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# Editorial Board

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ABSTRACT
Germination of Himalayan Balsam (*Balsaminaceae*) and Crossandra (*Acanthaceae*) seeds was made by irrigated with distillery spentwash of different concentrations. The spentwash i.e., primary treated spentwash (PTSW), 1:1, 1:2, and 1:3 spent wash were analyzed for their plant nutrients such as nitrogen, phosphorous, potassium and other physical and chemical characteristics. Experimental soil was tested for its chemical and physical parameters. Himalayan Balsam and crossandra seeds were sowed in different pots and irrigated with raw water (RW), 1:1, 1:2 and 1:3 spentwash. The nature of germination of seeds was studied. It was found that, the germination was very good (100%) in 1:3 SW irrigation, while very poor (25%) in 1:1 SW, moderate (80%) in 1:2 SW and 95% in RW irrigations.

**Keywords:** Distillery spentwash, Himalayan Balsam, Crossandra, Germination, Growth, Irrigation, Soil.

INTRODUCTION
Himalayan Balsam belongs to the family *Balsaminaceae*. It typically grows to 1 to 2 m high, with a soft green or red-tinged stem, and lanceolate leaves 5 to 23 cm long. The crushed foliage has a strong musty smell. The flowers are pink, with a hooded shape, 3 to 4 cm broad the flower shape has been compared to a policeman’s helmet, giving rise to the alternative common name Policeman’s Helmet. Although it does not range all over India and is by no means the only Impatiens native to that country, it is also known as Indian Balsam in countries where it is introduced. In India it is commonly called as *Karnakundala*. Himalayan Balsam, Impatiens glandulifera, is a large annual plant, native to the Himalayans (resulting in its colloquial name of Kiss-me-on-the-mountain in the UK. After flowering between June and October, the plant forms seed pods 2 to 3 cm long and 8mm broad. Which explode when disturbed, scattering the seeds up to 7 meters. The green seed pods and seeds can be eaten, and also the young leaves and shoots. Which is a method of controlling the plant’s spread. However, a recent study it may cause more harm than good. Destroying riparian stands of Himalayan Balsam can open up the habitat for more aggressive invasive plants. The Bionic Control of Invasive Weeds in Wiesbaden, Germany is trying to establish a self sufficient project to conserve their local biodiversity by developing several food products made from the Impatiens flowers. Eventually, if all goes well, this project will have the Himalayan Balsam financing its own eradication.
Crossandra is belongs to the family *Acanthaceae*, comprising 52 species that occur in Africa, Madagascar, Arabia and the Indian subcontinent. Some species, especially Crossandra infundibuliformis\(^5\), are cultivated for their brightly colored flowers. It is known as Kanakambara in Karnataka, southern states in India. Crossandra from the Greek, meaning fringed anthers. The male portion of the flower, the anthers, is distinctly fringed in this genus of plants. The firecracker flower, while relatively unknown to the general public as a houseplant, is just about the most prolific indoor flowering plant. A well tended specimen will bloom continuously for years. It is growing from four sided stalked spikes; the asymmetrical petals arise as a slender tube and then split in to their ends. Plant breeders, especially in Europe, have been hybridizing Crossandras. Cultivars with yellow and even red flowers are available. Crossandra is a sturdy, productive ornamental that should be more popular with indoor gardens.

Molasses (one of the important byproducts of sugar industry) is the chief source for the production of ethanol in distilleries by fermentation method. About 08 (eight) liters of wastewater is generated for every liter of ethanol production in distilleries, known as raw spentwash (RSW), which is known for high biological oxygen demand (BOD: 5000-8000mg/L) and chemical oxygen demand (COD: 25000-30000mg/L), undesirable color and foul odor.\(^7\) Discharge of RSW into open field or nearby water bodies results in environmental, water and soil pollution including threat to plant and animal lives. The RSW is highly acidic and contains easily oxidisable organic matter with very high BOD and COD.\(^8\) Also, spentwash contains high organic nitrogen and nutrients.\(^9\) By installing biomethenation plant in distilleries, reduces the oxygen demand of RSW, the resulting spentwash is called primary treated spentwash (PTSW) and primary treatment to RSW increases the nitrogen (N), potassium (K), and phosphorous (P) contents and decreases calcium (Ca), magnesium (Mg), sodium (Na), chloride (Cl), and sulphate (SO\(_4^{2-}\)).\(^10\) PTSW is rich in potassium (K), sulphur (S), nitrogen (N), phosphorous (P) as well as easily biodegradable organic matter and its application to soil has been reported to increase yield of sugar cane, wheat and rice.\(^11\) Quality of groundnut\(^12\) and physiological response of soybean.\(^13\) Diluted spentwash could be used for irrigation purpose without adversely affecting soil fertility,\(^14\) seed germination and crop productivity.\(^15\) The diluted spentwash irrigation improved the physical and chemical properties of the soil and further increased soil micro flora.\(^16, 17, 18\) Twelve pre-sowing irrigations with the diluted spentwash had no adverse effect on the germination of maize but improved the growth.\(^19\) Diluted spentwash increases the growth of shoot length, leaf number per plant, leaf area and chlorophyll content of peas.\(^20\) Increased concentration of spentwash causes decreased seed germination, seedling growth and chlorophyll content in Sunflowers (*Helianthus annuus*) and the spentwash could safely used for irrigation purpose at lower concentration.\(^21\) The spentwash contained an excess of various forms of cations and anions, which are injurious to plant growth and these constituents should be reduced to beneficial level by diluting spentwash, which can be used as a substitute for chemical fertilizer.\(^22\) The spent wash could be used as a complement to mineral fertilizer to sugarcane.\(^23\) The spentwash contained N, P, K, Ca, Mg and S and thus valued as a
fertilizer when applied to soil through irrigation with water. The application of diluted spentwash increased the uptake of Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn) in maize and wheat as compared to control and the highest total uptake of these were found at lower dilution levels than at higher dilution levels. Mineralization of organic material as well as nutrients present in the spentwash was responsible for increased availability of plant nutrients. Diluted spentwash increase the uptake of nutrients, height, growth and yield of leaves vegetables, nutrients of cabbage and mint leaf, nutrients of top vegetable, pulses, condiments, root vegetables, of some root vegetables in untreated and spentwash treated soil, yields of top vegetables (creepers). However, no information is available on sprouting and growth of Himalayan Balsam and Crossandra flowering plant irrigated by distillery spentwash. Therefore, the present investigation was carried out to study the influence of different proportions of spent wash on the sprouting and growth of Himalayan Balsam and Crossandra.

**MATERIALS AND METHODS**

Physico-chemical parameters and amount of nitrogen (N), potassium (K), phosphorous (P) and sulphur (S) present in the primary treated diluted spentwash (1:1, 1:2 and 1:3 SW) were analyzed by standard methods. The PTSW was used for irrigation with a dilution of 1:1, 1:2 and 1:3. A composite soil sample collected prior to spentwash irrigation was air-dried, powdered and analyzed for physico-chemical properties. Flowering plants selected for the present investigation were Himalayan Balsam and Crossandra. The sets were planted in different pots (30(h), 25(dia)] and irrigated (by applying 5-10mm/cm² depends upon the climatic condition) with raw water (RW), 1:1 SW, 1:2 SW and 1:3 SW at the dosage of twice a week and rest of the period with raw water as required. Cultivation was conducted in triplicate, in each case sprouting, growth were recorded.

**RESULTS AND DISCUSSION**

Chemical composition of PTSW, 1:1, 1:2, and 1:3 SW such as pH, electrical conductivity, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), settleable solids (SS), chemical oxygen demand (COD), biological oxygen demand (BOD), carbonates, bicarbonates, total phosphorous (P), total potassium (K), ammonical nitrogen (N), calcium (Ca), magnesium (Mg), sulphur (S), sodium (Na), chlorides (Cl), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), chromium (Cr) and nickel (Ni) were analyzed and tabulated (Table 1). Amount of N, P, K and S contents are presented (Table 2). Characteristics of experimental soils such as pH, electrical conductivity, the amount of organic carbon, available nitrogen (N), phosphorous (P), potassium (K), sulphur (S), exchangeable calcium (Ca), magnesium (Mg), sodium (Na), DTPA iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were analyzed and tabulated (Table 3 & 4). It was found that the soil composition is fit for the cultivation of plants, because it fulfils all the requirements for the growth of plants. Sprouting and growth of Himalayan Balsam and Crossandra plant leaves, uptakes of all the parameters were very good in both 1:2 and 1:3 spent wash as compared to 1:1, SW and raw water. In both 1:1, 1:2 and 1:3 spent wash irrigation, the uptake of the nutrients such as fat, calcium, zinc, copper and vitamins carotene and
vitamin c were almost similar but the uptake of the nutrients and parameters such as protein, fiber, carbohydrate, energy, magnesium and phosphorous were much more in the case of 1:1, 1:2, spent wash irrigation than 1:3, and raw water irrigations (Table-5).

CONCLUSION
It was found that, the germination of both Himalayan Balsam (Balsaminaceae) and Crossandra (Acanthaceae) was very good (100%) in 1:3 SW irrigation, while very poor (25%) in 1:1 SW, moderate (80%) in 1:2 SW and 95% in RW irrigations. This concludes that, the maximum absorption of nutrients by plants at more diluted spentwash irrigation. At higher concentrations, spentwash made the mask on the surface of soil and hence, decreases the sprouting of seeds. Growth of plants is also maximum in 1:3 SW irrigation than 1:1 SW, 1:2 SW and RW irrigations. This might be due to the

ACKNOWLEDGEMENT
Authors are grateful to The General Manager, N. S. L. Koppa, Maddur Tq., Karnataka, India, for providing spentwash.

REFERENCES
1. Wanted Himalayan Balsam, British isles.
3. Impatiens glandulifera plants for a future.
5. Yamunchi, M; Tsuruma, K; Imai, S; Nakanishi, T; Umigai, N; Shimajawa, M; Hara, H (2011).


Table: 1 Chemical characteristics of distillery Spentwash

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<th>1:3 PTSW</th>
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<tr>
<td>pH</td>
<td>7.57</td>
<td>7.63</td>
<td>7.65</td>
<td>7.66</td>
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<td>Electrical conductivity(^a)</td>
<td>26400</td>
<td>17260</td>
<td>7620</td>
<td>5330</td>
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<tr>
<td>Total solids(^b)</td>
<td>47200</td>
<td>27230</td>
<td>21930</td>
<td>15625</td>
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<tr>
<td>Total dissolved solids(^b)</td>
<td>37100</td>
<td>18000</td>
<td>12080</td>
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<td>Total suspended solids(^b)</td>
<td>10240</td>
<td>5380</td>
<td>4080</td>
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<tr>
<td>Settleable solids(^b)</td>
<td>9880</td>
<td>4150</td>
<td>2820</td>
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<td>COD(^b)</td>
<td>41250</td>
<td>19036</td>
<td>10948</td>
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<td>BOD(^b)</td>
<td>16100</td>
<td>7718</td>
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<td>2430</td>
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<td>Carbonate(^b)</td>
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<td>Nil</td>
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<tr>
<td>Bicarbonate(^b)</td>
<td>12200</td>
<td>6500</td>
<td>3300</td>
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<tr>
<td>Total Phosphorous(^b)</td>
<td>40.5</td>
<td>22.44</td>
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<td>10.80</td>
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<tr>
<td>Total Potassium(^b)</td>
<td>7500</td>
<td>4000</td>
<td>2700</td>
<td>1620</td>
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<tr>
<td>Calcium(^b)</td>
<td>900</td>
<td>590</td>
<td>370</td>
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<td>Magnesium(^b)</td>
<td>1244.16</td>
<td>476.16</td>
<td>134.22</td>
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<td>Sulphur(^b)</td>
<td>70</td>
<td>30.2</td>
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<td>Sodium(^b)</td>
<td>520</td>
<td>300</td>
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<td>Chlorides(^b)</td>
<td>6204</td>
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<td>3404</td>
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<td>Iron(^b)</td>
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<td>Manganese(^b)</td>
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<td>Zinc(^b)</td>
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<td>Copper(^b)</td>
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<td>Cadmium(^b)</td>
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<td>0.002</td>
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<td>Lead(^b)</td>
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<td>Chromium(^b)</td>
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<td>Nickel(^b)</td>
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<td>0.045</td>
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<td>Ammonical Nitrogen(^b)</td>
<td>750.8</td>
<td>352.36</td>
<td>283.76</td>
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<td>Carbohydrates(^c)</td>
<td>22.80</td>
<td>11.56</td>
<td>8.12</td>
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Units: \(a - \mu S\), \(b - mg/L\), \(c - \%\), PTSW - Primary treated distillery spentwash

Table: 2 Amount of N, P, K and S (Nutrients) in distillery Spentwash

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<th>Chemical parameters</th>
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<th>1:1 PTSW</th>
<th>1:2 PTSW</th>
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<td>283.76</td>
<td>160.5</td>
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<tr>
<td>Total Phosphorous(^b)</td>
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<td>Total Potassium(^b)</td>
<td>7500</td>
<td>4000</td>
<td>2700</td>
<td>1800</td>
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<tr>
<td>Sulphur(^b)</td>
<td>70</td>
<td>30.2</td>
<td>17.8</td>
<td>8.6</td>
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Unit: \(b - mg/L\), PTSW - Primary treated distillery spentwash

Table: 3 Characteristics of experimental soil

<table>
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<td>Coarse sand(^b)</td>
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<td>Fine sand(^c)</td>
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<tr>
<td>Silt(^c)</td>
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<td>Clay(^c)</td>
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<tr>
<td>Available Phosphorous(^b)</td>
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<tr>
<td>Available Potassium(^b)</td>
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<td>Fine sand</td>
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<tr>
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<td>DTPA Copper</td>
<td>12</td>
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<tr>
<td>DTPA Zinc</td>
<td>60</td>
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</table>

Units: a – μS, b – mg/L, c- %

Table: 4 Characteristics of experimental soil
(After harvest)

<table>
<thead>
<tr>
<th>Name of the plants</th>
<th>RW 15th 22nd 29th (Day)</th>
<th>1:1SW 15th 22nd 29th (Day)</th>
<th>1:2 SW 15th 22nd 29th (Day)</th>
<th>1:3 SW 15th 22nd 29th (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Himalayan Balsam (Balsaminaceae)</td>
<td>22, 25, 27</td>
<td>08, 09, 10</td>
<td>23, 24, 26</td>
<td>23, 27, 31</td>
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<tr>
<td>Crossandra (Acanthaceae)</td>
<td>23, 24, 26</td>
<td>06, 08, 09</td>
<td>22, 25, 26</td>
<td>24, 26 30</td>
</tr>
</tbody>
</table>

Table: 5 Growth of Himalayan Balsam and Crossandra plants at different irrigations (cm)
ABSTRACT
This paper have been produced and developed to study the factors affecting job satisfaction of university faculty members. Various concepts and sometimes conflicting views have been formed and developed concerning the definition of “job satisfaction”. Some experts such as Herzberg believes that it has two dimensions: one group is the factors and conditions that their absence may lead to dissatisfaction; however, they do not cause a strong motivation if provided, but only prevents the occurrence of dissatisfaction, that is the health factors or the ones influencing in maintaining the status quo or survival factor. According to Herzberg, these factors include: staff attitudes and perceptions, methods of administration, organization policies, nature and extent of supervision, job security, working conditions, status, salary level, the establishment of bilateral relations, supervisors, homogeneous, subordinate staff’s personal life. The lack of these factors may lead staff to dissatisfaction to the extent that employees leave the organization and endanger its existence. Hence, Herzberg maintains that these factors are necessary to provide and maintain the organization’s health. The second factors are the ones affecting in creating motivation which are lead to individual’s motivation and satisfaction, but their absence only makes a poor satisfaction. Therefore, the absence of the second group factors is the same as not having attitude. According to Herzberg, the factors affecting in creating motivation are: business success, recognition and appreciation of the people and their work, job and career development, personal growth and the nature of one’s work are called motivational factors.

Keywords: satisfaction, dissatisfaction, job satisfaction, needs, factors affecting job satisfaction

INTRODUCTION
From the early 1920s, the discussion about job satisfaction has been paid attention to different management schools and as Locke (1972) predicted, at least 3,350 articles were published in this field up to 1978, and over hundreds of articles are annually published on job satisfaction now. Robbins defines job satisfaction as person’s general attitude towards his job. Shertzer maintains that job satisfaction is considered as having interest the tasks required to have a job, the conditions where a job is performed and the reward obtained for. Graham conducted a research concerning job satisfaction.
Needs Theory
One of the most important theories in the field of human motivation proposed by Abraham Maslow entitled “hierarchy of needs” including: physiological needs, safety needs, need to love, esteem needs, self-actualization needs, the needs of research and freedom of speech and the need to acquire knowledge and understanding. Maslow maintained that once one level of needs is met they are no longer motivational and higher level of motivation are initiated for person’s motivation. In the end, Maslow did not maintain that his hierarchy of needs are comprehensive and total and the same goes for everyone and everywhere.

Health-Motivation Theory
One of the controversial theories in human nature was offered by “Frederick Herzberg” which is called Herzberg’s two-factor theory. Herzberg conducted a study of approximately 200 accountants and engineers employed in institutions located in the preview of Petersburg, Pennsylvania. He made use of “expressing the critical events” to present a substantive theory, then the responses have been analyzed and the reasons of staff’s satisfaction and dissatisfaction are derived, and came to this conclusion that bad feelings often related to job environment and/or job satisfaction is concerned with job content and dissatisfaction is the one with job environment. Satisfying factors are called “stimulators” and dissatisfaction factors are “health factors”.

Health factors or the ones maintain the status quo which their absence lead to dissatisfaction, but they are not lead to strong and powerful motivation if not existed provided and. Providing these factors only prevents dissatisfaction, but they are not lead to motivation if existed. Existing these factors maintain staff in the organization, otherwise they are affected to extreme satisfaction and may leave the organization.

There are other factors affecting in creating motivation and are lead to individual’s satisfaction and motivation if existed, while they are lead to weak dissatisfaction if not existed. Therefore, their absence is lead to lack of motivation. Herzberg maintains that the factors affecting in creating motivation are business success, recognition and appreciation of people and their works, job development and advancement. He called these factors “motivational factors”.

According to Herzberg, job satisfaction may be increased without job dissatisfaction decreased, and vice versa. Table (1) shows the theoretical relationship between motivational and health factors in job environment in Herzberg’s model.

<table>
<thead>
<tr>
<th>Health</th>
<th>Stimulator</th>
<th>Factor/Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of dissatisfaction</td>
<td>Satisfaction</td>
<td>Presence</td>
</tr>
<tr>
<td>Dissatisfaction</td>
<td>Lack of satisfaction</td>
<td>Absence</td>
</tr>
</tbody>
</table>

Factors Affecting Job Satisfaction
Five dimensions of job are as follows:
1. Automatic or nature of work: the amount where by a job provides interesting tasks, opportunities to learn and the possibility to accept responsibilities for the person.
2. Promotion opportunities: opportunities and chances for advancement in the organizational hierarchy.
3. Payment: the rewards and wages someone receives and the amount considers the payment as just and equitable against other staff’s wages. 
4. Monitoring and control: the ability to supervise in providing supportive behaviors and technical assistance. 
5. Partners: the extent to which partners are technically efficient and are socially supportive to the person. 

Concerning job satisfaction, Snider (1975) maintains that job satisfaction is one’s assessment of the current conditions and positions in the job and the results achieved from having the job. Poti and Khan maintain that one’s emotional aspects of working in an organization is called job satisfaction. 

In 1960s, Mrs. Smith and colleagues mentioned the following factors affecting job satisfaction:
1. Wages (income)
2. The nature of job
3. Opportunities for job promotion
4. Monitoring and supervision
5. The peer group

Wage Sand Income
It has a determining role on staff’s job satisfaction. In a research conducted on more than 2000 manager, Porter and Lowler found out that there is a relatively strong relationship between income and job satisfaction. 

Similar reports are offered by Smith and Kindal. They reckoned that there is a strong relationship between annual incomes of industrial workers in 21 factories and job satisfaction [2]. 

Automatic or nature of job: having published Herzberg’s research results in the book “Motivation at Work” in 1959, the role of automaticity in job satisfaction has been considered.

Three aspects of nature of job affecting job satisfaction are as follows:
1. High control on methods of work performed and its rapid measurement.
2. Variation in scientific management school, high emphasis on specialization and division of responsibilities and jobs to enhance efficiency.
3. Using skills and abilities.

**Instruments to Measure Job Satisfaction**
People do not clearly express their views to what they reveal, but preserve their attitudes towards the policies, laws and other issues which are widely relevant to them. The attitudes associated with the work are no exception. Most of these people reveal their attitudes related to job towards their close friends and relatives, but do not make it clear to their own supervisors and chiefs. Thus, contrary to initial impressions, assessing job satisfaction is a difficult task. However, there are several ways to evaluate job satisfaction that one is briefly explicated in this section[3].

Job Description Index (JDI): one of the most accurate and common means of measuring job satisfaction is Job Description Index which has been developed by Smith at Cornell University (Nanchian, Tavakoli, Mousavi and Trameshlou). In this index, respondents respond to some descriptive and short sentences about each five aspects of job position. These include regarded nature of work, supervision, coworkers, salary and wage and job promotions.

**Research on Job Satisfaction**
The research conducted by Frederick Herzberg in 1966 on 200 accountants and engineers of industrial and commercial organizations in Petersburg City, in Philadelphia State of America may be considered as one of the first ones performed in this field. Having studied in this regard, he concluded that job
satisfaction depends on five factors including job success, job identification, job attractiveness, job responsibilities and career advancement, and the lack of employee’s job satisfaction depends on some factors as below:

1. Regulations, policies and administrative regulations.
2. Proper monitoring and control.
3. Wage rights.
4. Private relations of corporate individuals.

From the beginning of human relations movement, all comments were concerned with the relationship between performance and satisfaction. Content theories are absolutely assumed that satisfaction causes improving performance; and on the contrary, dissatisfaction causes the lack of attention to performance. However, Porter and Lawler maintain that motivation is not the same as satisfaction and performance. Satisfaction, motivation and performance are separate variables that are related to each other differently, and there is a complex relationship between motivation, satisfaction and performance.

Porter and Lawler maintain that the rewards that are consecrated and how they received is determined by satisfaction and indicated that satisfaction is lead to performance and suggested that in practice, managers must step beyond what are traditionally thought and measure variables such as the values of possible rewards, understanding possible effort-reward and understanding the roles; these variables certainly helps management have a better understanding staff’s efforts and performance [4].

Woroum came to the following conclusions in his research:

- There is a negative relationship between job satisfaction and retirement from work.
- There is a negative relationship between job satisfaction and job absence.
- There is a negative relationship between job satisfaction, the extent of injuries and accidents resulted from working.
- There is a positive relationship between job satisfaction and performance.

Kimbel Wilez maintains, in his research lasted four years, that the following factors are the ones affecting job satisfaction among faculty members:

1. Assuredness and comfort in life
2. Fair and equitable treatment
3. Feeling love and attachment
4. Participating in determining working policy
5. Desirable working conditions
6. Support and assistance of managers towards personnel
7. Appreciation and acknowledgment to the services performed
8. Feeling of success and development

The above mentioned research hypotheses was confirmed by various correlation coefficients. Good satisfaction will increase the level of job satisfaction. Faculty member’s job dissatisfaction is more in men than women and there is an inverse relationship between job satisfaction and level of education.

Having reviewed the scientific writings, Ratsoury concluded that job satisfaction among faculty members in bureaucratic universities is low and there is also a correlation between job satisfaction and motivation [5]. Hersy and Blanchard found out in their studies that there is a positive relationship between leadership styles (ordering, understanding, participative and delegating personality) and job satisfaction of faculty members. They found out that faculty members are less satisfied with wages and facilities and are more pleased with cooperation and participation in leadership.
Kenly and Lerin Stone (1993) reported that experienced faculty member’s participation in re-designing job cause their job satisfaction and this has less effect on new faculty members. But increasing salary is effective in increasing job satisfaction in both groups. Kahn also points out four independent factors in his investigations that indicate employee’s satisfaction:

- Usefulness and satisfaction of duty.
- Certain elements of the task that are mutually beneficial, such as
  - Being satisfied by job’s physical value, the present salaries and those of the future.
  - Job satisfaction, having interest and enthusiasm to job and the dignity that it provides for the owner.
- Being satisfied by organization, working conditions and operation of device.
- Being satisfied by professional’s competency in the role of supervisors and leaders[6].

Roll and Kani (1993), Barry Foldokrouk Vadani. etc. conducted some studies concerning job satisfaction and as others, they discussed the factors affecting the employee’s job satisfaction and considered it as an effective factor in organizational efficiency and effectiveness and implicitly indicated that regarding it is one of administrative needs.

Maghaze found out in his study that faculty members are satisfied with their jobs but are not satisfied with the ones such as authorities’ performance, how to administrate university, working conditions, inadequate facilities and amounts of salaries[7].

Esmaili (2008) came to this general conclusion, in a research conducted in Tehran, that there is a positive and significant relationship between faculty member’s job satisfaction and their academic performance. That is to say, the academic satisfaction is increased when job satisfaction is increased and their performance is reduced when their satisfaction is declined[8].

Moradi (2010) came to a conclusion, regarding job satisfaction that was almost different from the ones obtained in other studies. He concluded that men have more job satisfaction than women[9].

Kontenz maintains that job satisfaction is lead to organizational effectiveness and efficiency[10].

According to the aforementioned studies, it seems that factors as nature of work, colleagues, opportunities for promotion and advancement, salaries and facilities and management is effective on faculty member’s job satisfaction.

Generally speaking, it can be said that people who are motivated to work are more required into high level needs of hierarchy of needs, such as the need to be respected, the need to be independent and the need for self-actualization. Regarding other’s needs is resulted to their satisfaction sand satisfaction is lead to consistency and commitment.

**Suggestions**

As the above-mentioned studies indicated, job satisfactions are among the factors that can be resulted into organizational effectiveness and efficiency and prevents from negative consequences of its absence. Therefore, the following cases are suggested to headmasters, assistants and administrators in universities and higher education:

1. Faculty members are often dissatisfied their jobs and currently do not involve in their working.

Being away from workplace help dissatisfied people avoid from unpleasant aspects of their workplace. On the contrary, satisfied faculty members are less inclined
to making complain; they are more acquired with health and longevity and quickly learn the tasks associated with their job. Therefore, it is required the workplace to be pleasant and desirable for faculty members to be benefited from increasing satisfaction as well as other consequences.

2. Manager’s support and is an important factor in faculty member’s satisfaction which can be effective in developing a good understanding of organization’s environment. If faculty members are assured they are acquired with managers and co-worker’s support, they will continue their work with greater confidence and interest and will be more satisfied.

CONCLUSION
Being satisfied with university’s goals and performances can increase people’s motivation and morale in higher education, and on the contrary, if someone does not satisfied with one’s workplace, hell will not be satisfied and his working motivation will be reduced. An appropriate and desirable atmosphere is so effective in creating motivation and satisfaction in people. It would be diligent to create such an atmosphere.

One of the important factors in creating motivation and an appropriate atmosphere is workplace satisfaction. If the workplace is inappropriate and people are not satisfied within, their motivation will be weakened. There are many factors involved in creating a suitable and satisfactory environment, including:
Creating intimacy and cordiality among people, establishing security, regarding rest times and classroom, the time to handle personal affairs and being reasonable.
Establishing facilities and healthy recreation: healthy and appropriate recreation must be arranged to create diversity and increasing work and life motivation with taking into account appropriate time and duration.
Creating a sense of mutual respect among people, regarding respect and considerate attention.
People are required to the opportunities for advancement and promotion in workplace, having rights and adequate facilities. Establishing these factors is highly significant in the profession of faculty and higher education members.
Creating a rewarding and encouraging system which is appropriate with providing objectives and organization’s missions is tremendously significant in employee’s advancement and personal development as well as creating job satisfaction within.

ACKNOWLEDGMENTS
The author wish to thanks from Prof. M.H. Pardakhtchi and Prof. K. Fathi due to their efforts, also thanks to assistants for cooperation in editing this paper.

REFERENCES
ABSTRACT

Recent reports have shown the increased emergence of bacteria resistance to many existing antimicrobial drugs. This has prompted the need to find alternative remedies, and plant products have proven to be vital in this search. *Vernonia colorata* has been reported to be active against syphilis, pneumonia, measles, dysentery and several skin infections in traditional medical practices. In the present study, aqueous and ethanolic extracts from the leaves of *Vernonia colorata* were evaluated in vitro for growth inhibitory activity on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using Agar diffusion method. Phytochemical analysis revealed the presence of reducing sugars, saponins, polyphenols, tannins, phlobatannins, alkaloids, sterols and triterpenes in both extracts. These classes of phytochemicals have widely been reported for their antibacterial properties. Of the several bacteria tested, only *S. aureus* and *P. aeruginosa* showed significant susceptibility to both ethanolic and aqueous extracts with concentrations range between 2.00 to 5.00mg/ml. The aqueous extract also showed the highest activity on *S. aureus* at concentration of 5mg/ml. The Minimum Inhibitory Concentration of the aqueous extract ranged between 4.00 and 6.00mg/ml while that of the ethanolic extract ranged between 5.00 and 6.00mg/ml. Following the results from the current study, it can be concluded that *V. colorata* has significant antibacterial activity and will be very useful in the discovery of novel antibiotics against *S. aureus* and *P. aeruginosa*.

Keywords: *Vernonia colorata*, Antibacterial activity, Minimum Inhibition Concentration, Phytochemical screening, Agar diffusion method

INTRODUCTION

Both ancient and modern men of all cultures have widely used medicinal plants for treating different ailments. Scientific discoveries have shown that plants produce a wide range of complex compounds (secondary metabolites) as part of their normal metabolic process. Several secondary metabolites have been reported to have significant therapeutic properties. Therefore, plants are model source of medicines as they contain many chemical agents with therapeutic properties. Despite increasing advancement in the field of medicine and molecular diagnosis, reports indicate that close to 80% of the world population still dependant on plant derived pharmaceuticals. There are also reports that suggest that nearly 28% of drugs available in the market are plant based products and its derivatives. (Newman et al., 2003). In recent times, scientists have extensively reported on bio-assays of several plants of nutritional and medicinal values. Furthermore,
a large proportion of compounds used as lead molecules in drug discovery are plant based compounds. This suggests that plant based compounds play a vital role in diversity oriented synthesis of natural product-like pharmaceuticals. (Marcaurelle and Johannes, 2008).

Bacterial infection treatment options include chemotherapy, radiation therapy and surgery. In chemotherapy, antibacterial drugs are usually employed in the treatment of various forms of bacterial infections. However, there are reports on increased emergence of multi-resistant bacterial strains of clinically important pathogens. This development has fetched the interest of scientist to develop newer broad spectrum antimicrobial agents. Due to the high cost and the less availability of new generation antibiotics, it is imperative to look for the substances from alternative medicines with claimed antimicrobial activity. A significant number of medicinal plants with significant antimicrobial activity have been reported in different traditional literatures. Vernonia is a genus of about 1000 species of forbs and shrubs in the family Asteraceae. The uses of several species of Vernonia, including Vernonia amygdalina, Vernonia auriculifera, Vernonia colorata, Vernonia galamensis, and Vernonia hymenolepis in traditional health care have been reported. There are many convergence in the usage of Vernonia spp. in its traditional use throughout West and Central Africa and North America as anti-inflammatory, analgesic, antibacteria, anticaner, antidiabetic, antifungal, antimalaria and antioxidant. (Abosi and Raseroka, 2003; Phillipson et al., 1993).

Previous studies on Vernonia spp. have largely been confined to Vernonia amygdalina. This species has been reported to contain glycosides, tannins, steroidal saponins, sesquiterpenes lactones flavonoids and vitamin C. (Ifeoma and Chukwunonso., 2011). V. Amygdalina is also known for its activity against syphilis, malaria, measles, dysentery and yellow fever. (Oluwalana and Adebunle.,1998).

V. colorata has also been reported to be used in traditional herbal medicine across many African countries for the treatment of bacteria, fungal, parasitic and inflammatory disorders. Despite the traditional uses of V. colorata in primary health care, reports on the phytochemical profile and bio-assay of V. colorata are limited.

In this regard, we aim to explore scientifically, the antibacterial potential of V. colorata to substantiate the reported claims. The current research seeks to screen for phytochemicals in the leaves of Vernonia colorata and also evaluate its potential as an antibacterial agent by analysing its growth inhibitory activity on E. coli, K. Pneumoniae, P. aeruginosa and S. aureus.

MATERIALS AND METHODS

Materials

Plant material

Fresh leaves of vernonia colorata were collected from a piece of land at Mampong-Akuapim in the Eastern Region of Ghana. The leaves were later taken to the herbarium Department of the Centre for Scientific Research into Plant Medicine, Mampong-Akuapim, for botanical identification.

Reagents

Ethanol, Fehling’s solutions, Chloroform, Acetone, Sodium Picrate paper, Ferric chloride, Ammonia, Chloramphenicol test paper, Dimethyl sulphoxide (DMSO), Hydrochloric acid (HCl), and Sulphuric acid(H$_2$SO$_4$) were of analytical grade and from BDH, UK. Other reagents used were of analytical grades and water used was glass distilled.

Bacteria strains

Four different microbes of standard strains were purchased at the Komfo Anokye Teaching Hospital (KATH) Kumasi, with standard codes. The microbes were; E.coli (ATCC25922), K. Pneumoniae (ATCC
Methods

Extraction from plant material
Adequate quantity of the leaves were collected, sun dried and then milled. The fine powder was then divided into two portions.

Preparation of ethanolic extract
About 500g of the powder were soaked in 5L of 70% ethanol for 24 hours. The suspension was then filtered. The filtrate was concentrated using rotary evaporator and then freeze dried.

Preparation of aqueous extract
Another 500g sample of the powdered leaves was soaked in 5L of distilled water and then boiled for 15 minutes after which the temperature was lowered to 60°C for another 15 minutes. This was then filtered and the filtrate evaporated using rotary evaporator at 60°C and then freeze dried.

Phytochemical analysis
About 5g of each freeze dried sample of crude extract was dissolved to 100ml and portions analyzed for phytochemical constituents using standard methods. (Stahl, 1969; Harborne, 1973)

Microbiology

Media
Muller Hilton Agar and Peptone Agar were used.

Sterilization
All other materials used in the microbiological work were sterilized before usage.

Preparation of Muller Hilton agar
About 15.2g of Muller Hilton agar was weighed and added to 400ml of distilled water, and then heated to dissolve. The media was sterilized at 121°C in an autoclave for 20 minutes and then cooled to about 60°C.
About 20ml of the media was poured gently into each plate and left to cool and then stored in an oven at 37°C for 16 hours.

Peptone water
Peptone agar was used as the broth for the culture of the microbes.

About 0.5g of peptone agar was weighed and added to 50ml of distilled water and then heated to dissolve. This was sterilised at 121°C for 20 minutes and cool to about 60°C. 5ml portions of the broth were pipetted into test tubes and then stored in an oven at 37°C for 16 hours.

Antimicrobial susceptibility test
The antibacterial test was performed using the agar diffusion method of Collins et al. (1995). The test microorganisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm in diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized by flaming. Different concentrations of the freeze extracts were prepared by dissolving various masses in 20% dimethyl sulphoxide (DMSO). To each well was introduced different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 mg/ml) of plant extracts. Control experiments were set up using Chloramphenicol and DMSO as positive and negative controls respectively.
The plates were allowed to stand for one hour at room temperature for diffusion of the substances to proceed before the growth of microorganisms commenced. The plates were made in triplicate and were incubated at 37°C for 24 h. Diameters of zones of inhibition in the triplicate plates were measured by calculating the difference between cork borer (5mm) and the diameters of inhibition (Hewett and Vincent, 1989; Singh et al., 2002; Adebayo and Adegoke; 2009). The zones of inhibition were then recorded.

Determination of minimum inhibitory concentration (MIC)
Various concentrations of both aqueous and ethanolic extracts ranging between 4.0 and 6.0 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of S. aureus, P. aeruginosa, E. coli and K. pneumonia diluted to give a final concentration of 10⁶ cells per ml. The
tubes were incubated at 37°C for 24 h. The least concentration of extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case (Collins et al., 1995).

RESULTS AND DISCUSSION

Phytochemistry of plant extracts
Preliminary phytochemical screening of crude aqueous and ethanolic extracts from leaves of *V. colorata* revealed the presence of triterpenes, sterols, alkaloids, reducing sugars, polyphenols, tannins, phlobatannins and saponins. Cynogenic glycosides, polyuronoids, anthracenocides and flavonoids were absent. (Table 1).

Antimicrobial activity
Both crude ethanolic and aqueous forms of the extracts of *V. colorata* exhibited varying degree of antimicrobial activities against the test organisms. (Table 2a,b) *E. Coli* and *K. pneumoniae* showed significant resistance against both aqueous and ethanolic extracts of *V. colorata* at concentrations below 5.00mg/ml. However, *S. aureus* and *P. aerugenosa* showed susceptibility to both extracts at concentrations between 4.00 and 5.00mg/ml. (Table 2a,b).

It was observed that antibacterial effectiveness increased with increasing concentration of extracts. This supports previous work by Kurosaki and Nishi (1983) who reported that higher concentrations of antimicrobial substances showed appreciable growth inhibition to microorganisms.

Although, phytochemical screening revealed similar results for both aqueous and ethanolic extracts, the compounds found in the classes of phytochemicals present in each extract may differ and this could account for their varying inhibitory activities. (Table 2a,b). Another possible reason for the varying degree of inhibition by the extracts could be the presence of other classes of phytochemicals that were not tested for in the ethanolic extract. Further to this, the boiling of leaves during the preparation of the aqueous extract may render some compounds inactive in the aqueous extract and this could also contribute to the observed variation in inhibitory activities.

Although the positive control (Chloramphenicol) showed significant growth inhibitory activity on all the bacteria tested, the aqueous extract was found to be more effective on *S. aureus* at concentration of 5mg/ml while the ethanolic extract was found to more effective on *P. pneumoniae* at the same concentration than the standard. (Table 2c).

The MIC of the aqueous extract in this study against the test organisms ranged between 4.0 and 6.0 mg/ml while those of the ethanolic extract ranged between 5.0 and 6.0 mg/ml. (Table 3).

Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. The present result shows that the aqueous extract is slightly more effective than the ethanolic extract.

Tannins, alkaloids saponins and phlobatannins have been reported for their antibacterial and antiviral activity (Enzo, 2007). Furthermore, alkaloids and saponins are classes of compounds that are known to be effective for the treatment of syphilis and other venereal diseases. (Sofowara, 1993). Steroids in modern clinical studies are known for their anti-inflammatory and analgesic properties. (Pithayanukul et al., 2007). The antibacterial activities demonstrated by extracts from *V. colorata* may be attributed to the presence of these phytochemicals and this supports the use of the plant for the treatment of syphilis, pneumonia and skin infections as reported in traditional folk medicine.

CONCLUSION
Although, a large number of medicinal plants are constantly screened for their antimicrobial effects, many plant species with potent antimicrobial properties are yet to be
discovered. The present study reveals the antibacterial potential of leaves of *V. colorata*. The antibacterial activities of the extracts against *S. aureus* and *P. aeruginosa* were comparable to those of the standard (Chloramphenicol). These results seem to justify the continued use of the plant in the treatment of microbial infections such as pneumonia, syphilis and skin diseases. In addition, the inhibition of growth of the test organisms (*S. aureus* and *P. aeruginosa*) that are known to display multidrug resistance to most antibiotics and nonantibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice.

### Table 1. Phytochemical analysis of aqueous and ethanolic extracts of *Vernonia colorata*

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cynogenic Glycosides</td>
<td>-</td>
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</tr>
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<td>Anthracenocides</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**KEY:** (+) = Presence of Phytochemical constituent; (-) = Absence of Phytochemical constituent

### Table 2a: *In vitro* Antimicrobial activity of *Vernonia colorata* aqueous Leaf extract

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Mean Diameter of Zone of Inhibition (mm±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
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<tr>
<td>1.0</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>2.0</td>
<td>0.00±0.00</td>
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<tr>
<td>3.0</td>
<td>0.00±0.00</td>
</tr>
<tr>
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</tr>
<tr>
<td>5.0</td>
<td>12.00±0.00</td>
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### Table 2b: *In vitro* Antimicrobial activity of *Vernonia colorata* ethanolic Leaf extract

<table>
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<tr>
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<td>E. coli</td>
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<tr>
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Table 2c: *In vitro* Antibacterial activity of Chloramphenicol

<table>
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<th>Mean Diammeter of Zone of Inhibition (mm±SEM)</th>
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<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
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<tr>
<td></td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>5.0</td>
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<tr>
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<td>20.00±0.20</td>
</tr>
<tr>
<td></td>
<td>18.00±0.30</td>
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<tr>
<td></td>
<td>20.00±0.00</td>
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</table>

Table 3: Minimum Inhibitory Concentrations of Extracts on organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (aqueous extract) mg/ml</th>
<th>MIC (ethanolic extract) mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>P. aerugenosa</td>
<td>4.5</td>
<td>5.0</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>4.0</td>
<td>6.0</td>
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REFERENCES
ABSTRACT

A 55 year old mild hypertensive presented with typical angina pain associated with giddiness and profuse sweating. His initial Electrocardiogram showed ‘Junctional rhythm’ with diffuse ‘T’ wave changes in leads II, III, avF, V4 to V6. 2D Echocardiogram, on the same day, showed regional wall motion abnormality of Left Anterior Descending (LAD) and Left circumflex (LCX) /or Right Coronary Artery (RCA) territories with moderate Left Ventricular dysfunction. Subsequently, Coronary Angiogram done two weeks later showed normal epicardial coronaries.

Keywords: Coronary Spasm; Prinzmetal; ECG abnormalities; Regional Wall Motion Abnormality (RWMA); Acute coronary syndrome

INTRODUCTION

Prinzmetal variant angina has always been a medical curiosity and thought to be a rare entity, nonetheless increasing number of cases are being reported every year. It was first described as "A variant form of angina pectoris" in 1959 by the American cardiologist Dr. Malcolm Prinzmetal.1 Prinzmetal angina most commonly affects a single site (usually the RCA) and presents as a focal spasm, which may vary for each attack (migratory spasm).2,3 We report a 55 year old man who presented with multi-vessel involvement as evidenced by his Electrocardiogram and Echocardiogram findings.

Case Report

A 55 year old man was brought with history of typical anginal pain associated with giddiness and profuse sweating in the early morning hours lasting for half an hour. He was euglycemic and a non smoker diagnosed to have hypertension 6 months prior to the present hospitalization, which was well controlled on Enalapril Maleate (5mg). His lipid profile was normal and the family history was non-contributory.

As he could reach the hospital only four hours later, his electrocardiogram that time showed ‘Junctional rhythm’ at rate of 50/min with deep “T” inversion in leads II, III, avF, V4 to V6.
Consequent 2D Echocardiogram showed regional wall motion abnormality involving the both Left coronary artery and Left Circumflex /or Right coronary artery domains with moderate LV dysfunction (Ejection Fraction ~ 38%).

A provisional diagnosis of Acute Coronary syndrome was made and was managed conservatively with low molecular weight Heparin, Statins and Anti-platelet drugs before he was referred to our hospital. On arrival at our hospital, he was totally asymptomatic, his ECG was within normal limits (Figure: 2) and his ECHO showed normal LV systolic function with no RWMA. A coronary Angiogram was done which showed normal epicardial coronaries with left dominant system (Figures: 3 & 4). Provocative tests could not be done due to ethical and safety concerns. Intra Vascular Ultra sonogram (IVUS) could not be performed on him due to lack of technical expertise and economical consideration. He was managed with Diltiazem hydrochloride 90 mg (sustained release) along with Asprin 75 mg and Rosuvastatin 10 mg. His hospital course was uneventful and there were no further angina attacks till date (on 10 months follow up).
Figure 2: Electrocardiogram recorded ten days later shows no significant changes

Figure 3: Right Coronary filling shows normal arteries
DISCUSSION

The typical presentations of Prinzmetal’s variant angina (PVA) are: Pain at rest, not related to any physical or emotional stress and associated ST segment elevation. Some studies say that not all ECG changes are accompanied by symptoms, sometimes there are ECG changes even in the absence of symptoms. Moreover the attacks tend to have a circadian rhythm usually between 12:00 am and 8:00 am and occur in clusters. They generally are not associated with any classical risk factors (except for heavy smoking). They are also found to be associated with other vasospastic disorders such as Migraine and Raynaud’s Phenomena. In our study we found that the subject had involvement of both coronary artery territories. The symptoms were present during the early morning hours and were accompanied by ECG and ECHO changes. In some cases, PVA can take a benign course without any complications but in others it can present with dreaded complications such as syncope, AV block, asystole, ventricular tachy-arrhythmias or MI. Although numerous reports are available on variant angina, very little is known about spasm involving multiple vessels.

Various theories have been proposed in the pathophysiology of Prinzmetal angina such as eNOS gene mutation, increased Phospholipase C activity etc. Basically it is an endothelial dysfunction causing increased vasomotor tone or vasospasm and repetitive vasospasm causes injury to the vasculature, which in turn leads to coronary stenosis. There are few provocative tests using Ergonovine and Acetylcholine that can induce coronary vasospasm. The test induced spasm can be relieved by intracoronary nitrates or calcium antagonists. This helps in diagnosing PVA.

Apart from these, 24 hours ECG monitoring can show episodic ST segment elevation, any associated arrhythmias or a silent MI. Exercise stress testing is not very contributory. Since Prinzmetal is not a “demand” induced symptom but
rather a vasospastic abnormality, it can not be induced by exercise. \[3\] Diagnostic hallmark of this disease has always been Angiogram; which may even be normal during an asymptomatic period. Nitrates and Calcium channel blockers (CCBs) are the mainstay of treatment for Variant angina. Nitroglycerin (in any route) effectively treats an episode of angina within minutes and CCB can be used as a prophylaxis to prevent future attacks. \[8, 9\] Diltiazem in particular produces coronary dilation but is a less potent peripheral vasodilator. Accordingly our patient was also started on Diltiazem hydrochloride 90 mg (sustained release) along with Asprin 75 mg and Rosuvastatin 10 mg. And there were no further anginal episodes in him at ten months follow up confirming the efficacy of the drugs on multi vessel coronary spasm. \[10\]

CONCLUSION
A variant of Prinzmetal Variant Angina with features of multi-vessel involvement involving normal or near normal coronary arteries, although transient and seemingly benign, can be life threatening. A multi-vessel presentation should not deter a physician from diagnosing a variant Prinzmetal angina because the condition can be successfully treated and prevented with Calcium channel blockers.

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REFERENCES
ABSTRACT

Aim: Previous studies show that neuropathic pain is refractory against conventional analgesics and thus novel medicaments are desired for the treatment. Activated K⁺ channels are associated with reducing inappropriate or excessive neuronal activity. The aim of this study was to investigate the possible analgesic effects of potassium channel opening on neuropathic pain. Therefore, the present study was designed to investigate whether potassium channel activator can generate qualitative analgesic effects on the acute pain induced by thermal and mechanical stimulation.

Methods: The effect of diazoxide at the dose of 200 mg/kg on acute thermal and mechanical nociception were assessed by sensory testing like spontaneous pain, mechanical hyperalgesia, tactile as well as cold allodynia in chronic constriction injury (CCI) induced pain in rat. Results: After CCI surgery, the rats developed neuropathic pain syndrome. Behavioral studies demonstrated that rats with the CCI experienced spontaneous pain, dynamic allodynia and mechanical hyperalgesia which were significantly different from the sham group. Treatment with diazoxide decreased significantly the withdrawal durations in all sensory tests.

Conclusion: The present study indicates that activity of K⁺ channel may contribute significantly to the development of central sensitization-mediated pain and suggests that K⁺ openers may be an important molecular target for the treatment of chronic pain of neuropathic origin.

Key words: Neuropathic pain, Diazoxide, Potassium channels, Chronic constriction injury, sensory test

INTRODUCTION

Neuropathic pain is one of the most significant health problems in the world. Neuropathic (neurogenic) pain is defined by IASP as pain caused by a lesion or dysfunction of the nervous system (1). It is characterized by inappropriate spontaneous or excessive neuronal activity in response to physiological stimuli. Chronic pain is one of most common reasons for hospital visits so it should be considered to be a disease rather than just a symptom. Recent advances in molecular biology techniques and the subsequent discoveries of key molecules involved in pain production, have clearly contributed to better understanding acute pain (2-5), by which the molecular multiple mechanisms underlying chronic pain can be fully clarified. Proper diagnosis and early
treatment are often found to be difficult in neuropathic pain, because it is quite different from other types of pain, such as nociceptive (or physiological) or inflammatory pain and it is irreversible, even when the underlying cause has been rectified (3). Also, the occurrence of neuropathic pain is commonly as a secondary symptom in diseases (e.g. diabetes, cancer, and herpes zoster infection) or as a side effect of chemotherapeutic treatments (4, 6-8).

The management of this disorder is achieved by various classes of drugs that are capable of dampening neuronal excitability. Examples may be voltage-gated sodium channel blockers (carbamazepine, phenytoin, lamotrigine and topiramate), voltage-operated calcium channel modulators (ethosuximide, gabapentin, levetiracetam) and modulators of inhibitory GABAergic neurotransmission (benzodiazepines, vigabatrin and tiagabine). Of these, the approved drugs for the treatment of neuropathic pain are gabapentin, and carbamazepine and lamotrigine has demonstrated efficacy for neuropathic pain in clinical trials (9). Various drugs with sodium channel blocking actions preferentially suppress thermal nociception which may be partly explained by the local anesthetic action of sodium channel blocking agents and differential sensitivities to local anesthetics of the fibers activated by thermal and mechanical nociception (10).

However, a number of issues regarding this treatment, including the effective, meaningful drug dose range, the durability of pain-relief effects leading a poor treatment to patient with currently available drugs. So there is a need for new agents with their novel mechanism for the treatment of neuropathic pain. Potassium (K+) channel opening is one potential mechanism that has not yet been exploited for neuropathic pain. Activated K+ channels are associated with reducing inappropriate or excessive neuronal activity (11).

Therefore, the present study was designed to investigate whether potassium channel activation generate qualitative analgesic effects on the acute pain induced by thermal and mechanical stimulation.

MATERIALS AND METHODS

Animals

Wistar-kyoto male rats of 8 weeks age with the body weight range from 250-300 gms were procured from Central Animal Facility, Nootan pharmacy college, Visnagar, India. They were maintained in essential condition of controlled temperature (<30˚C) and humidity (<70%) with 12 hour day and night cycle according to the norms of CPCSEA.

Chronic Constriction Injury (CCI)

CCI surgery was carried out as described (12). Rats were anesthetized with combination of ketamine and xylazine and a 7-mm segment of the left common sciatic nerve was exposed at the mid-thigh level, Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue and 4 ligatures (4-0 silk) were tied loosely around at about 1 mm spacing. Ligatures were tied loosely enough so that, on visual inspection, blood flow was not obstructed. The incision was closed in layers. Post seven days of healing nociceptive test was done followed by behavioural assessment.

Sham Surgery:

Same surgical procedure was followed in six animals except the removal part/whole of kidney and kidneys were touched with forceps and threads.

Drugs and Chemicals: Diazoxide was procured from Ranbaxy Research
laboratories, India. DMSO was procured from Veeraj Associates Ahmedabad, India. Ketamine and Xylazine were purchased from Visnagar, India.

**Grouping of animals:**
After one week of CCI in animals they divided in to three groups: Group 1: sham group, Group 2: CCI with drug (Diazoxide) and Group 3: CCI with vehicle (CCI group).

**Treatment with diazoxide:**
Animals in group 2 were treated with Diazoxide at the dose of 200 mg/kg (dissolved in DMSO 25mg/ml) by oral route once a day for 7 days, while that of group 3 were treated with vehicle (DMSO).

**Sensory testing using nociceptive assay in CCI rats:**
Four nociceptive assays aimed to determine the severity of neuropathic responses namely allodynia and hyperalgesia were performed. Estimation of parameters at basal, day 1, day3, day 5 and day 7.

1. **Spontaneous pain**
2. **Dynamic allodynia**
3. **Cold allodynia**
4. **Mechanical hyperalgesia**

**Spontaneous pain:**
Spontaneous pain was assessed for total period of 5 min as described (13). The operated rats were placed into the observation cage 5 cm from working place. An initial acclimatization period of 10 min was given to each of rats. From each group total six rats were assessed. The test is based on recording of the cumulative duration that the rats hold its ipsilateral paw off the floor. The paw lifts associated with locomotion or body repositioning was not counted. The paw lifts in the absence of any overt external stimuli are associated with spontaneous pain, and are correlated of ongoing pain are counted.

**Dynamic alodynia:**
All of the operated rats were assessed for dynamic allodynic response according to the procedures described (14). The operated rats were placed into the observation cage 5 cm from working place. Lifting of the affected paw for finite period of time in response to mild stroking on the plantar region using cotton-bud is a positive dynamic alodynia. This stimulus is non-noxious to normal- behaving rats. The latency to paw withdrawal was then counted. If no paw withdrawal was shown within 15s, the test was terminated and animal were assigned withdrawal time. Hence 15s effectively represented no withdrawal.

**Cold alodynia:**
Application of cold (an acetone drop placed on the paw) in the on the plantar region (15) was used for estimating the withdrawal duration of cold alodynia. If no paw withdrawal was shown within 15s, the test was terminated and animal were assigned withdrawal time. Hence 15s effectively represented no withdrawal.

**Mechanical hyperalgesia:**
The operated rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described (16). The initial set up was same as previous test. The measurement of hind paw withdrawal duration was done after mild pinprick stimulus to the mid plantar surface of the ipsilateral (left) hind paw. A withdrawal was defined as being abnormally prolonged if lasted at least 2s. The mean withdrawal duration was taken from a set of three responses.

**Statistical analysis**
All the data were expressed as mean ± s.e.m. The single treatment studies were analysed using unpaired t-test using graphPad Prism 5.0.
RESULTS

General observations
After CCI surgery, the rats developed neuropathic pain syndrome as previously described (12). Unusual gait and posture of the rats was observed as early as on the first day after surgery. The rats often raised the affected hind paws from the floor and hold them in a protected position. Frequent licks of the affected paws were seen. All the animals were in good health and the behavior was generally normal with no palsy or additional sensory dysfunction encountered. There was no significant difference among the weights of the three groups (data not shown).

Effects on Sensory testing:

Spontaneous pain:
After CCI surgery, the rats developed spontaneous pain which was found to be 191.67 ± 18.97 at the end of 7th day. There was a gradual elevation in the spontaneous pain from day 1 of surgery. Drug treatment at the dose of 200 mg/kg decreased the spontaneous pain. A gradual reduction was observed in animals of group 2 which was observed to be 58.33 ± 2.55 at the end of day 7 post treatment. There was a marked increase in the withdrawal duration in group 3 animals as the days progressed post surgery which was greatest at the end of day 7.

Dynamic allodynia:
Animals exhibited dynamic allodynia which was 72.67 ± 4.73 at the end of day 7, and was found to be reduced to 49.33 ± 0.51 in group 2. A significant difference was found in the withdrawal duration for dynamic allodynia between group 2 and group 3. Moreover sham operated animals of group 1 showed negligible duration of withdrawals, thus a significant difference was also observed between group 1 and group 3.

Cold allodynia:
Elevation in the withdrawal duration (57.67 ± 2.22) was observed in group 3 which was statistically different from group 1. Moreover the drug treatment decreased the duration gradually with maximum effect exerted at day 7 with a value of Cold allodynia 30.67 ± 3.67 which was approximately near that observed in sham operated group.

Mechanical hyperalgesia:
A significant rise was observed in withdrawal duration between group 1 and group 3. Drug treatment decreased the withdrawal duration in group 3 (72.33 ± 0.84) significantly as compared to that of group 2 (126.67 ± 6.94).

DISCUSSION

Painful stimuli are transferred to the CNS by the lateral spinothalamic tract. First order neurons transmitting pain impulses from the skin (Aδ and C fibres) enter the substantia gelatinosa of the dorsal horn via the dorsal roots. Second order neurons in the lateral spinothalamic tracts convey impulses associated with pain up to the nuclei of the ventroposterior thalamus where the painful impulses are integrated. From the thalamus, third order neurons convey the impulses up to the cerebral cortex, where subjective interpretation of pain is thought to occur (17).

Neuropathic pains are disorders characterized by excessive neuronal activity. These disorders are currently managed by drugs that are capable of dampening neuronal excitability, including voltage-gated sodium channel blockers, voltage-operated calcium channel modulators and modulators of inhibitory GABAergic neurotransmission. However, these drugs are rarely 100% efficacious and their use is often associated with limiting side effects. Thus, there is a clear medical
need for novel agents to treat these diseases. One potential mechanism that has not yet been exploited is potassium (K⁺) channel opening. A significant and growing body of genetic, molecular, physiological and pharmacological evidence now exists to indicate that KCNQ-based currents represent particularly interesting targets for the treatment of diseases such as epilepsy and neuropathic pain (11).

Moreover, ATP-sensitive potassium (K_ATP) channels may be linked to mechanisms of pain after nerve injury, but remain under-investigated in primary afferents so far. A study characterized these channels in dorsal root ganglion (DRG) neurons, and tested whether they contribute to hyperalgesia after spinal nerve ligation (SNL). Following SNL, this channel activity was suppressed in large neurons from hyperalgesic rats. In large neurons, selective inhibition of whole-cell ATP-sensitive potassium channel current (I_KATP) by glibenclamide depolarized resting membrane potential (RMP). The contribution of this current to RMP was also attenuated after painful axotomy. These findings indicate that functional K_ATP channels are present in normal DRG neurons, wherein they regulate RMP. Alterations of these channels may be involved in the pathogenesis of neuropathic pain following peripheral nerve injury. Their biophysical and pharmacological properties are preserved even after axotomy, suggesting that K_ATP channels in primary afferents remain available for therapeutic targeting against established neuropathic pain (18). A study has demonstrated that K_ATP channel opening results in decreased excitability, attenuated neurotransmission, and possibly antihyperalgiesia. Therefore, these channels in DRG neurons provide novel opportunities for therapeutic targeting using K_ATP channel openers (19) or CaMKII activators (20, 21) as analgesics in neuropathic pain.

We tested a hypothesis that K⁺ channel opener like diazoxide administration may decline the pain due to decreased excitability of the hyperactive nerves and improve the pain condition to which we obtained consistent results. While our ultimate goal was to elucidate the role of K⁺ channels in neuropathic pain, we employed a well-established chronic constriction injury model (CCI) in this study to investigate effects of our approach. CCI also known as Bennett model is a rat model of painful peripheral mononeuropathy (12). CCI rats show behavioral signs of spontaneous pain such as mild to moderate autotomy, guarding, excessive licking and limping of ipsilateral hind paw, and avoidance of placing weight on the injury side. Hyperalgesia due to noxious thermal and mechanical stimuli is detectable, as are cold allodynia and tactile (12, 22). All pain signs last for the entire duration of the study.

CCI induced spontaneous pain which was characterized by signs of paw guarding, lifting, and limping, excessive grooming and biting, changes in exploratory behavior, weight bearing. In addition, evoked pain (allodynia and thermal hyperalgesia) to thermal or mechanical stimuli was observed in CCI group although it was at different levels. There was a significant increase in the all the four Sensory parameters like spontaneous pain, dynamic allodynia, cold allodynia and mechanical hyperalgesia as compared to the animals of sham group. The parameters were noted at the interval of 1 day right from basal, day 1 post surgery, day 3, 5 and 7 post surgery. There was a gradual augmentation in the withdrawal duration as was observed from the data from day 1 to
day 7. No statistically significant difference was observed between recordings in sham group till day 7.
Treatment with diazoxide at the dose of 200 mk/kg per orally for 7 days significantly reduced spontaneous pain in animals. Moreover a significant change in the mechanical and thermal evoked allodynia/hyperalgesia was also observed as compared to the CCI group. The development of thermal hyperalgesia and tactile allodynia is known to involve separate pathways (23). While noxious thermal stimuli is thought to be mediated through high-threshold, thin unmyelinated primary afferent C-fibers, non-noxious tactile stimulation is believed to be mediated through large diameter, low threshold Ab afferent fibers, and processed at supraspinal sites receiving input through the dorsal columns (24-26). We observed that there was retardation in the progression of the disease in drug treated group as compared to CCI group. But it was not restored to a normal value instead a partial restoration of values were obtained. More extensive research is needed in this area for more specific answers to the questions regarding the involvement of K+ channels in neuropathic pain.

CONCLUSION
Neuropathic pain is thought to become worse with time, untreated appropriately inducing a vicious circle. The development of agents that may block enhanced pain transmission is an important therapeutic approach for research. Neuropathic pains are resistant to conventional narcotic therapies and often incapacitate patients. The search for novel treatments for this pain syndrome characterized by central sensitization has stirred numerous investigations in both the basic science and clinical arenas. The present study indicates that activity of K+ channel may contributes significantly to the development of central sensitization-mediated pain and suggests that K+ openers may be an important molecular target for the treatment of chronic pain of neuropathic origin.

REFERENCES


26. Willis WD, Al-Chaer ED, Quast MJ, Westlund KN. A visceral pain pathway in the dorsal column of the

Fig 1 (a)

Spontaneous pain

![Graph showing the change in withdrawal duration (sec) over days.](image)

Fig 1 (b)

Dynamic alldynia

![Graph showing the change in withdrawal duration (sec) over days.](image)

Fig 1 (c)

Cold alldynia

![Graph showing the change in withdrawal duration (sec) over days.](image)
**Fig 1:** Comparison of withdrawal duration (sec) in sham group and CCI group at the basal, day 1, 3, 5, and 7 days post surgery by sensory tests like (a) Spontaneous pain, (b) Dynamic allodynia, (c) Cold allodynia and (d) Mechanical hyperalgesia. Each data point represents the mean ± S.E.M. The significance of differences between the sham group and CCI group values was determined by unpaired t-test. *P<0.05 and **P<0.01, ***P<0.001 vs sham group in respective time.

**Fig 2:** Effect of Diazoxide (200mg/kg, p.o. once a day) treatment for 7 days in CCI induced chronic pain at the basal, day 1, 3, 5, and 7 days by sensory tests like (a) Spontaneous pain, (b) Dynamic allodynia, (c) Cold allodynia and (d) Mechanical hyperalgesia. Each data point represents the mean ± S.E.M. The significance of differences between the drug treated group and CCI group values was determined by unpaired t-test. *P<0.05 and **P<0.01, ***P<0.001 vs sham group in respective time.
ABSTRACT
The aim of the present study was to isolate Salmonella typhi from various water sources and identification of drug resistant genes cat P and tem using Multiplex PCR technique. Enteric fever is prevalent world over and continues to be a major public health problem in developing countries. Infection with Salmonella typhi, the causative organism of this disease, requires effective antimicrobial chemotherapy in order to reduce mortality. Antibiotic-resistant strains of Salmonella are now encountered frequently and the rates of multidrug-resistance have increased considerably in recent years. In the present study, 30 samples were collected from various water sources, of which Salmonella typhi was isolated from 12 samples. All the isolates were subjected to antimicrobial susceptibility testing by standard disc diffusion method against 10 antibiotics. Isolates resistant to chloramphenicol and ampicillin were selected and the drug resistant genes were identified as cat P and tem respectively by Multiplex PCR technique. There was a very good correlation between the genotypic analysis by PCR and the phenotype determined by standard methods of susceptibility testing.

Keywords: Multiple drug resistance, Salmonella typhi, Disc diffusion technique, DNA extraction, Agarose gel electrophoresis, Multiplex PCR

INTRODUCTION
Water meant for human consumption should be free from pollution and should be safe and acceptable. Indeed the microbial quality of potable water should not exceed limits specified in the water quality guidelines (APHA – 1998).1 The presence of bacteria and pathogenic (disease-causing) organisms is a concern when considering the safety of drinking water.2 Human and animal wastes are the primary source of bacteria in water. These sources of bacterial contamination include runoff from feedlots, pastures, dog runs, and other land areas where animal wastes are deposited.3,4 Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil or plant bacteria. Bacteria from these sources can enter wells that are either open at the land surface, or do not have watertight casings or caps. Pathogenic organisms can cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, and other illnesses.5
Salmone\textit{lla typhi} is the primary aetiological agent of typhoid fever which is responsible for significant morbidity and mortality, particularly in the developing countries. \textit{S. typhi} is an obligate human pathogen. It causes infection by the faeco-oral route. Typhoid fever is typically acquired by ingesting food or water that has been contaminated by faeces of typhoid-infected individuals. Many outbreaks, caused either by consumption of contaminated water or food have been report. Effective antimicrobial therapy is required for the treatment of typhoid fever to reduce morbidity and mortality. Historically, the drugs of choice were chloramphenicol, ampicillin, and co-trimoxazole. Antibiotic-resistant strains of \textit{Salmonella} are now encountered frequently and the rates of multidrug-resistance have increased considerably in recent years. Chloramphenicol resistance is known in \textit{Salmonella Typhi} since 1972, when plasmids of incompatibility group Inc H, coding for chloramphenicol resistance were found in \textit{S. typhi}. Multi drug resistance, (defined as resistance to all the first line antibiotics used to treat typhoid fever, i.e chloramphenicol, ampicillin, co-trimoxazole and tetracycline) has been endemic in India since 1984. MDR \textit{S. typhi} have emerged as the newer challenges to treatment of typhoid fever. So in this study we have made an attempt to isolate \textit{Salmonella typhi} from the water samples collected from in and around Chennai and to identify the resistant gene responsible for resistance to the conventional drug therapy of typhoid, which is a life threatening endemic infection.

**MATERIALS AND METHODS**

A total of 30 water samples were collected from various water bodies like sewage, stagnant, marine, well and ponds from different areas in and around Chennai. Six different samples were collected from above mentioned water sources and it was brought to the laboratory for further processing.

**ISOLATION OF SALMONELLA TYPHI**

For the isolation of \textit{Salmonella typhi}, 1ml water samples were inoculated in 9ml selenite-F-broth and incubated for 18hrs at 37°C for enrichment. The enriched samples were plated onto \textit{Salmonella-Shigella Agar} (Oxoid) and incubated for 24hrs at 37°C. Small colorless colonies suggestive of \textit{S. typhi} were subjected to preliminary tests like Gram staining and motility and biochemical reactions like Indole, Methyl red, Voges prauker, Citrate utilization, Urease test, Triple sugar iron test and further confirmed by slide agglutination test with specific antisera. The confirmed colonies of \textit{Salmonella typhi} were stored at 4°C in nutrient agar slant.

**ANTIBIOTIC SUSCEPTIBILITY TESTING**

The antibiotic susceptibility pattern of the isolated stains were found by disc diffusion technique [Kirby Bauer method] against the following antibiotics like Ampicillin(A30mcg/disc), Chloramphenicol(C30 mcg), Tetracycline(TE 30 mcg), Co-trimoxazole(CO 23.75 mcg), Ciproflaxacin(CIP 5mcg), Gentamycin(G 10 mcg), Ceftriazone (CI 30 mcg), Nalidixic acid (Na 30 mcg), Nitrofurantoin (NIT 300 mcg), Amoxycillin (AMC 30mcg). The antibiotic discs were purchased from Hi-media laboratories Pvt limited, Mumbai. Broth culture of the bacterial strains compared to Mac Farland’s standard 0.5 were prepared. Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs
impregnated with different antibiotics were placed on the plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeter diameter.

EXTRACTION OF DNA

Overnight broth cultures of *Salmonella typhi* was taken for DNA extraction following manufacturer’s instruction using LTRC kit MSS 12. The broth culture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet obtained after centrifugation was used for DNA isolation. The pellet was suspended in 300µl of solution A (200 µl 1 x TE buffer and of SDS) at room temperature and vortexed completely. It was then incubated at 60°C for 20 minutes and then solution B (300 µl of Phenol: Chloroform: Isoamyl alcohol) was added and completely vortexed. It was centrifuged at 10,000 rpm for 10 minutes. About 500µl of the aqueous supernatant solution was taken in a fresh vial and an equal volume of isopropanol was added and mixed thoroughly by inverting the vials. It was again kept for centrifugation at 10,000 rpm for 10 minutes. About 200µl of ethyl alcohol was added to the vial and mixed by inverting the tube till the white strands of DNA precipitation were seen. It was then centrifuged at 10000 rpm for 10 minutes and the supernatant was discarded. Then the alcohol was decanted without dislodging the pellet. It was completely air dried to remove ethyl alcohol smell from the vial. To the final pellet, about 20µl of TE buffer was added and mixed by tapping the tube till the solution settle at the bottom. The isolated DNA was separated and visualized with the help of agarose gel electrophoreses and viewed in the UV transilluminator.

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is the easiest and commonest way of separating and analyzing DNA. The DNA is visualized in the gel by addition of ethidium bromide. This binds strongly to DNA by intercalating between the bases and is fluorescent. 17 1% agarose gel, was prepared by weighing and mixing 1g agarose in 100ml of 1 x TBE buffer in a conical flask. The solution was heated until the agarose was completely dissolved. The gel casting tray was prepared by sealing ends of gel chamber with tape and appropriate number of combs were placed in gel tray. 5 ul of ethidium bromide was added to cooled gel and poured into gel tray and allowed to cool for 15-30 min at room temperature. The combs were removed and the gel is placed in electrophoresis chamber and covered with TAE buffer. 20 µl of digested DNA was loaded along with 3 µl of gel loading buffer onto gel and electrophoresed at 100V for 1 h. The DNA bands were visualized using UV transilluminator. 18,19

MULTIPLEX PCR

The multiplex PCR assay offers a rapid, simple and accurate identification of antibiotic resistance profiles and could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic resistance determinants in epidemiological studies. 20,21 The protocol developed by Asma Haque et al., 2005 was followed for the amplification of DNA. 22 The procedure was carried with the following PCR temperature cycling parameters: Initial denaturation at 95°C for 45 sec followed by 35 cycles of denaturation at 95°C for 45 s; Primer annealing at 51°C for 45 s, primer extension at 72°C for 1 min and 30 s and the final extension at 72°C for 10 min. After the reaction, 5 µl of loading dye was added to amplify PCR products and mixed
well. Then, 20 µl of total sample was loaded on 1% Agarose Gel Electrophoresis.

**The primers used in multiplex PCR are as follows**-

<table>
<thead>
<tr>
<th>Targeted genes</th>
<th>Primer sequences</th>
<th>Product size (bps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tem&lt;sup&gt;16&lt;/sup&gt;</td>
<td>F 5’ GCA CGA GTG GGTTAC ATC GA 3’ R 5’ CGT CCT CCG ATC GTT GTC AG 3’</td>
<td>311</td>
</tr>
<tr>
<td>CatP&lt;sup&gt;4&lt;/sup&gt;</td>
<td>F 5’ CCT GCC ACT CAT CGC AGT 3’ R 5’ CCA CCG TTG ATA TAT CCC 3’</td>
<td>623</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

A total of 30 samples were collected from various water bodies. Out of this, *Salmonella typhi* was isolated from 12 samples. The isolated organisms were confirmed by subjecting to preliminary and various biochemical tests, the results of which are given in table I. Confirmed organisms were subjected to antibiotic susceptibility testing using disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are interpreted as sensitive (S) and resistant (R) and given in table II. Out of 12 isolates, six were resistant to chlorampenicol, five were resistant to ampicillin also. The DNA from the samples was extracted and bands, were observed by performing Agarose gel electrophoresis. The isolated DNA was used as template in the PCR study. The protocol developed by Asma Haque et al., 2005 was followed for the amplification of DNA. Multiplex PCR was employed to generate genomic amplification products of *Salmonella* species. The results showed that there was a very good correlation between the genotypic analysis by PCR and the phenotype determined by standard methods of susceptibility testing and identification of *salmonella* species:

<table>
<thead>
<tr>
<th>PRILIMINARY TEST</th>
<th>Grams Staining</th>
<th>Gram Negative Bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanging Drop Method For Motility</td>
<td>Motile</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BIO CHEMICAL REACTIONS</th>
<th>Indole</th>
<th>Methyl Red</th>
<th>Voges Prausker</th>
<th>Citrate Utilization</th>
<th>Urease Test</th>
<th>Triple sugar iron test</th>
<th>Alkaline slant/Acid butt with H2S production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Alkaline slant/Acid butt with H2S production</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1 : Results for Preliminary and biochemical test for *Salmonella typhi***
Table II : Results of Antibiotic susceptibility testing of *Salmonella typhi*

<table>
<thead>
<tr>
<th>ISOLATES</th>
<th>A10</th>
<th>C10</th>
<th>TE30</th>
<th>Co25</th>
<th>CIP 5</th>
<th>Na30</th>
<th>G10</th>
<th>NF300</th>
<th>Ci30</th>
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<tr>
<td>S1 Sewage1</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S2 Stagnant1</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S3 Pond1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>S4 Well1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>R</td>
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<td>S</td>
</tr>
<tr>
<td>S5 Marine1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S6 Pond2</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<tr>
<td>S7 Sewage2</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S8 Sewage3</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>S9 Pond3</td>
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<td>S</td>
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<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S10 Well2</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
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</tr>
<tr>
<td>S11 Stagnant 2</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S12 Marine2</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*Salmonellae* are primarily intestinal parasites of humans and many other animals. They are found frequently in sewage, river and other waters, and soil. The presence of *Salmonella* in other habitats like water, food, natural environment is due to faecal contamination. Under suitable environmental conditions, they may survive for weeks in waters and for years in soils. The prevalence of salmonellosis depends on the water supply, waste disposal, food production and preparation practices, and climate. Treatment with an appropriate antibiotic is essential for Salmonellosis. Multi drug resistant *S. typhi* are now endemic in many developing countries and are responsible for significant morbidity and mortality. As a result of the proliferation of such strains, the use of chloramphenicol has been compromised, and that of ampicillin and trimethoprim similarly impaired. Our present study was to isolate *Salmonella typhi* from various water sources. Isolates resistant to chloramphenicol and ampicillin were selected and the drug resistant genes were identified as *cat P* and *tem* respectively by Multiplex PCR technique.
CONCLUSION

Though public health, sanitation and vaccines do have a role to play in control of typhoid fever, it is the antimicrobial therapy which plays a key role in management of typhoid fever. It is likely that S. typhi will continue to acquire genes responsible for antibiotic resistance. Restrictions on the irrational use of antibiotics, and public awareness activities should be undertaken to alert the public to the risks of the unnecessary use of antibiotics. The timely detection of these genes and prevention of their spread is the need of the hour to control antibiotic resistance among S. typhi. More research could also be directed towards factors responsible for their spread and means to prevent dissemination of such plasmids so as to limit drug resistance.

ACKNOWLEDGEMENT

The authors are grateful to Dr.I Seetha Lakshmi, Director of Life Tech Research Centre, Chennai-26 and the Principal, Valliammal Ammal College for Women, Chennai-102 for their kind support to carry out this project work.

REFERENCES

15. Rui Huang, Shuyan Wu, Xueguang Zhang and Yanyun Zhang. Molecular Analysis and Identification of Virulence Gene on pRST98 from Multi-Drug Resistant Salmonella typhi Cellular & Molecular Immunology, Volume 2 Number 2 April 2005
POLYANILINE COATED EXPANDED GRAPHITE ACTS AS A NEW ELECTRODE MATERIAL FOR ETHANOL FUEL CELL

Abhik Chatterjee¹, I Basumallick²

¹Department of Chemistry, Raiganj college (University college), Raiganj
²Electrochemical laboratory, Department of Chemistry, Visva-Bharati University, Santiniketan

E-mail of Corresponding Author: acpcju@yahoo.co.in

ABSTRACT
An expanded graphite electrode coated with a thin layer of polyaniline and loaded with platinum has been used to study electro-oxidation of ethanol. The graphite plate electrode was expanded by doping with potassium (K)-vapour using vapour incorporation technique developed at our laboratory. The expanded graphite was platinised by electro-deposition technique and its electrochemical behaviour was examined using a laboratory model fuel cell. The open-circuit potential (OCP) and shortcircuit current (SCC) values of laboratory model fuel cell in 1M H₂SO₄ comprising of normal and expanded graphite electrodes having same catalytic loading as working electrode were observed. The expanded graphite electrode was also coated with a thin film of polyaniline and then loaded with Pt catalyst as before. Cyclic voltammogram of polyaniline coated electrodes in H₂SO₄ solution was investigated. Electro-catalytic activity of the modified electrode is better than Pt deposited electrode due to the higher reaction area of Pt particles.

Keywords: Polyaniline, ethanol, electro-catalyst, expanded graphite

INTRODUCTION
Metal microparticles dispersed in polymer modified electrodes have been widely used as electro-catalyst. There are various polymeric films, such as polypyrrole (PPY), polyaniline (PANI), poly(3-methylethiophene) (PMT), poly(3,4-ethylenedioxythiophene) (PEDOT), and so forth, have been investigated as conducting catalyst supports.¹² These polymers are usually used as matrix to incorporate noble metal catalysts in the application for electro-oxidation of small molecules such as hydrogen, methanol and formic acid, etc.³⁴⁵ Among the various conducting polymers, polyaniline (PANI) is one of them most interesting material because of its moderately high conductivity, well behave electrochemistry, easy preparation and possible applications as electro-catalyst towards various electro-oxidation reactions. A thin film of a conducting polymer (CP) improves the interfacial properties between the electrode and the electrolyte. Conducting polymer can allow a facile flow of electronic charge during the electrochemical oxidation of alcohol on Pt. Generally, an electrochemically deposited conducting polymer develops three dimensionally on a substrate.⁶ Therefore, it introduces a high porosity and roughness;
as a result it generates a large surface area for electrochemical reactions.

The major problems of fuel cells are poisoning of the electro-catalyst even with Pt as the catalyst and its crossover from anode to cathode compartment. Besides electro-catalyst, the carbon material used as catalytic support also plays an important role in dictating fuel cell efficiency. In the present research an attempt has been made to improve catalytic activity of ethanol (EtOH) fuel cell using polyaniline coated expanded graphite as catalytic support material.

**MATERIAL AND METHODS**

H₂SO₄ (Merck), H₂PtCl₆•6H₂O (Arora Matthey Limited) were used as supplied. Aniline (Merck) and EtOH (Bengal chemicals) were distilled before use. The distillate was collected rejecting head and tail fractions.

The anode was a 1cm² graphite plate of thickness 3.8 mm obtained from the R&D of BHEL, India. Graphite plates were expanded using a simple technique developed at our laboratory. The experimental arrangement for expansion is shown in figure 1. The graphite plate was expanded to an extent of 20% by potassium vapour. Pt particles were deposited on the working electrode (2 cm²) under galvanostatic condition using +10 mA.cm⁻² current for 30 minutes from chloroplatinic acid solution (0.01M) in 0.5M H₂SO₄.

The air cathode was fabricated by loading a 1cm² graphite plate with Pt. For the sake of comparison a Pt loaded anode without expansion was used. All experiments were carried out at 30°C.

Polyaniline was prepared galvanostatically. Electrolyte solution was 0.5 M aniline in 1M H₂SO₄ solution. The solution was taken in a one compartment cell in which supporting material was used as working electrode and counter and reference electrode merged to Pt wire. -1 mA.cm⁻² current under galvanostatic condition was passed for 100 seconds to deposit a film of PANI. After polymerization the polymer coated electrodes were washed repeatedly with distilled water and used for electrochemical studies. Cyclic voltammogram of PANI coated electrodes in H₂SO₄ solution was investigated using a potentiostat-galvanostat (PAR Versastat™ II).

**Laboratory model fuel cell**

Laboratory model fuel cells were housed in two glass rectangular chambers (50ml capacity) connected by an inverted U-shaped bridge. Cathode and anode, as fabricated above, were inserted into the respective chambers through the openings of the lid. H₂SO₄(1M) were poured into cathode and anode chambers, respectively. Alcohol was added to the anode chamber to obtain the desired concentration. The U tube was plugged with foam, which had been soaked in the acid to avoid crossover of alcohol. Air was bubbled slowly through cathode chamber using an air pump.

**RESULTS**

Figure 2 shows the galvanostatic polymerization curve of aniline on support material surface from a deposition bath of 0.5M aniline in 1M H₂SO₄. From the figure, it is clear that the polymerization starts at about potential of 0.95 volts and continues in the potential range of 0.9 to 1.0 V for a period of 100 seconds. Figure 3 shows the cyclic voltammogram of expanded graphite-PANI electrode in H₂SO₄ solution (blank) at a scan rate of 50 mV.s⁻¹.

It is found that within the potential limits – 0.2 to 1.3 V vs. SCE, the response changed on cycling. After about the five cycles, the PANI film shows a steady response and
then it was used as an anode material for deposition of Pt. The open-circuit potential (OCP) and shortcircuit current (SCC) values of laboratory model fuel cell in 1M H₂SO₄ comprising of normal and expanded graphite electrodes having same catalytic loading as working electrode are shown in table1. Steady open-circuit potentials (OCP) were reached within 30 minutes. The potential remained undisturbed for an appreciable duration which indicates that these electrodes are stable in acidic medium. The shortcircuit current (SCC) values were measured after attainment of a steady OCP value. These data demonstrates the role of support material on the activity of catalyst.

To compare the performances of laboratory model fuel cells with different anodes, cells were discharged under 0.20 mA current for 180 minutes and profiles are presented in figure 4. The observed discharge profiles indicate that the cell with expanded graphite electrode exhibits better performances.

**DISCUSSION**

The CV of PANI coated electrode shows two peaks (figure 3). In the anodic scan, the peaks at about 0.57 V is due to SO₄²⁻ anion up taking. In the reverse scan, the peaks at about 0.23 V is due to SO₄²⁻ anion expulsion.

Generally, graphite electrodes are utilised for providing support only, no electro-catalytic activity has been observed for alcohol fuel cell. Pt deposited on support material is known as an efficient electro-catalyst for alcohol oxidation. But the catalytic activity of the electrode can be enhanced if the support material is modified. It is seen from the table that OCP value is higher for Pt loaded expanded graphite material electrode. This indicates better catalytic effect of expanded graphite.

Electro-oxidation of EtOH takes place via an initial absorption onto the anode surface followed by deprotonation. For expanded graphite the nanographite channels act as better absorption sites and the electro-catalytic activity of Pt within these channels is also enhanced as is reflected in their SCC values (table1). Basically, potassium metal as dopant changes the interlayer distance between graphite layers. Table1 also indicates the relative improvement of catalytic effect of normal and expanded graphite when loaded with Polyaniline and Pt. It is very interesting that when Pt particles were deposited onto PANI coated electrode, catalytic effect is further improved. This is due to high dispersion of Pt particles. As a result the specific reaction area of these electrodes is increased and thus improves catalytic efficiency.

The observed discharge profiles (figure 4) indicate that the cell with expanded graphite electrode exhibits better performance. The reason is the higher tolerance of the platinum particles to poisoning effect, in comparison with the serious problem of poisoning effect on bulk platinum electrodes. PANI may adsorb some of the poisonous intermediates and thus the adsorption prevents the dispersed Pt particles from becoming deactivated.

Thus this research introduces the use of polyaniline modified expanded graphite as an efficient electrode material in fuel cell.

**ACKNOWLEDGEMENT**

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors /publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.
REFERENCES


Table 1: Electrochemical parameters for a laboratory model cell comprising of normal/expanded graphite as anode material in 1 M ethanol in 1M H2SO4 vs Air electrode (fabricated cathode)

<table>
<thead>
<tr>
<th>Anode</th>
<th>OCP(Volt)</th>
<th>SCC(mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphite plate + Pt</td>
<td>0.710</td>
<td>0.230</td>
</tr>
<tr>
<td>Graphite plate + PANI + Pt</td>
<td>0.740</td>
<td>0.360</td>
</tr>
<tr>
<td>Expanded Graphite plate + Pt</td>
<td>0.728</td>
<td>0.320</td>
</tr>
<tr>
<td>Expanded Graphite plate + PANI+ Pt</td>
<td>0.754</td>
<td>0.470</td>
</tr>
</tbody>
</table>

Figure 1: Expansion of graphite plate Using K-vapour, (A) solid block of potassium; (B) graphite electrode.
Figure- 2: Plot of potential vs. time for PANI deposition onto expanded graphite electrode.

Figure-3: Cyclic Voltammogram (6th CV) of expanded graphite-PANI electrode in H₂SO₄ solution (blank). Scan rate 50 mV.s⁻¹.

Figure-4: Discharge study of laboratory model direct ethanol fuel cell with normal and expanded graphite electrodes under a steady current drain of 0.20 mA.
ABSTRACT

Methanol and aqueous extracts of four plant species namely *Nigella sativa*, *Acorus calamus*, *Myristica fragrans* and *Hemidesmus indicus* traditionally used in Indian folklore medicine for the treatment of various bacterial and fungal infections were investigated for antimicrobial activity against pathogens viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Escherichia coli* and *Candida albicans* by well diffusion and minimum inhibitory concentration (MIC) methods. Comparative study of the antimicrobial activity of the plant extracts and its synergistic effect with antibiotics was carried out. Maximum zone of inhibition was observed against *S. aureus*, *E. coli*, *Bacillus* sp and *P. aeruginosa* by methanol extracts of *Nigella sativa* and *Hemidesmus indicus*. *C. albicans* showed susceptibility to methanol extracts of *Acorus calamus* and *Hemidesmus indicus*. Phytochemical analysis of methanol and aqueous extracts of *Nigella sativa* and *Hemidesmus indicus* revealed the presence of alkaloids, flavonoids, triterpenes, polyphenols and sterols. Both the active extracts were separated by adopting analytical thin layer chromatography. Totally two spots were observed for *Hemidesmus indicus* and *Nigella sativa* showed only one spot, when chloroform: methanol (70:30) used as a solvent system. The active fraction was determined by bioautography by using *S. aureus* as test organism. Only *Nigella sativa* extract alone showed synergistic effect with antibiotics and the positive results were showed by *S. aureus* and *E. coli*. The results provide justification for the use of the plants in folk medicine to treat various infectious diseases.
Key words: *Nigella sativa, Hemidesmus indicus*, Antimicrobial Activity, Synergistic effect, Bioautography

**INTRODUCTION**

India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. According to World Health Organization, medicinal plants would be the source of obtaining a variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As preventive and curative measures, plants and their products are used in the treatment of infections for many centuries ago. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare1,2 and India happens to be the largest user of traditional medical cure, using 7000 plant species. The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity 1,3, 4. Antibacterial properties of various plants parts, such as leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades1,3. Antibiotic principles are distributed widely among angiospermic plants. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial activities 1,6. Within the recent years, infections have increased to a great extent and antibiotic resistance effects become an ever-increasing therapeutic problem. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is of great interest to carry out a screening of some plant materials in order to validate their use in folk medicine and to reveal the active principles by isolation and characterization of their constituents7.

The present study aimed to screening and evaluating the antimicrobial assay of *Nigella sativa, Acorus calamus, Myristica fragrans* and *Hemidesmus indicus* against five pathogenic microorganisms.

**MATERIALS AND METHODS**

**Collection of plant Material**

*Nigella sativa* seeds, Rhizome of *Acorus calamus*, Root of *Hemidesmus indicus* and *Myristica fragrans* seeds were collected from nearby areas in Vellore (Latitude ‘12° 55’ N North: Longitude 79° 11’ E East) Tamilnadu.

**Preparation of plant extracts**

Plant samples were air dried at room temperature and dried samples were coarsely powdered with pestle and mortar. Each 20 gms of powdered material were completely soaked in 100ml of sterile water and 70% methanol and then covered with aluminum foil. The Extraction was allowed to proceed for 48h. The extract was decanted and the solvents were removed by evaporation in vacuum by rotary evaporator. The air dried extracts were used for the antimicrobial and phytochemical analysis8.

**Microorganisms used**

A total of 5 clinical isolates of bacteria and a fungi belonging to 5 genera comprising of *S. aureus, P. aeruginosa, Bacillus sp., E. coli* and *C. albicans* isolated from various clinical samples were obtained from Government hospital, Vellore, Tamilnadu. The clinical isolates were identified by biochemical methods as recommended by Bergey’s Manual of Systemic Bacteriology9.

**SCREENING FOR ANTIMICROBIAL ACTIVITY**

**(i) Well diffusion method**

The 18 hrs bacterial and fungal suspensions were prepared with help of McFarland standard. The aliquot was spread evenly on Muller Hinton agar and Sabouraud’s Dextrose agar. On each plate, equidistant wells were made with a 6 mm diameter with help of sterilized cork borer. Then fifty micro liter of each plant extract containing 0.25mg was aseptically introduced into a respective agar well. Ciprofloxacin (5 μg/ml) and
amoxicillin (25 μg /ml) were used as positive controls and nystatin (5 μg/ml) and amphotericin B (25 μg /ml) were used for fungal isolates. The methanol and distilled water were used as negative controls and incubated at 37 °C for 24-48 hrs for bacteria and fungus. The formations of clear inhibition zone of ≥12 mm diameters around the wells were regarded as significant susceptibility of the organisms to the extract. The experiment was performed with duplicate.

(ii) Phytochemical Analysis
The solvent extracts which showed maximum antibacterial activity was subjected to qualitative test for the identification of various plant constituents such as carbohydrates, proteins, phytosterols and flavonoids.

(iii) Separation of active compound by TLC
The methanol extract of both plants which showed promising activity against the test pathogens were subjected to separation by thin layer chromatography (TLC) using silica gel coated TLC plates. About 3μl of active crude extract was spotted at the bottom of the sheet using capillary tube. To find out the better solvent system for separation, the sheet was placed in a beaker containing the different solvent systems, such as methanol, chloroform, acetic acid, n-butanol, n-hexane and water with following proportions: n-butanol: acetic acid: water (60:30:10), (70:20:10), (75:20:5) chloroform: methanol (70:30), (60:40), (40:60), (50:50), and n-hexane : chloroform (60:40). After running the chromatogram the sheet was kept in a developing chamber containing potassium iodide crystals. The compound present in the crude extracts were appeared as brown spots.

(iv) Detection of active compound by bioautography:
The bioautography method used for the detection of active compound separated in TLC using capillary tube. In the present study among the 4 plants screened, the methanolic extracts of N. sativa and H.indicus showed promising activity and found to be more effective than aqueous extract except for Nigella sativa. Maximum zone of inhibition were observed against S.aureus, P.aeruginosa and E.coli by aqueous and methanol extracts of N.sativa and H.indicus. Bacillus sp. Showed susceptibility to N. sativa, M. fragrans and H.indicus extract. C.albicans inhibited by methanol extracts of broth and Sabouraud’s Dextrose broth’s and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for bacterial isolates and 10⁶ cfu/ml for fungal isolates were introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and cotrimoxazole for bacteria and nystatin and amphotericin B for fungal isolates). A tube containing nutrient broth and Sabouraud’s Dextrose broth only were seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32°C). After incubation the tubes were then examined for microbial growth by observing for turbidity.

(vi) Evaluation of synergistic effect of antibiotics and plant extracts on resistant bacterial samples
Aliquots of 100 μl of resistant bacterial cultures (0.5 MacFarland Standard) grown in 10 ml of nutrient broth for 6 h were inoculated in nutrient broth supplemented with the respective antibiotics (50μg/ml) and 10⁶ cells/ml fungal cultures grown in Sabouraud’s Dextrose broth supplemented with 100 μg /ml coltrimazole with different concentrations of plant extracts. The concentration for plant extracts ranged from 10 to 500 μg/ml, based on MIC values that had previously been evaluated. Only streptomycin or gentamicin was used as the sub-inhibitory concentration (50μg / ml) and incubated at 37°C for 48 hours. After 48 h, the optical density of each sample was recorded and compared to those of MIC to verify the synergistic effect of the tested compounds.

RESULTS
(i) Well diffusion method
In the present study among the 4 plants screened, the methanolic extracts of N.sativa and H.indicus showed promising activity and found to be more effective than aqueous extract except for Nigella sativa. Maximum zone of inhibition were observed against S.aureus, P.aeruginosa and E.coli by aqueous and methanol extracts of N.sativa and H.indicus. Bacillus sp. Showed susceptibility to N. sativa, M. fragrans and H.indicus extract. C.albicans inhibited by methanol extracts of
A. calamus and H. indicus. The results of the antimicrobial activity of plant extracts tested against microorganisms by well diffusion method are shown in Table 1 & 2,2A.

(ii) Separation of active compound by TLC
Methanol extract of H. indicus has showed two clear spots, when chloroform: methanol (70:30) used as solvent system. Their Rf values are calculated as 0.56 and 0.88. Aqueous extract of N. sativa showed only one clear spot, when chloroform: methanol (70:30) used as a solvent system and its Rf value was calculated as 0.75.

(iii) Detection of active compounds by bioautography:
In bioautography, two spots of H. indicus and one spot of N sativa, totally three different spots separated in TLC were taken. In this the first spots of H. indicus and N sativa showed good activity against S. aureus with 15-26 mm of inhibition zone.

(iv) Phytochemical Analysis
Methanolic extract of H indicus showed presence of flavonoids, triterpenes, polyphenols and sterols. Aqueous and methanolic extracts of N. sativa only comprised of alkaloids (Table 3). The result will help in identification of various compounds through gas chromatography in the further studies.

(v) Minimum inhibitory concentration method
Aqueous extract of N. sativa and methanol extract of H. indicus was taken for MIC assay. N. sativa extract showed minimum inhibitory concentration to S. aureus, E coli, Bacillus sp. and P. aeruginosa at the concentrations of 20µg/ml, 80µg/ml, 20µg/ml and 40µg/ml respectively (Table 4). A. calamus extract showed minimum inhibitory concentration to C. albicans and Bacillus sp. at concentrations of 40 µg/ml and 80 µg/ml respectively (Table 5). The extract of M fragrans exhibited MIC to Bacillus sp alone at concentration of 20mg/ml (Table 6). H. indicus extract showed minimum inhibitory concentration to S aureus, Bacillus sp, E coli, P. aeruginosa and C. albicans at concentrations of 40µg/ml, 80µg/ml, 20µg/ml 160µg/ml and 80µg/ml respectively (Table 7).

(vi) Synergistic effects of antibiotics with plant extract
The comparative studies of the synergistic effect of antibiotics with plant extracts alone were studied. The effect of association of N.sativa extract with streptomycin and gentamicin on S aureus and E coli were shown in figure 1. S aureus showed MIC at concentration of 8µg/ml, when N sativa extract combined with streptomycin and Gentamicin, whereas N.sativa alone showed MIC only at 20µg/ml. E coli showed MIC at 40µg/ml concentration, When N sativa extract combined with streptomycin and gentamicin, whereas N.sativa extract alone showed MIC only at 80µg/ml. No other plant extracts showed synergistic effects with antibiotics to any other test organisms.

DISCUSSION
In the last few decades, there has been particular interest in the use of abundant naturally occurring antimicrobials (herbs, spices and plants) 17. N.sativa, A.calamus, M. fragrans and H. indicus are traditionally used in Indian folklore medicine. So far only a limited data is available regarding its efficacy against pathogenic microorganisms. Hence the present study was therefore designed to evaluate it’s antimicrobial activity. Methanolic extracts, hot and cold water extracts of N. sativa showed excellent antibacterial activity against pathogenic microbes18. Chloroform extract of H indicus showed promising activity against the clinical isolates of Helicobacter pylori19. In the present study the methanolic extracts of N.sativa and H.indicus also showed promising activity than the standard antibiotics and methanol extracts found to be more effective than aqueous extract except for N.sativa.

In TLC Petroleum-ether extract of N.sativa using benzene: ethyl acetate (6:1), showed five spots. In the chloroform extract, using benzene: ethyl acetate (4:1), five spots and in ethanol extract, using chloroform: methanol (93:7), six spots were observed13. Methanol extract of H.indicus has showed two clear spots and N. sativa showed only one clear spot, when chloroform: methanol (70:30) used as a solvent system.

The assay for bioautography demonstrated that the strong inhibition zones of H. indicus and N.sativa against the growth of S aureus. The clear zones were
located in separate places on the TLC plate, suggesting the potent antimicrobial effect.

In phytochemical analysis, *H indicus* showed presence of flavonoids, triterpenes, polyphenols and sterols. Aqueous and methanolic extracts of *N. sativa* only comprised of alkaloids. The antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β-pinene and limonene. The mechanism of action of terpene is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds.

In MIC assay, *N.sativa* extract showed minimum inhibitory concentration against *S. aureus* and *Bacillus sp.* at the concentrations of 20 µg/ml respectively. *H.indicus* extract showed minimum inhibitory concentration against *S. aureus* and *E.coli* at the concentrations of 40µg/ml and 20 µg/ml respectively. This antimicrobial activity may be due to the presence of alkaloids in plant extracts.

Synergistic effect was found by *S. aureus* and *E coli* in the combination of *N sativa* with streptomycin and gentamicin. No other plant extracts showed synergistic effects with antibiotics to any other test organisms.

In earlier studies, *N. sativa* seed extract has antimicrobial activity against MRSA and it was found to be active against ESBL producers. *H.indicus* showed good activity against *Propionibacterium acnes*. The antimicrobial activity and phytochemical analysis also carried out for *N. sativa* and *H.indicus* extracts. But the present study focused on purification, bioautography and comparative study of *N.sativa* and *H. indicus* synergestic effects.

The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in folk medicine, which could be of considerable interest to the development of new drugs.

**CONCLUSION**

Based on the results obtained in this study, it may be concluded that out of 4 plants screened, plant extracts of *N. sativa* and *H. indicus* have a stronger and broader spectrum of antimicrobial activity against pathogenic microorganisms and the extracts may be used to discover bioactive natural products that may serve as basic source for the development of new antimicrobial compounds to overcome the problem of increasing resistance to known traditional antibiotics. The further purification through HPLC and spectral analysis will be worthy study to identify the nature of compound present.

**ACKNOWLEDGEMENT**

The authors are thankful to Management of DKM College for Women, Vellore, Tamilnadu, India for providing all the facilities and also thankful to Vice Chancellor and Registrar, Periyar University, Salem, Tamilnadu, India. Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript.
Table – 1: Antimicrobial activity of 4 plants against clinical pathogens

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Nigella sativa</th>
<th>Acorus calamus</th>
<th>Myristica fragrans</th>
<th>Hemidesmus indicus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of inhibition (mm in diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
<td>Aqueous</td>
<td>Methanol</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>15</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>26</td>
<td>19</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
</tbody>
</table>

Note: (+) Susceptibility (inhibition zone ≥12mm)
(-) Absence of Susceptibility

Table – 2: Susceptibility pattern of 4 plants against pathogens

<table>
<thead>
<tr>
<th>MICROORGANISMS</th>
<th>Nigella sativa</th>
<th>Acorus calamus</th>
<th>Myristica fragrans</th>
<th>Hemidesmus indicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table: 2A Antibacterial Effect of Standard Antibiotics

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Ciprofloxacin / Zone of inhibition (mm in dia)</th>
<th>Amoxicillin / Zone of inhibition (mm in dia)</th>
<th>Nystatin/ Zone of inhibition (mm in dia)</th>
<th>Amphotericin B/ Zone of inhibition (mm in dia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>14</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>14</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>10</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>10</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Table-3: Phytochemical Analysis of *Nigella sativa* and *Hemidesmus indicus* extracts

<table>
<thead>
<tr>
<th>Test</th>
<th><em>N. Sativa</em></th>
<th></th>
<th><em>H. indicus</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Methanol</td>
<td></td>
<td>Methanol</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Polyphenols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Table – 4 Minimum inhibitory concentration of test organisms with *Nigella sativa* extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>MIC µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus spp.</em></td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>40</td>
</tr>
</tbody>
</table>

Table – 5: Minimum inhibitory concentration of test organisms with *Acorus calamus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Candida albicans</em></td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus spp.</em></td>
<td>80</td>
</tr>
</tbody>
</table>

Table – 6: Minimum inhibitory concentration of test organisms with *Myristica fragrans*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organism</th>
<th>MIC µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bacillus spp.</em></td>
<td>20</td>
</tr>
</tbody>
</table>

Table – 7: Minimum inhibitory concentration of test organisms with *Hemidesmus indicus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>MIC µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus spp.</em></td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td><em>Escherichia coli</em></td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>160</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida albicans</em></td>
<td>80</td>
</tr>
</tbody>
</table>
Figure – 1: Evaluation of synergistic effect of Nigella sativa with antibiotics against test organism

REFERENCES


ABSTRACT

Anti microbial photodynamic therapy is one of the most upcoming treatment approaches in field on dentistry. Applications of PDT in dentistry are growing rapidly: the treatment of oral cancer, bacterial and fungal infection therapies, and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions. Periodontitis as known is polymicrobial disease and this novel therapeutic approach sounds promising in eliminating the microorganisms when used in adjunct to conventional debridement methods. This technique involves application of a photosensitizer and activating it with a light source of specific wavelength. This system in presence of oxygen creates reactive oxygen species that exerts the classic photodynamic reaction. The advantage of this new approach includes rapid bacterial elimination, minimal chance of resistance development and safety of adjacent host tissue and normal microflora. This review discusses about the general principles, mechanism of photodynamic therapy with additional highlights on its application in periodontal diseases.

Keywords: Antimicrobial photodynamic therapy, periodontitis, photosensitizers, lasers.

INTRODUCTION

There have been many changes & developments in dentistry over the past decade than in the previous hundred years combined, and the pace is accelerating! Oral cavity is an abode of variety of microorganisms and periodontitis is an infectious disease caused by number of these organisms. Current treatment techniques involve either periodic mechanical disruption of oral microbial biofilms or maintaining therapeutic concentrations of antimicrobials in the oral cavity, both of which are fraught with limitations. The development of alternative antibacterial therapeutic strategies therefore becomes important in the evolution of methods to control microbial growth in the oral cavity. Numerous adjunct conventional antimicrobial therapies have been attempted. One disadvantage of these therapies is development of resistant strains. So alternate antimicrobial treatment modalities have been researched & one such promising alternative is antimicrobial photodynamic therapy.

The history dates back over 3000 years when Indians used psoralens in the treatment of vitiligo & the Egyptians employed it in the treatment of leucoderma.
Later it was rediscovered by Western civilization at the beginning of the twentieth century. In 1834, Kalbrunner isolated the chemical bergapten from bergamot oil but did not use it in any therapeutic application. In 1900 Prime, a French neurologist, used eosin orally in the treatment of epilepsy. He discovered that this induced dermatitis in sun-exposed areas of skin. This discovery then led to the first medical application of an interaction between a fluorescent compound and light. It was further developed by the Danish physician, Niels Finsen, who at the turn of the last century described the successful treatment of smallpox using red light. He then went on to use ultraviolet light to treat cutaneous tuberculosis and developed the use of carbon arc phototherapy in the treatment of this condition for which he was awarded a Nobel Prize in 1903.

History of photodynamic therapy began when Von Tappeiner, who along with dermatologist Jesionek, used a combination of topical eosin and white light to treat skin tumors. They demonstrated the requirement of oxygen in photosensitization reactions and in 1907 they introduced the term "photodynamic action" to describe this phenomenon. The German physician Friedrich Meyer–Betz performed the first study with what was first called photoradiation therapy (PRT) with porphyrins in humans in 1913. Meyer in 1989, First Paper on photodynamic therapy was presented at the Congress on photodynamic therapy of Tumours. This review elucidates the evolution & the current position of photodynamic therapy, its applications in dentistry, especially in periodontal treatments and its likely impact in future.

COMPONENTS OF PHOTODYNAMIC THERAPY

Photodynamic reaction is a physico-chemical reaction that basically involves 2 components: Photo sensitizer and the activating light source.

**Photosensitizers**

These are natural or synthetic photoactive compounds that have photosensitizing potential. They function by trapping the light in the form of photons and transferring the energy to other molecules resulting in the liberation of short-lived energetic species that interact with biological systems and produce tissue damage. Ideal properties of photosensitizers – this includes photo-physical, chemical, and biological characteristics.

- An ideal photosensitizer must be biologically stable.
- Photochemically efficient.
- Selectively retained in the target tissue.
- Low toxicity and fast elimination from the skin and epithelium.
- Absorption peaks in the low-loss transmission window of biological tissues.
- High quantum yield of singlet oxygen production in vivo.
- Cost-effectiveness and commercial availability
- High solubility in water, injection solutions, and blood substitutes.
- Storage and application light stability.

Generations – Three generations of photosensitizers have evolved over period of time. I generation includes Hematoporphyrins and phthalocyanines. II generation photosensitizers are meso-tetra(hydroxyphenyl)porphyrins marketed as foscan, 5-aminolevulinic acid, tin ethyl etiopurpurin, benzoporphyrin derivative monoacid ring (verteporfin, visudyne) and phenothiazone dyes.
Phenothiazine dyes includes toluidine blue and methylene blue. These are the recent major photosensitizers used in dental field. Both have similar chemical & physiochemical characteristics.

(1). Toluidine blue – It’s a blue-violet solution. It stains granules within mast cells, proteoglycans & glycosaminoglycans. Used to detect mucosal tumors or atypical epithelia.

(2). Methylene blue – Redox indicator that is blue in oxidizing environments & becomes colorless on reduction. It is used to identify dysplasia or precancerous lesions of oral mucosa. Recently because of its photocatalytic action it is used for virus inactivations before blood transfusions. Both dyes are effective against pathogenic bacteria & are hence used extensively in antimicrobial PDT. They are effective against both gram positive & gram negative periopathogens.

III generation dyes are modified by targeting with monoclonal antibodies or with non antibody-based protein carriers and protein/receptor systems, and conjugation with a radioactive tag. These include expanded metalloporphyrins, Metallochlorines, Metallophthalocyanines, Metallononphthalocyanines. Currently, only four photosensitizers are commercially available: Photofrin®, ALA, VisudyneTM (BPD; Verteporfin), and Foscan®. The first three have been approved by the FDA, while all four are in use in Europe.

**Light Sources**

Numerous light sources have been tried ranging from conventional light lamps, lasers to non laser light sources which have evolved recently. Conventionally lasers have been in use for photoactivation. Various lasers that have been used are argon dye lasers, KTP lasers, metal vapor and diode lasers and pumped dye lasers. Most commonly used lasers and wavelengths in intraoral aPDT are Helium-neon lasers – 663 nm, Gallium Aluminum arsenide diode lasers – 630-690 nm, Argon lasers – 488-514 nm. Non laser light sources used are Blue U light systems and light emitting diodes.

**TECHNIQUES OF ILLUMINATION**

3 basic illumination techniques are practiced that includes superficial, interstitial light delivery and intraoperative PDT.

**MECHANISM OF ACTION**

The bactericidal effect of photo-dynamic therapy can be explained by two potential, but different, mechanisms. One is DNA
damage and the other is the damage caused to the cytoplasmic membrane of the bacteria by cytotoxic species generated.

The mechanism of action of antimicrobial photodynamic therapy can be briefly described as follows:

After irradiation with light of a specific wavelength (lasers), the photosensitizer at ground state is activated to a highly energized triplet state. The longer lifetime of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of cytotoxic species produced during photodynamic therapy occurs in this state\(^{10,11,12}\).

The triplet-state photosensitizer follows two different pathways

**Type I Reactions**

Involve hydrogen-atom abstraction or electron-transfer reactions between the excited state of the photosensitizer and an organic substrate molecule of the cells, which produces free radicals and radical ions. These interact with endogenous molecular oxygen to produce highly reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide, which are harmful to cell membrane integrity, causing irreparable biological damage\(^{11,12}\).

**Type II Reactions**

Involves the production of a highly reactive state of oxygen, known as singlet oxygen (\(^{1}\)O\(_2\)), which reacts with the surroundings as a result of its high chemical reactivity. The free radicals and the singlet oxygen convey toxic or lethal effects by damaging the cell membrane and the cell wall\(^{10,12}\).

It seems that the primary cytotoxic agent responsible for the biological effects of the photo-oxidative process is singlet oxygen. Thus, the process of antimicrobial photodynamic therapy is generally mediated by a type II reaction, which is accepted as the major pathway in microbial cell damage\(^{11,12,13}\).

Microorganisms that are killed by singlet oxygen include viruses, bacteria, protozoa and fungi. Singlet oxygen has a short lifetime in biological systems (<0.04 ls) and a very short radius of action (0.02 lm). The reaction takes place within a limited space, leading to a localized response and making it ideal for application at localized sites without affecting distant molecules, cells or organs\(^{13}\).
PHOTOINACTIVATION OF BACTERIAL CELLS

Paul Erlich in the beginning of 20th century came up with the concept of “magic bullet”. The bullet is considered as a microbe-targeting drug. It reacts only with a germ, not with the host. He hypothesized that the incubation of bacteria with the methylene-blue dye should cause their death at light exposure.

In the 1990s, it was observed that there was a fundamental difference in susceptibility to PDT between Gram-positive and Gram-negative bacteria. The photosensitizer molecules bind in a greater extent to cell wall of gram positive cells whereas it binds to a lesser extent only to outer cell wall layer of gram negative cells. The affinity of negatively charged photosensitizers for Gram negative bacteria may be enhanced by linking the sensitizer to a cationic molecule, by the use of membrane-active agents, or by conjugating the sensitizer with a monoclonal antibody that binds to cell-surface-specific antigens. PS does not have to penetrate the bacterium to be effective, or, indeed, even come into contact with the cells. If singlet oxygen can be generated in sufficient quantities near to the bacterial outer membrane, it will be able to diffuse into the cell to inflict damage on vital structures.

PHOTOINACTIVATION OF FUNGI AND VIRUSES

Many studies conducted revealed that lipid-enveloped viruses are more susceptible to PDT than non enveloped strains. Membrane damage and the consequent increased permeability was the proximate cause of cell death after Methylene Blue-mediated PDT on yeast. Human pathogenic parasites have also been killed by combinations of photosensitizers and light.

USE OF PHOTODYNAMIC THERAPY IN PERIODONTICS

The main objective of periodontal therapy is to eliminate deposits of bacteria and bacterial niches by removing the supragingival and subgingival biofilm. It has been demonstrated that conventional mechanical therapy cannot completely remove all periodontal pathogens, as the bacteria invade soft tissues also. Systemic use of antibiotics may be recommended in certain situations as an adjunct to periodontal therapy. But the main disadvantage is increased emergence of antibiotic resistant bacterial strains. Photodynamic therapy is considered as one another novel approach to assist mechanical periodontal therapies in tackling the bacterial challenge. The photosensitizer is placed directly in the periodontal and peri-implant pocket and the liquid agent can easily access the whole root or implant surface before activation by the laser light through placement of the optical fiber directly in the pocket.

Photodisinfection of periodontal pocket is carried out in following sequence:
1. Initial mechanical debridement using hand curettes.
2. Photosensitizer is applied via syringe at the diseased site that contains residual bacteria. Occasionally, excess dye solution is removed using water spray.
3. Photosensitization is performed using an intensive light by a special tip applied in the pocket. Singlet oxygen and other very reactive agents that are toxic to bacteria are produced, resulting in photochemical disinfection of the periodontal pocket.

EFFECT OF PDT ON ORAL BIOFILMS

The antimicrobial activity of photosensitizers does not only target bacterial cells but also the oral biofilm
matrix. It has a direct effect on the polysaccharides on the extracellular matrix on biofilms making the bacterial cells more susceptible to photodamage. Such dual activity is not exhibited by antibiotics. Photodynamic Therapy in Treatment of Peri-Implantitis

The incidence of peri-implantitis in patients with chronic periodontitis is up to five times greater than in patients who are free of this disease. Treatment of peri-implantitis has become an interesting topic among clinicians and researchers. Conventional mechanical methods such as adjunctive application of systemic or local antibiotics and antiseptics has been generally recommended and are apparently ineffective for complete debridement of the bone defect as well as of the contaminated micro structured implant surface.

Recently lasers and aPDT has gained popularity in treating peri-implant diseases. Numerous studies have shown promising results.

Table: Systematic Review of Photodynamic Therapy

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>STUDY</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dobson et al, 1992</td>
<td>In vitro study to determine effects of toluidine blue and methylene blue mediated aPDT in biofilms containing P.g, F.n, A.a</td>
<td>Effectively eliminated periodontopathic bacteria from biofilm</td>
</tr>
<tr>
<td>Wilson et al, 1995</td>
<td>To determine whether bacteria in supragingival plaque samples could be killed by low-power laser light in the presence of a suitable photosensitizer.</td>
<td>Following irradiation, substantial reductions were achieved in the total anaerobic count as well as in the number of viable streptococci and actinomyces present in the samples</td>
</tr>
<tr>
<td>Packer S et al, 1999</td>
<td>To determine the effects of irradiating the organism with red light in the presence of TBO on its proteolytic enzyme activity in suspensions of P. gingivalis</td>
<td>On exposure to 126 J of red light in the presence of 12.5 µg/ml of TBO the proteolytic enzyme activity was reduced by 100%.</td>
</tr>
<tr>
<td>Dortbudack O et al, 2001</td>
<td>To examine the clinical effectiveness of PDT in reducing periodontal pathogens, such as A.a, P.g &amp; P.i</td>
<td>This combined treatment showed significant bacterial reduction, but complete elimination of all three microorganisms was achieved in none of the cases.</td>
</tr>
<tr>
<td>KomerSci et al, 2003</td>
<td>Inoculated P.g into maxillary molar of rats and did a study to determine whether PDT could be used to kill the organism in the oral cavities of rats and whether this would result in a reduction in the alveolar bone loss characteristic of periodontitis.</td>
<td>When toluidine blue was used together with laser light there was a significant reduction in the number of viable P. gingivalis. The bone loss in the animals treated with light and toluidine blue was found to be significantly less.</td>
</tr>
<tr>
<td>Oliviera RR, 2007</td>
<td>split mouth design to treat 10 patients with either PDT or SRP. Clinical assessment of PI, GI, PD, BOP, GR &amp; CAL were made at baseline &amp; 3 months after treatment.</td>
<td>Both PDT &amp; SRP showed similar results in non surgical treatment of aggressive periodontitis.</td>
</tr>
<tr>
<td>Qin YL, 2008</td>
<td>Conducted a study to investigate the in vivo photosensitization of periodontal bacteria in rats and to compare its efficacy with that of routine scaling and root planning</td>
<td>Both PDT &amp; SRP showed similar results</td>
</tr>
<tr>
<td>Author(s) and Year of Publication</td>
<td>Description of Study</td>
<td>Findings</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Anderson R (2007)</td>
<td>To compare the effectiveness of a photodisinfection process to that of scaling and root planing (SRP) for non-surgical periodontal treatment.</td>
<td>No difference in any of the investigated parameters was observed at baseline between the three groups. At 6 &amp; 12 weeks PDT group showed significant BOP &amp; PPD compared to SRP group. CAL showed no statistical difference between the 2 groups.</td>
</tr>
<tr>
<td>Braun et al, (2008)</td>
<td>Conducted a study to assess the effect of adjunctive antimicrobial photodynamic therapy in chronic periodontitis. Relative attachment level (RAL), probing depths (PDs) and gingival recession (GR) were evaluated at baseline and 3 months after treatment.</td>
<td>Values for RAL, PD, SFFR and BOP decreased significantly 3 months after treatment in the control, with a higher impact on the sites treated with adjunctive aPDT.</td>
</tr>
<tr>
<td>Christodoulides N, (2008)</td>
<td>To evaluate the clinical and microbiologic effects of the adjunctive use of PDT to non-surgical periodontal treatment.</td>
<td>At 3 and 6 months after treatment, there were no statistically significant differences between the groups with regard to CAL, PD, FMPS, or microbiologic changes. At 3 and 6 months, a statistically significantly greater improvement in FMBS was found in the test group.</td>
</tr>
<tr>
<td>Oliveria RR, (2009)</td>
<td>A split mouth study to investigate cytokine levels in GCF of patients with aggressive periodontitis, after treatment with PDT or SRP. GCF samples were collected &amp; concentrations of TNF α and RANKL were determined by using ELISA.</td>
<td>PDT &amp; SRP had similar effects on TNF α &amp; RANKL in aggressive periodontitis patients</td>
</tr>
<tr>
<td>Sigusch BW, (2010)</td>
<td>To clinically and microbiologically evaluate the effect of photodynamic therapy (PDT) as a full-mouth procedure in Fusobacterium nucleatum–infected patients with periodontitis.</td>
<td>Four and 12 weeks after PDT, the mean PD and CAL showed significant differences from baseline values and from those of the control group. In the PDT group, 12 weeks after treatment, the F. nucleatum DNA concentration was found to be significantly reduced compared to the baseline level.</td>
</tr>
<tr>
<td>Ramanoz et al, (2010)</td>
<td>To examine the effects of PDT on the periodontal bacteria in combination with scaling and root planing (SRP) in the same group of patients by randomly selecting PDT or SRP for use in different quadrants of the mouth. For the present study, PDT was compared with a diode laser (980 nm) and an Nd:YA G laser (1,064 nm). Microbiological samples were examined and evaluated over a period of three months</td>
<td>Significant bacterial reduction has been observed in all cases. The diode laser with SRP presented long-term positive results, while PDT showed a significant bacteria reduction during the entire observation period.</td>
</tr>
<tr>
<td>Liu J et al, (2011)</td>
<td>Split mouth Short-term clinical trial to evaluate the effects of a combination of photodynamic therapy with low-level laser therapy as an adjunct to nonsurgical treatment of chronic periodontitis. PI, BOP, PD &amp; gingival recession were recorded at baseline, 1 and 3 months after the treatment. Gingival crevicular fluid was collected for assay of interleukin-1β levels at baseline, 1 wk and 1 month.</td>
<td>A significant decrease in gingival crevicular fluid volume was observed in both groups at 1 week, with a further decrease at 1 mo in the test sites. The test sites showed a greater reduction of interleukin-1β levels in gingival crevicular fluid at 1 wk than the control sites. No significant differences in periodontal parameters were found between the test and control teeth at 3 months.</td>
</tr>
</tbody>
</table>

**REFERENCES**


ABSTRACT

Decision making in today’s social and business environment has become a complex task. High costs of technology, material, labor, competitive pressures, different economic, social as well as political factors and viewpoints greatly increase the difficulty of managerial decision making. For well handling of arising problem the decision maker must examine a problem from both qualitative as well as quantitative approach. In today’s global era Operations Research tools can help the decision maker to solve the arising problem. Operations Research represents the study of optimal resource allocation. In order to make the effective and efficient decisions, managers must have a fundamental understanding of the decision science tools utilized in developing set of recommendations to choose from. The Operations research is usually the mathematical treatment, analysis of a process, problem, or Operations to determine its purpose and effectiveness and to gain maximum efficiency. Quantitative methods which comprises of Simulation, Linear and nonlinear programming, Queuing Theory and Stochastic Modeling are well-accepted techniques by both research and practice communities. Project scheduling techniques: PERT and CPM are efficient tools for scheduling and monitoring lengthy, complex and expensive projects of that time. This research Paper focuses mainly on the process of Operations research and explains some of the applications of Operations Research, elaborates some of the applications and benefits that may be gained by incorporating Operations Research into the actual business framework. The researcher has a wide interest in the foresaid topic and their further research area is the same.

APPLICATION OF OPERATIONS RESEARCH WITH SPECIAL REFERENCE TO TRANSPORTATION PROBLEM

V. P. Deshmukh¹, S. P. Shinde²

¹Business Administration Department, Bharati Vidyapeeth Deemed University, Pune, Yashwantra Mohite Institute of Management Karad
²Computer Applications Department, Bharati Vidyapeeth Deemed University, Pune, Yashwantra Mohite Institute of Management Karad

E-mail of Corresponding Author: Vishal.deshmuk50@yahoo.com
Keywords: Operations Research, optimal resource allocation, optimum solution, Performance Evaluation and Review Techniques & Critical Path Method

1. INTRODUCTION
In the global age decision making in business environment has become a complex task. High costs of technology, materials, labor, competitive pressures & so many different economic, social as well as potential factors & view points, greatly increase the difficulty of managerial decision making [27].
Whenever some national crises emerges due to the impact of political, economic or cultural factors the talents from all walks of life join together to overcome the situation & solve the problems. These combined efforts always result in new discoveries & techniques. O.R is also outcome of such situations [28].
The term Operations Research describes the discipline that is focused on the application of information technology for informed decision-making. Operations Research represents the study of optimal resource allocation. In Operations Research much of the actual work is conducted by using analytical and numerical techniques to develop and manipulate mathematical models of organizational systems that are composed of people, machines, and procedures. Some of the problems in the area of hospital management, energy conservation, environmental pollution, etc. have been solved by Operations Research specialists and this is an indication that Operations Research can also contribute towards the improvement in the social life and areas of global need.

1.1 History of Operations Research
Operations Research as a new field started in the late 1930's and has grown and expanded tremendously in the last 30 years. The British army conducted exercises on the radar system for detecting the aircrafts. In July 1938, the Superintendent of Bawdsey Research Station, announced that although the exercise had demonstrated the technical feasibility of the radar system for detecting aircraft, its Operational achievements were not up to what was required. He therefore proposed that a crash program of research into the Operational - as opposed to the technical - aspects of the system should begin. The term "Operational Research" was coined as a suitable description of this new branch of applied science. On 15th May 1940, with German forces advancing rapidly in France, Stanmore Research Section was asked to analyze a French request for ten additional fighter squadrons. They prepared graphs for Winston Churchill (British Prime Minister), based upon a study of current daily losses and replacement rates, indicating how rapidly such a move would deplete fighter strength. No aircrafts were sent and most of those currently in France were recalled. This is held by some to be the most strategic contribution to the course of the war made by Operations Research as the aircraft and pilots saved were consequently available for the successful air defense of Britain, the Battle of Britain. In 1941 Operational Research Section (ORS) was established in Coastal Command which was to carry out some of the most well-known Operations Research work in World War II. Thus Operations Research was born as a separate field of specialization. In order to make the effective and efficient decisions, managers must have fundamental understanding of the decision science tools utilized in developing set
of recommendations to choose from. The Operations Research is usually the mathematical treatment, analysis of a process, problem, or Operations to determine its purpose and effectiveness and to gain maximum efficiency. The Operations technique is utilized by functional groups such as Industrial Engineering in effort to support Operations Managers to make economically feasible decisions on a range of systematic challenges. The main responsibilities of Operations management are to manage and operate as efficiently and effectively as possible with the given resources.

**Quantitative methods** which comprises of Simulation, Linear and nonlinear programming, Queuing Theory and Stochastic Modeling, are well-accepted techniques by both research and practice communities. Functional entities such as Industrial or Systems Engineering use methodologies to provide feasible alternatives for Operations managers to decide on. An important component of decision-making process is verifying and validating alternatives, which typically involve decision makers, engineers or analysts. Growth of Operations Research is to a large extent, the result of the widespread availability of computers. Most Operations Research involves carrying out a large number of numeric calculations and without computers this would simply not be possible.

In India, Operations Research came into existence in 1949 when an Operations Research unit was established at Regional Research Laboratory, Hyderabad. Also Prof. R.S.Verma set up an Operations Research team at Defense Science Laboratory to solve problems of store, purchase and planning. During the 1950’s there was substantial progress in the application of Operations Research techniques for civilian activities along with a great interest in the professional development and education in OR. Many colleges and universities introduced Operations Research in their curricula. They were generally schools of engineering, public administration, business management, applied mathematics, economics, computer science etc. In 1953, Prof. P.C. Mahalanobis [8] established an Operations Research team in the Indian Statistical Institute, Calcutta to solve problems related to national planning and survey. In 1958, project scheduling techniques: PERT (Program Evaluation and Review Technique) and CPM (Critical Path Method) were developed as efficient tools for scheduling and monitoring lengthy, complex and expensive projects of that time. The real development of Operations Research in the national field was carried out by Prof. Mahalanobis in India when he used it in national planning. It is also being used in Railway, waiting or queuing problems of passengers for tickets at booking windows or trains queuing up in marshalling yard, waiting to be sorted out are tackled by various Operations Research technique.

### 2.1 Operations Research Process

The actual Operations Research process can be described in the following steps [15]:

- Identification of problem
- Data collection and mathematical model construction
- Solving the mathematical model
- Identification of optimum solution
- Validity of solution
- Solution Implementation
- Model modification
- Establishing controls over the solution
Specific Application Area: Operations Research in Manufacturing

The term Operations in Operations Research may suggest that the manufacturing application category represents the original home of Operations Research. That is not quite accurate, as the name originated from military Operations, not business Operations. Nevertheless, it is a true statement that Operations Research’s successes in contemporary business pervade manufacturing and service Operations, logistics, distribution, transportation, and telecommunication. The myriad applications include scheduling, routing, workflow improvements, elimination of bottlenecks, inventory control, business process re-engineering, site selection, or facility and general Operational planning. Revenue and supply chain management reflect two growing applications that are distinguished by their use of several Operations Research methods to cover several functions. Revenue management entails first to accurately forecasting the demand, and secondly to adjust the price structure over time.
to more profitably allocate fixed capacity. **Supply chain decisions describe** the who, what, when, and where abstractions from purchasing and transporting raw materials and parts, through manufacturing actual products and goods, and finally distributing and delivering the items to the customers. The prime management goal here may be to reduce overall cost while processing customer orders more efficiently than before. The power of utilizing Operations Research methods allows examining this rather complex and convoluted chain in a comprehensive manner, and to search among a vast number of combinations for the resource. Optimization and allocation strategy are most effective and hence beneficial to the Operations.

**Example of Application of Operations Research in Manufacturing**

**MATHEMATICAL FORMULATION OF TRANSPORTATION PROBLEM**

Let there be three units, producing Motor Cycles, say, A₁, A₂ and A₃ from where the Motor Cycles are to be supplied to four outlets say B₁, B₂, B₃ and B₄. Let the number of Motor Cycles produced at A₁, A₂ and A₃ be a₁, a₂ and a₃ respectively and the demands at the outlets be b₁, b₂, b₃ and b₄ respectively.

We assume the condition

\[ a₁ + a₂ + a₃ = b₁ + b₂ + b₃ + b₄ \]

i.e., all Motor Cycles produced are supplied to the different outlets.

Let the cost of transportation of one Motor Cycles from A₁ to B₁ be c₁₁. Similarly, the costs of transportation in other cases are also shown in the figure 2 and Table 1.

Let out of a₁ Motor Cycles available at A₁, x₁₁ be taken at B₁ depot, x₁₂ be taken at B₂ depot and to other outlets as well, as shown in the following figure and table 1.

![Figure 2](image)

Total number of Motor Cycles to be transported forms A₁ to all destinations, i.e., B₁, B₂, B₃, and B₄ must be equal to a₁.

\[ x₁₁ + x₁₂ + x₁₃ + x₁₄ = a₁ \]

Similarly, from A₂ and A₃ the Motor Cycles transported be equal to a₂ and a₃ respectively.

\[ x₂₁ + x₂₂ + x₂₃ + x₂₄ = a₂ \]
\[ x₃₁ + x₃₂ + x₃₃ + x₃₄ = a₃ \]

On the other hand it should be kept in mind that the total number of Motor Cycles delivered to B₁ from all units must be equal to b₁, i.e.,

\[ x₁₁ + x₂₁ + x₃₁ = b₁ \]
\[ x₁₂ + x₂₂ + x₃₂ = b₂ \]
\[ x₁₃ + x₂₃ + x₃₃ = b₃ \]
\[ x₁₄ + x₂₄ + x₃₄ = b₄ \]
With the help of the above information we can construct the following Table 1:

<table>
<thead>
<tr>
<th>Unit</th>
<th>Outlet</th>
<th>To B₁</th>
<th>To B₂</th>
<th>To B₃</th>
<th>To B₄</th>
<th>Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>From A₁</td>
<td>$x_{11}$</td>
<td>$c_{11}$</td>
<td>$x_{12}$</td>
<td>$c_{12}$</td>
<td>$x_{13}$</td>
<td>$c_{13}$</td>
</tr>
<tr>
<td>From A₂</td>
<td>$x_{21}$</td>
<td>$c_{21}$</td>
<td>$x_{22}$</td>
<td>$c_{22}$</td>
<td>$x_{23}$</td>
<td>$c_{23}$</td>
</tr>
<tr>
<td>From A₃</td>
<td>$x_{31}$</td>
<td>$c_{31}$</td>
<td>$x_{32}$</td>
<td>$c_{32}$</td>
<td>$x_{33}$</td>
<td>$c_{33}$</td>
</tr>
</tbody>
</table>

Table 1

The cost of transportation from $A_i$ ($i=1,2,3$) to $B_j$ ($j=1,2,3,4$) will be equal to

$$S = \sum_{i,j} c_{ij} x_{ij},$$

eq. (8)

where the symbol $c_{ij} x_{ij}$ put before $c_{ij}$ signifies that the quantities $c_{ij} x_{ij}$ must be summed over all $i = 1,2,3$ and all $j = 1,2,3,4$. Thus we come across a linear programming problem given by equations (1) to (7) and a linear function (8). We have to find the non-negative solutions of the system such that it minimizes the function (8).

We can think about a transportation problem in a general way if there are $m$ sources (say $A₁, A₂, ..., Aₘ$) and $n$ destinations (say $B₁, B₂, ..., Bₙ$). We can use $a_i$ to denote the quantity of goods concentrated at points $A_i$ ($i=1,2,.., m$) and $b_j$ denote the quantity of goods expected at points $B_j$ ($j=1,2,..,n$). We assume the condition $a₁+a₂+...+aₘ=b₁+b₂+...+bₙ$ implying that the total stock of goods is equal to the summed demand for it.

The following terms are to be defined with reference to the transportation problems:

(A) Feasible Solution (F.S.): A set of non-negative allocations $x_{ij}$ which satisfies the row and column restrictions is known as feasible solution.

(B) Basic Feasible Solution (B.F.S.) : A feasible solution to a $m$-origin and $n$-destination problem is said to be basic feasible solution if the number of positive allocations are $(m+n–1)$. If the number of allocations in a basic feasible solutions are less than $(m+n–1)$, it is called degenerate basic feasible solution (DBFS) (otherwise non-degenerate).

(C) Optimal Solution: A feasible solution (not necessarily basic) is said to be optimal if it minimizes the total transportation cost.

SOLUTION OF THE TRANSPORTATION PROBLEM

Let us consider the numerical version of the problem stated in the introduction and the mathematical formulation of the same in the next section, as below in Table 2.
All terms are in hundred

Table: 2
In order to find the solution of this transportation problem we have to follow the steps given below. (A) Initial basic feasible solution, (B) Test for optimization. Let us consider these steps one by one.

(A) Initial Basic Feasible Solution

(VBFS): There are three different methods to obtain the initial basic feasible solution viz: Vogel’s approximation method, North-West corner rule, Least cost entry method. In the light of above problem let us discuss here Vogel’s approximation method.

Vogel’s approximation method

(a1) Write the difference of minimum cost and next to minimum cost against each row in the penalty column. (This difference is known as penalty).

(a2) Write the difference of minimum cost and next to minimum cost against each column in the penalty row. (This difference is known as penalty).

We obtain the table as given below.

<table>
<thead>
<tr>
<th>Unit \ Outlet</th>
<th>B₁</th>
<th>B₂</th>
<th>B₃</th>
<th>B₄</th>
<th>Stocks</th>
<th>Penalties</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>(2)</td>
<td>(3)</td>
<td>(5)</td>
<td>(1)</td>
<td>8</td>
<td>(1)</td>
</tr>
<tr>
<td>A₂</td>
<td>(7)</td>
<td>(3)</td>
<td>(4)</td>
<td>(6)</td>
<td>10</td>
<td>(1)</td>
</tr>
<tr>
<td>A₃</td>
<td>(4)</td>
<td>(1)</td>
<td>(7)</td>
<td>(2)</td>
<td>20</td>
<td>(1)</td>
</tr>
<tr>
<td>Requirement</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>15</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Penalties</td>
<td>(2)</td>
<td>(2)</td>
<td>(1)</td>
<td>(1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3

(b) Identify the maximum penalties. In this case it is at column one and at column two. Consider any of the two columns,(here take first column) and allocate the maximum units to the place where the cost is minimum (here the position (1,1) has minimum cost so allocate the maximum possible units ,i.e., 6 units to this position). Now write the remaining stock in row one. After removing the first column and then by repeating the step (a), we obtain as follows:

<table>
<thead>
<tr>
<th>Unit \ Outlet</th>
<th>B₂</th>
<th>B₃</th>
<th>B₄</th>
<th>Stocks</th>
<th>Penalties</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>(3)</td>
<td>(5)</td>
<td>(1)</td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td>A₂</td>
<td>(3)</td>
<td>(4)</td>
<td>(6)</td>
<td>10</td>
<td>(1)</td>
</tr>
<tr>
<td>A₃</td>
<td>(1)</td>
<td>(7)</td>
<td>(2)</td>
<td>20</td>
<td>(1)</td>
</tr>
<tr>
<td>Requirement</td>
<td>8</td>
<td>9</td>
<td>15</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Penalties</td>
<td>(2)</td>
<td>(1)</td>
<td>(1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4

(c) Identify the maximum penalties. In this case it is at row one and at column two. Consider any of the two (let it be first row) and allocate the maximum possible units to the place where the cost is minimum (here the position (1,4) has minimum cost so allocate the maximum possible units, i.e., 2 units to this position). Now write the remaining stock in column four. After removing the first row and by repeating the step(a), we obtain table 5 as given below.
Table 5
(d) Identify the maximum penalties. In this case it is at column four. Now allocate the maximum possible units to the minimum cost position (here it is at (3,4) position and allocate maximum possible units, i.e., 13 to this position). Now write the remaining stock in row three. After removing the fourth column and then by repeating the step (a) we obtain table 6 as given below.

<table>
<thead>
<tr>
<th>Unit \ Outlet</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>Stocks</th>
<th>Penalties</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>A3</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Requirement</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Penalties</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6
(e) Identify the maximum penalties. In this case it is at row three. Now allocate the maximum possible units to the minimum cost position (here it is at (3,2) position and allocate maximum possible units, i.e., 7 to this position). Now in order to complete the sum, (2,2) position will take 1 unit and (2,3) position will be allocated 9 units. This completes the allocation and with the help of the above information draw table 7 as under.

<table>
<thead>
<tr>
<th>Unit \ Outlet</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>Stocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>A2</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>A3</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Requirement</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>15</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 7
From the above facts calculate the cost of transportation as
6 \times 2 + 2 \times 1 + 1 \times 3 + 9 \times 4 + 7 \times 1 + 13 \times 2 = 12 + 2 + 3 + 36 + 7 + 26 = 86 \ i.e., \ Rs. \ 8600.

After calculating the cost of transportation, it is clear that Vogel’s approximation method gives an initial basic feasible solution which is much closer to the optimal solution.

**Algorithm for Optimality Test:** In order to test for optimality we should follow the procedure as given below:

| Step 1 | Start with B.F.S. consisting of m+n−1 allocations in independent positions |

(B) Test for Optimization: In part (A) of this section we have learnt how to obtain an initial basic feasible solution. Solutions so obtained may be optimal or may not be optimal, so it becomes essential for us to test for optimization.
Step 2: Determine a set of m+ n numbers \( u_i \) (i=1,2,...,m) and \( v_j \) (j=1,2,...,n) such that for each occupied cells \((r, s)\),
\[ c_{rs} = u_r + v_s \]

Step 3: Calculate cell evaluations (unit cost difference) \( d_{ij} \) for each empty cell \((i, j)\) by using the formula
\[ d_{ij} = c_{ij} - (u_i + v_j) \]

Step 4: Examine the matrix of cell evaluation \( d_{ij} \) for negative entries and conclude that

(i) If all \( d_{ij} > 0 \), then the Solution is optimal and unique
(ii) If all \( d_{ij} \geq 0 \) with at least one \( d_{ij} = 0 \), then the Solution is optimal and alternate solution also exists.
(iii) If at least one \( d_{ij} < 0 \), then the Solution is not optimal. If it is so, further improvement is required by repeating the above process. See step 5 and onwards.

Step 5 (i) See the most negative cell in the matrix \([d_{ij}]\).
(ii) Allocate q to this empty cell in the final allocation table. Subtract and add the amount of this allocation to other corners of the loop in order to restore feasibility.
(iii) The value of q, in general is obtained by equating to zero the minimum of the allocations containing \(-q\) (not + q) only at the corners of the closed loop.
(iv) Substitute the value of q and find a fresh allocation table.

Step 6: Again, apply the above test for optimality till you find all \( d_{ij} \geq 0 \). Computational demonstration for optimality test

Consider the initial basic feasible solution as obtained by Vogel’s approximation method in section (A) of this article [Table ()].

Step 1: (i) In this table number of allocations = 3+4–1=6.
(ii) All the positions of allocations are independent.

Step 2: Determine a set of \((m+n)\), i.e., \((3+4)\) numbers \( u_1, u_2, u_3 \) and \( v_1, v_2, v_3 \) and \( v_4 \) for each occupied cells. For this consider the row or column in which the allocations are maximum (here, let us take first row).

Now, take \( u_1 \) as an arbitrary constant (say zero) then by using \( c_{ij} = u_i + v_j \) try to find all \( u_i \) and \( v_j \) as

<table>
<thead>
<tr>
<th></th>
<th>B₁</th>
<th>B₂</th>
<th>B₃</th>
<th>B₄</th>
<th>( u_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>A₂</td>
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<td>3</td>
<td>4</td>
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<td>A₃</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td>( v_j )</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 8
Step 3: Cost matrix for the empty positions

\[
\begin{bmatrix}
3 & 5 & \bullet \\
7 & \bullet & \bullet \\
4 & \bullet & 7 \\
\end{bmatrix}
\]

Matrix \([u_i + v_j]\) for empty positions

\[
\begin{bmatrix}
0 & 1 & \bullet \\
5 & \bullet & 4 \\
3 & \bullet & 2 \\
\end{bmatrix}
\]

\[
\therefore\ c_{ij} = \begin{bmatrix} c_{ij} - [u_i + v_j] \end{bmatrix} =
\begin{bmatrix}
3 & 4 & \bullet \\
2 & \bullet & 2 \\
1 & \bullet & 5 \\
\end{bmatrix}
\]

Step 4: Here all \(d_{ij} > 0\) \(\Rightarrow\) Solution obtained by Vogel’s approximation method is an optimal solution.
4. CONCLUSION

The driving idea behind Operations Research is to collaborate with clients to design and improve Operations, make better decisions, solve problems, and advance managerial functions including policy formulation, planning, forecasting, and performance measurement. The goal of Operations Research is to develop information to provide valuable insight and guidance. By utilizing Operations Research methods, the objective is to apply to any given project the most appropriate scientific techniques selected from mathematics, any of the sciences including the social and management sciences, and any branch of engineering, respectively. The work normally entails collecting and analyzing data, creating and testing mathematical models, proposing approaches not previously considered, interpreting information, making recommendations, and aiding at implementing the initiatives that result from the study. Moreover, utilizing Operations Research methods allow developing and implementing software, systems, services, and products related to a clients methods and applications. The systems may include strategic decision-support systems, which play a vital role in many organizations today. After calculating the cost of transportation by all methods, researcher finds that Vogel’s approximation method gives an initial basic feasible solution which is much closer to the optimal solution than the other methods. It is always worth while to spend some time finding a “good” initial solution because it can considerably reduce the total number of iterations required to reach an optimal solution.

ACKNOWLEDGEMENTS

In writing this research paper many people have helped us a lot. I would like to acknowledge my teacher Prof.D.V.Patil for his great support and guidance during preparation of this manuscript.

REFERENCES

22. Dominique A. Heger, Fortuitous Technology, (dom@fortuitous.com), Austin, TX, 2006, An Introduction to Operations Research – Benefits, Methods & Application
ABSTRACT
Earth's climate is determined by complex interactions between the Sun, oceans, atmosphere, land, and living things. The composition of the atmosphere is particularly important because certain gases (including water vapour, carbon dioxide, methane, ozone, and nitrous oxide) absorb heat radiated from the Earth's surface. As the atmosphere warms, it in turn radiates heat back to the surface, to create what is commonly called the "greenhouse effect." Changes in the composition of the atmosphere alter the intensity of the greenhouse effect. Variations of Chennai’s average mean temperature and diurnal temperature range for a period of ten years are investigated and is compared with past hundred years data. There is an observed variability of average mean temperature and diurnal temperature range. The variation in the temperature can be influenced by various climate forcing. In this paper we have correlated the sunspot numbers and Carbon dioxide emission with DTR and have estimated which influences the DTR more. Global warming over land can be characterized by faster warming at nights. This observed recent trend should lead to considerable decrease in the diurnal temperature range (DTR). The decrease of the diurnal temperature range is approximately equal to the increase of mean temperature

INTRODUCTION
The work of the farmers, power company engineers, weather analysts and many others would be great deal more difficult without accurate information of careful recording of temperature data. The difference between the daily maximum and minimum temperature is called the diurnal temperature or range of temperature. The greatest variation in daily temperature occurs at the earth’s surface and becomes progressively smaller as we move away from the surface. This daily variation in temperature is larger on clear days than on cloudy days. The diurnal temperature range can be analyzed in four different categories like the deserts, plateau regions, cities and humid regions.

The largest diurnal range of temperature occurs on high desserts, where the air is often cloud free, and there is less carbon dioxide and water vapour above to radiate much infrared radiation back to the space. An elevated plateau region like Reno which is located at 1350m above sea level will have 35degree Celsius in the day and will cool down to 15 degrees at night time.
Thus, showing a temperature range of 25 degrees. The city region can be split into two categories coastal and inland. Coastal cities usually have a smaller diurnal temperature range than the inlands as the water vapour will heat and cool more slowly than land. The city inlands which also have a decreased DTR are caused due to urban heat islands.

The city inlands which also have a decreased DTR are caused due to urban heat islands. The urban heat islands are due to industrial and urban development. In rural areas a large part of the incoming solar energy is used to evaporate water from vegetation and soil. In the cities where less vegetation and exposed soil exits, the majority of the solar energy is absorbed by urban structures and asphalt. Hence during warm daylight hours, less evaporative cooling in cities allows the surface temperature to rise higher than in rural areas. And during night time the temperature does not reduce much due to radiation emitted from the urban structures.

Chennai is located on the thermal equator on the southeast coast (Coromandel Coast) of India at an average altitude of 6 metres from the sea level. The latitude and longitude of this city is 13°04 N 80°17 E respectively. It covers a total area of 174 sq km that is spread irregularly in the northeast corner of Tamil Nadu. Its proximity to the Bay of Bengal ensures a hot & humid climate for most of the year. The highest temperature is attained in late May and early June which is usually about 38 °C (100.4 °F) it exceeds 40 °C (104 °F) for a few days. Average daily temperature in Chennai during January is around 24 °C (75.2 °F), though the temperature rarely falls below 18 °C (64.4 °F). The lowest temperature recorded is 15.8 °C (60.44 °F) and highest 44.1 °C (111.38 °F). Thus Chennai falls under the fourth category of the diurnal temperature range.

In this paper we study the relationship between the solar activity, carbon dioxide emission and their influence on the daily temperature which may prove to be the evidence for the climate change over Chennai.

DATA AND METHODOLOGY
The maximum and minimum temperature data of Chennai for the month of March, April, May and June are taken in two parts. First, for a period of hundred years (1901-2000). Next, for a period of ten years (2001-2010). The data for maximum and minimum temperature are obtained from the Indian Meteorological department. The sunspot number data for the corresponding months are obtained from National Geophysical Data Centre, Boulder, Colorado, USA. Similarly the carbon dioxide data are obtained for a period of 1980 to 2009. From the NOAA website. Solar activity, green house gases and climate change are much of much interest these days.

The statistical analysis of the examined data series involves a non parametric rank correlation technique (Spearman Rank Correlation). The statistical reliability of the obtained correlations is evaluated by a significance test. The Diurnal Temperature Range (DTR) between the hundred years data and ten years temperature maximum and minimum data are determined. Followed by this a statistical correlation is worked out between diurnal temperature range and sun spot numbers. And the same is carried out for the carbon dioxide emission data.

The anomaly in diurnal temperature is shown in Table 1. And the correlation values are shown in Table 3. A graph is plotted between the temperature range and sun spot numbers and between the temperature range and carbon dioxide for
the months of March, April, May and June. It is shown in Figure 1 and Figure 2 respectively.

RESULTS
Comparing the value of maximum and minimum temperatures for March, April, May and June in column two and three of Table 1: we observe a slight rise in the maximum and minimum temperature individually. The diurnal temperature range shows a slight anomaly. The anomaly was expected to be more prominent as per the concept of global warming. As the global warming increases the night time temperature increases leading to decrease in the diurnal temperature range. Column 2 of Table 1 shows a decreased diurnal temperature range. But column 3 shows slightly higher values of diurnal temperature range.

This result is contrary to several observed trends in the other regions of the world. A city is characterized by a decrease in the DTR. Thus the anomaly is investigated further. For this we have considered the DTR in six other coastal cities of Tamil Nadu and compared it with the DTR of six Inland cities in the state. The results are tabulated in Table 2. From the data it is inferred that all the coastal cities exhibit an increased DTR. The Inland cities exhibit a decreased DTR effect. Thus the anomaly in Chennai City can be attributed to the effect of its coast line.

The change in DTR can be forced by natural and anthropogenic factors. Table 3 shows the correlation between the diurnal temperature range, sunspot numbers and the carbon dioxide emission. We can infer from table 3 the sunspot numbers shows a good correlation with the DTR than the carbon dioxide emission. The Figure 1 shows a prominent relevance between the sunspot numbers and the DTR than the carbon dioxide emission as in Figure 2.

DISCUSSION
The coastal parts of India often come under the warm and humid zones. The city of Chennai falls under this zone. The contradictory result obtained from the Table 1 can be due the increased aerosol concentration in the atmosphere in the city during the last ten years, apart for this the ocean currents, direction of prevailing winds, the El Nino effect, water vapour concentration all these factors affect the DTR in the Chennai region and thus we observe an increase in DTR.

The city inland are affected by the Urban Heat Island Effect (UHIE) which increase the night time temperature thus increasing the minimum Temperature at a faster rate than the increase in the maximum temperature. This leads to a decreased DTR in these regions.

In Table 3 the significance of correlation depends on the sample size. For large sample as in our case even the small relations between variables will be significant. Here we have $r = 0.2$ at a significant level $p<0.005$. Thus the sunspot numbers correlate well with the DTR than the Carbon dioxide emission. From Figure 1 we can infer that the sunspot numbers varies promptly with the temperature range except for the month of May. May is the peak summer month in Chennai so the temperature is not affected by sunspot numbers alone. The anomaly during this time is caused by various other anthropogenic agents. Figure 2 represents the graph between the carbon dioxide emission and the temperature range. We can infer that the trend of variation in
temperature range is moving well ahead than that of the carbon dioxide emission.

CONCLUSION
The solar forcing is more prominent on the diurnal temperature range. The contradictory result of Table 1 is due to the topographic status of Chennai city which poses a dynamic climate. The maximum and minimum temperature increases for all the twelve cities that were considered. For the coastal regions the increase in the maximum temperature is more than the increase in the minimum temperature leading to increased DTR. For the inland cities the increase in the minimum temperature is more than the increase in the maximum temperature leading to decreased DTR. The relationship between the regional surface air temperature and the sunspots shows a statistically significant value yet quite low. In conclusion at regional level the temperature changes are correlated with corresponding solar activity. Signatures of human activity are not yet distinguishable in the observations. Even though solar activity may not be the dominant factor in global warming, it is important enough that understanding how the climate responds to small changes in solar irradiance will help us to predict the climate changes caused by human activity.

Table 1: Anomaly In Temperature Range For 100 Years And Last Ten Years

<table>
<thead>
<tr>
<th>Year/ Month</th>
<th>1901-2000 (100 Years)</th>
<th>2001-2010 (10 Years)</th>
<th>Anomaly In Temperature Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max Temp</td>
<td>Min Temp</td>
<td>Range</td>
</tr>
<tr>
<td>March</td>
<td>32.6</td>
<td>23</td>
<td>9.6</td>
</tr>
<tr>
<td>April</td>
<td>34.7</td>
<td>25.8</td>
<td>8.9</td>
</tr>
<tr>
<td>May</td>
<td>37.4</td>
<td>27.6</td>
<td>9.8</td>
</tr>
<tr>
<td>June</td>
<td>37.3</td>
<td>27.4</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Table 2: DTR for Coastal cities and Inland Cities

<table>
<thead>
<tr>
<th>Coastal Cities</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
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<tbody>
<tr>
<td>Karaikal</td>
<td>7.4</td>
<td>8.5</td>
<td>6.8</td>
<td>8.5</td>
</tr>
<tr>
<td>Nagapattinam</td>
<td>7.4</td>
<td>7.8</td>
<td>6.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Chennai</td>
<td>8.9</td>
<td>8.9</td>
<td>7.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Pondicherry</td>
<td>8.4</td>
<td>8.8</td>
<td>7.5</td>
<td>8.4</td>
</tr>
<tr>
<td>Pamban</td>
<td>7.8</td>
<td>7.5</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Cadalore</td>
<td>9.5</td>
<td>9.1</td>
<td>8.3</td>
<td>8.7</td>
</tr>
</tbody>
</table>
Table 3: Spearman rank correlation between sunspot numbers, carbon dioxide and diurnal temperature range

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Kodaikanal</td>
<td>11.2</td>
<td>10.2</td>
<td>9.6</td>
<td>9.1</td>
<td>9.0</td>
<td>8.1</td>
<td>8.0</td>
<td>6.6</td>
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<tr>
<td>Trichy</td>
<td>12.8</td>
<td>11.9</td>
<td>12.0</td>
<td>11.0</td>
<td>11.2</td>
<td>11.3</td>
<td>10.5</td>
<td>10.4</td>
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<td>Vellure</td>
<td>14.8</td>
<td>14.8</td>
<td>13.5</td>
<td>13.8</td>
<td>13.0</td>
<td>14.0</td>
<td>11.2</td>
<td>12.0</td>
</tr>
<tr>
<td>Dharmapuri</td>
<td>15.2</td>
<td>13.9</td>
<td>13.4</td>
<td>12.2</td>
<td>12.0</td>
<td>11.4</td>
<td>11.0</td>
<td>9.8</td>
</tr>
<tr>
<td>Coimbatore</td>
<td>14.6</td>
<td>13.1</td>
<td>12.9</td>
<td>11.6</td>
<td>11.3</td>
<td>10.9</td>
<td>9.8</td>
<td>9.7</td>
</tr>
<tr>
<td>Salem</td>
<td>15.8</td>
<td>13.6</td>
<td>13.6</td>
<td>11.8</td>
<td>12.8</td>
<td>11.2</td>
<td>11.8</td>
<td>10.0</td>
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</table>

Table 3: Spearman rank correlation between sunspot numbers, carbon dioxide and diurnal temperature range

<table>
<thead>
<tr>
<th>MONTH</th>
<th>SSN</th>
<th>CO₂</th>
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<tr>
<td>MARCH</td>
<td>0.29371</td>
<td>0.1002</td>
</tr>
<tr>
<td>APRIL</td>
<td>0.27972</td>
<td>-0.0917</td>
</tr>
<tr>
<td>MAY</td>
<td>0.22028</td>
<td>0.3200</td>
</tr>
<tr>
<td>JUNE</td>
<td>-0.13287</td>
<td>-0.0409</td>
</tr>
</tbody>
</table>

Figure 1: variation of sunspot number with temperature range for March, April, May and June
Figure 2: Variation of Temperature range with carbon dioxide concentration
REFERENCES


3. NOAA/NESDIS/NGDC/STP, Boulder-Sunspot Number Data from NGDC http://www.ngdc.noaa.gov/stp/solar/ssndata.html


8. Variability of climate change in india, s.k. dash and j.c.r. hunt, current science, vol.93,no.6,2007,pg782-788.
ABSTRACT

A still born male foetus of 20 wks gestation was brought to Anatomy department as a part of routine research work from a private nursing home in vizianagaram. On gross examination foetus showed bilateral cleft lip with cleft palate, atresia of right external acoustic meatus with malformed right pinna and an abnormally distended abdomen. This prompted the author to take up an in depth study. On exploring the abdomen, we found two large cystic cavities filled with clear and watery fluid occupied the entire abdomen. Branchial arch anomalies, ear deformities and renal anomalies constitute Melnick-Fraser syndrome. The microscopic structure of both cystic kidneys is compared with histological sections of kidneys in 15 still born fetuses of 18-22 weeks gestation. The present work is an initial and preliminary attempt to analyse the growth pattern of kidney in human fetuses, which may prove useful in diagnosing all foetal kidney diseases such as agenesis, hypoplasia, multicystic kidney, polycystic kidney etc.

Keywords: cystic kidneys, ear anomalies, glomeruli, cortico-medullary junction.

INTRODUCTION

Melnick-Fraser syndrome is also known as Branchio-oto-renal syndrome (BORS). A typical case of Melnick-Fraser is not fatal according to Melnick et al. BORS, is an autosomal dominant disorder characterized by the presence of hearing loss, auricular malformations, branchial arch anomalies, and renal anomalies, first recognized by Melnick et al in 1975 & further delineated by Fraser et al. Approx 90% of individuals diagnosed with BORS have an affected parent, where as 10% of cases are caused by de novo mutations. Mutations in the EYA gene, a human homologue of the drosophila “eyes absent” gene, are identified as the cause of BOR syndrome. The diagnosis of BOR spectrum disorders is based on clinical criteria. The prevalence of BORS ranges from 1:700000 to 1:40000. The syndrome occurs in about 2% of profoundly deaf children. The kidney is developed from metanephric blastema& ureteric bud. Secretary part appears as small filtering units, nephrons. A diverticulum appears on mesonephric duct, which have an affinity to grow towards metanephros. The branches of ureteric bud develops communication with all the nephrons. The process of renal development begins at deeper regions and reaches the peripheral part of the cortex with the advancement of ampulla in that
region and terminates during the last month of gestation with subsequent interstitial growth.6,7,8

MATERIALS AND METHODS
1) Dead foetuses were collected and injected with 10% formalin and immediately immersed in 10% formalin.
2) The fetuses were dissected to observe and isolate kidneys. Isolated kidneys were fixed in 10% formalin.
3) Kidneys of one 20 weeks GA foetus with Melnick-Fraser syndrome has been collected from a private nursing home, was preserved in 10% formalin.
4) 30 kidneys from 15 stillborn fetuses ranged from 18-22 weeks GA (table 1) were also preserved.
5) Coronal sections of kidneys (both BORS foetus and control group),
6) Transverse sections of lung and liver of BORS foetus were subjected to routine histological preparation to observe for any related, or accompanying changes
7) The H&E stained sections were studied under light microscopy.

Observations:
Macroscopic:
Autopsy of one BORS foetus revealed bilateral cleft lip with cleft palate, anotia and pre auricular tags on the right side. The left pinna and external acoustic meatus were normal (Fig 1). No other external anomalies were detected. On opening of thorax, the lungs were normal in size, shape and colour. A large cyst of irregular shape occupied entire abdomen except left hypochondriac and epigastric region, which was occupied by coils of small intestine. Sub-hepatic vermiform appendix was noted. Another large irregular cyst was found in the left hypochondriac region under coils of intestine. The Ureters connected ‘cysts’ to the urinary bladder.

The Liver, stomach, pancreas and spleen were normal. Both right and left testes were intra abdominal overlying the right and left cysts (Fig 2). On palpation the wall of right cystic kidney was thin with minimum amount of nephrogenic tissue when compared with left cystic kidney.

The metanephric kidney appeared as an oval organ with a smooth surface and having no lobulation in the control group.

Microscopic:
Control Group
18 weeks:
Definite capsule with few & small septae were noticed. No evidence of lobulations were appreciated due to the presence of continuous cortex. Mesenchymatous tissue i.e., Nephrogenic zone at the periphery was noticed. The peripheral area was deeply stained indicating increased activity while medulla showed mesenchymatous tissue with some developing tubules. PCT & DCT with their characteristic cuboidal epithelium were noted. Papilla of the pyramid was clearly seen indicating tertiary division of renal pelvis i.e., minor calyx (Fig 3). Few deeper glomeruli were larger in size.

20 weeks:
Cortex appeared as an uniform zone. Nephrogenic zone was clear and decreased in thickness. Cortico medullary junction was well defined. Juxta glomerular apparatus was well defined with the evidence of existence of mesangial cells. Juxta medullary glomeruli were very large.

22 weeks:
Evidence of mesenchymatous tissue at the periphery i.e., Nephrogenic zone was still present, but the thickness decreased with increase in the gestational age. Clear demarcation of cortex & medulla along with well defined juxta medullary
glomeruli arranged in multiple rows were noted. Both limbs of loops of henle & the collecting ducts were noted. Arrangement of sectioned parts of renal tubule indicated increase in their length. Renal pyramid formation was complete with prominent papillae. (fig 5)

**BORS Foetus**

**Right Cystic kidney: (fig 6)**
Capsule was well defined without any septae.
Nephrogenic zone was not evident.
Cortico-medullary differentiation was not appreciated.
Very few glomeruli per field were noticed. Deeper glomeruli were large.
Proximal convoluted and distal convoluted tubules were not well differentiated.
No pyramid formation - as there was no differentiation of tubules.

**Left cystic kidney: (Fig 7)**
Capsule was well defined without any septae.
Thin nephrogenic zone was evident.
Cortico-medullary differentiation was not appreciated.
Relatively more number of glomeruli were noted when compared with right cystic kidney, but less when compared with control group. Proximal and distal convoluted tubules were appreciated. Presence of collecting ducts was evident, but no pyramid formation.

**Liver:**
Microscopic structure of liver revealed no abnormal features like biliary dysgenesis and periporal fibrosis, which were evident in an autosomal recessive polycystic kidney disease.

**Lung:**
Microscopic structure of both lungs corresponds with the gestational age. No evidence of pulmonary hypoplasia.

**DISCUSSION**
The mechanism of development of dysplastic kidneys is not always clear and may be variable. It is generally accepted that renal dysplasia can be the result of very early in-utero urinary tract obstruction, whether it be at the level of the urethra, bladder or at the ureter. The most severe dysplasia is the result of early obstruction with abnormal disappearance of nephrogenic blastema and subsequent arrest of nephrogenesis. In the present case histological examination revealed formation of renal corpuscles of secretory part and incomplete differentiation of collecting part.

Melnick Fraser reported ear deformities ranging from 77 to 89%, branchial fistulas 63%, and renal dysplasias 63%. Chang et al 2004 noted 67% renal anomalies in a study of 21 affected individuals, who underwent either Intravenous pyelography or retrograde pyelography. He presented the following renal anomalies -
  a) Renal agenesis (29%), Hypoplasia (19%), Dysplasia (14%)
  b) Uretero-pelvic junction (UPJ) obstruction (10%)
  c) Calyceal cyst/diverticulum (10%)
  d) Calycectasis, pelviectasis, hydronephrosis, and vesicoureteral reflux (5% each)

**CONCLUSIONS**
Absence of pulmonary hypoplasia and cystic fibrosis of liver ruled out autosomal recessive polycystic kidney disease, which is the commonest form of intranatal cystic kidney disease. The present case is a variant of Melnick Fraser syndrome, as the foetus presents palate anomalies, external auditory deformities and severe renal dysplasia must have resulted in the mortality of foetus in mid-term. Branchial cysts or fistulae were absent. Since it is an
autosomal dominant disorder, affected individuals have a 50% chance of transmitting this disorder to each child. Correct and timely antenatal diagnosis of cystic kidney disease with associated anomalies is essential and important. A proper counseling with appropriate obstetric and paediatric management, foetal mortality can be reduced along with parental psychological trauma.

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Authors acknowledge the immense help received from the scholars, whose articles are cited and included in references of this manuscript. The authors are also grateful to authors/ editors/ publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed. The authors are grateful to the management & Prof Dr T.A.V. Narayanaraju, dean of Maharajah’s Institute of Medical Sciences for their encouragement. We would like to thank EDP staff, all the administrative and academic staff for their assistance.

REFERENCES
Table I: Age and sex of investigated fetuses

<table>
<thead>
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<th>Foetal age (weeks)</th>
<th>Number</th>
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<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig 1: Showing anotia and pre auricular tags on the right side and normal left pinna and external acoustic meatus.

Fig 2: Showing bilateral cleft lip with cleft palate and bilateral cysts occupying entire abdomen.
Fig 3, 4 & 5 Showing the coronal sections of kidneys in 18 wks, 20 wks & 22 wks G.A foetuses, H&E.
Fig 6 & 7: Shows transverse sections of right & left cystic kidneys, H&E.

**Figure legend:**

Fig 1: Showing anotia and pre-auricular tags on the right side and normal left pinna and external acoustic meatus.

Fig 2: Showing bilateral cleft lip with cleft palate and bilateral cysts occupying entire abdomen.

Fig 3: Showing cortico medullary differentiation in coronal section of kidney, 18 wks G.A, H&E, 10X10

Fig 4: Showing larger deeper glomeruli, 20 wks G.A, H&E, 10X40.

Fig 5: Showing well defined pyramidal system, 22 wks G.A, H&E, 10x10.

Fig 6: Showing T.S of right cystic kidney in BORS foetus, 20 weeks GA, H&E, 10X40.

Fig 7: Showing T.S of left cystic kidney in BORS foetus, 20 weeks GA, H&E, 10X10.

**Abbreviations:**

1. BORS: Branchio-Oto-Renal Syndrome.
2. EAM: External auditory meatus.
5. T.S: Transverse section.
ABSTRACT
Medical education is undergoing tremendous change. This has been facilitated by medical education units set up in different colleges. The main aim of medical education is to bring about an upliftment in knowledge, skill and attitude of the students. Anatomy, being a basic medical science, is one of the pillars on which medical science stands. A need has therefore come up to increase the awareness of anatomy. This is possible if we are able to come out of the straight – jacket of traditional styles and incorporate new ideas, thoughts and technologies. This study is therefore an endeavor to initiate this process. It covered medical undergraduates in Nepal, whose feedback had been taken in form a questionnaire. An attempt has been made to understand the general attitude towards anatomy among the students. It also sought opinions and suggestions from participants about new ideas to bring about these changes. This study would explore new horizons for the development of anatomy and in the field of medical education.

INTRODUCTION
Anatomy forms the foundation for clinical medicine and its place in the medical school curriculum requires careful attention [1]. Patient diagnosis and treatment requires a knowledge of three dimensional within the human body. In Nepal, the seven basic science subjects (Anatomy, Physiology, Biochemistry, Pharmacology, Microbiology, Pathology and Community medicine) are taught during the first four semesters of the undergraduate medical (MBBS) course in an integrated fashion.[2] Integration is carried out at an organ system level. The teaching in the first semester starts with basic concepts and then moves on to Autonomic Nervous System (ANS) and then blood and hemotopoietic system. The lectures of different subjects take place within the time frame allotted to particular system. Integration using clinical problem solving and clinical case scenarios does not take place. In Anatomy dissection is an integral part of the course. There are sessions on gross anatomy and histology. Anatomy teaching and learning takes place through didactic lectures and practical sessions. Anatomy dissection occupies around 6 to 7 hours every week [2], while histology practicals occupy around 2 hours weekly. In each semester, there are around 50 hours of anatomy lectures, 70 hours of dissection, and 30 hours of histology.
practicals. In each week there are around 24 hours of didactic lectures, 6 to 7 hours of dissection and 10 hours of practical/problem based learning sessions. A previous study had shown that majority of students regarded anatomy dissection as a largely positive experience [2]. Dissection of cadavers has been used for anatomy teaching since the renaissance. Dissection gives students a three-dimensional view of the body and helps in integration of anatomy in a whole organism [3]. The dissection room is perceived as a good introduction to self-directed learning and team working [4]. Due to various reasons, the use of dissection is decreasing in medical schools. A number of initiatives are underway to use technology to facilitate the learning of anatomy and to make it more relevant to the needs of 21 century medical practice. Medical schools the world over are using virtual-reality imaging to support anatomy learning [5],[6]. A new medical school in the United Kingdom does not use cadaveric material for learning [7],[8]. Virtual anatomy provides significant advances over the cadaver,[9]. It can provide different views and perspectives, standardization, diversity and the opportunity to learn anatomy of the living human body. Problem based learning (PBL) is becoming increasingly common in medical education. The Arabian Gulf University College of medicine teaches anatomy in systems based PBL units [9]. A survey conducted in a medical college in Eastern Nepal had shown that students had a positive opinion about anatomy and were of the opinion that dissection helped them the most in learning the subject [10]. Information on the attitude of students towards anatomy are lacking in our institution. Hence the present study was carried out. The objectives were to

a) Obtain information on the student attitudes towards anatomy.
b) Note the association, if any, of the attitudes with demographic and personal characteristics of the respondents.
c) Obtain information on the strengths and weakness of anatomy teaching and assessment.
d) Get suggestions to improve the teaching and learning of the subject.

This study is done to incorporate newer ideas, thoughts and technologies to increase scope of teaching in Anatomy, with a view of finding ways to uplift knowledge, skills and attitude of students towards the subject.

MATERIALS AND METHODS
The study was carried out among the Basic Science Students of Manipal College of Medical Sciences, Pokhara, Nepal. The questionnaire we developed was circulated among the students of four semesters (Table/Fig1). Each semester has 60 students. Student opinion was assessed using Likert scales. Detailed qualitative Analysis of Student perceptions was not carried out. The internal consistency of the Questionnaire was examined using Cronbach’s Alpha. We laid the technical expertise to carry out factor analysis.

DISCUSSION
The students overall had a high subject and improvement scores. Majority of the students had favorable attitude towards animal dissection. In a previous study in our institution, students regarded anatomy dissection as a significant life experience and one which was largely positive [2]. In a study in Ireland most students found the prospect of their first visit to the anatomy room to be exciting and found it to be a positive learning experience [11]. A similar result was reported in a study in the United Kingdom [12].
In the United States, anatomy dissection was regarded as a positive experience by the vast majority of students but a small minority regarded it as a traumatic experience [13]. In our institution a previous study had shown that student attitude towards dissection was largely positive.

The second semester students felt that the subject required greater improvement compared to the fourth semester. It could be that by the time students reached the fourth semester they were more comfortable with the teaching and learning methodology in Anatomy. It will be difficult to explain why students of doctor parents had lower subject and improvement scores compared to students with non-doctor parents. It is to be expected that students of doctor parents would be more exposed to Medical Scenarios and have a more favorable attitude towards the subject.

A previous study has found no significant differences between students of Doctor and Non-Doctor parents with regard to the emotional impact of Cadaver dissection. [1]

A problem noted was that students who had a high subject score also had a high improvement score. It could be that students who had a more positive attitude towards the subject also had a higher expectations from the subject and expected a greater degree of improvement. A similar phenomenon was noted in our institution when student attitudes towards Pharmacology was surveyed.[14]

Self-learning Behavior in dissection was regarded as an important strength of the teaching, learning methodology by the students. The 3 common instructional strategies used to teach Anatomy are Lecture, Discovery or inquiry based learning and co-operative learning [15].

Reciprocal peer teaching (RPT) refers to circumstances where students alternate roles as teacher and student. A study in Medical School in United States had shown that RPT increased student understanding of the topics. They taught and also increased their retention of information [16]. It also improved their communication skill. We are exploring the possibility of introducing RPT in Anatomy and other subjects in our institution.

It was heartening to note that students were of the opinion that Anatomy teaching is clinically oriented. In a Medical School in Germany dissection, a survey conducted among students just before graduation ranked Anatomy as a Key-stone for their Medical Course [17]. A medical school in Turkey had introduced an Orientation Lesson on Moral and Psycho-Social issues for Second Year students before Anatomy Dissection,[1] They concluded that consideration of Psychosocial factors would allow Students to interpret their Laboratory Experience in the context of their Professional development as Physicians [1]. In a Medical School in Bahrain the examination system encourages Anatomists to formulate Clinically Oriented Inter-disciplinary questions[9]. At MCOMS there are Hospital visits to expose the basic science students to a clinical environment.

The clinical has a skills laboratory and this should be used more for anatomy teaching. This will expose the students to models and various clinical procedures. The use of more visual aids, models CD-ROMs, interactive programs and the internet for learning was suggested. Many medical schools use virtual imaging to support anatomy learning [5], [6]. The Chinese visible human project [18] and the visible Korean human [19] provide a medium that can be used for training students in anatomical practice. It can also be used for the interpretation of model medical images.
A web-based three-dimensional anatomy training system based on national library of medicine (NLM) visible human male datasets provides a real time self-guided virtual tour of the entire body and detailed information about structures. Substructures and proximal structures.[20]. The system facilitates learning of visuospatial relationships [20].

In the US, course materials for a human anatomy and development course were placed on the world wide web. Most students used the website to prepare for examinations but not for daily studying[21]. We are in the process of starting an e-learning model module for all basic science subjects and this finding may be of importance for us. The department of Anatomy has not introduced Problem-Based learning for the students. In the United States and Canada common clinical cases had guided the selection of content for an abbreviated First Year Anatomy Course [22]. The students are more benefited when multiple Problem Oriented Modalities are integrated including Dissection Computer Exercises, Radiology and Small Group Discussions [22]. This may have implications for Future Introduction of Problem based sessions in Anatomy in our institution.

The visible human Project has not taken off in South Asia. The use of Internet Based training models is also difficult in our institution. However CD-ROMs and other models can be used to teach Anatomy. Peer-Examination Life Models, Body Projection and Body Painting has used in a UK Medical School[8]. can be easily adopted for use in our institution.

CONCLUSION

The overall student opinion towards Anatomy was Positive. Various suggestions for improvement of Anatomy teaching and learning were put forward such as making the teaching clinically oriented, using living anatomy, X-ray anatomy, presentation of patients, problem based learning and skills laboratory. The use of more visual aids, models CD-ROMs, interactive programs and the internet for learning was suggested.

Incorporation of some of these ideas and use of newer technologies, hope to widen the scope of teaching in Anatomy, making it more interesting and clinically relevant, uplifting the knowledge of students. However, the present study was restricted to only one medical school and studies are underway to survey colleges across India including postgraduates and faculty opinions to introduce higher studies and research in Anatomy.

REFERENCES

5. Rosse c. the potential of computerized representations of anatomy in the training of health care providers. Acad Med 1995; 70; 499-505
6. Zirkel JB, Zirkel PA. Technological alternatives to actual dissection in
anatomy instruction; a review of the research. Edu Tech 1997; 52-56

Appendix
Table 1: Student attitudes towards Anatomy

Semester:
Sex: Nationality: Religion:
Graduation: Yes/No If yes then main subject:
Profession of parents: Father:
Mother:
Medium of instruction at School: English/Vernacular
Your attitude towards Biology at School: Liked it/ Neutral/ Hated it
Have you been exposed to animal dissection at School: Yes/No
If yes, then what is your attitude towards animal dissection at School: Liked it/ Neutral/ Hated it
What is your present attitude towards cadaver dissection:
Liked it/ Neutral/ Hated it
For the following statements score using the following key: 1-strongly disagree with the statement, 2-disagree, 3-neutral, 4-agree, 5-strongly agree.
1. My first look at the cadaver was scary.

2. The formalin smell of the cadaver is irritating.

3. My eating habits have got changed because of anatomy dissection.

4. Our seniors say that anatomy is difficult to study/understood.

5. I feel the embryology models in the museum are beneficial for learning embryology.

6. The gross anatomy specimens in the museum are beneficial for learning gross anatomy.

7. The time allotted for anatomy dissection is adequate.

8. The time allotted in the dissection class for studying osteology is sufficient.

9. I feel bedside teaching in the hospital is necessary in learning anatomy.

10. I would like more seminars to be organized in anatomy subject.

11. I refer to CDs and internet for making anatomy interesting.

12. I feel group studying will help in learning anatomy.

13. The anatomy teachers teach the subject well.

14. I will consider anatomy as one of my subjects for post graduation.

15. I feel that cadaver dissection will help to develop my surgical skills.

16. I feel that we should be taken to surgical wards to make anatomy learning more clinically oriented.

17. The skills lab will help to make anatomy learning interesting.

18. The department ensures proper utilization of the skills lab.

19. Anatomy is my favourite subject in the basic sciences.

20. Methods of histology teaching and assessment are good.

21. I find the anatomy lectures interesting and stimulating.
22. I would like anatomy to be more closely integrated with the surgical/clinical sciences.

23. The assessment system in anatomy is fair.

24. The assessment process is transparent.

25. I would like MCQ's to be included in the assessment.

26. The anatomy teachers have inculcated in me a capacity for self-directed learning.

27. I prefer clinical discussions should be used in teaching anatomy.

List what in your opinion are the **TWO** most important strengths and weaknesses of the department with regard to Teaching and Assessment.

**Teaching:**

**Strengths:**
1) 
2) 

**Weaknesses:**
1) 
2) 

**Assessment:**

**Strengths:**
1) 
2) 

**Weaknesses:**
1) 
2) 

Mention **Two** important suggestions to improve the teaching and learning of Anatomy at MCOMS:
ABSTRACT

Owing to ideal climatic and socio-economic condition, the oak tasar silk is one of the major agro based industries of the north eastern states of India. Tasar silk reeling industry in this region has the advantage of availability of abundant nature grown host plant and skilled reellers and weavers. However, due to the hard and compact nature of these wild non-mulberry cocoons, the reellers are facing constant problems during reeling of silk from the cocoons mostly because of lack of easy, economic and environmentally friendly cooking method. A number of methods (both chemical and enzymatic) developed by various worker are used by various reeling units according to their convenience. The present study investigates the comparative reeling performances of most commonly used cocoon cooking method adopted by the silk reeling units in this region. It is observed that enzymatic cocoon cooking has advantages over chemical method. The method involving pineapple extract (with or without 9.8mM sodium carbonate) is a relatively better method regarding its overall better reeling performances and also mostly due to the abundant availability of pineapples grown in the oak tasar belt in India. Moreover the method is less labour intensive, fuel efficient and is readily accessible to the common tasar silk reellers and weavers.

Keywords: Single silk filament reeling, Sericin, Fibroin.

INTRODUCTION

As a prerequisite to reeling, the cocoon has to be softened by decomposing or partially solubilising the sericin component, the proteinaceous silk gum, which binds the protein fibroin strands from which the silk thread is reeled (Krishnaswami et al., 1972). The silk proteins differ considerably in their chemical composition e.g. in mulberry proteins, fibroin has roughly 76 mol% of amino acids having non-polar side chain, the main among these being glycine and alanine, and only about 21 mol% of amino acids having polar groups. In sericin, however, the ratio is the other way round, with about 25 mol% of amino acids having non-polar groups and about 75 mol% of amino acids having polar side chains mainly serine, aspartic acid, glycine and threonine (Komatsu, 1985). This difference in the composition makes sericin more water soluble than fibroin and it serves as the basis of removal of sericin from the cocoons.
Unlike the mulberry (*Bombyx mori* L.) silk cocoons, the oak tasar (*Antheraea proylei* J.) cocoons cannot be satisfactorily softened by boiling in plain water due to its hard and compact nature and also because of presence of relatively low amounts of sericin and high amounts of protein-tannin complexes in the form of proanthocyanidins (Pandey, 1990). These cocoons have to be softened by more drastic boiling off techniques. Generally the cocoons are cooked in presence of strong alkali agent or other harsh chemicals. Since the chemical methods reduce the quality of the tasar silk thread in many ways, an alternative method for the oak tasar cocoon cooking based on enzymes have been developed by number of workers.

Oak tasar silk production is one of the major agro based industries of the North Eastern region of India due to abundant availability of the oak tasar (*Antheraea proylei* J.) silkworm, the larvae of which feed on leaves of oak tree *Quercus* species (Family-Fagaceae). In the present investigation, a study was undertaken to compare the reeling performances using various chemical and enzymatic method commonly adopted by the tasar silk reelers and weavers in the NE states of India.

**RESEARCH METHODOLOGY**

The cocoons produced by the oak tasar silkworm *Antheraea proylei* J. fed on *Quercus serrata* (Thunb.) leaves, hot air stifled for 6-7 hrs at 70ºC, and then stored for 2-3 months were used in the present investigation. The following commonly adopted methods were used for cocoon cooking:

**Method 1:** The cocoons were cooked by commonly adopted chemical procedure using soda developed by Tikoo and Goel (1987). The oak tasar cocoons were boiled for 30 mins in distilled water containing 1% sodium carbonate and were washed with hot water to remove sodium carbonate and semi dried for dry basin reeling.

**Method 2:** Another commonly adopted enzymatic method using commercial protease preparation viz Biopril-50 which was developed by Jolly *et al.* (1979) was used in the present investigation. The cocoons were boiled in plain water for 60 min and then steamed for 30 min at 15 lbs/sq inch pressure in a pressure cooker. After the steaming, the cocoons were left in the cooker until the pressure gradually dropped to normal. Then, the cocoons were loosely wrapped in a porous cloth and soaked in 0.050% Biopril-50 solution for 20 hr initially at 40-50ºC and thereafter at room temperature. The soaked cocoons were semi-dried by spreading them on an ash-bed and then deflossed for dry-basin reeling.

**Method 3:** The cocoons were cooked using pineapple extract method (Devi, 2004) in which 150 g of the pineapple (mature and yellow) fruit pulp was homogenized with 1 litre of distilled water and the resulting homogenate was strained through a coarse cotton cloth. The supernatant having high proteinase activity was used for softening of the oak tasar cocoons. The cocoons were subjected to 30 minutes pressure cooking at 15 lbs/sq inch pressure and were then soaked in the above pineapple extract at room temperature (26-31ºC) for 12 hours and were washed repeatedly with tap water until the associated brown colour and proteinase activity were washed out. The cocoons were then semi-dried on blotting papers, deflossed for dry basin reeling.

**Method 4:** Alternatively the cocoons were cooked as described in method 3 above and were soaked in the pineapple extract containing 9.8mM (0.125%) sodium carbonate for 6 hours at room temperature.
The parameters viz concentration of soda and the time of soaking were determined by studying the enzymological characteristics of the proteinase activity in the pineapple extract (Singh et al., 2003).

**Method 5:** Alternatively the cocoons were also cooked by method developed by Sinha et al. (1989) using papaya extract in combination with chemicals. The oak tasar cocoons were boiled for 30 min in a solution containing 0.1% washing soda and 0.1% soap and then steamed for 1 hr. The cocoons were then soaked overnight in raw papaya extract (200 g of fresh raw and immature papaya fruit homogenized with 1 L of water) in combination with sodium sulphite (5 g/L) and washing soda (1 g/L) at initial temperature of 50°C, pH of the bath being maintained at 8.0. The cooked cocoons were semi-dried for reeling.

For all the methods, single filament reeling was performed on an epprouvette machine and mass yarn reeling was performed on a modified CTR&TI pedal reeling cum twisting machine. The fibres were also subjected to tensile and elongation tests at 64% humidity and 25°C using Instron Tensile Strength Tester 6021.

**RESULTS AND DISCUSSION**

The reeling parameters for determining the reeling performances was evaluated as indicated by FAO - Silk Reeling And Testing Manual (1999). The results of the single filament reeling performances are given in Table 1 and the results of the mass yarn reeling performances are given in Table 2. The following results on the single filament reeling parameters were recorded as Mean of 30 cocoons ± S.D and of mass yarn filament reeling performance as average of 5 replications of 50 cocoons each.

Data reveals that cocoon cooking with 1% Na₂CO₃ for 30 mins gives good reeling performances, however, it was found to reduce the strength of the fibre (Tenacity of 1.67 and 1.55 in case of single filament and mass yarn reeling). The silk filament was found to have reduced lustre and smoothness causing naps, consequently presenting an obstacle to the oak tasar silk reeling and weaving efficiencies. Moreover, soda may cause irritation to the workers of silk industry and may also cause effluent environmental problems.

The use of commercial enzymatic preparation Biopril-50 also gave good reeling performances however, the proteolytic action weakens the tasar silk yarn (Tenacity of 1.81 and 1.97 in case of single filament and mass yarn reeling). Also, it was found that the silk sericin is extensively hydrolysed, reducing the cohesion of silk filaments ultimately resulting in brills of the filament. Due to the prolonged enzymatic treatment, this method was however time consuming.

The reeling performance of the cocoons cooked with pineapple extract was relatively better with that of soda and biopril-50 method, moreover, it was observed that there is an increase in the tenacity (2.15 and 3.02 in case of single filament and mass yarn reeling). The method was found to be more fuel efficient, less labour intensive and involves less time as compared to biopril-50 method. Also similar reeling performances were also observed with addition of sodium carbonate (0.125%) to the pineapple extract, however, there is less time involvement (6 hours as compared to 12 hours). The pineapple fruit contains a cysteine protease known as fruit bromelain, an acidic protein having a broad range of specificity towards polypeptides (Rowan and Buttle, 1994). Pineapple extract is found to be an effective oak tasar cocoon cooking agent (Devi, 2011). Sodium
carbonate is known to have an enhancing effect on the proteinase activity of pineapple fruit (Singh et. al., 2003).

Reeling performances of papaya extract with soda, soap and sodium sulphite is almost the same as that of soda method. Papaya contains a serine protease (a sulphhydryl enzyme) known as papain which exhibits a wide range of specificity in its action towards polypeptides. Peptide bonds formed by the carboxyl groups of arginine and lysine residues are highly susceptible to attack by papain. In addition, cleavage occurs readily at the carboxyl group of histidine, and also at glycine, glutamic acid, glutamine, leucine and tyrosine (Gulrajani, 1992). Maybe because of the combined action of the proteolytic activity of the enzyme along with the action of chemicals the silk yarn might have weakened giving decreased tenacity (Tenacity of 2.01 and 1.75 in case of single filament and mass yarn reeling).

CONCLUSION

Even though, each method described above has its own merits and demerits, from the present comparative study it can be concluded that the method involving pineapple extract (with or without soda) is a relatively better method regarding its overall better reeling performances and also mostly due to the abundant availability of pineapples grown in the oak tasar belt in India. Moreover it is less labour intensive and is a fuel efficient method.

Table 1: Comparison between the different commonly adopted method for cooking oak tasar (Antheraea proylei J.) cocoons with respect to single filament reeling performance

* Each value is an average of 30 replications

<table>
<thead>
<tr>
<th>Method of cocoon treatment</th>
<th>Method 1 (Soda)</th>
<th>Method 2 (Biopril-50)</th>
<th>Method 3 (Pineapple extract)</th>
<th>Method 4 (Pineapple extract with soda)</th>
<th>Method 5 (Papaya extract with soda, soap and sodium sulphite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ends feeding/cocoon</td>
<td>3.4± 2.0</td>
<td>4.1 ± 1.6</td>
<td>2.5± 1.4</td>
<td>2.7 ± 0.9</td>
<td>3.1±2.8</td>
</tr>
<tr>
<td>Filament length (m)</td>
<td>666.2±179.1</td>
<td>764.4±121.0</td>
<td>678.6± 140.5</td>
<td>685.0± 202.9</td>
<td>709.4±187.2</td>
</tr>
<tr>
<td>Recovery %</td>
<td>64.7±5.6</td>
<td>77.6 ± 4.2</td>
<td>69.7 ± 9.2</td>
<td>67.3 ± 8.8</td>
<td>62.9±9.1</td>
</tr>
<tr>
<td>Denier (D)</td>
<td>5.6±0.5</td>
<td>6.5 ± 0.8</td>
<td>6.7 ± 1.0</td>
<td>7.1±2.4</td>
<td>6.9±1.6</td>
</tr>
<tr>
<td>Reelability %</td>
<td>29.41</td>
<td>24.39</td>
<td>40.0</td>
<td>37.0</td>
<td>32.25</td>
</tr>
<tr>
<td>NBFL (m)</td>
<td>195.9</td>
<td>149.8</td>
<td>271.4</td>
<td>253.4</td>
<td>228.7</td>
</tr>
<tr>
<td>Tenacity (g/D)</td>
<td>1.67±0.04</td>
<td>1.81± 0.04</td>
<td>2.15 ± 0.07</td>
<td>2.02± 0.03</td>
<td>2.01±0.09</td>
</tr>
<tr>
<td>Elongation %</td>
<td>34.40±7.96</td>
<td>38.21 ± 8.67</td>
<td>36.94 ± 8.13</td>
<td>37.47±11.24</td>
<td>35.16±9.42</td>
</tr>
</tbody>
</table>
Antheraea tract

110

5.

4.

3.

2.

and discussed. The literature for this article has been reviewed articles, journals and books from where the authors/editors/publishers of manuscript. The authors are also grateful to all those received from the scholars whose articles are cited and included in references of this Authors acknowledge the immense help authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Table 2: Comparison between the different commonly adopted method for cooking oak tasar (Antheraea proylei J.) cocoons with respect to mass yarn filament reeling performance

<table>
<thead>
<tr>
<th>Method of cocoon treatment</th>
<th>Method 1 (Soda)</th>
<th>Method 2 (Biopril- 50)</th>
<th>Method 3 (Pineapple extract)</th>
<th>Method 4 (Pineapple extract with soda)</th>
<th>Method 5 (Papaya extract with soda, soap and sodium sulphite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking efficiency %</td>
<td>90</td>
<td>99</td>
<td>95</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>Raw silk %</td>
<td>60.1</td>
<td>64.6</td>
<td>64.4</td>
<td>64.0</td>
<td>64.8</td>
</tr>
<tr>
<td>Yield/1000 cocoons (g)</td>
<td>347</td>
<td>331</td>
<td>380</td>
<td>320</td>
<td>412</td>
</tr>
<tr>
<td>Production/8 hr/reeler (g)</td>
<td>200</td>
<td>201</td>
<td>175</td>
<td>183</td>
<td>276</td>
</tr>
<tr>
<td>Tenacity (g/D)</td>
<td>1.55</td>
<td>1.97</td>
<td>3.02</td>
<td>3.36</td>
<td>1.75</td>
</tr>
<tr>
<td>Elongation %</td>
<td>25.01</td>
<td>29.35</td>
<td>31.06</td>
<td>28.07</td>
<td>29.05</td>
</tr>
</tbody>
</table>

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REFERENCES


INCIDENCE OF URINARY TRACT INFECTION ALONG WITH ITS KNOWN ASSOCIATED RISK FACTORS AMONG SELECT NOMAD TRIBAL POPULATIONS OF RAJASTHAN, INDIA

Bandana Sachdev

Birla Institute of Technology and Science (BITS), Pilani, Rajasthan

E-mail of Corresponding Author: sachdev.neha@rediffmail.com

ABSTRACT

Background: Urinary tract infection (UTI) is the second most common infectious complaint in clinics overall, and the most common outpatient complaint caused by bacteria. Urinary Tract Infections are a serious health problem affecting millions of people every year. Objective: Screening of Select nomad tribal populations for urinary tract infection and also to determine the association with others risk factors like sex, age and genetic factor. Methods: 1034 persons (472 males and 562 females) aged ≥18 years were examined from a cluster of three districts i.e. Jhunjhunu, Sikar and Churu. The urine examination emphasized the collection of mid-stream clean catch urine for chemical analysis by urine Xpress dipstick method. Results: Prevalence of urinary tract infection identified by chemical analysis was 35.8 % (Pyuria) and 5.8% (Nitrite pos+) in overall population. The prevalence rate of urinary tract infection screened showed high infection among the nomad tribal women i.e. 43.4% as compared to males (26.7%). It further shows that the infection increases with increase in the age and the unmodified factor i.e. ABO Blood group showed positive association with the infection in both the sexes. Chi-square analysis showed that sex, age and genetic factor were associated significantly with the prevalence (p<0.01). Conclusion: Prevalence rate of urinary tract infection is very high as found in rural and urban population.

Key words: Screening, Incidence, UTI, ABO Blood Grouping, Nomads

INTRODUCTION

The ghumantoos, or nomads, of India are mobile tribes, each characterized by a distinct culture that is reflected in their beliefs, customs and traditional practices. Each jati practices a hereditary occupation and often speaks a specialized dialect. The exact number of the nomadic population is unknown because a formal census has never been conducted of these nomadic communities in India. However, informal studies indicated that about five hundred endogamous groups make their home in India, two dozen of which can be found in Rajasthan. At present, nomadic communities are found in the states of Andhra Pradesh, Maharashtra, Gujarat, Karnataka, Tamil Nadu, Haryana and Punjab. Pastoral nomadic communities also live in the Himalayan region in India (Malhotra, 1982). Unsafe potable water, insufficient food, lack of health care and
educational facilities left them vulnerable to diseases and ignorance.

Urinary tract infection (UTI) is the second most common infectious complaint in clinics overall, and the most common outpatient complaint caused by bacteria.\(^1\)

Urinary Tract Infection is the most common bacterial infection prevalent in both male and female patients, causing discomfort in elderly patients, thus representing bacterimia, septic shock, respiratory disease syndrome and death.\(^2,3\)

Hardly any screening process for diagnosing urinary tract infection among these populations has done so far. So keeping in mind the above reasons this study has been undertaken to study the prevalence rate of urinary tract infection among these populations.

METHODS AND MATERIALS

Cross-sectional, Tribal populations-based study, consisting of a total sample of one thousand and thirty-four (1034) persons (472 males and 562 females) aged \(\geq\) 18 years were examined from a cluster of three districts i.e. Jhunjhunu, Sikar and Churu. Men and women \(\geq\) 18 years of age were considered eligible except pregnant women, seriously ill subjects and those who were on some medication or Antibacterial drugs etc. The initial contact with tribals’ living conditions and life styles was through elders of tribal community and other people living in and around tribal habitations. As the exact percentage and location of nomadic populations of Shekhawati region was not known snow and ball method of sampling was used for data collection. It was not easy though to locate their places of living because they had been living away from easily accessible locations or open public view. Statistical calculations were done using the chi-square test to find out if there was a significant association between the different groups and tribal populations.

Urinalysis Procedure

The urinalysis test involves the collection of urine sample in a specimen cup. The proper collection of a sample is very important in order to avoid contamination of urine. The collection technique is different for men and women. Proper instructions were given to both the sexes for collecting their urine sample. For men, it was told that the opening of the urethra (tip of the penis) should be wiped clean with a cleansing wipe before collection is begun. In women, it was instructed that the area around the urethra should be wiped clean with a cleansing wipe. Then she was asked to spreads the labia of the external genitalia and wipes from front to back. After the urethra was properly cleaned, the collection begins by discarding the initial stream of urine into the toilet. Then, 10-15 milliliters (ml) of urine was collected in the sterile specimen cup by directly urinating into the cup. Once an adequate amount was collected, then the remaining urine was voided in the toilet. This technique is called the mid-stream clean catch urine sample collection.

Urine Dipstick Chemical Analysis

The urine dipstick is a rapid test used to analyze urine. A dipstick (Xpress) is a thin plastic strip which has several small squares of different colors attached to it. There are about 10 squares on each strip with each square measuring about a quarter of an inch. The dipstick is simply inserted into the specimen cup containing the urine briefly (for 60 seconds) and then it is inserted into accurex machine for interpretation. The squares on the dipstick contain specific chemicals that react differently when they come in contact with urine. Each color
measures one important quality of the urine.

Presence of white blood Cells in the Urine: The presence of these cells in the urine is suspicious for a urinary tract infection (UTI). Other supportive evidence of a UTI may include bacteria in the urine, leukocyte esterase and nitrite on the dipstick. The study was approved by the institutional human ethics committee at BITS, Pilani, Rajasthan, India and performed according to the Declaration of Helsinki. All study members were given detailed explanation of the study in their regional language before obtaining their written consent.

**RESULTS**

The frequency distribution of urinary tract infection among select nomad tribal populations were as shown below in Table 1. Three hundred and seventy cases identified through urinalysis as positive for urinary tract infection. Similarly 5.8% of the individual diagnosed as positive for the infection on the basis of nitrite being positive.

<table>
<thead>
<tr>
<th>Urinary tract infection</th>
<th>Pyuria N (%)</th>
<th>Nitrite N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>370 (35.8%)</td>
<td>60 (5.8%)</td>
</tr>
<tr>
<td>Negative</td>
<td>664 (64.2%)</td>
<td>974 (94.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>1034</td>
<td>1034</td>
</tr>
</tbody>
</table>

Further it was analyzed to see the amount of pus present in the urine on the basis of the presence of quantity of leucocytes in urine as shown below in Fig 1.
Fig 1: Frequency distribution of quantity of pyruia in urine among the tribal populations of Rajasthan

Presence of leucocytes in urine

It was seen that the females (43.4%) were found to be more prone to urinary tract infection as seen from the Table 2 as compared to males (26.7%). Chi-square test was calculated to see the association between sexes and urinary tract infection and it was found to be significant (chi-square=31.216, p=.001).

Table: 2 Sex-wise distribution of urinary tract infection among select nomad tribal populations

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>126</td>
<td>244</td>
<td>370</td>
</tr>
<tr>
<td>26.7%</td>
<td>43.4%</td>
<td>35.8%</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>346</td>
<td>318</td>
<td>664</td>
</tr>
<tr>
<td>73.3%</td>
<td>56.6%</td>
<td>64.2%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>472</td>
<td>562</td>
<td>1034</td>
</tr>
<tr>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square=31.216, p=.001
Very small numbers of cases were found to be positive for urinary tract infection based on the nitrite present in the urine. It is also well known fact that the presence of nitrite in urine might be due to the presence of bacteria that results in UTI infection. Six percent of the females found to be positive for the presence of nitrite in the urine and whereas 5.5% of the males found to be positive.

It was observed that at any age one gets the urinary tract infection as shown below in the Table.3. Further it was analyzed to find out the association between age and the urinary tract infection. It was found to be statistically significant. (Chi-Square=25.275, p=0.001). The positive association reveals that the infection increases with increase in the age of respondents.
Table 3. Age-wise distribution of urinary tract infection among the Nomad tribal populations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>73</td>
<td>56</td>
<td>65</td>
<td>38</td>
<td>79</td>
<td>40</td>
<td>17</td>
<td>0</td>
<td>2</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>19.7%</td>
<td>15.1%</td>
<td>17.6%</td>
<td>10.3%</td>
<td>21.4%</td>
<td>10.8%</td>
<td>4.6%</td>
<td>.0%</td>
<td>.5%</td>
<td>100.0%</td>
</tr>
<tr>
<td>negative</td>
<td>178</td>
<td>138</td>
<td>86</td>
<td>77</td>
<td>110</td>
<td>57</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>664</td>
</tr>
<tr>
<td></td>
<td>26.8%</td>
<td>20.8%</td>
<td>13.0%</td>
<td>11.6%</td>
<td>16.6%</td>
<td>8.6%</td>
<td>2.4%</td>
<td>.3%</td>
<td>.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
<td>194</td>
<td>151</td>
<td>115</td>
<td>189</td>
<td>97</td>
<td>33</td>
<td>2</td>
<td>2</td>
<td>1034</td>
</tr>
<tr>
<td></td>
<td>24.3%</td>
<td>18.8%</td>
<td>14.6%</td>
<td>11.1%</td>
<td>18.3%</td>
<td>9.4%</td>
<td>3.2%</td>
<td>.2%</td>
<td>.2%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Chi-Square=25.275, p=0.001

ABO Blood grouping was also done to see the prevalence of urinary tract infection. And it was found that the percentage distribution of B blood group (41.6) was higher in infected group as compared to non infected group individuals (38.9) as shown in below Table 4.

Table 4 Distribution of ABO Blood group in select Nomad tribal populations (diagnosed positive for pyuria)

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>A</th>
<th>AB</th>
<th>B</th>
<th>O</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells positive</td>
<td>95</td>
<td>31</td>
<td>154</td>
<td>90</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>94.1</td>
<td>32.2</td>
<td>147.4</td>
<td>96.3</td>
<td>370.0</td>
</tr>
<tr>
<td></td>
<td>25.7%</td>
<td>8.4%</td>
<td>41.6%</td>
<td>24.3%</td>
<td>100.0%</td>
</tr>
<tr>
<td>negative</td>
<td>168</td>
<td>59</td>
<td>258</td>
<td>179</td>
<td>664</td>
</tr>
<tr>
<td></td>
<td>168.9</td>
<td>57.8</td>
<td>264.6</td>
<td>172.7</td>
<td>664.0</td>
</tr>
<tr>
<td></td>
<td>25.3%</td>
<td>8.9%</td>
<td>38.9%</td>
<td>27.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>263</td>
<td>90</td>
<td>412</td>
<td>269</td>
<td>1034</td>
</tr>
<tr>
<td></td>
<td>263.0</td>
<td>90.0</td>
<td>412.0</td>
<td>269.0</td>
<td>1034.0</td>
</tr>
<tr>
<td></td>
<td>25.4%</td>
<td>8.7%</td>
<td>39.8%</td>
<td>26.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Chi-Square=1.173, p=0.759
When it was seen only in the infected group even then the prevalence rate of B blood group was higher as compared to the other ABO Blood groups among the select nomad tribal populations as shown below in the Fig.3
DISCUSSION

Urinary Tract Infection, commonly known as UTI, affects as many as 50% women at least once during their lifetime. All individuals are susceptible to Urinary Tract Infection (UTI); however the prevalence of infection differs with age, sex and certain predisposing factors. The findings of the present study confirmed the presence of urinary tract infection among these populations. The prevalence rate was also very high. Owing to various reasons such as remote location and many other socio-economic factors nomads could not access health-related or educational facilities of the state government. Women belonging to nomad groups had faced the major brunt of such problem. The problems varied to the extent of lack of nutritional food, potentially unsafe conditions of pregnancy and delivery, after-delivery of the child and lack of proper sanitation condition. These might be the reasons for the prevalence rate of urinary tract infection were high among the select nomad tribal population of females as compared to the males. Similar findings have been seen in many other studies that females are more prone to urinary tract infections. Women are more susceptible to UTI because a woman’s urethra is short, allowing quick access of bacteria to the bladder. Also a woman’s urethral opening is near sources of bacteria from the anus and vagina. This study further confirmed that the incidence increases with age and reaches maximum at postmenopausal age group as documented in other studies.

Findings from the present study also lend support to the hypothesis that genetic factors related to the distribution of some blood groups may play a role in the development of elevated urinary tract infection. It was seen the increased
prevalence of urinary tract infection in B Blood group individuals. Those carrying the B blood group were more susceptible to infection as compared to blood group A and O. Whereas AB blood group had less chance of getting infection. This could suggest that B blood group individuals might genetically more prone to infection as compared to other ABO Blood groups. Although this is preliminary study, a clear trend is seen which needs further investigations on large scale epidemiological studies. Although this findings showed agreement with some of the studies. 9, 10

ACKNOWLEDGEMENTS
Author acknowledges the immense help received from the scholars whose articles are cited and included in references of this manuscript. The author is also grateful to authors/ editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed

REFERENCES
ABSTRACT
Leaf spring is one of the key components of vehicle suspension system. A lot of research has been done for improving the performance of leaf spring. In this paper the authors reviewed nine papers on use of alternate materials, effect of material are process properties on leaf spring performance and fatigue life prediction of leaf spring. Five papers were about performance of epoxy fiber glass materials used in leaf spring and four about various methods of fatigue life prediction and correlation between their outputs. In general, it was found that fiber glass materials have better strength characteristic and lighter in weight as comparison is steel for leaf spring. Different papers discuss different aspect and procedures for fatigue life prediction.

Keyword: leaf spring, fiberglass material, material, fatigue life.

INTRODUCTION
The large vehicle needs a good suspension system that can deliver a comfortable drive. The leaf spring should be light weight and have excellent fatigue life it must with stand numerous number of cyclic before it can fail past researched on leaf spring mainly focused on improving fatigue resistance weight reduction to as measured of to reduce un-sprung weight of leaf spring.

1 Prediction and improvement of fatigue life – A few papers where discussed about developing and validating procedures for predicting the fatigue life.
R. W. Landgraf and R. C. Francis (1) have presented Procedure for assessing the influence various material factors (using deferent properties such as microstructure monotonic and cyclic properties of SAE 5160 steel) And processing factor (heat treatment, presetting shot, peening strain peening) on the fatigue Performance of leaf spring. Level of cyclic stability of residual stresses resulting from mechanical processing as well as permanent deformation associate with presetting operations is determine on the basic of cyclic deformation consideration. a damage parameter incorporating material properties, residual stress effects and applied stressing condition, provides a technique for predicting failure location and lifetime as of spring and failure location Predicted results are conform by experimental bending results.
M.L Aggarwal V.P.Agrawal, R.A.Khan (2) has calculated fatigue strength of shot peening leaf spring from laboratory
samples of EN45A spring steel specimen. A lot of research has been done improved fatigue strength of material by creating compressive residual stress field in their surface layers through shot peening. Optimum shot peening condition for specimen is found and S-N curve of the specimen are correlated with leaf spring curve a mathematical model for full scale correlated factor (FSCF) based on relation between FSCF and stress. Linear relation is found between FSCF and stress.

\[ \text{Stress} = 370 \times \frac{\text{fatigue life of actual spring/fatigue life of specimen} + 566}{\text{fatigue life of specimen} + 566} \]

The prediction found agreed with experimental result the author found different in fatigue life between specimen and full scale leaf spring testing is mainly due to fretting fatigue between mating leaves.

For prediction of fatigue life, M Senthil Kumar and S. Vijayarangan (2007) (3) adopted the analysis model of Hwang and Han.

\[ N = \{B(1 - r)\}^{1/2} \]

On the line of above discussed research paper this paper also compared the load carry capacity stiffness and weight saving of composite leaf spring with that of steel leaf spring. They also found use weight reduction with composite leaf spring however for the same deflection more load was found required to deflection composite leaf spring the reason for increased stiffness was lower density of composite materials during full load bump test the spring constant for composite leaf spring fast found greater then steel leaf spring. However it was found that maximum longitudinal compressive stress for composite leaf spring was approximate 1/3 of the same for steel leaf spring similarly compressive strength of composite material was also found less then yielding strength of steel. It show that composite leaf spring is steeper that composite leaf spring is steeper then steel less spring they also conform that natural frequency of composite leaf spring is much more then maximum road frequency.

The same data obtain experimental by testing on electro hydraulic test ring for composite leaf spring. The fatigue life of composite leaf spring is predicted as higher then steel leaf spring. In their research, M Senthil Kumar, Sabhapanth Vijayarangan (2007) (4) have conducted static and fatigue analysis of steel leaf spring and composite multi-leaf spring. They found that for the same spring length the experimental stress was very low is composite leaf spring as compare to steel leaf spring they also found that for the same spring length magnitude longitudinal stress where much lower than composite spring in comparison to steel leaf spring In their research it is again confirm that the natural frequency of composite leaf spring was much higher (14.3 Hz) for composite springs than for steel spring (6.3 Hz) in comparison to that for road bumps due to road irregularities, 12 Hz). The result of the accelerated test for fatigue life was that life of composite leaf spring is was much higher than that of the steel leaf spring.

F.N.Ahmad Refngah, S. Abdullah, et al. (5) wrote about fatigue life prediction based on finite element analysis and variable amplitude loading. FEM gives the prediction of creation area from the view point of static loading meanwhile, using the variable amplitude loading the fatigue damage and life of the parabolic spring has been predicted, represent the actual scenario when the vehicle run on the road they found that experiment results were found to higher then FEA but most of the FEA stress value was higher than experimental result is may due to residual...
stress by shot peening, inter leaf friction and nip stress.

S. Abdullah, C.K.E. Niz Wan et al. (6) discussed the development of the STFT based fatigue technique was performed by removing low amplitude cycles contained in the original signal in order to produce a shortened signal using the short-Time Fourier Transform (STFT) parameter. SAE Random fatigue data are the strain time histories named as SAESUS (these data were obtained from the data based SAE) this method is very improved in the fatigue design criteria, specially for the task of accelerated fatigue testing. This method is suggested as an alternative technique in fatigue durability study.

2 Considerations of Composite Materials has an Alternative for Spring Steel

Gulur Siddaramanna Shiva Shankar, et al. (7) The Automobile industry has shown increased interest in the replacement of steel spring with fiberglass composite leaf spring due to high strength to weight ratio. Fiber glass leaf spring performance is compared with steel spring experimentally as well as by FEA. The author found their adhesively bonded joint and hands the performance of composite leaf spring in composite with bolded joint as stress constraints at holes are absorb in bolted joints over view of results for load deflection and stresses indicated that composite leaf spring which is 8.7 time liter in weight can carry more static load with more deflection and less maximum stresses. Even in between the three type of composite leaf spring performance of E-glass/Epoxy was found based then other in harmonic analysis, the natural frequency of composite leaf spring is higher than that of the steel leaf spring and is far enough from the road frequency to avoid the reducing.

<table>
<thead>
<tr>
<th>Modes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequencies(HZ)</td>
<td>33</td>
<td>135</td>
<td>192</td>
<td>288.5</td>
<td>368.7</td>
</tr>
</tbody>
</table>

Table 1.-describes the comparison of deflections and bending stresses

<table>
<thead>
<tr>
<th>Material</th>
<th>Static load (N)</th>
<th>Maximum deflection (mm)</th>
<th>Maximum stress(Mpa)</th>
<th>Weight(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEA</td>
<td>Experimental</td>
<td>FEA</td>
<td>Experimental</td>
</tr>
<tr>
<td>Steel</td>
<td>3980</td>
<td>90</td>
<td>107.5</td>
<td>511</td>
</tr>
<tr>
<td>E-Glass/Epoxy</td>
<td>4250</td>
<td>94</td>
<td>105.0</td>
<td>466</td>
</tr>
<tr>
<td>Graphite/Epoxy</td>
<td>-</td>
<td>68</td>
<td>-</td>
<td>422</td>
</tr>
<tr>
<td>Carbon/Epoxy</td>
<td>-</td>
<td>62</td>
<td>-</td>
<td>413</td>
</tr>
</tbody>
</table>

Table 2.-Composite results of load, deflection and stresses
M. M. Patunkar1, D. R. Dolas2 In this paper a comparison analysis of steel leaf spring is done with a virtual model of a composite leaf spring under static load condition only under the same static load condition deflection and stresses of steel leaf spring and composite leaf spring are found with the great deferent deflection of composite leaf spring is less as compare to steel leaf spring with the same loading condition conventional steel leaf spring was found to weight 23kg where is composite leaf spring weight 3.6kg indicating reducing in weight by 84.4% for same level of performance conventional leaf spring show failure ions end only. Is maximum load condition composite leaf spring shows the minimum deflection as compare to steel leaf spring .composite leaf spring can be used on smooth road with very high performance expectation on rough road condition due to lower chipping resistance failure form chipping of composite leaf spring is highly probable.

K. K. Jadhao, Dr. R.S Dalu In this paper comparison is made between steel leaf spring and glass fiber composite leaf spring. Stress and deflection result were verified for analytical and experiment result they were found all most similar area comparable result show that the composite spring has stress much lower than steel leaf spring and weight of composite spring was nearly reduced up to 85% they found the major disadvantage of composite leaf spring as same time breaking of fibers due to heavy by stone from road also confirm that natural frequency of composite leaf spring is much more then maximum road frequency.

CONCLUSION

In this paper various methods which are developed for prediction of fatigue life in conventional steel leaf spring are discussed. To trend were found

First trend was for prediction of fatigue life under influence of various methods processing factor and second trend was prediction of fatigue life analytically through FEA, coffin manson and Hwang and Han relation and verification through various experimental producer. In this paper performance of composite material is also studies for leaf spring result of various researched were similar and confirmed that composite material spring is much lighter in weight than steel leaf spring and equally strong as steel leaf spring.

Composite leaf spring is costly compare to conventional leaf spring. The chipping resistance is composite leaf spring is low compare to conventional leaf spring

REFERENCES


8. M. M. Patunkar, D. R. Dolas “Modelling and Analysis of Composite Leaf Spring under the Static Load Condition by using FEA” International Journal of Mechanical & Industrial Engineering, Volume 1 Issue 1-2011

ABSTRACT

**Objective:** The present study was designed to find out prevalence of methicillin resistant *Staphylococcus aureus* in community acquired pyoderma cases and their antibiotic susceptibility patterns. **Method:** Two pus swabs from each 130 clinically diagnosed cases of pyoderma were collected for gram staining and culture. All swabs were processed and growth was identified by using standard microbiological techniques. Antibiotic susceptibility testing of isolates was done as per CLSI guidelines. Methicillin resistance was detected in *Staphylococcus aureus* by using 30µg cefoxitin disc. **Results:** Out of 130 cases, 60.77% were of primary pyoderma and 39.23% of secondary pyoderma. Folliculitis (22.31%) was the commonest cause of primary pyoderma. Single bacterial isolate obtained in 73.85% swabs, polymicrobial growth in 10% swabs and no growth observed in 16.15% swabs. Total numbers of bacterial isolates obtained were 123. *S. aureus* was the most common bacterial isolate detected in 84 (68.29%) pyoderma cases. Out of 84 isolated strains of *S. aureus*, methicillin resistance was detected in 34 (40.48%). Most of the strains of *staphylococci* were resistant to penicillin and co-trimoxazole while resistance to gentamicin, ciprofloxacin and erythromycin was less. No strain of *S. aureus* was found to be resistant to vancomycin. **Conclusion:** MRSA is prevalent and increasing in community-acquired infections. The emergence of antibiotic resistant strains and increase incidence of MRSA poses a significant problem in deciding empirical treatment. Antibiotic susceptibility testing of bacterial isolates is essential to ensure effective treatment and to avoid further antimicrobial resistance.

**Key words:** pyoderma, *S. aureus*, MRSA.

INTRODUCTION

Pyoderma is quite common in India and constitutes a major portion of patients in dermatology department. Cutaneous bacterial infection is divided into primary and secondary type. Primary infections have a characteristic morphology and course, caused by a single organism, and arise in normal skin. Primary infections are most frequently incited by *Staphylococcus aureus* or *β*-haemolytic streptococci. They are also the most common invaders in secondary infection \(^{1,2,3}\). Antibiotic sensitivity pattern differs from region to region, and even within the same region, with progress of time. Methicillin resistant *Staphylococcus aureus* (MRSA) has established itself as a significant cause of...
hospital and community-acquired infections worldwide \[4\]. MRSA are being increasingly reported from India as a coloniser in healthy individuals without risk factors and even in community acquired infections including pyoderma \[4,5,6,7,8\]. Resistance to methicillin compromises clinical treatment options, as it results in cross resistances to all other β-lactam antibiotics, which are the most commonly prescribed antibacterial agents \[4\]. Increasing resistance to antibiotics seen in microorganism poses a big problem to the clinicians. It is therefore essential to determine the susceptibility pattern of clinical isolates of \textit{S. aureus} in different communities across our diverse country. The present study was undertaken to determine the prevalence of methicillin resistant \textit{Staphylococcus aureus} in community acquired pyoderma and their antibiotics susceptibility pattern.

**MATERIAL AND METHODS**

This was a cross sectional study carried out in the Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, a tertiary care hospital, Sawangi (M), Wardha from July 2010 to June 2011. The study was started after approval from ethical committee of Jawaharlal Nehru Medical College Sawangi (M), Wardha. One hundred and thirty cases of pyoderma were included in the study. Specimens were collected aseptically from skin lesions with the sterile cotton swabs. Such two swabs were collected from each patient for gram staining and culture. All swabs were processed as per standard microbiological protocol for isolation of bacteria. Swabs were inoculated on 5% sheep blood agar and Mc conkey agar and incubated aerobically at 37°C overnight. Organisms grown were identified by standard microbiological techniques. \textit{S. aureus} were identified on the basis of their morphology, cultural characters and positive catalase and coagulase tests \[9\]. Antibiotic susceptibility of isolated organism was performed by modified Kirby Bauer disc diffusion method on Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guideline \[10\]. The antimicrobials tested included penicillin G (10 units), erythromycin (15µg), cotrimoxazole (1.25/23.75µg), ciprofloxacin (5µg), gentamicin (10µg) and vancomycin (30µg). \textit{S. aureus} ATCC 25923 was used as control. Methicillin resistance was detected by using 30µg cefoxitin disc.

**RESULTS**

Amongst 130 patients of pyoderma studied, 88 were males and 42 were females and male to female ratio was 2.1:1. Their ages ranges from 4 to 86 years. The maximum patients 56 (43.08%) were in the age group of 20 years to 40 years. Amongst 130 cases of pyoderma, primary pyoderma constituted (79) 60.77% of cases and (51) 39.23% were of secondary pyoderma with different clinical presentation (Table I). Folliculitis 30 (23.08%) was the commonest clinical diagnosis in primary pyoderma followed by furunculosis 16 (12.30%). Amongst 130 swabs from dermal lesions subjected to culture aerobically, single bacterial isolate (monomicrobial) was obtained in 96 (73.85%) swabs, polymicrobial growth obtained in 13 (10%) swabs and no growth obtained in 21 (16.15%) swabs. Total numbers of bacterial isolates obtained from monomicrobial and polymicrobial growth were 123. The bacteria isolated were shown in fig I. The most common bacterial isolate was \textit{S. aureus} 84 (68.29%) Oxacillin resistance was detected in 34 out of 84 (40.48%).
strains of S. aureus and 5 out of 9 (55.55%) strains of CONS isolated from pyoderma lesions. Antibiotic susceptibility pattern of isolated S. aureus is given in table II.

DISCUSSION

One hundred and thirty cases of pyoderma were investigated for bacterial aetiology. Primary pyoderma (60.77%) forms major group of cases and 39.23% of cases were of secondary pyoderma. Higher incidence of primary pyoderma was reported by others [1,2,3,5]. Folliculitis (22.31%) was the commonest cause of primary pyoderma followed by furunculosis, ecthyma impetigo and carbuncle. Parikh et al [1] and Patil et al [11] also reported folliculitis as the commonest clinical condition in their studies. Kakar et al [3] reported that impetigo (48.61%) was the commonest lesion in the study group which comprised of children. The majority of patients in the present study were in the adult age group (20 years – 40 years), which could account for high frequency of folliculitis and furunculosis.

In the bacteriological analysis of pyoderma, it is observed that S. aureus (84 isolates, 68.29%) was the commonest isolate (fig I). Ghadge et al [2] and Kakar et al [3] reported S. aureus most common isolate from pyoderma lesions with prevalence of 67.34% and 48% respectively. While Parikh et al [1] detected S. aureus in all the pyoderma cases. Prevalence rate of S. aureus in the present study is comparable with the observations of other workers [4,5,6,11,12].

The methicillin resistance among isolated S. aureus was found to be 40.48% (34 out of 84 strains). The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 [13]. Prevalence of MRSA significantly differs among various clinical specimens [14]. Prevalence of MRSA also varies worldwide among pyoderma cases. Tan et al [12] reported 7% MRSA from Japan; 41% MRSA was reported by Carbera et al [4] from Philippine and 61% MRSA was reported in skin and soft tissue infections by Forcade et al [8] from Texas.

Patil et al [11] reported very low (1.4%) prevalence of MRSA from Mumbai, in the study group consisting of primary pyoderma only. In other study from New Delhi reported 9.6% MRSA were reported where secondary pyoderma cases were 12% [5]. Comparative higher prevalence of MRSA (40.48%) in the present study may be attributed to large number of secondary pyoderma cases (39.23%).

In present study all strains of S. aureus (100%) were sensitive to vancomycin consistent with the other studies [4,5,7,8,11]. Vancomycin resistance (20.27%) was reported in S. aureus isolated from different clinical specimen [14]. Most of the strains were resistant to penicillin G (95.24%) and cotrimoxazole (84.52%). Cotramoxazole was reported as a drug of choice [2,8,12]. Increasing resistance is noted to ciprofloxacin (40.48%) and erythromycin (51.19%). Increasing resistance to erythromycin and ciprofloxacin was reported by others [6,11]. Most of the strains were sensitive to gentamicin (resistance 17.86%). Most of the strains were resistant to one or more antibiotics. This increasing resistance to antibiotics remains a cause of concern to clinician as it poses significant problem both in community as well as hospital practice in deciding empiric therapy. Studies like the present one help in establishing etiological agents and deciding empirical treatment time to time. However, we have not been able to check the sensitivity of these isolates to some other popular topical antimicrobial agents like
sodium fusidate, mupirocin and nadifloxacin as our study was focused on the prevalence of MRSA. In spite of this drawback our findings indicate importance of testing antibiotic susceptibility of bacterial isolates considering variable resistance pattern observed time to time.

CONCLUSION
The increasing prevalence of MRSA in the community acquired infections is alarming. MRSA poses a significant problem in deciding empirical treatment because of resistance to various antibiotics. Antibiotic susceptibility pattern of bacterial isolates varies with time and region. So, we recommend that frequent monitoring of susceptibility patterns of MRSA and the formulation of a definite antibiotic policy may be helpful in decreasing the incidence of MRSA infection and its spread in the community.

ACKNOWLEDGEMENT
Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES


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Table I: Clinical presentation of pyoderma cases (n=130)

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Clinical diagnosis</th>
<th>No of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Folliculitis</td>
<td>30 (23.08%)</td>
</tr>
<tr>
<td>2</td>
<td>Furunculosis</td>
<td>16 (12.31%)</td>
</tr>
<tr>
<td>3</td>
<td>Impetigo</td>
<td>9 (6.92%)</td>
</tr>
<tr>
<td>4</td>
<td>Ecthyma</td>
<td>9 (6.92%)</td>
</tr>
<tr>
<td>5</td>
<td>Abscess</td>
<td>8 (6.15%)</td>
</tr>
<tr>
<td>6</td>
<td>Acute paronychia</td>
<td>3 (2.31%)</td>
</tr>
<tr>
<td>7</td>
<td>Carbuncle</td>
<td>2 (1.54%)</td>
</tr>
<tr>
<td>8</td>
<td>Acne vulgaris</td>
<td>2 (1.54%)</td>
</tr>
<tr>
<td>9</td>
<td>Secondary pyoderma</td>
<td>51 (39.23%)</td>
</tr>
</tbody>
</table>

Table II: Antibiotic resistance pattern of S. aureus

<table>
<thead>
<tr>
<th></th>
<th>Penicillin (10U)</th>
<th>Erythromycin (15µg)</th>
<th>Cotrimoxazole (1.25/23.75µg)</th>
<th>Ciprofloxacin (5µg)</th>
<th>Gentamicin (10µg)</th>
<th>Vancomycin (30 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus (n=50)</td>
<td>46(92%)</td>
<td>17(34%)</td>
<td>40(80%)</td>
<td>12(24%)</td>
<td>3(6%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>MRSA (n=34)</td>
<td>34(100%)</td>
<td>26(76.47%)</td>
<td>31(91.18%)</td>
<td>22(64.71%)</td>
<td>12(35.29%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Total (n=84)</td>
<td>80(95.24%)</td>
<td>43(51.19%)</td>
<td>71(84.52%)</td>
<td>34(40.48%)</td>
<td>15(17.86%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>
Fig I: Bacteria isolated (%) from pyoderma lesions (n=123)

- S. aureus: 68.29%
- S. pyogens: 11.38%
- CONS: 7.32%
- Enterococci: 2.44%
- E. coli: 2.44%
- K. pneumoniae: 0.00%
- Pr. Merabilis: 2.44%
- Pr. Vulgaris: 0.81%
- Ps. aeruginosa: 2.44%
ABSTRACT

Objectives: To compare the effect of balance exercises and standardised exercises in children with Down’s syndrome. Methods: 30 children with Down syndrome aged 5-15 years were assigned to the experimental and control group using block randomisation. Children in the experimental group underwent balance exercise program whereas children in the control group underwent standardised exercise program, twice per week for a total of 6 weeks. Results: There was significant improvement in both the groups on the Pediatric Balance scale and the gross motor function measure scores. However the experimental group showed a greater improvement than the control group. Conclusion: Balance exercises are better than standardised exercises in improving the dynamic balance and gross motor function in children with Down’s syndrome.

Keywords: Down’s syndrome, Balance exercises, Gross Motor Function

INTRODUCTION

Down’s syndrome is caused by the presence of the whole or part of an extra copy of chromosome 21.1 Global estimation of the incidence of Down Syndrome is 1 in 1,000 to 2,000 live births.2 Down’s Syndrome (DS) is associated with neuromuscular abnormalities leading to a consistent delay in acquisition of gross and fine motor skills and deficits in the automatic postural control system leading to functional balance problems.3,4,5 Children with DS perform poorly in running speed, strength, visual motor control, agility and balance tasks especially static balance.6,7,8 Early intervention with the child and the family is a critical first step in the long term program of children with DS.9,10 Muscle strengthening for adults with DS has shown to improve physical and kinetic abilities when compared to the control group with no training.11 Treadmill walking in adults with DS has significantly improved the dynamic balance performance.12 Although research has been done on routine treatment strategies using strength training and fitness programs, studies on specific balance exercises and their effect on DS could not be retrieved. Thus, the purpose of this study was to compare the effect of balance exercises and standardised
exercises in children with Down’s syndrome.

METHODS
30 children with DS, aged between 5-15 years were recruited for the study from tertiary care hospitals and special schools in Mangalore and were assigned to the experimental and control group by block randomisation. The experimental group (mean age 8.3±2.6 years) received balance exercises and the control group (mean age 11.2±2.6 years) received standardized exercise program. Institutional Ethical Committee approval was obtained. Informed consent was taken from parents/caregivers following which the child was screened for the inclusion and Exclusion Criteria. Children were included in the study based on the scores of Modified Mini-Mental Scale for Cognitive Functions in children, i.e. children of 5 years must have a score >24, 6-8 years >28, 9-11 years > 30 and children of 12-15 years must have a score >35. Children were excluded if they underwent any surgical procedures during the study period, history of untreated cardiac problems and any musculoskeletal problems that prevented the child to maintain upright stance.

The purpose and the procedure of the study were explained to the children and their parents/caregivers. Children in the experimental group underwent balance exercise program along with routine special education. The balance exercise program included 5 minutes of warm up activities followed by balance exercise in ‘all fours’ position on the floor, therapy ball exercises, activities in standing with emphasis on hip, ankle and stepping strategy training, single leg stance, reaching activities and tandem walking. This was followed by ‘5 minutes of ‘cool down’ exercises.12,13,14 Children in the control group underwent standardized exercise program along with routine special education. The standardised exercise program included 5 minutes of ‘warm up exercises followed by strength training for upper limb, trunk and lower limb, cardiovascular fitness exercises and ‘cool down’ exercises.11,13 The duration of the exercise program for both the groups was twice per week for a total of 6 weeks. Each session lasted for 45 minutes which was supervised by the tester. Exercises were started at the level of the child and progression was done subsequently. A child was excluded if he/she missed 6 sessions or more. The outcome measures i.e. Pediatric Balance Scale and Gross Motor Function Measure were administered on day one i.e. prior to the commencement of the intervention and at the end of 6 weeks.

Data was analysed using SPSS version 13. Wilcoxin Signed Ranks Test was used to analyse within-Group effect for the Gross Motor Function Measure score and the total and the individual subtests scores of Pediatric Balance Scale. Mann-Whitney Test was used to compare the outcome measures between the two groups.

RESULTS
Demographic data for age and gender is as shown in Table 1.
Wilcoxin signed rank test was used to study the effect of exercise within the group, pre and post intervention with ‘p’ value set at <0.05 as shown in table 2. As shown in table 2, there was a significant difference in the total scores of Pediatric Balance Scale within both the Experimental and Control group. The GMFM scores showed a significant difference within the Experimental group in Crawling & Kneeling, Standing, Walking, Running and Jumping, and no
significant difference was found in Lying & Rolling and Sitting. The GMFM scores showed a significant difference within the Control group in Crawling & Kneeling, Walking, Running & Jumping and no significant difference was found in Lying & Rolling, Sitting & Standing. Mann Whitney test was used to compare the scores of PBS and GMFM between the experimental and Control group as shown in table 3. As shown in table 3, there was a significant difference between the experimental and control group in Pediatric Balance Scale and Gross Motor Function Measure with ‘p’ value set at ≤0.05.

**DISCUSSION**

In the present study, the results showed significant improvement between the pre and post exercises for both the experimental and control group in the Pediatric Balance Scale and Gross Motor Function Measure scores. However, the experimental group showed better improvement in both the outcome measures when compared to the control group. Our results were similar to the previous studies which had shown improvement in balance following exercises which included strength training and fitness programs.  

In the present study, both the experimental and control group included a series of exercises of progressive nature unlike the previous studies which had limited and non-progressive exercises. Significant improvement seen in the experimental group could be attributed to the emphasis on core stability exercise, and moving in and out of the stable posture thus placing greater demand on postural control mechanisms. 

Research on Down’s Syndrome has highlighted inadequate co-contractions and insufficiency of stabilizing myogenous contractions around the joints. According to Bobath, this co-contraction is important for the development of postural and movement patterns. It was also reported that a lack of postural tone was accompanied by a lack of co-contraction which leads to stabilization problems. In our study, the child had to maintain the posture on both stable and unstable surface, thus improving co-contractions and proprioceptive feedback, leading to the improved balance. 

The current study also included strategy training as one of the components of balance exercises program followed by maintenance of single leg stance. Studies had shown that transferring weight to one leg made an increased demand on the postural control system. The above factors may be the reason for improvement seen in the balance exercise group. There was significant improvement in the control group also. Our results were similar to the previous studies where children with Down’s Syndrome responded well to the circuit training programs that were aimed at increasing aerobic capacity and muscular strength. It is shown that extensive practice over a wide variety of movement tasks, exercise and strength training can be applied to improve quality of movement in individuals with Down’s Syndrome. The cause for improvement may be related to the increase in the intensity of activation of motoneuron pools. 

The Experimental group showed highly significant difference in the total scores of Pediatric Balance Scale and Gross Motor Function Measure when compared to the control group. The maintenance of stability requires the execution of fast automatic postural responses with onset latencies below those of voluntary reaction time responses. Techniques that rely on voluntary or consciously acquired balance
responses probably will not ensure stability unless the learned response becomes automated i.e. not requiring conscious processing.3 The above reason could have led to a better improved balance and gross motor functions in the experimental group. There was no specific home program given to the children in both experimental and control group. They were permitted to carry out normal everyday activities. Most of the children were engaged in play activities after school hours, however, the levels of functional activities were not considered.

Both the experimental and control group showed significant improvement in the GMFM scores and total scores of PBS following the exercise program, however, the experimental program showed a better improvement in both the outcome measures when compared to the control group. The stratification of the sample according to age group was not done because of small sample size. The follow up/carryover effect following exercise program was not assessed.

Balance exercises should be implemented in clinical programs for children with Down’s syndrome so as to improve the dynamic balance and gross motor function.

REFERENCES


Table 1: Demographic data for age & gender

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Gender</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Mean age± S.D in years</td>
<td>8.3± 2.6</td>
<td>11.2± 2.6</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>15</td>
</tr>
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</table>

Table 2: Within group effect for total score of PBS and GMFM

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean± S.D</th>
<th>‘Z’ value</th>
<th>‘p’ value</th>
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</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Pre</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.5±3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Post</td>
<td>44.3±1.7</td>
<td>3.4</td>
</tr>
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<tr>
<td>GMFM</td>
<td>Experimental</td>
<td>85.1±1.4</td>
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<td>Control</td>
<td>88.6±1.3</td>
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<td>&lt;0.024</td>
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<tr>
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<td>PBS</td>
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<td>85.5±0.7</td>
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<tr>
<td></td>
<td>Control</td>
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<td>2.2</td>
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<tr>
<td></td>
<td></td>
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<td>&lt;0.001</td>
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Table 3: Between group effect for the total score of PBS and GMFM

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Difference±S.D</th>
<th>‘Z’ value</th>
<th>‘p’ value</th>
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<tr>
<td>PBS</td>
<td>Experimental</td>
<td>11.0±3.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.2±2.1</td>
<td></td>
</tr>
<tr>
<td>GMFM</td>
<td>Experimental</td>
<td>3.6±0.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.5±0.4</td>
<td></td>
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ABSTRACT

Aims and objectives: The aim of the study was to estimate the levels of vascular endothelial growth factor (VEGF) in gingival crevicular fluid (GCF) of diabetic and non-diabetic patients with periodontal disease. Materials and methods: A total of 40 subjects in the age group of 30-65 years were selected. The subjects were divided into four groups as Group I (Healthy), Group II (Gingivitis), Group III (Chronic periodontitis) and Group IV (Chronic periodontitis with Type II Diabetes Mellitus with a HbA1c level of ≥8). Samples of GCF were collected in all groups and the concentration of VEGF was determined using a human VEGF enzyme immunometric assay (EIA) kit. Results: The highest mean VEGF concentration in GCF (74.11 pg/ml) was observed in Group IV and the lowest mean VEGF concentration (11.71 pg/ml) was observed in Group I. The VEGF levels in GCF increased proportionally with the progression of periodontal disease with a further increase in those with type II DM as well as periodontitis. Conclusion: The results observed in this study indicate that VEGF could serve as a potential biomarker of periodontal disease progression.

INTRODUCTION

Periodontitis is a chronic inflammatory disease of the vascularized supporting tissues of the teeth. Many studies have documented the highly vascular nature of the lesion and have indicated that in the region of the periodontal pocket, there is a relationship in the number of blood vessels and progression of the disease, especially affecting capillaries and venules.\(^1\)\(^2\) Angiogenesis (neovascularisation) is the budding of new capillaries and is thought to be an essential process of development of chronic inflammatory diseases. Angiogenesis contributes to the severity of the inflammation as a result of the ability of the new blood vessels to transport pro-inflammatory cells to the lesion and supply nutrients and oxygen to the inflamed tissues.\(^2\) In addition, the increased endothelial surface area forming the new blood vessels increases the potential substrate for production of cytokines, adhesion molecules and the progression factors for inflammation.\(^2\)
The most potent agent that acts specifically on vascular endothelium is the vascular endothelial growth factor (VEGF).\textsuperscript{3,4} VEGF potently increases microvasculature permeability, stimulates endothelial cell proliferation, induces proteolytic enzyme expression and the migration of endothelial cells, monocytes and osteoblasts all of which are essential for angiogenesis.\textsuperscript{4}

Periodontitis is frequently mentioned among the oral problems observed in diabetes mellitus (DM), and there are many reports investigating the relationship between periodontitis and DM.\textsuperscript{5} It has been reported that DM may contribute to periodontitis by mechanisms such as vascular changes, neutrophil dysfunction, altered collagen synthesis, and genetic disposition.\textsuperscript{6} In addition, oxygen supply and diffusion removal of metabolic end products, leukocyte migration, and diffusion factors are impaired in diabetic patients because of the gingival microangiopathy, consequently leading to tissue repair and regeneration inability.\textsuperscript{6}

Recent reports have suggested the major role of VEGF in DM, since microangiopathy and increased angiogenic response are main characteristics of this disease.\textsuperscript{7}

Analysis of specific constituents in the GCF provides a quantitative biochemical indicator for the evaluation of the local cellular metabolism that reflects a person’s periodontal health status and has been used to evaluate risk for an individual to develop periodontal disease.\textsuperscript{8,9}

Many studies have shown that in patients with periodontitis, the volume of GCF and the total amount of VEGF collected from diseased sites were greater than that collected from clinically healthy sites.\textsuperscript{10} VEGF was increased in tissue and GCF samples in periodontal sites in healthy subjects and in patients with diabetes mellitus.\textsuperscript{10} Levels of VEGF in GCF differed significantly between healthy and diseased sites.\textsuperscript{11} This extensive cellular distribution with detectable levels of VEGF in GCF suggests that VEGF plays a predominant role in the maintenance of periodontal health and in chronic inflammatory periodontal disease.\textsuperscript{11}

This biochemical study was designed to estimate the levels of vascular endothelial growth factor (VEGF) in gingival crevicular fluid (GCF) of diabetic and non-diabetic subjects with periodontal disease.

**MATERIALS AND METHODS**

The study population consisted of 40 subjects in the age group of 30-65 years attending the outpatient section, Department of Periodontology, Meenakshi Ammal Dental College, Chennai, based on the criteria for subject grouping as given below. Ethical clearance for the study was obtained from the ethical committee of the MAHER University.

Exclusion criteria were: aggressive periodontitis, hypertension, psoriasis, tumors, smoking, alcoholism, pregnancy, cardiovascular disease, delayed hypersensitivity, Sjogrens syndrome, rheumatoid arthritis, previous periodontal treatment, use of antibiotic drugs/neovascularization inhibitor use in the last 6 months.

Subjects underwent brief case history recording which included patient’s chief complaint, medical and dental history, oral examination with full-mouth periodontal probing and charting and radiographic examination. Clinical measurements recorded were, Gingival Index (Loe H, 1967)\textsuperscript{12}, clinical attachment level and radiographic bone loss.

The subjects were categorized into 4 groups, each group comprising of 10 patients based on gingival index (GI) and
clinical attachment level (CAL), and radiographic evidence of bone loss. Group I consisted of 10 subjects with clinically healthy periodontium and with no evidence of disease with a GI score = 0, no attachment loss (CAL=0mm) and with no evidence of radiographic bone loss. Group II (Gingivitis) consisted of 10 subjects whose gingiva showed clinical signs of inflammation, GI score between 1 and 3, no evidence of attachment loss i.e., CAL=0mm and with no bone loss as determined from radiograph. Group III (Chronic Periodontitis) consisted of 10 subjects, who showed clinical signs of gingival inflammation i.e., GI score between 1 and 3, CAL ≥1mm and with bone loss as determined from radiographs. Group IV (Chronic periodontitis with type II diabetes mellitus) consisted of 10 subjects who had type II Diabetes mellitus with a HbA1c level of ≥ 8 and showed clinical signs of gingival inflammation i.e., GI score between 1 and 3, CAL≥1mm and with radiographic evidence of bone loss. GCF was collected by the same examiner, the next day, to prevent contamination of the sample with blood associated with the probing of inflamed sites. The patients were explained about the study and written informed consent was obtained from those who agreed to voluntarily participate in this study.

Site selection and GCF collection:
Clinical and radiological examinations, group allocation and sampling-site selection were performed by one examiner and the samples were collected on the subsequent day by the same examiner. Only one site per subject was sampled. In the healthy group, to standardize site selection and obtain adequate fluid volume, sampling was predetermlined to be from the mesio-buccal region of the maxillary right first molar. In subjects with gingivitis, sites were selected showing the most severe clinical inflammatory signs in the absence of CAL. In chronic periodontitis patients with and without DM, sites with highest clinical attachment loss confirmed with radiographic evidence of bone loss were selected. On the subsequent day the selected test site was air dried and isolated with cotton rolls. Without touching the marginal gingiva, the supragingival plaque was removed to avoid contamination of the GCF sample and blocking of the microcapillary pipette. Then GCF was collected using the black colour-coded 1-5μl calibrated volumetric microcapillary pipettes obtained from Sigma-Aldrich Chemical Company, USA. By placing the tip of the microcapillary pipette extracrevicularly (unstimulated) for 5-20 minutes, a standardized volume of 1μl GCF was collected using the calibration on the microcapillary pipette. The test sites which did not express standardized volume (1μl) of GCF were excluded from the study and the micropipettes contaminated with blood or saliva was discarded. The GCF collected was immediately transferred to plastic vial and stored at -70°C till the time of the assay.

VEGF Assay: The concentration of VEGF was determined using a human VEGF enzyme immunometric assay (EIA) kit (Koma Biotech Inc.) as instructed by the manufacturer. The kit uses a monoclonal antibody to human VEGF immobilized on a microtiter plate to bind the human VEGF in the standards or sample. All standards, controls and samples were run in duplicate. 200μl of washing solution was added to each well. The wells were aspirated to remove liquid and the plate was washed 3 times using the 300μl of washing solution.
per well. After the last wash, the plate was inverted to remove residual solution and blotted on paper towel. 100µl of standard or sample was added to each well in duplicate. The wells were covered with the plate sealer that was provided and the wells were incubated at room temperature for 2 hours. The wells were aspirated to remove liquid. After washing the plate 4 times, 100µl of the diluted detection antibody (0.25µg/ml) was added per well. The wells were covered with the plate sealer provided and incubated for 30 minutes at room temperature. The wells were aspirated and washed 4 times again, after which 100µl of color development solution was added to each well and was incubated at room temperature for a proper color development (5-15 minutes). To stop the color reaction, 100µl of the stop solution was added to each well (or 37°C for 30 minutes). The color development was visually monitored to optimize the incubation time. Using a microtitre plate reader, the plate was read at 450nm wavelength.

**Statistical Analysis:**
One way ANOVA test was used to calculate the P-value. Independent t-test was used for pair wise comparison between groups. Pearson correlation test was used to correlate VEGF levels and the clinical parameters within each study group.

**RESULTS**

**Clinical parameters:**
A comparison of the mean values of the clinical parameters in the various study groups is depicted in table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>Overall P-value</th>
<th>Significant Groups</th>
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<tbody>
<tr>
<td>GI</td>
<td>I</td>
<td>0.00</td>
<td>0.00</td>
<td>&lt;0.001 (Sig.)</td>
<td>I vs. II</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.54</td>
<td>0.49</td>
<td></td>
<td>I vs. III</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2.57</td>
<td>0.386</td>
<td></td>
<td>I vs. IV</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>2.66</td>
<td>0.430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL(mm)</td>
<td>I</td>
<td>0.00</td>
<td>0.00</td>
<td>&lt;0.001 (Sig.)</td>
<td>I vs. III</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td>I vs. IV</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5.50</td>
<td>0.527</td>
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<td>II vs. III</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>5.70</td>
<td>0.823</td>
<td></td>
<td>II vs. IV</td>
</tr>
</tbody>
</table>

The mean GI score in Group II (2.54), Group III (2.57) and Group IV (2.66) are significantly higher than the mean GI score in Group I (0.00) ( p <0.001). The mean CAL in Group III (5.50mm) and Group IV (5.70mm) are significantly higher than the mean CAL in Group I (0.00mm) and Group II (0.00) (p<0.001).
The VEGF levels in the study groups are depicted in table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>P - Value</th>
<th>Significant Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10</td>
<td>11.71</td>
<td>1.940</td>
<td>&lt;0.005</td>
<td>I vs II, I vs III, I vs IV</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>31.38</td>
<td>16.950</td>
<td></td>
<td>II vs III, II vs IV, III vs IV</td>
</tr>
<tr>
<td>Group III</td>
<td>10</td>
<td>55.66</td>
<td>9.934</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>10</td>
<td>74.11</td>
<td>24.348</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean VEGF level of Group IV (74.11 pg/ml) was significantly higher than Group I (11.71 pg/ml), Group II (31.38 pg/ml) and Group III (55.66 pg/ml) (p<0.005). The mean VEGF level of Group III (55.66 pg/ml) was significantly higher than Group I (11.71 pg/ml) and Group II (31.38 pg/ml) (p<0.005). Also, the mean VEGF level of Group II (31.38 pg/ml) was significantly higher than Group I (11.71 pg/ml) (p<0.005).

Table 4: Relationship between VEGF level and various clinical parameters within each study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean VEGF levels(pg/ml)</th>
<th>Clinical Parameters</th>
<th>Correlation Coefficient*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.71</td>
<td>GI</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>31.38</td>
<td>GI</td>
<td>0.830</td>
<td>0.003(sig.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>55.66</td>
<td>GI</td>
<td>0.611</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAL</td>
<td>0.714</td>
<td>0.020(sig.)</td>
</tr>
<tr>
<td>IV</td>
<td>74.11</td>
<td>GI</td>
<td>0.514</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAL</td>
<td>0.785</td>
<td>0.007</td>
</tr>
</tbody>
</table>

The relationship between VEGF levels and various clinical parameters within each study group is shown in table 4, which showed a significant correlation between GI score and VEGF levels in Group II. Also, there was a significant correlation between CAL and VEGF levels in Group III. However, the remaining clinical parameters did not show significant relationship when compared with their corresponding VEGF levels.

**DISCUSSION**

Angiogenesis is thought to be an essential process in the development of chronic inflammatory diseases. In one such disease, chronic periodontitis, aberrant angiogenesis and persistent granulation in gingiva have also been observed and these phenomena may contribute markedly to its progression.

It was reported that VEGF is strongly associated with some angiogenesis dependent diseases, such as diabetic retinopathy, psoriasis and rheumatoid arthritis and also has the ability to increase vascular permeability, which contributes to incrementation and extension of inflammation. Therefore, there is possibly
an association of VEGF with the pathogenesis of periodontitis, which is accompanied by heavy neovascularization.\textsuperscript{13}

The role of diabetes mellitus in various periodontal diseases has been extensively investigated, with many studies indicating an impact of periodontal inflammation on diabetic balance.\textsuperscript{14} DM and periodontitis represent common chronic diseases that may have a reciprocal influence.\textsuperscript{15} The structural changes characterizing diabetic microangiopathy, such as abnormal growth and impaired regeneration, strongly suggest a role for a number of aberrantly expressed growth factors, possibly acting in combination, in the development of these complications. This has been supported by the detection of increased concentrations of several growth factors consequent to the activation of biochemical pathway linking hyperglycemia to microvascular changes: the polyol pathway, non enzymatic glycation of proteins, vasoactive hormones, oxidative stress and hyperglycemic pseudohypoxia.\textsuperscript{16}

A relationship was also found between an increased number of blood vessels and progression of periodontitis.\textsuperscript{17} Asperiello et al (2009)\textsuperscript{18} observed an increased epithelial VEGF expression in patients with type I and II DM compared to controls, which confirmed that DM has an inductive effect on periodontal VEGF. VEGF acts a potent and pleiotrophic inflammatory agent in periodontitis, especially when further aggravated by diabetes.\textsuperscript{18}

VEGF was detectable in periodontal tissues within vascular endothelial cells, plasma cells, macrophages and in junctional, sulcular and gingival epithelium. This extensive cellular distribution combined with detectable levels of VEGF in GCF from diseased and healthy sites suggests that VEGF plays a role in both maintenance of periodontal health and chronic inflammatory periodontal disease.\textsuperscript{19}

GCF provides a quantitative biochemical indicator for the evaluation of the local cellular metabolism and reflects a person’s periodontal health. Findings from diabetic patients illustrate how the measure of GCF inflammatory mediator levels seems to be highly correlated with the overall individual’s systemic inflammatory response.\textsuperscript{8,20} PGE\textsubscript{2} in GCF was associated with active destruction of periodontal attachment\textsuperscript{21} and it is of interest that PGE\textsubscript{2} is a potent stimulator of VEGF synthesis. In addition to PGE\textsubscript{2}, IL-1 and TNF-\alpha are also implicated in the induction of VEGF.\textsuperscript{22} The present study was aimed to evaluate the VEGF concentrations in GCF of diabetic and non-diabetic subjects with periodontal disease.

The mean GI score in Group II (2.54), Group III (2.57) and Group IV (2.66) are significantly higher than the mean GI in Group I (0.00) (p< 0.001). This clearly indicates that the severity and quantity of gingival inflammation progresses with increasing severity of disease. The mean CAL in Group III (5.50mm) and Group IV (5.70mm) are significantly higher than the mean CAL in Group I (0.00mm) and Group II (0.00mm) (p<0.005). The mean VEGF level of Group IV (74.11pg/ml) was significantly higher than Group I (11.71pg/ml), Group II (31.38 pg/ml) and Group III (55.66 pg/ml) (p<0.005). The mean VEGF level of Group III (55.66pg/ml) was significantly higher than Group I (11.71pg/ml) and Group II (31.38 pg/ml) (p< 0.005). The mean VEGF level of group II (31.38 pg/ml) was significantly higher that Group I (11.71 pg/ml) (p<0.005).

With respect to the general trend, the results of the present study is in accordance
with those of Guneri et al (2004) who also reported increased VEGF levels in GCF samples in periodontal sites both in healthy subjects and in patients with DM. They also evaluated VEGF levels in tissue samples and found that there was an increased amount of tissue VEGF in healthy sites of DM patients, though this finding was not observed in GCF samples of the same sites. It was suggested that low VEGF levels in those samples, may be due to bonding of most VEGF to the tissue receptors, leading to a lesser degree of release in GCF.

But contrary to the present study, the study done by Sakallioğlu EE et al (2007) showed that VEGF levels were significantly higher in the gingival supernatants of the DM group than that of the control group. However, there was no statistically significant difference in the VEGF levels of GCF between the study groups and confirms that DM affects the VEGF levels of periodontal soft tissues in periodontal disease.

The present study showed that the mean concentration of VEGF in GCF increased progressively from healthy to periodontitis subjects, with the mean concentration in gingivitis subjects falling between the two values, which is in accordance with the study done by Prapulla et al (2007). However, contrary to our findings, Booth et al (1998) have suggested that VEGF was generally upregulated even in relatively healthy sites, probably reflecting subclinical levels of inflammation/healing after microbial assault, or revealing the presence of VEGF as a component of physiological angiogenesis in the gingival/periodontal environment. This finding was not observed in the present study, probably due to differences in patient selection criteria and comparison methods.

Pearson correlation test showed there was a significant relationship between GI and VEGF level in Group II. Also, there was a significant relationship between CAL and VEGF level in Group III. However, the remaining clinical parameters did not show significant relationship when compared with their corresponding VEGF levels. This is in contrast to the study done by Prapulla et al (2007) in which they reported significant positive correlation between GCF VEGF concentration and all clinical parameters within each study group. However, there was a significant relationship between CAL and VEGF level in Group III which is in accordance with the study done by Prapulla et al (2007).

Booth et al (1998) and Guneri et al (2004) did not include the gingivitis group. Having additional groups of gingivitis helped us to better evaluate the role played by VEGF in different stages of periodontal disease. Also Prapulla et al (2007) did not include a diabetic group, but they did however, assess the GCF levels after performing scaling and root planing in the chronic periodontitis groups, which confirmed the role of VEGF in attachment loss.

Having an additional diabetic group in the present study helped us further evaluate the role of systemic conditions on VEGF level. Treatment, however was not carried out which is one of the limitations in the present study.

Few samples from Group II (Gingivitis) showed values nearing those of Group III (Chronic periodontitis), which could be attributed to near conversion of a gingivitis lesion to a chronic periodontitis lesion that is not clinically detectable or it could be due to a limited sample size. A few of the samples from Group I (healthy) also
showed values nearing those of Group II (gingivitis), which can be attributed to the subclinical levels of inflammation in clinically healthy tissues. The presence of significant differences between the VEGF levels in GCF from diabetic patients as compared to non-diabetic patients may indicate the possible effect of systemic conditions on the level of VEGF. The spillover of these increased GCF VEGF levels from diseased periodontal tissues could lead to a concomitant increase in serum VEGF. The increase in VEGF concentration in circulation, apart from being associated with periodontal inflammation, also has been linked to an increased incidence of rheumatoid arthritis and psoriasis. The increase in serum VEGF levels that are due to progressive periodontal disease could act as a risk factor for RA and psoriasis.

Within the limitations of this clinical study, the role of VEGF as a diagnostic biomarker of periodontal disease progression in gingival crevicular fluid could be proposed. To reach a more precise conclusion about the effect of VEGF in periodontal pathogenesis in systemically healthy and diseased people, application of more specific methods such as PCR, to larger study populations, may be suggested. However, further long-term prospective studies with larger sample sizes are needed to fully establish the role of VEGF as a predictor of periodontal tissue destruction, disease activity, and response to therapy in periodontitis patients, which could lead to development of chair-side diagnostic kits and VEGF-specific therapeutic intervention strategies to arrest periodontitis associated alveolar bone destruction.

**CONCLUSION**

The results indicates that the mean concentration of VEGF in GCF progressively increased from healthy to periodontitis subjects, with a further increase in those with type II diabetes mellitus showing a systemic influence on VEGF levels and it clearly indicates its role as a potential biomarker as a predictor of disease activity.

**ACKNOWLEDGEMENTS**

The authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors, editors, and publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

**REFERENCES**


Legends:
Fig 1 - Collection of GCF using black colour-coded 1-5μl calibrated volumetric microcapillary pipette.
Fig 2 - A view of microtitre plate after adding the substrate solution and final incubation
Fig 3 - A view of microtitre plate while adding the stop solution
Fig 4 – Elisa reader
ABSTRACT

Purpose: To report a rare case of keratoacanthoma of bulbar conjunctiva.

Materials and methods: Detailed clinical examination of 19 yr old female, with slit lamp biomicroscopy, Ophthalmoscopy, complete blood picture, Elisa for HIV, Bleeding time and Clotting time, excision biopsy and histopathological examination.

Results: O/E - Patient was moderately built and moderately nourished. No pallor or lymphadenopathy.

Ocular examination: Va 6/6 both eyes. Lids normal both eyes. In Right eye there was a conjunctival nodule raised 2 mm above the surface and 2 mm medial to limbus of about 3x4mm size, surface looking dry and silvery, freely mobile, with dilated and tortuous blood vessels around the lesion. The rest of the anterior and posterior segments examination was normal. We suspected it to be a unattended foreign body granuloma or chronic conjunctival phlycten. With ulceration. We posted the patient for excision biopsy.

HPE Report: sections studied showed hyperkeratotic and edematous squamous epithelium with crater formation and keratinization. The cells showed atypical with mild to moderate amount of mitosis. suggesting the lesion as keratoacanthoma.

Conclusion: the HPE report confirmed the excised nodule to be keratoacanthoma in contrast to our clinical suspicion. Keratoacanthoma(KA) of skin is a common finding, keratoacanthoma of the conjunctiva is very rare. Risk of progression of KA to squamous cell carcinoma is still under study. There was no recurrence in our case in 2 mnths follow up. Since surgery. Since first reported by FREEMAN et al in 1961 only 15 cases were reported in literature till 2003. We here by are reporting a rare case of bulbar conjunctiva.

INTRODUCTION

Keratoacanthoma is a squamous epithelial neoplasm characterized by a very rapid growth phase, followed by gradual involution (1-5). It generally occurs on skin and is rarely found on the conjunctiva. The tumour is most commonly seen on sun exposed and hair bearing areas of elderly patients, mainly on the face, forearms, and hands (3,4). We report a rare case of conjunctival keratoacanthoma, indistinguishable from squamous cell carcinoma clinically, which was treated with complete excision.

MATERIALS AND METHODS

Detailed clinical examination of 19 yr old female, with slit lamp biomicroscopy,
Ophthalmoscopy, complete blood picture, Elisa for HIV, Bleeding time and Clotting time, excision biopsy and Histopathological examination

CASE REPORT

A 19-year-old female from Khammam attended the eye department with a sore and red right eye. She had noticed a nodule on the nasal conjunctiva of her right eye for 2 months, which had gradually increased in size, and recently become inflamed and uncomfortable. There was no history of injury or previous ophthalmic surgery and she was systemically well.

Patient was moderately built and moderately nourished. No pallor or lymphadenopathy

Ocular examination:
Visual acuity is 6/6 in both eyes. Lids are normal in both eyes. In Right eye there was a conjunctival nodule raised 2 mm above the surface and 2mm medial to limbus of about 3x4mm size, surface looking dry and silvery, freely mobile, with dilated and tortuous blood vessels around the lesion. The lump was mobile and there was contact bleeding from fine surface vessels. The rest of the anterior and posterior segments examination was normal. (FIG: 1)

Case was suspecting it to be an unattended foreign body granuloma or chronic conjunctival phlycten with ulceration, infected pingueculum, and intraepithelial neoplasia/carcinoma. The case was posted for excision biopsy. The lesion was subsequently excised under local anaesthesia leaving bare sclera, and cryotherapy was applied to the conjunctival margins.(FIG:2). The lesion was not adherent to underlying tissues. The mass was sent for Histopathological examination.

HPE Report:
Sections showed hyperkeratotic and edematous squamous epithelium with crater formation and keratinization. The cells showed atypical with mild to moderate amount of mitosis suggesting the lesion as keratoacanthoma. (FIG: 3)

CONCLUSION

Keratoacanthoma is a squamous epithelial neoplasm characterized by the rapid growth of a painless firm keratotic nodule and regresses completely with out treatment (6). keratoacanthoma(KA) of skin is a common finding, keratoacanthoma of the conjunctiva is very rare . Since first reported by FREEMAN et al (6, 7) in 1961 only 15 cases were reported in literature till 2004(6- 8). Risk of progression of keratoacanthoma to squamous cell carcinoma is still under study. Unlike cutaneous lesions, the natural history of conjunctival keratoacanthoma is obscure because they are excised early. These tumors are usually excised because of similar clinical presentation of squamous cell carcinoma (11). There was no recurrence in our case in 6 months follow up since surgery. Conjunctival keratoacanthoma preferentially occurs in the limbic region, which is similar to squamous cell carcinoma. The KA lesion occurs more frequently at the temporal limbus(6,9) but in our case it occurred at the nasal limbus. However, a noteworthy case, described by Grossniklaus et al,(10, 11) of a rapidly growing limbal lesion with keratoacanthoma features was excised after 3 weeks with evidence of invasion on histology. There was subsequent rapid recurrence with intraocular invasion requiring enucleation. It is unclear whether the lesion in their report was a keratoacanthoma or a very rapidly growing squamous carcinoma, but clearly early
excision of a suspected conjunctival keratoacanthoma is recommended. The exact pathogenesis of conjunctival keratoacanthoma remains unknown but the possible proposed risk factors for the cutaneous keratoacanthoma include immunosuppression, exposure to chemical agents, human papilloma virus infection, chronically injured skin (ulcers, burns, sinus tracts, vaccination scars, and chronic skin diseases), and genetic aberrations. Histologically, mature lesions demonstrate a central keratin-filled crater with a surrounding buttress of epidermis. They are composed of tumour islands made of enlarged keratinocytes with a pale cytoplasm, arranged in concentric layers with increasing keratinisation centrally. The large cells with pink cytoplasm towards the centre of the tumour cell nests are generally more characteristic of keratoacanthoma. Inflammatory cells are usually found in the stroma, at the base of the lesion. The HPE report of the present case confirmed with the above histological study suggesting keratoacanthoma in contrast to our clinical suspicion.

The distinction between keratoacanthoma and SCC is not always simple. Various markers such as involucrin and lectins were not specific enough, and could not be used in clinical practice. In a recent report by Cribier et al., the authors studied 296 fully excised tumours, previously classified as keratoacanthoma or SCC, and analysed the histopathological criteria differentiating the two tumours. They concluded that the most important criteria were an epithelial lip and a sharp outline demarcation between the tumour and the stroma (favouring keratoacanthoma), and ulceration, pleomorphism/anaplasia, and numerous mitoses (favouring SCC). Perineural invasion (PNI) is an uncommon finding in keratoacanthoma, and when present, it does not affect prognosis or the risk of metastatic disease. Early excision of periocular keratoacanthoma is essential, not only to maintain normal eyelid function, but also to prevent further tissue destruction and invasion into deeper tissues. The role of alternate treatments such as curettage and electrodessication, cryotherapy, radiotherapy, or intralesional chemotherapy, is probably limited to patients unable to tolerate surgery. Hence, it is generally accepted that surgical excision with margin control is the most appropriate method for solitary tumours.

**REFERENCES**

Keratoacanthoma of the conjunctiva E H Hughes¹, L Intzedy², A D Dick¹ and D M Tole¹

FIG: 1- CLINICAL PICTURE OF KERATOACANTHOMA
FIG: 2 – POSTOPERATIVE PICTURE OF KERATOACANTHOMA.

FIG: 3 - HISTOPATHOLOGICAL PICTURE OF KERATOACANTHOMA