent treatment with α-TOC (Grp.5) indicated strong suppressive effect of α-TOC compared to the other two interventions (in Grp.3 and Grp.4). In contrast, the results in the groups supplemented with annatto-TCTs (Grp.6 & Grp.7) and α-TOC (Grp.8) alone (without nicotine treatment) showed the expressions of *PTEN*, *atm*, *Trp53cdkn1a*and*cdkn1b* were either remained unchanged or down regulated. These are known as an important condition because it facilitates in the normal progression of G1/S transition in preimplantation mouse embryos [57] besides being necessary for the maintenance of karyotypic stability [58].

*PTEN, pdpk1, akt1*and*GSK3β* are among the most important genes in the pathway. *PTEN*, a tumour suppressor gene plays an important role in the control of PI3K/Akt pathway. *PTEN* is a negative regulator of phosphatidylinositol-3, 4, 5, triphosphate (PtdIns(3,4,5)P3) or PIP3 production. *PTEN* removes the phosphoric acid from the 3′ and 5′ of PIP3*,* converts PIP3 into PIP2 and further degrades the PIP2 substrate [59,60]. This degradation is necessary if there is a need to control the unlimited cell proliferations of damaged cells. PIP3 is an important second messenger involved in many pathways of cell growth, proliferation and survival [61]. It functions in facilitating the recruitment of pleckstrin homology (PH) domain containing proteins, for example in this study they were PDK1 (encoded by *pdpk1*) and Akt (encoded by *akt1*) [62].

PDK1 binds to the PIP3 substrate. It is known as a 'master kinase', whose activation is required in mouse embryonic development through phosphorylation and activation of several other kinases including Akt [63]. Phosphorylation of Akt by PDK1 results in the translocation of the activated (phosphorylated) Akt to the nucleus where many of the Akt's substrates are localized [64,65] including the glycogen synthase kinase-3 (GSK3). GSK3 is constitutively activated in mammals, but its activity is significantly reduced by the phosphorylation of an N-terminal serine, Ser9 in GSK3β[66]. GSK3β is a multifunctional kinase that involves in many signalling pathways that regulate cell fate. In addition, GSK3β also functions to phosphorylate cyclin D1 (encoded by *ccnd1*) which will result in the rapid proteolytic turnover of cyclin D1 protein [67].

Present study is the first to attempt to study on the regulation of the PI3K/Akt-Cyclin D1 gene expressions by annatto δ-TCT and α-TOC in nicotinic 4-cell murine preimplantation embryos. Obtained results showed significant changes in the fold-change values following treatment with annatto δ-TCT and α-TOC in nicotinic and normal 4-cell embryos. And to enhance our understanding on the effects of these changes on the normal cell regulations, we are currently conducting detailed studies using proteomics and *in silico* approaches.

CONCLUSION

Present preliminary study showed that annatto δ-TCT and α-TOChave caused significant changes in the expressions of the PI3K/Akt-Cyclin D1 genes in nicotinic 4-cell murine preimplantation embryos.

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Table 1. List of the analyzed PI3K/Akt-Cyclin D1 genes

Genes	Accession No.
Pik3ca	NM_008839
Pik3cb	NM_029094
Pdpk1	NM_011062
Akt1	NM_009652
PTEN	NM_008960
GSK3β	NM_019827
ATM	NM_007499
Ccnd1	NM_007631
Ccne1	NM_007633
Cdk2	NM_016756
Cdk4	NM_009870
Cdk6	NM_009873
Cdkn1a	NM_007669
Cdkn1b	NM_009875
Trp53	NM_011640
Reference genes	
Hprt1	NM_013556
Gapdh	NM_008084
Actb (β-actin)	NM_007393

Table 2. Fold change values of the analyzed genes in each group (4-cell embryos)

Genes	Grp.1	Grp.2	Grp.3	Grp.4	Grp.5	Grp.6	Grp.7	Grp.8
Pik3ca	1	ϒ0.33**	-0.21	-21.21	-3.79	-1.67	-0.09	-1.25
Pik3cb	1	ϒ0.37**	ϒ0.33**	Φ3*	ϒ0.34**	1	ϒ0.36**	ϒ0.3**
PTEN	1	-0.33	ϒ0.21**	Φ15.15*	Φ3.79*	-3	-0.09	-0.03
Pdpk1	1	ϒ0.25**	ϒ0.2**	1	ϒ0.25**	ϒ0.33**	ϒ0.33**	ϒ0.43**
Akt1	1	-0.33	ϒ0.61**	Φ15.15*	-0.76	ϒ0.33**	ϒ0.15**	ϒ0.03**
GSK3β	1	-0.11	Φ1.82*	Φ27.27*	Φ11.36*	-1.67	-0.03	-0.07
Atm	1	ϒ0.33	ϒ0.61**	-1.52	-0.12	-0.33	-0.05	-0.05
Ccnd1	1	-0.11	Φ1.82*	Φ9.09*	ϒ0.36**	Φ2*	Φ1.62*	Φ1.15*
Ccne1	1	-0.11	ϒ0.55**	Φ27.27*	Φ1.09	ϒ0.33**	ϒ0.23**	ϒ0.15**
Cdk2	1	-1	ϒ0.2**	Φ7*	ϒ0.2**	ϒ0.33**	ϒ0.85**	ϒ0.15**
Cdk4	1	-0.11	-3	-9.09	-0.36	1	ϒ0.69**	ϒ0.25**
Cdk6	1	-0.11	ϒ0.64**	Φ45.45*	-0.36	Φ1.67*	1	ϒ0.75**
Cdkn1a	1	ϒ0.11**	Φ11.5*	Φ118.18*	Φ4.72*	-0.33	ϒ0.23**	ϒ0.15**
Cdkn1b	1	ϒ0.56**	ϒ0.06**	-1.79	ϒ0.14**	ϒ0.33**	ϒ0.23**	ϒ0.85**
Trp53	1	ϒ0.11**	-0.2	-3	-0.12	-1	-0.08	ϒ0.25**

Groups

Grp.1 serves as a control, and received 0.1mL of tocopherol stripped corn oil, Grp.2 was given 3 mg/kg/day of nicotine, Grp.3 was concurrently treated with 3 mg/kg/day nicotine and 60 mg/kg/day of mixed δ-TCT (90% delta:10% gamma), Grp.4 was concurrently treated with 3 mg/kg/day nicotine and 60 mg/kg/day of pure δ-TCT

(>98% purity), Grp.5 was concurrently treated with 3 mg/kg/day nicotine and 60 mg/kg/day of α-TOC, Grp.6 received 60 mg/kg/day of mixed δ-TCT alone, Grp.7 received 60 mg/kg/day of pure δ-TCT alone, and Grp.8 received 60 mg/kg/day α-TOC alone.

Mode of regulation is indicated based on the reference value of one (1). Values of less than 1 (γ) = downregulated genes; more than 1 (□) = upregulated genes; non-significant values (negative (−) values) and the value '1' = unchanged value of gene expressions ratio.

* Indicates significant ($p < 0.05$) increase in the fold change value (corresponds to up-regulation).

**Indicates significant ($p<0.05$) decrease in the fold change value (corresponds to down-regulation).

Abbreviations

Akt1-V-Akt Murine Thymoma Viral Oncogene-Like Protein 1; *Atm*-Ataxia Telangiectasia Mutated; *Ccnd1*-Cyclin D1; *Ccne1*-Cyclin E1; *Cdk2*-Cyclin Dependent Kinase 2; *Cdk4*-Cyclin Dependent Kinase 4; *Cdk6*-Cyclin Dependent Kinase 6; *Cdkn1a*-Cyclin Dependent Kinase Inhibitor 1A; *Cdkn1b*-Cyclin Dependent Kinase Inhibitor 1B; *GSK3β*-Glycogen Synthase Kinase 3 Beta; *Pdpk1*–3-Phosphoinositide Dependent Protein Kinase 1; *Pik3ca*-Phosphatidylinositol–4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; *Pik3cb*-Phosphatidylinositol–4,5-Bisphosphate 3-Kinase Catalytic Subunit Beta; *PTEN*-Phosphatase And Tensin Homolog Deleted On Chromosome 10; *Trp53*-Transformation Related Protein 53.

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RESEARCH ARTICLE

Current Status of Surgical Planning for Orthognathic Surgery: Traditional Methods Vs 3D Virtual Surgical Planning

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ABSTRACT

Orthognathic surgery has traditionally been performed using stone model surgery. This involves translating desired clinical movements of the maxilla and mandible into stone models that are then cut and repositioned into class I occlusion from which a splint is generated. Model surgery is an accurate and reproducible method of surgical correction of the dent facial skeleton in cleft and non-cleft patients, albeit considerably time-consuming. With the advent of computed tomography scanning, 3D imaging and virtual surgical planning (VSP) have gained a foothold in orthognathic surgery with VSP rapidly replacing traditional model surgery in many parts of the country and the world. What has yet to be determined is whether the application and feasibility of virtual model surgery is at a point where it will eliminate the need for traditional model surgery in both the private and academic setting. Traditional

model surgery was compared with VSP splint fabrication to determine the feasibility of use and accuracy of application in orthognathic surgery within our institution.VSP was found to generate acrylic splints of equal quality to model surgery splints in a fraction of the time. Drawbacks of VSP splint fabrication are the increased cost of production and certain limitations as it relates to complex craniofacial patients. It is our opinion that virtual model surgery will displace and replace traditional model surgery as it will become cost and time effective in both the private and academic setting for practitioner.

Keywords: Orthognathic surgery, traditional planning, virtual planning, 3D**.**

INTRODUCTION

Orthognathic surgery requires exact assessment of complex dentofacial deformities of the craniofacial skeleton[1]. The achievement of the surgical arrangement is not only dependent on the accuracy of the skeletal and dental diagnosis of the deformity but also is unequivocally dependent on pre surgical prediction of the proposed jaw movements. The success of orthognathic surgery planning depends on careful physical examination, correct diagnosis, and accurate treatment planning[2]. It is the task of the surgeon to first characterize the original position of the dent facial skeleton and after that to evaluate the desired final position and finally to develop a 3-dimensional portrayal of the movements important to achieve the expected goal. Conventional surgical planning (CSP) for orthognathic surgery uses 2-dimensional (2D) cephalometric analysis, photographs, dental casts mounted in an articulator with face-bow transfer, and model surgery to simulate the movements of the jaw. After facial and cephalometric analysis, the conventional procedure implements a 2D treatment plan based on the facial analysis and cephalometric tracing[3]. On the basis of the planning, model surgery is performed on an articulator and a surgical splint is fabricated as guidance during the operation.. This conventional model surgery organizes the quantitative information and permits exchanges of the normal 3D movements directly to the patient to encourage the intra operative position of the maxilla and/or the mandible. This procedure has stood the trial of time and has taken into consideration for precise and reproducible surgical correction of the dentofacial skeleton. This strategies requires abroad procedure of analytical and radiographic examination, dental model fabrication and splint preparation which require abroad time commitment, and a firm handle of dental materials and has the potential to have inaccuracies during the process. Over the past few years, the development of 3-dimensional (3D) virtual surgical planning (VSP) has become more important for planning orthognathic surgery. VSP for orthognathic surgery has been greatly enhanced by the incorporation of 3D imaging methods, such as cone beam computed tomography (CBCT), facial scanning, and digital dental casts, as well as the use of computer-aided design (CAD) and computer-aided manufacturing (CAM) technology[4]. These advances have been shown to facilitate equivalent or better outcomes when compared with standard 2D planning and conventional fabrication of splints. The remarkable progression takes after its foundation back to 1906 when the first principal surgery to correct at prognathic mandible was performed on a Washington University medical studentby plastic surgery pioneer Vilray Blair. This introduced in decades of jaw surgery obscured by Obwegeser's introduction of the sagittal split osteotomy in the1950s and Bell's research on the vascularization of the upper jaw leading to the safe downfracture of themaxilla in a LeFort I osteotomy. Various software programs are available for VSP and the manufacture of surgical splints using CAD-CAM technology. In an office-based workflow, the surgeon and his or her team perform data acquisition, image fusion, 3D cephalometric and soft tissue analysis, virtual osteotomies, and virtual surgery to work toward the ideal esthetic and occlusal outcome. In review of recent literature, one can distinguish various articles defining and commending the use of computer-aided design/computer-aided manufacturing(CAD/CAM) in development of surgical planning for the treatment of complex craniomaxillofacial deformities. In addition to the gaining popularity of VSP within orthognathic surgery, a series of recent investigations performed at multipleinstitutions have confirmed the accuracy of this technique. As VSP is showing both very exceptionally correct and capable, the future of traditional model

surgery comes into question. It is our objective in this articleto characterize both traditional model surgery and VSP and determine the accuracy and relevance of the 2 techniques[5].

Virtual Surgical Planning

With the appearance of computed tomography (CT) scanning and, in specifically, cone beam dental CTs in the course past 10 years, 3D imaging and VSP have picked up a foothold in orthognathic surgery. VSP, or CAD/CAM, is quickly replacing traditional model surgery in numerous parts of the country and the world.VSP envolves getting a maxillofacial CT with 3D reproduction, which gets unrivaled quality than plain radiographs, and naturally modify the maxilla and the mandible in the fitting relationship, taking out the requirement for a face-bow transfer and adjusting the Frankfort horizontal plane to the maxilla and the mandible. The quality of the images and the applications are great; however, it is yet to be resolve dregard less of whether or not the significant advantages and time-saving technologic innovations of medical modeling and splint fabrication are at a point where they will dispose of conventional cephalometric analysis and analytical model surgery. VSP through restorative indicating utilizes the stone models aquired from the patient as is done in traditional model surgery and a standard therapeutic maxillofacial CT with 1-mm cuts[6]. The models are laser checked and the computerized information are joined with that obtained from the maxillofacial CT scan of the patient to make a precise 3-dimensional model of the patient's maxillofacial and mandibular anatomy, including the dental arches[7]. These techniques fuse information obtaining through cone beam CT, usage of a face-bow jig with a joined gyroscope, and patient-specific bite registration mounted to a face bow to produce numerical values for the pitch, roll, and yaw of the patient's head.

As technology has advanced in CT imaging, it is currently comceivable toutilize medical CT images with 3D reconstruction as the patient's Frankfurt horizontal is standardized through this procedure. For CT-guided splint fabrication, 2 original stone models are given to the splint maker[8]. If multi segment LeFort surgery is planned, the maxillary stone model is sliced and repositioned to make the desirable occlusion by articulating it to the mandibular teeth. The splint producer requires that the a balanced maxillary model and the mandibular model are settled in the desired final occlusion and delivered to them along with the original maxillary model. In cases with no segmentation of the maxillary arch, 2 models articulated in final occlusion and stabilized with bite registration material are required. A virtual splint planning session is then arranged. The session begins by conformation of patient information. This is trailed by orientation of a superimposed soft-tissue profile aquired from the CT scan or digital clinical photographs. After setting the maxillary position virtual surgery starts. The software is able to recognize areas of bony overlap and impedances and allocate numerical values in millimeters[9]. The new position of the maxilla is seen in frontal, profile, and worms eye view positions. The impact of the planned surgical movement on the 3-dimensional position of the previously mentioned anatomical landmarks is assessed.

Now, change of the position and angulation of the maxillomandibular complex is conceivable to allow for exact control of key anatomical landmarks, like ANS, and thus accomplishing the most desirable aesthetic outcome. This is followed by simulation of the mandibular surgery. The relationship of the proximal segments to the distal segment of the mandible is then imagined and any gaps or overlaps are identified. This enables the surgeon to envision any required alteration or bone grafting of the segments intra operatively to guarantee a stable fixation. The symmetry and final position of the mandible are assessed from a worm's-eye view and frontal and profile views. Similarly, the final position of the pogonion is eassessed to ensure ideal aesthetic results[10]. After finishing the virtual surgery, the company then manufactures stereo-lithographic intermediate and final occlusal splints to be used during orthognathic surgery.

TRADITIONAL METHOD

Model Surgery – Principles

Model surgery is an extremely important and intricate process, which permits the preoperative preparatory orthognathic surgical 'work up' to be undertaken by the maxillofacial technologist using models of the patient's dental occlusion[11]. The model surgery can be categorised by its complexity, which is directly related to the complexity of the planned surgical procedure. The simplest type of model surgery is for a planned bilateral sagittal split osteotomy (BSSO) of the mandible. The degree of difficulty increases for a bimaxillary Le Fort I osteotomy and BSSO, and the highest degree of complexity is fora multi-segment maxillary osteotomy with differential movement of the segments combined with a mandibular symphyseal osteotomy and BSSO[12].

Stages of Model Surgery

There are essentially four sequential stages in model surgery:

1. Dental impressions, facebow record (if required) and occlusal registration.
2. Articulation – transfer of the dental models and registration information to the articulator.
3. Model surgery – to evaluate and verify the feasibility of the planned surgery and to fabricate the wafer splints.
4. Wafer splint construction. Each stage will vary somewhat depending on the type and complexity of the proposed surgical procedure.

Isolated Mandibular Surgery

If isolated mandibular surgery is being performed, it is usually possible to hand articulate the dental models into the desired occlusion[13]. A simple hinge articulator will suffice for articulator mounting, and the wafer splints may then be made from the articulator[14]. If there is an obvious and well-interdigitating proposed postoperative dental occlusion, a wafer splint is not absolutely essential. The osteotomized mandible may be secured intraoperatively into the postoperative position using the dental occlusion as the guide to the new position[15]. However, most surgeons still prefer a final splint to be prepared for surgery.

Isolated Maxillary or Bimaxillary Surgery

The principal role of model surgery in preparation for isolated maxillary surgery or bimaxillary surgery is to reproduce the correct maxillary repositioning determined at the preoperative clinical and cephalometric planning stages[16]. Ultimately, it is the accurate repositioning of the maxilla that will dictate the subsequent mandibular repositioning. If a maxillary osteotomy is being performed, the dental models should be mounted on a semi-adjustable articulator using a facebow transfer and accurate registration of the patient's occlusion. These three-dimensional measurements may be used to reproduce the maxillary model's exact location and to determine the proposed new position. The distances the maxilla will move in the three planes of space and in relation to the three axes of rotation will have been determined at the clinical and cephalometric planning stages. The occlusal portion of the maxillary cast is separated from its base using a saw. As much plaster is removed from the cast as is required to accommodate the new position of the maxilla. Once the measuring device verifies the maxilla to be in the new position, the model is secured with sticky wax or plaster to the mounting ring and placed on the articulator. At this stage, the maxillofacial technologist will have a mounting of the postoperative maxilla related to the preoperative position of the mandible. An acrylic splint is fabricated at this point – the intermediate splint– and is used intraoperatively to determine the new sagittal and transverse position of the maxilla to the preoperative position of the mandible. Asecond mounting with the dental models in the desired postoperative occlusion is used to fabricate the final splint that represents the new position of the mandible to the repositioned maxilla.

Model Surgery – Technique

Dental impressions, facebow record and occlusal registration Casting the dental impressions. Due to the possibility of damage to the models, plaster of Paris is no longer advisable for casting of the impressions. A polyurethane resin (Hit Model, Euro Resina, Italy) produces a scratch resistant model, and metal retention rings (Retention Washers, Skillbond Direct Ltd. UK) are placed in the resin as it sets to allow for the retention of the plaster

Transferring the Maxillary Model to the Articulator

The maxillary model is placed onto the bite fork and secured in place using plaster[17]. Once the plaster has set, the base of the model is trimmed parallel to the true horizontal plane (the Frankfort plane if it is horizontal). Magnets are placed into the base of the model (Eclipse Magnets, UK), which allows the sections to be freely removed and repositioned and grooves are cut into the base of the model to stop rotational movement. A plaster separator is used as an insulating material to prevent the plaster surfaces sticking together. There has to be an easily recognizable colour difference between the preoperative and postoperative positions. For example, a peach colour may be used for the preoperative position and blue for the postoperative position. The peach plaster 'spacer' is trimmed to a specific thickness, in this case 10 mm. The maxillary model and the plaster spacer are placed back onto the bite fork and articulated to the upper arm of the articulator using a zero expansion articulating plaster. If the planned surgical procedure is a mandibular BSSO, then the maxillary model can be articulated to the upper arm of the articulator arbitrarily as no facebow record is required.

Articulating the Mandibular Model to the Maxillary Model

Using the occlusal registration, the maxillary and mandibular models should be held together to check the relationship[18]. Once satisfied that the position is correct and that there are no premature contacts, the mandibular model can be articulated to the maxillary model using the same process as previously described for articulating the maxillary model. If the planned surgical procedure is an isolated maxillary Le Fort I osteotomy, then the mandibular model can be articulated to the maxillary model without the need for a removable plaster spacer as there will be no movement of the mandible.

Maxillary Movement

The planned maxillary movement is carried out using the model repositioning instrument which is specific to this articulator system[19]. Holes are drilled into the maxillary model using the drill bit in the area of the mesiobuccal cusp of the first molars bilaterally, with an additional hole drilled in the anterior region, in line with the midline of the maxillary central incisors. Plastic sleeve inserts (MRI 210) are placed into the holes using the insert tool (MRI 212). The maxillary model is placed back onto the articulator ensuring that it is in the correct position in relation to the mandibular model. The mandibular model and articulating plate are then removed and the model repositioning instrument attached to the lower arm of the articulator. The repositioning unit has three pins, one right, one left and one central. The locking nuts are released, which allow the pins to be placed into the maxillary model. Once secure, the locking nuts are tightened and the plaster spacer between the model and articulating plate can be removed. The arms on the model repositioning instrument are calibrated in millimetres; the reading for each arm should be noted prior to repositioning the maxillary model. The MRI will allow the full range of movement: superior repositioning (impaction), inferior repositioning (set-down), advancement, set-back, and rotation round the three axes of rotation, such as a differential impaction for the correction of a maxillary cant. The maxillary model may now be repositioned to the movement requested by the surgeon and orthodontist. When satisfied that the position is correct, plaster is used to secure the model in place, using magnets to retain the segments.

Mandibular Movement

The maxillary and mandibular models are securedtogether in the postoperative position using wax and placed back onto the articulator[20].This postoperative position should be determined bythe orthodontist and surgeon. The

maxillofacial technologist subsequently secures the mandibular model in place using plaster. Occasionally the surgeon may perform a mandibular symphyseal split in order to constrict the mandible and correct a cross bite.

Construction of the Occlusal Wafer Splints

Once the model surgery has been completed, the occlusal splints may be fabricated[21]. As described earlier, the facebow record and jaw registration are used to transfer the patient's preoperative position to the dental articulator. The constructed occlusal splints are then used to transfer the postoperative position to the patient at the time of surgery. With bimaxillary surgery there will be a requirement for two splints. An intermediate splint is used to reposition the maxilla relative to the unmoved mandible, and this splint forms the prescription for the repositioning of the mobilized maxilla to the unmoved mandible in the sagittal and transverse planes (the vertical position of the maxilla is decided using some form of skeletal reference guide intraoperatively). A final splint is used to reposition the mandible relative to the new maxillary position. This technique allows one jaw at a time to act as the stable reference structure for the repositioning of the surgically mobilized opposing jaw. The intermediate splint uses the initial, preoperative position of the mandible in relation to the maxilla to transfer the maxillary movement. This is why it is imperative that the preoperative position on the articulator is identical to the patient's preoperative position. Any discrepancy will result in the maxillary movements being incorrect. The intermediate and final splints may be colour coded to avoid intraoperative confusion. The intermediate position is when the maxillary model is in the postoperative position and the mandibular model still in the preoperative position.

It is important to block out the undercuts on both models using wax in order to prevent the acrylic from locking into the undercuts, which will result in damage to the model when removing the splints. A separator is used to prevent the acrylic from sticking to the models which must be sprayed on both models to allow the acrylic to be freely removed from the models once cured. The acrylic is then placed on the dental occlusal surface of both the maxillary and mandibular models and the articulator arms closed together into the intermediate position. An elastic band is wrapped tightly round the articulator arms to stop any expansion of the acrylic during the setting process. The articulator is then placed into a pressure vessel for 30 minutes at 3 bars or 43.5 psi to cure the acrylic. The same procedure is carried out with the mandibular model in the postoperative position. Once set, the acrylic splints are removed from the articulator and trimmed and polished. The splints should be tried in on the patient to ensure accuracy and allow any minor adjustments to be undertaken prior to surgery. The splints should fit securely and passively on the patient's dental arches without rocking. However, in cases where there has been a mandibular constriction the splint cannot be tried on the mandible preoperatively. The movements should be written clearly on the models, including the direction of the movement, and whether rotational movement or bodily movement has been undertaken. Due to the instability of the acrylic at medium to high temperature it is not possible to sterilise the splints, though they can be disinfected.

DISCUSSION

Orthognathic surgery is an exceptional undertaking in facial surgery: a patient's appearance and occlusal function can be enhanced significantly, affecting the patient's sense of self and well-being[22]. Successful outcomes in modern orthognathic surgery rely on close collaboration between the surgeon and the orthodontist across all stages of treatment, from preoperative planning to finalization of occlusion. The traditional procedure of clinical quantitative examination, cephalometric and radiographic examination, systematical model analysis, and model surgery for the making of intermediate and final splints used in double-jaw surgery has withstood the test of time. This process, however, is extremely time-consuming. Without having a multidisciplinary group and a highly effective and efficient system in place, it turns out to be difficult for the solo private practitioner to continue to provide orthognathic surgical care especially in light of the significant insurance limitations as it relates to compensation for the surgical procedure, not to mention the lack of compensation for the presurgical workup and splint fabrication.

This had driven numerous clinicians away from practicing orthognathic surgery as their time could be more cost-effective doing different techniques. By contrast, at large academic institutions that have access to oral surgery, plastic surgery, and craniofacial residents and fellows, the traditional route has still proven to be highly effective and educational. Virtual surgical planing has challenged the current state of presurgical orthognathic preparation and workups. As, VSPis significantly time-effecient tools that are assumed to be highly accurate in terms of imaging, quantitative analysis, and predictability of aesthetic outcomes from the planned surgical movements of the maxillofacial and mandibular skeleton and their overlying soft-tissue components. VSP splints,which have been seen to be as exact as acrylic splints in a 2003 study, actually allow surgeons toeliminate the use of acrylic splints that (1) can often have warping issues leading to poor intraoperativefits and (2) are obtained from model surgery stones that undergo an no negligible amount of deteriorationand blunting of the occlusal surfaces from overuse and control of the models to acquires splints. Not only are the splints highly reliable in their construction and accuracy but also surgeons are reporting results from CAD/CAM surgery within2 mm of predicted maxillary and mandibular positions when comparing the planned and postoperative outcomes [23].

Although there are many obvious advantages to VSP, there are still significant limitations. As of now, treating clinician must complete the initial steps of traditional model surgery of taking dental impressions in the clinic and pouring modelstones for securing of occlusal surfaces. Another contentious area VSP identifies with is multisegmentLeFort Iosteotomies. Organizations giving VSP items advise against virtual fragmenting with respect to the maxilla as it is not Food and Drug Administration approved.The present recommendations are for the clinician to take an impression of the maxilla, pour theimpression in stone, and accordingly perform the3-piece LeFort I osteotomy. The segments are thenrepositioned and affixed into 1 piece and placed intofinal occlusion with the mandibular model and sentto the company for registration of the desired final bite. This adequately includes all of the time-consumingtraditional methods of model surgery excluding face-bow transfer, mounting of the model, and generation of the intermediate and final splints. Thisis fundamental to consider as many patients treated in acraniofacial center are cleft patients who by virtue ofthe cleft undergo multisegment Le Fort techniques.

CONCLUSION

Through the appearance and surgical advances of orthognathic and craniofacial surgical arranging, it establishes the connection that soon VSP and medical modeling will take out investigative model surgery and conventional pre surgical workup. The inquiry now is regardless of whether that time has come and if, truth be told, the days and hours spent in the dental research facility performing model surgery and creating acrylic supports are behind us. Concerning jaw surgery and the utilization of VSP, it is best to depict which surgeries are best served by conventional model surgery versus 3D surgical arranging. For single-jaw surgery, it is our assessment that traditional model surgery in a reasonably supported environment still better than VSP. An expert oral and maxillofacial specialist surgeon who is basic at taking dental impressions, pouring the stones, and mounting maxillary and mandibular stones on a fundamental hinge articulator can set the final occlusion and manufacture any acrylic splint in well under an 60 minutes. A surgeon can cut that time venture to 15 minutes by having an assistant aquire the impressions and pour the models. The straight forwardness, minimal time commitment, and cost savings in preoperative arrangement for single-jaw surgery dictate the practice of traditional model surgery in this situation. This is additionally valid for clear bilateral sagittal split osteotomies surgical procedures. The genuine application and potential prevelance of VSP lies in the double-jaw methods, where a LeFort I and a bilateral sagittal split osteotomy are important. In the conventional course, this would require the clinician to take an impression of the upper and lower jaw, get a face-bow exchanger, pour plaster stone models, mount the maxilla on the mandible per the face bow, to make an intermediate splint for LeFort1 osteotomy on a semi adjustable articulator, and finally mount the surgically changed maxilla onto the mandible for the desired occlusion to fabricate the final splint. Even if the practitioner is specialist, this is extremely time-consuming and restrictive in a solo practice in terms of compensation. It is in this setting that VSP and medical modeling end up being the method of choice. In bigger

academic organization with residents and fellows, the traditional technique is not time nor cost restrictive and observed to be highly educational and instructive. It empowers the beginner specialist surgeon to have an excellent spatial relationship of the 3-dimensional movements important to perform successful jaw surgery, which will encourage their real intra operative experience. As stated, however, this requires institutional organization and support. On the other hand, in a world becoming more and more dependent on technology, the accuracy of VSP and its educational possibilities cannot be overlooked. It is, however, important to note the limitation in VSP osteotomies which should be addressed: 1. Right now, the Food and Drug Administration has not approved VSP for multiple-piece Le- Fort planning. This may or may not discourage practitioners from using the program as it brings into account risk mitigation. 2. The inability of VSP to join occlusal surfaces into the CT (computed tomography) images; in like manner, the expert still should take dental impressions and pour stone models to submit to VSP expert technician so they may check the occlusal surfaces of the teeth by scan. 3. The necessity of using a intermediary technician to encourage VSP. 4. A splint manufacture time lag from finish of VSP to splint delivery. 5. Splint production by an outside lab as rather than an in-house 3D printer or milling device. 6. Cost confinement of VSP incorporate san revealed protection advantage. 7. Absence of long-term follow-up information recording the viability of VSP.

Exactly when these limitations are relieved, craniofacial centers treating syndromic and cleft patients with complex dentofacial abnormalities will be in a position where VSP replaces traditional model surgery in its completely. In summary, it is our assessment that virtual model surgery will clearly dislodge and supplant traditional model surgery. The obstacles that need to be overcome for this to happen are not difficult, and this will likely happen in the near future. Sooner or later, VSP will be cost and time effective in the clinical and academic setting and professional providing orthognathic and maxillofacial surgical care will no longer have to endure the tedious application of traditional model surgery. With all of this being said, however, it seems appropriate to end on a quote from Hausamen5 in a 2001 article: "(one) has to realize that every step forward, every allegedly new development as well as our entire knowledge and technical know-how in medicine are only temporary; they have only a transitory character; they are certain to change."

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REVIEW ARTICLE

A Review on Variations in Lingual Foramina of the Mandible Midline

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ABSTRACT

The sublingual, Submental arteries or their anastomosis perforate the lingual cortical plate through the lingual foramen. Both the arteries are branches of facial and lingual arteries respectively which either arises independently from the external carotid artery or arises from a common lingual facial trunk. The anterior mandibular midline at which the lingual foramen is frequently present is subjected to various procedures like dental implants, genioplasty, tori removal, block graft harvesting, screwing with or without plating following trauma or osteotomy. There is a wide range of anatomical variations of lingual foramen among individuals. Cone-beam computed tomography (CBCT) has been shown to be superior to panoramic radiographs in displaying mandibular lingual foramen and its variations. Numerous studies have been carried out to examine the frequency, diameter, and other anatomical features of the lingual foramen and its canals. There is a paucity of the data which compares the different groups of the population based on the position and the occurrence of the lingual canal and very few studies compared the gender and population. The main purpose of the review is to examine the anatomical variations of the lingual foramen.

Keywords : Lingual foramen, Lingual canal, Accessory foramen, Anatomy, Cone beam computed tomography ,Sublingual artery , Submental artery

INTRODUCTION

The use of implants and the grafting procedures were increasing specifically in the anterior jaw bone results in the raising reports of postoperative complaints(1). Many dental anatomy books or reports have failed to report the existence of the lingual foramen. Perhaps the lingual foramen is well-defined through oral radiographs(2). The awareness of lingual foramen plays a major role in pre-surgical consideration before the installation of midline mandible implants. Anterior mandible includes many anatomical structures which include mandibular incisive canal (MIC) and lingual foramen(3–5). The lingual foramen is situated in the midline of the mandible and at the equal level, inferior or superior to the mental spines(6). These anatomical structures in the anterior jaw plays a major role in optimizing the plans for dental surgeries and also to avoid further complications(7).

The structure of the lingual foramen, dimension and the location of the bony canals have to be considered with greater importance during any anterior dental surgeries such as grafting procedures, implant placement and genioplasty so as to avoid the major complications. Some of the complications involved in these kinds of dental surgeries include pulp canal obliteration, intraoperative bleeding, neuropraxia of the mandibular incisive nerve, and nerve injury. The short term and long term disturbances involve alteration or loss of pulp sensitivity in the lower front teeth(8). Many studies assume the vascular content, an anastomosis of the sublingual branch of the right lingual arteries and left lingual arteries(9). The size of the artery should be sufficient enough to aggravate the haemorrhage in the soft connective tissues or intraosseously which may be very crucial to control(10). Numerous studies have been carried out to examine the frequency, diameter, and other anatomical features of the lingual foramen and its canals(11–13). There is a paucity of the data which compares the different groups of the population based on the position and the occurrence of the lingual canal and very few studies compared the gender and population. The main purpose of this review is to search the literature on possible anatomical variations of the lingual foramen

Clinical Considerations

The lingual mandibular foramen is a special anatomical variation on the inferior jaw bone. It consists of both vascular and nervous anastomosis which is derived from the branches of the submental and lingual artery and mylohyoid nerve. Various studies reported that during implant interventions, the intraoperative massive bleeding occurs in this area(14). Bernardi(15) reviewed the frequency and anatomical features of the mandibular lingual foramina on the midline of the inferior jaw. The finding highlighted that there exist high frequency of variation with a quite important diameter, a sign of significant calibre of the related vessels. If the variation is underestimated in the modern textbooks of oral anatomy, the radiological screening is necessary during preoperative planning. Further appropriate risk management is mandatory to minimize injury in the anterior floor of the oral cavity. In the current scenario, dental implants are considered as the standard options for prosthetic rehabilitation for edentulous patients. In many cases, the placement of the implants is a routine and predictable technique(16). Certain situations lead to hemorrhagic episodes in the lingual cortex, this is perhaps due to dental implants in the anterior mandibular region.

Previous studies showed that rupturing of the lingual periosteum leads to hematoma in this region and results in the swelling of the floor of the mouth and causes upper airway obstruction(17). Hence, preoperative planning should include radiological imaging to avoid these complications(7).

Oettlé.(18), determined the exact location and the occurrence of the midline mandibular canal (MLC) in the different age groups in both gender and dentition groups. There exists a significant difference in dentition pattern among gender shows that edentulous females were at higher risk of vessel injury in the MLC in the process of implantation.
In a retrospective study to improve the safety of the mandibular surgical procedure, Wang(19) evaluated the occurrence, location, and the diameter of the mandibular lingual canals using CBCT. The study finding emphasized the presence of mandibular lingual canals and lateral lingual canals, among which MLC is common, detected using CBCT. Majority of the samples showed at least one lingual perforation (97.0%) and all the samples showed at least one lingual perforation in the mandible. The further significant difference was observed among the gender in both

MLC and LLC. The mandible is the strongest bone in the face. In newborn the two halves of the mandibles are joined together by fibrous symphysis in the median plane and it has been replaced by the bone after a year. Natekar(20) showed that the posterior symphyseal surface shows a small elevation and is divided into upper and lower part in the mental spines. Mandibles having the lingual foramen open into the canal which crosses the bone with the branch of the lingual artery. The location and the variations in the lingual foramen help in the preoperative decision during mandibular surgeries. Bilaterally on the lingual surface of the mandible the genial tubercle, a group of four bony extensions, are situated between the superior and inferior borders of the mandible(21). Although its development is uncertain, it forms a useful radiological landmark. In 28% of mandibles, radiographically lingual foramen was observed, however, 49% was observed through periapical radiographs. Earlier studies emphasized that the superior canal is derived from the lingual artery and nerve end and for arterial origin, it was submental or sublingual, however, the innervation is branched from the mylohyoid nerve. Though the midline pit was observed on the lingual surface(22)a persistent foramen has been seen radiographically. In general the frontal part of the mandible is considered a safe place for surgical intervention; however, it has to be given more attention in the aspect of vital structures which is passing through the lingual foramen.

In the treatment of fracture, the genial tubercles are highly controversial, as many believe in the conservative treatment(23), while some believe in the fracture bone fragment removal and the muscles inserted into them(21).A single foramen on each side was observed by Nagar *et al*.2016. No anatomical books have shown mentioned the intermediate and the paramedian foramen on the lingual surface of the mandible. Sheikhi et al , assessed the anatomical variations in the lingual foramen of 102 patients along with the bony canals with Cone-Beam Computed Tomography (CBCT). The finding highlighted that around 52% of the study population had at least one foramen and shows that there is an increased prevalence of the anatomical lingual foramen. Further, the finding showed that upto four lingual foramen have been detected. A similar finding was observed by Katakami (24). In a retrospective study, using CBCT imaging, examined the regional frequency along with the anatomical properties of lateral lingual mandibular foramina. The study reported a higher regional frequency of lingual foramina in the premolar area. Hundred and fifty-four lingual foramina from 181 patients, among which 31 of the 154 lingual foramina exhibits anastomosis to the mandibular canals in the premolar area through well-defined lingual canals.

Von(25) evaluated the location and dimensions of lingual foramina by using limited CBCT, and 217 were detected among 1054 sites. The high frequency (96.2%) of foramina was observed in the midline of symphysis followed by the right first premolar area (27.5%). The sizes of the midline and posterior foramina differ significantly. The lingual foramina present along with the bony canal originate from the lingual surface, 40% of which communicated with other anatomic structures, most frequently with the incisive canal. Liang(7), in a cadaveric study, assessed the anatomic of the anterior mandible and its relative hemorrhage risk during implant dentistry. The finding highlighted that all the 118 mandibles detected had at least one lingual foramen above the genial spines. The single foramen was most frequently observed and the patients with a single lingual foramen will benefit from the inferior location of this foramen, which facilitates deeper flap surgery and implant placement that reduces the risk of damage to the canal. Therefore, cautious preoperative planning is required for the implant positioning at mandibular midline, wisely choosing an even number of implants in the interforaminal region, which avoids the risk of surgical complications. Thus CBCT imaging can be recommended as a preoperative evaluation before dental implants.

The average diameter of the artery was measured at 1.41mm and 0.31 mm with the largest diameter of 1.6mm, this indicates that the size of the artery entering(12,13)the lingual foramen is sufficient enough to cause hemorrhage in the floor of the mouth when the lingual cortex is perforated. The bleeding may increase the risk of obstructing the airway. The trauma to the artery in MLC leads to the serious hemorrhage, if the artery size increases. Airway obstruction is considered as the major complication due to hematoma formation on the floor of the mouth which leads to swelling and pushes the tongue against the palate(26). Further, the risk of hemorrhage may also result in the edentulous patients who were having atrophic mandibles with resorption of the alveolar ridge.

The placement of the implant in the thin ridges is often unrestricted as the implant strength where the surface area for load distribution is greatly reduced. Whereas when reducing the thin alveolar ride obtain a width of 6mm, in such cases decreased the vertical dimension of the implant site. Thus, the vertical reduction might also be required to create the sufficient vertical restorative space. The decrease in the alveolar bone might pose a major risk when the midline implant is considered, as it will encroach on the MLC position. The examination of the mandibular lingual region is frequently done by using gross anatomical dissections through cadavers, CT, radiographic imaging, and CBCT. The CBCT is more superior to other radiographic techniques in visualizing the lingual foramina and its canal(27)Kawai(28) investigated the frequency, location, and angulations of the lingual foramina along with the bony canals in the median region of the mandible using CBCT in the Japanese population. Most frequently observed was the MLF and the arteries which pass through the canal. Since midline superior to the mental spine was the most frequent and consisted of the artery and located superior to the other MLFs, clinicians have to identify such foramina during preoperative images.

In the description of the surgical procedures which involves the anterior mandible, the occurrences of lingual foramina along with its afferent neurovascular bundles are frequently neglected and it is associated with a negligible clinical and surgical risk. The surgical procedures in chin foramina were generally considered as free from major neurovascular complications, this might be due to the absence of the major neurovascular structures. Perhaps the interforaminal implant intervention is a routine procedure which is free from vascular complications due to the decent bone density in the median mandibular region and devoid of life-threatening endosseous vessels. Loukas(11) showed the anatomical variation of sublingual artery in relation to the mandible, and provided preoperative information to avoid hemorrhagic complications of implant placement. The lingual artery branches into sub lingual artery and provides vascular supply to the anterior mandibles. Specifically, 73% of the sublingual artery originates from the lingual artery and 27 % from the submental artery. These data suggested that single midline lingual foramen (24.5%) is widely seen above the genial spine. From a clinical perspective, the location of the foramen is important rather than the number and the midline lingual foramina have to be focused to avoid complications.

The clinical relevance of this anatomic feature is underlined by the growing diffusion of implant treatments at the mandibular midline and by reports of complications deriving from such procedures. Inter foraminal section is the best choice for the placement of the implant which supports the fixed partial dentures or overdentures. One of the autologous areas in the oral cavity is the symphysis which required excessive ridge augmentations. Lingual artery supplies the arteries from the submental branch and the sublingual branch which includes genioglossus muscles, geniohyoid, sublingual gland, mylohyoid, lingual gingiva and mucous membranes in the floor of the mouth floor.
The inferior alveolar artery gives rise to the complex branches and then separated into the mental artery and incisive branches, which communicates with the sublingual artery in the region of the internal mandible(29). Even though the interforaminal is comparatively a safe region to place the implants, the perforation in the lingual cortex can lead to the severe haemorrhage during the placement of the implant. Further, if drilling ruptures lingual periosteum, the bleeding might be enhanced due to damage to the anatomical structures in the sublingual spaces, these results in the hematoma in the mouth floor. Apart from the interforaminal region, the lingual foramen present in the molar area is also well reported(30). The cadaveric studies showed that the sublingual and submental arteries both were perforated through the lingual foramina in the mandible(31)

Subsequent to the tooth extraction, the horizontal bone loss is primarily on the labial side. This pattern of resorption leads to the lingually angulated trajectory of the mandible. If the atrophying mandible is not noticed prior to the implant placement, the lingual perforation complication will increase. The bony architecture and its surrounding anatomical structures were well depicted by the CT which is a frequently used imaging technique. The 3D imaging of the particular area is extremely suggested to achieve favourable prosthetic angulations which also excludes the complications(32). The recent studies emphasize that the structures which increase the risk of complications include the anterior dilation of inferior alveolar neural tubes, the concavity of lingual bones, lingual foramina, and lingual tubes. However, there are cases where atypical hemorrhage has been caused due to lingual plate perforation. The

mucosal branches are present along with the lingual side of the mandible requiring special care pre surgical period, as they are known to deposit lingual cortical bone into the mandible. The bleeding along with severe edema, in the process of the implant surgery due to the direct damage of the sublingual arteries followed by lingual cortical bone perforation. If the bleeding is delayed the possibility of the bleeding in the sublingual artery branch has to be considered. However, the risk of bleeding should be assessed in patients with hypertension/patients who were on anticoagulation drugs. The pre surgical assessment is mandatory if foramen's diameter is higher than 1mm in CT scan. The increased risk is prevalent among the elderly patients who are in the need of alveoloplasty (for dental procedures) and patients with severe alveolar bone atrophy. As in these patients, lingual foramina are closer to alveolar ridge and the frequency of appearance of lingual foramina is higher.

Anatomical Considerations

The anatomical feature and its clinical relevance are underpinned by the increasing implant treatments in the mandibular midline and the increasing report of the complications during such procedures. Secondary to the implant treatment, life threatening hemorrhage and hematoma formation in the floor of the mouth were recorded in many earlier reports(33). In humans, three major subdivisions namely superior, inferior and the middle sublingual alveolar branches have been identified. In general facial artery is the fourth successive and third anterior branches of the external carotid artery, except it originates along with the lingual artery through the common linguofacial trunk. This anatomical attention lays the basic foundation for the role of the submental artery is either a major vessel or a supplementary vessel in this region and deserving the consideration so as to understand the nature of hemorrhages descending from the perforation of the mandibular lingual cortical during implant surgery. The mechanical injury in the branches of the arterial plexus might possibly lead to the dangerous hemorrhage. The elaborate knowledge of the anatomy of the fine arterial structures is necessary for the implant surgeries. From the level of the hyoid bone, the lingual artery is the third sequential and second anterior branch of the external carotid artery. This lingual artery provides the body and the top of the tongue through the terminating deep dorsal branches along with the lingual artery. At the frontal border of the hyoglossus muscle, the lingual artery leads to the sublingual artery.

Recommendations

Clinically attention has to be given to recognize the situation where this risk might occur. Subsequently, following recommendations has to be followed. An appropriate preoperative planning is mandatory before any surgical procedures concerning the median mandible, bearing in mind the degree of osseous atrophy along with the mandibular inclination. If necessary radiographic examination of these intraosseous canals through computed tomography can be considered. An accurate knowledge of the anatomy of the region is necessary. The positioning of implants in the mandibular midline has to be given most priority. A wise opting of an even number of implants in the interforaminal region can avoid the risk of trauma to the lingual cortical plate of the mandibular midline.

CONCLUSION

The present review showed that the variations in the anatomical landmarks and the measurements of lingual foramen vary in every individual, thus it is important to think about the lingual foramen during the planning session for surgery and particularly during the placement of anterior mandibular implants, to avoid post-operative related complications. The clinicians have to note the position of the midline mandibular lingual canal and should approach with precautions, specifically if the alveolar ridge has to be decreased prior to the placement of the implant. To conclude there are various kinds of lingual foramen that have been identified based on their position and their neurovascular contents. Future studies should focus on the micro-dissections, which in light of the neovascularization of the anterior mandible, during surgical interventions. This kind of knowledge on the anatomical variations is necessary during the surgical process involves all potential risk which is related to the anatomical variations in the lingual foramen. Further, it also helps in planning the oral implants in terms of both

aesthetic points and also to avoid neurovascular complications. The current review provides the immense contribution to clinical and surgical understanding.

Conflicts of interest
Nil

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RESEARCH ARTICLE

Assessment of Relationship of ABO Blood Groups among Oral Squamous Cell Carcinoma Patients Treated in Saveetha Dental College, Chennai

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ABSTRACT

The possibility of association between ABO blood groups and malignancy was first discussed by Anderson DE & Haas C. The association between blood group and oral cancer is least explored and hence this study was undertaken to evaluate relationship of ABO blood groups with an increased risk for oral cancer. The present study was conducted at Saveetha dental college Chennai. The study samples comprised 106 oral cancer (OSCC) patients. The information regarding the socio demographic profile, type of oral cancer and ABO blood group profile was obtained from the case sheets of the patients In the study of 106 patients, the frequency of squamous cell carcinoma was significantly higher in men (79%)

than women (21%). It was predominantly more in the age group of 45-65 years. 42 patients were of blood group B+ve, 32 of O +ve, 19 of A+ve, 4 of O-ve, 3 of AB-ve and 6 had the blood group AB+ve. There was higher potential risk in B+ve blood group followed by O+ve. Among locations of oral cancers, Oral squamous cell carcinoma of buccal mucosa (52%), tongue (30%) and Carcinoma of floor of mouth (18%). The present study reveals that there is an inherited element in the susceptibility against different types of oral cancers like OSCC. The people with blood group B+ve and O+ve are more at risk to develop oral cancer than people with other blood groups.

Keywords: ABO blood groups, oral cancer, oral squamous cell carcinoma, buccal mucosa, tongue.

INTRODUCTION

The membrane, which defines the extent of the cell, is not only a physical boundary but also has many specific functions, among which is the capacity to react with other cells and the intracellular matrix [1]. Carbohydrates are structures found on the cell surface bound to either lipid or protein embedded in the membrane [2]. Changes in the carbohydrate structure of these cell-surface glycolipids and glycoproteins have been demonstrated during development, during cell maturation in adult tissue, and in relationship to malignant development [3]. The cell-surface carbohydrates have an enormous potential for serving as informational molecules [4]. Monosaccharides can be linked together in different sequences and by different glycosidic linkages, thereby generating a vast complexity of saccharide chains [5]. The variety of structures, which can be formed by a limited number of units, is thus far larger in oligosaccharide chains than in peptides [6]. Each monosaccharide unit has 3 or 4 different sites that can be substituted by the next sugar, in contrast to amino acids, which have only one site for linkage with the next monomeric unit. Although carbohydrates are extremely complex structures, the cellular expression is highly regulated, and, within a particular organ, the carbohydrates may be expressed in a way that correlates with cell differentiation [7]. When carbohydrates are bound to proteins, changes in glycosylation may affect the conformation and function of the protein and thereby influence the interaction between the cell and its environment [8]. It has been demonstrated, for example, that changes in glycosylation of integrins alter their function, and that the Notch receptor, which is a transmembrane protein that mediates communication associated with cell differentiation, is regulated by alteration inthe glycosylation of the protein [9]. Cell-surface carbohydrates are built up in a stepwise fashion when monosaccharides are tranferred from their sugar nucleotide derivatives to appropriate acceptors [10]. Each particular type of transfer is catalyzed by a unique specific glycosyltransferase [11]. Thus, a missing or changed enzyme will block further synthesis of the carbohydrate structure, which will remain as a precursor structure [12]. Additionally, the appearance of new enzymes that either compete for common substrate or lead to elongation of previously terminated structures will result in alteration in the carbohydrate that is finally expressed [13]. In tumors, changes in glycosylation are found in both glycolipids and glycoproteins [14]. Most studies have dealt with alteration of carbohydrates at the cell surface. However, several recent studies have shown that altered glycosylation plays a major role inmost aspects of the malignant phenotype, including signal transduction and apoptosis. These studies have recently been reviewed.

MATERIALS AND METHODS

The present study was conducted at Saveetha dental college Chennai. The study samples comprised 106 oral cancer (OSCC) patients. The information regarding the socio demographic profile, type of oral cancer and ABO blood group profile was obtained from the case sheets of the patients

RESULTS

In the study of 106 patients, the frequency of squamous cell carcinoma was significantly higher in men (79%) than women (21%). It was predominantly more in the age group of 45-65 years. 42 patients were of blood group B+ve, 32 of O +ve, 19 of A+ve, 4 of O-ve, 3 of AB-ve and 6 had the blood group AB+ve. There was higher potential risk in B+ve blood group followed by O+ve. Among locations of oral cancers, Oral squamous cell carcinoma of buccal mucosa (52%), tongue (30%) and Carcinoma of floor of mouth (18%).

DISCUSSION

Lipid or protein-bound carbohydrates (glycolipids or glycoproteins) are found on cell membrane, which may undergo some changes during cell maturation or malignant transformation as shown by some previous studies [15]. Most of the time, the outer part of such glycoconjugates includes carbohydrates like ABO and Lewis blood group antigens [16]. A high incidence of various carcinomas are found in patients having A/B blood groups, which may be due to higher affinity of these antigens to some micro-organisms known to develop cancer [17]. Several reasonable mechanisms have been proposed to explain the relationship between ABO blood groups and risk of cancer such as inflammation, immunocompetency to detect malignant cells, intercellular adhesion and membrane signaling [18]. Down regulation of glycosyl transferase that is involved in the biosynthesis of A and B antigens, and linkage disequilibrium between ABO genes with other genes may help promote carcinogenesis [19]. ABO antigens can also be present on key receptors such as EGF receptors, integrins, cadherins and CD-44, which control cell proliferation, adhesion and motility. As the expression patterns of these receptors vary in normal and cancerous cells, the role of ABO antigens in tumerogenesis may be different as well. In this study, among oral cancer cases, male: female ratio was 2:1,and most of the patients (75%) were older than 50 years of age. Cawson also suggested that oral cancer is an age-related disease, and 98% of patients are over the age of 40 [20]. However, in recent decades an upward trend has been observed in the number of oral cancer cases among women and younger age groups. On the basis of the present study, blood group B was significantly more frequent in oral cancer patients than control group. Furthermore, Logistic Regression demonstrated that people with blood group B and those older than 50 years of age had 3.5 and 19.4 times risk of developing oral cancer, respectively. In accordance to our findings, Akhtar showed a high incidence of blood group B (37.5%), in patients with oral cancer, which was followed by A (35%), O (20%) and AB (7.5%) blood groups. Sharma and colleagues also demonstrated that in cancer of the buccal mucosa blood group B had the highest frequency (34.1%), followed by A (30.4%), O (28.0%), and AB (7.3%). Jovanovic-Cupic et al. (2008) evaluated the relationship between ABO blood groups and malignant tumors of the digestive tract in Bosnia and Herzegovina, and found a significantly higher frequency of blood group B in cancer patients. Jaleel in another study from India showed that people with blood group A had 1.4 times higher risk of developing oral cancer followed by B blood group (1.1 times), AB (0.9 times) and O (0.6 times). Other studies, which were done in India, have demonstrated individuals with blood group A have predisposition for oral cancer. These differences in findings may be related to diversity in sample size, study design and races of participants. Yu, Guleria, and Xie noted that association between ABO blood groups and the risk of cancer may vary among different geographic localizations and races or ethnicities. Unfortunately, there is lack of information about association between ABO blood groups and oral cancer in Iranian population to compare with our study. In addition, Xie in a study from US, evaluated the relationship between ABO blood groups and skin cancers, and showed that the risk of developing SCC was significantly lower for patients with blood group A. The same results were found in patients with BCC, but they did not observe a statistically significant decreased risk of developing melanoma in participants with non-O blood groups. Our study also showed that blood groups O and B were more frequent in squamous and non-squamous cell originated oral cancers, respectively. But there were no significant differences between these two types of oral cancer in terms of ABO blood groups and Rh factor. Risk of Developing Squamous and NonSquamous Originated Oral Cancer in Terms of Age, Sex, Blood Group, and Rh Status.

CONCLUSION

The present study reveals that there is an inherited element in the susceptibility against different types of oral cancers like OSCC. The people with blood group B+ve and O+ve are more at risk to develop oral cancer than people with other blood groups. By employing a simple blood grouping test during community field programs, we can target the people with blood group B in the age group of 40-59 years having tobacco chewing habits and educate them that they are more at risk to develop oral cancer than people with other blood groups. But since this was a retrospective study based on hospital records, it may not be truly representative of all oral cancer cases in the community. Hence, further study in this regard is recommended.

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SHORT COMMUNICATION ARTICLE

Elucidation of the Antimicrobial Capabilities of Spirooxindole Fused Heterocycles Synthesized Via Catalyst Free Method

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ABSTRACT

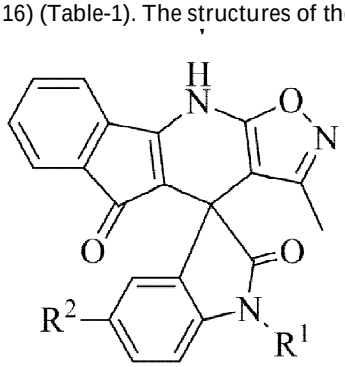
In this study, catalyst free synthesis of isoxazolo [5,4-*b*]pyridine/ quinoline derivatives *via* one-pot three-component condensation, was replicated by using the previously reported components (isatins, 3-methylisoxazol-5-amine and cyclic enolizable carbonyl compounds in ethylene glycol at 80 ^{o}C. Microbicidal activities of the 16 spirooxindole derivatives (SD) were evaluated against both bacterial (*E. coli*) and fungal (*C. albicans*) pathogens, wherein it was found that the derivatives showed better antifungal activity as compared to antibacterial activity. Out of these, compounds SD11, SD12, SD13 and SD2 displayed impressive antifungal activity, whereas only compound SD14 showed significant antibacterial potential. This study provides us useful information on the antimicrobial potential of the synthesized isoxazole analogs and paves the way for synthesis of better and eco-friendly analogs with potent therapeutic properties.

Keywords: Isoxazolo[5,4-*b*]pyridines/quinolines, Minimum inhibitory concentration, Antifungal, Antibacterial.

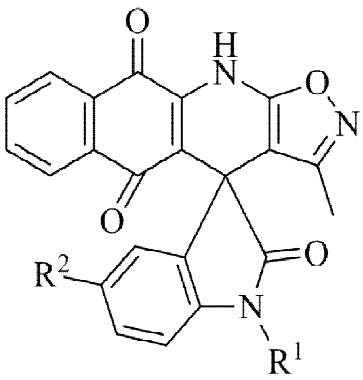
Microbial pathogens remain the most common cause of infectious diseases and account for millions of mortality and morbidity in humans [1]. *Escherichia coli* (*E. coli*: bacterial pathogen) and *Candida albicans* (*C. albicans*: fungal pathogen) are microbial pathogens which exist as part of human microbiota. But under certain conditions, these become pathogenic and cause severe infections such as *E. coli* causes food poisoning, diarrhea, urinary tract infection, septic shock, meningitis and *C. albicans* causes oropharyngeal candidiasis, vulvo vaginal candidiasis and invasive candidiasis [1-3]. Both pathogens are the leading causes of bloodstream nosocomial infections in hospitalized patients [3-4]. Although, several conventional therapeutic compounds are available, these microbes often develop resistance to conventional drug therapies and increase the risk of prolonged illness, expensive treatment and later, even mortality [5-7]. Thus, there exists a constant need for new compounds to mitigate the recurrence of microbial infections and drug resistance.

In the field of organic chemistry and development of novel compounds for therapeutics, the native molecule of isoxazole has been used to derive several compounds such as sulfonamide derivatives of isoxazolo[5,4-b]pyridine (spirooxindoles) [8]. Isoxazoles are monocyclic heteroarene compounds with an oxygen atom next to nitrogen and have been extensively exploited for their versatile chemotherapeutic characters [8-9]. Isoxazoles are known to display anti-inflammatory properties by inhibiting the COX-2 enzymes [10] and they exhibit an impressive range of biological properties such as hypoglycemic, muscle relaxant, anticonvulsant [11]. The isoxazole ring plays key role in drug designing and may increase the efficacy along with improved pharmacokinetics profiles and decreased cytotoxicity [12]. Sulfonamide derivatives are present among natural as well as synthesized compounds and are exploited for new drug discoveries [13]. Many conventional and traditional methods have been developed to synthesize sulfonamide derivatives of isoxazolo[5,4-b]pyridine. One such compound spirotryprostatins, a naturally occurring spirooxindole alkaloid has been found to exhibit anti-cancer property [14-15]. This study is therefore an endeavor to use such compounds and establish their broad spectrum microbicidal potential against common bacterial and fungal pathogens.

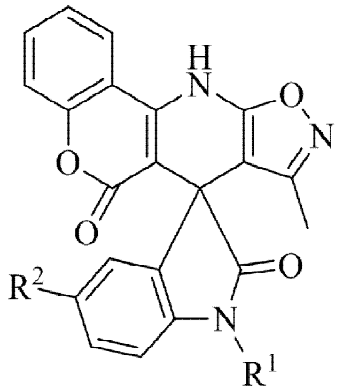
Khurana and co-workers have already reported the successful synthesis of novel isoxazolo [5,4-b]pyridine derivatives that have less reaction time, easy work-up and high yields [16]. The study led to synthesis of the corresponding spirooxindole derivatives (SD) namely3-methylspiro[indeno[2,1-*e*]isoxazolo[5,4-*b*]pyridine-4,3'-indoline]-2',5(10H)-diones (SD1-5); 3-methyl-5Hspiro[benzo[g]isoxazolo[5,4-b]quinoline-4,3'-indoline]-2',5,10(11H)-triones (SD6-10); and 8-methylspiro[chromeno[3,4-e]isoxazolo[5,4-b]pyridine-7,3'-indoline]-2',6(11H)-diones (SD11-16) (Table-1). The structures of the derivatives are as follows:



SD1-5



SD6-10



SD11-16

In this study, this multi component cyclocondensation process was efficiently duplicated at original conditions of temperature 80 ^{O}C in ethylene glycol as solvent and the derivatives were further evaluated for their antimicrobial potency.

The anti-fungal activities of these 16 derivatives were evaluated against *C. albicans* (SC5314) [17] by determination of minimum inhibitory concentration (MIC) using broth microdilution assay as described elsewhere [18-19], wherein cells were allowed to grow with all derivatives, separately for 48 hours at 30 ^{o}C. Then, the absorbance at λ=600 nm of the *C. albicans* cells grown in the absence (growth control) and presence of these compounds were recorded in a microtiter plate (Fig. 1A). The MIC values were determined through calculation of the percentage of growth inhibition by comparing the absorbance of *C. albicans* cells grown in the presence and absence (growth control) of these test compounds (Fig. 1B). A solvent control (DMSO) was used for all test compounds and the percentage of growth inhibited by the respective compounds were calculated at the concentration where there was no inhibitory effect of the solvent and the growth inhibition was only due to the presence of the compound.

The percentage of growth inhibition of *C. albicans* cells at the respective concentrations of the compounds tested is given in (Table 2). Of the 16 compounds tested, the best results were obtained for compounds in the order SD11> SD12>SD13>SD2 at concentrations 400, 400, 400 and 250 µg/ mL, respectively, wherein they showed respective growth inhibition of 79, 77, 73 and 62 % for *C. albicans* cells. Spirooxindole derivatives SD3, SD7 SD16, SD8 and SD15 at respective concentrations of 400, 300, 200, 500 and 236 µg/ mL exhibited growth inhibitions of 57, 50, 41, 40, and 40 %, respectively for *C. albicans* cells. The compounds SD4, SD5, SD1, SD14, SD9, SD6 and SD13 showed less than 40 % growth inhibition of *C. albicans* cells at respective concentrations of 400, 250, 100, 400, 400, 400, 200 µg/mL and inhibited the growth of fungal cells by only 36, 24, 23, 14, 19, 19 and 9 % respectively. The experimental method adopted gave reproducible results for positive control since MIC determined by broth microdilution assay for fluconazole (positive control) was similar to the values indicated in the literature [20] and all positive controls demonstrated almost complete inhibition of *C. albicans* cells at a concentration of 0.5 µg/ mL fluconazole. In addition, all growth controls *i.e.* media containing only cells but no inhibitory compound showed proper fungal growth and thus confirmed the accuracy and reproducibility of the methodology in the study.

The anti-bacterial activity of the derivatives was evaluated against *E. coli* (DH5α) [21] by determination of MIC using broth microdilution assay as described earlier [22], wherein the cells of the pathogenic Gram-negative bacterium, *E. coli* were grown separately in the presence and absence (growth control) of these compounds and after 48 hours the absorbance at 600nm were recorded in microtiter plate reader (Fig. 2A). The MIC was determined by the percentage of growth inhibition calculated by comparing the absorbance values of the *E. coli* cells grown in the presence and absence (growth control) of these derivatives (Fig. 2B). A solvent control (DMSO) was used for all compounds and the percentage of growth inhibited by the respective compounds were calculated at the concentration where there was no inhibitory effect of the solvent and the growth inhibition was only due to the presence of the compound. Reproducible results were obtained for positive control in the experiments conducted and MIC determined by the broth microdilution method for ampicillin (positive control) was similar to the values indicated in the literature [23]. All positive controls demonstrated almost complete inhibition of *E. coli* cells at a concentration of 2 µg/ mL ampicillin and all negative controls (growth control containing only media and cells but no inhibitory compound) displayed proper growth of bacterial cells, confirming the accuracy and reproducibility of the methodology in this study.

The percentage of growth inhibition of *E. coli* cells along with the respective concentrations of the spirooxindole derivatives tested is given in Table 3. Of the 16 derivatives tested, compound SD14 showed 71 % growth inhibition of *E. coli* cells at a concentration of 100 µg/ mL. Compounds SD1, SD9, SD8, SD4 and SD6 showed growth inhibition of *E. coli* cells by 39, 39, 38, 34 and 33 % respectively at 100 µg/ mL concentration of each. Compounds SD12, SD13, SD7, SD16, SD15, SD11, SD3, SD10, SD5 and SD2 exhibited still lower inhibition in growth of *E. coli* cells *i.e.* by 29, 20, 16, 15, 13, 11, 9, 8, 7 and 2 %, respectively at 100 µg/ mL of each.

The literature studies have already confirmed that native as well as various derivatives of isoxazolo possess a wide range of biological activities. This encourages chemists to synthesize different new compounds bearing isoxazolo nucleus with low toxicity. In congruence to this, we replicated the synthesis of isoxazolo [5,4-b] pyridines/quinolones

derivatives and were able to get moderate to high yields. The antimicrobial potential of these derivatives were evaluated. This study suggests that such synthesized isoxazoloquinoline derivatives can be further modified to improve their biological activities and may replace the indiscriminately used conventional antibacterial compounds such as penicillins, quinolones, sulfamethoxazole and antifungal drugs such as azoles and polyenes, for which many bacterial and fungal strains already exhibit resistance [5-6]. To conclude, we would like to add that extensive *in vitro* and *in vivo* anti-microbial research must accompany the synthesis of novel chemical synthesis and such combinatorial study could save us from the looming scarcity of effective antimicrobial drugs and eventual emergence of multidrug resistant microbial pathogens.

Conflict of interest

The authors declare no competing conflict of interest.

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Table 1: Detailed structure of isoxazolo [5,4-*b*] pyridine derivatives

Product	R_1	R_2
SD1	H	H
SD2	$-CH_2-CH=CH_2$	H
SD3	H	Br
SD4	H	Cl
SD5	H	NO_2
SD6	H	H
SD7	$-CH_2-CH=CH_2$	H
SD8	H	NO_2
SD9	H	Cl
SD10	$-CH_2-C\equiv CH$	H
SD11	H	H
SD12	$-CH_2-CH=CH_2$	H
SD13	$-CH_2-C\equiv CH$	H
SD14	H	Br
SD15	H	Cl
SD16	H	NO_2

Table 2: Percentage of growth inhibition of *C. albicans* cells by compounds (SD1-SD16) tested

S. No.	Compound	Concentration (μg/ mL)	% of growth inhibition
1	**SD11**	400	79
2	**SD12**	400	77
3	**SD13**	400	73
4	**SD2**	250	62
5	**SD3**	400	57
6	**SD7**	300	50
7	**SD16**	200	41
8	**SD8**	500	40
9	**SD15**	236	40
10	**SD4**	400	36
11	**SD5**	250	24
12	**SD1**	100	23
13	**SD9**	400	19
14	**SD6**	400	19
15	**SD14**	400	14
16	**SD10**	200	9

Table 3: Percentage of growth inhibition of *E. coli* cells by compounds (SD1-SD16) tested

S. No.	Compound	Concentration (μg/ mL)	% of growth inhibition
1	**SD14**	100	71
2	**SD1**	100	39
3	**SD9**	100	39
4	**SD8**	100	38
5	**SD4**	100	34
6	**SD6**	100	33
7	**SD12**	100	29
8	**SD13**	100	20
9	**SD7**	100	16
10	**SD16**	100	15
11	**SD15**	100	13
12	**SD11**	100	11
13	**SD3**	100	9
14	**SD10**	100	8
15	**SD5**	100	7
16	**SD2**	100	2

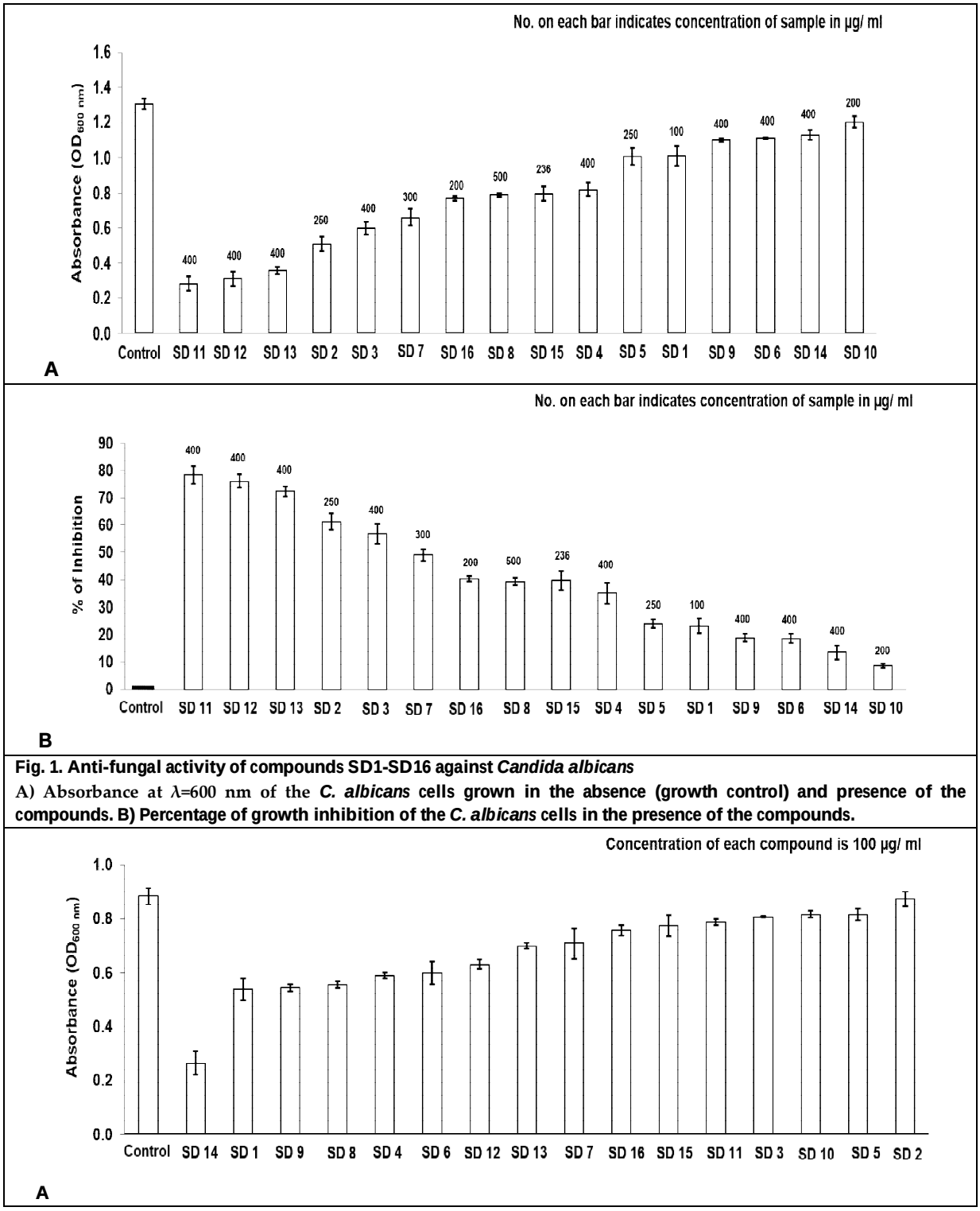

Fig. 1. Anti-fungal activity of compounds SD1-SD16 against *Candida albicans*

A) Absorbance at λ=600 nm of the *C. albicans* cells grown in the absence (growth control) and presence of the compounds. B) Percentage of growth inhibition of the *C. albicans* cells in the presence of the compounds.

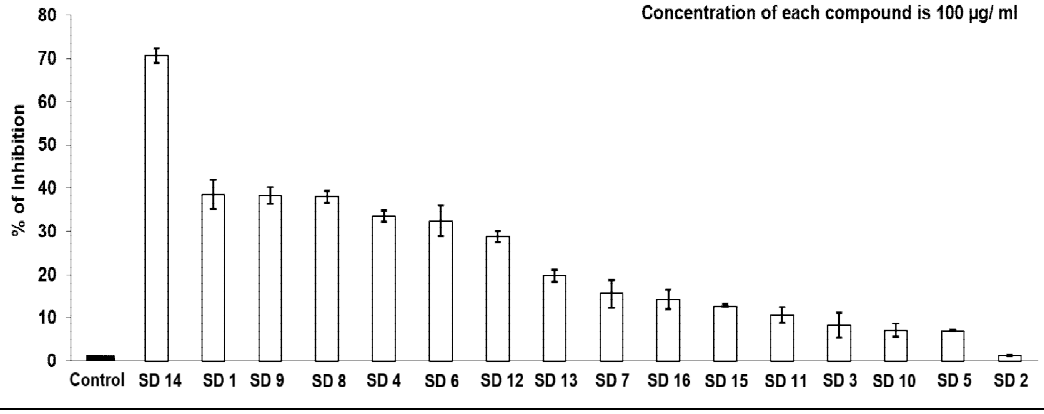

Fig. 2. Anti-bacterial activity of compounds SD1-SD16 against *Escherichia coli*

A) Absorbance at λ=600 nm of the *E. coli* cells grown in the absence (growth control) and presence of the compounds. B) Percentage of growth inhibition of the *E. coli* cells in the presence of the compounds.

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RESEARCH ARTICLE

A Comparative Study of Postoperative Pain in Patients Undergoing Surgical Removal of Impacted Third Molars Tooth using Diclofenac Transdermal Patch against Oral Diclofenac Tablet

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ABSTRACT

The objectives of this study was to evaluate subjectively the analgesic efficacy of Oral Diclofenac Sodium 100 mg (voveran 100 mg) against Diclofenac Sodium Transdermal patch 100 mg (Diclo PLAST 100mg) in the management of postoperative pain following surgical removal of impacted mandibular third molars. Twenty healthy subjects belonging to both the sexes in the age group of 21–40 years with bilateral mesioangular impactions of mandibular third molar teeth underwent surgical removal on two different occasions with a minimum interval of 1 week in-between the procedures. The postoperative pain was recorded on visual analog scale. Readings were taken at 2 hours, 4 hours, 8 hours, 12 hours and 24 hours

postoperatively. Patients received the study medication i.e. Diclofenac Sodium 100mg once a day for 3 days after performing surgery on one side and the same patients were given Diclofenac Sodium Transdermal Patch 100mg once a day for 3 days after performing surgery on the contralateral side. Both the statistical analysis and clinical observation showed that on the first postoperative day diclofenac sodium 100 mg administered orally has slightly more significant efficacy when compared to the drug administered transdermally. However, on the second and third postoperative days there was no statistical or clinical difference in the pain control by either route of administration. The investigation concludes that diclofenac sodium transdermal patch 100 mg can be utilized as an elective type of pain relief following removal of impacted mandibular third molars, however considering that the analgesic potency might be lesser in the immediate postoperative period, it might be prudent to use oral diclofenac sodium for immediate postoperative pain relief, following which transdermal route can be used for pain control.

Keywords: Diclofenac sodium, Oral, Transdermal, Third molar extraction, Pain

INTRODUCTION

Pain is one of the most commonly experienced symptoms in surgery and as such is a major concern to the surgeon. It is often spoken of as a protective mechanism, since it is usually manifested when an environmental change occurs that causes injury to responsive tissue [1]. One of the most important aspects of the practice of dentistry is the control or elimination of pain. In the past, pain has been so closely associated with dentistry that the words pain and dentistry have become almost synonyms [1].The third molar surgical experiences are desirable pain models to evaluate the analgesic efficacy and tolerability of oral analgesics due to the following characteristics, the surgeries are elective; patients are young and healthy and ambulatory. The performed procedures are consistent and are generally completed within 25 minutes. Patients undergoing removal of third molars, are considered standardized mode for the evaluation of acute surgical pain and especially those who present with bilaterally similar impacted lower third molars provide an opportunity to carry out two similar surgical procedures on separate occasions such patients act as their own controls in cross over trials.

An estimated 63.5% of patients experience severe pain at sometime during the first day for this reason oral analgesic is provided as standard care for postoperative time periods for atleast 24 hours. Non steroidal anti-inflammatory drugs work well to relieve mild to moderate intense postoperative pain caused by 3rd molar surgery [2]. Analgesic drugs can be administered in a variety of routes, including oral, parentral, inhalation as well as transdermal. Oral route carries the risk of first pass metabolism and loss of substantial quantities of the drug before it is absorbed systemically. Parentral administration of drugs can be extremely painful and sudden increase in drug concentration in the plasma could lead to certain adverse effects. Transdermal administration has the advantages of being a very easy, simple route of administration without the disadvantages of the routes mentioned above and also comparatively fewer side effects and complications. This study attempts to compare two different drug delivery systems in the management of postoperative pain following surgical removal of impacted mandibular third molars using diclofenac sodium as a standard drug which was administered in two different routes, oral and transdermal.

MATERIALS AND METHOD

Twenty healthy subjects belonging to both the sexes in the age group of 21–40 years, without any systemic diseases or previous drug allergy, who presented with bilaterally impacted mesioangular mandibular third molars to the

department of Oral and Maxillofacial Surgery, Saveetha Dental College and Hospital Chennai were selected for the study. The patients were informed about the nature and purpose of the study and the likely adverse effects and complications from the drugs being investigated. A written informed consent was obtained from all patients. Preoperative radiographs and routine medical history was taken. Analgesics and Antibiotics were administered postoperatively. Following removal of the impacted mandibular third molar on one side the patients were given oral Diclofenac (Voveran SR) 100mg once daily and when the same patients returned back for the surgery on the contralateral side the drug administered was Diclofenac sodium transdermal patch (DicloPLAST) 100mg once daily. The study drugs were administered to the patients 30 minutes after the surgery and the sides and drugs were randomly chosen. The teeth were either sectioned or removed in toto.

Standardization was maintained by either removing both the teeth in the same patient in toto or both the teeth were sectioned and removed. Of the twenty cases in this study, in ten cases, the teeth were sectioned and removed and in ten cases the teeth were removed in toto. The Transdermal diclofenac patch (DicloPLAST) is one of the few drugs, which can be administered via the skin. It is used to relieve mild to moderate postoperative pain. It is applied once for duration of 24hrs and produces rapid pain relief with minimal or no side effects. The patch is to be applied on the skin, preferably in an area devoid of any hair. It comes in strengths of 100mg, this particular patch is a product of Zuventus health care limited. The 100mg patch is 58x87mm in size It is a flat and transparent device and is packaged in hermetically sealed, foil-lined packets. The patch achieves plasma levelsranging between 20 and 50ng/ml, which is lesser when compared to the oral route, but these levels are sustained for a longer time. Oral formulation of diclofenac sodium (Voveran SR) was used in the dosage of 100mg and was administered once daily for a period of three days. Four subjective scales were used to record the pain postoperatively. The Visual Analog Scale (VAS) and one other four point scales. A simple category scale, such as the four point 'none, mild, moderate and severe' scale is acceptable for recording pain magnitude, but need numbers to be assigned to each level (0,1,2,3) to quantify the data. The four point scales used was Pain Relief Scale (PRS) [2] . Further a scale to record any adverse effects was also used. The patients had to assign scores for each parameter at intervals of 2hrs, 4hrs, 8hrs, 12hrs and 24 hrs postoperatively.

Statistical Methods

Descriptive analysis has been carried out with mean and Standard deviations were computed. Wilcoxons signed rank test has been used to find the significance of VAS and PRS between two drug delivery techniques namely, ORAL and TRANSDERMAL PATCH

1. Significant figures

+ Suggestive significance 0.05<P <0.10

* Moderately significant 0.01<P <0.05

** Strongly significant P <0.01

Statistical software*:* The Statistical software namely IBM SPSS 20.0,was used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Statistical Analysis

Four scales were used to collect the data for this study. The Visual Analog Scale, The Verbal Rating Scale, The Pain Relief Scale and The Pain Intensity Scale. The VAS provides a simple, efficient, and non invasive measure of pain intensity that has been used widely in clinical and research settings where a quick index of pain is required and to which a numerical value can be assigned. The VAS consists of a l0 cm horizontal or vertical line with the two endpoints labeled 'no pain' and 'worst pain ever.' The patient is required to mark the l0 cm line at a point that corresponds to the level of pain intensity he or she presently feels, The distance in centimeters from the low end of the VAS and the patient's mark is used as a numerical index of the severity of pain. The Pain Relief Scale is also a four point scale, again with values from 0–3. In this scale, the interpretation for the values was complete relief for a score of 0 and no relief for a score of 3. Based on the statistical data, for the pain relief scale, the P value suggested

significantly better pain relief on oral administration compared to transdermal at the 2 hour interval. There was no statistical difference in p values obtained in the efficacy of either route of administration over the subsequent two days of administration. On the whole, evidence points to the fact that diclofenac sodium administered orally is slightly more effective compared to diclofenac sodium administered transdermally over the first twenty four hours. However, on the second and third days, there is no significant statistical difference in the pain control by either mode of delivery. No adverse effects were reported by the patients following any of the procedures performed.

Statistical analysis and results

Twenty healthy patients in the age group 18–40, with a mean age of about 23 were considered in the study undertaken. Thirteen of them were male and the remaining female. Descriptive analysis was carried out with mean and Standard deviations were computed. Wilcoxon signed rank test was used to find the significance of VAS and PRS between two drug delivery routes. Two scales were used to collect the data for this study. The Visual Analog Scale and the Pain Relief Scale Scale. Based on the statistical data, for the visual analog scale, the P value suggested significantly better pain relief on oral administration compared to transdermal at the 12 hour interval. The p values obtained suggested no difference in the efficacy of either route of administration over the subsequent two days of administration. Based on the statistical data, for the pain relief scale, the P value suggested significantly better pain relief on oral administration compared to transdermal at the 2 hour interval. There was no statistical difference in p values obtained in the efficacy of either route of administration over the subsequent two days of administration.

On the whole, evidence points to the fact that diclofenac sodium administered orally is slightly more effective compared to diclofenac sodium administered transdermally over the first twenty four hours. However, on the second and third days, there is no significant statistical difference in the pain control by either mode of delivery. No adverse effects were reported by the patients following any of the procedures performed.

DISCUSSION

Patients typically relate dental care with pain and an experience of poorly managed pain related to dental treatment procedure can lead patients to avoid or neglect the treatment, as well as make them more difficult to treat. Third molar removal represents a major part of oral and maxillofacial surgical practice. It is essential that anaesthesia be obtained during removal, but effective postoperative analgesia is equally important for good patient care [5]. Impaction of third molar teeth is a common disorder which often necessitates their removal [6]. Surgical removal of an impacted third molar is a model used commonly to test the efficacy of analgesics for acute dental pain [4]. It is well documented that pain after removal of third molars is of short duration and reaches its maximum intensity in the early postoperative period [6]. Most young, healthy adults may expect to experience some symptoms and limitation of activity for 5 days or less after third molar surgery. Interference with routine activities and work a school may be expected to be restricted to the first 3 days after surgery, with pain decreasing steadily over the first 5 days [7]. Bilaterally symmetrical impacted mandibular third molars are a useful mode of comparison because the surgical procedures for both the sides remain the same, the individuals who will be studied are the same, the depth and degree of impaction will be the same, the pain perception also remains the same, since the parameters assessed will be the same bilaterally symmetrical impacted mandibular third molars provide an opportunity to carry out two similar surgical procedures on two different occasions. In the present study two drug delivery routes - oral against transdermal were compared using the drug, diclofenac sodium, 100 mg as a standard and the comparative analgesic efficacy trail was done on 20 patients undergoing surgical removal of bilateral mesioangularly impacted mandibular third molars. The statistical analysis and the clinical observation indicate that diclofenac sodium ministered per orally provides slightly better analgesia than when administered transdermally, on the immediate postoperative day. sstatistical difference observed.

CONCLUSIONS

The results of the study, both statistically and clinically showed that diclofenac sodium when administered orally showed slightly better pain control in the first 24 hours when compared to the transdermal form. However, over the next two postoperative days, there was no difference was observed in either form of administration. Transdermal administration has its role in pain control following minor surgical procedures, especially in patients who are susceptible to gastritis and in whom compliance is a problem. Considering the small size of the sample, it would need a larger study to validate the above findings

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Conflict of interest: None declared.

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Table 1 Comparison of VAS scores in oral and transdermal patch in the Post op periods:

VAS	Oral		Patch		
DAY 1	**Mean**	**SD**	**Mean**	**SD**	**P value**
2 hour	4.7	2.73	5.5	2.71	0.178
4 hour	4.4	2.8	4.9	3.1	0.502
8 hour	3.5	2.92	4.3	3.34	0.284
12 hour	2.8	2.7	4.1	3.2	0.256
24 hour	2.41	2.26	3.1	3.12	0.256
DAY 2					
2 hour	2.8	2.4	2.91	3.07	0.838
4 hour	2.32	2.4	2.6	2.8	0.679
8 hour	2.1	2.33	2.4	2.62	0.699
12 hour	1.8	1.9	2.3	2.4	0.325
24 hour	1.6	1.5	2.02	1.9	0.322
DAY3					
2 hour	1.5	1.97	1.3	1.78	0.519
4 hour	1.2	1.8	1.3	1.7	0.804
8 hour	1.2	1.8	1.34	1.6	0.725
12 hour	1.3	1.8	1.3	1.6	1.000
24 hour	1.2	1.53	1.21	1.61	0.789

Table 2 Comparison of PRS score in oral and transdermal patch

VAS	Oral		Patch		
DAY 1	**Mean**	**SD**	**Mean**	**SD**	**P value**
2 hour	1.50	0.89	2.10	0.69	0.014
4 hour	1.35	0.88	1.70	0.78	0.181
8 hour	1.12	0.92	1.45	1.05	0.217
12 hour	1.01	0.89	1.20	0.95	0.359
24 hour	0.85	0.67	1.05	0.94	0.258
DAY 2					
2 hour	1.00	0.65	1.15	0.93	0.453
4 hour	0.90	0.72	1.00	0.86	0.577
8 hour	0.80	0.70	0.95	0.83	0.459
12 hour	0.70	0.73	0.90	0.72	0.297
24 hour	0.73	0.57	0.70	0.57	1.002
DAY3					
2 hour	0.25	0.55	0.25	0.55	1.00
4 hour	0.25	0.55	0.23	0.57	0.331
8 hour	0.30	0.56	0.25	0.55	0.577
12 hour	0.25	0.45	0.35	0.57	0.577
24 hour	0.25	0.57	0.27	0.55	1.020

Figure 1 – Diclofenac tablet (Voveran SR 100)

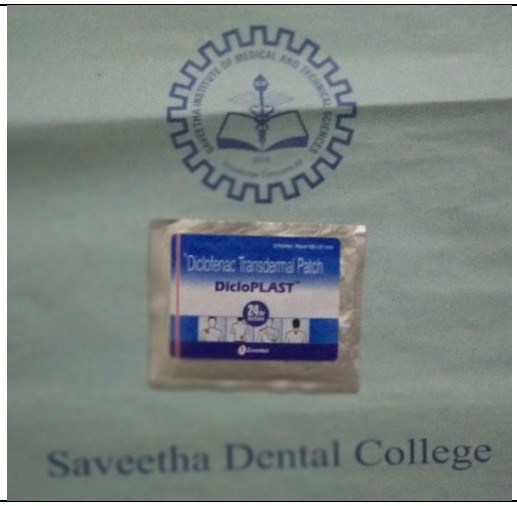

Figure 2- Diclofenac Transdermal patch (Dicloplast 100 mg)

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RESEARCH ARTICLE

Models of Conflict Prevention

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ABSTRACT

In this Paper, we have applied operational research technique to conflict prevention. We provide a brief introduction to conflict analysis and modeling, with special attention to quantitative approaches based on operational research methods. We have studied two causal methods for studying conflicts dynamics based on simulation techniques, lateral pressure's model and Gurr's model. Our study is devoted to two approaches to the analysis of the conflict's cycle, based on grammars and Markov chains. The use of clustering techniques in early warning is analyzed, in particular two approaches are discussed, the first based on single criterion optimization, and the second based on multicriteria optimization.

Keywords: conflict prevention , simulation , operations research.

INTRODUCTION

Security is one particular global challenge. Within this context, one of the areas in which operations research can give an important contribution is the area of conflict prevention. This is a challenging research area requiring a multidisciplinary approach, which has enjoyed an increased interest in recent years. Here, our endeavour is to highlight some of the problems related to conflict prevention, in which operations research techniques can find useful applications. The research community is paying more and more attention to security related problems since their importance as a global challenge has been growing in the last years. Political, demographic, social and economic evolutions have given life to new threats, more diverse, less visible and more technologically sophisticated than those faced in the past. In the next future we will have to deal with always less stable regions and it is thus necessary to adopt a more coordinated approach to ensure that a high level of security is established across this new, more diverse territory. There is a great need of instruments to deal with the threats such as terrorism, proliferation of weapons of mass destruction, failed states, regional conflicts and organized crime. In our view it is essential that our

attention and research be directed, more than to the development of more sophisticate defense technologies, to gaining a better understanding of the root reasons of such new threats, and to the development of effective tools for conflict prevention. One of the goals we deem particularly important for the research in this area is the capability for anticipating the explosion of violent conflicts several months before they occur in order to effectively plan and implement appropriate conflict prevention and peace building activities. In this chapter we describe some possible applications of Operations Research techniques to conflict prevention. In section 2, we provide a brief introduction to conflict analysis and modeling, with special attention to quantitative approaches based on operations research methods. Then, two causal models for studying conflicts dynamics based on simulation techniques are described in section 3: lateral pressure's model and Gurr's model. Section 4 is dedicated to two approaches to the analysis of the conflict's cycle, based on formal grammars and on Markov chains, respectively. In section 5, the use of clustering techniques in early warning problems is analyzed; in particular two approaches are discussed, the first based on single criterion optimization, and the second based on multicriteria optimization. Some final remarks are presented in last section.

Conflict Analysis and Modeling

The study of conflicts and of their dynamics is now a wide multidisciplinary research area, with a large body of literature and with many specialized journals. Although most of the approaches to conflict analysis one can find in the international studies literature remain at qualitative level, it is not rare the use of analytical and quantitative models, in some cases highly formalized and with a certain degree of mathematical sophistication. Here our interest is mainly in this latter type models, which we will partition in two broad sets. In one set we put the models whose main goal is the study of the effects on conflicts (their origin and their dynamic behavior) of single elements (arms, technology, resources, environment, economic development, ethnic and cultural differences, ...) or of subsets of such elements. These models often bear a strong resemblance to the kind of models developed in the economics theory area. Because of their relatively high degree of formalization we will call them analytical models. In the other set we place model which represent general frameworks which can be applied to the analysis and representation of any particular conflict (or at least of large classes of conflicts), in order to determine who are the actors really involved in the conflict or affected by it (the stackeholders), and to fully understand all the elements which define the conflict (interests, needs, etc.) and their interactions. These models are typically less formalized (and in general less prone to formalization) of the analytical models, and bear many similarities with some techniques for problem structuring developed within the Operations Research area [18] Although also in the approaches of the second type OR techniques may find applications, namely the type of techniques which go under the name of Soft Operations Research, here we will restrict our attention to the analytical models. Possibly the first attempt to approach the analysis of conflicts via mathematical tools is due to the British mathematician L. F. Richardson, in the years between World War I and World War II. In a book published after his death, Richardson [17] analyzes the dynamics of the arms race by means of a model based on differential equations. It is interesting to note that Richardson's model has much in common with another model developed about at the same time: the well known Volterra equations. In his model the Italian mathematician Volterra [23, 24] studied a particular conflict situation in which there are two populations, one of predators and one of preys sharing the same habitat. Richardson model, for the first time, shows analytically how the desire to be more secure may lead to an increase in insecurity and eventually to war. The study of analytical models of conflicts has grown after World War II within the new born area of peace studies. One can find many examples of such models in specialized journals of the area. Worth mentioning are the attempts to build a systematic analytical theory of conflict, due to Isard [12], Isard and Smith [13] and Rapoport [16]. Isard and Smith present not only analytical models but also concrete procedures (algorithms) for the management and resolution of conflicts.

Simulation Models

In the following we will describe in detail two models which show clearly how computer simulation, one of the most widely used OR techniques, can give a substantial contribution to conflict analysis. The first model is due to Wils et al. [25] Starting from a theory developed by Choucri and North [3], the authors present a system dynamics model which relates the conflict to factors affecting the possibility of sustainable development such as population growth, resources and technology. Considering the type of variable used, which change rather slowly over time, this model can evidenciate situations of possible instability, but can hardly be used to anticipate the outbreak of a violent conflict. It can be used to analyze both, inter-state and infra-state conflicts.

The second model is due to Gurr [8] and focusses on infra-state conflicts, namely on conflicts involving minority groups within a state. Although this does not appear to be the original intention of Gurr, it is interesting to note that his model maps in a almost one-to-one way into a system dynamics model, and hence also in this case computer simulation can apply.

Lateral Pressure Model

The theory developed by Choucri and North [3] argues that the causes and the consequences of conflicts have to be searched analyzing the interconnections between variables such as population (*P*), technology (*T*), and resources (*R*), which are called master variables. A function called Lateral Pressure (LP) describes those pressures related to the master variables, which lead to international conflicts:

$$LP = f\left(\frac{T \cdot P}{R}\right)$$

The *LP* function can be considered as an increasing function of population and technology, and a decreasing function of resources. The lateral pressure function is characterized by diminishing marginal contributions from the three variables, that is, the larger are the values of *P*, *T* and *R*, the smaller is the effect of further increase in their values. The theoretical meaning beyond the mathematical expression is that if the level of population and technology is high as opposed to a low level of resources, then there can rise the need for the country of expanding beyond its national boundaries, which can lead to violence and conflicts. The system dynamics model of Wils et al. [25] can be used to understand the process through which factors related to the master variables turn into international armed conflict. Since international armed conflict may emerge from conditions of national, or even local, human insecurity, a function, not developed in the original lateral pressure theory, has been added in the model: the Internal Tension (*IT*) function. Like *LP*, the *IT* function is related to the master variables, and it describes those pressures that can increase internal tensions and bring to internal conflicts:

$$IT = f\left(\frac{P}{R} \cdot \frac{1}{T}\right)$$

For a given quantity of accessible resources, an high density of population needs an high level of technology to have an internally stable society while a low density of population require only a low level of technology. The objective of the theory is to build behavioral models that describe the dynamics relations among the variables, and the causal chains and the feedback loops involved.

The main structure of the model is shown in figure 1.1, were we have drawn the main causal relations among the variables.

In addition to the master variables, *P*, *R* and *T*, two other important variables are included, military force and trade bargain leverage. The level of military force is affected by the level of technology and affects the intensity of the conflict and hence the impact of the conflict on the country. The presence of a strong military force makes more easy paths to violent confrontation. On the other side, resources can be accessed in a less violent way by means of trade, negotiations and bargaining; this fact is represented by the variable trade bargain leverage, which has the effect to

reduce the potential for conflict. The model has been tested on 13 countries at different level of development and in different geographical areas: seven African countries, six European countries and the USA. It has been run over a time horizon of about one century, from 1960 to 2050 for the African countries, and from 1950 to 2050 for the European countries and for the USA. The available data, going from 1960 (1950) to 1990, have been used to calibrate the model. The results obtained are mixed. As an example, consistent with the historical record, the simulations for Botswana and Zambia have shown no conflict, while the simulations for Burundi and Rwanda have produced multiple eruptions of domestic conflict with high values of internal tension, as has historically been the case. Instead, the simulation for Angola and Mozambique has not shown the conflicts which have historically characterized these countries; that is most likely due to the fact that these conflicts had external (the intervention of South Africa) more than internal motivations. Some additions to the model are due to Oner et al. [14] who have enlarged the model to include new variables such as the regime type and the income equality. Suggestions for further improvements can be found a technical report by Gallo and Sodini [6] where the importance of considering the interactions between pairs of neighbooring countries is stressed.

Gurr's Model

Ted Robert Gurr, in the Minorities at Risk project1, has monitored and analyzed the status and conflicts of politically-active communal groups in the larger countries of the world from 1946 to 1989: the objective was to explain these social phenomena through causal mechanism. Although not conceived as a system dynamics model, Gurr model can be easily translated into a system dynamics one. In figure 1.2 it is shown the structure of a possible system dynamics model 2 based on the processes of communal mobilization for protest and rebellion according to Gurr. In the model, the potential of communal rebellion is seen as a function of structural or situational variables (group incentives, group capacity, opportunities for collective action) that influence the ethnic's group decision to rebel or not. The independent variables are usually divided in three groups. The first group consist of incentives for collective action and include history of political autonomy, collective disadvantages, repression by the state: incentives derive, for instance, from the fact that past losses can create resentments, anticipation of future losses can create fears, and the potential for future gains can create hopes (incentives include both rational and emotive components). The second group of factors reflects the group's capacity for collective action and include the strength of group identity and the degree of militant mobilization : Gurr argues that, having a territorial base, a preexisting organization and an authentic leadership, makes rebellion more feasible. The third category regards factors affecting the groups opportunities for collective action, such as recent major changes in the political structure of the political regime: state power, regime instability, and regime type, but also transitions, various types of foreign support for the group (or the state) [9, 10, 11]. On the basis of these indicators, it might be possible to find the politically active groups which are at greatest risk of ethno-political rebellion. According to Gurr's model, serious future rebellions are most likely among groups with high incentives and medium to high capacity and opportunities. If it is clear that the beginning of a conflict is ethnically motivated, or if ethnicity is a real factor, then the model can provide some useful insights. But the main drawback is that the model contains only structural conditions that do not make easy to derive predictions from the model. And, indeed, the author, has tried to introduce some dynamic indicators/accelerators and trigger events. In fact, Gurr and Harff [10] have formulated eight accelerators of ethno-rebellion, like, "Occurrence of violent opposition by kindred groups in neighboring countries", "Elite instability : Disunity within the state elite, conflict and inefficiency in the conduct of routine government", … . These new addictions, plus a study on the dynamics of the conflict can be a good basis for a system dynamics model that allow a better understanding of how incentives, capabilities and opportunities translate into action, that is, into an ethno-political rebellion.

Conflict Cycles

The models presented in the previous section are mainly causal models, i.e. models in which the focus is on the causal relations among the different variables which characterize the situations of potential conflict. Different, although complementary, types of models are those in which the focus is on the analysis of the so called cycles of the

conflict. A conflict is in itself something dynamic which develops along a succession of cycles which constitute often a pattern common to different conflicts. The capability of discovering such patterns and to detect the transitions from one phase to another in specific situation is key to any early warning method. An example of different forms of conflict which can be viewed as successive phases in a conflict escalation path are the ones described by Schmalberger and Alker [19] who introduce "three variables that constitute conflict in its generic form, and can help us to identify and distinguish different forms of conflict. The first variable describes an oppositional relation between groups. As a second variable they choose the use of violence. The use of violence is the single most common variable used in the literature to distinguish types of conflicts. As a third variable they introduce opposition-relevant sequential expectations. Expectations is a most useful variable because it not only allows one to distinguish violent and nonviolent forms of conflict, it connects them. For instance, a dispute is characterized by the expectation of a possible, subsequent crisis. If a crisis does occur, it continues the underlying opposition of the dispute, and in fact is premissed on its nonresolution, so that a different mode of behavior is justified" [19].Accordingly to the three main variables, the four main types of conflicts and hence of conflict phases of table 1.1 are defined.

To be able to describe the dynamics of a conflict, abatement phases and settlement phases are introduced. An abatement phase is not the solution of the conflict, but a phase in which there is no expectation of an escalation. For instance the abatement phase corresponding to a dispute is one in which the first two variables are the same as in the dispute (the divergent claims are still there, and there is non violence), but there is no expectation of threats of using violence. The same for the other three types of conflict. "An abatement phase is the transitional stage from where a conflict can move toward its resolution or a renewed escalation". A settlement phase instead is the conclusion of a conflict, a situation in which the "conflicting parties resolve the opposition underlying the conflict in a noneliminative fashion, establish or reconstitute mutually recognized actors and/or institutional processes in which opposition claims are accommodated, and end intergroup violence and the expectation that other such episodes of the same conflict might begin again" [19].

Conflict Grammars

A conflict is characterized by a sequence of phases (types of conflict) and of transitions from one to the other. Not all the transitions are possible. As suggested by Schmalberger and Alker [19], we can define a grammar of possible conflict phases sequences. In figure 1.3 a grammar of this type is represented: the arrows from one phase to another represent feasible transitions.Note that from an abatement phase we can go to any of the preceding phases, so that we may have for instance a sequence dispute - crisis – limited violence - massive violence - abatement, followed by a new sequence of the same type or shorter (e.g. limited violence - massive violence - abatement). These two sequences represent different episodes of the same conflict, and it is possible that passing from one to the other the foundational opposition of the conflict has changed; for instance it can be the claim for autonomy of a minority in the first episode and the claim for complete independence in the second. During the conflict the actors may undergo political or social evolutions which may change their expectations. An application to the Burundi case of this conflict modeling formalism is given in figure 1.4 (Schmalberger and Alker [19]).The objective of such types of models "is to develop an early warning capability that provides reliable estimates of conflict potential; generates "warnings" months in advance of serious escalation; and enables analysts to differentiate among different types of conflict" [19]. Of course, to this purpose it is essential to have a good data base of events relevant to the conflict, to be updated continuously.

Hidden Markov Chains

In the conflict cycle model of the previous subsection the structure was based on a kind of formal language: the phases were the elements or words of the language and a grammar defined the feasible sequences of phases/words. A different approach to analyze the structure of a conflict cycle, which has been proposed by Schrodt [20], is based on the theory of Markov chains, or what is called Hidden Markov Models (HMM), which are Markov chain models, in which the states are not directly observed; they are rather inferred from the presence or absence of a given

sequence of symbols. A typical Markov chain model is characterized by a finite set of n possible states, $S = \{x_1, x_2, \ldots, x_n\}$, and by $n \times n$ matrix $a = [a_{ij}]$ of transition probabilities, where the element a_{ij} is the probability that, assuming the system to be in state x_i, a transition from x_i to the new state x_j occurs (see figure 1.5). Here, the states are the diverse conflict phases, and the transition matrix provides the probabilities of transition from one phase to another. The problem is that we do not know exactly in which state/phase the system is; our only knowledge is about a set of elements (events, variable values, …) which characterize the particular phase in which the conflict is. For instance, taking the phases defined in section 4, we may be in a situation in which the information we have suggests that the conflict is either in a dispute phase or in a crisis one, but it is not easy to say more precisely which of the two phases we are facing.

So, what is done is to codify a finite set of symbols representing events, facts and variables relative to the conflict, and for each subset of symbols and for each state, we define the probability b_i, $i = 1 \ldots n$ that a system in that state produce that subset of symbols (y_i, $i = 1 \ldots n$). That allows us, given a set of observed symbols to infer the state (or the set of most likely states) of the system (conflict situation). Since the state paths are hidden, we cannot find the most likely model parameters (parameters estimation) analytically: a training algorithm is needed. For this purpose the Baum-WelchRabiner (1989) algorithm can be used: it tries to find the model that assigns the training data the highest likelihood.

Beginning with some model (S, a), random or preselected, a set of observed sequences from a variety of countries are run through the current model to estimate the expectations of each model parameter. Then, the model is changed to maximize the values of the paths more used. The steps are then repeated in order to converge to optimal values for the model parameters (S, a). This estimation allows to build a set of consistent scenarios (between 1000 and 5000) to be used for comparing the observed sequences with unknown sequences: if we have some sequences of events for a critical country, our aim is to find the model that better represents the situation of that country. The model chosen is that which maximize the probability of generating the observed sequence.

Clustering Based Methods

In the previous section we have assumed the different conflict phases to be given. The phases are defined, e.g. as in table 1, and the problem is to recognize which phase fits better the characteristic of a particula situation. A different approach would be to let the characteristic of the different phases to come out from the data by means of clustering techniques. Clustering techniques can be useful also to address a different, although related, kind of problem, the problem of classifying countries according to their degree of risk of experiencing internal conflicts. This seems to be a classical problem of classification/discrimination: given a set of n alternatives (the countries), we want to classify them into q classes $C_1, \ldots, C_q$ (country at risk of civil war, unstable country, stable country ,...) where each alternative is described (evaluated) along a set of m evaluation criteria $G = \{g_1, \ldots, g_m\}$. We present next two examples of clustering approaches, one based on single criterion clustering and the other on multicriteria clustering.

Single Criterion Clustering

Schrodt and Gerner [21] have used a clustering algorithm to analyze the Middle East conflict. To this purpose they have built an event data set derived from the Reuters headlines by means a computerized coding system. In particular, for the period from April 1979 to July 1996, they have considered all the events involving any two countries (dyads) among Egypt, Israel, Jordan, Lebanon, the Palestinians, Syria, the United States and USSR/Russia, with the exception of the dyads USA-USSR and USSRUSA. That means that they have considered all the events in which someone belonging to one of the countries has done something jointly, or against someone (or something) belonging to another country in the area; as an example, a US envoy meeting the Israeli first minister, a Israeli airplane hitting a target in South Lebanon, or a Palestinian suicide bomber blowing himself inside a bus in Jerusalem. As for the USA-USSR and USSR-USA dyads, the events involving them were deemed only rarely of interest to the

Middle East conflict. The basic assumption in this study is that within each conflict phase there is a relative stability of the interactions among the different actors, while sudden changes happen in the transition from one phase to the next. The objective of the study was to detect those critical patterns of event flows which characterized the transitions from one phase to another. In order to perform the study, 80,000 events have been extracted from Reuter reports, and properly coded. Then, for each month and each dyad, the events have been aggregated, using the Goldstein scale4, into one single value. Being 54 the dyads considered ($7 \times 8 - 2$), the aggregation resulted in a sequence of points $x_t \in \Re^{54}$, one for each month from April 79 to July 96. The analysis of the sequence $\{x_t\}$ allows to determine the behavior of the system, identifying the transition points between two consecutive phases. In order to do so, a "stability measure", LMLt, has been introduced:

$$LML_i = \frac{1}{k}\sum_{i=1}^{k}\left\|x_i - x_{i-k}\right\| - \frac{1}{k}\sum_{i=1}^{k}\left\|x_i - x_{i+k}\right\|$$

where k is an integer parameter and $\left\|x - y\right\|$ is the distance between x and y.

If $LML_t > \Delta$ where Δ is a properly chosen threshold, then the point x_t is closer to the k points following it in time than to the k preceding points: a new phase/cluster is thus formed. In figure 1.6 it is shown the results for the clustering algorithm in the case of $k = 4$, correlation metric and $\Delta = 0.3$. The vertical lines derive from an a priori analysis in which a rough distinction of the phases is done. The clusters defined a priori are then used as a reference point.

Multicriteria Clustering

One of the main drawbacks of the single criterion approach we have just described is that aggregation leads to the loss of a large part of the information contained in the event data set. Two situation very different one from the other might happen to share the same value in the Goldstein scale. This difficulty may be overcome using multicriteria analysis, and so avoiding the need of aggregating the event data. Multicriteria analysis can deal with a large amount of data, a large number of criteria and different data types (qualitative, quantitative, fuzzy, linguistic parameters), with the possibility to combine quantitative methods with qualitative (judgement) ones. Moreover, multicriteria analysis has the ability to deal with the multiple dimensions of a problem (social, economic, ecological, institutional, cultural, ethical, etc) and has a certain level of transparency, since criteria, options and scores are made explicit.

In addition to the problem already seen to determine the different phases of a conflict, multicriteria analysis can be used to address another relevant problem, the problem to determine which countries are at risk of violent internal conflict, and how serious is the risk they face. This is a kind of problems which bears strong similarities with the problem of analyzing the financial risk of countries or of firms, which is widely studied in the field of finance. This is a very complex problem, with a great number of economic and financial data to analyze, among which the most relevant have to be identified and chosen. A typical multicriteria clustering method is the one described by De Smet and Montano Guzmán [4], which can find application both in classifying states according to risk criteria and in discriminating among the phases in a conflict cycle. We give here a brief description of the former type of application, while for the second, a research is under way [7].

We can assume that each state be represented by a vector, x_i, i = 1, 2, …, n, with m components, where each component corresponds to one of the criteria/variables which are deemed relevant to the risk level evaluation. Such variables can be macro level variables which vary slowly over time, such that population, income per capita, inequality indices like the Gini one, literacy index, …, or variables representing events which may change more rapidly over time, such as the monthly number of violent confrontation, the number of casualities in violent clashes during the past year, … . We can allow also for qualitative variables such as the level of freedom of the media (high, medium, low, poor), or the level of respect of the rights of minorities. If m is the total number of the variables, we have that $x_i = (x_i, 1, x_i, 2, \ldots, x_i, m)$, for all i. In a scale which goes from a maximum of stability and of welfare for the population to a maximum of instability/violence and a minimum of welfare, we can assume that the vectors xi can be

ranked, although the ranking might not be a total order. We can think of defining three relations on $X = \{x_1, x_2, \ldots, x_n\}$, the set of the vectors representing the states, namely a relation of preference, P, a relation of indifference, I, and one of incomparability, J, so that for each pair x_i and x_j, we say:

x_iPx_j if x_i is preferred to x_j,
x_iIx_j if x_i is indifferent to x_j,
x_iJx_j if x_i is incomparable to x_j.

As an example one might prefer a situation in which there is a reasonably high life standard for all, no violence and in which the freedom of the media is low rather one in which the wealth distribution is highly unequal, there is violence but the press is completely free. While a state characterized by high inequalities (with large segments of the population starving) and no violence might be considered to be incomparable to one in which nobody is starving but there is a widespread and high level of violence; i.e. they cannot be considered as equivalent but it is not possible to say which of the two is better. To define such three relations, we assume that a total order can be defined on the domains of the single components of the vectors x_i. That seems to be reasonable: the Gini index can be ordered from 1 (the worst case) to 0, the literacy index can be ranked from 0 (no literate adult) to 1 (100% of literate adults), violence from the highest value to a no-violence situation, and so on. If that is true, then outranking methods, such as for instance ELECTRE, can be used to define the relations on X [1, 22]. Once the order relations have been defined, each element x_i in X is characterized by a set $P(x_i) = \{J(x_i), P-(x_i), I(x_i), P+(x_i)\}$, where

$J(x_i) = \{x_j : x_iJx_j, j = 1, 2, \ldots, n\} = P_1(x_i)$,
$P-(x_i) = \{x_j : x_jPx_i, j = 1, 2, \ldots, n\} = P_2(x_i)$,
$I(x_i) = \{x_j : x_iIx_j, j = 1, 2, \ldots, n\} = P_3(x_i)$,
$P+(x_i) = \{x_j : x_iPx_j, j = 1, 2, \ldots, n\} = P_4(x_i)$.

The set $P(x_i)$ is called the profile of x_i. Based on the profiles, a distance in the data space can be defined as follows:

$$d(x_i, x_j) = 1 - \frac{\sum_{k=1}^{4} \left| P_k(t_i) \cap P_k(t_j) \right|}{n} \qquad (1.1)$$

The algorithm proposed by De Smet and Montano Guzm´an [4], partitions the set of data in q subsets or clusters, $C_1, C_2, \ldots, C_q$, trying to minimize the intra-cluster distances. In this approach we do not need to know in advance the characteristics of the clusters; such characteristics are in fact the result of the algorithm. At the end, each cluster, C_j, will be represented by a centroid, c_j with a profile $P(c_j)$. The centroid does not need to be one of the elements in the original data set, and is defined as that element which minimizes the sum of the distances from all the elements in C_j. The algorithm is an iterative procedure. We start from a set of centroids chosen either randomly or based on a first inspection of the data, and assign each vector to the nearest centroid. Then the centroids are recomputed and a new assignment is done. The algorithm iterates until a stable assignment is found, that is one that does not lead to further changes in the centroids. The result is a set of clusters $C_1, C_2, \ldots, C_q$, which can be ranked, by outranking methods, from the one containing the less risky states to the one containing the states with higher failure/conflict risk. Again the final rank does not need to be a complete one.

Interesting aspects of an approach of this type is that all the different criteria/variables are maintained separate during the computation, and that the classification is based on the real data and not on some a priori definition of which variable values define a stable state or a failed one. Other interesting and potentially useful multicriteria approaches can be found in the literature; among them we recall the one proposed by Doumpos et al. [5]. An interesting use of multicriteria techniques embedded in a simulation approach based on system dynamics has been proposed by Brans et al. [2].

CONCLUSION

In this chapter, we have reviewed some of the main problems arising in modeling conflicts for early warning and conflict prevention purposes, and we have tried to highlight some points in which operations research techniques can find useful application. In particular we have singled out two methodologies which appear quite promising for the area of early warning and conflict prevention. The first is system dynamics. System dynamics might result an effective tool to analyze the dynamics of a conflict, and to determine the causal chains that explain such dynamics. We believe that there is space for a wider use of this technique well beyond the limited applications done so far. The second is multicriteria analysis. This is a methodology which, as far as we know, has not found yet use in early warning and conflict prevention, but which, in our opinion, might provide a way to overcome some of the most serious limits in the way event data are used today. A possible use of multicriteria clustering is the object of a research work which is currently carried on by the authors.

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Table 1 : Conflict Phases

Form of conflict	Opposition	Use of Violence	Expectations
Dispute	divergent claims are accommodated within existing institutional processes	None	possible threats of using violence
Crisis	divergent claims are accommodated within existing institutional processes	incidental and sporadic	possible use of limited or massive violence
Limited violence	divergent claims are expressed by the use of violence	regular, sistematic, and restrained	possible use of massive violence
Massive violence	divergent claims are expressed by the use of violence	regular, systematic, and unrestrained	destruction, elimination, unconditional surrender

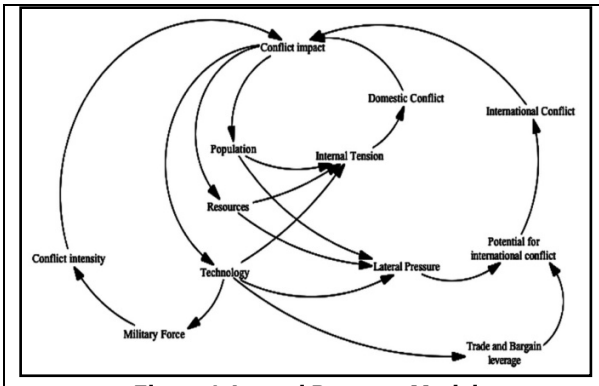

Figure 1: Lateral Pressure Model

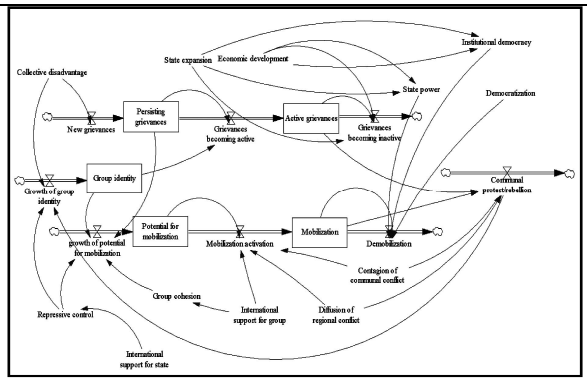

Figure 2: Gurr's system dynamic model

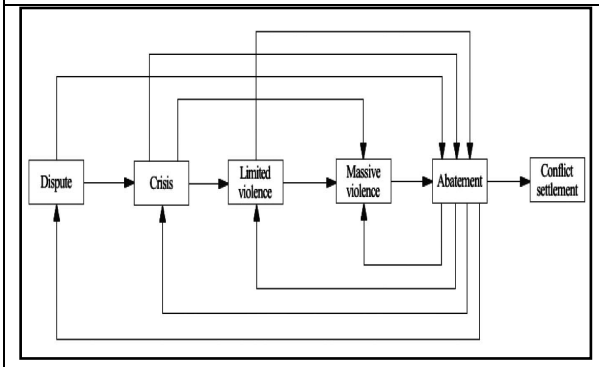

Figure3 : A Grammar of Possible Conflict Phases Sequences

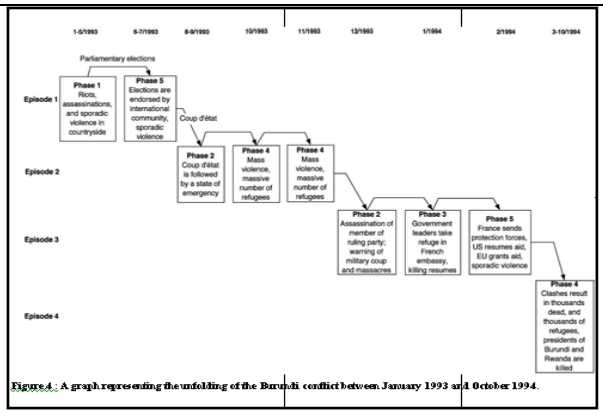

Figure 4 : A graph representing the unfolding of the Burundi conflict between January 1993 and October 1994.

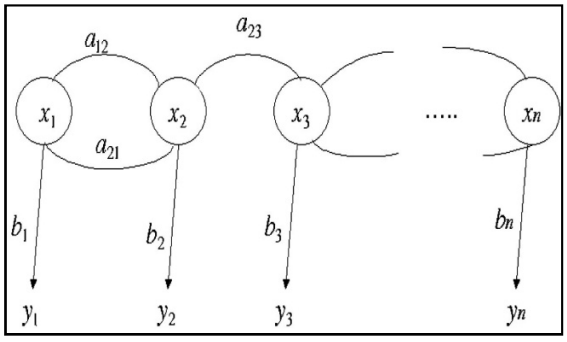

Figure 5 : Hidden Markov Model

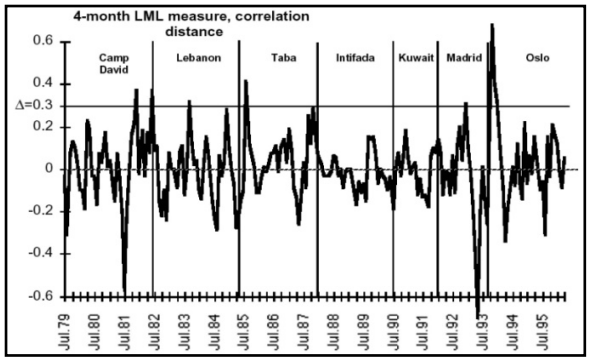

Figure 6 : LML values for the Middle East conflict (Schrodt and Gerner, 1996)

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RESEARCH ARTICLE

Management of Two Time Failed TMJ Reankylosis –An Surgical Challenge and Charisma

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ABSTRACT

Temporomandibular joint (TMJ) ankylosis is fusion or a bony union of the head of the condyle and the glenoid fossa by bony or fibrotic tissues. Due to the immobility of the TMJ, all mandibular movements get affected. Treatment goals are to allow nearly normal TMJ movements, restore symmetry of the face and occlusion, and promote growth and correction of deformity in children. The surgical techniques used to treat TMJ ankylosis are a gap or interpositional arthroplasty, joint reconstruction, and distraction osteogenesis. Appropriate interposition materials include autogenous tissues, allogeneic tissues, and alloplastic and xenograft tissues. This report presents the treatment of a patient with a diagnosis of TMJ ankylosis, who had failed surgery. Interpositional autogenous dermis-fat graft was used to manage TMJ ankylosis of the right side.

Keywords: Ankylosis, case report, gap arthroplasty, temporomandibular joint

INTRODUCTION

The temporomandibular joint (TMJ) ankylosis involves fusion of the mandibular condyle to the base of the skull [1]. It is a debilitating condition usually effecting children and young adults. It causes problems in mastication, digestion, speech, appearance, and oral hygiene. In growing patients; it may result in deformities of mandible and maxilla causing malocclusion. Due to the growth deformity, the child may become shy and reclusive and have a low self-esteem. TMJ ankylosis earlier in 1938 was classified into two types by Kazanjian as intra-articular and extra-articular ankylosis [2]. Present classification includes bony, fibrous, fibro-osseous, complete, and incomplete [3]. Patients with longstanding bilateral temporomandibular joint (TMJ) ankylosis caused by joint trauma during the active growth period in early childhood present mainly with: (a) severe bird face deformity, (b) inability to open the mouth and (c) manifestations of upper airway obstruction in the form of either snoring during sleep or obstructive sleep apnoea[4]. The effective treatment of TMJ ankylosis is based on a detailed preoperative radiographic assessment of the type and extent of ankylosis [5].

CASE

12-year-old boy, reported to Saveetha Dental College and Hospital, Chennai, with a chief complaint of inability to open his mouth. History revealed that the patient had a fall when he was 5-years-old. He had pain and swelling on right TMJ area which progressively subsided. However, there was also gradual reduction of mouth opening seen, as a result of which he was unable to eat properly. History of TMJ gap arthroplasty surgery 5 years back and Distraction Osteogenesis with Gap arthroplasty 4 years back. The initial clinical examination revealed an obviously hypoplastic mandible [Figure 1, 2, 3]. Extraoral examination revealed facial asymmetry with fullness of cheek on the right & left side. The patient had almost nil mouth opening [Figure 4]. Radiographic investigation included orthopantomogram and computed tomography (CT) that revealed a lack of structural organization and obliteration of right and left TMJ space. Based on these finding, a diagnosis of Bilateral bony ankylosis of TMJ was confirmed [Figure 5, 6, 7]. Preop OPG of 2014 shows Distraction Osteogenesis in Right side [Figure 8]. After complete clinical and radiographical evaluation, a surgical treatment of gap arthroplasty with interpositional dermal graft on right and left TMJ was planned under general anesthesia. The postoperative course was uneventful. A mouth opening of 25 mm was noted postoperatively. Vigorous postoperative physiotherapy was started to maintain the mobility of the joint. After 2 weeks of physiotherapy using wooden spatula, mouth opening was noted to be 35 mm. Later mouth opening exercises were given by using Hister's mouth gag

DISCUSSION

The causative factors of TMJ ankylosis are trauma, systemic and local inflammatory conditions, and neoplasm in the TMJ area [6]. In the present case, the history revealed that the patient had a fall from the stairwell and got injury on the face. In addition, he informed that he has undergone a previous bilateral TMJ ankylosis surgery in another department. One of the main causes of the failed TMJ surgery is inadequate heterotopic bone removal [7]. To prevent possible re-ankylosis, ankylotic bone was aggressively removed during operation. According to the management protocol for TMJ ankylosis reported by Kaban et al,[8] an ipsilateral coronoidectomy was performed to maintain optimum mouth opening.. Other factors that can lead to re-ankylosis include wound infection, and a foreign body reaction caused by interpositional materials. In the present case, no wound infection or foreign body reaction associated with the autogenous dermis-fat graft was observed. The treatment protocol entails surgical approach by releasing and correction of ankylosis. Three main surgical techniques are gap arthroplasty, interpositional arthroplasty, and TJR [9]. The interpositional materials such as skin, dermis, and flaps of the temporal muscle/fascia, silicone, and cartilage for arthroplasty in TMJ ankylosis treatment have been widely discussed [10]. At the present

time, there is no gold standard for interpositional graft. Alloplasts have their advantages such as avoiding donor site morbidity, reducing operation time, reducing the chance of recurrent ankylosis, and allowing a closer reproduction of the normal anatomy of the joint [11]. They also have some disadvantages such as displacement, failure and fracture of the prosthesis, infection, and extrusion. Reconstruction with custom-made prosthesis for the management of ankylosis has a lot of advantages. These are no requirement for the second surgical field, reduced operative time, no need for vascularization around prosthesis, and rapid recovery period for mastication [12]. On the other hand, the high cost of custom-made prosthesis for TMJ joint replacement was not an option in our case because of the poor socioeconomic situation of the patient. Therefore, dermis-fat graft was advised as an interpositional material after the removal of the ankylotic bone.

Possible complications of abdominal fat graft harvesting include hematoma, seroma, infection, ileus, and inadvertent peritoneal perforation [13]. Wolford suggested inserting a suction drain and leaving it in position for approximately 3 days to prevent hematoma or seroma formation. In the present case, no complications were observed at the abdominal site, and we did not prefer to use any drain at surgery because hemostasis was achieved during operation.

CONCLUSION

In the present case, approximately 30 mm of mouth opening was maintained. No postoperative complication was observed regarding the use of the graft. A minimal donor site morbidity was observed, and the manipulation of the graft to the gap was not a complex procedure during operation. In this report, the importance of the multidisciplinary approach including surgical management and physiotherapy was also emphasized.

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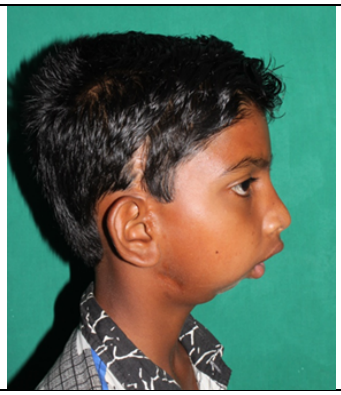

Figure 1– Right Profile

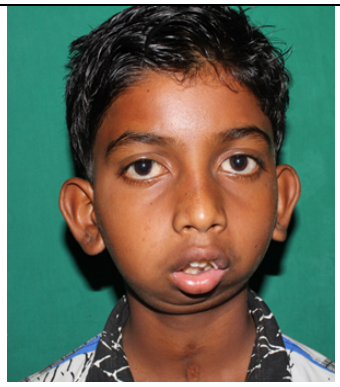

Figure 2– FrontalProfile

Figure 3- Left Profile

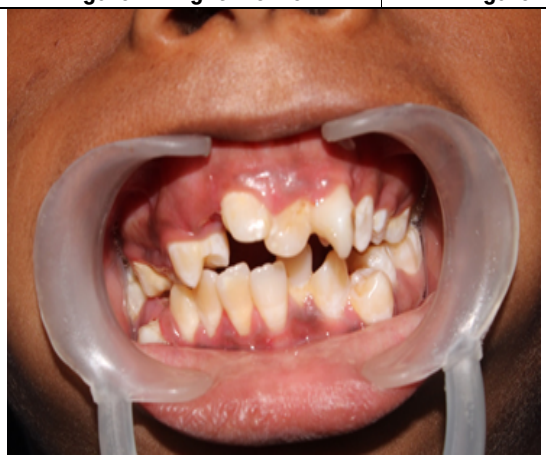

Figure 4- Pre op mouth opening

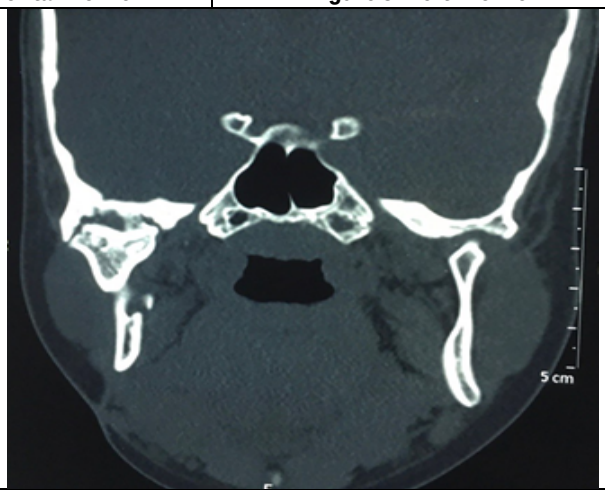

Figure 5- Coronal CT scan showing Right TMJ Ankylosis

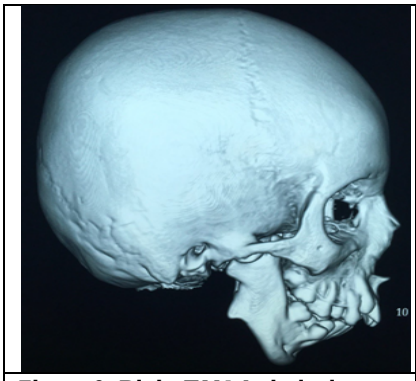

Figure 6- Right TMJ Ankylosis

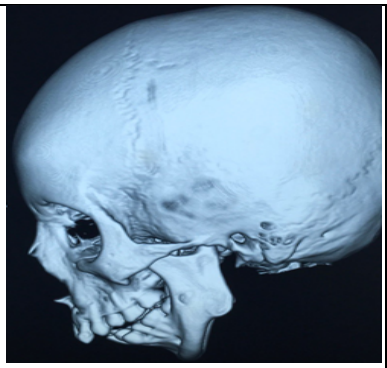

Figure 7- Left TMJ Ankylosis

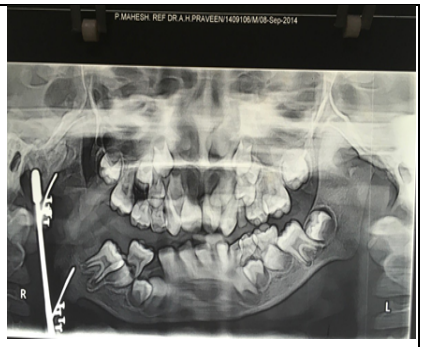

Figure 8- OPG taken5 years back showing Right side Distraction Osteogenesis but got failed in post Distraction period.

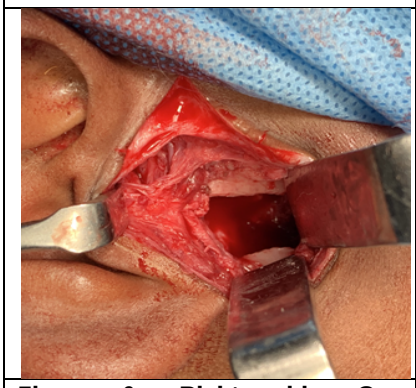

Figure 9- Right side Gap Arthroplasty done

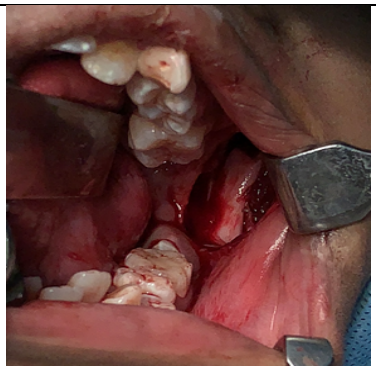

Figure 10-Left Side Coronoidectomy done

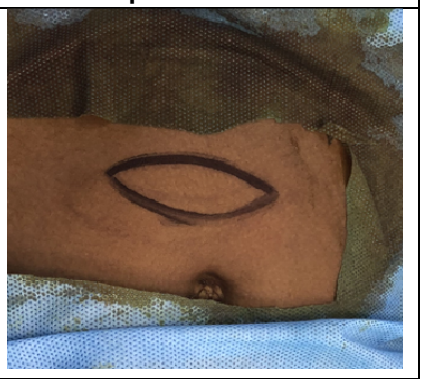

Figure 11 -Marking on abdomen for Dermis Graft

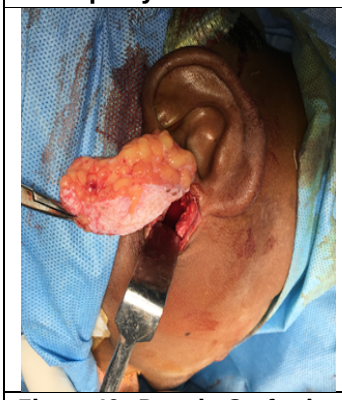

Figure 12- Dermis Graft placement in left TMJ region.

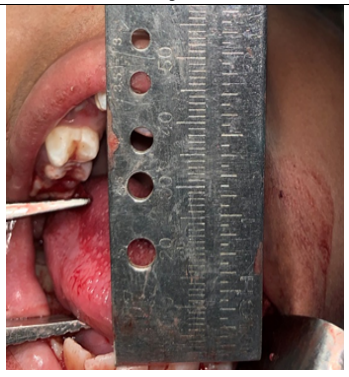

Figure 13- Intraoperative 40 mm mouth opening achieved

Figure 14- Post op – 30 mm mouth opening

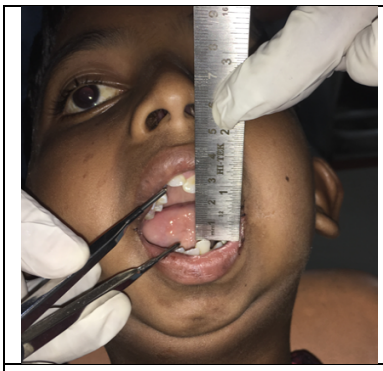

Figure 15- 1 Year post op 30 mm mouth opening

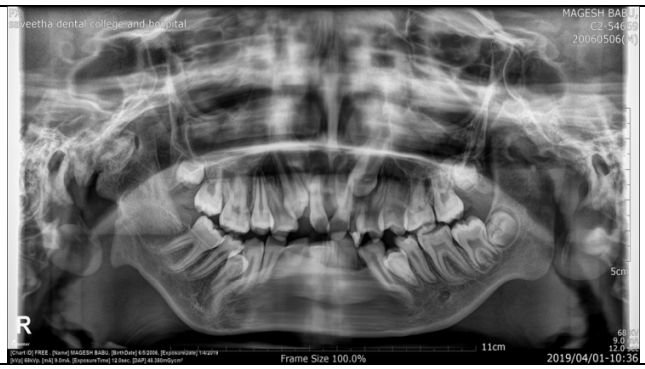

Figure 16- Preop OPG

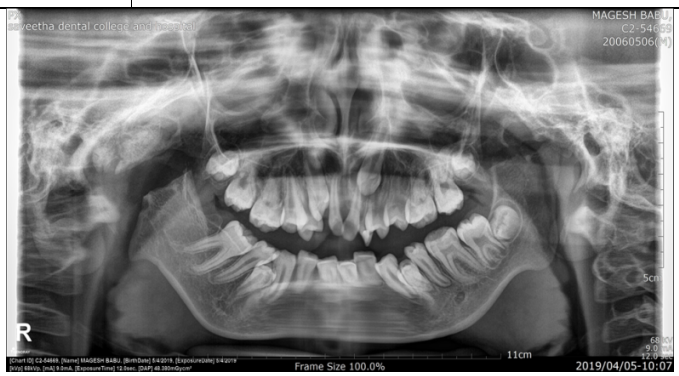

Figure 17- Postop OPG- Bilateral Gap Arthroplasty

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RESEARCH ARTICLE

Screening and Characterization of Antibacterial Compounds from Selected South Indian Medicinal Plants

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ABSTRACT

Plants have been known to be a reservoir of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes. The fact that some of these plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of these plants further strengthens the search for pharmacologically active compounds from plants. The herbals *Asparagus racemosus, Zingiber officinalis* and *Picrorhiza kurooa* were characterized by phytochemical, TLC and FTIR analysis. The extract of *A. racemosus* contains saponin, steroid, tannin, and flavanoids. In TLC analysis there was spots observed with the R_f value of 0.164, 0.275 and 0.611. This revival of interest in *A. racemosus* plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs.

Keywords: Plants, Secondary metabolites, Phytochemical, drugs

INTRODUCTION

Natural products play a major role as active substances, model molecules for the discovery and validation of drug targets. Medicinal plants are defined as those which produce one or more active constituents capable of preventing or curing an illness. These plants contain a range of effective compounds and can produce very different effects according to the way in which the drug is treated. Numerous studies have been carried out to screen extracts from

medicinal plants for the presence of novel compounds and an investigation of their biological activities. Research and developmental work in herbal medicine is essential because of its social and economic benefits and it has become persistent part of present day healthcare in developing countries [1]. Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants [2]. Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [3]. The need of the hour is to screen a number of medicinal plants for promising biological activity. The purpose of this study was to screen for the aqueous and ethanolic extracts of these medicinal plants that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases. The present study focuses on screening and characterization of the selected Indian immune stimulant herbals *Asparagus racemosus, Zingiber officinalis* and *Picrorhiza kurooa* extracts.

MATERIALS AND METHODS

Selection of Medicinal Plant for this Study

The antibacterial and immunostimulant plant extracts of *Asparagus racemosus* (methanol)*, Zingiber officinalis* (methanol) and *Picrorhiza kurooa* (methanol), were selected for this study. The active fractions of the selected extracts were selected based on the immunostimulant activity against the fish bacterial pathogen, *Aeromonas hydrophila.*

Phytochemical Screening

The Phytochemical screening was determined by the method [4-5]. This screening was carried out with the methanolic extracts using chemical methods and thin-layer chromatography (TLC) as per standard protocol [6].

Thin-layer Chromatography

Thin-layer chromatography (TLC) was performed using silica gel, solvent system used for, *Asparagus* extract is chloroform: methanol (18:2) V/v. Plates were sprayed with 5% vanillin 50% sulfuric acid and heated for 10 min at 120˚C to visualize saponins, and heated for 10 min to visualize all substances.

Fourier-Transform Infrared (FT-IR):

FT-IR was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. All spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a FTIR spectrophotometer. A small amount of powdered leaves was placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm–1 to 675 cm–1and computerized for analyses). The ultraviolet (UV) spectra were recorded on Shimadzu UV spectrophotometer.

Antibacterial Activity

The fractions eluted from the column purification were screened by agar disc diffusion method [7]. Disc of filter paper (5 MM) impregnated with herbal extracts were placed on an agar plate that was heavily and uniformly inoculated (lawn) with an actively growing culture of the organisms. The medium of choice was Muller Hinton Agar for Aeromonas “medium 228” (75 % seawater) and “Growth medium 53” (nutrient broth with 3% NaCl) for *Aeromonas hydrophila*. The dynamics and timing of antimicrobial agent diffusion to establish a concentration gradient coupled with the growth of organisms over 16 – 24 hour duration is critical for reliable results.

RESULTS

Characterization of the Phytochemical From Selected Plants

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals including saponin, terpenoids, tannin, flavonoids and steroids were present in the samples. The result of the phytochemical analysis showed that the *A. racemosus* had the presence of tannin, saponin, steroids and flavanoids, The *Z. officinalis* had the presence of Alkaloid, saponin, terpenoids, steroids and flavanoids and *P. kurooa* had the presence of Alkaloid, tannin, saponin, steroids and flavanoids (Table 1).

Active Compound Characterization of Plants by Thin Layer Chromatography.

Thin layer chromatographic analysis of the hot water extract of *A. racemosus* revealed that, the spot was confirmed as the active compounds. The R_f value of the fractions are 0.164, 0.275 and 0.611 in the *A. racemosus* respectively (Figure 1a,b,c). The R_f value of the fractions are 0.426, 0.571 and 0.602 in the *Z. officinalis.* The R_f value of the fractions are 0.523 and 0.572 in the *P. kurooa* respectively.

Functional Group Analysis

Fourier Transform Infrared Spectroscopy analysis for the antibacterial extract *A. racemosus* active fractions are given in the figure 2. The possible functional groups of active principles were analyzed between in wave number 500- 4000 /cm. The active fraction of hot water extracts of *A. racemosus* gave the following peaks in the I-R spectrum. The peaks represented the various functional groups in the molecule. The broad peak around 2922 cm^{-1} may be the –OH stretching or -NH stretching the one peak at 1041 cm^{-1} may be due to C-O stretching. The one at 673 cm^{-1} may be due to C≡C-H. The one at 1624 cm^{-1} may be due to $RONO_2$. The observation revealed that it may be inferred that the compound is alkenes or ketones. Thus the extract may contain a free carbonyl group where the OH group is hydrogen bonded. The extract is also suspected to contain a carbonyl species in conjugation with O= bond (Fig 2)

Antibacterial Screening for agar well and Disc Diffusion Method

The antibiogram studies of the selected strain *Aeromonas hydrophila* against the selected Antibiotics (zone of inhibition in cm) are given in the Fig 3. For these, four antibiotics were used such as Chloramphenicol, Streptomycin, Neomycin, Gentamycin. Among these antibiotics the maximum values were got for all strains by using the antibiotic Neomycin at 1.5 cm in diameter. The minimum values for *A. hydrophila* at 0.5 cm were got by using the antibiotic Streptomycin. The antibacterial activities of the herbal extract against the selected strain *A. hydrophila* (zone of inhibition of mm diameter) were given in the Fig 3. Among this *A. racemosus* were effectively suppressed the pathogens at 0.9, 1.3, 1.6 and 1.4 cm of zone of inhibition to *A. hydrophila.*

DISCUSSION

The chemical and synthetic vaccines have some demerits including high cost and some side effects. The antibacterial compound from herbal origin are advisable in aquaculture operations due to its versatile characterizers are safety, eco-friendly and create no side effects. In the present study the phytochemical analysis of the *A. racemosus* root extracts revealed the presence of saponin, steroid, tannin and flavanoids. The major active constituents of root extract *A. racemosus* are steroidal saponins namely shatavarins apart from alkaloids, flavonoids, sterols and terpenes [8]. The hot water extract of *A. racemosus* was separated into its constitutive fractions by preparative thin layer chromatography (TLC). The R_f value obtained was 0.164, 0.275 and 0.611 and the fractions may be active compounds. The FTIR study revealed that, the hot water extract of *A. racemosus* had primary or secondary amine or an amide or substituted amide, olefininc band, cumulated system, C-F and C-Br bond. A stretching of C-O-C, C-O at 1000–1200 cm^{-1} corresponds to the presence of carbohydrates [9]. Absorption peaks centered on 910–665 and 690–515 cm^{-1}

correspond to N-H wag of primary amine and C-X stretch of alkyl-halides, respectively. IR peak observed in the range of 2350–2360 cm^{-1} may be because of CO_2 adsorption [10] or asymmetric stretching of group –N=C=O- [11]. In the present study, the antibacterial extract of *P. kurooa* and *A. racemosus* was effectively suppressed the bacterial growth *in vitro* and crude of the above extracts were suppress the bacterial growth by *in vivo* [12]. Similar work done by Direkbusarakom,[13] sixteen kinds of Thai traditional herb; *Ocimum sanctum* white and red strains, *Cassia alata, Tinospora cordifolia, Eclipta alba, Tinospora crispa, Psidium guajava, Clinacathus nutans, Andrographis paniculata, Momordica charantia, Phyllanthus reticulatus, Phyllanthus pulcher, Phyllanthus acidus* were tested for their antibacterial efficacy against 10 strains of *Vibrio spp*. The present work, the active principle natures such as alkaloids, flavanoids, pigments, phenolics, terpenoids, starch, steroids and essential oils of the herbals they are the immense use in the antibacterial feed making in the aquaculture.

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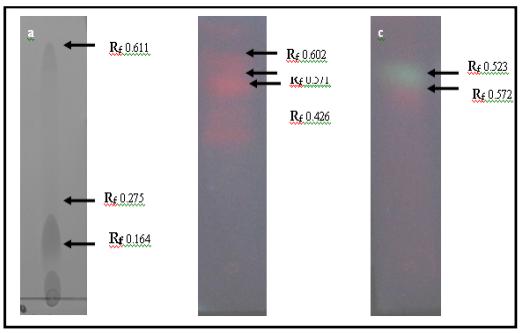
Table 1. Phytochemical analysis of antibacterial herbals by standard protocols

Sl. No	Phytochemical constituents	*Asparagus racemosus*	*Zingiber officinalis*	*Picrorhiza kurooa*
1	Alkaloid	-	+	+
2	Saponin	++	++	+
3	Steriods	++	+	+
4	Tannin	+	-	+
5	Terpenoids	-	+	-
6	Flavonoids	+	+	+

+ = Present, ++ = Present in large quantity, - = Absent



a. Asparagus racemosus b. Zingiber officinalis c. Picrorhiza kurooa

Fig 1. Thin Layer chromatogram of the plant extract by iodine development

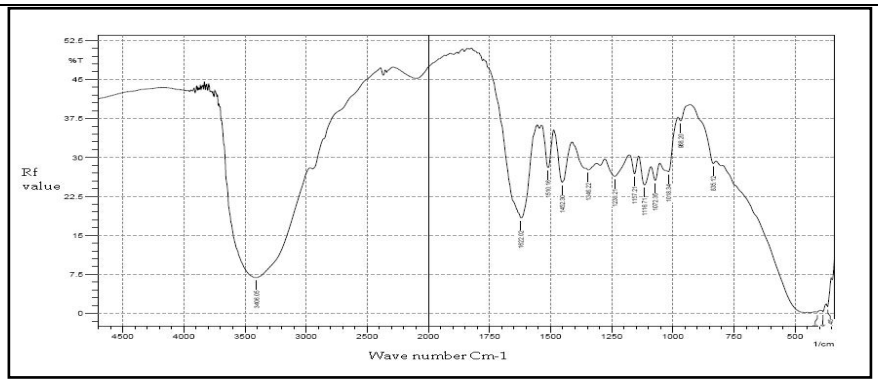


Fig 2. Fourier Transform Infrared Spectroscopy analysis for the *Asparagus racemosus* extract

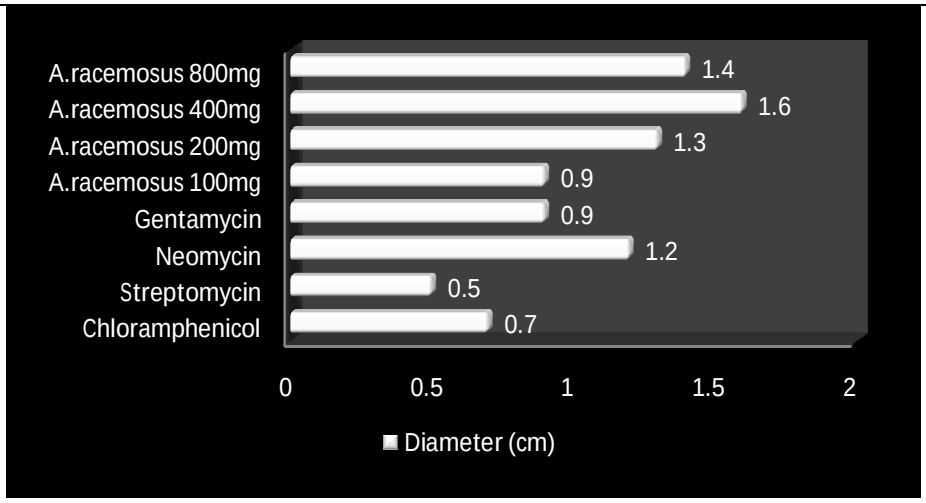


Fig 3.Antibiotics and *A. racemosus* extract against *A. hydrophila* pathogen

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RESEARCH ARTICLE

The Effect of Neutralized Specific Egg Yolk Antibodies from *Gallus domesticus* in the Growth Inhibition of *Aeromonas hydrophila*

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ABSTRACT

The IgY antibodies can have broad applications from developing immunoassays to treating various diseases. In the present study, a well acclimatized female hen (*Gallus domesticus*) was immunized by the *Aeromonas hydrophila* and the egg yolk immunoglobulins (IgY) were separated and tested for the growth inhibition of the same pathogen. The result showed a significant diminution ($p<0.05$) of growth in *A. hydrophila* when compared to control.

Key words: IgY antibody, *A. hydrophila,* immunization, Growth inhibition

INTRODUCTION

The discovery and use of antibiotics and vaccination in animal agriculture have evolved from the management of small poultry flocks in the era prior to 1890s (Wehman, 1892) to the large consolidated units of today (Cook, 2000). Hatta *et al.,* (1990) termed the antibodies present in egg yolk as IgY. Thus, it is possible to obtain pathogen-specific IgY antibody from eggs laid by hens immunized against antigen (Shimizu *et al.,* 1988). Since poultry farming is carried out worldwide, eggs may be a suitable source of antibody for passive immunization. Chicken egg yolk antibody (IgY) has received much attention in recent years because it can be easily prepared in high concentration and is both affordable and safe (Gassmann *et al*., 1990). IgY is successfully used in medical immune testing, diagnosis, heterograft and therapy. Du-Plessis *et al.* (1999) reported that the chicken IgY was used in a double antibody sandwich ELISA for detecting African horse sickness virus. Immunization of chickens for pAb production is comparable to that of rabbits with respect to route of injection, the amount of antigen used and the kinetics of specific antibody generated (Haak-Frendscho, 1994). Polyclonal antibodies (PAb) against *A. hydrophila* antigen were produced and used for passive immunisation in shrimp, *Penaeus monodon*, but the application for detecting *A.*

hydrophila has not been established (Lee *et al.,* 1970). Researchers have used egg antibodies in passive immunotherapy to treat a range of other diseases from bovine rotavirus in cattle to Mastitis in dairy cattle (Coleman, 1998). Antibody production in eggs is particularly advantageous because hens can be effectively immunised, antibodies are readily deposited in the yolk and eggs are a convenient and inexpensive food source. New vaccine technology has led to vaccines containing highly purified antigens with improved tolerability and safety profiles. Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers (Gottstein and Hemmeler, 1985). There are meagre reports available on the effect of egg yolk antibodies against pathogenic *A. Hydrophila.* So the present study has made an attempt to analyse the growth inhibition of pathogenic A*eromonas hydrophila* utilizing the egg yolk immunoglobulin (IgY) from G*allus domesticus***.**

MATERIALS AND METHODS

Preparation of extracellular product (ECP) of *A. hydrophila sp.*

A. hydrophila culture was grown on tryptic soy broth (TSA) supplemented with 1.5% NaCl for 16 hrs at 25^{0}C. Swabs of this bacterial culture was suspended in 5ml phosphate buffered saline (PBS) pH 7.2 and it was then washed with PBS for 3 times and spread plated onto TSA plate (+1.5% NaCl) overlaid with sterile cellophane and kept for 24 hours at 25^{0}C (Lee and Ellis, 1990). Then 15 ml of PBS added onto the surface of the cellophane overlay TSA plate. Make it to spread completely. The harvested bacterial suspensions were then centrifuged at 25,000 g for 60 min at 4ºC the pellet was discarded the supernatant fluids was passed through a 0.22µm filter (Millipore Corp., Bedford, Mass) and the ECP collected was stored at -70^{0}C.

Immunization

Healthy female lay-off hen, *Gallus domesticus* having the weight of approximately 1.8 kg and 1 year old stage were selected and reared in separate cages. After acclimatization, the hens were injected the inactivated *A. hydrophila*. Injections were repeated to the breast of the hen every 7 days for the same respective dose of each groups up to one month. The eggs collected were separately marked and stored at 4ºC until immunoglobulin isolated.

Preparation of Yolk Immunoglobulin

During extraction, the eggs stored at 4ºC were taken, and are cracked for the removal of albumin. The intact yolk was washed gently with distilled water to remove as much albumin as possible. The yolk sac was cut open and the yolk materials were mixed 1:3 with pre cooled (-20ºC) isopropyl alcohol at 5ºC for 20-30 min. The precipitate was allowed to settle for 5 min before the supernatant was decanted. This procedure was repeated three times with isopropyl alcohol and twice with pre-cooled (-20ºC) acetone, for complete removal of lipids. The final residue was filtered through Whatman filter paper no.1, and washed with a small amount of acetone, and left to dry at room temperature. The resultant powder was a mixture of yolk proteins (Bradford, 1976) including anti-*A. hydrophila*IgY characterized by the method of Laemmli, 1970. Thus obtained IgY were stored at 5ºC until used.

Growth Inhibition Assay (Guimarães *et al.,* 2009)

This assay shows the binding activity of anti-*A. hydrophila* IgY which could inhibit *A. hydrophila* growth in a liquid medium. The same strain of *A. hydrophila* used as an antigen for immunizing chickens was subculture on tryptic soy agar plates supplemented with 1.5% of NaCl and is suspended in TSB. Two millilitres of prepared bacterial culture were mixed with 2 ml of TSB and incubated at 37ºC with shaking. The turbidity of the culture (optical density at 600 nm) was measured by a spectrophotometer at 1-h intervals. The growth curve was plotted until the station stationary phase was reached. The extracted IgY solution was centrifuged at 1,500 X g at 4ºC for 20 min. The supernatant was taken and sterilized by using a 0.22 μm membrane filter. Two millilitres of specific or nonspecific IgY solution were then added to the same volume of prepared *A. hydrophila* culture. The bacteria and IgY mixtures were incubated at 37ºC with shaking. Aliquots of samples (100 µl) were taken at 0, 2, 4, and 6 h of incubation. The inoculated plates

were incubated at 37ºC overnight. The number of colony-forming units per plate was counted to determine the total number of bacteria colony-forming units per ml of sample.

RESULTS

Total Protein Estimation for Antibody Anti-*A. hydrophila* IgY By Bradford's Assay

Using Bradford's method, the total protein for purified antibody was calculated from Control IgY and Specific Anti-*A. hydrophila* IgY are furnished in the Table 1. Overall, the protein level in the Control IgY was lower in comparison to that of the Specific anti-*A. hydrophila* IgY in all the concentrations. The optimum proteins acquired by the Specific anti-*A. hydrophila* IgY in 100µg/ml was 179.58 µl and the Control IgY in100µg/ml was 122.20 µl.

IgY and anti Vibrio- IgY Characterization by SDS-PAGE

The Control IgY and Specific anti-*A. hydrophila* IgY obtained from *A. hydrophila* antigen were characterized by SDS-PAGE and given in the Fig (1). There was a lower level expression of antibody in the control IgY of the egg of hen *G. domesticus* as per the SDS –PAGE report. Surprisingly, in the two types of immunization of the hen yolk *G. domesticus*, a specific anti *Vibrio* IgY was detected with high molecular weight around 99.12 k Da.

Growth inhibitory effect of anti-*A. hydrophila*–IgY

In the colony counting method, the growth curves of *A. hydrophila* consists of lag phase (0-2 hours of incubation time), exponential (2-6 hours of incubation time), and stationary phase. *A. hydrophila* along control and specific anti *A. hydrophila* IgY were incubated for 6 hours during which the samples were taken at ever 2 hour incubation intervals to see its growth incubation assay. In this assay, different concentration of control IgY and specific anti *A. hydrophila* IgY were used (100 and 200 mg), while considering the importance of minimum inhibitory concentration of antibody interact with the antigen. After 4 hours of incubation, the growth of *A. hydrophila* incubated with specific IgY showed a significant reduction in bacterial growth when compared to Control IgY, which maintained its lag phase and exponential phase from 0-2 hours and 2-6 hours of incubation. In Control, the low effect of bacterial growth was noted and it ranged from 34 – 14 and 36 – 7 during 2-6 hours at 100 and 200 mg respectively. Whereas in the Specific IgY, the minimum reduction was noted as 24 during 2 hours of incubation and the growth was completely eradicate after 6 hours of incubation in both concentrations (Table.2 and 3). The similar trend was noted in the Optical density method too (Fig.2).

DISCUSSION

In this study, we demonstrated the ability of attenuated anti-*A. hydrophila* antibody to deliver the vaccine orally against shrimp in an invertebrate animal for aquaculture. It has been revealed that vaccination could induce protective response in shrimp against *A. hydrophila.* The bacterial diseases in maricultured animals in China are very common and lead to extensive economical losses in maricultural industry (Woo *et al.,* 2001). Although some live attenuated vaccine and killed vaccine have been developed for the prevention or cure of the bacterial disease in marine animals, live vaccine against bacterial infection in marine animals are still not available (Zhu *et a*l., 2004). Most of the bacterial diseases in marine animals in China are found to be caused by *A. hydrophila*.

The present study was conducted to determine the effectiveness of egg-yolk antibody (IgY) obtained from hens immunized against *A. hydrophila.* The yolk antibody against *Vibrio harveyi* study showed a remarkable preventive effect against *Vibrio harveyi* infection (Kumaran *et al.,* 2014). It has been known that serum IgG of the hen is transferred to its egg yolk and provides its offspring with acquired immunity (Patterson *et al.,* 1962), Hatta *et al*., (1990) termed the antibody in egg yolk as IgY. It was that the Avian immunoglobulins will be soon accepted as a viable alternative to mammalian ones, particularly with respect to specific applications such as those discussed in this research. Moreover, a laboratory that is ready to use non-mammalian, e.g. chicken antibodies, will be better able

to adhere to stricter rules coming in the near future with regard to experimental animal handling (Kumaran *et al.,* 2014). In the present study, the growth of *A. hydrophila* incubated with specific IgY showed a significant reduction in bacterial growth when compared to Control IgY. In Control, the low effect of bacterial growth was noted and it ranged from 34 – 14 and 36 – 7 during 2-6 hours at 100 and 200 mg respectively. Whereas in the Specific IgY, the minimum reduction was noted as 24 during 2 hours of incubation and the growth was completely eradicated after 6 hours of incubation in both concentrations. Sunwoo *et al.,* (2002) reported that the binding of Anti- Vibrio IgY to bacterial surface components could cause some structural alterations of the bacterial surface, which may block the opportunity to take the nutrients and proliferate. This could be because of the generation of the antibodies against the whole bacterial cells possessing the binding activities against various epitopes of the bacterial surface as is the characteristics of a polyclonal antibody. Therefore, binding activities of IgY against bacterial surface components, including fimbriae and outer membrane protein may cause the inhibition of growth. Similar result was stated by Guimarães *et al.,* (2009). The present study clearly indicates that the specific IgY obtained from hens, immunized by *A. hydrophila,* may provide a novel approach to the management of *A. hydrophila*.

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Table - 1: Total Protein Estimation (μl) of Control IgY and Specific Anti- *Aeromonas hydrophila* IgY by using Bradford's Assay

S.NO	CONCENTRATION (μg/ml)	TOTAL PROTEIN (μl)	
		Control IgY	Specific Anti- *A. hydrophila* IgY
1	10	8.75[a]	11.25[b]
2	20	19.04[a]	22.13[b]
3	30	27.54[a]	31.14[b]
4	40	38.46[a]	43.13[b]
5	50	48.33[a]	55.07[b]
6	60	56.34[a]	66.22[b]
7	70	71.36[a]	118.07[b]
8	80	77.56[a]	135.22[b]
9	90	99.36[a]	157.47[b]
10	100	122.20[a]	179.58[b]

Means with the same superscripts (a-b) do not differ from each other (P < 0.05)

Table - 2: Growth Inhibition assay of Control IgY and Specific Anti- *A. hydrophila* IgY (100mg) by using Colony counting Method (CFU)

0	Too numerous to count	Too numerous to count
2	34	24
4	20	13
6	14	-

Table-3. Growth Inhibition assay of Control IgY and Specific Anti- *A. hydrophila* IgY (200mg) using by Colony counting Method (CFU)

Incubation Hours	Control IgY	Specific Anti- *A. hydrophila* IgY
0	Too numerous to count	Too numerous to count
2	36	24
4	24	13
6	7	-

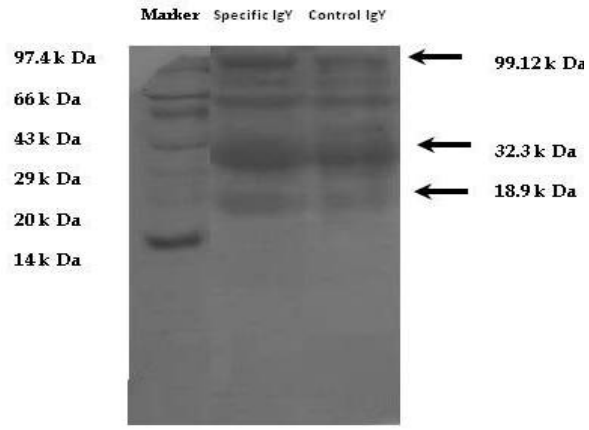

Fig – 1. SDS PAGE for the Yolk antibody IgY, Control and Specific anti- *A. hydrophila* IgY.

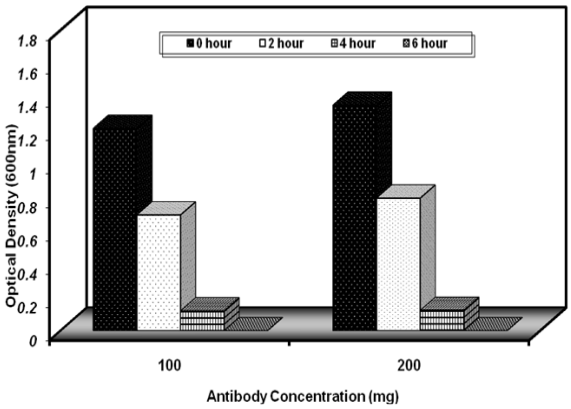

Fig 2. Growth inhibition assay of Cotrol IgY and Specific Anti- *A. hydrophila* IgY by using Optical Density Method

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RESEARCH ARTICLE

Quality of Work Life, Job Satisfaction and Employees Performance in Textile Industries in 'COVID 19'

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ABSTRACT

The aim of this paper is to find out the linkage between quality of work life, job satisfaction and employees performance in textile industries in 'COVID 19' at Tiruppur. The China market is closed due to the Corona pandemic, Tiruppur textile manufacturers' targets these international market. A sample of 389 employees identified using systematic sampling method. The result indicates that employee performance in this COVID 19 period was in the expected mark. i.e., revenue per employee, productivity (Output), and quality of the output are in high standard. Social support from supervisors, fair treatment, good working relationships, and respect from colleagues may also improve the QWL of employees.

Keywords: Quality of work life, Job satisfaction, Employees performance, COVID, Textile, Tiruppur.

INTRODUCTION

The quality of work-life (QWL) is a process by which employees' quality of work-life and organizational effectiveness is improved together by taking different measures in an organization. In this QWL concept, the well being of the employee is assuring about a holistic approach instead of job-related features (Daubermann and Tonete, 2012). QWL includes compensation, working conditions, health, and safety, security organizational and personal

relations (Mosadeghrad, Ferlie, and Rosenberg, 2011; Raj Adhikari and Gautam,2010). When on an organization is providing a right QWL environment, it fetches the improved performance of the employees, reduce absenteeism and work-related injuries, stress and increase the job morale and satisfaction of the employees (venkateswaran,2018; Blaauw, Ditlopo, and Rispel,2014; Goudarznand-Chegini and Mirdoozandeh,2012) and enhanced productivity (Delgoshyii, Riahi, and Motaghi,2010).QWL is associated with productivity, health care, job security, appropriate working time; and an appropriate salary (Pandey, M.K.; Tripathi, P (2018). QWL is a construct that concerns the well-being of employees and is conceptually different from job satisfaction. QWL enhances employees' dignity through job satisfaction and humanizing work (Adhikari et al., 2011). The scope of QWL not only affects employees' job satisfaction but also their lives outside of work, such as family, leisure, and social needs. The Textile industry of Tiruppur provides employment opportunities to 6 lakhs people. Due to the COVID pandemic, the maximum number of units is working with less than 50 percent of employees in May –June period. The challenges faced by the Tiruppur units are getting new orders from the US and European countries. However, they have excellent opportunities in healthcare-related orders such as face masks, hand gloves, and cotton tissues. This present paper studied the quality of work-life, job satisfaction, and employee performance in textile industries, Tiruppur.

REVIEW OF LITERATURE

Quality of Work Life

Quality of work life is an important criterion which focused by the organizations in order to achieve higher productivity and organizational goals, reduce employee turnover at large. It observed that a significant impact of the quality of work-life dimensions on employee satisfaction in the organization. To enhance the quality of work-life for employees that has a positive impact on organizational goals and objectives (Ashwini et al., 2014). Quality of work-life includes health and well-being, job security, competency development, a balance between work life and non-work, and further a significant direct correlation between job satisfaction and health well-being, which is intervened by job satisfaction variable (Hossein, 2012). Nanjundeswaraswamy (2013) stated that High quality of work-life and proper Leadership style is essential to retain employees and organizational effectiveness and performance. Quality of work-life dimensions, basic extrinsic job dimensions, intrinsic job dimensions, managerial style, and the job itself are the essential dimensions that influence the level of quality of work-life of employees (Parameshwari and Suresh, 2015). The following variables are identified from the previous studies and used for the current study. These are Wage and salary (QWL1), Training and Development (QWL2), Professional growth (QWL3), Recognition (QWL4), Safety measures (QWL5), Work schedule (QWL6), Interpersonal relationship (QWL7), Job security (QWL8), Job content (QWL9) and Working environment (QWL10).

Job Satisfaction

Job satisfaction includes an assessment of whether an individual feels that his or her needs and expectations met within their particular job. Overall, the job satisfaction literature suggests that it is a crucial workplace attitude necessary for the proper functioning of a correctional facility (Lambert et al., 2009). The extant scholarly thought substantiates a positive relationship among employee satisfaction, customer satisfaction, and corporate performance (Harter, Schmidt, & Hayes, 2002; Huang, Li, Meschke & Guthrie, 2015; Symitsi, Stamolampros, & Daskalakis, 2018). The moderating effect of the customer-employee interaction during the consumption experience (Brown & Lam, 2008, for a review and meta-analysis).Employees with a low level of satisfaction have less incentive to excel and, as such, may deliver lower service quality (McPhail, Patiar, Herington, Creed, & Davidson, 2015), affecting, in turn, corporate performance through the service satisfaction-profitability link (Lam, Baum, & Pine, 2003). Previous research (Lu and Gursoy, 2013; Ziegler et al., 2012) has highlighted job satisfaction due to its positive effect on job performance and the firm's performance. Hence, researchers need to capture the essential antecedents of employee job satisfaction. The following variables are identified from the previous studies and used for the current study. These are Satisfaction from the wages and bonuses (JS1), Satisfaction from the rewards (JS2), Satisfaction from

fringed benefits (JS3), Satisfaction from non-financial rewards (JS4), Teamwork (JS5), Collective decision making (JS6), Perception of being valued and respected (JS7), Personal recognition (JS8), Promotion opportunities (JS9), Training and development (JS10) and Effective communication (JS11).

Performance

Employee performance is essential in the effort to achieve the goal (Rivai, 2004). Another view describes the performance as a series of activities undertaken through the inputs, processes, outputs, outcomes, benefits, and impact on the performance of activities (Thompson, 2003). Results of the study provide evidence that there were inconsistencies in the explanation of the effect of job satisfaction on individual performance, that job satisfaction has a positive and significant impact on employee performance (Al-Hussami, 2008; Al-Ahmadi 2009; Khan et al., 2010). The evidence contradicted by other researchers that job satisfaction is not significant to the performance of the individual (Chen & Zhong, 2007). The following variables are identified from the previous studies and used for the current study. These are, Revenue per Employee (EP1), Cost Effectiveness (EP2), Productivity (EP3), Efficiency (EP4), Turnaround Time (EP5), Quality (EP6), Budget Variance (EP7) and Customer Satisfaction (EP8).

RESEARCH METHODS

This study applied a quantitative method to explore the QWL and its impact on job satisfaction and employee performance. A list of all employees working in the selected 20 textile industries acquired from the concerned industry's employee's wage and salary list. The list contained the names and contacts of 1705 employees. The data were collected using the self-administered questionnaire, which is in their native language (Tamil). A descriptive research design was adopted. Systematic random sampling used to identify 389 samples by selecting every 4th item from the employee's wage list. (i.e.4, 8, 12, 16, 20 and so on). The questionnaire consists of two parts. The first part consists of socio-economic or demographic variables of the respondents; the second part consists of questions related to QWL, JS, and EP. The researcher used Likert's five-point scale for the questionnaire. The data collected from May 2020 to June 2020 in the COVID pandemic period. The statistical tools used for the study are reliability analysis, t statistics, correlation, and SEM.

Hypothesis Testing

Null Hypothesis H_{01}- QWL has no influence on JS.
Null Hypothesis H_{02}- JS has no influence on EP.
Null Hypothesis H_{03}- QWL has no influence on EP.

Analysis and Interpretation

Demographic Analysis

A maximum of 57.81 percent of the employees in the present study is female. The vital age group of the employees is 26 to 35 and less than 25 years, which constitutes 36.8 and 29.3 percent to the total, respectively. 40.6 percent of the employees are unmarried'. The most vital educational qualifications among the employees are under graduation and diploma, which constitute 42.05 and 38.24 percent to the total, respectively. The vital level of personal income per month among the employees is Rs.10, 000 – 15,000 and Rs.15, 001 – 25,000, which constitute 46.8 and 21.2 percent to the total, respectively. The experience of the employees is 5 to 10 years and 1 to 5 years, which constitute 28.8 and 32.3 percent. In total, a maximum of 53.90 percent of the employees follows the nuclear family system. The vital family sizes of the employees are less than 3 members and 3 to 4, which constitute 44.9 and 31.2 percent to the total, respectively. The nature of employment among the employees is 'Contract' and 'Temporary ', which constitute 40.4 and 31.6 percent to the total, respectively. The hours worked per day by the employees are 8.00 to 10.00 hours and 10.00 to 12.00 hours, which constitute 54.6 and 30.4 percent. The working shift among the employees is a day shift and night shift, which constitute 47.5 and 33.8 percent to the total, respectively.

Table 1 indicates that the internal consistency of the Quality of work-life variables is right because the reliability co-efficient (Cronbach Alpha) is more significant than its minimum threshold of 0.70. The analysis reveals that the reliability and validity of variables in QWL are usable for further analysis. The mean scores of the variables on QWL among the male and female employees along with its 't' statistics and reliability score. The highly viewed QWL variables by the male employees are 'wage and salary' and 'recognition' since their mean scores is 3.890 and 3.884, respectively. Among the female employees, these are 'working environment' and 'safety measures' with a mean score of 3.638 and 3.621, respectively. Regarding the view on variables in QWL, the significant difference among the male and female have been noticed in the case of eight variables out of ten variables since their respective 't' statistics are significant at five percent level.

Table 2 indicates that the internal consistency of the Job satisfaction (JS) variables is suitable because the reliability co-efficient (Cronbach Alpha) is more significant than its minimum threshold of 0.70. The analysis reveals that the reliability and validity of variables in JS are usable for further analysis. The mean scores of the variables on JS among the male and female employees, along with its 't' statistics and reliability score. The highly viewed JS variables by the male employees are 'satisfaction from the wages and bonuses' and 'satisfaction from fringed benefits' since their mean scores is 4.314 and 4.093, respectively. Among the female employees, these are ''satisfaction from the wages and bonuses' and 'satisfaction from fringed benefits' since their mean scores are 4.314 and 4.093, respectively. Regarding the view on variables in JS, the significant difference among the male and female identified in seven variables out of eleven variables since their respective't' statistics are significant at five percent level. Table 3 indicates that the internal consistency of the employee performance (EP) variables are right because the reliability co-efficient (Cronbach Alpha) is more significant than its minimum threshold of 0.70. The analysis reveals the reliability and validity of the variables in the EP are usable for further analysis. The table shows the mean scores of the EP among the male and female employees, along with 't' statistics and reliability score. The highly viewed EP variables by the male employees are 'productivity' and 'revenue per employee' since their mean scores is 4.0are and 3.980, respectively. Among the female employees, these are ''quality' and 'productivity' since their mean scores are 3.850 and 3.832, respectively. Regarding the view on variables in EP, the significant difference among the male and female have been noticed in the case of five variables out of eight variables since their respective 't' statistics are significant at five percent level.

Bivariate Correlation Analysis

Bivariate correlation helps to explore the relationship between the two continuous variables. A positive correlation indicates that as one variable increases, so does the other, while a negative correlation indicates that as one variable increases, the other decrease. The relationship between quality of work-life, Job satisfaction, and employee performance was measured using Pearson product-moment correlation coefficient. Table 5 shows the moderate positive relationship between QWL and JS variables (r=.549**, p<.01), which indicates that QWL has a significant influence on the job satisfaction of the employees. It shows that when one unit of QWL increases simultaneously. Job satisfaction increased positively. JS and EP have a moderate positive correlation (r=0.499**, p<.01) between them. It shows that satisfied employees are performing well. i.e., when one unit of JS increases correspondingly, the EP increases positively. The weak positive correlation between QWL and EP (r=0.395**, p<.01) shows that QWL has a weak and significant influence on the performance of the employees. The standardized direct, indirect, and total effects of the linkage between the Quality of work-life, Job Satisfaction, and Employee Performance in Textile industries, Tiruppur is shown in figure 1. QWL had a direct effect on employee performance (β = .175, p = .013). JS has a direct effect on employee performance (β = .382, p = .001). QWL had an indirect effect on employee performance (β = .382, p = .001). QWL had an indirect effect on employee performance (β = .725, 0.175+0.277), p = .001). Therefore, the modified model effectively explained the linkage between the Quality of work life, job satisfaction, and employee performance.

DISCUSSION

This paper aims to explore the linkages among quality of work-life, job satisfaction, and employee performance in the textile industries in the COVID 19 at Tiruppur. The findings of the research support existing research and provide new insights, especially in the COVID 19 pandemic period. The means of the study variables indicate that textile employees have a moderate level of job satisfaction, QWL, and employee performance. The correlation analyses explain the extent to which QWL and JS influence employee performance. Correlation between QWL & JS, JS & EP was moderate, and the correlation between QWL and employee performance was weak. These results suggest that both QWL & JS, JS & EP have a positive and significant relationship with employee performance. The results of SEM highlights that QWL has a significant relationship with the JS in the textile industry, which is significant at 0.001 level (Null hypothesis H01 rejected). Hence there is a strong influence of QWL on JS (venkateswaran,2015). The employees who have an optimistic perception of the level of QWL may exhibit a higher degree of job satisfaction. Further, the results also reveal that employees experience better QWL when they have good wages and salary, proper recognition, high safety measures, and a congenial work environment. The results also showed the significant relationship between QWL and JS, which is significant at the 0.05 level (Null hypothesis H02 rejected). It indicates that employees are satisfied with their job when they have good wages & bonuses and proper fringe benefits. Similarly, the results show that QWL has a significant relationship with employee performance in the textile industry (Null hypothesis H03 rejected). It reveals that employee performance in this COVID 19 period was in the expected mark. i.e., revenue per employee, productivity (Output), and quality of the output are in high standard. Additionally, the result also establishes the partial mediating effect of JS on the relationship between quality of work-life and employee performance.

CONCLUSION

Quality of work-life includes wage and salary, training and development, professional growth, recognition, safety measures, work schedule, interpersonal relationship, job security, job content, and working environment directly influence employees' job satisfaction and performance. When employees are satisfied with the wages and bonuses, satisfaction from the rewards, satisfaction from fringe benefits, satisfaction from non-financial rewards, teamwork, collective decision making, perception of being valued and respected, personal recognition, promotion opportunities, training and development, and effective communication, they tend to be more productive and maintain excellent performance.

Managerial Implications

An organization must pay a good wage and salary based on their work experience and education so that they will be more productive and loyal to the firm. By providing a good QWL will enhance the employees more committed and engaged towards the work. The textile units should certainly focus on improving the quality of work life. They must also organize training programs for the development of the skills of employees. The support received from the colleagues and supervisors in the form of advice, feedback, and constructive criticism will help them to act as a cohesive unit in this COVID 19 period. This study provides a conceptual framework for employee performance enhancement in this pandemic period. The textile units must consider the QWL of employees as an essential HR strategy and develop guidelines and policies to enforce the same. By providing a safe work environment, flexible schedules, and ensuring sufficient staff to cover workload ensure the health safety of the employees. The employees assisted in managing work pressure, avoid long working hours, and occupational stress. Social support from supervisors, fair treatment, good working relationships, and respect from colleagues may also improve the QWL of employees.

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Table 1 View on Quality of work-life variables

S.No.	Quality of work-life variables (QWL)	Reliability Co-efficient	Mean score of Employees		't' statistics
			Male	Female	
1.	Wage and salary (QWL1)	0.837	**3.890**	3.479	6.214*
2.	Training and Development (QWL2)	0.876	3.641	3.217	7.323*
3.	Professional growth (QWL3)	0.902	3.857	3.385	9.542*
4.	Recognition (QWL4)	0.868	**3.884**	3.528	6.805*
5.	Safety measures (QWL5)	0.859	3.841	**3.621**	6.329*
6.	Work schedule (QWL6)	0.829	3.827	3.346	8.472*
7.	Interpersonal relationship (QWL7)	0.841	3.533	3.480	0.961
8.	Job security (QWL8)	0.837	3.654	3.457	9.524*
9.	Job content (QWL9)	0.808	3.803	3.344	7.708*
10	Working environment (QWL10)	0.875	3.724	**3.638**	1.482

Source: Primary data

Table 2 View on Job satisfaction variables

S.No	Job satisfaction variables	Reliability Co-efficient	Mean scores among employees		't' statistics
			Male	Female	
1.	Satisfaction from the wages and bonuses	0.879	**4.314**	**3.903**	7.651*
2.	Satisfaction from the rewards	0.897	3.738	3.461	7.647*
3.	Satisfaction from fringed benefits	0.906	**4.093**	**3.818**	3.976*
4.	Satisfaction from non-financial rewards	0.882	3.865	3.714	1.054
5	Teamwork	0.853	3.790	3.357	5.357*
6	Collective decision making	0.827	3.526	3.281	4.883*
7	Perception of being valued and respected	0.881	3.671	3.448	3.979*
8	Personal recognition	0.836	3.714	3.686	1.012
9	Promotion opportunities	0.785	3.808	3.739	1.506
10	Training and development	0.862	3.706	3.437	5.940*
11	Effective communication	0.865	3.473	3.316	1.213

Source: Primary data

Table 3 View on employee performance variables

S.No	Employee performance variables	Reliability Co-efficient	Mean scores among employees		't' statistics
			Male	Female	
1.	Revenue per Employee (EP1)	0.874	**3.980**	3.652	6.784*
2.	Cost Effectiveness (EP2)	0.856	3.876	3.655	3.527*
3.	Productivity (EP3)	0.824	**4.016**	**3.832**	3.420*
4.	Efficiency (EP4)	0.786	3.967	3.690	4.418*
5	Turnaround Time (EP5)	0.794	3.786	3.549	3.722*
6	Quality (EP6)	0.868	3.961	**3.850**	1.099
7	Budget Variance (EP7)	0.887	3.745	3.679	1.014
8	Customer Satisfaction (EP8)	0.843	3.726	3.611	1.085

Source: Primary data

Table.4 –Correlations between QWL, JS and EP (N=389)

	QWL	JS	EP
QWL	1	.549**	.395**
JS	.549**	1	.499**
EP	.395**	.499**	1

Table.5 Model Fitness Index for the Hypothesized Model and Modified Model (*N* = 389)

	$\chi 2$	*df*	$\chi 2/df$	*p* Value	GFI	AGFI	CFI	TLI	RMSEA
Model Fit Measure	1116	342	3.263	0.000	.469	.474	0.980	.973	0.145
Hypothesized model	1209	342	3.535	0.000	.387	.401	0.985	.967	0.112

Note. GFI = goodness-of-fit index; AGFI = adjusted goodness-to fit-index; CFI = comparative fit index; TLI = Tucker–Lewis index; RMSEA = root mean square error of approximation.

Table 6 shows the path analysis

Model path			Unstandardized Estimate	S.E.	Standardized Estimate	't' statistics	P	Hypothesis (P<0.05)
JS	<---	QWL	.724	.054	.547	13.335	0.001**	H_{01} rejected at 0.001 level
EP	<---	QWL	.175	.070	.128	2.493	0.013*	H_{02} rejected at 0.05 level
EP	<---	JS	.382	.053	.370	7.224	0.001**	H_{03} rejected at 0.001 level

*Significant at 0.05 level. ** Significant at 0.001 level.

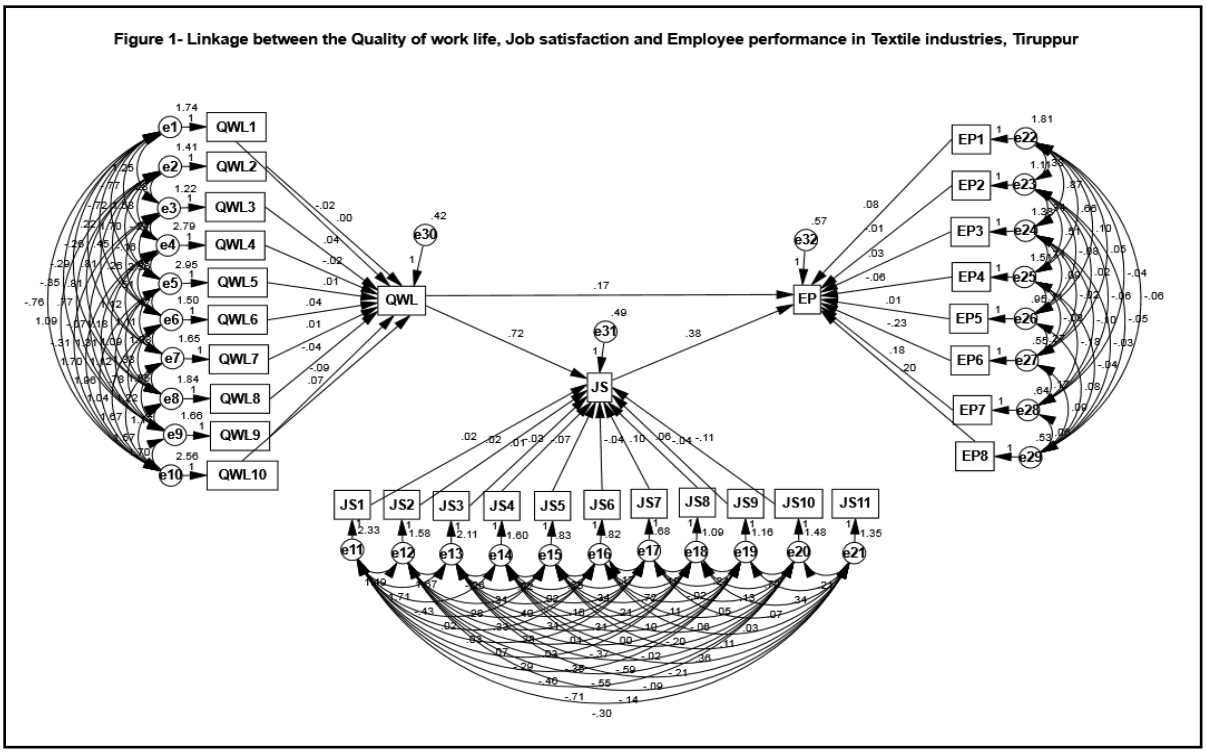

Figure -1: The linkage between the QWL, JS and EP

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RESEARCH ARTICLE

Cytotoxic Activity of Different Solvent Extracts and Fractions of *Clerodendrum thomsoniae* Balf on Different Cancer Cell Lines

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ABSTRACT

Clerodendrum is a genus of about 500 species belongs to the family Lamiaceae. Many species of this genus have been proved for the treatment of various diseases including cancer. This study aimed to evaluate the cytotoxic effect of different solvents and their most active fractions of *Clerodendrum thomsoniae* Balfin different human cancer cell lines. In this study Aerial parts of the plant were subjected to Soxhlet extraction. Phytochemical analysis was done. *In vitro* anticancer activity on MCF-7, Hep-G2, A549, HT-29, MOLT-4,Hela and Vero cell lines were evaluated by MTT assay. GC-MS analysis was used to identify compounds present. Phytochemical analysis confirmed the presence of most of the phytoconstituents in ethyl acetate extracts and the same extracts were found to be more cytotoxic activity to cancer cell lines MCF-7, Hep-G2, A549, HT–29, MOLT-4 and Hela with IC_{50}values 29.43±1.44μg/mL, 43.22±1.02μg/mL, 56.93±1.41μg/mL, 60.68±1.05μg/mL, 69.83±1.33μg/mL, 40.02±1.14 μg/mL respectively, while it had no cytotoxic effect on normal Vero cell IC_{50}=367.5±1.03 μg/mL. Ethyl acetate extracts were selected for the fractionation and MTT assay was performed with MCF-7 cell line and found that fraction F5 was the most active fraction with IC_{50} 17.33±0.54μg/mL. These findings have proved that *Clerodendrum thomsoniae* Balf have anticancer activity especially for breast cancer. Further studies required for the isolation of constituents and to explore the mechanism of action.

Keywords: Anticancer potential, Cancer cell lines, Ethyl acetate fractions, *In vitro* anticancer, *Clerodendrum thomsoniae* Balf

INTRODUCTION

Cancer is considered as one of the life-threatening ailments, which include the abnormal cell growth with the capacity to continuously multiply and invade from one tissue to the other in the body [1]. Cancer had continuously been the most serious disease in humans around the world due to its high morbidity and mortality [2]. Breast cancer is an significant global health issue and one of the foremost causes of deaths in females [3]. The World Health Organization (WHO) expects about 15 million new cases of cancer by 2020 [4]. Chemotherapy, radiation therapy, hormonal therapy and surgery are the common treatments for all kinds of cancer, and due to resistance and adverse or toxic side effects of these treatments it has become necessary to hunt for an alternative anticancer treatment [5]. Natural products preserve vast pharmacological significance and have been considered as a key source of potential chemotherapeutic treatments [6]. Natural products obtained from plants are well-accepted sources and also a many drugs are presently used from plant basis for the treatment of numerous human ailments including cancer. Over the past few decades, there has been a certain interest in the role of medicinal plant extracts in cancer prevention. Plants are rich sources of chemically diverse compounds, several with beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents identified are derived from plants [7]. Several plant-based molecules that consist of vinblastine,vincristine, taxol and camptothecin derivatives are used clinically to treat various types of cancers [8]. Various drugs were derived from the natural sources such as plants and microorganisms are evidenced for their ability to cure several types of diseases such as prostate, breast, lung and colon cancers. In addition, anticancer activities of numerous natural products are presently being studied to identify potential anticancer agents which could improve the efficacy of specific targeted remedies against cancer [9-13]. Most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied toward battling cancer [14-15]. *Clerodendrum thomsoniae Balfis* a twining, rambling, vine like shrub native to tropical West Africa. Bleeding-heart vine or Bag-flower is the collective name of *C. thomsoniae* [16-17].The leaves and flowers of *C.thomsoniae* are the main bases of several medicinally significant phytocompounds. These phytocompounds protect human body from oxidative stress by its own capable defense mechanism and curing diseases like bruises, cuts, skin rashes and sores [18]. The objective of the study to evaluate anticancer activity of *C. thomsoniae* and its active fractions on different cancer and normal cell lines.

MATERIALS AND METHODS

Chemicals and Reagents

3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), DMEM (Dulbecco's modified Eagle's medium) and DMSO (Dimethyl sulfoxide) were purchased from Sigma Chemicals Co. (St.Louis, MO,USA). Fetal Bovine Serum (FBS) were purchased from Gibco (USA). All other chemicals and reagents used for the experimentation were all of analytical grade and were purchased from Merck (India).

Cell Lines and Maintenance

MCF-7 (Human breast cancer cells), Hep-G2 (Human liver cancer cells), A549 (Humanlung cancer cells), HT–29 (Human colon cancer cells), MOLT-4 (Human acute T lymphoblastic leukemia cells), Hela cells (Human cervical cancer cells) and Vero (African green monkey kidney cells) cell line were cultured separately in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS),100 µg/mL penicillin and 100 µg/mL streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC. All cell line was purchased from the National Centre for Cell Sciences (Pune, India).

Preparation of Extracts

Aerial parts of *C. thomsoniae* were obtained locally from Calicut district (Kerala, India). The plant materials were identified and authenticated by Dr.A.K.*Pradeep, Assistant Professor* -Department of Botany, Calicut University

(Calicut, India). Voucher specimens were deposited in the same department herbarium as specimen No.148249. The aerial parts of *C.thomsoniae* was dried properly in shade for 3 weeks, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. About 1 kg of air-dried plant material was extracted in soxhlet assembly successively with petroleum ether, chloroform, ethyl acetate, ethanol and water (order of increasing polarity). Each time before extracting with the next solvent, the powdered material was dried at room temperature. Each extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The color and consistency of the extract were also noted. All the solvents used for this entire work were of analytical grade (Merck, Mumbai).

Phytochemical Analysis

Phytochemical tests were carried out using standard procedures to identify constituents, as described by Harborne [19], Trease and Evans [20] and kokate [21]

Fractionation of Ethyl Acetate Extract

The ethyl acetate extracts were subjected to column chromatography using silica gel(mesh size 60-120).Fifty grams of extract was submitted to flash chromatography using silica gel (mesh size 60-120)as stationary phase. The silica column was prepared using ethyl acetate by wet packing method and the column was washed using 100 ml of ethyl acetate. Then, the ethyl acetate extract was mixed with silica gel and made fine powder for easy distribution of sample. The powdered sample mass was placed on the top of the pre-packed silica column. The elution was done using increasing solvent polarity made of hexane: ethyl acetate and methanol mixtures Each 10 ml of fractions were collected in vials and further analyzed by thin layer chromatography. Similar fractions were pooled together to produce seven fractions (F1-F7), evaporated to dryness and kept in the dark for subsequent analysis.

In vitro Anticancer Activity Assay by MTT Method

The test sample was evaluated for *in vitro* cytotoxicity on selected cell lines by 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well(200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with different concentrations of the test sample in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT(20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT(220 µL)were aspirated off the wells and washed with 1X PBS(200 µl). Furthermore, to dissolve for mazan crystals, DMSO(100 µL)was added and the plate was shaken for 5 min.The Absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA)and the percentage cell viability and IC_{50} value was calculated using Graph Pad Prism 6.0 software (USA).

RESULTS

Extraction Yield

The percentage yield and color of all five extracts are listed in following(Table 1).Highest percentage was observed in ethyl acetate extract (13.12%w/w)and the lowest was noted in water extract (2.78%w/w).

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis showed that most of the phytochemicals are present in ethyl acetate extracts. Detailed reports are listed in the following table(Table 2). The results suggest that ethyl acetate is the best solvent for the extraction of phytoconstituents from *C.thomsoniae*. Tannins are present in petroleum ether extract at minor

quantity. Chloroform extract contain flavonoids and phenols at minor extend. Ethyl acetate extracts showed high presence of steroids, flavonoids and phenols, also showed the presence of terpenoids, alkaloids, carbohydrate and alkaloids at lower extend.

Evaluation of IC_{50} using MTT assay

The IC_{50} value was determined based on cell viability rates. The results are listed in the following table (Table No. 3). IC_{50} values showed that ethyl acetate extracts showing more toxicity on all cancer cell lines used, especially more toxicity selectively to MCF -7 (IC_{50}=29.43±1.44 µg/ml). The IC_{50} observed for ethyl acetate extract on MCF-7 was 43. 22±1.02, 56.93±1.41, 60.68±1.05, 40.02±1.14 and 69.83±1.33 for Hep-G2, A549, HT-29, Hela and MOLT-4 respectively. The results showed Vero cells were not showing toxicity any of the extracts treated which reveals the safety of this plant. Ethyl acetate extracts was selected for fractionation because of its specific toxicity on MCF-F7 when compared to other cell lines. IC_{50} value of ethyl acetate extract on MCF-7 was 29.43±1.44. IC_{50} value of ethyl acetate extract fractions showed that fraction F5 was more cytotoxic to MCF-F7 then F6, F7, F4, F3, F2 and F1 respectively(Table 4). All extracts and fractions showed non toxicity on Vero cell line.

Statistical Analysis

All the data expressed as mean ± SEM were analyzed by one-way analysis of variance (ANOVA), using Prism Graph Pad and values of $P<0.05$ were considered as statistically significant

DISCUSSION

Cancer is the foremost cause of death worldwide and as reported by WHO it caused more deaths than AIDS, tuberculosis and malaria in 2012. Among the 14 million occurrences of cancer in 2012, commonly identified cancers were lung, breast and colorectal and the most common causes of cancer death were lung, liver, and stomach cancer (WHO, 2013). The management of cancer relies on surgery, radiotherapy, chemotherapy or a combination of these methods [22]. Numerous important antitumor drugs have been isolated from plants [23]. Almost one-third of prescribed drugs in the world are derived from plants and anticancer properties of over 3000 plant species have been identified [24]. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has studied around 114,000 extracts for anticancer activity [25]. Over 3000 species of plants with antitumor properties have been reported [26]. The genus *Clerodendrum* (Verbenaceae more recently placed in the Lamiaceae) is a diverse genus with 580 species [27] of small trees, shrubs or occasionally perennial herbs, typically in the tropical and subtropical region around the world [28]. A number of species from this genus have been used in traditional systems of medicine by several tribes in many countries like China, Japan, India, Korea and Thailand [29]. This genus has been found to contain terpenoids as the major secondary metabolites [30-31]. The genus also contains neo clerodanediterpenes [32], triterpenes [33] and iridoids [34]. Phenolic compounds have been frequently reported with phenylpropanoids and flavonoids as a principal class [35-36]and few species have been reported to have macrocyclic alkaloids and cyanogenic glycosides [37-38]. Some of these compounds have been assessed for a number of activities mostly anti-inflammatory [39-40], anti-asthmatic [41-42], hepatoprotective [43], antioxidant [44], cytotoxicity [45], antitumor [46]and for the effects on the central nervous system [47]. The *Clerodendrum* genus has been a good source of herbal medicinal products. Pharmacological investigation has shown that these compounds and extracts from the *Clerodendrum* genus have wide-ranging activities, such as anticancer, anti-inflammatory, analgesic, antioxidant, antihypertensive, antimicrobial, antidiarrheal, hepatoprotective, hypoglycemic, hypolipidemic, memory enhancing and neuroprotective activities [47]. Earlier studies on some *Clerodendrum* species such as *Clerodendrum quadriloculare* [48], *Clerodendrum trichotomum* [49]*Clerodendrum bungei* [50], *Clerodendrum trichotomum* [51], *Cleroodendrum kiangsiense* [52] and *Clerodendrum serratum* [53]have reported for cytotoxic activity against tumor cell lines.

The percentage yield of extracts will be different in plant parts as well as among plant species. It also varies with solvent systems too. In the present study, ethyl acetate extract yield was better when compared with other extracts. The plant parts possessed varying levels of secondary metabolites. Preliminary phytochemical analysis revealed the presence of secondary metabolites in the selected extracts of the plant. Most of the phytoconstituents are present in ethyl acetate extracts such as phenols, flavonoids, steroids, and alkaloids. In this study ethyl acetate, extracts showed more cytotoxicity on breast cancer cell line (MCF-7) when compared to the other cell lines. It has been noted that ethyl acetate extract also showing cytotoxicity to other cell line used in the present study such as Hep-G2 (IC_{50}=43.22 μg/mL), A549 (IC_{50}=56.93 μg/mL), HT-29 (IC_{50}=60.68 μg/mL), Hela (IC_{50}=40.02 μg/mL) and MOLT-4 (IC_{50}=69. 83 μg/mL). Because of the selective toxicity of ethyl acetate extracts on these cell lines, we had performed the fractionation of ethyl acetate to find the more active fraction on the MCF-7 cell line and observed that fraction F5(IC_{50}=17.33±0.54 μg/mL) is more active when compared to other fractions tested. It has been reported that phenolic compounds possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, as well as inhibition of angiogenesis and cell proliferation activities [54]. So the presence of this compound may be responsible for the cytotoxicity observed in our study and farther experiment is going on in our laboratory to isolate the active compound responsible for the toxicity on the cancer cell line.

CONCLUSION

This study concluded that *C.thomsoniae* having anticancer activity and it is more selective to breast cancer cell line. This study also concluded that ethyl acetate is the best solvent to extract phyto constituents from this plant. Further studies required for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity and these works are going on in our laboratory.

List of Abbreviations

MTT: 3- (4 5-Di methyl thiazol-2-yl) -2,5-Diphenyl tetrazolium Bromide, WHO: World Health Organization, AIDS: Acquired Immunodeficiency Syndrome, ANOVA: Analysis of Variance. MEM: Dulbecco's Modified Eagle *Medium*, FBS: Fetal Bovine Serum

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Table 1 Percentage yield and color of various solvent extracts from *Clerodendrum thomsoniae* Balf

S. No	Extracts	Color	Yield % (W/W)
1.	Petroleum ether	Dark Green	5. 44
2.	Chloroform	Brownish Green	3. 67
3.	Ethyl acetate	Light Green	13. 12
4.	Ethanol	Brown	6. 76
5.	Water	Chocolate Brown	2. 78

Table 2 Phytochemical analysis of *Clerodendrum thomsoniae* Balf

S. No.	Phytochemical constituents	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Alkaloids	-	-	+	++	-
2.	Corbohydrates	-	+	-	+	+
3.	Glycosides	-	-	+	+	++
4.	Terpenoids	-	-	++	-	-
5.	Proteins	-	-	-	-	++
6.	Amino acids	-	-	+	+	+
7.	Steroids	-	-	++	+	+
8.	Flavonoids	-	+	++	+	-
9.	Phenols	-	++	+++	-	-
10.	Tannins	+	-	-	+	-
11.	Quinones	-	-	-	-	-
12	Anthraquinones	-	-	-	-	-
13	Saponins	+	-	-	-	++
+++ :highly present, ++:moderately present, +:low, -:absence						

Table 3 IC_{50} of different solvent extracts of *Clerodendrumthomsoniae*Balfon different cell lines

Extracts	IC_{50} value in µg/ml						
	Hep-G2	A549	MCF-7	HT-29	Hela	MOLT– 4	Vero
Petroleum ether	213. 12±2.14	312. 43±1.42	174. 43±3. 45	287. 21±3.56	259. 22±2. 44	317. 11±1 67	522. 45±2. 21
Chloroform	112. 43±2.48	213. 54±2. 34	256. 23±1. 72	157. 44±2.84	312. 32±2. 65	247. 33±2.34	425. 45±2. 14
Ethyl acetate	43. 22±1.02	56. 93±1. 41	29. 43±1. 44	60. 68±1.05	40. 02±1. 14	69. 83±1. 33	367. 5±1. 03
Ethanol	178. 23±1 09	183. 87±2. 45	119. 22±3. 47	213. 11±2.34	229. 12±2. 67	311. 24±3. 47	574. 29±2. 27
Water	389. 22±2.46	299. 11±2. 89	234. 39±2. 94	253. 39±3.56	211. 23±3. 78	159. 34±3. 61	>600
All values are in mean ± SEM (n=3)							

Table 4 IC_{50} of fractions of ethyl acetate extract of *Clerodendrum thomsoniae* Balfon MCF-7

Cell line	MCF-7						
Fractions	F1	F2	F3	F4	F5	F6	F7
IC_{50}	85. 50±0. 33	48. 58±1. 43	44. 87±1. 45	26. 12±1. 43	17. 33±0. 54	19.49±1. 4	26.53±1. 94
All values in mean ± SEM(n=3);F=Fraction							

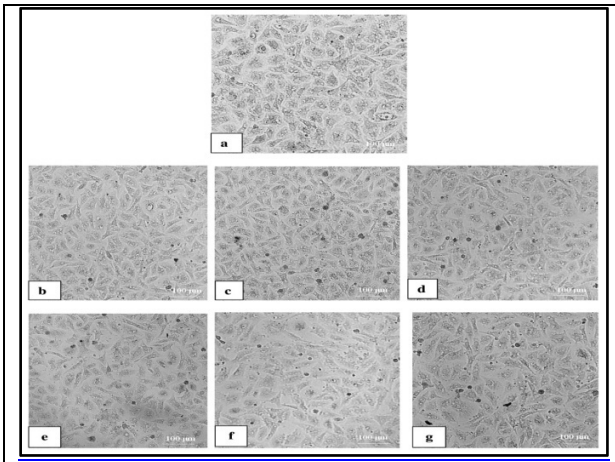

Fig. 1. Morphological profile of the Vero cells after treated with ethyl acetate extract of *Clerodendrum thomsoniae* Balf1. 0 µg/mL (b) , 10 µg/mL (c) 50 µg/mL (d) 100 µg/mL (e) 300 µg/mL (f) and 500 µg/mL **(g) compared to control (a) for 24 h. (100 x enlargement) .**

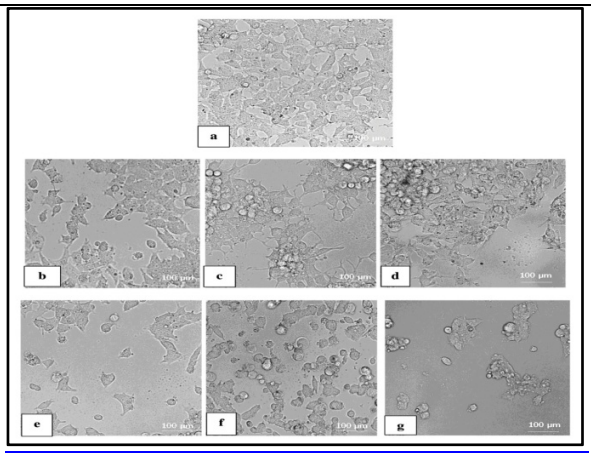

Fig .2. Morphological profile of the MCF-7 cells after treated with ethyl acetate extract of *Clerodendrum thomsoniae* Balf 1.0 µg/mL (b) 10 µg/mL (c) 50 µg/mL (d) 100 µg/mL (e) 300 µg/mL (f) and 500 µg/mL (g) **compared with control (a) for 24 h (100 x enlargement).**

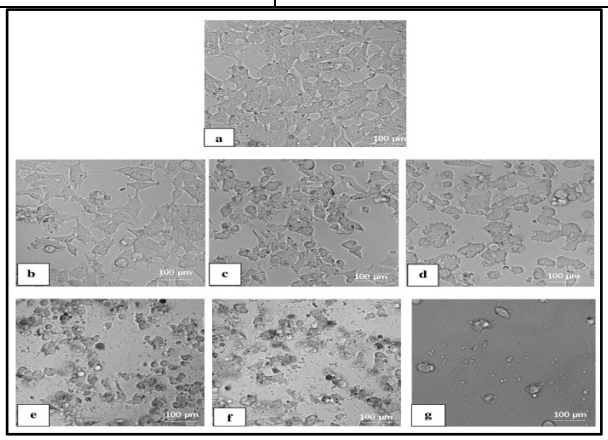

Fig. 2. Morphological profile of the MCF-7 after treated with fraction 5 (F5) of ethyl acetate extract of ClerodendrumthomsoniaeBalf1. 0 µg/mL (b) , 10 µg/mL (c) 50 µg/mL (d) 100 µg/mL (e) 300 µg/mL (f) and 500 µg/mL (g) compared to control (a) for 24 h. **(100 x enlargement)**

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RESEARCH ARTICLE

Phytoplanktonic Composition in Matturu Pond of Shivamogga District, Karnataka

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ABSTRACT

The present study deals with the phytoplankton diversity in Matturu pond of Shivamogga district composed of four major groups viz., Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. Among phytoplankton Bacillariophycee group were dominated followed by Chlorophyceae, Cyanophyceae and Euglenophyceae. A total of 19 species of chlorophyceae, 12 genera and 22 species of Bacillariophyceae , 07 species each of Cyanophyceae and Euglenoids were recorded in Matturu pond. Phytoplankton abundance and physico-chemical characteristics of the Matturu pond indicate that this pond is Oligotrophic in nature.

Key Words: Phytoplankton, Matturu pond, Shivamogga district, Diversity indices.

INTRODUCTION

Small water bodies are found all over the world, although no accurate estimates of their number are available but most likely their number shows in millions. Most small water bodies were built for irrigation, water storage for drinking, livestock and to replenish water tables, decrease the severity of flash-flooding, reduce soil erosion and increase vegetative cover especially trees (Roggeri, 1995). Plankton occur in all natural water as well as in artificial impoundments like ponds, tanks, reservoirs, irrigation channels etc. Phytoplankton being the lowest trophic level in the food chain of fresh water ecosystem and plays key role in fish culture. Water is necessary for all the living beings. It is a valuable resource presented in very partial quantities to man and other living things. Pond is a natural water sources exploited by human being at various time to meet needs, or may have been formed for a different functions (Rajagopal et al., 2010; Rashmi and Somashekar Malammanavar,2013).

Phytoplankton of fresh water bodies have been studied extensively in India. Phytoplankton are play an important role in maintaining equilibrium between living organisms and abiotic factors. The magnitude and dynamics of phytoplankton population have become an essential tool to assess the general health of an aquatic ecosystem. However, they are ecologically significant as they form the basic link in the food chain of all aquatic animals (Misra *et al*., 2001). Phytoplankton assumes a noteworthy biological role and are by and large widely utilized as a marker of water contamination, since they are characteristic occupants of water. Aquatic biological system harbors an assortment of networks, which comprise the qualities and working of the environment, as far as keeping up creation and natural way of life. The utilization of thickness and assorted variety of phytoplankton and their relationship as natural pointers in the appraisal of water quality or trophic status has been made by a few workers (Chaturvedi and Sharma, 1999). A few animal types then again can be hurtful to human and different vertebrates by discharging toxic substances into water. In this way, phytoplankton studies and observing are helpful for control of the physico-compound and natural states of the water in any water system venture. In this way, certain gatherings of phytoplankton, particularly blue green algae can corrupt recreational estimation of surface water, especially thick scum, which decreases the utilization of civilities for physical sports, or huge fixations, which cause deoxygenation of water prompting fish mortality.

MATERIALS AND METHODS

Study Area

Matturu pond is a perennial fresh water body situated about 7.5 km away from Shivamogga town (Figure 1). It lies in between 75° 24'longitude and 13° 48'latitude. The catchment are of this water body is 1.84 sq. km. Tunga channel water is the main source for this water body. The water spread area during the study period was about 75.40 ha. The water is mainly used for agricultural practices. Besides this, water is also used for washing cloths, cattle bathing and other domestic activities. The catchment area of the water body is covered by are can't, paddy and natural vegetation. Government allowed public to conduct fishing activities in this water body. A part of water body has been encroached for agricultural practices.

Preservation and enumeration techniques of plankton

For the qualitative and quantitative analysis of plankton, two liters of composite water samples at the surface level were collected at an interval of 30 days. One liter of each sample was fixed with 20 ml of 1% lugol solution. After sedimentation 100 ml of sample is subjected to centrifugation at 1500 rpm for 20 min and used for further investigation. Microscopic observations were carried out and diagrams were drawn with the help of camera lucida technique under suitable magnification. Enumeration of plankton were made using sedgewick rafter cell and organism expressed as org/L^{-1}. Plankton were identified with the help of standard monographs upto generic and species level (Fritch, 1945; Desikachary, 1959; Hegde and Bharati,1985;Adoni et al.,1985).

Statistical analysis

The data obtained during the study period has been subjected to statistical analysis. Karl Pearson's correlation co-efficient has been calculated to understand the relation between the plankton densities. Diversity indices, such as Shannon-Weiner and evenness were calculated by using following formulae. The diversity indices of Shannon-Wiener H^1 Hurlbert PIE, Margalef D (Washington, 1984) and Simpson diversity a and b were employed to describe the temporal and monthly changes in community structure were used to investigate the relationship between phytoplankton diversity and environmental parameters. Margalef Shannon-Wie indices was employed in this study because it is most widely used index.

Shannon-Wiener

$$H = -\sum_{i=1}^{s} (pi)(\log_2 pi)$$

Where, H = Shannon-Wiener Diversity
SUM represents a capital epsilon
s = number of species
pi = proportion of individuals belonging to the ith species calculated as ni/N for each ith species with ni being the number in species i and N being the total number of individuals in the sample

Evenness

$J = H / H_{max}$

Where, H = Shannon-Weiner Diversity
H_{max} = H maximum, what H would be if all species had the same number of individuals calculated as
$H_{max} = \ln S$
(natural log of the number of species.)

Margalef

Diversity = (S - 1) / ln N

Where, Diversity = Margalef's Diversity
S = Total number of taxa represented in sample
N = Total number of individuals in sample

Neither text I am using as reference gave a base for the log in this formula. I arbitrarily chose to use the natural log because I think it looks better. If you know what it should be let me know.

Simpsons Dominance

$$\text{Dominance} = \sum_{i=1}^{s} (pi)^2$$ (read Sum from i=1 to s of p sub i squared)

Where, Dominance = Simpsons Dominance
s = number of species (or taxa)
pi = proportion of individuals in sample that belong to the ith species calculated as ni / N
Where ni = number individuals in spp i
N = total number of individuals

Simpsons Diversity A

Diversity = 1 / C

Where, Diversity = Simpsons Diversity A
C = Simpson's Dominance

Simpsons Diversity B

Diversity = 1 - C

Where, Diversity = Simpsons Diversity B
C = Simpson's Dominance

RESULTS AND DISCUSSION

Phytoplankton groups in Matturu pond of Shivamogga district comprises four major groups viz., Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. Bacillariophyceae members dominated followed by Chlorophyceae, Cyanophyceae and Euglenophyceae respectively.

Chlorophyceae (Table 1)

The Chlorophyceae occur in fresh cold water where they seem to have their majority evolution. They either form greenish scum on the surface of stagnant or quiet water or grow firmly attached to the submerged rock, pieces of wood and other objects in water. Chakraborty *et al*. (1959) recorded *Pediastrum* sp. in river Yamuna, throughout the year. But more abundant during April and July. Venkateshwarlu (1969) observed that in tropical waterbodies, the green algae are abundant at high temperatures. Agbeti and Smol (1995) have recorded that Chlorophyceae that they have studied and attributed that the temperate waters with low range of temperature do not support the growth of Chlorophyceae. In the present investigation, temperature recorded above 21°C in all the waterbodies. Therefore, maximum temperature favoured the presence of chlorophyceae. Similarly, chlorophycean groups is also recorded more or less similar in Matturu pond. In Matturu pond during the period of study, 19 species of Chlorophyceae constituting 36% of total group with maximum density during April 2006 4666 org/L^{-1} and minimum 2307 org/L^{-1} during December 2005.

Bacillariophyceae

Bacillariophyceae constitute an important part of fresh water, which forms the basic food of the aquatic animals and possesses chlorophyll a and c. The ecology of diatoms has been studied by several workers like Zafar (1967); Ramakrishnaiah & Sarkar (1982) and Venkateshwaralu (1986). pH is one of the factors has definite influence on the diatom population. Patrick (1948), Singh and Swarup (1979) have stated that, alkaline pH favours the abundance of diatoms. In the present study, pH of all waterbodies is slightly acidic to alkaline. A total of 12 genera and 22 species of bacillariophyceae (Table 2) were recorded viz., *Amphora lindhemeri, A. ovalis, Cymbella tumida, C. turgidula, C. lanceolata, Cyclotella kuezingiana, Fragillaria* sp., *Gomphonema gracili, G. intricatum, Gyrosigma accuminatum, Melosira granulata, Navicula radiosa, N. gracili, N. Pupila, Nitzschia acuta, N. obtuse, Pinnullaria major, P. viridis , P. gibba, Synedra ulna, Stauroneis anceps* and *Diatoma* sp. were frequently observed throughout the year. In Matturu pond species diversity showed maximum with 22 species comprising of 42% of the total group of Bacillariophyceae. Despite seasonal variations showed maximum density reached upto 6545 org/L^{-1} in the month of January 2007 and minimum density reached upto 2466 org/L^{-1}. Whereas, seasonal variation is considered, diatoms showed high during post-monsoon than monsoon seasons.

Cyanophyceae

The Cyanophyceae provide good example of the adaptability of life to extremes of environment, high temperature of hot spring and low temperature of polar regions. The fresh water blue greens occur in all clean or polluted water reservoirs, ponds, open tanks and lakes exhibit cyclic growth. Hegde and Bharathi (1985), Unni (1985) and Swarnalatha & Narasingarao (1993) were of the opinion that high temperature favours the luxuriant growth of Cyanophyceae. In the present investigation, Matturu pond showed maximum density during pre-monsoon season. Hence, present study is witnessed the findings of above mentioned authors. Whereas, Matturu pond reported 7 species (Table 3) comprising of 12.05% of total group of Cyanophyceae. Density of Cyanophyceae recorded maximum of 2636 org/L^{-1} in the month of October 2006 and minimum of 666 org/L^{-1} in the month of February 2007, seasonal changes exhibited maximum during pre-monsoon season and minimum during monsoon season.

Euglenophyceae

Many investigators reported that free carbon dioxide, dissolved oxygen and phosphate as the chief factors that regulate the distribution of euglenophyceae members in the fresh waterbodies (Hegde & Bharathi, 1985 and Puttaiah

& Somshekar, 1987). The euglenophyceae occur larger number in organic rich water bodies. Kumar *et al*. (1974) observed the blue green algae and euglenoid flagellates were mostly associated with organically rich effluents with low in dissolved oxygen. Seenaya (1971) and Hegde and Bharathi (1985) are of the opinion that, temperature in between 30-35°C is highly congenital for the luxuriant growth of Euglenoids. In the present investigation, in selected waterbodies exhibited, high temperature ranged between 23-38.5°C. On the other hand, less number of euglenophyceae were recorded. Interestingly, in Matturu pond only 7 species (Table 4) comprising 10% of total group of euglenoids was recorded. While, maximum density of euglenoids showed 3181 org/L^{-1} in the month of January 2007 and very low density 250 org/L^{-1} in the month of May 2005 was encountered. Seasonal abundance occur maximum euglenoids during post-monsoon compared to pre-monsoon season.

Correlation coefficient relationship among phytoplankton

The association among the phytoplankton is studied by calculating the correlation coefficient among different groups. The population of chlorophyceae is positively influenced by cyanophyceae except in Matturu pond (Table 8)..

CONCLUSION

Desmids are the indicator of water quality. Desmids population are also sensitive to physical and chemical characteristics of water.To educate the stake holders about the scientific, aesthetic and recreational importance of water body. Proper awareness must be necessary to prevent excessive use of fertilizers in the catchment area. Other alternative systems to be developed nearby waterbody. Agricultural runoff should be avoided. The government authorities must strictly follow the environmental regulations in relation to management of lake. Season-wise desiltation is necessary, so that water level can be increased to meet the demand of stake holders.

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Table 1: Diversity of Chlorophyceae in Matturu pond during April 2005 to March 2007

Sl. No.	Name of the organisms	Occurrence
1.	*Ankistrodesmus falcatus*	+
2.	*Cosmarium angulatam*	+
3.	*C. spinuliferum*	+
4.	*C. alatum*	+
5.	*C. marginatum*	+
6.	*Coelastrum microporum*	+
7.	*Closterium lunula*	+
8.	*C. acutum*	+
9.	*Desmidum bengalicunm*	+
10.	*Pediastrum simplex*	+
11.	*P. duplex* Var. reticulata.	+
12.	*Spirogyra singularis*	+
13.	*S. subsalsa*	+
14.	*Scenedesmus quadricauda*	+
15.	*S. arcuatus*	+

16.	*S. accuminatus*	+
17.	*Staurastrum sebaldi*	+
18.	*S. wilde*	+
19.	*Tetraedon* sp.	+

Table 2: Diversity of Bacillariophyceae in Matturu pond during April 2005 to March 2007

Sl. No.	Name of the organisms	Occurrence
1	*Amphora lindhemeri*	+
2	*A. ovalis*	+
3	*Cymbella tumida*	+
4	*C. turgidula*	+
5	*C. lanceolata*	+
6	*Cyclotella kuezingiana*	+
7	*Fragillaria* sp.	+
8	*Gomphonema gracili* Ehr.	+
9	*G. intricatum*	+
10	*Gyrosigma accuminatum*	+
11	*Melosira granulata*	+
12	*Navicula radiosa* Kutz.	+
13	*N. gracilis*	+
14	*N. pupila*	+
15	*Nitzschi aacuta*	+
16	*N. obtuse*	+
17	*Pinnularia major*	+
18	*P. viridis* Smith.	+
19	*P. gibba*	+
20	*Synedra ulna* (Nitzsch) Her	+
21	*Stauroneis anceps*	+
22	*Diatoma* sp.	+

Table 3:Diversity of Cyanophyceae in Matturu pond during April 2005 to March 2007

Sl. No.	Name of the organisms	Occurrence
1	*Aphanocapsa biformis*	+
2	*A. naviculoides*	+
3	*A. tennuis*	+
4	*Microcystis aeruginosa*	+
5	*M. incerta*	+
6	*Oscillatoria subbrevis*	+
7	*Spirulina major*	+

Table 4:Diversity of Euglenophyceae in Matturu pond during April 2005 to March 2007

Sl. No.	Name of the organisms	Occurrence
1	*Euglena acus*	+
2	*E. desus*	+
3	*E. proxima*	+

4	*Phacus tortus*	+
5	*P. pleuronectes*	+
6	*P. indicus*	+
7	*P. carvicauda*	+

Table 5: Monthly variations of phytoplankton groups (org/L^{-1}) in Matturu pond

Group	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
2005-06												
Chlorophyceae	2900	2933	3666	4384	4600	3000	2800	2833	3090	2307	2916	3600
Bacillariophyceae	3500	2466	3833	2615	3800	4600	3800	4166	5272	3538	5666	5400
Cyanophyceae	1900	1133	1666	769	800	1500	733	1083	1363	1000	833	1200
Euglenophyceae	300	600	250	692	400	1700	866	416	1272	1153	3083	500
Total	8600	7132	9415	8460	9600	10800	8199	8498	10997	7998	12498	10700
2006-07												
Chlorophyceae	3666	4666	3833	3000	4166	4066	4166	3909	4000	2800	3454	3333
Bacillariophyceae	3933	4133	4166	3000	4250	2733	3583	3909	3000	3466	6545	4833
Cyanophyceae	1200	1466	1250	800	833	1200	916	2636	1250	800	1090	666
Euglenophyceae	800	866	750	466	916	333	1000	1363	1000	733	3181	1416
Total	9599	11131	9999	7266	10165	8332	9665	11817	9250	7799	14270	10248

Table 6. Seasonal variations of phytoplankton diversity in Matturu pond

Groups	2005-06			2006-07			2005-07		
	Monsoon	Pre-monsoon	Post-monsoon	Monsoon	Pre-monsoon	Post-monsoon	Monsoon	Pre-monsoon	Post-monsoon
Chlorophyceae	3696	3274	2786	3849	3874	3540	3772	3574	3163
Bacillariophyceae	3703	3799	4660	3391	4266	4230	3547	4032	4445
Cyanophyceae	950	1474	1069	937	1145	1444	943	1309	1256
Euglenophyceae	914	412	1481	678	958	1569	769	685	1525

Table 7: Diversity indices for phytoplankton in Matturu pond

Sites	Shannon-Wiener	Margalef's	Evenness	Simpson's Dominance	Total Numbers
Mattur	1.18	0.23	0.85	0.35	9933.72

Table 8: Correlation coefficient among phytoplankton of Matturu pond

Phytoplankton group	Chloro-phyceae	Bacillario-phyceae	Cyano-phyceae	Eugleno-phyceae
Chlorophyceae	1.00			
Bacillariophyceae	-0.13	1.00		
Cyanophyceae	0.09	0.01	1.00	
Euglenophyceae	-0.19	0.71	-0.06	1.00

Bold values indicate significant at 0.05

During pre-monsoon season

In post-monsoon season

During monsoon season

Figure 1: Views of Matturu pond during different seasons

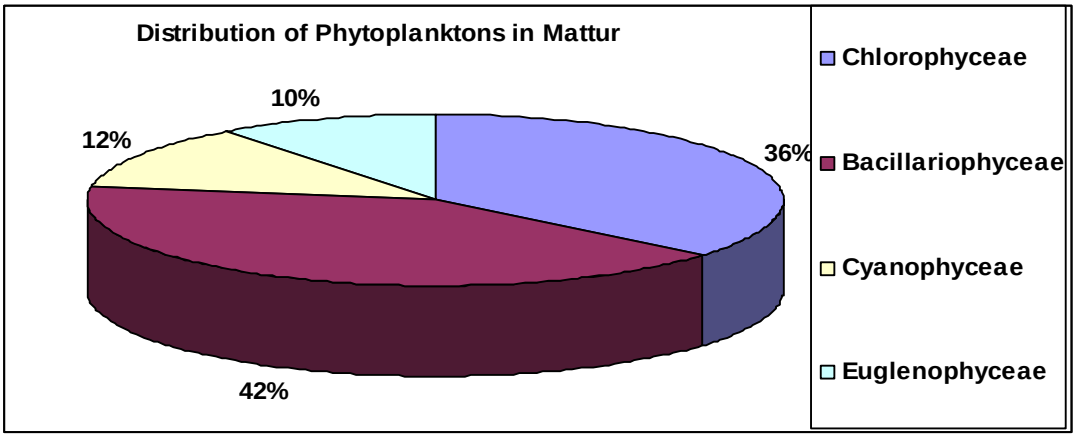

Fig. 2 Distribution of phytoplankton percentage observed in Matturu pond

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RESEARCH ARTICLE

Histopathological Changes Induced by Confidor in the Gill of the Fresh Water Fish, *Cirrhinus mrigala*

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ABSTRACT

The histopathological effects of Confidor on the gill tissue in *Cirrhinus mrigala* were determined. The fishes were treated with the sublethal concentrations (0.505 and 1.01mg/l) of Confidor for 10, 20 and 30 days with parallel untreated control. There were no histopathological effects recorded in control group. Confidor induced marked pathological alterations such as hyperplasia, necrosis, desquamation, aneurysm, blebs, rapture of gill epithelium, vacuolation in gill lamellae and reduced gill rakers were noted in the gill tissue exposed to Confidor.

Key words: Confidor. *Cirrhinus mrigala*. Histopathology.

INTRODUCTION

The most important problems faced by the globe nowadays are Population and pollution. They both are interrelated to each other. As the population goes on increasing year by year, an enormous pressure is put on the land in order to feed this population. Therefore, there is an urge to boost the agricultural productivity. And so, countries are producing and using more pesticides. Over the past forty years, pesticides have become a central and essential part of world agriculture. The pesticides usage is popular for the control of pests; on the other hand, these are causing environmental pollution (Fanta *et al.,* 2003; Dixit, 2005). These pesticides, through surface run off, reach unrestricted areas like ponds and rivers, thus changing the physico-chemical properties of water and consequently affecting aquatic organisms (Kamble and Muley, 2000, Bhachandra *et al.,* 2001; Madhab Prasad *et al.,* 2002 and Sindhe *et al.,* 2007). Fish is highly nutritious, easily digestible and much sought after food, but the nutritional value of fish depends on their biochemical composition, which is affected by the water pollution (Prado *et al*., 2009).

Fishes are susceptible to different concentrations of pesticides and their tissues are prone to pathological effects (Matos,2007). Toxicant weakens the metabolic and physiological activities of the organisms. Such studies alone do

not satisfy the complete understanding of pathological conditions of tissues under toxic stress though. Hence, it is valuable to have an insight into histological analysis. These histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Tilak *et al.,*2005). In the present study, an attempt has been made to know the extent of damage to the general architect of gill of the test fish, *Cirrhinus mrigala* under the exposure to the sub-lethal concentrations of Confidor.

MATERIALS AND METHODS

The candidate species, *Cirrhinus mrigala* (Ham.) were collected from private fish farms. They were then transported to laboratory in polythene bags containing oxygenated water, and special care was taken to reduce hyperactivity and physical injuries to the fish. They were then stocked and maintained in large cement tanks containing chlorine free bore-well water. Before stocking, the tank was washed with 0.1% KMnO4 to free the walls from fungal infections of dermal infection. Well acclimatized *Cirrhinus mrigala* fishes were selected from the stock and individually exposed to different concentrations of Confidor for the bioassay test. The experiments were conducted in 10 litre tanks with 10 fishes each, starved for 24 hrs prior to the experiments for the maintenance of bio assay. The experimental medium was renewed daily till the end of the experiment. The mortality of fishes in different concentration was noted at 12, 24, 48, 72 and 96 hrs and the dead animals were removed immediately. LC_{50} values of Confidor were computed using software by transforming mortalities (percentage values) into probit scale (Finney, 1971). Simultaneously ten fishes were reared in pesticide-free medium and are treated as control.

From the 96 hours LC_{50} values three sub-lethal concentrations, viz 1.01 ($1/4^{th}$) and 0.505 mg/l ($1/8^{th}$) were chosen to expose the fish for histological study. Both the experimental and control fish were sacrificed at the end of 10, 20 and 30 days of experiment. Gill tissue was removed and put in aqueous Bouins fluid. After fixation for 24-30 h, tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections of 4-6 µm were prepared from paraffin blocks by using a rotary microtome. These sections were then stained with Hematoxylin-Eosin. Histopathological lesions were examined and photographed.

RESULT AND DISCUSSION

The computed LC_{50} values for 12, 24, 48, 72 and 96 hours were found to be 7.738, 6.977, 5.254, 4.812 and 4.043 mg/l respectively. Similar results were also reported by Muhammad *et al.*, (2013); Ansari and Ansari (2011); Nannu *et al.*,(2015). The data clearly shows the relationship between the concentration of the pesticide and the percentage mortality. The mortality rate was enhanced with the increase in the concentration of the pesticide at different duration of exposure. Acute toxicity studies are the very first step in determining the water quality requirements of the fish and also reveal the toxicant concentrations that cause the mortality of the fish even at short exposure (pandey *et al*., 2005). It has been indiacted that the toxicity of a pesticide can be modified by various factors including the physico-chemical characteristics of the medium, the biological behaviours and the status of test animal (Holcomb *et.al.,* 1982).

There are five pairs of gill arches in Teleosts. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries separated by pillar cells run parallel along the surface The primary gill lamellae consists of centrally placed rod like supporting axis with blood vessels on either side. The secondary lamellae, also termed as respiratory lamellae, are highly vascularised and covered with a thin layer of epithelial cells, blood vessels are extended into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have a single nucleus which is flattened in appearance. The individual filaments of gills that arise from the rachis of gill have a core of cartilaginous skeleton covered by a connective tissue sheath. Many layers of cells are arranged around the core. These cell layers form the soft parts of the gill. The outermost layer is known as the gill epithelium. There is a branch of an artery and a vein supplying blood to the gill adjacent to the skeleton. The gill filaments through which

the small capillaries run into are also seen arranged diagonally. Connective tissues and epithelial cells in several layers envelop the rachis. The outermost epithelial layer forms the respiratory surface of each finger shaped gill filament. Their epithelial cells are mostly isodiametric and are cubical in certain places and have large nuclei. In the epithelial layer, there are large mucous cells found scattered all over the filament. The blood capillaries associated with the arteriole and venule form the core of the respiratory filament. A prominent structure in any section of the gill is the branchial artery which extends up to the tip of the filament. The mucous cells are goblet shaped and their vacuole- like cavities are filled by mucus (plate.1) .

Confidor has induced marked pathological changes in fish gill. A severe damage was seen in the fish gill exposed to higher concentrations. There were no remarkable changes noted in the gill filaments when the fish exposed up to 10 days at both concentration. After 20 days of exposure to 0.505 and 1.01 mg/l, the fish showed desquamation, aneurysm, hyperplasia, necrosis, blebs, vacuolation in gill lamellae, rapture of gill epithelium, enlarged blood vessel with packed RBC Hypertrophic and enlarged mucocytes. The treated fish showed collapsed secondary lamellae and curling of secondary lamellae, peeling of epithelial lining (Plate.2) corrosion of lateral extensions, damaged gill filaments, extruded RBCs at 0.505 mg/l after 30 days of exposure. In another area, lamellar fusion, a longitudinal section showed hypertrophied and enlarged mucocytes under high magnification. The fishes exposed to 1.01 mg/l for 30 days showed aneurysm in the gill filaments, a portion of a gill rachis facing the pharyngeal region showed reduced gill rakers. Adjacent to this reduced gill rakers, the gill rachis showed disintegration of cartilage (Plate 3).

Histopathological investigations have proven to be a sensitive tool to detect direct effects of chemical compounds within the targeted organs of fish in laboratory experiments. The quantity of pathological intensity is fully dependent on the dose and duration of exposure. In the present study, the *Cirrhinus mrigala* showed an extensive damage to their gill architecture when exposed to toxicant and this is in consent with the earlier observations (Ramesh Kumar *et al.,* 1988; Srivastava and Maurya, 1991; Sujatha, 2006). The gill is the most important site for the entry of toxicant that provokes lesions and gill damage (Bols *et al.,* 2001).A number of pathological changes have been noted in the fishes exposed to different organochlorine, organophosphate and synthetic pyrethroid pesticides (Anitha Sussan,1994; Vijayalaksmi and Tilak,1996 ; Ramanakumari, 1999; Veeraiah, 2001; Tilak *et al.,* 2001a; Tilak *et al.,* 2001) and Tilak and Yacobu, 2002).

Wannee *et al.* (2002) noted the filament cell proliferation, lamellar cell hyperplasia, lamellar fusion and aneurysm in the nile tilapia, *Oreochromis niloticus* under exposure to glyphosate for 96 hr. Mazhar Sultana and Dawood Sharif (2004) recorded that these pathological changes in the gills might have been due to a shift from aerobic to anaerobic pathway in tissues of fish under the toxic exposure. Tilak *et al.,* (2005) also reported the same in fishes exposed to different pesticides. Thus, irreparable architectural changes were caused in the vital organs like gill, making the fish less fit for better survival. when fish is happened to be exposed to pesticides. These histopathological changes can modify various physiological activities of fish such as release of various enzymes and consequently metabolism is affected.

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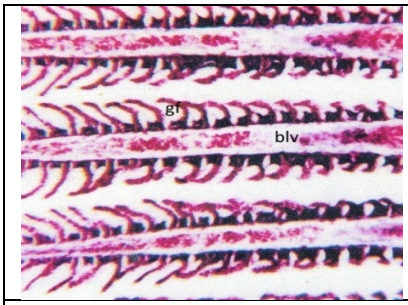	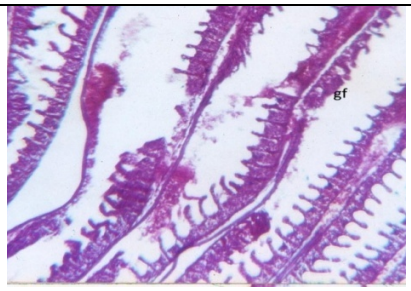	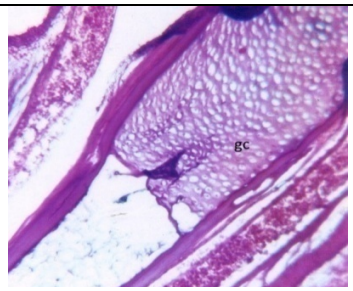
Plate: 1 Gill- Control Fish gf-gill filament blv-blood vessel	**Plate: 2 Gill- Exposed (0.505mg/l; 30 days) Fish gf-gill filament**	**Plate: 3 Gill- Exposed (0.01mg/l; 30 days) Fish gc-gill cartilage**

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RESEARCH ARTICLE

Predicting the Long-Term Survival after Liver Transplantation Using Deep Learning

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ABSTRACT

The main challenge in organ transplantation is the shortage of donated organs. A significant number of organs are being rejected due to suboptimal match between the graft and the patient. The demand for organ transplantation is increasing and the number of donors remains the same, the longer lists of patients waiting for transplantation. In such a setting, outcome prediction is becoming increasingly important in medicine. Prediction of survival is clinically important but a challenging problem. To predict the Long term survival of liver patients after Liver Transplantation by using Deep Learning Method.

Keywords: Deep learning, UCI ML Respository dataset, Data preprocessing, Dimensionality reduction, Classification, CNN.

INTRODUCTION

To designed manually features are often over-specified, incomplete and take a long time to design and validate learned features are easy to adapt and fast to learn Deep learning provides a very flexible ,universal, learnable framework for representing world, visual and linguistic information which can learn both unsupervised and supervised effective end-to-end joint system learning and utilize large amounts of training data. To predict the survival of patient after the Liver Transplantation which depends on the appropriated Donor-Recipient Match[1].

Recipient and donor have set of features and these features matchingare very important.Manual method for getting matching donor receipt feature is acomplicated method.There are three parameters such as Creatinine, InternationalNormalized Ratio (INR) and Bilirubin from MELD score.It may be vary according to body weight of liver recipient.Mostly medical experts depends on the MELD score of survival prediction.The survival rates are occurring due to the inappropriate selection of the parameters[2].The model for End-Stage Liver Disease (MELD)system follows the policy sickest first policy. Mostly they are using a FIFO (First In First Out) policy to allocate the livers without considering the characteristics of the recipient and donor.

Most of the medical experts depend on the MELD score of survival prediction. The survival rates are occurring due to the inappropriate selection of the parameters. The Artificial Neural Networks (ANN) is a computing system which is implementing either the software or hardware. Neuron takes information from the artificial neurons or the sensor it perform operation on data and it pass results on to the other artificial neuron. ANN also operates on real time. Neural networks are also used in much real time application like Sonar Signal Processor, Hand Written Character Recognition, Robotics, Nucleic Acid Sequence Prediction and Image Reconstruction**.** Neural Networks also has impact on the Clinical Medicine.

Deep Learning

The deep learning is used when lots of data (ie10k + examples). The problem is complex. The data is unstructured. Need the absolute best model. Deep Learning is taking off quickly in recent times[3]. Access to large amount of digitized data. Deep learning which is resulted an significant improvements in important applications, such as online advertising, speech recognition and image recognition. Availability of more computational power. Deep means many hidden layers. In deep learning, increasing the training set size and also increasing the size of a neural networks does not hurt any algorithm's performance.

Surgical Risk Challenging Task

The surgical risk depends on the nature of the surgical procedure and presence of co morbid conditions[4]. Once the liver disease is identified in a patient and need of surgery. An assessment of the severity of liver disease must be undertaken. The following are assessing Surgical risk challenging task:

1. Removes potentially toxic byproducts of certain Medications.
2. Prevents shortages of nutrients by storing vitamins, minerals and sugar.
3. Produces most proteins needed by the body.
4. Metabolizes or break down nutrients from food to produce energy when needed .
5. Helps our body fight infection by removing bacteria from the blood Produces most of the substances that regulating blood clotting.

Surgical Risk

Liver transplantation is life-saving for patients with end-stage liver disease (MELD)and there is no established alternative to provide support such as dialysis and artificial ventilation available for kidney and respiratory failure[5].The timing of the transplant is very important, a patient needs to be sick enough to derive benefit from transplantation.

C. Living Donor Liver Transplantation

LDLT which provides life-saving therapy for many patients, otherwise die awaiting a cadaveric organ. In recently, LDLT has been shown to be a clinically safe of addition to deceased donor liver transplantation (DDLT) and has been able to significantly extend the scarce donor pool[10]. The donor shortage continues to increase, LDLT which play an important role of LT. The following are decision-Making Process for LDLT:

1. Donor's Emotional Reaction
2. Available Personal and Social Resources

3. Risk Perception
4. Time Factor
5.Motivation(including donor-recipient relationship)
6. Family Situation/Expectations
7. Medical System
8. Recipient Aspect
9. Moral- Societal Expectations

Related Work

ANN and MELD

Here donor characteristics like height, age donation after the cardiac death and causes of death are considered. ANN techniques which measure the rate of mortality risk of the patient with cirrhosis more accurate than the MELD system[6]. For the prediction of survival with the ANN and MELD system considered the same database. Key aspect of the donor which are impacting graft failure are highlighted. The Model for End Stage Liver Disease is using for the allocation of optical argons and MELD system follows sickest first policy.

Three Classifiers

Orthotropic liver transplantation treatment approach using for begins to end stage liver disease. For the accuracy survival prediction three classifiers are used for liver transplantation patients[7].These Adaptive Resonance Theory (ART) ,Classifiers Multilayer Perception and RBF MAP are used in the survival prediction. For the liver transplantation donor characteristics are important factors.

Operative Risk

The operative risk in patients with liver disease derive from single-center, retrospective cohorts which involve patients with cirrhosis[8].The operative morbidity and mortality increase with increasing the severity of liver disease whether measured by the MELD score. In general, patients with cirrhosis who have normal synthetic function have a low overall risk and the risk increases for patients with decompensate cirrhosis[9].

Acute Hepatitis and Alcoholic Hepatitis

Acute hepatitis which is either self-limited or treatable and elective surgery can be undertaken after the patient improves clinically and biochemically [11]. Alcoholic hepatitis is a contraindication for elective surgery and increases preoperative mortality after urgent or emergent surgery.

Acute Liver Failure

Patients with acute liver failure defined as the development of coagulopathy, jaundice and hepatic encephalopathy in a patient with acute liver injury and without pre-existing liver disease are critically ill[12]. All surgery other than the liver transplantation is contraindicated in patients[13].

Proposed System

In proposes work, deep learning which predict the long term survival after liver transplantation. In this System, we consider UCI ML Respository dataset as medical input data set and data preprocessing with Normalization techniques ,Classification using CNN techniques.

IV UCI ML Respository dataset

The dataset has been used as a Medical, Scientific, and Tex-Exempt and Educational Organization. The dataset consists to the Pre-transplant and Post-Transplant multi organ data. The UCI ML Respository dataset is a multi

organ dataset since 1987 from which extracted the liver patient records. The dataset also consists of male and female liver patient records

Liver Transplant Dataset

The transplant experience in a particular region over a time period in which liver transplant became much more widely recognized as a viable treatment modality. The number of liver transplants rises over the period, the number of subjects added to the liver transplant waiting list grew much faster. The data are the change in waiting time, who waits, and whether there was an consequent increase in deaths while on the list. Blood type is an another important consideration. Donor livers with blood type O can be used by patients with A, B, AB or O blood types, whereas an AB liver can only be used by an AB recipient. (Table 1 Important attributes of Liver patients)it has little relevance to current practice. Liver allocation policies have evolved and now depend directly on each individual patient's risk and need, assessments of which are regularly updated while a patient is on the waiting list. The overall organ shortage remains acute. The transplant data set was a version used early in the analysis, transplant has several additions and corrections and was the final data set and matches the paper. Subjects on a liver transplant waiting list from 1990-1999and their disposition received a transplant, died while waiting, withdrew from the list or censored.

Data Preprocessing

The technique for dealing with very different feature ranges is applying normalization. During normalization, We must treat our feature **X** as a simple mathematical vector. This is not a problem at all, since all values of **X** are numeric and every value in **X** would represent an entry in a vector. Normalization is a rescaling of the data from the original range so that all values are within the range of 0 and 1.Normalization which is able to accurately estimate the minimum and maximum observable values. To estimate these values from the available data and the value is normalized as follows: y = (x - min) / (max - min) Where the minimum and maximum values pertain to the value x being normalized. This means the training data will be used to estimate the minimum and maximum observable values. This is done by calling the fit() function. Apply the scale to training data which means that can use the normalized data to train the model. This is done by calling the transform() function. This means that can prepare new data in the future on which want to make predictions.

Classification

Deep learning architectures have been applied to the prognosis of Acute Hepatitis and Acute failure and monitoring of complex conditions such as death, transplant, withdrawl. For the classification of multiple hepatic structures from biopsy images, based on convolution neural networks (CNNs). Particularly, In medical image analysis, CNN architectures can overcome the problems caused by the hand-crafted features used in traditional techniques due to their fully automated feature extraction. To find the feature mapping among Donor-Recipient matching. Based on the Donor-Recipient matching classify the post-operative survival of the patient.

EXPERIMENTAL RESULTS

We have analysis the data from the dataset of liver patients by pre-processing method and classify the conditions such as death, transplant and withdrawl using CNN architectures in order to predict the long term survival of the patient after Liver Transplantation. We have analysis with important attributes of liver patients such as age, serum bilirubin, serum creatinine, Blood group, MELD Scor, primary diagnosis alcoholic liver disease, cholestatic liver disease, hepatitis B, hepatitis C or other. These two figures shows experimentally that liver transplantation prediction by using blood group and also classify the death rate, transplant rate and withdrawal rate respectively.

CONCLUSION

In this, we are going to proposes the Deep learning techniques which predicts the survival analysis of the patient. And also choosing the appropriate attributes of donor, recipient, and transplantation. Liver Translation is a definitive treatment for the Acute and Chronic end-stage disease of Liver. The collection and sharing of liver organs are the most important aspects of LT. The survival rate of a patient who undergoes in LT treatment had been found out using several machine learning techniques So as to help the doctors to predict the best survival of patients who undergo LT with long term mortality.

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Table 1 Important attributes of Liver patients

Attributes	Description
Age	Age at addition to the waiting list.
Sex	M or F
ABO	Blood type: A, B, AB or O.
Year	Year in which they entered the waiting list.
Futime	Time from entry to final disposition.
Event	Final disposition: censored, death, ltx or withdraw.
Creat	Serum creatinine
Bili	Serum bilirubin

MELD	Calculated MELD score.
INR	International Normalized Ratio, a measure of the blood's clotting ability.
Diag	Primary diagnosis: alcoholic liver disease, cholestatic liver disease, hepatitis B, hepatitis C, or other.

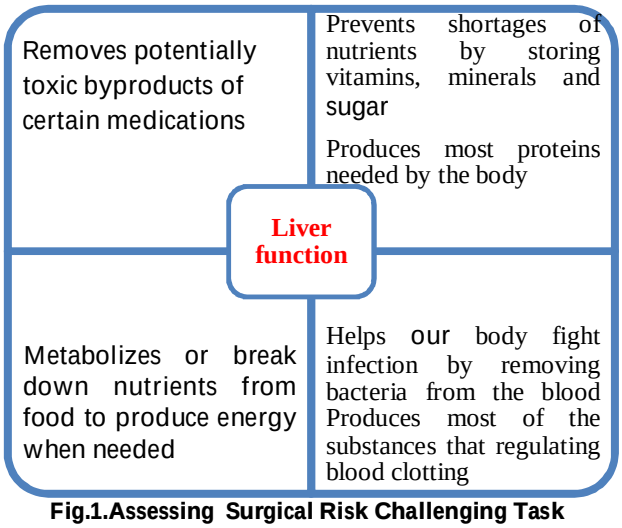

Fig.1.Assessing Surgical Risk Challenging Task

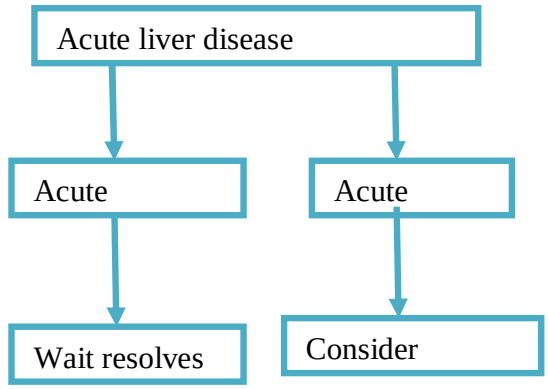

Figure 2. Surgical Risk

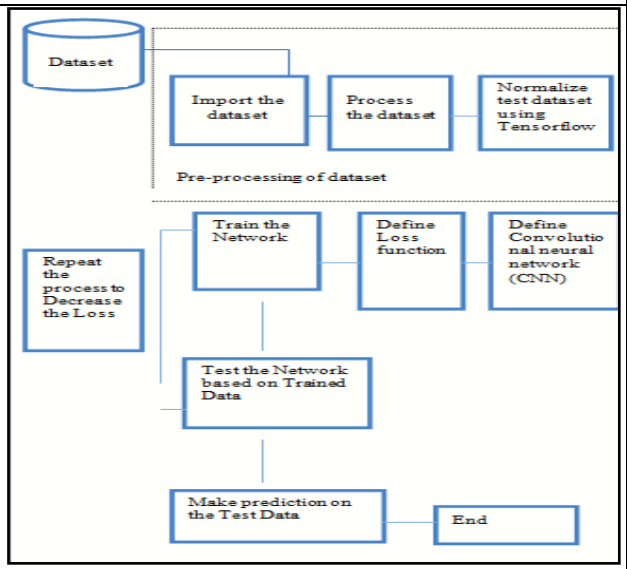

Fig. 3. Architecture of the Model

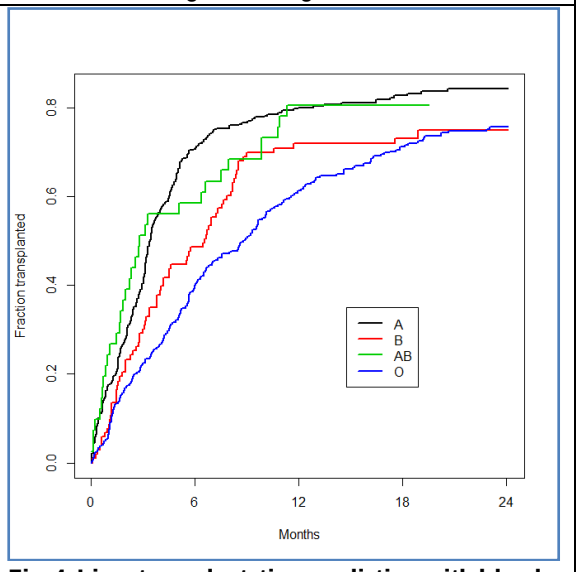

Fig. 4. Liver transplantation prediction with blood group

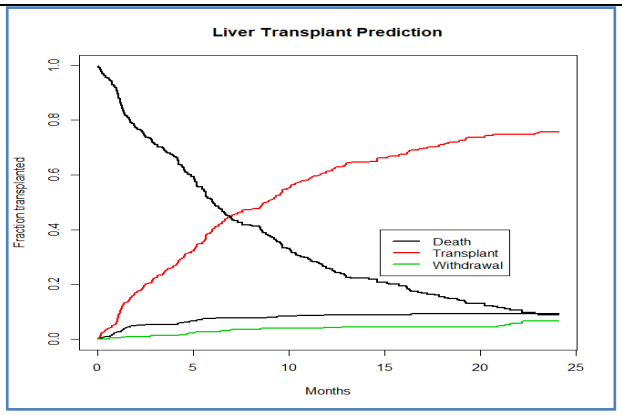

Fig. 5. Classification of death, transplant and withdrawal

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RESEARCH ARTICLE

Pathogenicity of the Extra Cellular Products of *Aeromonas hydrophila* Isolated From Diseased *Cyprinus carpio*

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ABSTRACT

In the present study, two strains of Aeromonas hydrophila were observed for the pathogenic activities. The result showed that the Aeromonas hydrophila strain-2 caused 90% mortality rate and was found to be more virulent.

Keywords: ECP, A. hydrophila, C. carpio.

INTRODUCTION

Aeromonas hydrophila is well-known in nature and is generally found in fresh water, brackish water, moist oil, and non-fecal organic material (Chuang and ko, 1995). Outbursts of motile aeromonad septicemia usually take place whether fish are immune compromised due to unpleasant environment or predisposing factors leading to stresses such as temperature, overcrowding, organic pollution, and hypoxia. In China, with the mortality of 80 % upward, an outbreak of imported koi (*Cyprinus carpio*) was examined and *A. hydrophila* was one of two bacteria that were causative agents (Liu *et al.,* 2002). Potential virulence factors of *A. hydrophila*, which add to their pathogenicity, comprise the production of extra cellular enterotoxins, hemolysin, cytotoxins and protease, the ability to stick on the cells, and the control of certain surface proteins (Howard and Buckley, 1985). Extracellular products (ECPs) include the Siderophore for iron acquirement and the selection of exoenzymes and exotoxins (Santos *et al.,* 1987). These virulent determinants, most of whose mechanisms of action remain to be determined, are implicated sequentially in facilitating the bacteria to colonize, gain entry, establish, replicate and cause damage in host tissues and to escape the host defense system and spread, ultimately killing the host (Yu *et al.,* 2004). Also, in causing infection and pathogenicity to the host, the outer membrane plays an important role (Tsolis, 2002). It is basically composed of

protein, lipid and sugar. Therefore, the host's immunological defense systems could easily recognize them as foreign substances. The major step in the development of the disease is the capacity of a pathogen to locate, to attach, and subsequently contaminate a susceptible host. The important elements of bacterial virulence are the factors produced by motile aeromonads, which can facilitate contagion. The motile aeromonads that were taken from lesions on diseased fish have a greater chemotactic response to skin mucus than the isolates that were acquired as free-living organisms from pond water (Cipriano, 2001). Khalil and Mansour (1997) reported the lethal effects of the extracellular products of A. hydrophila several species of fish. This paper, therefore, presents the alterations induced by A. hydrophila or its extracellular products.

MATERIALS AND METHODS

Sample Collection, Isolation and Identification

The samples of the disease affected *C. carpio* were collected from the infected farm for the isolation of *A. hydrophila*. The gathered shrimp samples were kept in an icebox and transported to the laboratory and stored at -20^{0}C until the further work. The infected samples were washed 3 times with 100ml of sterile sea water on sterile filters. It was homogenized in a sterile glass homogenizer with sterile water and the samples were serially diluted up to 10 fold. One hundred micro liters of these samples were plated on TCBS agar medium. All the plates were incubated between 28 and 30°C. After 2 to 7 days, colonies growth was observed and was selected based on their morphological appearances and further purified by pure culture technique. The selected isolates were identified by morphological, physiological and biochemical confirmations (Farmer and Hickman-Brenner, 1992) as well as based on the characteristics described in Bergey's Manual of Systematic Bacteriology.

Chitinase Activity of *A. hydrophila*.

For measuring the enzyme activity, enzyme mixture was prepared using enzyme sample 100 µl, 500 µl colloidal chitin, 400µl of 125 mM Sodium acetate buffer (pH 5) and it was mixed well followed by shaking incubation at 37ºC with 200 rpm for 1 hour. After incubation, the enzyme mixtures were boiled at boiling water bath for 3 minutes followed by centrifugation at 10,000 rpm for 5minutes. The supernatant of each sample was collected and from each supernatant sample, 250µl was taken and mixed with 250 µl of colour reagent and was boiled at boiling water bath for 10minutes sample with high enzyme activity cleaned the colour reagent, and the absorbance was read as 420 nm in spectrophotometer (Harman *et al*., 1993).

Haemagglutination Assay

This assay was performed in U – shaped micro well plates. Two fold serial dilution of serum samples were made in TBS. An equal volume of 1.5% RBC was added to each dilution of serum sample. The plates were incubated at 25 ^{0}c for 30 min. Haemagglutination titer was recorded as the reciprocal of last dilution, which results in agglutination after 30 minutes incubation. Negative controls comprised mixed equal volume of RBC & TBS.

Preparation of Extracellular Product

Bacteria cultures were incubated at 28°C for 24 hr in TSB with shaking. Twenty-five ml of each culture was centrifuged at 10,000 x g for 30min to remove bacterial cells. Supernatant was passed through 0.45mm syringe filter. The filtered supernatant was taken as sample 1, the syringe was washed reversely with PBS (pH 7) and the soup said to be sample 2 and sample 3. Aliquots (2 ml) of each preparation were determined by the protein.

Survival of *A. hydrophila* Challenged Species

Pathogenic *A. hydrophila* culture was kept in a shaker for 24 h. The culture was centrifuged at 10,000 rpm for 10 minutes. Pellet was collected and mixed with Phosphate Buffered Saline (PBS pH 7.4) challenged with of *C. carpio* at

the rate of more than 10^7 Cfu/ml in the culture tank. The overall survival rate after challenge were assessed every 24 hours interval of the culture period.

RESULTS

Morphological and biochemical confirmation of pathogenic *A. hydrophila*

The pathogenic *A. hydrophila* collected from two localities were isolated from *C. carpio* and were marked as *A. hydrophila* strain A-1 and *A. hydrophila* strain A-2. They were confirmed by morphological and biochemical tests and the colony morphology were given in the Table 1.

Screening for Chitinolytic activity

The isolated bacterial strains were screened for ½ LB medium supplemented with colloidal chitin which forms a zone of enzyme activity. The highest zone activities were A-2 strain and it indicates virulence of the microorganism. The results were recorded in Fig 1.

Hemeagglutination assay

Haemagglutinin assay was performed by human blood groups against *A. hydrophila* pathogen by Microwell titre plate assay technique. The agglutinin takes place in strain A-1 1:1, 1:2, 1:4 and 1:8 dilutions and is completely disappeared in O^{+ve}, whereas in strain – 2, it was there till 1:32(Table.2)..

Extra Cellular Protein Characterization

The details of the Extra Cellular Protein profile were given in the Fig 2. The maximum level of proteins obtained by the isolates of *A. hydrophila* A-1 and A-2 at 100 ml were 95.12 and 99.52 µg/ml respectively(Fig.2).

Cumulative Mortality studies against pathogenic *A. hydrophila* challenge

The virulence of *A. hydrophila* isolates were determined by challenging with *C. carpio* and the mortalities were recorded for 48, 72 and 96 hours. In *A. hydrophila* strain A-1, the mortalities in *C. carpio* within 96 hours of injection was 70%. In *A. hydrophila* strain A-2, the cumulative mortality after 96 hours of injection was 90%. It was said to be due to virulence (Fig 3).

DISCUSSION

In the present study, the extracellular products induced notable morphological and biochemical changes. Similar results were obtained with the extracellular products of V anguillarum in rainbow trout by Lamas *et al.,* (1994). Fouz *et al.,* (1995) observed more severe clinical signs and histological lesions with the extracellular products of *V. damsel*. Gooday (1990) reported that the Chitins can vary by the arrangement of *N*-acetylglucosamine strands, degree of deacetylation, and presence of cross-linked structural components, such as proteins and glucans. The end products of chitin hydrolysis, e.g., *N*-acetylglucosamine, glucosamine and chitobiose are known to induce chitinase synthesis in *A.hydrophila*. Chopra *et al.* (2000) reported that the hemolysins released by Aeromonas sp. were cytotoxic and would cause lysis of erythrocyte and play important roles in pathogenesis. The extracellular products from selected pathogenic *A. hydrophila* strains were lethal for rainbow trout and displayed proteolytic, hemolytic and cytotoxic activities by Santos *et al*., (1968). Multiple virulence-associated biological activities, including enterotoxic, cytotoxic, cytolytic and proteolytic have been detected in culture supernatant fluids of clinical and environmental isolates of *A. hydrophila*. Cicmanec and Holder (1979) stated that the role of protease enzyme is to provide nutrients by breaking down host proteins into small molecules that are capable of entering the bacterial cell. Thus, the present study showed that the virulent activity was found to be stronger in Strain A-2 than in A-1.

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Table 1. Morphological and biochemical confirmation of the pathogenic *A.hydrophila* (A-1, *A.hydrophila* strain 1 and A2, *A.hydrophila* strain 2)

S. No	Biochemical Tests	Isolated strains	
		A-1	A-2
1.	Gram staining	-Ve	-Ve
2.	Motility	motile	motile
3.	Oxidase	+Ve	+Ve
4.	Catalase	+Ve	+Ve
5.	Indole	+Ve	+Ve
6.	Methyl red	+Ve	+Ve
7.	Voges proskauer	+Ve	+Ve
8.	Citrate	+Ve	+Ve
9.	Starch	+Ve	+Ve
10.	Glucose	+Ve	+Ve
11.	Sucrose	+Ve	+Ve
12.	Lactose	-Ve	-Ve
14.	Mannitol	+Ve	+Ve
15.	Maltose	+Ve	+Ve

A-1: *A.hydrophila* strain 1, A-2: *A.hydrophila* strain 2

Table 2. Haemagglutinin assay performed by *A.hydrophila* on human blood (O group).

Dilutions	Agglutination	
	A-1	A-2
- ve control	-	-
+ve control	+	+
1:1	+	+
1:2	+	+
1:4	+	+
1:8	+	+
1:16	-	+
1:32	-	+

- : No agglutination takes place; + : Agglutination takes place

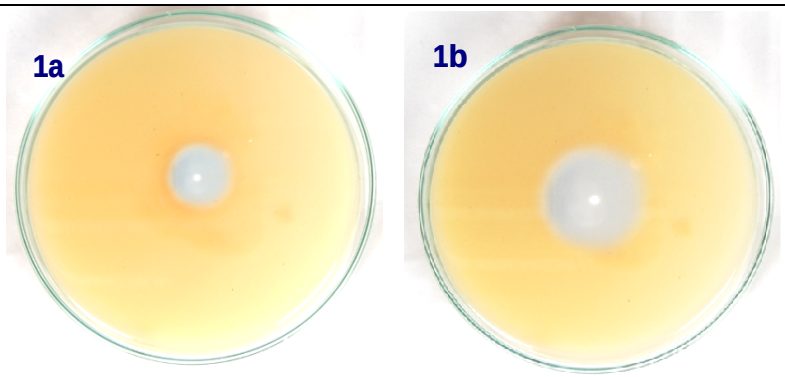

Figure 1. Chitinase activity of pathogenic *A.hydrophila* strain.
(1a - *A.hydrophila* strain 1, and 1b - *A.hydrophila* strain 2)

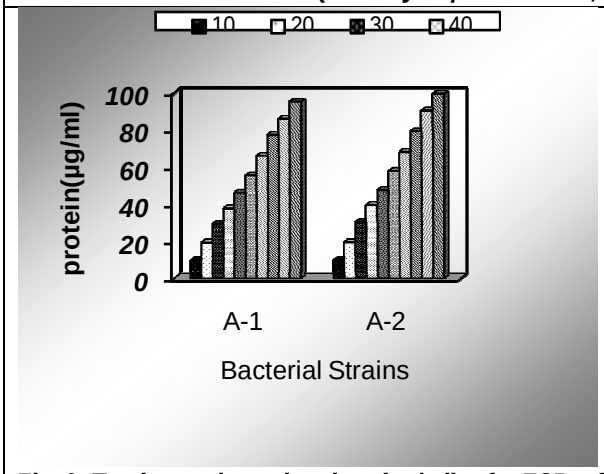

Fig 2 Total protein estimation (µg/ml) of ECP of pathogenic *A.hydrophila* using Bradford's assay.

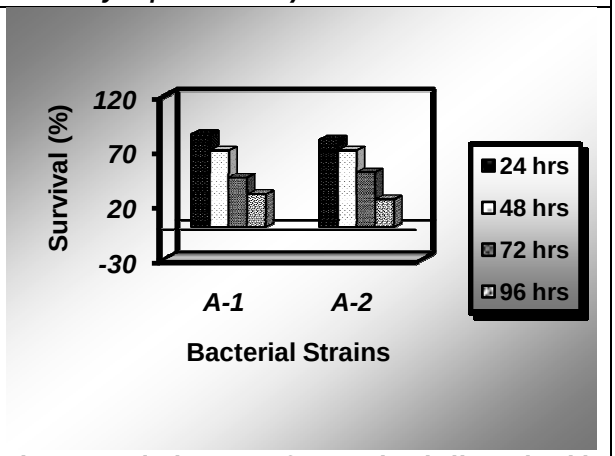

Fig 3. Survival rate of *C.carpio* challenged with pathogenic *A.hydrophila* strain 1(A – 1) and *A.hydrophila* strain 2 (A – 2) at different time intervals

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RESEARCH ARTICLE

Complex Dental Rehabilitation with Vestibuloplasty and Implant Supported Hybrid Denture- A Case Report

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ABSTRACT

One of the greatest challenges when rehabilitating fully edentulous patients, is to manufacture a denture to be placed and mainly it should be retentive enough that too on an alveolar ridge with advanced bone resorption and reduced or no vestibular depth. This requires performing preprosthetic surgery and prepares groundwork on bone and surrounding soft tissues to receive a tissue-borne prosthesis, as well as providing suitable retention and support. Surgical reconstruction needs to include the restoration of masticatory function so that the quality of life after the operation is optimal. The case presented here is that of a 66 year old male, with a history of Squamous Cell Carcinoma of floor of mouth and treated with wide local excision and reconstructed with Radial forearm free flap in 2005. Intra-oral clinical examination revealed edentulous lower alveolar ridge and reduced vestibular depth. Obwegesers modification technique vestibuloplasty was performed to deepen the vestibule. six weeks after

surgery,the tissues were completely healed, the vestibule had recovered 10 mm depth in the labial and lingual region. Rehabilitation was completed after 6 months with hybrid denture. It was thus concluded that vestibuloplasty confers depth to the vestibule.

Keywords: patients, mandibular, mouth, vestibuloplasty, Carcinoma

INTRODUCTION

Neoplasms, which are associated directly or indirectly with the mandible usually require surgical removal of the lesion and extensive resection of the bone and surrounding soft tissues [1]. Smaller lesions, which are removed without discontinuity of the bone are relatively simple to restore with a prosthesis [2]. Larger lesions that extend into the floor of the mouth may be more difficult to restore with prosthesis, even though the continuity of the mandible is maintained [3]. Frequently, the edentulous mandible requires reconstructive plastic surgery to create a buccal or lingual sulcus depth to provide a favorable attached tissue foundation for an acceptable mandibular denture [4]. We present a case of Obwegesers modification technique vestibuloplasty and prosthetic rehabilitation with implant supported hybrid dentures.

Case Report

A 66 year oldmale patient reported to Saveetha dental college dissatisfied with esthetic appearance as well as mastication and speech.She was having a history of SCC of floor of mouth and treated with wide local excision and reconstructed with Radial forearm free flap in 2005. Intraoral examination revealed clinical absence of all teeth in the lower jaw and reduced vestibular depth labially and lingually(figure 1 & 2).

Surgical Technique

After all per operative investigations and anesthetist fitness for surgery under general anesthesia ,patient was taken for surgery.Naso endotracheal intubation done,local anesthesia infiltrated in the lower vestibule.Incision placed over the crest of the alveolar ridge from second premolar on one side to another side.Mucosal flap without disturbing the periosteum reflected.on the lingual side mylohyoid muscle attachment dissected and only superficial fibres of Genial muscles dissected.The reflected buccal and lingual flaps were sutured and as a modification of Obwegeser'stechnique,instead of skin graft,collagen membrane is used to avoid donor site morbidity.the prefabricated stent with collagen sheet placed over the surgical site and fixed with two 2mm X 12 mm screws(figure 3,4).Patient extubated and discharged uneventfully and recalled for review after 2 weeks,4 weeks,6 weeks which revealed complete healing of the surgical site with desirable vestibular depth of 10 mm achieved and the appearance of the mucosa sutured over the edge of the gums was inspected; however, it more resembled the buccal mucosa.and after two weeks Noble bio care implants placed two in the anterior region and two in the premolar regions.Then after 3months, second stage of rehabilitation done with Implant supported Hybrid Denture(figure 5,6,7). After 6 months a prosthesis rehabilitation was done with Implant supported hybrid denture. The patient is comfortable, feeding is satisfactory, improved speech , and he was satisfied with the result both functionally and aesthetically.The patient was highly satisfied with his facial appearance.

DISCUSSION

Vestibuloplasty is a surgical procedure designed to deepen oral vestibule by changing soft tissue attachments [5]. Various surgical modalities have been used for vestibuloplasty including sub mucosal vestibuloplasty, secondary epithelialization vestibuloplasty (Kazanjian technique, Edlan-Mejcharvestibuloplasty, Obwegesers modification) and

soft tissue grafting vestibuloplasty [6]. For vestibuloplastyprocedure , some factors should be evaluated before surgery, especially the height and thickness of the residual ridge, which are essential conditions, and attachment level and integrity of the underlying musculature and quantity of mucus, which should be sufficiently healthy to create a new groove offering greater stability to the prosthesis [7].Malignant tumors of the oral cavity can cause more destruction to innumerable adjacent tissues when compared to any other parts of the body [8]. The surgical management for neoplastic lesions of the oral cavity often requires resection involving several anatomical structures such as mandible, floor of the mouth, tongue and palate etc [9].

In our case of SCC of floor of the mouth, composite resection and reconstruction done with radial forearm free flap was done in 2005 which leads to decreased vestibular depth in lower alveolar region both labially and lingually. Patients may have aesthetic deformities, functional compromise and psychological sequel difficulty in speech. The patient may be given a conventional or implant-borne dental prosthesis, but in most cases this requires a vestibuloplasty procedure to deepen the buccal and lingual sulci and to enable the fabrication of an adequately functional prosthesis [10]. Vestibuloplasty is a surgical modification of the gingival-mucous membrane relationships including deepening of the vestibular trough, altering the position of the frenulum and muscle attachments thereby widening of the zone of attached gingiva [11]. Prior evaluation of the maxillofacial patient reveals that this procedure is required in most of the cases for optimal preparation of the patient and planning of the treatment. This surgical procedure to restore alveolar ridge height by lowering muscles attaching to the buccal, labial and lingual aspects of the jaws can greatly enhance the patients' post-surgical adjustment to the prosthesis [12]. Prior to the fabrication of a dental prosthesis, many patients need pre-prosthetic surgical procedures including vestibuloplasty. The goal of vestibuloplasty is to increase the vestibular depth of the reduced region. This increased depth aids in denture retention by limiting traction produced by muscular and fibrous attachments. A variety of surgical techniques have been used for vestibuloplasty including submucosal vestibuloplasty, secondary epithelization vestibuloplasty, and soft-tissue grafting vestibuloplasty [13]. Several types of grafts such as split-thickness skin grafts, buccal mucosal grafts and palatal grafts can be used for these procedures [14].

CONCLUSION

The results of this study suggest that in the case described in the present paper special attention is paid to the preserving of the vestibule depth, which renders the method successful for the full recovery of the masticatory apparatus. Rehabilitation done with Implant supported Hybrid denture. Patients' follow-up review checks reveal good clinical results for those undergoing vestibuloplasty with early prosthetic loading. So it is observed from this study that even though PreProshetic surgical techniques seems to be very old fashioned outdated,till we have to consider its role in certain conditions like the one reported in this study.So it's proven that Vetibuloplasty is not vanishing procedure but it still has a role in complicated dental rehabilitation.

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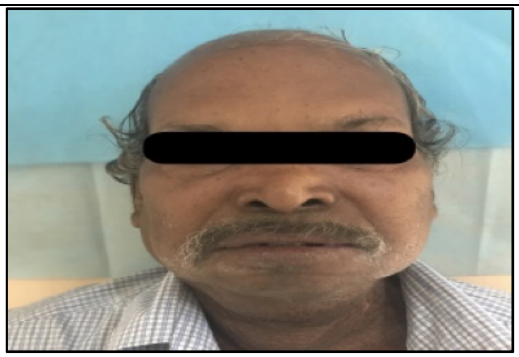

Figure 1- Profile pic

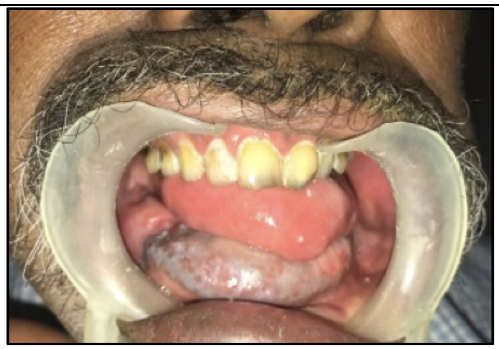

Figure 2- Intraoral pic

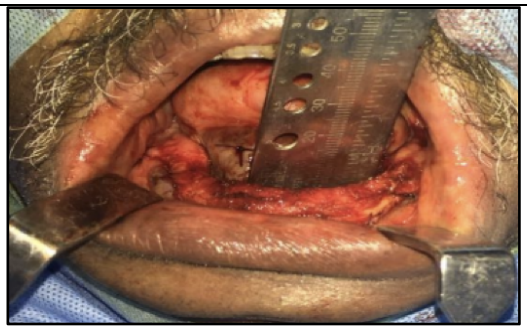

Figure 3- 6 mm labial & lingual depth achieved.

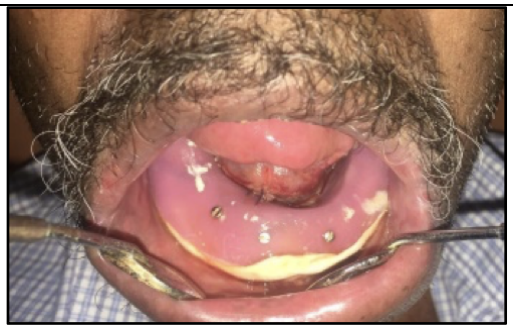

Figure 4- Stent placed with screws

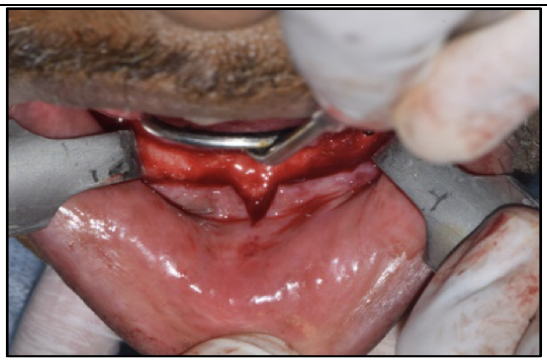

Figure 5- Crestal Incision placed for implantplacement

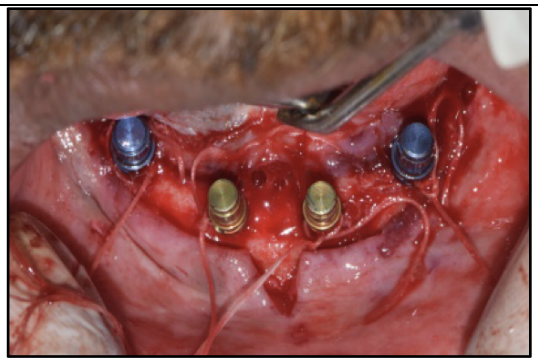

Figure 6- 4 Implant placement the mandible

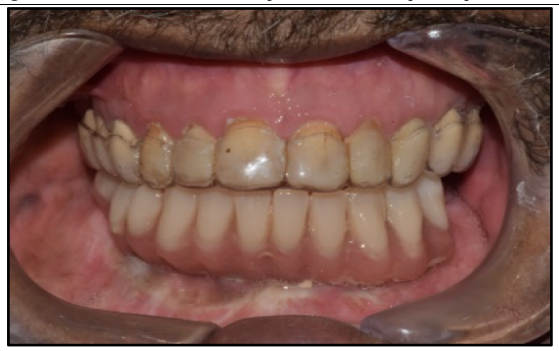

Figure 7- Implant supported Hybrid denture placed in mandible

Figure 8- Post op profile pic.

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RESEARCH ARTICLE

A Comparative Analysis of Text Categorization Using Deep Learning Techniques

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ABSTRACT

Text categorization may be a fundamental and traditional task in Natural Language Processing (NLP), which may be applied in various applications like sentiment analysis, question classification and topic classification. Nowadays, one among the foremost commonly used methods to handle the task is to represent a text with a low dimensional vector, then feed the vector into a softmax function to calculate the probability of every category. Recurrent Neural Network (RNN) has achieved remarkable performance in text categorization. RNN can model the entire sequence of process and capture long-term dependencies, but it doesn't have best in extracting key patterns. In contrast, Convolutional Neural Network (CNN) is sweet at extracting local and position-invariant features. In this paper, we present a comparative study on Recurrent Neural Network and Convolutional Neural Network. In that, CNN achieves good performance on the benchmark datasets for text categorization.

Keywords: Deep Learning, Convolution Neural Network, Recurrent Neural Network

INTRODUCTION

Recurrent Neural Network (RNN) and Convolution Neural Network (CNN) are two sorts of Deep Neural Networks frequently approached to represent the text. RNN can model the entire sequence and capture long-term dependencies [1]. However, modeling the whole sequence sometimes can be a burden, and it's going to neglect key parts for text categorization. In contrast, CNN is in a position to extract local and position-invariant features well. Figure 1 is an example of Text Categorization, where based on the sentences that present in the documents, it may be classified as Food, Sports and Politics. The key phrase that determines the category is unsolved mysteries of mathematics, which may be extracted by CNN to position-invariance. RNN, however, doesn't address such issues well because the representation of the key phrase relies on all the previous terms and therefore the representation

changes because, the key phrase moves. In this paper, we incorporate position invariance into RNN and we disconnect the information transmission of RNN and limit the maximal transmission step length as a hard and fast value k, so that the previous k-1 words and the current word. To take care of the position-invariance, we utilize max pooling to extract the important information, which has been suggested in [2]. Therefore, the maximal transmission step length also can be considered because the window size in CNN is high. Another difference to RNN is that CNN can increase the window size k arbitrarily without increasing the number of parameters. We also find that there's a trade-off between position-invariance and long-term dependencies within RNN and CNN. When the window size is just too large, the position-invariance will disappear like RNN. Against this, when the window size is just too small, we can lose the power to model long-term dependencies a bit like CNN. This paper discovers that the optimal window size is said to the sort of task, but affected little by training dataset sizes. Thus, we will search the optimal window size by training on a little dataset. We conduct experiments on seven large-scale text classification datasets also.

Related Works

Deep neural networks have shown great success in many NLP tasks like Machine Translation [5], reading comprehension [7], sentiment classification [4], etc. Nowadays, nearly most of deep neural networks models are supported by CNN or RNN. Below, we can introduce some important works about text classification supported them. Convolutional Neural Networks CNN has been utilized in tongue processing due to the local correlation and position-invariance.[4] first utilize 1D CNN Parts Of Speech (POS), Named Entity Recognition (NER) and participant role labeling (SRL). [6] proposes to classify sentence by encoding a sentence with multiple sorts of convolutional filters. To capture the relation between words, [11] propose a completely unique CNN model with a dynamic k-max pooling. [9] introduce an empirical exploration on the utilization of character-level CNN for text classification. Shallow CNN cannot encode long-term information well. Therefore, [3] propose to use very deep CNN in text classification and achieve good performance.

Similarly, [10] propose a deep pyramid CNN which both achieves good performance and reduces training time. Recurrent Neural Networks RNN is suitable for handling sequence input like natural language. Thus, many RNN variants are utilized in text classification. [12] utilize LSTM to model the relation of sentences. Similarly, [8] propose hierarchical attention model which includes attention mechanism into hierarchical GRU model in order that the model can better capture the important information of a document. [13] incorporate the residual networks into RNN, which makes the model handle longer sequence. [12] propose a completely unique LSTM with a cache mechanism to capture long-range sentiment information. Hybrid model some researchers plan to combine the benefits of CNN and RNN. [14] extract local and global features by CNN and RNN separately. [9] firstly model sentences by RNN, and then use CNN to urge the ultimate representation. The most differences are as follows. Firstly, they regard their models as CNN and set a little window size of three, while we use an outsized window size. We discussed that tiny window size makes the model lose the power to capture long-term dependencies. Secondly, we utilize max pooling but not mean pooling, because max pooling can maintain position-invariance better.

METHODOLOGY

Recurrent Neural Network (RNN)

RNN may be a class of neural network which models a sequence by incorporating the notion of your time step [10]. Figure 2 shows the structure of RNN. Hidden states at each step depend upon all the previous inputs, which sometimes are often a burden and neglect the key information. A variant of RNN has been introduced by [13] with the name of gated recurrent unit (GRU). GRU may be a special sort of RNN, capable of learning potential long-term dependencies by using gates. The gating units can control the flow of data and mitigate the vanishing gradients problem. GRU has two sorts of gates: reset gate r_t and update gate z_t. The hidden state h_t of GRU is computed as

$h_t = (1 - z_t) \square h_{t-1} + z_t \square \hat{h}_t$ .....Equ(1)

where h_{t-1} is that the previous state, $\hat{h}_t$ is the candidate state computed with new input information and □ is that the element wise multiplication. The update gate z_t decides in what proportion did new information is updated. z_t is computed as in equation 2.

$z_t = \sigma(W_z x_t + U_z h_{t-1})$ .....Equ(2)

here x_t is that the input vector at step t. The candidate state $\hat{h}_t$ is computed by the equation 3.

$\hat{h}_t = \tanh(Wx_t + U(r_z \square h_{t-1}))$ .....Equ(3)

Where r_t is the rest gate which controls the flow of previous information. Similarly to the update gate, the rest gate r_t is computed as in equation 4.

$r_t = \sigma(W_r x_t + U r\, h_{t-1})$ .....Equ(4)

Let see the representation of step t depends upon all the previous input vectors. Thus, we will also express the t^{th} step state shown in Equation 5.

$h_t = GRU(x_t, x_{t-1}, x_{t-2}, ..., x_1)$ .....Equ(5)

Convolutional Neural Network (CNN)

CNN are often considered as a special 1D CNN which replace the convolution filters with recurrent units. Let x_t denote the t^{th} input word vector. Then for every position t we will get a window vector c_t from equation 6.

$c_t = [x_t, x_{t-1}, x_{t-2}, ..., x_{t-k+1}]$ .....Equ(6)

here, we concatenate k word vectors and generate vector ct. Then we will get the output of convolution as in equation 7.

$h_t = Wc_t + b$ .....Equ(7)

where W may be a set of convolution filters and b may be a bias vector. Then a pooling operation is often applied after the convolutional layer and generates a hard and fast size vector. Similarly to RNN, CNN also represent the context vector using equation 8.

$h_t = Conv(x_t, x_{t-1}, x_{t-2}, ..., x_{t-k+1})$ .....Equ(8)

The left model only applies dropout in input and output layers, but the proper model applies dropout in hidden states. Context vector are often considered as a representation of a text fragment. Then we feed the context vectors into a Multi Layer Perceptron (MLP) to extract high-level features as illustrated in Figure 3. Before feeding the vectors into MLP, we utilize Batch Normalization [3] after CNN, in order that the model can alleviate the interior covariate shift problem. To urge the text representation vector, we apply max pooing after MLP layer to extract the foremost important information and position-invariant features [14]. Finally, We feed the text representation vector into an MLP with Rectified Linear Unit (ReLU) activation and send the output of MLP to a softmax function to predict the probability of every category. We use cross entropy loss function as follows:

$H(y, \tilde{y}) = \sum_i y_i \log \tilde{y}_i$ .....Equ(9)

By contrast, for CNN the parameters don't increase with the rise of window size. Hence, CNN can mitigate over fitting problem caused by the increase of parameters.

EXPERIMENTS AND RESULTS

Datasets Used

We use 5 large-scale text classification datasets which are proposed by [15]. AG corpus is news and DBPedia is an ontology which comes from the Wikipedia. Yelp and Amazon corpus are reviews that we always used to predict the sentiment. Yahoo! Answers may be a question answering dataset. We have taken datasets contain various domains and sizes, which might be credible to validate our models

EXPERIMENTAL RESULTS

Experimental results show that the Deep learning techniques RNN and CNN significantly outperforms for all 5 datasets. RNN doesn't have too many hyper parameters. The most hyper parameter is that the window size which may be determined by an empirical method. In this section, we mainly study what factors affect the optimal window size. Additionally to the recurrent units and pooling methods discussed above, we believe the optimal

window size could also be also associated with the quantity of coaching data and therefore the sort of task. In order to review the factors that affect the optimal window size, we conduct experiments on seven datasets. To eliminate the influence of different training data sizes, we conduct experiments with an equivalent training data size. This shows that the amount of training data has little effect on the selection of the optimal window size. It also provides an honest empirical way for us to settle on the optimal window size. That is, conducting experiments on a little dataset first to pick the optimal window size. Here, modeling the entire sequence with RNN results in a loss of position-invariance. Compared with RNN, CNN can better maintain the position-invariance by max pooling. Table 1 show that CNN model achieves 10-50% relative error reduction compared with RNN in these datasets. From Figure 4 we can see that the sort of task features a great impact on the optimal window size.

CONCLUSION

In this paper, we incorporate position-invariance into RNN, in order that CNN can both capture key phrases and long term dependencies. We conduct experiments to match the effects of various recurrent units and pooling operations. Additionally, we also analyze what factors affect the optimal window size of RNN and present an empirical method to look it. The experimental results show that CNN model outperforms the RNN models on all of those datasets and achieve the simplest performance in seven large-scale text categorization.

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Table 1: Comparison of RNN and CNN using Error Rate

Datasets	Error Rate %	
	RNN	CNN
AG Corpus	9.51	8
DBPedia	10.55	9.12
Yelp	23.9	19.4
Amazon	28.8	21.6
Yahoo	19.56	13.8

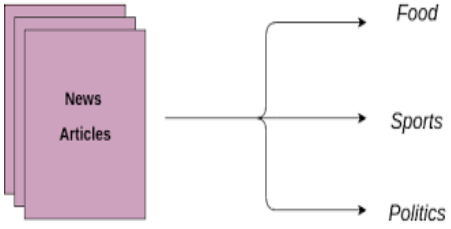

Figure 1: Text Categorization

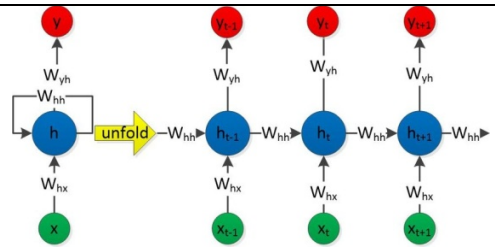

Figure 2: Functional Architecture of Recurrent Neural Network

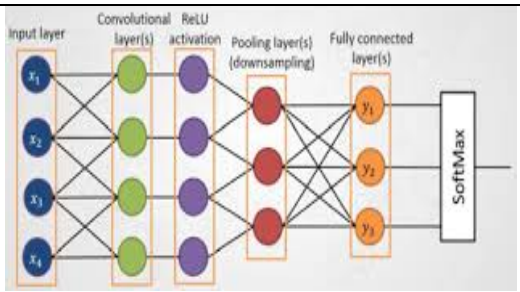

Figure 3: Basic Architecture of Convolutional Neural Network.

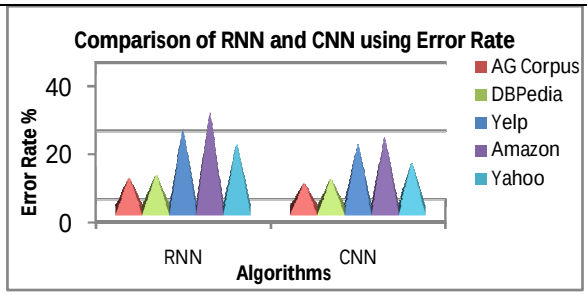

Figure 4 : Comparison of RNN and CNN using Error Rate

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RESEARCH ARTICLE

Knowledge, Awareness of Comprehensive Cleft Care Management among Dental Interns in Chennai

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ABSTRACT

The purpose of the study was to assess the knowledge, awareness, and attitude on comprehensive cleft lip and palate care management among interns in dental college of Chennai. A questionnaire with 16 questions were asked in an online survey and distributed among dental Intern students and knowledge, awareness, and attitude on comprehensive cleft lip and palate management was assessed. The results were interpreted in pie chart, and the Dental Intern students were aware of the condition but were not completely aware of the classification, treatment procedure, treatment timing, the sequential timeline followed for each procedure and aware of feeding & position of feeding in cleft patients. The knowledge, awareness, and attitude on comprehensive cleft lip and palate management among dental students is necessary for proper timely care and efficient treatment.

Keywords: procedure, management, dental, cleft

INTRODUCTION

Clefts of the upper lip and palate are the most common major congenital craniofacial abnormality and are present in approximately 1 in 700 live births [1]. At approximately 6 weeks of human embryological development, the median nasal prominence fuses with the lateral nasal prominences and maxillary prominences to form the base of the nose, nostrils, and upper lip [2]. The adhesion of these anterior components becomes the primary palate. When this mechanism fails, clefts of the lips and/or maxilla occur [3]. At approximately 8 weeks the palatal shelves elevate and fuse with the septum to form the intact secondary palate. When one palatal shelf fails to fuse with the other components, then a unilateral cleft of the secondary palate occurs [4]. If both of the palatal shelves fail to fuse with each other and the midline septum, then a bilateral cleft of the palate occurs [5]. Clefts of the lip occur more commonly in males than in females [6]. In addition left-sided cleft lips are more common than right-sided cleft lips, and unilateral cleft lips are more common than the bilateral cleft of the lip [7].

Clefting of both the primary and secondary palates are often associated with bilateral clefts of the lip. Cleft palate alone is seen in approximately 1 in 2,000 live births and this incidence is similar in all racial groups [8]. Comprehensive and coordinated care from infancy through adolescence is essential in order to achieve an ideal outcome, and surgeons with formal training and experience in all of the phases of care must be actively involved in the planning and treatment. Successful management of the child born with a cleft lip and palate requires coordinated care provided by a number of different specialties including oral/maxillofacial surgery, otolaryngology, genetics/dysmorphology, speech/language pathology, periodontist, orthodontics, prosthodontics, and others [9]. The typical classification system used clinically to describe standard clefts of the lip and palate is based on careful anatomic description. Clefts can be unilateral or bilateral; microform, incomplete, or complete; and may involve the lip, nose, primary palate, and/or secondary palates [10]. Knowledge about the various treatment procedures involved and the timing of the procedures among the dental students is vital for them to refer the patient at the correct stage to the appropriate specialist, who will provide the best of patient.

MATERIALS AND METHOD

Questionnaire

A questionnaire consisting of 16 questions was asked in an online survey and distributed among dental students and knowledge, awareness, and attitude on Cleft lip and palate were assessed.

1. Does Consanguinity marriage can cause Cleft Lip and Palate?
2. Do you think Folic Acid have role in Cleft Lip and Palate
3. Are you aware of classification of Cleft Lip and Palate?
4. Are you aware of NAM APPLIANCE?
5. Are you aware of feeding options for new born Cleft Lip and Palate Patient?
6. Are you aware of position of feeding in cleft patient?
7. Does Pediatrician have role in Cleft care management?
8. Does Orthodontist have role in Cleft care management?
9. Does ENT surgeon have role in Cleft care management?
10. Ideal age for Cleft Lip treatment
11. Ideal age for Cleft Palate treatment
12. Ideal age for Secondary Alveolar Bone Grafting
13. Are you aware of Rule of 10?
14. Are you aware of any surgical procedure for Cleft Lip Treatment?
15. Are you aware of any surgical procedure for Cleft Palate Treatment?
16. Ideal bone graft for Cleft Alveolus management

RESULTS

A questionnaire consisting of 16 questions was distributed among dental interns and knowledge, awareness, and attitude on CL/P management were assessed and total 194 responses were received (Figure 1). The responses deduced were, a question on Does Consanguinity marriage can cause Cleft Lip and Palate? 93.8% were aware of it. (Figure 2) Do you think Folic Acid has a role in Cleft Lip and Palate, 51% were unaware of it. (Figure 3) On awareness of classification of Cleft Lip and Palate, 67.5% were aware of it. (Figure 4) On awareness of NAM appliances, 64.9% were unaware of it. (Figure 5) On awareness of feeding options for new born Cleft Lip and Palate patients, 58.2% were unaware of it. (Figure 6) On awareness of the position of feeding in cleft patients, 63.8% were unaware of it. (Figure 7) Question on does Pediatrician have a role in Cleft care management 97.9% said yes. (Figure 8) Question on does Orthodontist have a role in Cleft care management 96.9% said yes. (Figure 9) Question on does ENT surgeons have a role in Cleft care management 49% said no. (Figure 10) On asking for an Ideal age for Cleft Lip treatment. 52.1% said after 6 months, 37.15% said 3-6 months. (Figure 11) On asking for Ideal age for Cleft Palate treatment. 49% said 10-18 months, 37.6% said after 2 years, 13.4% 5-9 months. (Figure 12) On asking for Ideal age for Secondary Alveolar Bone Grafting.55.7% told 6-7 years, 32% 8-11 years, 12.4% 2-5 years. (Figure 13) On awareness of Rule of 10. 77.3% responded yes (Figure 14) onawareness of any surgical procedure for Cleft Lip treatment. 51.5% were unaware of it. (Figure 15) On awareness of any surgical procedure for Cleft Palate treatment. 51% were unaware of it. (Figure 16) On asking for Ideal bone graft for Cleft Alveolus management. 89.7% responded for auto graft. (Figure 17)

DISCUSSION

The Cleft lip and palate is a congenital defect which can be treated and requires multidisciplinary team approach and good patient and doctor rapport and parental cooperation. The cleft and craniofacial team involves nurses, general dentists, orthodontists, oral surgeons, otolaryngologists, geneticists, prosthodontists, orthodontists, speech therapists, radiologists, psychologists, feeding specialists, and plastic surgeons. The children's affected with clefts needs are multifactorial, it is often simple to appreciate when one starts to list out the functional and anatomic areas affected by the dentofacial deformity. The craniofacial team is composed of nursing and physician specialists with specific interest and special training in the care of children with cleft and craniofacial deformities [11]. A study suggests that the prepubertal midface growth in sagittal vertical and transverse planes (9–13 years) remained unaffected by presurgical NAM and Gingivoperiosteoplasty, [12] the need for early nose and lip correction is emphasized for better molding of soft tissues and increase self- esteem of the patient, adhesion approach achieves the main goal of moving the palate into a preferred position and stabilizing the arch with an osseous bridge that attracts teeth. It prevents the emergence of anterior fistulae and presents a symmetrical platform on which the lip and nose correction can be brought out [13]. The use of NAM and gingivoperiosteoplasty is more preferred than SABG since it is cost-effective in the management of unilateral cleft management [14].

Presurgical infant orthopedic treatment is indicated in patients with a wide unilateral CL/P and bilateral CL/P with an anteriorly and superiorly displaced premaxilla [15]. Early management is crucial and should begin preferably within the first two weeks of life. Primary consultations starts with the feeding specialist, who assists families with managing the special feeding needs of cleft newborns. The geneticist plays a role in diagnosing associated syndromes and counsels parents regarding genetic risks and future possibilities of inheriting it. A specialty nurse coordinator acts as a communicator between the patient and family and the craniofacial team. The timing for completing the SABG surgery is critical. This surgical procedure is commonly performed approximately at 8–10 years of age, when the permanent canine in the cleft area presents half to two thirds of root formation and before it erupts into the cleft defect [16][17]. Parents of newborns with cleft lip and palate should be informed about basic information in the immediate newborn period, especially feeding instructions and identifying illness [18]. Grayson and Maull reveals the primary objective of presurgical nasoalveolar molding (NAM) is to decrease the severity of the

initial cleft deformity [19]. The role of an orthodontist in cleft lip and palate management is emphasized in this article along with the critical decision-making and sequelae of treatment performed by the orthodontist during the adult dentition stage [20]. The most frequently used surgical cleft repair techniques are the Furlow Palatoplasty and the Bardach style with intravelar veloplasty [21]. If possible, a dedicated clinic space with examination rooms, dental examination and treatment areas, dental laboratory, radiology facilities, photographic room, and adequate waiting area for children, staff offices, and electronic record maintenance unit are preferred. Although not a significant part of the craniofacial team, the patient's local pediatrician and community dentist also play a critical role in coordination and dispersal of primary care needs. Orthodontic treatment is essential for all Cleft lip and palate cases, and the decision to proceed in a surgical or nonsurgical manner is critical to the overall successful outcome for the patient. Since the main objective of pre-surgical orthodontics is to decompensate the existing malocclusion, therefore a detailed and timed sequelae of treatment plan should be developed before execution of actual treatment. To be developed before any actual treatment is provided.

Orthognathic surgery that involves maxillary advancement, mandibular setback, maxillary distraction osteogenesis, a combination of both mandibular setback, maxillary advancement and, occasionally, an isolated mandibular setback [22]. The selection of the optimal treatment protocol for a specific patient depends on physiological and functional parameters including the rate of advancement needed, amount of the maxillomandibular discrepancy, velopharyngeal insufficiency, retention/relapse/stability relationships, esthetic outcome, and the consideration of the possible complications. Children born with an isolated cleft lip can feed quite well and even have the opportunity to breastfeed in most instances. However, infants with cleft palate can have difficulty feeding due to the inability to form an adequate seal between the tongue and palate for creation of sufficient negative pressure to suck fluid from a bottle [23]. Nasal regurgitation and inefficient handling of secretions and foodstuffs may also be observed during early development. Immediately after birth specialized nipples and bottles are necessary to improve feeding. The most useful devices combine oversized nipples with reservoir spaces and large openings, a squeezable bottle to push fluid into the nipple assembly, and a one-way valve that allows the bolus of fluid to pass from the bottle to the nipple only in order to minimize the amount of work the child must perform to feed. These include a variety of nipples with reservoirs that collect a variable volume of liquid that can be expressed more easily when sucking is inefficient or not possible. Bottles that can be squeezed to allow for manual flow of liquid to the infant are helpful for improving feeding. No single bottle and nipple combination tends to work better than another, but trials with a variety of types using different techniques are helpful in optimizing feeding early in life.

Close attention to weight gain is necessary for these children. Generally, in 24 hours each infant should have approximately 2 to 3 ounces of milk for each pound of weight [24]. Feeding sessions should last no longer than 35 minutes as longer sessions are fatiguing and burn more calories than the baby can consume. Infants should be weighed at least weekly using the same scale, preferably at their pediatrician's office. The subject of breast-feeding an infant with a cleft palate is controversial, with some practitioners encouraging the practice and others strongly opposed to it. There are clear advantages to breast-feeding a newborn, including passive immunologic contribution of the mother to the child in the form of secretory immunoglobulin [25]. Presently, innovations in distraction osteogenesis have decreased the need for conventional osteotomies as the important treatment for correction of maxillary and mandibular discrepancies. Regardless, conventional osteotomies still play a key role in the management of the very complex and multiphasic CLP patients [26]. The use of folic acid has been shown to decrease the incidence of neural tube defects by almost 70%, and may be higher than the influence of consanguineous marriages as a single entity [27]. In prevention of Cleft lip and palate disorders, prophylactic therapy with folic acid may reduce the incidence by almost 30% [28]. Long-term follow-up is much in need to achieve the maximum outcome of secondary alveolar grafting, the age of the patient should be within the mixed dentition period, there is no sex predilection, varied socioeconomic status. It can be either unilateral or bilateral. The knowledge, awareness, and attitude of dental interns in Cleft lip and palate management were considerably low, and they were aware of the Cleft lip and palate but most of them were not aware of treatment procedures and treatment time, patient management and the role of each specialty. Awareness of the scope of Cleft lip and palate management

should lead to improved access and efficient delivery of quality service. Our medical and dental colleagues need to have the necessary knowledge to make informed decisions about their patient's management. Equally, the public would benefit from knowing what the dental team offers them so that they can request an appropriate referral.

CONCLUSION

The Cleft Lip and Palate management requires a multidisciplinary team approach and special care, and attention should be given. In this study, we have assessed the knowledge, attitude, and awareness of Cleft lip and palate management among dental interns, in which they were aware of Cleft lip and Palate but not aware of various procedures involved and was not aware of the timing of different procedures, the multidisciplinary team involved and role of each specialty at different phases of treatment.

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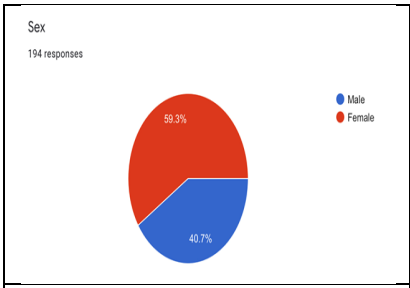

Figure 1. Pie diagram showing gender distribution of this study. This shows that female participants(59.3%) are more when compared to males.

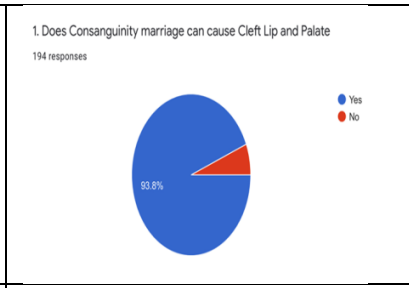

Figure 2. Pie diagram showing results of awareness of consanguinity. From this graph, it is understood that most of the participants were aware that consanguinity can cause cleft lip/cleft palate

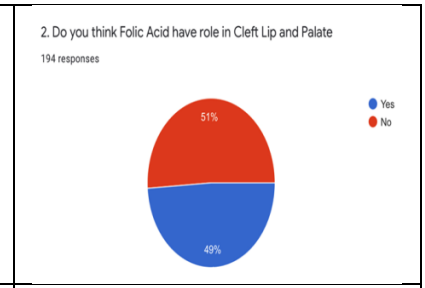

Figure 3. Pie diagram showing results of awareness of Folic acid. From this graph, it is understood that 51% of participants were aware that Folic acid can cause cleft lip/cleft palate.

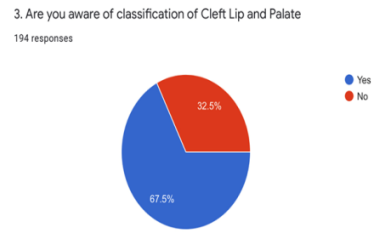	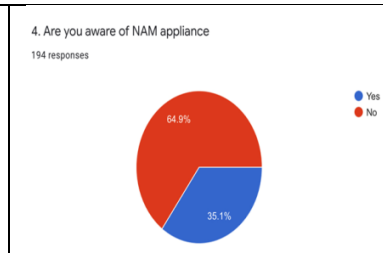	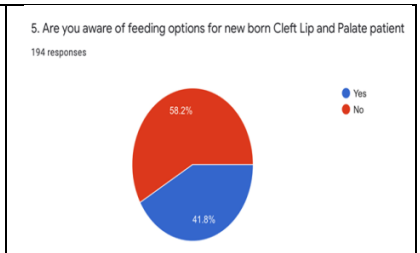
Figure 4. Pie diagram showing results of awareness of classification. From this graph, it is understood that most of the participants (67.5%) were aware of the classification of cleft lip/cleft palate.	**Figure 5. Pie diagram showing results of awareness NAM appliance. From this graph, it is understood that most of the participants (64.9%) were unaware of the NAM appliance .**	**Figure 6. Pie diagram showing results of awareness of feeding options. From this graph, it is understood that most of the participants (58.2%) were unaware of the feeding option for cleft lip/cleft palate.**
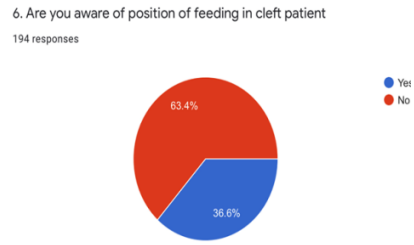		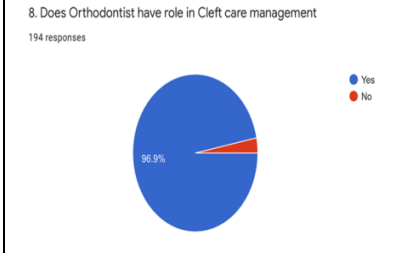
Figure 7. Pie diagram showing results of awareness of position of feeding. From this graph, it is understood that most of the participants (63.4%) were unaware of the position of feeding in cleft lip/cleft palate.	**Figure 8. Pie diagram showing results of awareness of Pediatrician role in Cleft care management . From this graph, it is understood that most of the participants (97.9%) were aware of the role of paediatrician in cleft lip/cleft palate**	**Figure 9. Pie diagram showing results of awareness of Orthodontist role in Cleft care management. From this graph, it is understood that most of the Orthodontist (96.9%) were aware of the role of Orthodontist in cleft lip/cleft palate.**
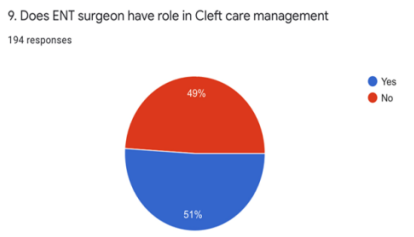	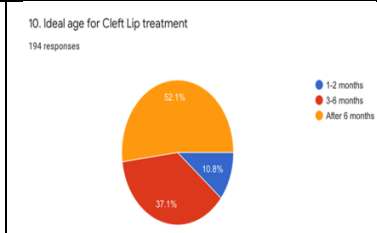	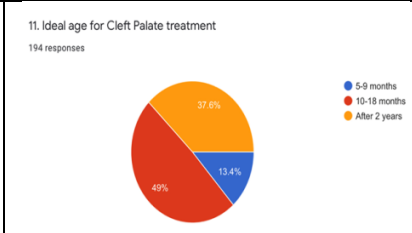
Figure 10. Pie diagram showing results of awareness of ENT surgeon role in Cleft care management . From this graph, it is understood that most of the ENT surgeon (49%) were unaware of the role of ENT surgeon in cleft lip/cleft palate.	**Figure 11. Pie diagram showing results of awareness of Ideal age for cleft lip treatment . From this graph, it is understood that 52.1% told after 6 months, 37.1% told 3-6 months, 10.8% told 1-2 months.**	**Figure 12. Pie diagram showing results of awareness of Ideal age for cleft palate treatment . From this graph, it is understood that 49% told after 10-18 months, 37.6% told after 2 years, 13.4% told 5-9 months.**

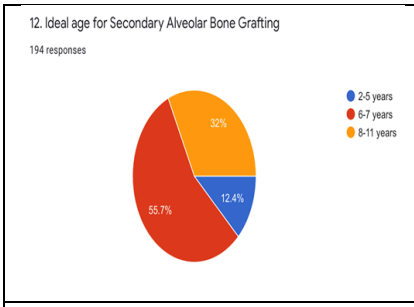

Figure 13. Pie diagram showing results of awareness of Ideal age for Secondary alveolar bone grafting. From this graph, it is understood that 55.7% gave answer for after 6-7 years , 32% told 8-11 years, 12.4% told 2-5 years .

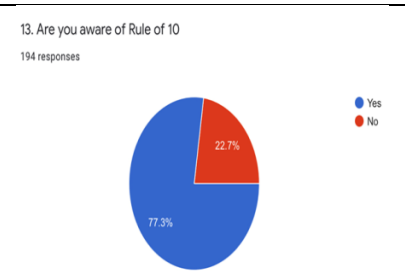

Figure 14. Pie diagram showing results of awareness of Rule of 10. From this graph, it is understood that most of the participants (77.3%) were aware of the Rule of 10 for cleft lip/cleft palate.

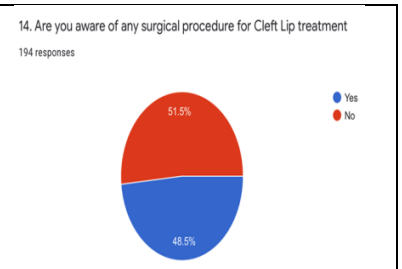

Figure 15. Pie diagram showing results of awareness of surgical procedure for cleft lip treatment. From this graph, it is understood that most of the participants (51.5%) were unaware of the surgical procedure for cleft lip treatment

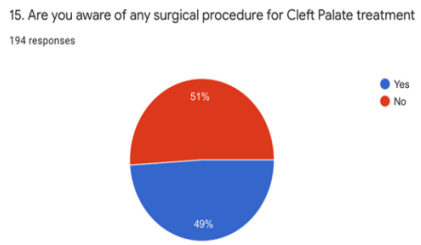

Figure 16. Pie diagram showing results of awareness of surgical procedure for cleft palate treatment. From this graph, it is understood that most of the participants (51.%) were unaware of the surgical procedure for cleft palate treatment

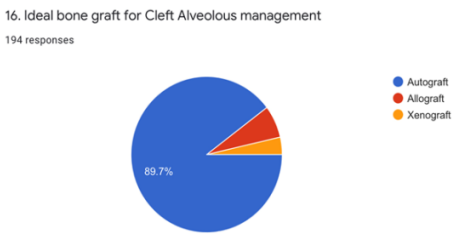

Figure 17. Pie diagram showing results of awareness of Ideal bone graft for cleft alveolousmanagement . From this graph, it is understood that 89.7% gave answer for autograft.

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RESEARCH ARTICLE

Application of Operations Research in Tax Revenue

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ABSTRACT

In this paper, a model of participation in lottery games designed to address the optimisation of tax revenue in state-sponsored lotteries. The model treats participants dynamically and examines a long-run equilibrium. A novel high frequency approximation is used to turn the problem into a static, state-contingent deterministic programming problem. We demonstrate that the solution of this problem has qualitatively plausible properties.

Keywords: Operations research , Kuhn-Tucker Method ,Simulations.

INTRODUCTION

State-sponsored lotteries are extensively used to raise revenue for good causes such as education and the arts. Worldwide the annual value of tickets bought in 1998 exceeded $51bn (see La Fleur and La Fleur [1] for references). States often contract out the running of such games to an operating company with a proportion of the cost of each ticket going to good causes or to general tax revenue and the contractor taking a further proportion as operating costs; most of the remainder is returned to participants as prizes. For example, in 1998 out of every pound spent on the UK National Lottery (UKNL), about 50 pence went to prizes, 28 pence to good causes, 13 pence to Customs and Excise as excise duties, 5 pence to the retailer as commission, 3 pence to cover the operating company (Camelot) operating costs, and Camelot kept 1 pence as profits. The tax rate and the level of participation will affect the total good-causes revenue. Participation depends on the tax rate and the design of the lottery, in particular, the probability of winning a jackpot prize. In order to study the effects of changing such parameters on the good-causes revenue it is necessary to model their effect on participation. In this topic, we present such a model and use it to address the issue of whether the current tax rate and jackpot probability are the appropriate ones to maximize good-causes revenue in the UKNL. A common feature of lotteries is that, if there are no winners in a given draw, the jackpot prize pool from that draw is added to the pool for the next draw: a rollover. The increased pool typically induces a higher level of participation. This suggests good-causes revenue may be increased by making rollovers more likely and this can be achieved by reducing the probability of winning a jackpot. However, doing so may also have a disincentive effect on

participation in draws that do not feature a rollover and thus offset any increase in revenue. To capture such a trade-off effectively requires a dynamic model of individual participation. One possible approach would be to fit a 'black box' model (eg., regression-based) using standard statistical methods. However, the similarity and stability of lottery designs means that the available data offers little natural variation and poor prospects of a good fit, particularly if one controls for differences in country-specific propensities to gamble. Our approach is to work from first principles and build a model of individual participation based on standard inter-temporal models of individual behavior under uncertainty. Lack of variation in the data forces us to adopt the simplest model parameterization. Nonetheless, this is sufficient to capture the principal features of observed behavior. Since quantitative verification of the model is not possible, we check that the qualitative predictions of the model are plausible and consistent with casual observation. Our final step is to aggregate the behavior of individual agents into a full model of demand for lottery tickets, which enables us to examine the effects of changing lottery design and tax rates.

There is little literature on the issues discussed here and (to our knowledge) none which bases the optimal design problem on a dynamic model of individual behavior. Farrell et al [4] equate the effective price of a ticket in the UKNL to the ticket price less the expected value of the prizes and use the variation in the latter in rollover weeks to estimate the elasticity of demand. Farrell et al [5] analyze the same data using use time-series methods. Although both these papers find values for the short- and long-run elasticities roughly consistent with revenue maximisation, they implicitly assume risk neutrality and neither addresses inter-temporal substitution by participants. Furthermore, they do not disentangle the effects of the tax rate and the lottery design and, using point estimates, they cannot investigate non-local changes of the parameters.

Scoggins [13] offers rank-dependent utility as an explanation of the complex prize structure. An interesting paper by Simon [14] attempts to construct a model that fits the first couple of years of participation in the UKNL. Although it is too tuned to UK data to be useful as a general tool for addressing design problems, perhaps its most interesting contribution is an attempt to model how observed attrition in participation could be counteracted by the high probability of winning small fixed prizes. This is usually offered as justification for the presence of small fixed prizes. We have not explicitly included this (small) effect in our model as it will not show up in the high-frequency limit we employ.

We conclude the Introduction by giving an informal description of our model. Full details of each component of the model are then set out in subsequent sections.Lottery draws take place at regular intervals and, at each draw, consumers decide how many tickets to purchase. The cost is measured in foregone expenditure on other goods. Each ticket displays a set of positive integers (1 to 49 for the UKNL) from which participants must choose a 'combination' i.e., a subset of the available integers (of size 6 for the UKNL). After all purchases of tickets have been made, the Lottery organizers randomly draw a combination according to a uniform distribution (see Haigh [7], [8] and references therein). A ticket holder whose chosen combination matches that drawn is a jackpot winner and his/her prize is determined by dividing the jackpot prize pool equally amongst all the jackpot winners. The jackpot prize pool is a fixed proportion of the prize pool augmented by any amount rolled over from previous draws. Such rollovers occur if there are no jackpot winners in a given draw, in which case the jackpot prize pool is held over and added to the jackpot prize poo1 in the next draw. A run of draws with no winners can lead to such a large jackpot prize pool that behavior regarded as undesirable (such as attempting to buy all combinations) can occur. To avoid this, a limit on the number of consecutive draws that can be rolled over is imposed. If this limit is reached, we assume, for expositional convenience, that the money in the accumulated jackpot prize pool is simply lost from the system. The remainder of the prize pool is allocated by looking at partial matches of the subsets chosen with that drawn. In our model, we concentrate on jackpot prizes where winning is rare (1 in 14 million chance for the UKNL) with values large enough to change the life of winners and other prizes that are low in value and common (more than 1 in 60 chance for fixed-value prizes in the UKNL).

Knowing the prize structure, we model participants' decisions of how many tickets to purchase as a dynamic optimisation problem. Since the number of tickets bought is very large (over 60 million per draw in the UKNL), we assume that consumers treat rollovers as exogenous, ignoring the minuscule effect their individual actions have on their occurrence. Nevertheless, aggregate levels of participation determine (stochastically) the rollover process. This results in a circular model in which the rollover process affects participation and the latter affects the rollover process. Rather than specify the dynamics of the adjustment process, we analyse the equilibrium in which we have rollover-contingent participation levels that are optimal given the rollover process determined by those participation levels. Once the full network of terminals was installed, participation levels rapidly stabilized in the UKNL, suggesting that equilibrium had been reached.[5].

In section 1.1, we develop our model of consumer choice as a stochastic dynamic optimisation problem. In the section 1.2, we analyse the limit as the time interval between draws approaches zero. By regarding this limit as a first order approximation to the dynamic problem, we are able to recast the consumer's optimization problem as a static, deterministic, non-linear programming problem. In section 1.3, we study the Kuhn–Tucker conditions for this problem and show that the unique optimal solution captures several qualitative properties of the behavior we are seeking to model. In section 1.4, closes the model by relating rollovers to participation levels. This necessitates the construction of a simple sub-model of how combinations are chosen. Finally, we apply the model to the UKNL and draw some tentative conclusions on the current values of the parameters.

Individual Behavior

We will suppose participants live indefinitely (or participants leaving the system are replaced with others having similar tastes) and divide their expenditure in period t between y_t on lottery tickets and x_t on other goods so as to maximise the expected value of the lifetime utility:

$$\sum_{t=1}^{\infty} \Delta t \delta^{t-1} u(x_t, y_t), \qquad (1.1)$$

where $\delta = e^{-\beta_{\Delta t}}$ is the discount factor, β is the instantaneous rate of discount, Δt is the time interval between draws and u is the instantaneous, von Neumann–Morgenstern rate-of-utility function in period t. We have included lottery expenditure as a continuous variable, which will permit the use of calculus-based methods in the sequel. As tickets are indivisible, this raises the question of the interpretation of non-integral y. If restricted to purchasing whole numbers of tickets, it is not hard to see that, even in the absence of rollovers, a participant's optimal number of tickets will typically vary from week to week. However, if purchases are made more frequently (as we shall assume), the expected utility will be nearly the same as that of a stationary policy in which the (non-integral) long-run average number of tickets is purchased in each draw. Friedman and Savage [6] explain how gambling can be reconciled with risk averse behaviour in a static expected utility framework by including convex sections in the utility function. Dynamic versions of this approach based on [11] are easy to write down but Farrell and Hartley [3] show that repeated purchase of lottery tickets cannot be explained using expected utility functions unless consumers derive direct value ('fun') from their purchases of tickets (or capital markets are imperfect). We shall also assume an absence of income effects in lottery purchases since this allows us to simplify the utility function and is broadly consistent with observation. The most general form of utility function consistent with these observations is

$$u(x, y) = v[x + f(y; a^e, a^h)],$$

where v captures the consumer's attitude to risk and will be assumed increasing and concave, whilst the 'fun function' f expresses the direct consumption value of lottery purchases in terms of expenditure on other goods. We also permit the consumption value to depend on external factors, a^e, such as a rollover draw, as well as past history, a^h, including the consumer's past winnings, if any. We will specify these variables more fully in the sequel. Under such a specification, participation is induced both by the direct enjoyment gained from purchasing tickets (which is independent of whether a prize is won) as well as the extra wealth in the event of winning.

Rollover Technology

We allow expenditure choices to be contingent on the number of weeks that the jackpot has been rolled over, to reflect the observed increase in ticket sales in rollover weeks.[4] We choose this way of allowing for rollovers to simplify the model. In fact making purchases contingent on the actual amount of money rolled over makes no difference in the high frequency limit used in the sequel. Furthermore, measured levels of participation contingent on weeks rolled over is subject to rather little variation. [4] .

Let the (non-negative) integer-valued random variable R_t denote the number of weeks the jackpot has been rolled over into week t. More precisely, $R_t = k$ if and if only the jackpot was won in week $t – k – 1$ but not in weeks $t – k$, ..., t 1 (or, if the jackpot has never been won and $t = k + 1$). It follows that the transition probability $P(R_t + 1 = j | R_t = k)$ is zero if $j \notin \{0, k + 1\}$. Thus, if K is the maximum permitted value of R_t, the latter follows a Markov chain with Transition Probability Matrix (TPM):

$$\mathbf{Q} = \begin{pmatrix} 1-\pi_0 & \pi_0 & 0 & 0 & \cdots & 0 \\ 1-\pi_1 & 0 & \pi_1 & 0 & \cdots & 0 \\ 1-\pi_2 & 0 & 0 & \pi_2 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 1-\pi_{k-1} & 0 & 0 & 0 & \cdots & \pi_{k-1} \\ 1 & 0 & 0 & 0 & \cdots & 0 \end{pmatrix}$$

where $\pi_k = P(R_t + 1 = k + 1 | R_t = k) > 0$ for $k = 0, ..., K – 1$, and the rows and columns are entered in the order 0, 1, 2, ..., K – 1, K. Since **Q** is uni-chained and acyclic, there is a unique limiting probability vector $\mathbf{p} = (p_0, p_1, ..., p_K) \geq 0$ which satisfies $\mathbf{Q}^T\mathbf{p} = \mathbf{p}$ and $\sum_{j=0}^{K_{p_j}} = 1$. It is readily verified that $p_0 = \sigma^{-1}$ and $p_k = \sigma^{-1}\pi^0 ... \pi_{k-1}$ for $k \geq 1$, where $\sigma = 1 + \sum_{j=0}^{K_{p_j}} \pi_0 ... \pi_j$.

Our assumptions on how a^e and a^h affect f are designed to reflect certain observations. Participation is typically higher in rollover draws than in normal draws. Higher potential winnings are an obvious explanation but it is also possible that the increased publicity and general excitement of participation associated with rollover draws increase the direct consumption value. To maintain the simplicity of the model, we ignore other environmental factors so that a^e depends only on R_t and write $f_k(y)$ for the value of expenditure y on tickets when $R_t = k$. We also assume that the fun associated with purchasing tickets derives predominantly from contemplation of winning a prize large enough to change the winner's lifestyle substantially. We reflect this by including only a past jackpot win in the history a^h and, in particular, setting $f \equiv 0$ for a consumer who has won the jackpot in the past. This will mean that such a consumer no longer participates. Since participation and other consumption will typically depend on the rollover state and past winnings, if any, they are random variables. We write $Y_t[X_t]$ for expenditure on lottery tickets [other goods] in period t where the process $\{X_t, Y_t\}_{t=0}^{\infty}$ is adapted to the rollover process $\{R_t\}_{t=0}^{\infty}$ and the individual winning process.

Thus, consumers maximise:

$$\Delta tE\left\{\sum_{t=1}^{T}\delta^{t-1}v\left[X_t+f_{R_t}(Y_t)\right]\sum_{t=T+1}^{\infty}\delta^{t-1}v\left[X_t\right]\right\}, \quad (1.2)$$

where the stopping time T denotes the period in which the consumer wins the jackpot. Note that T is a random variable that depends (probabilistically) on $\{Y_t\}_{t=0}^{\infty}$, and takes the value $+\infty$ for a non-participant.

Constraint

A consumer's expenditure is subject to the lifetime budget constraint:

$$\sum_{t=1}^{\infty}\frac{\Delta t\left(X_t+Y_t-m\right)-W_t^{*}\left(Y_t\right)}{\left(1+r\right)^{t-1}}\leq 0, \quad (1.3a)$$

$x_t \geq 0, y_t \geq 0$, for t = 1, 2, ..., (1.3b)

where $r = \exp(\beta\Delta t) - 1$ is the rate of interest, m is income per period (assumed constant) and $W_t^{*}\left(y\right)$ is a non-negative random variable representing winnings in period t (taking the value {0} if the consumer does not win in period t) if y is spent on lottery tickets. We interpret the constraint as holding with probability one. This prevents borrowing on the strength of future lottery wins since for any $\varepsilon > 0$ and pattern of participation, there is a positive probability that the net present value at $t = 1$ of future winnings is less than ε.

For $t > T$, lottery winnings cease so that (1.3a) can be written:

$$\sum_{t=1}^{T}\frac{\Delta t\left(X_t+Y_t\right)-G_t\left(Y_t\right)}{\left(1+r\right)^{t-1}}-\frac{W^{k(t)}}{\left(1+r\right)^{T-1}}$$

$$+\sum_{t=1}^{\infty}\frac{\Delta tX_t}{\left(1+r\right)^{t-1}}-\Delta tm\left(1+\frac{1}{r}\right)\leq 0, \quad (1.4)$$

where $G_t(y)$ is the random variable representing the value of a non-jackpot prize in period t when y is spent on lottery tickets and takes the value 0 if no such prize is won. Similarly, ignoring the fact that a ticket cannot win more than one type of prize, W^k is a random variable representing the value of a jackpot prize in state k.

To solve the consumer's problem, we first look at the situation faced by a jackpot winner. This involves examining the optimisation problem for a consumer conditional on $T = t^{\wedge}$ and $W^{k(t^{\wedge})} = w$. Discounted to the first period, this yields optimal utility of $\Delta t\delta^{t^{\wedge}-1}\psi(w;\Delta t)$ where:

$$\psi(w;\Delta t)=\max_{x_1,x_2,\ldots}\sum_{t=1}^{\infty}\delta^{t-1}v(x_t)$$

$$\text{Subject to } \sum_{t=1}^{\infty}\frac{\Delta tX_t}{\left(1+r\right)^{t-1}}-\Delta tm\left(1+\frac{1}{r}\right)+w,$$

$x_t \geq 0$, for $t = 1, 2, \ldots$

We have assumed that winners continue to receive per-period income m (or its equivalent from quitting a job in monetary value). Since $v(\cdot)$ is concave, we may use the fact that $\left\{r/(1+r)^t\right\}_{t=1}^{\infty}$ is a probability distribution, and that the constraint says that the mean value of $\left\{X_t\right\}_{t=1}^{\infty}$ is $x^- = m + wr/\Delta t(1+r)$, to apply Jensen's inequality and deduce that the optimal solution is $x_t = x^-$ for every t. Hence

$$\psi(w;\Delta t) = \max_{x_1,x_2,\ldots} \sum_{t=1}^{\infty} \delta^{t-1} v(\bar{x})$$

$$= v\left[m + w\frac{1-e^{-\beta\Delta t}}{\Delta t}\right]\frac{1}{1-e^{-\beta\Delta t}}.$$

We shall focus on the case when Δt is small and note that, as $\Delta t \to 0^+$, $\Delta t\psi(w;\Delta t) \to (1/\beta)v(m+\beta w)$. The consumer's problem can therefore be written as:

$$CP \qquad \text{Maximise } \Delta tE = \left\{\sum_{t=1}^{T} \delta^{t-1} v\left[x_t + f_{k(t)}(y_t)\right] + \delta^{T-1}\psi(W^{k(T)};\Delta t)\right\}$$

$$\text{Subject to } \sum_{t=1}^{T} \frac{\Delta t\left(X_t + Y_t\right) - G_t\left(Y_t\right)}{\left(1+r\right)^{t-1}} - \sum_{t=1}^{T} \frac{\Delta t m}{\left(1+r\right)^{t-1}} \le 0,$$

$x_t \ge 0$, $y_t \ge 0$, for t = 1, 2, …

An Approximation to the Consumer S Problem

Since lottery draws occur at least weekly, the discount factor 3will be close to one. In a Markov reward process with this property, a good approximation to the expected discounted reward per period is the long-run average reward obtained by taking the expectation of the reward per period with respect to the long-run stationary probabilities. (See, for example, Whittle, [15] p. 123). However, the objective function and constraint in CP are complicated by the presence of additional terms representing the possibility of exiting the reward process upon winning the jackpot. Since the probability of winning the jackpot is so low, for a consumer who buys the same small number of tickets each week, wins can be regarded as events in a (discrete approximation to a) Poisson process with expected inter-event times of the order of 107 periods. When considering such rare events, it is quite inappropriate to approximate the expected discounted value of the jackpot by the expected average reward as the discount rate β, though small, is much larger than the quit rate ρ. To cope with these two distinct components of the reward, we adopt a hybrid approach in which consumers receive the long-run average reward per period until they win the jackpot, the value of which is discounted to the current period.

The consumer's problem CP is actually slightly more complicated than the preceding description suggests, for the probability of winning the jackpot will depend on the number of tickets purchased and this is contingent on the rollover state. Hence, quitting probabilities are state-dependent and the corresponding process is not Poisson. Nevertheless, the expected number of periods until a jackpot win will still be very large and, if we assume that the pre-quit process can be regarded as in the steady state, it is plausible that exiting can still be well-approximated by a

Poisson process. In this process, we take the probability of a jackpot win leading to a quit as the expected probability of winning, taken with respect to the steady state probabilities. A formal statement of these results, with proofs, is given Lemma 1.1.

Objective Function

For consumers who have not yet won the jackpot we concentrate on stationary decision rules in which consumers' decisions depend on the rollover state k but not on t and write $y^k[x^k]$ for the rate of expenditure on lottery tickets [other goods] when $R_t = k$. To allow for winning the jackpot, the environment of any particular consumer can be summarized by the extended state space:

$$\{(0, 0), (0, 0), \ldots, (K, 0), (0, 1), (1, 1), \ldots, (K, 1), \varpi\}$$

where, at period t, the extended state is $(k, 0)$ if the consumer has not yet won the jackpot and $R_t = k$ is $(k, 1)$ if the consumer won the jackpot in period $t - 1$ and $R_{t-1} = k$ and is ϖ if the consumer won the jackpot in a period before t –1. Writing **D** for the order-$(K + 1)$ diagonal matrix with $d_{kk} = y^k$ and ρ for the probability that a single ticket wins the jackpot, the transition matrix for the extended state space becomes:

$$\mathbf{P} = \begin{pmatrix} (1 - \rho\Delta t\mathbf{D})/\mathbf{Q} & \rho\Delta t\mathbf{D} & 0 \\ 0 & 0 & \mathbf{e} \\ 0 & 0 & 1 \end{pmatrix},$$

where every component of **e** is 1 and the 0 s represent appropriately dimensioned zero matrices or vectors. (Note that since **Q** is a transition matrix, the elements in each row sum to one). We have used the fact that the probability of winning the jackpot when $R_t = k$ is $\rho\Delta t\, y^k$ to a very good approximation. Define the $(K + 1)$-vectors:

$u = (v(x^0 + f_0(y^0)), \ldots, v(x^K + f_K(y^K)))$,

$v(\Delta t) = (\mathrm{E}\psi(W^0; \Delta t), \ldots, (\mathrm{E}\psi(W^K; \Delta t))$,

Then the expected value of the objective function of the consumer's problem CP is the (0, 0)th (in the extended state space) component of:

$$\mathbf{A}\begin{pmatrix} n \\ v(\Delta t) \\ 0 \end{pmatrix} \qquad (1.5)$$

where $\mathbf{A} = \sum_{t=1}^{\infty} \Delta t \delta^{t-1} \mathbf{P}^{t-1}$.

In Lemma 1.1, we demonstrate that this component is well approximated by:

$$\beta^{-1}\left\{\sum_{K=0}^{K} p_k v(x^k + f_k(y^k)) + \frac{\rho}{\beta}\sum_{K=0}^{K} p_k y^k \mathrm{E}v(m + \beta W^k)\right\} \qquad (1.6)$$

This result demonstrates our earlier discussion. The first term is the net present value of receiving the long run expected reward every period for a long time. The second term is the expected utility of winning the jackpot when the rollover variable is in the steady state, discounted to the initial time.

Constraint

We shall treat the budget constraint in (CP) in a similar way. We assume that, conditional on $R_t = k$, $G_t(y^k) > 0$ with probability $\Delta t^{\phi}(y^k)$ where $\Delta t^{\phi}(y^k)$ is the probability of winning a non-jackpot prize. Similarly, conditional on $G_t(y^k) > 0$ and the rollover state being k, we assume that $G_t(y^k)$ is distributed as G^k: the non-jackpot prize.

We write g_k for $E[G^k]$. In Lemma 1.1, we demonstrate that the left-hand side of the constraint in CP is well approximated by:

$$\sum_{K=0}^{K} p_k\{x^k + y^k - \phi(y^k)g_k\} - m \qquad (1.7)$$

which says that the budget constraint must be satisfied on average using the limiting probabilities.

Since v is increasing in x^k, the consumer's optimisation problem becomes:

$$CP^* \qquad \text{Maximise} \sum_{k=0}^{K} p_k v(x^k + f_k(y^k))$$

$$+\frac{\rho}{\beta}\sum_{K=0}^{K} p_k y^k \mathrm{E}v(m + \beta W^k)$$

$$\text{Subject to} \sum_{K=0}^{K} p_k\{x^k + y^k - \phi(y^k)g_k\} - m$$

$x^k \geq 0$, $y^k \geq 0$, for $k = 1, 2, \ldots, K$.

Now, we will use the following ergodic results for which we offer an elementary proof.

Lemma 1.1. Suppose $\{\mathbf{a}_t\}_{t=1}^{\infty}$ is a sequence of independent and identically distributed random $(K + 1)$-vectors and Ea1 exists. Let **Q*** be the transition matrix in which every row is **p** (the stationary distribution of **Q**). Then, as $\Delta t \to 0^+$

$$\Delta t\sum_{t=1}^{\infty}\delta^{t-1}[\mathbf{I} - \rho\Delta t\mathbf{D}]\mathbf{Q}]^{t-1}\mathbf{a}_t \to \frac{1}{\beta}\left(\mathbf{I} + \frac{\rho}{\beta}\mathbf{Q}^*\mathbf{D}\mathbf{Q}\right)^{-1}\mathbf{Q}^*E\mathbf{a}_1$$

with probability one.

Proof. It is well known [1] that $\mathbf{Q}_t \to \mathbf{Q}^*$ as $t \to \infty$. Hence, $\mathbf{QQ}^* = \mathbf{Q}^*\mathbf{Q} = \mathbf{Q}^*\mathbf{Q}^* = \mathbf{Q}^*$. It follows inductively that, if $\mathbf{S} = \mathbf{Q} - \mathbf{Q}^*$, then $\mathbf{Q}^t = \mathbf{S}^t + \mathbf{Q}^*$ for $t \geq 1$. Consequently, $\mathbf{S}^t \to 0$ as $t \to \infty$, which means that $\mathbf{I} - \mathbf{S}$ is non-singular. Thus, using $\delta = e^{-\beta\Delta t}$,

$$\Delta t\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^{t-1} = \Delta t\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^* + \Delta t\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{S}^{t-1}\Delta t\mathbf{Q}^*$$

$$= \Delta t(1 - e^{\beta\Delta t})^{-1}\mathbf{Q}^* + \Delta t(\mathbf{I} - e^{\beta\Delta t}\mathbf{S})^{-1}\Delta t\mathbf{Q}^*$$

$$\to \frac{1}{\beta}\mathbf{Q}^* \text{ as } \Delta t \to 0^+$$

Write

$$\mathbf{A}\Delta t = \Delta t\sum_{t=1}^{\infty}\delta^{t-1}(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}]^{t-1}$$

$$= \Delta t(\mathbf{I} - \delta(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}]^{-1}$$

The latter inverse exists since $\delta(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}$ is a sub-stochastic matrix. Note that

$$\left(\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^{t-1}\right)\delta(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{Q}]$$

$$=\mathbf{I}+\rho\Delta t\delta\left(\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^{t-1}\right)\mathbf{DQ}$$

from which it follows that

$$\mathbf{A}\Delta t=\left[\mathbf{I}+\rho\Delta t\delta\left(\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^{t-1}\right)\mathbf{DQ}\right]^{-1}\Delta t\left(\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{Q}^{t-1}\right)$$

$$\rightarrow\mathbf{A}^{\infty}=\frac{1}{\beta}\left[\mathbf{I}+\frac{\rho}{\beta}\mathbf{Q}*\mathbf{DQ}\right]\mathbf{Q}*\frac{1}{2}\text{ as }\Delta t\rightarrow 0^{+}$$

For $n \geq 1$, write $S_n = (1/n)\sum_{t=1}^{n}\mathbf{a}_t$. By the strong law of large numbers, for almost all sample paths ω, $s_n(\omega) \rightarrow \mathbf{E}\mathbf{a}_1$. For such an ω, we shall write

$$\mathbf{S}\Delta t=\Delta t\sum_{t=1}^{\infty}\delta^{t-1}\left[\left(\mathbf{I}-\rho\Delta t\mathbf{D}\right)\mathbf{Q}\right]^{t-1}\mathbf{a}_t(\omega)$$

and prove that $\mathbf{S}(\Delta t) \rightarrow \mathbf{A}^{\infty}\mathbf{E}\mathbf{a}_1$ as $\Delta t \rightarrow 0^{+}$. Note that by rearranging the sums,

$$\mathbf{A}(\Delta t)\mathbf{S}(\Delta t)=(\Delta t)^2\sum_{t=1}^{\infty}\delta^{t-1}(\mathbf{I}-\rho\Delta t\mathbf{D})^{t-1}\sum_{t=1}^{\infty}\delta^{t-1}$$

$$\times\left[(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{Q}\right]^{t-1}\mathbf{a}_t(\omega)$$

$$=(\Delta t)^2\sum_{t=1}^{\infty}\delta^{t-1}(\mathbf{I}-\rho\Delta t\mathbf{D})^{t-1}[\mathbf{a}_1(\omega)+\ldots+\mathbf{a}_t(\omega)]$$

A similar argument gives

$$=(\Delta t)^2\sum_{t=1}^{\infty}\delta^{t-1}(\mathbf{I}-\rho\Delta t\mathbf{D})^{t-1}\mathbf{s}_t(\omega)$$

$$\mathbf{A}(\Delta t)^2\mathbf{E}\mathbf{a}_1=(\Delta t)^2\sum_{t=1}^{\infty}\delta^{t-1}\left[(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{D}\right]^{t-1}\mathbf{E}\mathbf{a}_1$$

Taking the difference of these results gives

$$\mathbf{S}(\Delta t)=\mathbf{A}(\Delta t)\mathbf{E}\mathbf{a}_1+\left[\mathbf{A}(\Delta t)\right]^{-1}$$

$$\times\left\{(\Delta t)^2\sum_{t=1}^{\infty}t\delta^{t-1}\left[(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{Q}\right]^{t-1}\left[\mathbf{S}_t(\omega)-\mathbf{E}\mathbf{a}_1\right]\right\}$$

and the proof is completed by showing that the term in braces approaches 0 as $\Delta t \rightarrow 0^{+}$.

To achieve this, we use the vector and matrix norms $\|x\| = \max_k |x_k|, \|\mathbf{A}\| = \max_{k'} \sum_k |a_{k'k}|$, and the fact that $\|\mathbf{Ax}\| \le \|\mathbf{A}\|\|\mathbf{x}\|$ together with the standard results on matrix norms. We have $\|\mathbf{Q}\| = 1$ and $\|\mathbf{I} - \rho\Delta t\mathbf{D}\| = 1 - \rho\Delta t\underline{y}$, where $\underline{y} = \min_k y^k$. Since

$$\left[1 - \delta(1 - \rho\Delta t\underline{y})\right]/\Delta t \to \beta + \rho\underline{y} > 0 \text{ as } \Delta t \to 0^+$$

there exists a $\mu > 0$ and $\delta_1 > 0$ such that, if $0 < \Delta t < \delta_1$, then $1 - \delta(1 - \rho\Delta t\underline{y}) > \mu\Delta t$.

Let $\varepsilon > 0$. Since $s_t(\omega) \to \mathbf{E}\mathbf{a}_1$ as $t \to \infty$ there is an N such that $\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\| < \mu^2(\varepsilon/2)$ for all $t > N$. Define

$$\delta_2 = \left\{\frac{\varepsilon}{2}\sum_{t=1}^{N} t\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\|\right\}^{-\frac{1}{2}}$$

If $\Delta t < \min\{\delta_1, \delta_2\}$, then

$$\left\|(\Delta t)^2\sum_{t=1}^{\infty} t\delta^{t-1}\left[(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}\right]^{t-1}\left[\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\right]\right\|$$

$$\le (\Delta t)^2\sum_{t=N+1}^{\infty} t\delta^{t-1}\|(\mathbf{I} - \rho\Delta t\mathbf{D})\|^{t-1}\|\mathbf{Q}\|^{t-1}\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\|$$

$$+(\Delta t)^2\sum_{t=1}^{N} t\delta^{t-1}\|(\mathbf{I} - \rho\Delta t\mathbf{D})\|^{t-1}\|\mathbf{Q}\|^{t-1}\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\|$$

$$< \frac{1}{2}(\Delta t)^2\sum_{t=N+1}^{\infty} t\delta^{t-1}(1 - \rho\Delta t\underline{y})^{t-1}\varepsilon\mu^2$$

$$+(\Delta t)^2\sum_{t=1}^{N} t\delta^{t-1}(1 - \rho\Delta t\underline{y})^{t-1}\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\|$$

$$\le \frac{\varepsilon}{2}\mu^2(\Delta t)^2\sum_{t=1}^{\infty} t\delta^{t-1}(1 - \rho\Delta t\underline{y})^{t-1} + (\Delta t)^2\sum_{t=1}^{\infty} t\|\mathbf{S}_t(\omega) - \mathbf{E}\mathbf{a}_1\|$$

$$= \frac{\varepsilon}{2}\left\{\mu\Delta t\left[1 - \delta\left(1 - \rho\Delta t\underline{y}\right)\right]^{-1}\right\}^2 + (\Delta t)^2\frac{\varepsilon}{2\delta_2^2} < \varepsilon,$$

which concludes the proof.

For $i \ge 1$,

$$\mathbf{P}^i = \begin{pmatrix} \left[(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}\right]^i & \rho\Delta t\left[(\mathbf{I} - \rho\Delta t\mathbf{D})\mathbf{Q}\right]^{i-\mathbf{I}}\mathbf{D} & \cdot \\ 0 & 0 & \mathbf{e} \\ 0 & 0 & 1 \end{pmatrix} \quad (1.8)$$

where the missing term is chosen to make the rows sum to 1. It follows that the (0, 0)th component (in the extended state space) of (7.5) is the 0th (in the original state space) component of

$$\sum_{t=1}^{\infty}\delta^{t-1}\left[(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{Q}\right]^{t-1}\left[(\mathbf{u}+\rho\Delta t\mathbf{D}\mathbf{v}(\Delta t)\right]$$

(1.9)

$$\overset{\Delta t\to 0^{+}}{\to}\frac{1}{\beta}\left[\mathbf{I}+\frac{\rho}{\beta}\mathbf{Q}^{*}\mathbf{D}\mathbf{Q}^{*}\right]^{-1}\mathbf{Q}^{*}[\mathbf{u}+\rho\mathbf{D}\lim_{\Delta t\to 0^{+}}\Delta t\mathbf{v}(\Delta t),$$

using the lemma. (We can drop the 'almost surely' qualification in this case.) For the UKNL ρ^{-1} is close to 14×106 and if interest rates are less than 10% then does not exceed 1/500. Hence for purchases of a few tickets a week, the inverse matrix in the expression above is close to **I**. Furthermore, since *v* is concave and increasing, it is bounded above by an affine function and below by a constant, and this allows us to apply the dominated convergence theorem when taking the limit of ΔtE ψ(*W*^{k}; Δt) as $\Delta t \to 0^{+}$. Expression (1.6) is a rewriting of the limit in (1.9) and in using it in our model, we are assuming that draws are sufficiently frequent for us to apply this limit. One observation that supports this assumption is that winning small prizes has little effect on expenditure. We can use the same approach for the constraint. In time period *t*, if the rollover state is *k*, the probability of winning a non-jackpot prize is $\Delta t\phi(y^{k})$ and if won, the prize is g_t^k, an independent copy of g_1^k. Thus the left-hand side of the constraint of CP* is the (0, 0)th component of

$$\Delta t\sum_{t=1}^{\infty}\delta^{t-1}\mathbf{P}^{t-1}\begin{pmatrix}\tilde{\mathbf{u}}_t\\0\\0\end{pmatrix},$$

where $\tilde{\mathbf{u}}_t=(x^0+y^0-m-\phi(y^0)g_t^0,\ldots,x^K+y^K-m-\phi(y^K)g_t^K).$

We can use the expression (1.8) for **P**^{t} above to rewrite this as the 0th component of

$$\sum_{t=1}^{\infty}\delta^{t-1}\left[(\mathbf{I}-\rho\Delta t\mathbf{D})\mathbf{Q}^{*}\right]^{t-1}(x+y-me-\tilde{\mathbf{D}}g_t)$$

$$\overset{\Delta t\to 0^{+}}{\overset{a.s.}{\to}}\frac{1}{\beta}\left[\mathbf{I}+\frac{\rho}{\beta}\mathbf{Q}^{*}\mathbf{D}\mathbf{Q}\right]^{-1}\mathbf{Q}^{*}[x+y-me-\tilde{\mathbf{D}}\mathbf{E}g_1],$$

where **D**~ is the diagonal matrix with d~$_{kk}$ = $\phi(y^{k})$. By the same argument as above, we can replace the inverse matrix in this expression with I, thus justifying (1.7).

Properties of the Optimal Solution

An important test of our model of individual participation is that the optimal solution of CP* exhibits plausible qualitative properties if reasonable assumptions are made about the preferences. We now turn to such a set of assumptions. These are stated in terms of derivatives so we suppose throughout that *v*, f_0, f_1, ..., f_K and ϕ are twice differentiable for positive arguments.

Assumptions

1. For all $x > 0$, we have $v'(x) > 0$ and $v''(x) < 0$.
2. For $k = 0, \ldots, K$, we have $f_k(0) = 0$ and $0 < f'_k(y) < 1$, $f''_k(y) < 0$ for all $y > 0$.

3. For $K \geq k > j \geq 0$ and all $y > 0$, we have $f'_k(y) \geq f'_j(y)$.
4. We have $\phi < (0) = 0$, and $\phi'(y) > 0$, $\phi''(y) \leq 0$ for all $y > 0$.
5. For $K \geq k > j \geq 0$, we have $g_k \geq g_j$, and $E_\psi(W^k) \geq E_\psi W^j$).

Assumption 1 says that 'other goods' are desirable and participants are risk averse. The sign constraints on the derivative in Assumption 2 state that lottery tickets are desirable but that the additional fun derived from the purchase of an extra ticket falls as the number of tickets purchased rises. The requirement $f'_k(y) < 1$ implies that the 'fun' generated by purchasing tickets does not exceed the foregone consumption of other goods. Hence, both fun and the prospect of winning prizes are required to induce lottery participation; neither alone is sufficient. Assumption 3 specifies that marginal fun is non-decreasing in the size of the rollover, and Assumption 4 says that ϕ is concave. This will hold if participants choose the numbers on their tickets optimally. When the number of tickets purchased is small, we expect ϕ to be (nearly) linear. Assumption 5 requires the expected size of prizes to be non-decreasing in the rollover state variable. The total prize pool always increases but the number of participants and therefore, on average, the number of winners sharing this pool will also increase. Our assumption, which is supported by empirical evidence, is that this effect is not large enough to fully offset the increase in the prize pool.

Assumptions 1 and 2 mean that $v(x + f_k(y))$ is a strictly concave function of (x, y) for $k = 0, ..., K$. Hence, this is also true for the objective function in CP*. Assumption 4 means that the left-hand side of the equality constraint is a convex function of (x, y). Since the objective function is increasing in x^k the constraint may be replaced by a weak inequality. Furthermore, this inequality can be satisfied strictly (by setting x and y small and assuming $m > 0$). It follows that the first-order KuhnTucker conditions are necessary and sufficient and have a unique solution. [12] For $k = 0, ..., K$, these conditions can be written in the form:

$$v'(x^k + f_k(y^k)) \leq \lambda \qquad \text{with equality if } x^k > 0,$$

$$f_k'(y^k)v'(x^k + f_k(y^k)) + \frac{\rho}{\beta}Ev(m + \beta W^k)$$

$$\leq \lambda[1 - \phi'(y^k)g_k] \quad \text{with equality if } y^k > 0,$$

where λ is a Kuhn–Tucker multiplier for the first constraint. We have cancelled p_k from both sides of these inequalities, using the fact that $p_k > 0$ for all k.

We shall consider only solutions that are interior for other goods. Thus, all $x_k > 0$ and, since v' is strictly decreasing by Assumption 1, we derive:

Optimality Condition 1

$$x^0 + f_0(y^0) = x^1 + f_1(y^1) = ... = x^k + f_k(y^k).$$

It follows from Assumption I that $\lambda > 0$ and, hence, that the second part of the optimality condition can be written:

$$\xi_k(y^k) \leq 1 \text{ with equality if } y^k > 0, \qquad (1.10)$$

where

$$\xi_k(y) = f_k'(y) + \phi_k'(y)g_k + \lambda^{-1} + \frac{\rho}{\beta}Ev(m + \beta W^k).$$

Assumptions 2, 3 and 5 imply that:

1. $\xi_k(y)$ is strictly decreasing in y for a given k,
2. $\xi_k(y)$ is strictly increasing in k for a given y.

From (a) and (1.8), we deduce that, if $\xi_k(0) > 1$, then $y^k > 0$ and $\xi_k(y^k) = 1$; otherwise, $y^k = 0$. When $\xi_k(0) > 1$ for some $k = 0, ..., K$, we can define J to be the smallest k such that $\xi_k(0) > 1$. Then, (a) and (b) lead to:

Optimality Condition 2

If $\xi_K(0) \leq 1$, then $y^0 = \cdots = y^K = 0$, (Case NP) otherwise, $y^k = 0$ for $k < J$ and $y^k > 0$ with $\xi_k(y^k) = 1$ for $k \geq J$. (Case P)

Case P corresponds to lottery participation (when the rollover state variable is at least J) and NP to non-participation for any rollover state.

Properties (a) and (b) further imply that $y^k > y^j$ when $k > j \geq J$. Now observe that, by integrating the inequality in Assumption 3 from 0 to y, and using Assumption 2, we obtain $f_k(y) \geq f_j(y)$ for all $y \geq 0$ when $k > j$. Hence,

$$f_k(y^k) > f_k(y^j) \geq f_j(y^j)$$

using Assumption 2. In view of Optimality condition 1, we can conclude that $x^k < x^j$.

If $k > j$, then it follows from Assumption 2 that

$$0 < \int_{y^j}^{y^k} [1 - f_k^j(y)]\mathrm{dy} = y^k - y^j - \{f_k(y^k) - f_k(y^j)\}.$$

Suppose, further, that

$$f_k(y) = f_j(y) \text{ for } j, k = 0, \ldots, K, \text{ and all } y \geq 0,$$

i.e., additional participation following an increase in the rollover state is induced solely by the enlarged prize pool rather than by any additional fun. Then, using Optimality condition 1,

$$x^k + y^k - x^j + y^j = y^k - f_k(y^k) - [y^j - f_j(y^j)]$$
$$= y^k - f_k(y^k) - [y^j - f_j(y^j)] > 0.$$

Theorem 1.1. The optimal solution of CP* has one of the following forms:

Case NP: For all $k = 0, \ldots, K$, we have $y^k = 0$ and $x^k = m$.

Case P: There exits a $J \in \{0, \ldots, K\}$ such that

$0 < y^J < y^{J+1} < \ldots < y^K$ and $y^k = 0$ if $k < J$, and

$x^J > x^{J+1} > \ldots > x^K$ and $x^k = x^j$ if $k, j < J$, and

$x^J + y^J < x^{J+1} + y^{J+1} < \ldots < x^K + y^K$ when $f_k = f_j$ for j,

$k = 0, \ldots, K$.

For a potential participant the theorem says that positive participation will take place if the number of rollovers is at least J, as the rollover state increases, more is spent on lottery tickets, less on other goods. Nevertheless total expenditure increases if the fun function is independent of the rollover state.

Equilibrium/consistency

In this section we recognize that the collective decisions of all consumers will determine aggregate lottery expenditure and this influences both the transition matrix **Q** and the (random) jackpot prize **W** = (W^0, ..., W^K). Current lack of suitable data on heterogeneous purchases forces us to consider a homogenous population of identical individuals who engage in the gamble whatever the rollover state. Nonetheless, the arguments and formulae we develop below are readily extended to a population of heterogeneous agents. We assume that there are n individuals sharing the same preferences, reflected in the utility function v and the fun functions f_k, and the same income m. In rollover state k, the number of tickets sold is $n^k = ny^k$ and this affects both **Q** and **W** through the probability distribution of the number of prizewinners; if this number is 0 the jackpot prize pool is rolled over. To obtain this distribution, the simplest assumption to make would be that each combination of numbers (6 out of 49 for the UKNL) is chosen independently and with equal probability (i.e. = ρ). In this case, the number of winners would follow the binomial distribution $B(\rho, n^k)$ or, to a very good approximation, the Poisson distribution $P(\rho n^k)$. However, the equal probability assumption conflicts with observation in that it significantly under-predicts the probability that there are no winners [4, 8] and a consequent rollover. We will therefore assume that any ticket design induces a probability distribution χ over the set I of permitted combinations, such that the probability of the combination $i \in I$ being chosen on a randomly selected ticket is $\chi(i)$, independently of all other choices. Since all combinations are

drawn with probability $|I|^{-1}$, the distribution $Z_k(\chi)$ of the number of winners when the rollover state variable is k is an equally weighted combination of Poisson distributions. In particular,

$$\text{Prob}[Z_k(\chi)] = |I|^{-1} \frac{[\chi(i)n^k]^z}{z!} \exp\{-\chi(i)n^k\}. \quad (1.11)$$

The uniform case corresponds to $\chi(i) = \rho = |I|^{-1}$ for all $i \in I$; a simple, two-parameter generalisation is discussed in the next section. The probability of a rollover is given by

$$\pi_k = \text{Prob}[Z_k(\chi) = 0] = |I|^{-1} \sum_{i \in I} \exp\{-\chi(i)n^k\}, \quad (1.12)$$

and the expression for the stationary probabilities implies that

$$P_k \alpha \tilde{P}_k = \prod_{i=0}^{k-1} \pi_I \text{ if } k > 0, \text{ and 1 if } k = 0. \quad (1.13)$$

We now turn to the distribution of W^k, the jackpot prize from the perspective of a participant who will use $\chi(\cdot)$ to choose their combination. The total jackpot prize pool in rollover state k is a proportion α of the expenditure on tickets net of taxes and operating costs augmented, if $k \geq 1$ by the jackpots rolled over from the previous k-1 draws. Hence the total jackpot prize in this state is $\alpha(1- \tau - \kappa)n^{-k}$, where $n^{-k} = \sum_{l=0}^{k-1} n^l$, and κ is the proportion of revenue attributed to operating cost and profit. For any ticket, conditional on the combination $i \in I$ being drawn, the number of winning tickets excluding that ticket is a random variable Z_i^k where $Z_i^k \square P\{\chi(i)(n^k - 1)\}$. It follows that a winning ticket will receive a jackpot prize of $\alpha(1- \tau - \kappa)n^{-k} / (Z_i^k + 1)$. Since the probability that i is chosen, given that a ticket wins, is $\chi(i)$, we deduce that the probability distribution of the size of jackpot prize is:

$$\text{Prob}[W^k = w] = \sum_{i \in I} \chi(i) \text{Prob}\left[\frac{\alpha(1-\tau-\kappa)n^{-k}}{Z_i^k + 1} = w\right]. \quad (1.14)$$

(We have ignored the highly unlikely possibility that any participant wins two or more jackpot prizes in a given draw.) Using [2], we can write, by rearranging the series, for $k = 0, \ldots, K$:

$$Ev(m + \beta W^k) = \sum_{i \in I} \chi(i) \exp\{-\chi(i)(n^k - 1)\}$$

$$\times \sum_{z=0}^{\infty} \frac{[\chi(i)(n^k - 1)]^z}{z!}$$

$$\times v\left(m + \beta \frac{\alpha(1-\tau-\kappa)n^{-k}}{z+1}\right).$$

Since non-jackpot prizes are not subject to rollovers, a proportion $(1 - \alpha)(1 - \tau - \kappa)$ of the total expenditure on tickets is allocated to non-jackpot prizes and the average prize is worth g_k in state k. As all tickets are equally likely to win, the probability of winning must be $(1 - \alpha)(1 - \tau - \kappa) / g_k$. We shall further assume that ϕ is linear (since y is small):

$$\phi(y) = \frac{(1-\alpha)(1-\tau-\kappa)}{g_k} y. \quad (1.15)$$

in which case the term $\phi(y^k)g_k$ in the constraint of CP* can be replaced with $(1 - \alpha)(1 - \tau - \kappa)y^k$. We can therefore write the (necessary and sufficient) first-order conditions for households in state k as:

$v'(x^k + f_k(y^k)) = \lambda$ for $k = 0, ..., K$, (1.16)

$$\Delta^k \equiv f_k'(y^k) - [1-(1-\alpha)(1-\tau-\kappa) + \frac{\rho}{\lambda\beta} Ev(m+\beta W^k) \quad (1.17)$$

$$\leq 0, \text{ and } \Delta^k y^k = 0 \text{ for } k = 0,...,K,$$

$$\sum_{k=0}^{K} \tilde{P}_k \{x^k + [1-(1-\alpha)(1-\tau-\kappa) + y^k - m\} = 0. \quad (1.18)$$

Note that our assumption of homogeneity forces $y^k > 0$ for all k so that the inequality in [1] is satisfied as an equation. The equilibrium solutions (x, y) are the solutions of equations [1], [9] [10], [12] and [15].

Simulations

In this section, we apply the model to the UKNL and so we need to select specific functional forms for the utility function, fun functions, and the distribution χ. The limited variability in the data dictates the use of parsimoniously parameterized functions and, for the utility and fun functions, we choose functions with a single parameter. Individual preferences are assumed to exhibit constant relative risk aversion (CRRA):

$$v(x) = \frac{x^{1-\theta}}{1-\theta}. \quad (1.19)$$

The parameter θ characterizes relative risk aversion (defined as $-xv''(x)/v'(x)$) and is assumed constant. The literature tends to favor CRRA as a parsimonious and accurate description of individual behaviour.[2], [9]

A convenient model for the fun function is a power function.[10] However, in order to satisfy Assumptions 2 and 3, we need to transform it into the form

$$f_k(y) = (1+y)^{\eta_k} - 1, \quad (1.20)$$

where $0 < \eta_j \leq \eta_k < 1$ for $K \geq k > j \geq 0$. For simplicity, in the simulations we assume that $\eta_k = \eta$, $^{\forall}k$. These are the conditions in the last part of Theorem 1.1, which allow us to deduce that expenditure increases with the rollover.

The distribution χ needs to be parsimoniously parameterised and not tied too closely to a particular ticket design (since changing ρ entails changing the design). A simple way to do this is to assume that a proportion $1 - \xi$, $0 < \xi \leq 1$, of the $|I|$ possible combinations are never chosen and that the remaining combinations are equally likely to be chosen, with probability ρ/ξ. A slightly more sophisticated version assumes that some participants use this distribution and others choose uniformly. Indeed, in many lotto games, computerized pseudo-random generation of combinations is offered to participants (called 'Lucky Dip' in the UKNL). Let $v \in (0, 1)$ be the proportion of tickets on which combinations are chosen in this way. Then $\chi(i) = v_\rho(= \xi_1)$ if i is a forbidden combination, and $\chi(i) = (1 - v + \zeta v)(\rho/\xi)(= \xi_2)$, otherwise. It follows from [15] that the probability of a rollover in state k is:

$\pi_k = (1 - \zeta) \exp\{\xi_1 n^k\} + \zeta \exp\{-\xi_2 n^k\}$

and [9] can be written

$Ev(m + \beta W^k) = (1 - \zeta)\gamma v_1 + (1 - \gamma + \zeta\gamma)v_2$,

where, for $i = 1, 2$,

$$v_i = \exp\{-\xi_i(n^k - 1)\}\sum_{z=0}^{\infty}\frac{[\xi_i(n^k-1)]}{z!}$$

$$\times v\left(m + \beta\frac{\alpha(1-\tau-\kappa)n^{-k}}{z+1}\right).$$

We take α and κ, the jackpot proportion and operating costs, from the current design and consider values of τ, the tax rate, and β in a range of 4% either side of the current values, while we put income equal to the average UK weekly household expenditure and *N* to the number of UK households. We assume an annual interest rate of 10% and set the maximum number of rollovers *K* to 2 reflecting the maximum observed value. This leaves setting the values of the parameters: ξ, the proportion of combinations available for selection, θ, the coefficient of relative risk aversion, and η, the fun function parameter, to complete the calibration. The values are chosen such that (i) sales in normal (non-rollover) weeks (ii) sales in single rollover weeks, and (iii) the long run proportion of normal weeks agree with observed values. We note that the fitted value of β falls within the commonly observed range of values (i.e., between 0.5 and 3).

In Figure 1 we present contour plots in the (τ + κ, ρ) plane for the parameter values in Table 1. The panels illustrate how local changes in the design affect the expected tax revenue (top left panel), the expenditure per household on the lottery during a non-rollover week (top right panel) and during a single rollover week (middle right panel), the expected number of jackpot winners during a non-rollover week (middle left panel), the expected size of a jackpot in a non-rollover week (bottom right panel), and the long run proportion of non-rollover weeks (bottom left panel). With the parameters set at the calibrated values, we see that increasing ρ, and more surprisingly, decreasing τ, leads to an increase in expected tax revenue. In the former case, although the number of rollovers and therefore of potential additional revenue associated with more rollovers falls, this is more than offset by the increased participation induced by the increased chance of a jackpot win; the number of winners also increases, indeed sufficiently so that expected (normal week) jackpots fall in spite of the enlarged jackpot prize pool. Decreasing τ also increases participation sufficiently to offset the reduced tax take per ticket.

Normal and rollover week expenditure rise (in contrast to increasing ρ), the number of rollovers falls and the expected number of winners and the expected jackpot both increase. Changing the parameters from the current values in a direction of steepest ascent of the expected tax revenue function has the same effect on all the graphed functions as increasing ρ. (The expected jackpot decreases slightly).Before drawing policy conclusions from these results, it is appropriate to examine the sensitivity of the results to the parameter values and functional forms. Indeed, it is precisely because they will be particularly sensitive to such model details that we have refrained from suggesting optimal values. To investigate these issues we have experimented by varying the parameter values (re-calibrating where appropriate) and with different functional forms such as a constant absolute risk aversion utility function. The results of these experiments are too numerous to include in the chapter but most of the qualitative features of the functions plotted in Figure.1 remain robust to these changes (details are available from the authors). In particular, the expected tax revenue function always appears to be upward sloping at current parameter values in a west to northwest direction. We suggest that this evidence is sufficient to prompt further investigation into whether the tax rate in the UKNL may be too high and whether the probability of winning the jackpot may be too low.

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Table 1 - Simulation parameters.

$K = 2$	$\kappa = 0.1$	$\beta = (1+0.1)^{1/52} - 1$	$\theta = 0.767$
$N = 29 \times 10^6$	$\alpha = 0.5$	$\rho = (7.15 \pm 0.35) \times 10^{-8}$	$\eta = 0.819$
$m = 270$	$\gamma = 0.15$	$\tau = 0.4 \pm 0.02$	$\zeta = 0.684$

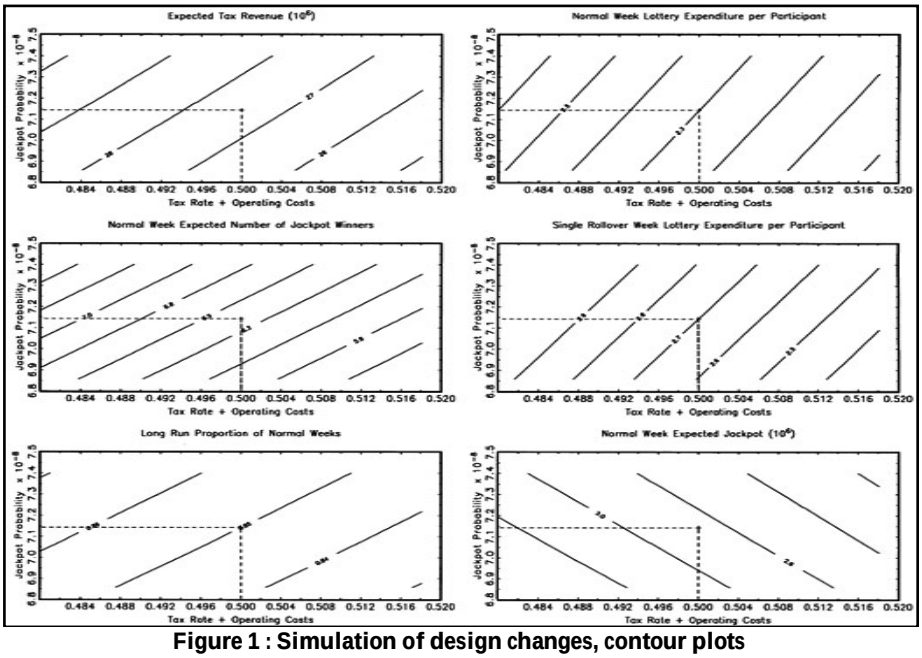

Figure 1 : Simulation of design changes, contour plots

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REVIEW ARTICLE

Aetiopathogenesis and Management of Dry Socket (*Alveolar osteitis*): A Review

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ABSTRACT

One of the most common postoperative complications following the extraction of permanent teeth is a condition known as alveolar osteitis (AO). The objective for this article is to harmonize descriptive definitions for the condition known as alveolar osteitis and to critically review and discuss managenent of alveolar osteitis. The requirement for the identification and elimination of risk factors and in addition the preventive and symptomatic management of the condition are talked about. The aim of this critical review is to provide a better basis for clinical management of the condition. Ameta-analysis of data was not done.

Keywords: Dry socket, alveolar osteitis, management, complications, pain

INTRODUCTION

A standout among the most common postoperative complications following the extraction of permanent teeth is a condition known as dry socket[1]. Crawford first used the term "dry socket" in 1896[2]. From that point forward, a couple of various terms have been used in referring to this condition, such as localized osteitis, alveolar osteitis, postoperative alveolitis, alveolalgia, alveolitis sicca dolorosa, septic socket, necrotic socket, localized osteomyelitis, and fibrinolytic alveolitis. Up until now, authors do not agree on terminology for this condition. In his fundamental articles, Birn labelled the complication 'fibrinolytic alveolitis' which is presumably the most accurate of all the terms, but is also the minimum utilzed in the literature[3].In this article, the condition will be referred to as alveolar osteitis, AO. The incidence of dry socket is higher in the mandible, happening up to 10 times more often for mandibular molars compared with maxillary molars[4].One likewise observes an extraction socket devoid of clot with exposed bone that may be filled with food debris, edema of the surrounding gingiva[5], and local lymphadentitis. Fever is uncommon, and the condition is usually self-limiting unless the patient has had radiotherapy or is immunocompromised[6]. The pain that is experienced by patient can be very debilitating, exceptionally crippling causing loss of sleep and affecting day by day work. This pain poorly responds to over-the-counter and narcotic analgesics and can radiate to the temple, ear, and neck. Dry socket is not characterized by redness, fever, swelling, and pus or discharge formation. The treatment of AO depends on each professional's clinical experience mainly due to the fact of its complex etiology, although many authors have published research on the management of dry socket[7].

Etiology

A couple of hypotheses have been shown on the etiology of dry socket. They consolidate bacterial infection, injury, and biochemical agents. Birn in 1973 demonstrated expanded fibrinolytic action and activation of plasminogen to plasmin within the site of of tissue activators indry sockets. This fibrinolytic action is thought to tinfluence the integrity of the post extraction blood clot. Despite the fact that AO is for the most part acknowledged to be multi factorial origin, the accompanying have been embroided most commonly as aetiological, aggravating and encouraging factors:

1. Oral micro-organisms and AO -the role of bacteria in AO has long been postulated. This concept was supported by various reports of the increased frequency of AO in patients with poor oral hygiene, pre-existing local infection such as pericoronitis and advanced periodontal. There have been numerous attempts to isolate a specific causative organism. The possible association of Actinomyces viscosus and Streptococcus mutans in AO was featured by ROZANIS et al, where they showed delayed healing of the extraction socket following the inoculation of these organisms in animal models[8]. NITZAN et al showed a possible significance of anaerobic organisms(which are also the predominant organisms in pericoronitis) in relation to the aetiology of AO. NITZAN et al. observed highplasmin-like fibrinolytic activities from cultures of the anaerobe Treponemadenticola, which is also known to be aputative micro-organism in the development of periodontal disease.

2. Difficulty and trauma during surgery and AO Most authors agree that trauma and difficulty of surgery play an important role in the development of AO. Surgical extractions that involve the reflection of a flap and sectioning of the tooth with some degree of bone removal have also been reported to be more likely to cause AO. One interesting study indicates that less experienced surgeons caused a significantly higher incidence of complications after the removal of impacted third molars; the most common complication being AO[10].Excessive trauma has been known to result in delayed wound healing. This has been attributed to the compression of the bone lining the socket, which impairs its vascular penetration. Alternatively, excessive trauma sometime results in thrombosis in the underlying blood vessels. BIRN proposed that trauma during extraction damages the alveolar bone cells, causing inflammation

of the surrounding alveolar bone marrow and the release of direct tissue activators into the alveolus, where they may precipitate fibrinolytic activity, thus playing a major role in the pathogenesis of AO.

3. Roots or bone fragments remaining in the wound and AOBIRN suggested this complication as a possible cause of AO. Such fragments are usually present after normal extraction or surgical removal of teeth, and that small bone and tooth remainders do not really cause complications during healing as they are often externalized by the oral epithelium. Despite a lack of scientific evidence for these remnants to be the causative factor for AO, it seems logical that fragment and debris remnants could lead to disturbed wound healing, and thereby possibly contribute to the development of AO[11].

4. Excessive irrigation or curettage of the alveolus after extraction and AO It has been postulated that vigorous repeate dirrigation of the alveolus socket might interfere with clot formation and give rise to infection, and that violent curettage might injure the alveolar bone. However, scientifically sound investigations confirming these contributions in the development of AO are lacking.

5. Physical dislodgement of the clot and AO-It has not been substantiated that the physical dislodgement of the blood clot either by manipulation or negative pressure, such as sucking on a straw, would be a major contributory factor to AO.

6. Local blood perfusion, anesthesia and AO - Three aspects of blood supply have been confused in the literature; the vascular architecture, the circulation, and the integrity of the blood clot[12]. KRUGER associated poor local blood supply with an increased incidence of AO in mandibular molar extractions. The presence of thick cortical bone, it was suggested, resulted in the poor perfusion of blood and it was suggested that minor perforations into the alveolar marrow cavity would allow blood vessels to grow in more easily. The vasoconstrictors in local anaesthetic solutions have been suggested as alternative factors in the pathogenesis of AO. On the other hand, AO also follows tooth extractions carried out under general anaesthesia where novaso constrictor was used. Some investigators claimed an increase in the incidence of AO when periodontal intraligamental (PDL) injections were used rather than block or infiltration injections. These findings have been attributed to the spread of bacteria, especially with multiple injections to the affected site.

7. Smoking and AO have reported that among patients with a total of 400surgically removed mandibular third molars, the individual who smoked a half-pack of cigarettes per day had a four- to five-fold increase in AO (12% vs 2.6%)contrasted with non-smoking patients. The occurence of AO extended to more than20% among patients smoking more than a pack per day, and to 40% among patients who smoked on the day of surgery, or on the first postoperativeday. This phenomenon could be because of thepresentation of a foreign substance that could act as a contaminant in the surgical site, and/or the suction applied to the cigarette which might dislodge theclot from the socket and interrupt healing. No references exist in the literature correlating the effects of heat from burningtobacco, contaminants in the smoke, or the systemic effects of the ingredients in cigarettes with AO.

Pathogenesis

Clinical and laboratory studies have shown the significance of locally increased fibrinolytic activity in the pathogenesis of AO[13].Blum claimed that partial or completely is and destruction of the blood clot was caused by tissue kinases liberated during inflammation by a direct orindirect activation of plasminogen in the blood. "At the point when coordinate tissue activators are discharged after injury to the alveolar bone cells, plasminogen (which is set down in the fibrin arrange as it is framed) is converted to plasmin, resultingin the breakup of the clot by disintegrating the fibrin. In trinsicactivators originate from plasma components whereas extrinsic activators originate outside of the plasma/blood perse. Direct intrinsic activators include FactorXII (Hageman factor)-dependent activator and urokinase, which are mediatedby leukocytes. Direct extrinsic activatorsincorporate tissue

plasminogen activators and endothelial plasminogen activators. Tissue plasminogen activators are foundin most tissue composes, including alveolarbone. Indirect activators incorporates substances such as streptokinase and staphylokinase, which are delievered by bacteria and bind to plasminogen to form an activator complex that then cleaves other plasminogen molecules toplasmin[14]. This strengthens the theory ofthe involvement of micro-organisms in the development of AO.

Preventive Measures

Antibiotics

The prevention of dry socket has in the past included both pharmacologic and surgical approaches. Pharmacologic techniques used in the prevention of dry socket have included use of antibiotic preparations placed into the socket after extraction and antiseptic rinses. Use oftetr acycline-impregnated gelatin sponges or Gelfoam,clindamycin-impregnated Gelfoam, lincomycinin Gelfoam, Placement of tetracyclinein 200 postextraction sockets was recently shown to reduce post extraction pain and trismus, although there was no significant effect on incidence of drysocket in that study[15]. Caution must be taken with drytetracyclin powder, because it has been linked to agiant-cell reaction in 1 case, although aqueous tetracycline has not shown such a reaction. In a recent review, Blum stated that although penicillin, clindamycin, and erythromycin have had positivereports, of all systemic antibiotics metronidazole has shown the greatest promise in randomized double-blind studies. Blum went further to express that metronidazolehas a narrower spectrum and targets essentially anaerobes, therefore decreasing the possibility of bacterialresistance as well as being associated with fewer side effects than erythromycin, penicillin, or clindamycin. Astudy by Rood and Danford demonstrated critical painrelief within 24 h of utilization of metronidazole.These findings support that anaerobic organisms are potentially associated with the pathogenesis of dry socket.However, a number of authors, including Fazakerley and Field prescribed that the use of systemic antibioticsis not necessary in nonimmunocompromised patients unless there are symptoms of malaise and lymph adenopathy. They recommend that the use of antibiotics in the extraction socket be reserved for those with history of multiple dry sockets or for immune compromised patients. Chlorhexidine rinses In view of the risk of aimless use of antibiotics,research was coordinated into looking at the effects of chlorhexidine rinses on dry socket. Rango and Szkutnik59 showed a50% reduction in dry socket in patients who prerinsed for 30 s with a 0.12% chlorhexidine gluconate solution. Hermesch et al. found a 38% diminishment in the rate of incidence of dry socket in patients who rinsed 30 s with 15 mL 0.12% chlorhexidine gluconate for 1 week prior and 1 week after the extraction, with no side effects because of chlorhexidine use reported. Similarly Tjern bergfound that good plaque control and oral hygiene candiminish the rate of incidence of dry socket after removal of mandibular third molars.

Prophylactic Management

In an era of evidence-based care, fewareas of clinical debate pose as substantial a dilemma to clinicians, as the topic of the alleged factors that are focuses for the different preventive regiments, and the topic of what prophylactic medicaments and materials, if any, should be placed in an alveolar socket following exodontia. References in the literature associating to the prevention of AO can be divided into non-pharmacological and pharmacological preventive measures. Successful non-pharmacological preventive measures incorporate a thoroughhistory of the patient with identification, and if conceivable, elimination of risk factors relate with an increased riskto develop AO. Moreover, besides the possible elimination of risk factors, it is imperative for active nonpharmacological preventive measures to be implemented. Because AO is probably the most common local post-extraction complication that exists, a successful method of pharmacological prevention has long been sought. These pharmacological prophylactic interventions are related to one or more of the following groups:

1. Antibacterial agents
2. Antiseptic agents and lavage
3. Antifibrinolytic agents

4. Steroid anti-inflammatory agents
5. Obtundent dressings
6. Clot support agents

Antibacterial agents

Prophylactic antibacterials, either given systemically or used locally, are considered to reduce the incidence of AO. Systemic antibacterials reported to be effective in the prevention of AO include the penicillins, clindamycin erythromycin and metronidazole. In addition, it has been reported that the preoperative administration of antibacterial agents is more effective in reducing the incidence of AO than when given post operatively. However, of all systemic antibacterials referred to in the literature for the prevention of AO, the only that stood trial successfully inrandomized double-blind studies was metronidazole. Owing to its narrow antibacterial spectrum (an aerobicidal), metronidazole is associated with fewer and more infrequent side-effects than the high resistance developing penicillins and erythromycin, and the pseudomembranous colitis inducing clindamycin [16]. Caution should however be taken with metronidazole in patients taking warfar in, disulfiram, phenytoin and possibly antihypertensives because of possible drug interactions. Concurrent alcohol should be avoided. Nowadays, the routine use of systemic pre- and/or postoperative antibacterials given prophylactically is highly disputed and by many considered to be controversial because of the development of resistant bacterial strains and possiblesystemic side effects, such as hypersensitivity and unnecessary destruction of host commensals.

Myriad studies have been carried out to evaluate the effectiveness of topical (intra-alveolar) medicaments in preventing AO including various types of antibacterials used alone or in combinationin varying formulations and dosages. However, very few studies are in agreement. CHAPNICK & DIAMOND investigated in a double-blind study the viability of topical clindamycin and they reported a significantly lessened rate of AO in mandibular third molar sockets following light socket irrigation with Betadine and the topical use of clindamycin in Gelfoam. They ascribed their findings to the viability of clindamycin however the irrigant utilized by them before wound closure is an iodophore with its own antimicrobial properties. Moreover, the subjects of their examination also received multiple oral doses of systemic antibiotics postoperatively; thus making it difficult to attribute their findings to either of the antibacterials alone used in their study. TRIEGER & SCHALAGEL inspected in a double-blind crossover study including 86 patients with 172 bony impacted mandibular third molars, the effect of the topical placement of clindamycin saturated gel sponge embedded into randomized unilateral extraction sites promptly following surgery. Every patient received a placebo in the contralateral site and served as his or her own control. The authors detailed 7 cases ofAO in the control group and none in the test group. Based on the significant differencein AO rates at a significance level of $P<0.5$ they inferred that the etiology of AO is related to an infection with anaerobic bacteria and that clindamycin applied topically can be effective in the prevention of AO. Many studies with topical tetracycline powder, aqueous suspensions of tetracycline, tetracycline on gauze drain ortetracycline-soaked Gelfoam sponges have been reported to be effective in significantly reducing the incidence of AO. The latter mixture is thought to provide a firm clot inaddition to preventing infection. However, side-effects including foreign body giant-cell reactions have been reportedin association with topically applied tetracycline.

Antiseptic agents and lavage Chlorhexidine (CHX) is a bisdiguanide germicide with antimicrobial properties. The use of CHX as both a mouthrinse and as a preoperative irrigant of the gingival crevice has been shown to significantly decrease the amount of oral microbial populations. Several studies have reported that the pre- or perioperative utilization of CHX mouthrinse significantly reduces the incidence of AO after the surgical removal of mandibular third molars. RAGNO & SZKUTNIK noted nearly a 50% reduction in the incidence of AO in patients who prerinsed for 30 s with a 0.12% CHX solution. FOTOS et al. analyzed in a randomized double-blind placebo-controlled study involving 70 patients with 140 uncomplicated non-infected third molars, the effect of the topical insertion of an intra-alveolar chlorhexidine gluconate solution-soaked Gelfoam into an extraction site and contrasted

it with an intra-alveolar saline-soaked Gelfoam inserted on the contralateral side. They detailed that the use of the previous significantly reduced postoperative inconvenience, but the incidence of AO was not particularly recorded. They additionaly detailed that the 0.1% chlorhexidine solution did not essentially diminishpostoperative discomfort whereas the use of the higher 0.2% concentration was significantly efficacious in reducing these symptoms. The authors recognized that Gelfoam displays a degree of hydrophobicity that precludes efficient absorption of chlorhexidine before intraalveolar placement. Also, pre-shaped Gelfoam morphology does not allow its placement to the full depth of the socket. No reference was found in the literature correlating neither the local applications of the biodegradable chlorhexidine Periochip_ nor that of chlorhexidine Corsodyl_ gel with AO. In a crossover study the antiseptic agent, 9-aminoacridine, saturated in Gelfoam was placed in mandibular third molar extraction sites, and was compared with the use of Gelfoam alone placed in the contralateral mandibular third molar extraction sites. The authors concluded that 9-aminoacridine was incapable in lessening the incidence of AO

Antifibrinolytic agents - Earlier investigations into the fibrinolytic nature of AO indicated that the topical use of para-hydroxybenzoic acid (PHBA), in extraction wounds significantly decreased the incidence of AO in a dose-dependent fashion. However, as PHBA is available on the market as a component of Apernyl_ (Bayer AG, Germany)—an alveolar cone with a formulation of 32 mg acetylsalicylic acid, 3 mg propyl ester of PHBA and 20 mg obscure tablet mass, it is not conceivable to credit the detailed findings to PHBA alone or maybe to the anti inflammatory properties of acetylsalicylic acid. In addition, PHBA has also been reported to have some antibacterial properties which may also have contributed to the reported findings [17]. Subsequent histological studies16 however, demonstrated that acetylsalicylic acid in contact with bone causes a local irritating effect joined by serious inflammation of the extraction socket, potentialy resulting in AO. The antifibrinolytic agent Tranexamic acid (TEA) has been reputed to prevent AO when applied topically in the extraction socket following exodontia, but controlled investigations with special reference to impacted mandibular third molar extraction wounds have not shown a significant reduction in the incidence of AO when compared to a placebo group [27]. Given the lack of a scientifically confirmed advantage, and many possible problems, it seems to be no rationale for the use of these agents.

Steroid anti-inflammatory agents - Only one reference was found in the literature regarding the individual use of topical corticosteroids in the prevention of AO. Even though the corticosteroid has been reported to decrease immediate post-operative complications, it failed to reduce the occurrence of AO after extraction. The topical utilization of a hydrocortisone and oxytetracycline mixture however, has been appeared to significantly diminish the frequenvy of AO after the removal of impacted mandibular third molar surgery.

Obtundent dressings A current crossover study12 on the prevention of AO following the bilateral removal of 200 mandibular molars asserted a significant decline in the incidence of AO, following the prompt placement of an eugenol containing dressing into arbitararily selected unilateral extraction sockets. The contralateral sockets were not packed and served as the patients own controls. However, the irritant local effect of eugenol and the delay in wound healing due to elective prophylactic packing is well reported in the literature.

Clot support agents - In the 1980s, a biodegradeable ester polymer, polylactic acid (PLA) was broadly advanced as the ultimate solution for preventing AO, and it is still accessible today under the brand name of DriLac (Osmed, Inc, Costa Mesa, CA, USA). It was recommended that PLA would give a biological stable support for the blood clot and for the future granulation and osteoid tissue. Zinc oxide eugenol (ZOE)-Gauze or ointment formulation. Antiseptic and anesthetic properties, as it depresses sensory receptors associated with pain perception[18]. Alvogyl (Septodont, Cambridge, Canada) - Includes eugenol as a pain relieving, iodoform as an antimicrobial and butamen as anesthetic[19]. Vitamin C Tablet formulation-Wound healing promoter and antioxidant action that decreases infection and inflammation[20]. SaliCept Patch (Carrington Laboratory, Irving, USA) - Contains Acemannan hydrogel, obtained from the clear inner gel of Aloe Vera ,which advances wound healing, augments reticuloendothelial function,

regulates the immune response and acts as an anti-inflammatory and antibacterial agent. Plasma rich in growth factors (PRGF) -Contains platelets and fibrinogen, so it advances wound healing as well as osteogenesis. Platelet-derived Growth Factor (PDGF) and Tissular Growth Factor (TFG) are some of the Growth Factors in this plasma[21]. Topical anesthetic gel Oraqix (Dentsply Pharmaceutical, York, USA). Contains 2.5% prilocaine, 2.5% lidocaine, thermosetting agents, hydrochloric acid and purified water. Antiseptic and anesthetic properties. Low level laser therapy (LLLT) (Lambda Laser Products, Vicenza, Italy)- Antimicrobial potential and increases the speed and quality of wound healing. 808 nm, 100-mW continuous mode gallium aluminium arsenide diode laser

SUMMARY

The event of dry attachment in an conventional oral surgery or dental practice is unavoidable. The risk factors for this temporary and cripling condition areclearly identified. However, adherence to sublime surgical technique in a young, healthy, and non-smoking male patient still conveys a 1%-4% incidence of dry socket. Surgeons must precieve additional risk factors in patients with particular medical conditions and include this information as a part of the informed consent. Treatment options for this condition are generally limited and directed toward palliative care. The surgical site should be irrigated with copious saline irrigation, avoiding curetting the extractionsocket. Packing with a zinc oxide– eugenol pasteon iodoform dressing can be considered to cause relieve acutepain episodes. Eventually it is the host's healing potential which determines the severity and duration of the condition. Prevention methods incorporate avoiding smoking 24h before and after surgery and a traumatic surgery with removal of bone and tooth pieces under copioussaline irrigation. Placement of topical antibiotics, suchas tetracycline, lincomycin, or clindamycin, on Gel-foam can be considered, while systemic antibiotics should be reserved for patients who are immune compromised.

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RESEARCH ARTICLE

A Novel Isolation and Spectral Characterization of Protein Isolates from Red Marine Sea Weed

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ABSTRACT

Marine organisms represent a major valuable and novel source of the new compounds. The distribution and biodiversity of the marine source along with chemical diversity, based on this prolonged and practically unlimited resource of new active substance in the inversion and development of bioactive products. Moreover, *Hypnea valentiae* red algae, selected to identify its chemical constituent by using spectral analysis. The potent *Hypnea valentiae* macerated by using various solvent, from this more potent Phyto constituent extract were selected. Aqueous extract of *Hypnea valentiae* , further subjected into SDS PAGE technic, for the isolation of protein. The aqueous extract of *Hypnea valentiae* were stained with comassie blue dye at pH7.2 turn out and produced 4 band within the gel Teflon. The separated biomolecule isolates were named as *Hypnea valentiae* 1 (HV1), *Hypnea valentiae* 2 (HV2), *Hypnea valentiae* 3(HV3), Hypneavalentiae(HV4). Spectral analysis of the isolated compounds were carried out, to find out the Chemical nature of isolated compound. From the spectral studies *Hypnea valentiae* 1 - Lecithin, Hypneavalentiae 2 - Tryptophan, *Hypnea valentiae* 3-Astaxanthin and *Hypnea valentiae* 4-Hesperidin.

Keywords: Red sea weed, *Hypnea valentiae* , Isolation and Spectral Analysis

INTRODUCTION

Seaweed research has been carried out for more than seven decades by many research workers. Research has been done separately in different aspects accordingly to our need. The main objective of the present review is to gather information relating to nutritional, pharmacological, clinical, biochemical, industrial uses and its application to human welfare. To date, researchers have isolated approximately 7000 marine natural products, 25 percent of which are from algae [1] The antimicrobial properties of seaweed extracts are well documented over the years. Hence the present work was focused to the isolation of protein from the sea weeds containing whole plant parts of *Hypnea valentiae* with the proteomic studies [2].

MATERIALS AND METHODS

The whole plants of *Hypnea valentiae* were shade dried and coarsely powdered. About 1000 gm of the coarsely powdered plant materials were extracted separately by cold maceration procedure successively with various solvents in the range of increasing polarity (Hexane, Chloroform, Ethyl acetate, Ethanol, and water). After 20 days of extraction, the solvent was filtered and distilled off. Finally traces of solvent was removed under vacuum. The colour of the extract were noted and its percentage yield were calculated [3].

Preparation of Polyacrylamide Gel

The glass plate was cleaned and assembled with the help of the strip and clips. The separating solution was poured into the gap with the help of pipette. The n-butanol was carefully over laid and the plate was kept in vertical position at room temperature. After polymerization completed, n- butanol was poured out. Then the top the gel was washed several times to remove any un-plymerized acrylamide [4]. The freshly prepared stacking gel solution was poured directly on to the surface of the polymerized resolving gel. Immediately inserted a clean Teflon comb into the stacking gel solution. Then some stacking gel solution was added to fill the space of the comb completely. The gel was placed in vertical position for 30 min at room temperature[5].

Running the Gel

After completion of polymerization the Teflon comb was removed carefully. The gel was mounted in the electrophoresis apparatus. The triglyceride electrophoresis buffer was added to the top and bottom reservoirs. Low molecular weight market was loaded into the bottom of the walls and also the protein sample was added into the well. The SDS – PAGE apparatus was attached to an electric power supply [6]. When the dye front came to 0.25cm above the bottom of the gel the power was turned off. Then the gel plates were removed and were gently separated out. The gel was rinsed with 100ml of distilled water. Then the gel was covered with staining solution (Coomasisie Brilliant Blue) and was kept for overnight on a rocker. The gel was washed with distilled water for 5 minutes and the bands pattern were observed. The gel was photographed and analyzed [7].

Precipitation of Protein by Salting out Method

The isolated were subjected to precipitation by using 60% saturated ammonium sulfate solution and stirred with magnetic stirrer. The pellets obtained was separated by centrifugation and used for further studies [8].

1. Purification of Isolated Proteins :
2. Partial purification was done by Dialysis method :
3. The isolated were purified by dialysis using PBS (pH 7.2)

Purification by Column Chromatography: [9,10]

The isolates was purified by column chromatography using column packed with Biogel P30 slurry and Band formation shown after development time, shown in Fig. no 1.

Spectral Analysis of Isolated Biomolecules:[11]

IR spectrum was recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν max in4000-400cm-1). 1 H NMR (500 MHz) spectrum was recorded on a Bruker Avance II 500 spectrometer, with 5 mm TUBES, using $CDCl_3$ as a solvent and TMS as internal standard (chemical shift in δ ppm). Mass analysis was carried out by using Q-T of Mass Spectrometer (Micro mass) with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI). Daltons. Source options: Electron impact (EI); Chemical ionization (CI) [12].

DISCUSSION

Based on phytochemical investigation, aqueous extract of *Hypnea valentiae* shown the presence of more no of phyto compounds such as carbohydrate, Glycosides, Flavonoids, Tannins, Phenolic compound and protein, as compared to other extracts. Generally the protein molecules are denatured in the remaining polar solvents [13. The potentcyof marine sea weeds, is due to the presence of protein along with the amino acids. Based on the screening analysis report, the potent extract were selected and consider for the isolation of biomolecules. The isolated biomolecules were screened for the endometrial cytotoxicity and compared with the potent extract. In this regard, the aqueous extract of *Hypnea valentiae* was selected for the further isolation of biomolecules and its spectral analysis [14]. In this method the aqueous extract of *Hypnea valentiae* were stained with comassie blue dye at pH7.2 turn out and produced 4 band within the gel Teflon. The separated biomolecule isolates were named as *Hypnea valentiae* 1 (HV1), *Hypnea valentiae 2* (HV2),*Hypnea valentiae 3*(HV3), *Hypnea valentiae*(HV4). The above biomolecules will be screened for the *in-vitro* cytotoxicity studies against the endometrial cancer cell line HEC 1A [15].

CONCLUSION

The synergetic significant activity was due to the presence of Phytoconstituents such as Lecithin, Tryptophan, Astaxanthin and Hesperidin was concluded by the spectral analysis. Based on that, the marine sea weed *Hypnea valentiae*cure the endometrial cancer due to the presence of Lecithin, Tryptophan, Astaxanthin and Hesperidin.

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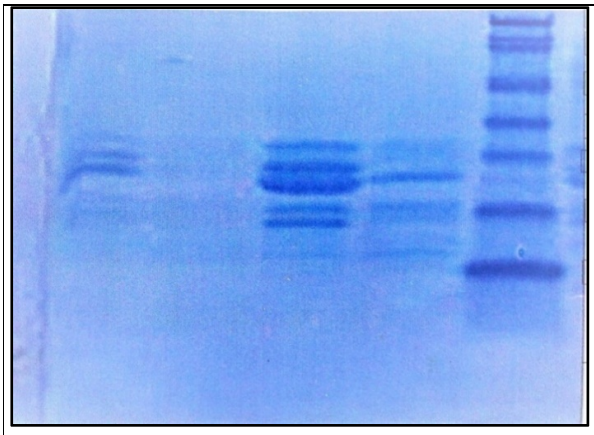
RESULTS

Table No.1 Phytochemical Investigation of *Hypnea valentiae*

S.No	Chemical Test	Extracts				
		Hexane	Chloroform	Ethyl Acetate	Ethanolic Extract	Aqueous Extract
1.	Alkaloids	-	-	-	-	-
2.	Glycosides	-	-	-	-	+
3.	Carbohydrate	-	-	-	-	+
4.	Protein	-	-	-	-	+
5.	Amino acid	-	-	-	-	+
6.	Saponin	+	+	-	-	-
7.	Flavonoid	-	-	+	+	+
8.	Phenolic Compound	-	-	-	-	+
9.	Tannin	-	-	-	-	+
10.	Terpenoids	-	-	-	-	-
11.	Oils and fats	+	+	-	-	-
12.	Steroids	+	+	-	-	-

+ Presence - Absence



Fig,1 Band Formation in Gel column of SDS PAGE

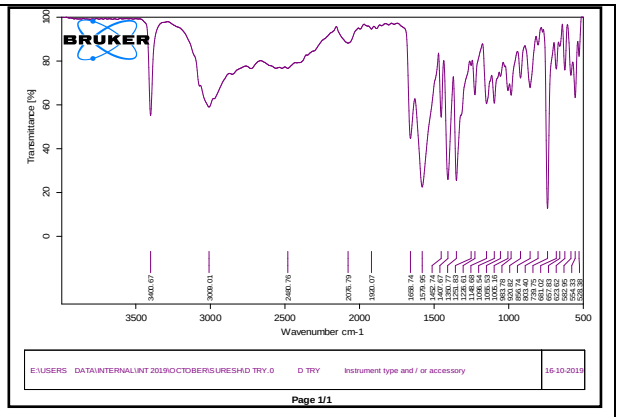


Fig:2-IR Spectral analysis of Isolated Biomolecule HV1

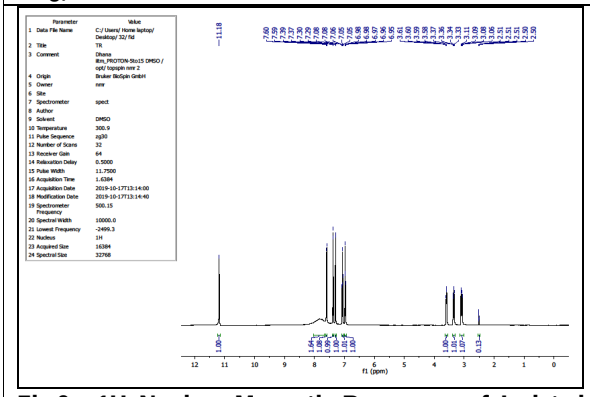


Fig:3 -1H Nuclear Magnetic Resonance of Isolated Biomolecule – HV1

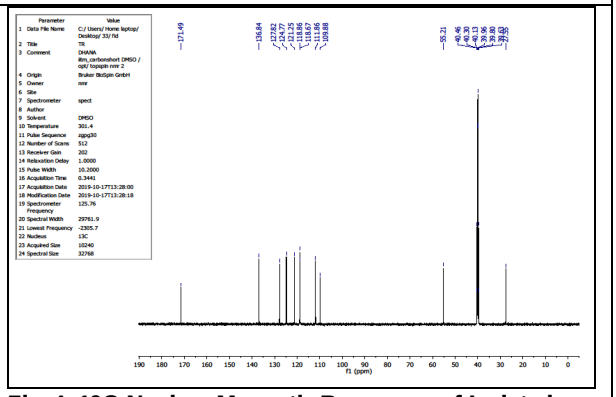


Fig.:4- 13C Nuclear Magnetic Resonance of Isolated Biomolecule – HV1

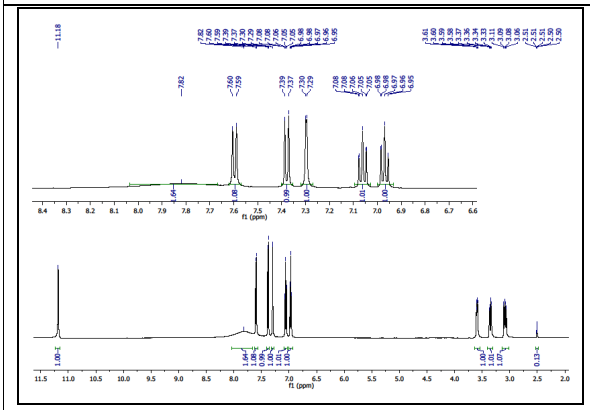
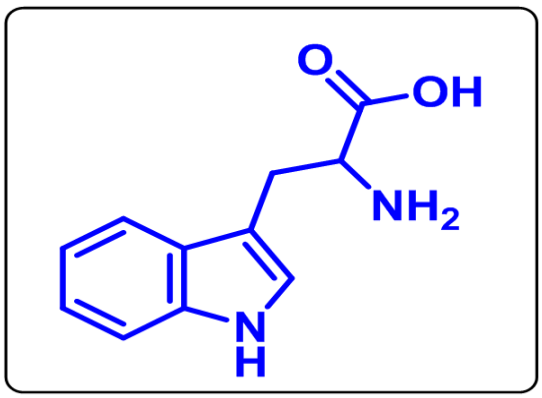


Fig:5-Mass Spectroscopy of Isolated Biomolecule – HV1



HV1

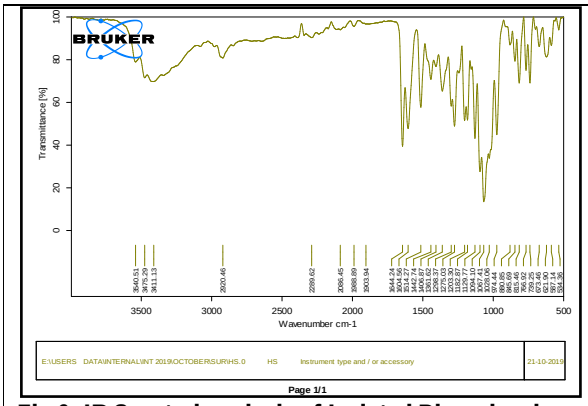


Fig:6 -IR Spectral analysis of Isolated Biomolecules HV2

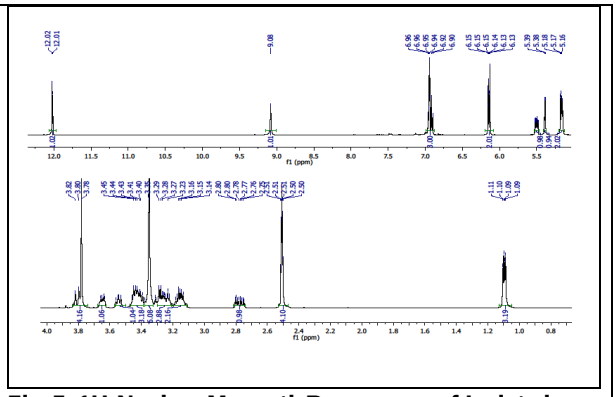


Fig: 7- 1H Nuclear MagenticReasonance of Isolated Biomolecule HV2

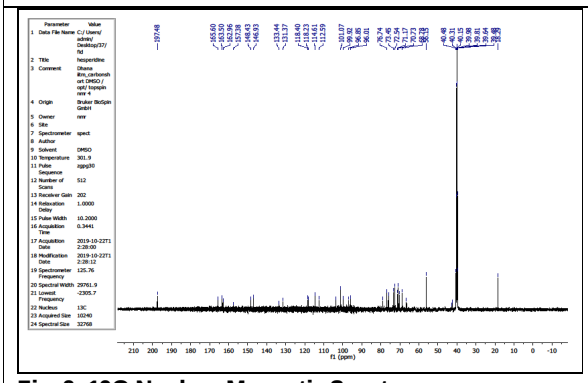


Fig. 8- 13C Nuclear Magnetic Spectroscopy Biomolecule Isolate HV2

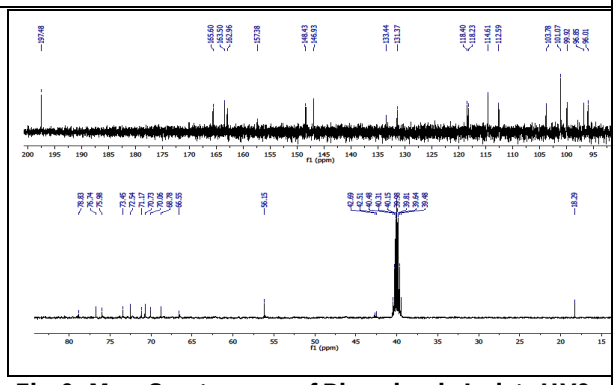
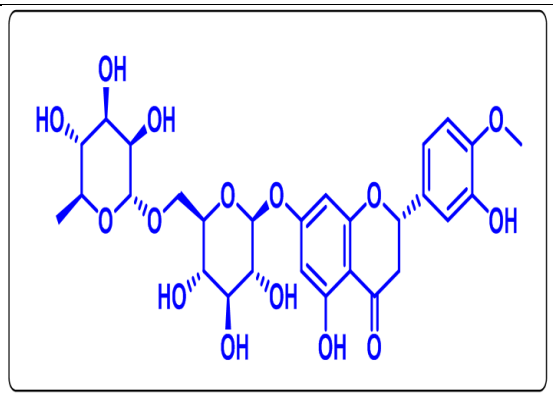


Fig. 9- Mass Spectroscopy of Biomolecule Isolate HV2



HV2

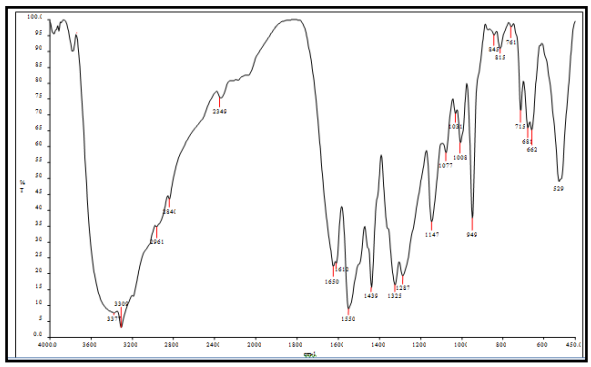


Fig. 10- IR Spectral Analysis of Isolated Biomolecules HV3

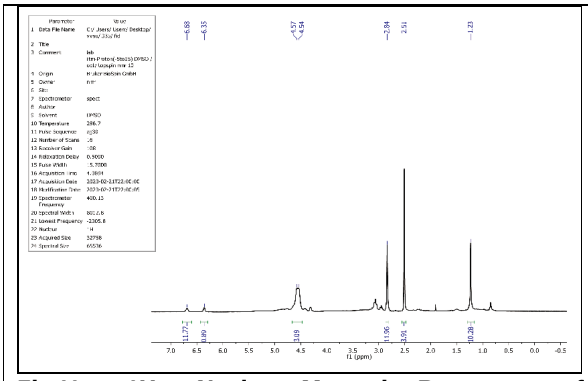


Fig.11- 1H Nuclear Magnetic Reasonance of Isolated Biomolecule HV3

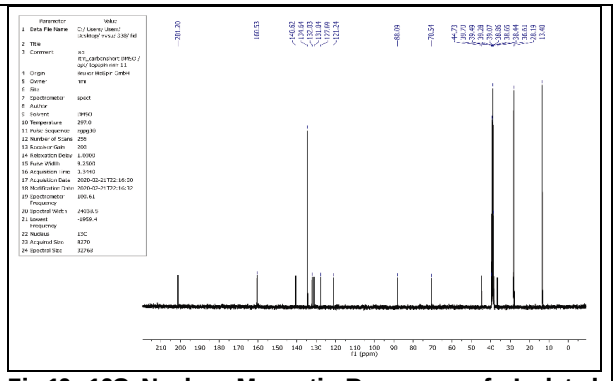


Fig.12- 13C Nuclear Magnetic Resonance of Isolated Biomolecules HV3

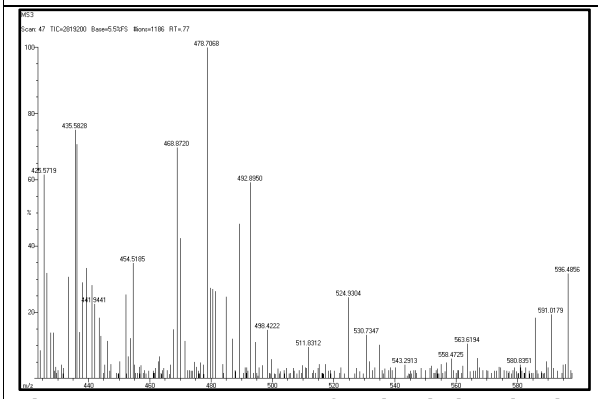
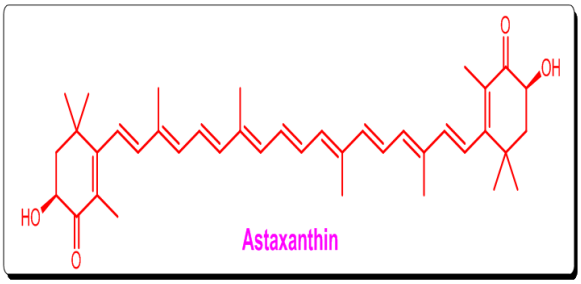


Fig. 13- Mass Spectroscopy of Isolated Biomolecules HV3



HV3

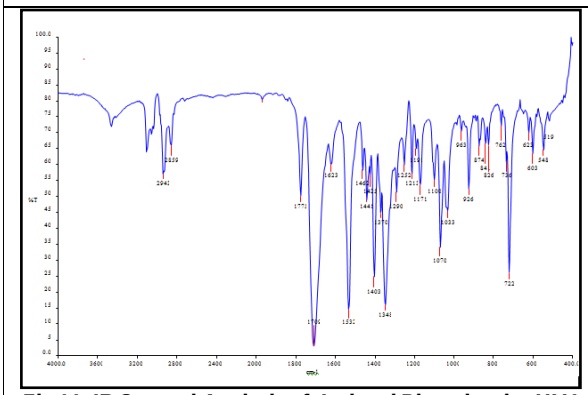


Fig.14- IR Spectral Analysis of Isolated Biomolecules HV4

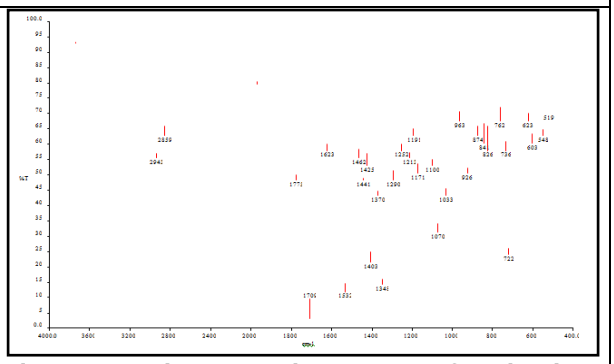


Fig.15- 1H Nuclear Magnetic Resonance of Isolated Biomolecules HV4

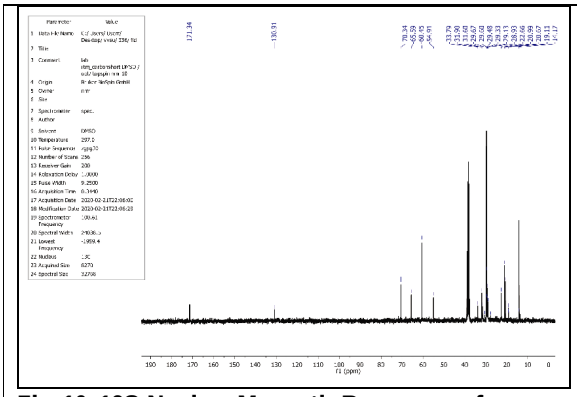


Fig. 16- 13C Nuclear Magnetic Resonance of Isolated Biomolecules HV4

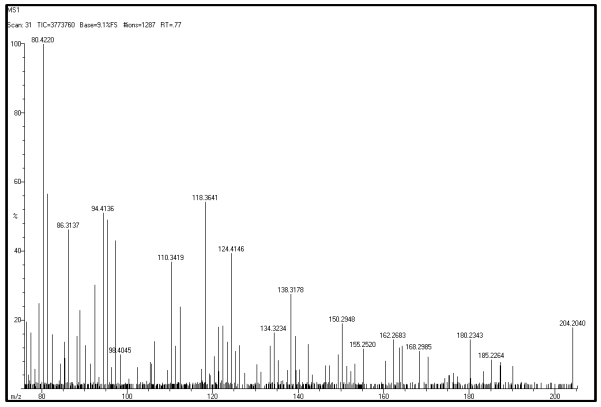
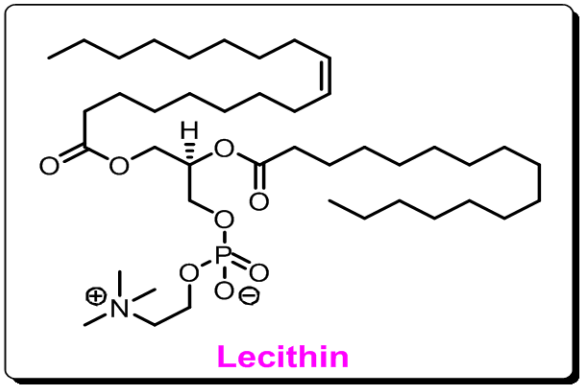


Fig. 17- Mass Spectroscopy of Isolated Biomolecules HV4



HV4.

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RESEARCH ARTICLE

Production of Biodiesel from Blue Green Algae - *Oscillatoria*

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ABSTRACT

Algal biodiesel has enormous potential as a future fuel source. Algae require minimal land resource and have minimal impact on the food supply. In this work, we have studied that blue green algae *Oscillatoria* has a great potential for biofuel and it has renewable energy source. The features of microalgae with their abundant supply of algal biofuel have the potential to be produced on a large scale to be an alternative source for petro diesel and it reduces the global warming. This technique may be new and uneconomic for the harvesting and processing systems.

Keywords: Biodiesel, microalgae, renewable energy source, global warming

INTRODUCTION

Algae are a diverse group of aquatic organisms that have the ability to conduct photosynthesis. Algae as a source of biofuel because of their growth rate and yield they could produce other energy crops. Microalgae possess several attractive characteristics in the context of energy and biofuels.

Oscillatoria

Oscillatoria is a cyanobacteria and it conducts photosynthetic activities. It has a long unbranching filamentous morphology and it has green colour due to the presence of chlorophyll. It is an organism that reproduces by fragmentation and it forms long filaments of cells which can break into fragments called hormogonia. The hormogonia can grow into a new, longer filament. It is usually occurred in ponds.

Biodiesel has good potential and alternative resource for petroleum fuel from the vegetable oils of terraneous oil plants such as soybean, sunflower and palm oils. Microalgal biotechnology appears to possess high potential for biodiesel production because a significant increase in lipid content of microalgae is now possible through heterotrophic cultivation and genetic engineering approaches [1]. Biodiesel from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products. Most productive oil crops, such as oil palm, do not come close to microalgae in being able to sustainably provide the necessary amounts of biodiesel [2]. Algae based technologies and in particular algae based sewage treatment provides an opportunity for recovery of water for recycle and re-use, sequestration of greenhouse gases, and generation of biomass [3]. Microalgae represent a sustainable energy source because of their high biomass productivity and ability to remove air and water born pollutants [4]. Due to their high biomass productivity, rapid lipid accumulation, and ability to survive in saline water, microalgae have been identified as promising feedstock's for industrial-scale production of carbon-neutral biodiesel [5].

Microalgae have long been considered as a promising feedstock for biomass production and it is used as a renewable feedstock for biodiesel production as they have some advantages when compared to traditional biofuel feedstocks [6]. Biodiesel has important in recent years due to its ecofriendly nature, non-toxic characteristics, biodegradability and lower net carbon cycle compared to conventional diesel fuels [7]. Efficient oil extraction from *Oscillatoria annae* was achieved by ultra-sonication in combination with various organic solvents than homogenization. Efficient conversion of triglycerides of *Oscillatoria annae* into biodiesel was obtained by alkali mediated transesterification when compared with lipase-mediated transesterification [8]. Biodiesel has attracted intensive attention as an important biofuel. Microalgae have numerous advantages for biodiesel production over many terrestrial plants. There are a series of consecutive processes for biodiesel production with microalgae as feedstock, including selection of adequate microalgal strains, mass culture, cell harvesting, oil extraction and transesterification [9]. Biofuels is an important source for renewable energy. The algal biofuel production is to stabilize the concentration of carbon dioxide in the atmosphere and decrease global warming impacts [10].

MATERIALS AND METHODS

Collection of Sample

The microalgae which were collected from open pond at Krishnapuram, Tamilnadu in India. The samples were spread under sun in an open area for 48 hours to evaporate the amount of water associated with biomass. The large scale production of microalgae is generally performed with solar energy in open ponds.

Identification of Sample

The morphology and microscopic structures observed under a light microscope. The algae cultivate in chu's medium. Preparation of chu's medium in 100ml conical flask. That media sterilize at 120°c for 30 minutes. After a 1ml of culture added in to chu's media, that culture incubate at 30°c ± 2°c for one week in an alternate light and dark regime of 12 hours.

Algae Mass Cultivation Systems

Most microalgae are strictly photosynthetic, i.e., they need light and carbon dioxide as energy and carbon sources, this culture mode is usually called photoautotrophic. Some algae species, however, are capable of growing in darkness and of using organic carbons such as glucose or acetate as energy and carbon sources, this culture mode is termed heterotrophic and they need a light source, carbon dioxide, water and inorganic salts. The water temperature should be between 15°c and 30^0c (approximately 60^0f to 80^0f) for optimal growth, the growth medium must contribute the inorganic elements that help make up the algal cell, such as nitrogen.

Harvesting of Microalgae

Separation

A fraction of the pond water is generally harvested every day and the algal biomass within the water is concentrated. Flocculation may precede the harvesting methods to increase the effective particle size and hence ease sedimentation. Centrifugal separation uses the same principles as gravity sedimentation but enhances the settling rate by centrifuging the particles. Filtration may be used to recover algal cell from a broth.

Extraction

Gram of grinded algal biomass was treated with 20 ml of solvent. Solvents n- hexane and di ethyl ether were used for the extraction of oil from algal biomass. Both solvents were used alone as well as a blend of n- hexane and diethyl ether was employed for the extraction. The mixture was kept at room temperature for 24 hours. A layer of oil on the solvent surface was formed, which was separated from the residue. We obtained a higher fraction of oil extracted by suing the combination of both the solvents. The extracted oil was separated by evaporating the solvent in a rotary evaporator. Initially, the evaporator was kept at 34 c for 15 minutes to evaporate diethyl ether. Temperature was then raised to 69°c to remove n – hexane. This process left behind solvent free oil in the evaporation flask. The different steps involved in extraction of oil from algal biomass.

Transestrification

About 400 ml oil was leached and separated through the extraction process from 20 kg of algal biomass sample. The extracted oil was converted to biodiesel through transestrification reaction in the presence of methanol. In this process triglycerides react with the alcohol to form the fatty acid ester (biodiesel) and the glycerol. During this reaction the algal oil was allowed to react with the methanol in the presence of alkali.

Analysis of phytocompounds

The qualitative analysis of lipids and proteins were performed with confirmative tests. The phytochemical test was carried out to identify the secondary metabolites.

RESULTS AND DISCUSSION

Sample Collection

The microalgae were obtained from the open pond and to identify the microalgae osillatoria sp by the morphology and microscopic structure were observed under light microscope. The effect of solvent to algae ratio on percent yield of extraction oil. It was observed that the percent yield of oil increases as the solvent to algae ratio increases. The higher yield at solvent to algae ratio is attributed to the excess solvent available to extract oil from the algal biomass. The size of algal biomass also effect the amount of oil extracted. The size of biomass decreases. This can be justified by the improved interaction between the algae specie and solvent, due to large surface area of smaller algal specie. The smaller sized particles have a good interaction with solvent as compared to large particles and thus enhance the yield.

Transestrification Reaction

Yield of biodiesel depends on the amount of extracted oil as well as the methanol used in the reaction. It was observed that biodiesel yield increases almost linearly by increasing oil to methanol ratio. At higher molar ratio, the excess amount of oil promotes the forward reaction. The range of oil to methanol ratio was 2 to 8, with a maximum yield of 95% of biodiesel.

CONCLUSION

From these results, we observed that algae shows great potential as a biofuel source and it has major advantages over other sources. Some of them are low impact on land use and the food chain ability to utilize waste streams for nutrients .Rapid growth rate and high oil content. Due to the positive characteristics of algae and an abundant supply of algal biofuel has the potential to be produced on a large scale enough to replace petro diesel and thereby reduce global warming. This algal biofuel production technology is still somewhat new and uneconomic. The efficiency of growth, harvesting and processing systems must be further improved before the technology can be commercialized. As a result, many researchers and companies are currently developing ways to make this technology more economical. It is forecasted that algal biodiesel will become competitive with petro diesel by 2020.

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Table 1. Phytochemicals present in *Oscillatoria*

Analysis	Result
Carbohydrates	-
Protein	++
Lipids	++
Alkaloids	+
Flavanoids	+
Saponins	-
Tannins	-

++ Present in high concentration + Present in low concentration
- Not present in the sample

Fig 1: Algal sample

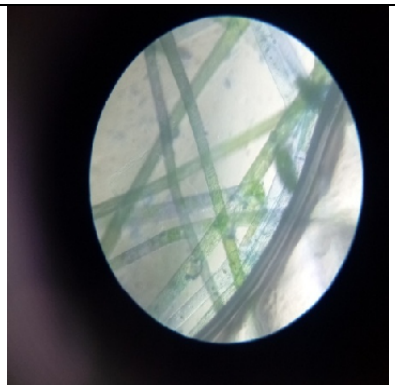

Fig2: Microscopic view of *Oscillatoria*

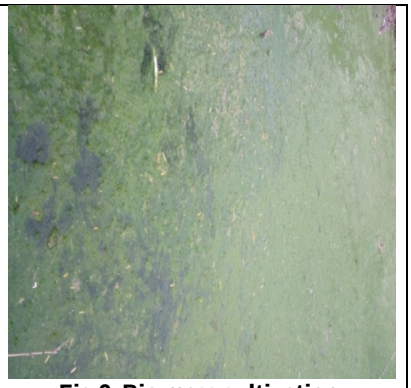

Fig 3: Bio mass cultivation

Fig 4: Dry sample

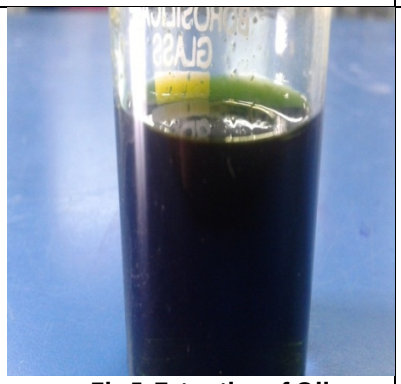

Fig 5: Extraction of Oil

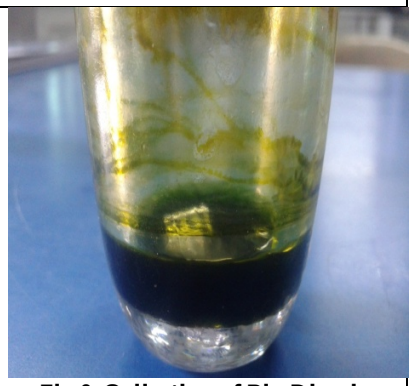

Fig 6: Collection of Bio Diesel

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RESEARCH ARTICLE

Utilization Pattern of Information Sources and Different Extension Agency Contact by Vechur Cattle Farmers of Kerala

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ABSTRACT

The study was conducted in Thrissur, Palakkad and Kottayam districts of Kerala state to assess the utilization pattern of information sources and different extension agency contacts by Vechur cattle farmers. By using chain referral sampling a total of 60 Vechur cattle farmers were selected, twenty from each district. Data were collected through personal interview using a structured pretested interview schedule. Analysis of communication variable revealed that veterinarians were the most preferred extension agency and according to farmer's perception newspaper was the most credible information source followed by social media. Majority of the respondent shad high information utilization.

Keywords: Information Source, Extension agency, Credibility of information, Vechur cattle, Farmers

INTRODUCTION

Vechur cattle are the only recognized native cattle breed of Kerala. The small size and adaptation of this animal to the hot and humid climate which gives high milk yield as compared to other native dwarf breeds have made the Vechur cattle the highest-ranking animal in the state. It produces a larger amount of milk compared to the feed it consumes. Consequent to the implementation of programmes like Intensive Cattle Development Programme and Operation Flood, to increase the milk production in the country, the number of this breed gradually reduced in the state and the breed was under the critical status of risk (Singh and Sharma, 2017). But now due to the intervention of many people, government institutions and nongovernmental organisations, the farmers realised the importance of

rearing indigenous animals and they started to rear it, as a result the number of Vechur cattle in the state increased. Even then, according to the latest report by Srivastava *et al.* (2019), the Vechur cattle is under the endangered status of risk or the breeds which need attention for the conservation.

At present more and more people are enthusiastic to do indigenous cattle rearing but they faced with the problem of not getting proper knowledge inputs about the farming practices of indigenous cattle. There should be proper connections between farmers and the extension agencies to facilitate the farmers to avail and utilize appropriate information for the successful farming. The extension information regarding various operations in agriculture and animal husbandry has been viewed as one of the important inputs for the production process (Phand *et al*. 2009). The cattle farmers need information on good animal husbandry practices, new emerging technologies, market-related information and information on agriculture policies (Kumari *et al.* 2017). Therefore, keeping in view of the above facts, the utilization pattern of information sources and contact of different extension agency by Vechur cattle farmers were studied.

MATERIALS AND METHODS

An *Ex post facto* research was conducted in Thrissur, Palakkad and Kottayam districts of Kerala to assess the utilization pattern of information sources and different extension agency contacts by Vechur cattle farmers. By using chain referral sampling a total of 60 Vechur cattle farmers were selected, twenty farmers from each district. Data were collected through personal interview schedule by using a structured pretested interview schedule.

Extension agency contact: This is referred to as the perception of the respondents about various input service agencies like veterinarians, agricultural and veterinary university scientists, Vechur conservation trust, livestock inspectors and officials/ staffs of the milk co-operative society. Data were collected using a structured interview schedule. Each respondent was then asked to rank the even common extension agencies on a seven-point continuum where the first rank would be assigned to the most useful and last rank to the least useful agency. By using Garret ranking technique the perception of the respondents about various input service agencies were identified.

Garret Ranking Technique

To find out the most significant actor which influences the respondent, Garrett's ranking technique was used. As per this method, respondents have been asked to assign the rank for all factors and the outcomes of such ranking have been converted into score value with the help of the following formula:

$$\text{Percent position} = \frac{100(Rij-0.5)}{Nj}$$

Where

Rij=Rank given for the i^{th} variable by j^{th} respondents

Nj=Number of variable ranked by j^{th} respondents

With the help of Garrett's Table, the percent position estimated is converted into scores. Then for each factor, the scores of each individual are added and then the total value of scores and mean value soft the score is calculated. The factors having the highest mean value is considered to be the most important factor (Dhanavandan, 2016).

Credibility of information source: It was operationalised as the credibility of the different information source like newspaper, radio, television, magazines, social media, co-operative society, members of Vechur Conservation Trust

in providing information regarding dairy farming. Data were collected using a structured interview schedule. Each respondent was then asked to rank the seven common information sources on a seven-point continuum where the first rank would be assigned to the most credible and last rank to the least credible source. By using Garret Ranking technique the most credible information source which influences the respondents identified.

Information source utilization pattern: It was operationalised as the utilization of a number of sources providing information regarding dairy farming and the frequency of exposure of sources. It was measured by assigning scores of 5, 4,3,2,1 to daily, weekly, fortnightly, monthly and occasional contact respectively with various sources of information. For qualitative analysis of the Utilization pattern of information sources and different extension agency contact by Vechur cattle farmers Focus Group Discussion (FGD's) were used.

RESULTS

Extension Agency Contact

The results showed that the veterinarians were the most preferred extension agency (97.14 per cent) followed by livestock inspector (79.05 per cent), milk cooperative society secretary (69.76 per cent), university scientists (27.62 per cent), Vechur Conservation Trust members (18.33 per cent), dairy farm instructors (4.76 per cent) and dairy extension officers (2.14 per cent).

Credibility of Information Source

Table 2 illustrates the farmer's perception about the credibility of the information source, it revealed that as newspaper ranked first (78.57 per cent) followed by social media (73.81 per cent), television (69.76 per cent), co-operative society (65.24 per cent), magazines (55.71 per cent), radio (28.33 per cent) and lastly Vechur Conservation Trust members (20.71 per cent).

Information Source Utilization

The result in Table 3 indicated that a major proportion of the farmers (36.67 per cent) had high information utilization by farmers followed by moderate (33.33 per cent) and low (30.00 per cent).

DISCUSSION

The results of this study point to the high frequency of the local veterinary surgeon were being contacted by the respondents. The respondents' frequent visit to veterinarians could be due to the fact that the veterinarians are being contacted during the critical stages of farmers like treatment of animals as it would affect the economy and livelihood of the respondents. Their interventions at the critical stages make the farmers to believe the veterinarians as the authority of in matters related to livestock rearing. This is in accordance with the findings of Gopi *et al.* (2018), who opined that among personal cosmopolite source, veterinarians were most frequently contacted and most useful contact agency followed by para veterinarians, dairy cooperative personals and private dairy personals. Opera (2008), who reported that the majority (70.00 per cent) of the farmers preferred the extension agents over the other sources (including radio, friends & relatives, and television). The interviews with veterinarians, milk cooperative society members and livestock inspectors revealed that farmers contacted them for guidance and advice about farming and also for information on subsidies and government policies.

In case of the credibility of an information source newspaper ranked first followed by social media and television this is in contrary with the findings of Kakade (2013), who reported that farm radio programmes are second credible, next to agriculture extension workers. Since majority of farmers in literate states like Kerala were subscribed to newspapers and publication of articles by resource persons of Animal Husbandry Department and Veterinary/agricultural universities it would have secured highest credibility rate. In contrast Zhao *et al.* (2009)

reported in their survey that television was the main channel by which farmers received the information, followed by information from village government officials, neighbours and milk stations, with very little information coming from radios, newspapers and the Internet. Demiryurek *et al.* (2008) found that in most economically advanced countries, the Internet and farmers' associations have become important information channels. Another important finding of the present study was that many of the Vechur cattle owners were members of different social media groups regarding the conservation of Vechur cattle or other indigenous cattle. They are using these groups to transfer different information regarding Vechur cattle, fixing of different group meetings and also it helps them in marketing produces and purchasing of live animals, Vechur cattle and other indigenous animals.

Analysis of information utilization pattern revealed that most of the farmers studied had a medium level of contact with extension agencies and mass media utilization which was in consonance with the findings of Sabapara *et al.* (2016) and Thesinguraja *et al.* (2017) who had reported that the majority respondents studied had a medium level of extension agency contact. However, the finding among the Vechur cattle farmers was contrary to the findings of Kalaivani *et al.* (2017) who observed a low level of extension agency contact among more than half of farmers studied. The increased frequency of utilization of information source would improve the adoption of new practices and technologies by the farmers, as the adoption of improved practices directly proportional to the frequency of utilization of communication sources.

CONCLUSION

Analysis of communication variable revealed that veterinarians were the most preferred extension agency and according to farmer's perception the newspaper was the most credible information source followed by social media. Majority of the respondents had high information utilization. The increased frequency of utilization of information source will improve the adoption of new practices and technologies by the farmers and the adoption of improved practices directly proportional to the frequency of utilization of communication sources. The information source should provide unbiased, up-to-date and factual information, at the right time. Further investigation into the reasons for Vechur cattle farmers not to have higher levels of information sources needs to be explored as this variable is crucial in enabling the transfer of technology and the viability of this system.

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Table 1. Distribution of Vechur cattle farmers based on extension agency contact

Sl. No.	Extension Agency	Total Score	Rank	Percentage
1	Veterinarians	408	I	97.14
2	Livestock Instructors	332	II	79.05
3	Milk cooperative Society Secretary	293	III	69.76
4	University Scientists	116	IV	27.62
5	Vechur Conservation Trust (VCT)	77	V	18.33
6	Dairy Farm Instructors	20	VI	4.76
7	Dairy Extension Officers	9	VII	2.14

Table 2. Distribution of information source based on credibility

Sl. No.	Information Source	Total Score	Rank	Percentage
1	Newspaper	330	I	78.57
2	Social Media	310	II	73.81
3	Television	293	III	69.76
4	Cooperative Societies	274	IV	65.24
5	Magazines	234	V	55.71
6	Radio	119	VI	28.33
7	Vechur Conservation Trust Members	87	VII	20.71

Table 3. Distribution of Vechur cattle farmers based on information source utilization

Sl. No.	Category	Frequency (f)	Percentage (%)
1	Low (1.58-2.17)	18	30.00
2	Medium (2.17-2.58)	20	33.33
3	High (2.58-3.83)	22	36.67
Total		60	100

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REVIEW ARTICLE

Comparative Evaluation of Efficacy of Ketoprofen versus Acetaminophen in Management of Post-Operative Pain in Third Molar Surgery: A Systematic Review

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ABSTRACT

The most commonly performed procedure in oral and maxillofacial surgery is third molar removal. It takes several days to recover following removal of the third molar. The most common postoperative complications following third molar removal are pain, swelling, trismus, alveolar osteitis. The aim of this systematic review was to analyze the existing literature to Comparative evaluation of efficacy of Ketoprofen versus Acetaminophen in management of post-operative pain in third molar surgery. The Data Bases of PubMed, Cochrane and Google scholar were searched for the related topics along with a complimentary manual search of all oral surgery journals till October 2018.The studies considered were based on the data extraction and analysis of the studies for quality and publication bias. The data collection form was customized. The primary outcome measure was post-operative pain and the secondary outcome measures were post-operative swelling & post-operative mouth opening. Two articles were selected based on the inclusion criteria. They included randomized controlled trials. The reviews found some clinical evidence that, there is significant difference between Ketoprofen and Acetaminophen on postoperative pain following dental

extractions. The clinical evidence in this review shows that Ketoprofen 25 mg and paracetamol 1000 mg are effective and safe analgesic for controlling post-operative pain after third molar surgery. Ketoprofen offers a slight advantage over Paracetamol by providing a earlier onset of relief.

Keywords: Ketoprofen, osteitis, surgery, maxillofacial, clinical

INTRODUCTION

Ketoprofen is a propionic acid derivative which possesses analgesic, anti-inflammatory and antipyretic properties. The drug is widely used in the management of musculo-skeletal disorders and evidence from clinical trials suggests that Ketoprofen is as effective as other non-steroidal anti-inflammatory drugs in reducing the pain and discomfort associated with these disorders [1]. A few studies have evaluated the efficacy of Ketoprofen in post-operative pain after third molar surgery. [2]. These single dose studies have demonstrated that Ketoprofen at dosages of 25, 50 and 100 mg is more efficacious than either placebo or codeine phosphate 90 mg [3] or aspirin 650 mg [4] or paracetamol 1000 mg. A further Method study has shown that a single dose of Ketoprofen 25 mg and ibuprofen 400 mg provide similar dose-effect curves in patients with post-operative pain after third molar parallel design was carried out on 206 patients who had surgery [5]. Evidence for a dose-response for Ketoprofen in post-operative dental pain is inconclusive, with one study demonstrating a clear dose-response [6] whilst the other failed to substantiate this finding [3]. There is no evidence that dosage of ketoprofen below 25 mg is efficacious after third molar surgery [6]. Thus, the aims of the present study were to evaluate the efficacy of doses of racemic ketoprofen 25 mg in patients with post-operative pain after removal of their impacted third molars, and to compare pain relief obtained with the two doses of ketoprofen with that obtained after paracetamol 1000 mg.

Structured Question

Ketoprofen have better efficiency on pain control than acetaminophen in treatment of postoperative complications after removal of third molar?

PICO Analysis

Population: Patients undergoing upper & lower third molar surgery
Intervention: Ketoprofen
Comparison: Acetaminophen
Outcome: Postoperative complication – pain.

MATERIALS AND METHODS

Sources Used

The Data Bases of PubMed Advanced, Cochrane and Google scholar were searched until October 2018 using controlled vocabulary and free text terms.
We used free-text terms to search the following journals
·British Journal of Oral and Maxillofacial Surgery
·International Journal of Oral and Maxillofacial Surgery
·Journal of Oral and Maxillofacial Surgery
·Journal of Cranio Maxillofacial Surgery
·Journal of Applied Oral Science
Only articles in English and human species were applied during the electronic search to include all the randomized control studies that are relevant for the search phase of the systematic review. Reference list of the identified randomized trials were also checked for possible additional studies.

Inclusion Criteria

Criteria for considering studies for the Review

Types of studies -

·Randomized controlled trials

·Clinical trials.

Types of Participants –

Patients undergoing surgical removal of impacted upper & lower third molar.

Types of Intervention

Ketoprofen 25 mg

Types of Comparison

Acetminophen 1000mg

Types of Outcome Measures

To evaluate severity of pain in ketoprofen and acetaminophen on pain control after third molar surgery.

Exclusion Criteria

The following studies were excluded,

- Studies with combination of Ketoprofen with other drugs are excluded.
- Studies which use other modes of application of ketoprofen

SEARCH METHODOLOGY

Electronic search was carried out using the keywords in the Search engines- PubMed, Cochrane and Google Scholar which yielded a total of 16 articles. Hand search yielded 2 articles. Based on pre-set inclusion and exclusion criteria, the titles of the studies identified from the search were assessed independently by three review authors (Dr. Rahul Kumar, Dr. Abdul Wahab, and Dr. Senthil Murugan.P). Conflicts concerning inclusion of the studies were resolved by discussion. Eight were identified from the search after excluding duplications. Nine articles were excluded after reading titles and abstracts. Full text articles were retrieved for two relevant studies. The reference list of the full text articles was reviewed for identifying additional studies. Titles of articles relevant to the review were selected by discussion. Difference of opinion concerning inclusion of a study was resolved by discussion. Quality Assessment criteria to evaluate the studies were decided by two review authors in accordance with CONSORT guidelines. The risk of bias for each study was independently assessed by the review authors and conflicts concerning risk of bias were sorted by discussion.

Data Extraction

Data extraction for general characteristics of studies and variables of outcome was done

For each trial the following data were recorded:

- Author and Journal
- Study Design
- Sample Size
- Participants and Group
- Methodology
- Parameters
- Statistical Analysis
- Results

Quality Assessment

(Higgins and Green. Cochrane reviewer's hand book 2009)

The quality assessment of included trials was undertaken independently as a part of data extraction process. Four main quality criteria were examined.

1. **Method of Randomization**, recorded as
a) YES- Adequate as described in the text
b) NO- Inadequate as described in the text
c) Unclear in the text

2. **Allocation Concealment**, recorded as
a) YES- Adequate as described in the text
b) NO- Inadequate as described in the text
c) Unclear in the text

3. **Outcome assessors Blinded to intervention**, recorded as
a) YES- Adequate as described in the text
b) NO- Inadequate as described in the text
c) Unclear in the text

4. **Completeness of Follow up** (was there a clear explanation for withdrawals and dropouts in each treatment group) assessed as
a) YES- Dropouts were explained
b) NO- Dropouts were not explained
c) None- No Dropouts or withdrawals.

Other methodological criteria examined included:
1. Presence or Absence of sample size calculation.
2. Comparability of Groups at the start.
3. Clear Inclusion or Exclusion criteria.
4. Presence or Absence of estimate of measurement error.

Risk of Bias in Included Studies

The study was assessed to have a "High risk" of bias if it did not record a "Yes" in three or more of the four main categories, "Moderate Risk "if two out of four categories did not record a "Yes", and "Low Risk" if all the four categories recorded if randomization assessor, Blinding and Completeness of follow up were considered Adequate. In case of non-randomized and clinical trials without control group, it is recorded as not applicable.

DISCUSSION

The analgesic effectiveness of NSAIDs is claimed to be the main reason for their popularity in inflammatory conditions [19]. It has also been proposed that the anti-swelling efficacy of NSAIDs is dissociated from their analgesic efficacy [20]. The somewhat diffuse concept of "anti-inflammatory effect," however, is widely used to categorize NSAIDs as therapeutic agents, market NSAIDs, and justify their use in inflammatory conditions. A clinical demonstration of anti-inflammatory drug effect on acute and chronic noninfectious inflammation would include drug effects on all of the traditional cardinal symptoms of inflammation (e.g., erythema, pain, local temperature elevation, swelling, and reduced function). Evidence from various clinical trials suggests that ketoprofen is an effective analgesic. The therapeutic effect was usually achieved at doses ranging from 100 to 300 mg day−1 [8]. The findings from this single dose study suggest that ketoprofen at a dosage as low as 12.5 mg still provides significant analgesia in an acute pain model [9]. Furthermore, the pain responses in patients taking the low dose ketoprofen

were similar to the other active treatment groups.According to Seymour et al - Both ketoprofen treatments provided significantly better pain relief with respect to placebo, at 1 h after dosage, whereas the difference in pain scores between placebo and both paracetamol treatments was not significant at this time point [10]. This would suggest that ketoprofen is providing a more rapid reduction in pain when compared with paracetamol. Such differences may be related to the anti-inflammatory properties of both drugs and the nature of acute post-operative dental pain or to differences in the pharmacokinetics between the two analgesics. Ketoprofen is a potent inhibitor of eicosanoids, blocking both cyclo-oxygenase and lipoxygenase pathways [11]. Further anti-inflammatory actions of the drug may be related to its anti-bradykinin action [12], a stabilizing effect on lysosomal membranes [13] and an inhibitory effect on PMN chemotaxis [14]. By comparison, paracetamol has weak anti-inflammatory properties [15]. After third molar surgery the postoperative sequelae of pain and swelling are driven by the local inflammatory response [16]. A drug which has an established anti-inflammatory action is likely to be of more value in the early post-operative phase, than one which has weak properties. Based upon the median time to taking such medication, it can be seen that both ketoprofen treatments and paracetamol 1000 mg provided sufficient pain control for up to 4 h after dosage. This is similar to the duration of analgesia obtained after ibuprofen [17]. Such findings may be related to the nature of acute pain after removal of impacted third molars, or a true reflection of the drug's efficacy. Pain intensity in this model usually reaches its maximum in the first 12 h after surgery and declines rapidly thereafter. Since non-steroidal anti-inflammatory drugs are most widely used and efficacious in the treatment of post-operative dental pain [18], it is important that patients are re-medicated every 4 h to ensure effective pain control in the first 12 h.

CONCLUSION

The clinical evidence in this review shows that Ketoprofen 25 mg and paracetamol 1000 mg are effective and safe analgesic for controlling post-operative pain after third molar surgery. Ketoprofen offers a slight advantage over Paracetamol by providing an earlier onset of relief.

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Table: 1 Variables of Interest

S NO	VARIABLES OF INTEREST
1.	Post-operative pain measured by VAS score

Table 2: Literature Matched the Inclusion Criteria

S. No	Author and year	Title of article
1.	R.A.Seymour et al, 1996	Onset of analgesia for acetaminophen, ketoprofen and placebo in Extraction of Impacted Mandibular Third Molars
2	Nancy z. Olson etal , 2001	Effects of ketoprofen and acetaminophen following third molar surgery

Table 3: Results and Summation

Sr. No.	Author	Year	Country	Study design	Sample size	Age	Set - up	Technique Used	Method of Evaluation
1.	R.A.Seymour et al, 1996	199	United Kingdom	Randomized controlled clinical trial	41	18 – 40 Years	College	Ketoprofen and Acetaminophen	Pain were measured using visualAnalogue scale.
2.	Nancy z.Olson etal , 2001	2001	United State of America	Randomized Controlled clinical trial	67	16-65 years	College	Ketoprofen and Acetaminophen	Pain were measured using visual analogue scale. 4 point categorical pain severity scale.

Table: 4 Evidence Level Of Selected Articles

(The United States department of health and human services 2016)

S no	Author & Year	Study Design	Level of Evidence
1	R.A.Seymour et al, 1996	Randomised trial	2
2	Nancy z.Olson etal , 2001	Randomised trial	2

Table: 5 For Individual Parameters

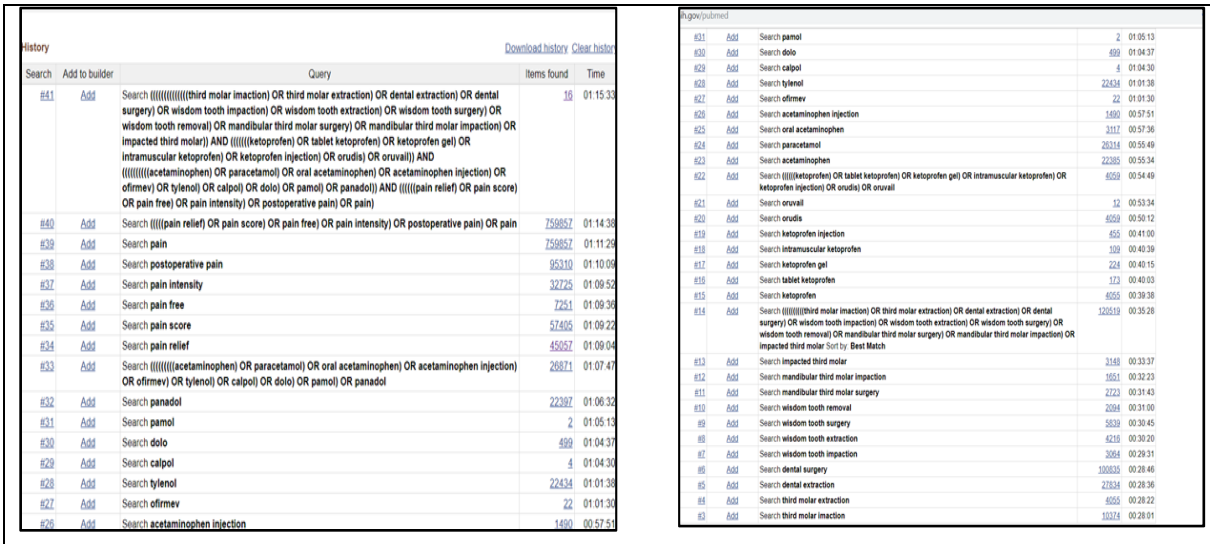
S. No	Author	Year	Evaluation period	Outcome
1.	Nancy z. Olson etal ,	**2001**	**2 hr, 6hr**	**PAIN** There was significant difference in pain control between two groups
2.	R.A.Seymour et al	**1996**	**15 min, 30 min 45min, 60 min, 120 min, 180 min, 240 min, 300 min , 360 min.**	**Pain –** There was a significant difference in Pain between two groups.

Table 6: Results

	Author	Materials	Metho	Mean Values	Outcomes
1.	Nancy z. Olson etal , 2001	Ketoprofen and Acetaminophen	Pain were measured using visual analogue scale.	% with complete relief: Ketoprofen- 58.2 Acetaminophen -48.5	There was significance difference in pain control between two groups.
2.	R.A.Seymour et al, 1996	Ketoprofen and Acetaminophen	Pain were measured using visual analogue scale.	% of pain relief at 1 hour: Ketoprofen- 50.9 Acetaminophen -43.7 Maximum pain relief (mm): Ketoprofen- 42.3 Acetaminophen – 38.3	There was significant difference in pain control between two groups.

Table 7: Risk of Bias – Major Criteria

study	Year	Randomization	Allocation Concealed	Assessor Blinding	Dropouts Described	Risk of bias
R.A.Seymour et al,	**1996**	Yes	Yes	No	No	Moderate
Nancy z. Olson et al	**2001**	Yes	Yes	No	No	Moderate



History — Download history Clear history

Search	Add to builder	Query	Items found	Time
#41	Add	Search ((((((((((((third molar imaction) OR third molar extraction) OR dental extraction) OR dental surgery) OR wisdom tooth impaction) OR wisdom tooth extraction) OR wisdom tooth surgery) OR wisdom tooth removal) OR mandibular third molar surgery) OR mandibular third molar impaction) OR impacted third molar)) AND (((((((ketoprofen) OR tablet ketoprofen) OR ketoprofen gel) OR intramuscular ketoprofen) OR ketoprofen injection) OR orudis) OR oruvail)) AND ((((((((((acetaminophen) OR paracetamol) OR oral acetaminophen) OR acetaminophen injection) OR ofirmev) OR tylenol) OR calpol) OR dolo) OR pamol) OR panadol)) AND ((((((pain relief) OR pain score) OR pain free) OR pain intensity) OR postoperative pain) OR pain)	16	01:15:33
#40	Add	Search (((((pain relief) OR pain score) OR pain free) OR pain intensity) OR postoperative pain) OR pain	759857	01:14:38
#39	Add	Search pain	759857	01:11:29
#38	Add	Search postoperative pain	95310	01:10:09
#37	Add	Search pain intensity	32725	01:09:52
#36	Add	Search pain free	7251	01:09:36
#35	Add	Search pain score	57405	01:09:22
#34	Add	Search pain relief	45057	01:09:04
#33	Add	Search (((((((((acetaminophen) OR paracetamol) OR oral acetaminophen) OR acetaminophen injection) OR ofirmev) OR tylenol) OR calpol) OR dolo) OR pamol) OR panadol	26871	01:07:47
#32	Add	Search panadol	22397	01:06:32
#31	Add	Search pamol	2	01:05:13
#30	Add	Search dolo	499	01:04:37
#29	Add	Search calpol	4	01:04:30
#28	Add	Search tylenol	22434	01:01:38
#27	Add	Search ofirmev	22	01:01:30
#26	Add	Search acetaminophen injection	1490	00:57:51

ih.gov/pubmed

#31	Add	Search pamol	2	01:05:13
#30	Add	Search dolo	499	01:04:37
#29	Add	Search calpol	4	01:04:30
#28	Add	Search tylenol	22434	01:01:38
#27	Add	Search ofirmev	22	01:01:30
#26	Add	Search acetaminophen injection	1490	00:57:51
#25	Add	Search oral acetaminophen	3117	00:57:36
#24	Add	Search paracetamol	26314	00:55:49
#23	Add	Search acetaminophen	22385	00:55:34
#22	Add	Search (((((ketoprofen) OR tablet ketoprofen) OR ketoprofen gel) OR intramuscular ketoprofen) OR ketoprofen injection) OR orudis) OR oruvail	4059	00:54:49
#21	Add	Search oruvail	12	00:53:34
#20	Add	Search orudis	4059	00:50:12
#19	Add	Search ketoprofen injection	455	00:41:00
#18	Add	Search intramuscular ketoprofen	109	00:40:39
#17	Add	Search ketoprofen gel	224	00:40:15
#16	Add	Search tablet ketoprofen	173	00:40:03
#15	Add	Search ketoprofen	4055	00:39:38
#14	Add	Search ((((((((third molar imaction) OR third molar extraction) OR dental extraction) OR dental surgery) OR wisdom tooth impaction) OR wisdom tooth extraction) OR wisdom tooth surgery) OR wisdom tooth removal) OR mandibular third molar surgery) OR mandibular third molar impaction) OR impacted third molar Sort by: Best Match	120519	00:35:28
#13	Add	Search impacted third molar	3148	00:33:37
#12	Add	Search mandibular third molar impaction	1651	00:32:23
#11	Add	Search mandibular third molar surgery	2723	00:31:43
#10	Add	Search wisdom tooth removal	2094	00:31:00
#9	Add	Search wisdom tooth surgery	5839	00:30:45
#8	Add	Search wisdom tooth extraction	4216	00:30:20
#7	Add	Search wisdom tooth impaction	3064	00:29:31
#6	Add	Search dental surgery	100835	00:28:46
#5	Add	Search dental extraction	27834	00:28:36
#4	Add	Search third molar extraction	4055	00:28:22
#3	Add	Search third molar imaction	10374	00:28:01

Figure 1-Screenshot of Pub Med search

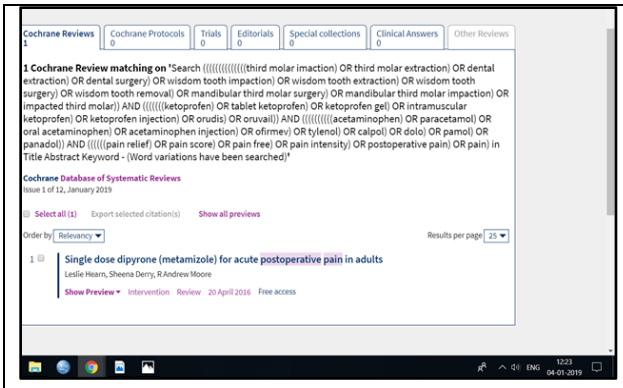


Figure 2-Screenshot of Cochrane search

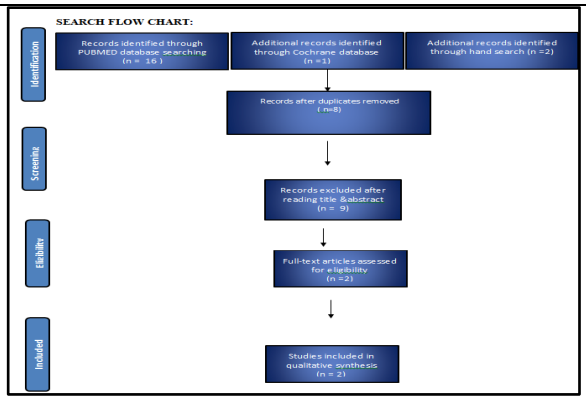


Figure 3 - Search Flow Chart