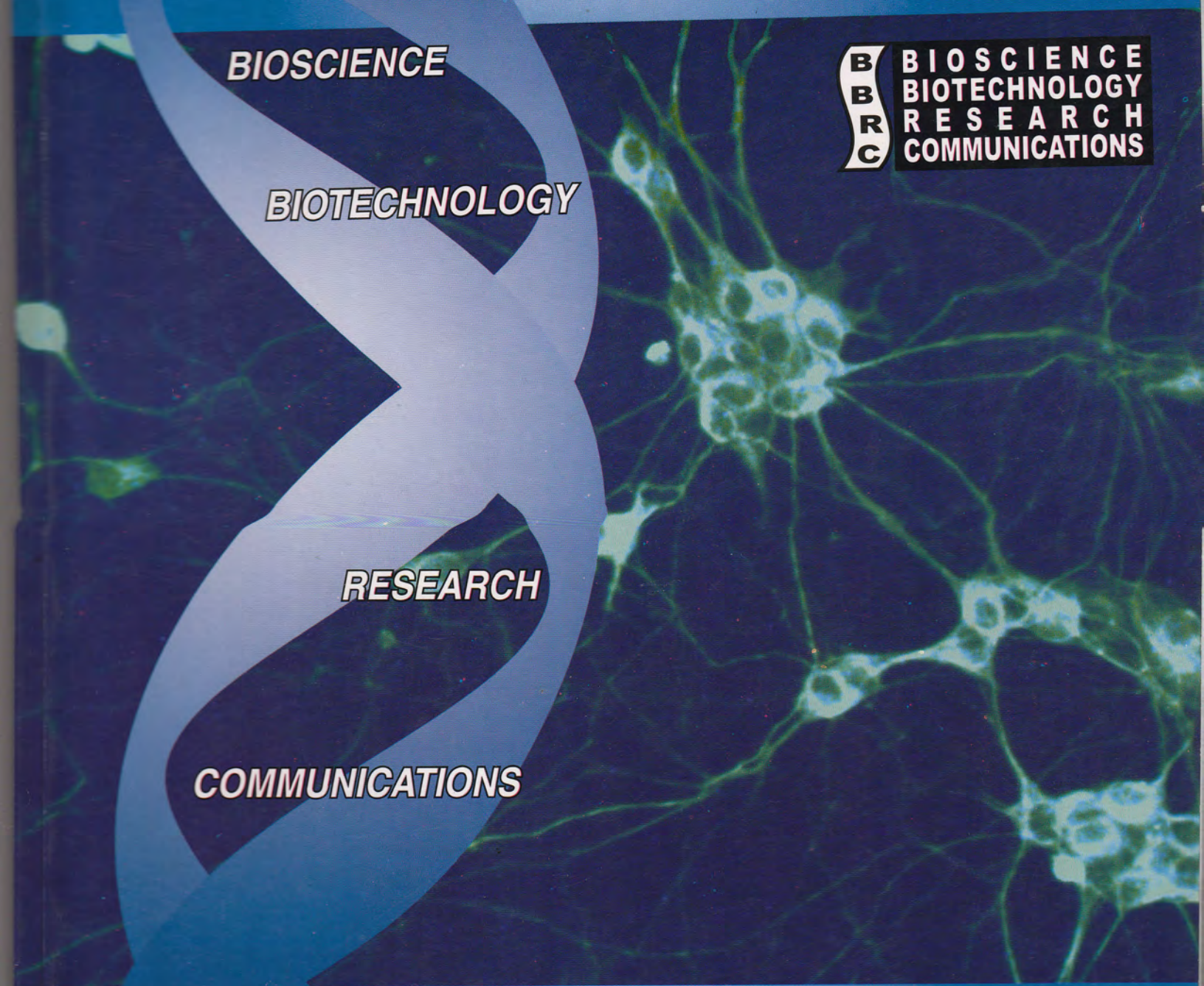
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INVITED COMMUNICATION

BIOTECHNOLOGY ENCAPSULATES THE DNA OF DEVELOPMENT

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ABSTRACT:

Biotechnology, globally recognized as a rapidly emerging and far-reaching technology, is aptly described as the "technology of hope" for its promise to deliver food security, life saving drugs, alternate energy and environmental sustainability. Human capital is perceived to be the key driver for global competitiveness. India has many assets in its strong pool of scientists and engineers, vast institutional network and cost effective manufacturing. The biotech sector also faces the same infrastructural hurdles that have affected Indian industry. The country continues to fall short on critical enablers such as quality roads and uninterrupted supply of power and potable water. However, beyond these common issues, the sector has its own problems that the government must address these in order to work symbiotically for the nations progress. Already a major hub, India has all that it takes to become a global biotech leader. This will not only spur economic growth and provide much-needed jobs, but will also ensure that we find answers to modern-day challenges in healthcare, energy, food security, and for the environment. However, biotechnology's promise and India's potential can be realized only if the government and industry work together and draw up a roadmap to facilitate innovation.

Keywords: *Biotechnology / DNA / Development*

Biotechnology, globally recognized as a rapidly emerging and far-reaching technology, is aptly described as the "technology of hope" for its promise to deliver food security, life saving drugs, alternate energy and environmental sustainability. Human capital is perceived to be the key driver for global competitiveness. India has many assets in its strong pool of scientists and engineers, vast institutional network and cost effective manufacturing. There are over a hundred National Research Laboratories employing thousands of scientists. There are more than 300 college level educational and training institutes across the country offering degrees and diplomas in biotechnology, bio-informatics and the biological sciences, producing nearly 500,000 students on an annual basis. About 300,000 postgraduates and 1500 PhDs qualify in biosciences and engineering each year. According to reports, outside of the US, India ranks the highest with 61 USFDA-approved plants and in excess of 200 GMP certified pharmaceutical manufacturing facilities.

The Indian Government has rolled out its National Biotechnology Development Strategy which is a well thought out, comprehensive road map for this emerging sector. The document is cognizant of the challenges of building both scale and critical mass in pursuing a global leadership profile. The Biotech Industry is poised to deliver a size of $5 billion by 2010 with Biopharma driving the growth trajectory. However, funding, infrastructure, regulation and skill competency mapping pose obstacles in this path to the future. Conversely, India is also uniquely placed to build a strong competitive edge in Biotechnology. For one, India offers an attractive cost arbitrage in Research & Development at roughly a third of that in the western hemisphere. Key enablers include a large, qualified English-speaking workforce, a network of reputed research laboratories and state-of-the-artpharmaceutical labs and

manufacturing facilities. Further, the patient population offers a large and diverse pool for effective clinical researc and development. As for external stimuli, the ever-increasing cost and time involved in drug discovery an development, fierce competition and pricing pressure are all spurring Western Pharmaceutical companies to hav an India strategy. A large number of blockbuster drugs are also set to go off-patent, giving the sector hea tremendous opportunities for growth. The industry is collaborating with global giants in clinical trials, discovery an development research, and manufacturing, thus rapidly moving up the value chain. In this age of hyper competitio and wafer-thin margins, India's biotech sector is poised at an inflection point.

In spite of these significant advances, the Indian industry continues to face numerous challenges. Foremost amon these is finance where venture funding has abstained from investing in this sector on account of its high risk profile This aversion to risk is also seen within the biotech sector itself which prefers to opt for low risk ventures based o services and generics shying away from an innovation led business model. The Department of Biotechnology has plugged this deficiency through a number of funding schemes viz. SBIRI, BIPP, BIRAP and others. It is now fo entrepreneurs to avail of these funds and rise to the challenge of innovation.

The woes do not end there. The biotech sector also faces the same infrastructural hurdles that have affected Indian industry. The country continues to fall short on critical enablers such as quality roads and uninterrupted supply o power and potable water. However, beyond these common issues, the sector has its own problems that the government must address.

The sector requires a streamlined regulatory framework to accelerate the commercialization of products. At present numerous regulatory agencies pulling in different directions slow down the process of growth and weaken the cause of the industry. Bt Brinjal is a good example of how years of intensive research investment are unable to guarantee commercial returns. First in man clinical trials is still an unresolved aspect of Schedule Y. Further, for a knowledge- intensive industry like biotechnology, essential strong industry-academia connections are sadly lacking.

The government can lead the way in facilitating growth by treating biotechnology as a priority sector. R&D is the bedrock upon which biotechnology rests. The government must enable international patenting, which curiously does not qualify for tax deduction, and encourage investment. A regulatory environment that helps the drug discovery process and approves products without delays is urgently required. A five-year tax holiday on new products developed in-house can be a great incentive to encourage R&D. Profits on such products can be mandated for re-investment in R&D to encourage development of newer and better drugs at lower costs. This will set in motion a virtuous cycle of discovery and growth that can benefit the sector in particular and the economy at large.

The Indian biotechnology sector truly needs creativity to aggressively harness its potential and assume global leadership. There exists a huge opportunity for growth – but only if innovation becomes part of the business ethic and a primary enterprise driver. It is no longer enough to produce clones of pharma products that have saturated the market, which do nothing to add value. Benchmarks must be set high and out-of-the-box thinking must become the norm. Profit margins can be maintained through contract discovery and manufacturing for foreign firms.

Extensive research, however, requires expensive equipment that needs to be imported. It is here that the government can step in again by exempting or reducing import duties on such equipment. It can also approve the Association of Biotech Led Enterprise's (ABLE) recommendation to set up a Biotech Fund to support first- generation biotech entrepreneurs up to the 'proof of concept' stage. ABLE has also urged the government to exempt venture funds investing in the biotech sector from capital gains tax. That can act as a reward for the longer-term investment cycles.

Already a major hub, India has all that it takes to become a global biotech leader. This will not only spur economic growth and provide much-needed jobs, but will also ensure that we find answers to modern-day challenges in healthcare, energy, food security, and for the environment. However, biotechnology's promise and India's potential can be realized only if the government and industry work together and draw up a roadmap to facilitate innovation.

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REVIEW ARTICLE:

MEDICINES AND METHOD OF THERAPY IN THE ARAB AND ISLAMIC WORLD

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ABSTRACT:

Arab and Islamic medicine is widely used in most Arab and Islamic world which form about one fifth of the world's population. The Arab and Muslim world refers in geopolitical sense to Muslim majority countries or countries in which Islam dominates politically. This community is spread across many different nations and ethnic groups connected only by religion. Medicine in general is considered to be one of the best known facets of Arab-Islamic civilization. Islamic medicine or Arab-Islamic medicine refers to medicine developed in the medieval Islamic civilization and written in Arabic, the lingua franca of the Islamic civilization. The development of Arab-Islamic medicine was based on, Practical knowledge developed traditional healers in the pre-Islamic period based on a long history of trial and error, by theoretical and practical knowledge developed in the Greek, Indian and Persian medical systems, which became available to Arab and Muslim scholars after translation of their medical scripts. These include natural products mentioned in the holly Quran or in the Hadith of the Prophet Mohammad (PBUH), Theoretical and practical innovations introduced by Arab and Muslim scholars. To keep within the scope of the present review, we will highlight contributions of Islam and innovations introduced by Arab and Muslim scholars in the fild of lerbal medicine.

Key words: *Arab-Islamic medicine; Herbal medicine, Tibb al Nabawi.*

INTRODUCTION

The development of Arab and Islamic medicine was simultaneous to Prophet Mohammad's (peace be upon him) (570-632 AC) journey from the Quraysh tribe in Mecca, when at the age of 40 he began to receive the Holy Quran in verses. He was able to unite the Arab tribes who had been isolated by revenge, rivalry, internal fights, and created a strong nation, able to defeat the Persian and Byzantine Empires. By the time of his death, all of Arabia had adopted Islam and a century later his followers had conquered half of Byzantine Asia, all of Persia, Egypt, the Maghreb (North Africa), and Spain. The Muslims not only conquered new lands and preserved their cultures but also became scientific innovators through originality and productivity. In temporal terms this age (Arab and Islamic golden age) covered a period of roughly nine centuries – from the middle of the seventh to the end of the fifteenth century, by which time the empire was divided into three distinct empires, the Safavid empire in Persia (modern Iran), the Mughal empire in India, and the Ottoman empire centered in Turkey. The Islamic Empire one of the most advanced and civilized nation in the world. This is because Islam stressed the importance and respect of learning, forbade destruction, and tolerance for other religions. Medicine

flourished because it was promoted by Caliphs, while Baghdad, Seville, Toledo, Granada, and other cities were well known as main centers for Arab-Islamic medical sciences and culture. By the tenth century their zeal and enthusiasm for learning resulted in the translation of essential Persian, Indian and Greek medical writings into Arabic in Damascus, Cairo, and Baghdad. Arabic became the international language of learning and diplomacy. The center of scientific knowledge and activity shifted eastward, and Baghdad emerged as the capital of the scientific world. Greco-Arab and Islamic medicine was a result of Roman, Greek, Persian, and Indian theories and practices, within the general context of Islam's system of ethics (Figure 1). Arabs then established and promoted their own medical sciences in theories and practices that became highly influential in Western science and teaching. Physicians, whether they were Muslims, Christians, or Jews, under the umbrella of Islam raised the dignity and caliber of the medical profession. During the Islamic golden age, the medical sciences rose in esteem from that of a menial calling to the rank of a learned profession. Arab and Islamic medicine had advanced from ephemeral talisman and theology to tangible hospital wards, mandatory testing for doctors and the use of technical terminology. Baghdad and Cairo had hospitals open to both male and female patients; staffed by attendants of both sexes. These medical centers contained libraries, pharmacies, intern systems, externs and nurses. There were mobile clinics to reach the disabled, the disadvantaged and those in remote areas. There were regulations to maintain quality control on drugs. Pharmacists became licensed professionals and were pledged to follow the physician's prescriptions (Saad *et al.*, 2005, 2008(a), Said *et al.*, 2002 , Albitar *et al.*, 1974, Alturkmani *et al.*, 1930, Sena *et al.*, 1994).

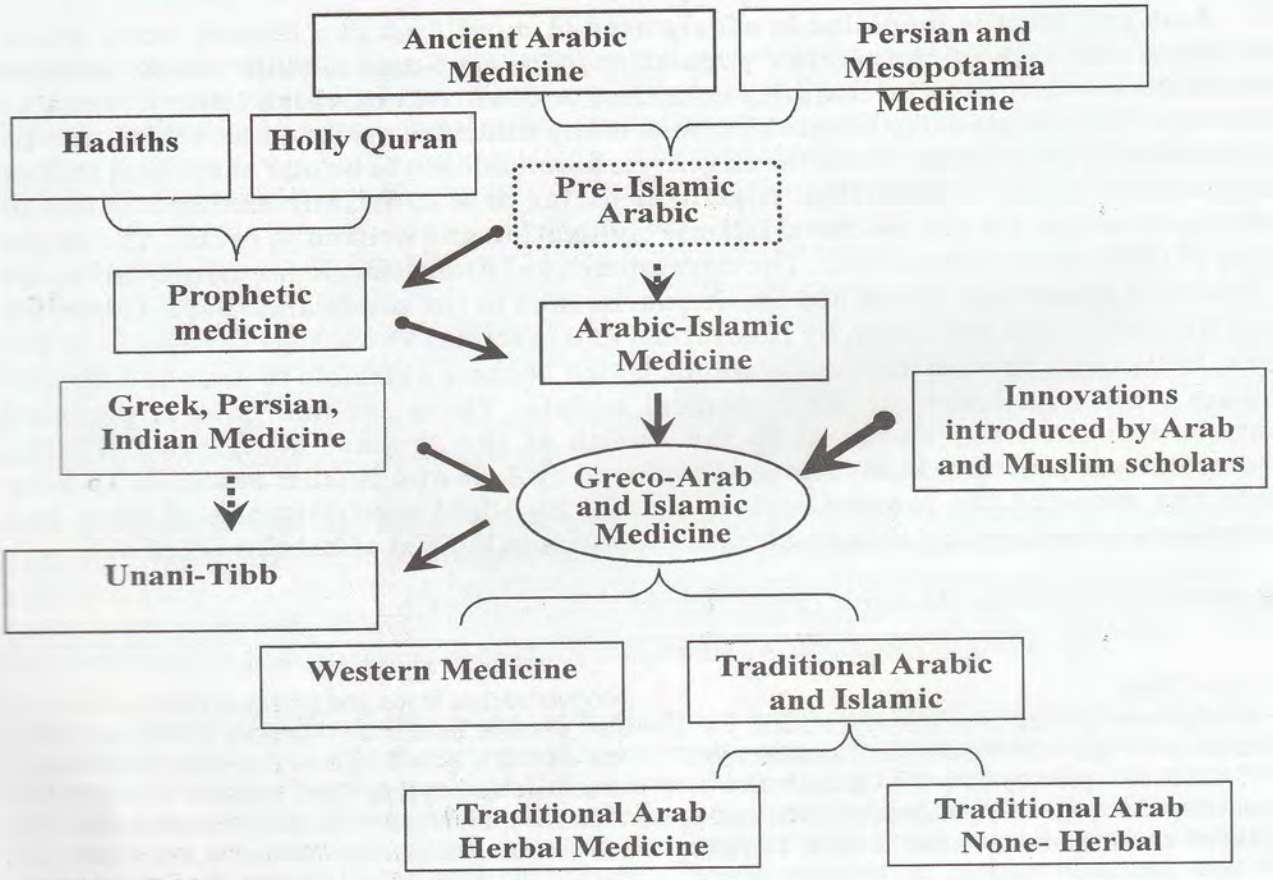


Figure 1. Development of Arab and Islamic medicine*. Arab and Islamic medicine became one of the most famous and best known achievements of Arab-Islamic civilization. Arab and Islamic medicine was a result of Roman, Greek, Persian, and Indian theories and practices, within the general context of Islam's system of ethics. Arabs then established and promoted their own medical sciences in theories and practices that became highly influential in Western science and teaching. Use of the Arabic language enabled a mix of diverse peoples speaking different languages to communicate with one another.*

Theoretical and practical knowledge introduced by the Islam.

In Islamic tradition, the first Arab-Muslim physician is believed to have been the Prophet Mohammad (PBUH) himself, as a significant number of Hadiths concerning medicine are attributed to him. The three methods of healing known to have been mentioned by him were honey, Hijamah (cupping), and Key (cauterization), though he was generally opposed to the use of cauterization unless it "suits the ailment." In addition, many plants and animal products are mentioned in the holly Quran and in the Hadith of the Prophet (PBUH), e.g., dates, black seeds, olive leaf and olive oil, and camel's milks. These products were used by the Prophet as diet as well as in treatment of various diseases (Figure 2). Later on these products were utilized by the medicine of the Prophet (Al-Tibb al-Nabawi), which includes medical treatments, prescriptions, prevention, health promotion, and spiritual aspects that were recommended by Prophet (PBUH) to his companions. As aforementioned, this medical practice adopted many medical knowledge and practices existed in pre-Islamic period (Figure 1). Two Hadiths of the Prophet (PBUH), *"The one who sent down the disease sent down the remedy."* and *"For every disease, Allah has given a cure"* encouraged Muslims to search for remedies for each diseases and greatly influenced the development of Arab-Islamic medicine during the golden age of the Arab-Islamic civilization (Saad *et al.*, 2005, 2008(a), Said *et al.*, 2002 , Albitar *et al.*, 1974, Alturkmani *et al.*, 1930, Sena *et al.*, 1994).

The book of Medicine (*kitab al-tibb*) of Sahih al-Bukhari by Imam Bukhari (810-870 A.D) is considered by the majority of the Muslim scholars to be one of the most credible collections of what had been said and practiced (Hadith and Sunnah) by the Prophet (PBUH). The scope of the Prophetic medicine has been explained in the very well known commentaries of Sahih al-Bukhari by Ibn Hajar Al-Asqalani (d. 1449 A.D.) and Abu Muhammad Al-`Ayni (d. 1452 A.D.), both were living in the golden age of the Arab-Islamic civilization when medical literature over flowed with all sorts of medical disciplines. It was probably in consequence of reading those literatures that they were in preference to hold that medicine of the Prophet, as other medical sciences, does not only refer to what had been said and practiced in the time of Prophet but it reaches and includes every field of human medical research, practices and thought at all time. Ibn Hajar divides the science of medicine into two types namely physical medicine and spiritual medicine, which are associated through a symbiotic relationship. Therefore, physicians should be fully aware of spiritual and physical medicine because, in Islam, the breath and the body, the soul and matter, the faith and the world have been accorded equal importance. The importance of the relationship between both types of medicines was highlighted by Ibn Sina who stated *"We have to understand that the best and effective remedy for the treatment of patients should be through the improvement of the power of the human body in order to increase its immune system, which is based on the beauty of the surroundings and letting him listen to the best music and allow his best friends to be with him"*. Hence, there is equilibrium in the nature of the body with the nature of heart, the body stays healthy. When equilibrium is lost, things become contra-natural and disease is produced (Syed, 2003 and Deuraseh, 2006).

The Prophetic medicine is not based on medical experiments but rather on inspiration, experience from the previous culture and tradition. Diet plays a central role in the Prophetic medicine. According to a Hadith, the stomach is the central basin of the body and origin of many diseases *"The stomach is the central basin of the body, and the veins are connected to it. When the stomach is healthy, it passes on its condition to veins, and in turn the veins will circulate the same and when the stomach is putrescence, the veins will absorb such putrescence and issue the same"*. Indeed, the prophet (PBUH) used to recommend food for ailments even more than herbs or animal-based medicines. He used everything from barley soup to honey to camel's milk to heal his followers and advised them to eat certain foods to prevent or cure other diseases. Honey, camel's milk, dates, olive oil, and black seeds were the favored foods by the Prophet (PBUH) who regarded food as part of an overall holistic approach. Concerning olive oil, he said *"Eat olive oil and massage it over your bodies since it is a holy tree"*. Black seeds were regarded as a medicine for that cures all types of diseases. He once stated, *"The black seed can heal every disease, except death"*. Dates are mentioned in 20 places in the Quran. Prophet (PBUH) is reported to have said: *"if anyone of you is fasting, let him break his fast with dates. In case he does not have them, then with water. Verily water is a purifier"*. In the following we will highlight the uses and pharmacological significance of some of diets used and recommended by the Prophet (PBUH) (Syed, 2003 and Deuraseh, 2006).

Camel's milk: Milk is rich in proteins and peptides, which play a crucial role in innate immunity when transferred to the offspring and may accelerate maturation of the immune system in newborns. The immune properties of these molecules prompted investigators to investigate their potential application in prevention and therapy for newborns and adults. Camel's milk is different from other ruminant milk; having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C, B2, A and E, low protein and high concentrations of insulin. It has no allergic

properties and it can be consumed by lactase deficient persons and those with week immune systems. The milk is considered to have medicinal properties. In Sahara, fresh butter is often used as a base for medicines. The products developed also include cosmetics or pharmaceuticals.

A series of metabolic and autoimmune diseases are successfully being treated with camel's milk. In India, camel's milk is used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes. Beneficial role of raw camel's milk in chronic pulmonary tuberculosis patients has been observed. In repeated trials, it was observed that there was 30-35% reduction in daily doses of insulin in patients of type 1 diabetes receiving raw camel's milk. The Prophet (PBUH) mentioned that camel's milk and urine have medical effects, so Islam encourages and permits the drinking of camel milk and camel urine is permitted in case of necessary medical treatment. Bedouins treat many diseases and disorders with camel milk. This range from osteoporosis, rickets, hepatitis, digestive ulcers and disorders, spleen problems, tuberculosis, asthma, flu, and other respiratory diseases to controlling heartbeat, hypertension and diabetes (Saad *et al.,* 2005, 2008(a), Said *et al.,* 2002 , Albitar *et al.,* 1974, Alturkmani *et al.,* 1930, Sena *et al.,* 1994).

Honey: In Arab-Islamic medical system, as in other medical systems, including Ayurvedic, Chinese, and Roman traditions, honey is considered as healthy drink. The holy Qur'an describes its potential therapeutic value. "*And thy Lord has inspired the Bees, to build their hives in hills, on trees and in man's habitations, from within their bodies comes a drink of varying colours, wherein is healing for mankind, Verily in this is a Sign, for those who give thought".* Imam Bukhari entitled chapter four of his Kitab al-Tibb (book of medicine) as "*al-Dawa'bi al-Asal wa Qawlihi Ta`ala 'Fihi Shifa li al-Nas* (treatment with honey and the statement of Allah: where is healing for men)." At this place, Imam al-Bukhari mentioned three ahadith on honey together with its value which is emphasized in many verses of the Quran. Al Razi (Rhazes, 864-932 A.D), claimed that a mixture made of flour and honey vinegar was good for skin disease and sports nerve injuries and recommended the use of honey water for bladder wounds. His book, *Al Hawi* (Encyclopedia of Medicine) he stated: *"Honey is the best treatment for the gums. To keep the teeth healthy mix honey with vinegar and use as mouth wash daily. If you rub the teeth with such a preparation, it will whiten the teeth. Honey does not spoil and could also be used to preserve cadavers".* Ibn Sina (Avicenna, *980-1037A.D*) recommended to use honey as part of an overall holistic approach to health and should be incorporated into one's everyday diet. He stated in his Canon: *"Honey is good for prolonging life, preserve activity in old age. If you want to keep your youth, take honey. If you are above the age of 45, eat honey regularly, especially mixed with chestnut powder. Honey and flour could be used as dressing for wounds. For lung disease, early stage of tuberculosis, use a combination of honey and shredded rose petal. Honey can be used for insomnia on occasions".* (Sena *et al.,* 1994, Syed, 2003, Deuraseh, 2006, Bogdanov *et al.,* 2008, Al-Quassemi and Robinson, 2003, Irish *et al.,* 2006, Al-Waili and Boni ,2003).

Olive oil: Olive oil and olive leaf are cited in the Bible as a natural healer:*" The fruit thereof shall be for meat and the leaf thereof for medicine".* In Islam, olive oil is mentioned in the Quranic verse:

"God is the light of heavens (paradise) and earth. An example of His light is like a lantern inside which there is a torch, the torch is in a glass bulb, the glass bulb is like a bright planet lit by a blessed olive tree, neither Eastern nor Western, its oil almost glow, even without fire touching it, light upon light" . The Qur'an also mentions (tells) olives as a sacred plant: *"By the fig and the olive, and the Mount of Sinai, and this secure city."* Prophet Muhammad (PBUH) said, *"Eat olive oil and massage it over your bodies since it is a holy (mubarak) tree".* He also stated that olive oil cures 70 diseases. In the Arab-Islamic world, olive oil has been commonly used in cooking, cosmetics, pharmaceuticals, and soaps and as a fuel for traditional oil lamps.

Olive oil, the main source of fat in the Mediterranean diet, has been associated with a low cardiovascular and cancer mortality. The beneficial effects of olive oil on coronary heart disease (CHD) risk factors are now recognized attributed to the high MUFA content and other minor compounds found in the olive oil. Evidences from epidemiological studies suggest that a higher proportion of MUFA in the diet is linked with a reduction in the risk of coronary heart disease. There is a large body of clinical data that show that consumption of olive oil can provide heart health benefits such as favourable effects on cholesterol regulation and LDL oxidation. It exerts also anti-inflammatory, antithrombotic, antihypertensive as well as vasodilatory effects both in animals and in humans. Olive oil, however, besides having a high MUFA level, the oleic acid, contains multiple pharmacology active components. Olive oil phenolics have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation. They are prevent endothelial dysfunction by decreasing the expression of cell adhesion molecules, and increasing nitric oxide (NO) production and inducible NO synthesis by quenching vascular endothelium intracellular free radicals. In addition, olive oil phenolic compounds inhibited platelet-induced aggregation and have been reported to enhance the expression the gene of the antioxidant enzyme glutathione peroxidase. Other potential properties include anti-inflammatory and chemopreventive activity. In animal models, olive oil-derived phenolics retained their antioxidant properties

in vivo and delayed the progression of the atherosclerosis. So far, most of the cardioprotective effect of olive oil in the context of the Mediterranean diet has been attributed to its high MUFA content. It must be noticed, however, that oleic acid is one of the predominant fatty acids in widely-consumed animal foods in Western diets, such as poultry and pork. A direct association of meat intake with the plasma oleic acid concentration was observed in a Swedish female population. In this population, oleic acid plasma concentrations were higher than those of females of Granada in Spain, without differences in polyunsaturated (PUFA) levels. Thus, perhaps a high oleic acid intake is not the sole primary responsible agent for the healthy properties of olive oil. In spite of the promising role for health displayed in experimental studies, evidence of the benefits of olive oil phenolic compounds consumption in humans is still on the debate. If the beneficial properties of olive oil in humans can be attributed solely to its MUFA content, any type of olive oil, rapeseed/canola oil, or MUFA-enriched fat would provide the same health benefits. Thus, public health implications are involved in order to specifically recommend olive oil, and which type of olive oil, (*i.e.*, virgin olive oil rich in phenolic compounds) as individualized nutritional strategies for CHD prevention (Saad *et al.,* 2005, 2008(a), Sena *et al.,* 1994, Covas , 2008, Goulas *et al.,* 2009).

Dates: Dates are an important traditional crop in Iraq, eastern region of the Mediterranean, and Maghreb countries and are mentioned in 20 places in the Quran. Prophet Mohammad (PBUH) is reported to have said: "*if anyone of you is fasting, let him break his fast with dates. In case he does not have them, then with water. Verily water is a purifier*". Therefore, in Islamic countries, dates and yogurt or milk is a first meal when the sun sets during Ramadan. Dry or soft dates are eaten out-of-hand, or may be pitted and stuffed with fillings such as almonds, walnuts, or marzipan Dates were favored food of the Prophet who said, "*Whoever takes seven dates in the morning will not be effected by magic or poison on that day.*" Dates contain high amounts of dietary fibers, which are important for the health of the digestive tract. Dietary fibers consist of the edible plant material, which is not hydrolyzed by the human digestive tract. Many studies recommend the public to consume adequate amounts of dietary fiber from a variety of plant foods. Dates also contain useful quantities of antioxidants. Antioxidants are thought to play an essential role in the prevention of cardiovascular disease, cancers, and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases, as well as inflammation and continuous ageing. A dietary antioxidant is defined as a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen, on normal physiological function in humans. Antioxidants markedly delay or prevent oxidation of the substrate when they are present in foods or in the body at low concentrations. Natural antioxidants consist primarily of plantphenolics, vitamin C, carotenoids, and selenium. Examples of common plant phenolic antioxidants include flavonoid compounds (anthocyanins), cinnamic acid derivatives, coumarins, and tocopherols (vitamin E). The average contents of phenolics ranged from 193.7 mg/100 g for fresh dates to 239.5 mg/100 g for dried dates (Saad *et al.,* 2005, 2008(a), Al-Shahib and Marshall, 2003, Al-Farsi and Lee, 2008, Al-Rawahy, 2007, Almana and Mahmoud, 1994 and Vayalil, 2002).

Black seed (Nigella sativa): Black seed is one of the most commonly used medicinal plants throughout the Middle East. Black seeds are known to have many medicinal properties and are widely used in Arab and Islamic medicine. *Black* seeds have been used for centuries as a spice and food preservative, as well as a protective and curative remedy for numerous diseases. The seeds are the main source of the active compounds of the plant. Black seeds were used by ancient Egyptian and Greek physician to treat nasal congestion, toothache, as a diuretic to promote menstruation, and increase milk production. The seeds, known as black seed, black cumin or "Habatul-Barakah" in Arabic, have long been prescribed in Greco-Arab and Islamic medicine as well as in Indian and Chinese traditional medicine for prevention and treatment of a wide range of diseases, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems. It is the black seed referred to by the prophet Mohammad (PBUH), who once stated, *"The black seed can heal every disease, except death"*. Avicenna (980-1037 AC) refers to black seed in his *Canon of Medicine*, as the seed that stimulates the body's energy and helps recovery from fatigue and dispiritedness. In the Unani Tibb system of medicine, seeds are regarded as a valuable remedy for a number of diseases. The seed's oil has been used to treat skin conditions such as eczema and boils and to treat cold symptoms. In conclusion, its many uses have earned black seed the Arabic name 'Habbatul barakah', meaning the *seed of blessing*. The aforementioned statement by the Prophet Mohammad describing black seed, as "*having a remedy for all illnesses*" may not be so exaggerated as it at first appears. Recent research has provided evidence, which indicates that black seed contains an ability to significantly boost the human immune system - if taken over time. The prophetic phrase, "*hold onto the use of the seed*," also emphasizes consistent usage of the seed. Therefore, one important is that black seed should be regarded as part of an overall holistic approach to health and should be incorporated into one's everyday diet. In this way, nutritional values and

therapeutic properties contained in the black seed can help in maintaining a healthy condition and supplying the immune system with the optimum resources it needs to help prevent and treat diseases. Therefore, in cognizance of black seed's substantial nutritional components, as well as its specific medicinal properties, the body's ability to maintain health and promote healing of a lasting nature is best increased through regular use of black seed.

The black seed is traditionally used in eastern Mediterranean as an enhancer of milk production during breastfeeding. Black seed is an excellent form of added nutrition for both mother (black seeds mixed with toasted flour, toasted sesame, and honey and prepared as cakes) and the growing child while its immune system boosting properties serve as a natural, safe way to build resistance against illness. In addition, as studies have shown, black seed helps increase milk production during breastfeeding. Therapeutic potential and toxicological properties of the seeds have been extensively studied A Medline and Google Scholar search using "*Nigella sativa*" and "medicine" reveals more than 1600 citations, including anti-oxidant, anti-inflammatory, anti-microbial, hypotensive, anti-nociceptive, choleretic, uricosuric, choleretic, anti-diabetic, and anti-histaminic, immunomodulatory, anticancer, and anti-fertility effects (Saad *et al.,* 2005, 2008(a), and Salem, 2005).

Pomegranate: The fruit, commonly known as Rumman in the Arab world, can be divided into three structural compartments: seed, juice, peel. Pomegranate molasses (known as dibs rumman in Arabic) is an essential Middle Eastern ingredient. The pomegranate has long been used in traditional Arab-Islamic medicine to treat a variety of ailments, including sort throat, inflammation and rheumatism. These uses of the pomegranate are common throughout the Middle East, Iran, and India, where the fruit is common. Additional traditional uses include treatment of diarrhea and colic and to remove intestinal worms in children. The fruit is also used for treating bladder disturbances, strengthening gums and soothing mouth ulcers. In India, the leaf of the pomegranate is used to treat cuts, as it contains a natural healing and soothing agent. Pomegranates feature prominently in all religions, Judaism, Christianity, Islam, Buddhism and Zoroastrianism. According to the Qur'an, pomegranates grow in the gardens of paradise. Pomegranates, along with dates and olives, are also mentioned in the following verse from the Holy Qur'an, which speaks of the dues that have to be paid upon each harvest, as well as the evil of wastefulness. *"And it is He Who produces gardens trellised and untrellised, and date-palms, and crops of different shape and taste and olives, and pomegranates, similar (in kind) and different (in taste). Eat of their fruits when they ripen, but pay the due thereof on the day of its harvest, And waste not by extravagance. Verily, He likes not those who waste by extravagance".* The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called punicalagins, which are powerful anti-oxidants. Punicalagins are absorbed into the human body and may have dietary value as antioxidants; other phytochemicals include beta-carotene, and polyphenols catechins, gallocatechins, and anthocyanins such as prodelphinidins, delphinidin, cyanidin, and pelargonidin. The fruit contains also Vitamin C at 0.47 mg/100 g. The pharmacological uses of the pomegranate, as was seen with the two other plants of the Qur'an, dates and olives, are numerous. These include anti-oxidant, hormone replacement therapy, resolution of allergic symptoms, cardiovascular protection, oral hygiene, ophthalmic ointment, weight loss soap, and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging. Pomegranate mediated antioxidant activity can be considered a means of lowering the threshold for inflammation. Antioxidant activity, as well as suppression of inflammation, may contribute to chemotherapeutic and chemopreventive utility against cancer (Saad *et al.,* 2005, 2008(a), Lansky and Newman, 2007).

Innovations introduced by Arab and Muslim scholars.

Arab and Muslim scholars established and promoted their own medical sciences in theories and practices that became highly influential in Western science and teaching. Like in other fields of science, Arab-Muslim physicians developed the first scientific methods for the field of medicine. This included the introduction of experimentation, quantification, experimental medicine, evidence-based medicine, clinical trials, dissection, animal testing, human experimentation and postmortem autopsy by Muslim physicians, whilst hospitals in the Arab-Islamic world featured the first drug tests, drug purity regulations, and competency tests for doctors.

Arab-Muslim scholars were not guided by a long history of trial and error, but mainly by scientific methods, which has led to production of evidence based medication. Avicenna (Ibn Sina) (Figure 2) discussed in his second book (on simple drugs or *materia medica*) the nature and quality of drugs, and the way that mixing them influences their effectiveness. He stated *"You can tell the potency of drugs in two ways, by analogy* (qiyas) *and by experiment* (tajribah). *We say experimenting leads to knowledge of the potency of a medicine with certainty after taking into consideration certain conditions."*

Abu Ali al-Husayn ibn Abd Allah ibn Sina (980-1037), known as Avicenna, began his studies in Bukhara under the guidance of several well-known scholars of the time. He studied logic, philosophy, metaphysics, and natural sciences, and gradually developed an interest in medicine. Ibn Sina is remembered in Western medical history as a major historical figure who made fundamental contributions to medicine and the European reawakening. About 100 treatises were ascribed to Avicenna. The best-known amongst them is his 14-volume The Canon of Medicine, which was a standard medical text in Western Europe for seven centuries.



Responding to circumstances of time and place, Arab-Islamic physicians and scholars developed a large and complex medical literature exploring and synthesizing the theory and practice of medicine. They introduced many new ideas and upgraded the knowledge about herbs and their potential medical efficacy and safety. The first encyclopedia of medicine in Arabic was *Firdous al-Hikmah* ("Paradise of Wisdom") by Ali ibn Sahl Rabban al-Tabari written in seven parts around 860 A.D. It was the first to deal with pediatrics and child development, as well as psychology and psychotherapy. Al-Tabari emphasized strong ties between psychology and medicine, and the need of psychotherapy and counseling in the therapeutic treatment of patients. Al Razi (Rhazes) wrote the Comprehensive Book of Medicine in the 9th century. The Large Comprehensive was the most sought after of all his compositions, in which Rhazes recorded clinical cases of his own experience and provided very useful recordings of various diseases. The Comprehensive Book of Medicine, with its introduction of measles and smallpox, was very influential in Europe. Abu al-Qasim al-Zahrawi (Abulcasis), regarded as the father of modern surgery, contributed greatly to the discipline of medical surgery with his Kitab al-Tasrif ("Book of Concessions"), a 30-volume medical encyclopedia published in 1000 A.D., which was later translated to Latin and used in European medical schools for centuries. He invented numerous surgical instruments and described them in his al-Tasrif. Ibn Sina (Avicenna, (980-1037)) was another influential figure. He is regarded as the father of modern medicine. His medical encyclopedia, The Canon of Medicine (c. 1020 A.D), remained a standard textbook in Europe for centuries. Among other things, Avicenna's contributions to medicine include the introduction of systematic experimentation and quantification into the study of physiology, the discovery of the contagious nature of infectious diseases, the introduction of quarantine to limit the spread of contagious diseases, the introduction of experimental medicine, evidence-based medicine, clinical trials, randomized controlled trials, risk factor analysis, and the idea of a syndrome in the diagnosis of specific diseases, the contagious nature of phthisis and tuberculosis, the distribution of diseases by water and soil, and the first careful descriptions of skin troubles, sexually transmitted diseases, perversions, and nervous ailments, as well the use of ice to treat fevers, and the separation of medicine from pharmacology, which was important to the development of the pharmaceutical sciences. Herbal medicine was a central part of Islamic civilization. Numerous encyclopedias on botany were written, with highly accurate precision and details of medicinal plants. For

instance, Al-Dinawari (828-896) is considered as the founder of Arabic botany for his *Book of Plants*, in which he described about 640 plants and described the phases of plant growth and the production of flowers and fruit. Ibn Abil-Bayan in 1161 A.D in Spain published *The Bimaristan Law in Pharmacopoeia, Materitenses.* This book contains 607 detailed medications. Ibn Zuhr (Avenzoar) who lived in Seville, Spain (1091-1161 A.D) wrote the *Al Kitab Al Jami*, about liquids and creams.

This book includes 230 medications that are mostly herbal and a few are of animal and mineral origin. This book gives a full description of the uses of herbs whether they are roots, seeds, or leaves. In the early 13th century, the Andalusian-Arabian biologist Abu al-Abbas al-Nabati published several books and dictionaries on the use of medicinal plants describing each plant species, the plant parts used, the preparation procedure used for each remedy, and the treatment procedure of certain diseases. Ibn al Baitar who lived in Damascus, Syria (1197-1248 AD) published *The Book on Drinks and Foods,* which is a collection of different drinks and foods. It is the most prestigious book in the Arabian pharmacopeia; it contains 260 references. The medications were classified in alphabetical order. Daoud Al-Antaki used different herbs for treating patients and published a book on medicinal herbs summarizing the knowledge of his predecessors (Saad *et al.,* 2005, 2008(a), Said *et al.,* 2002 , Albitar *et al.,* 1974, Alturkmani *et al.,* 1930, Sena *et al.,* 1994, Syed, 2003 and Deuraseh, 2006).

Method of Therapy used in the Arab and Islamic Medicine

In Islam, the breath and the body, the soul and matter, the faith and the world have been accorded equal importance. Similarly, many Arab and Muslim physicians proposed that the body must be treated as a whole and not just a series of parts, and that it was endowed with an ability of natural healing, which depended on rest, a good diet, fresh air and cleanliness. They noted that there were individual differences in the severity of disease symptoms, and in the individuals' ability to cope with their disease and to heal. Hippocrates thus laid the foundations of the modern theory that thoughts, ideas and feelings, which he proposed to originate in the brain, can influence health and the process of disease. Rhazes supported this concept by the his recommendation: "*The physician, even though he has his doubts, must always make the patient believe that he will recover, for state of the body is linked to the state of the mind*". Later on, Avicenna who defined medicine as "*Medicine is the science from which we learn the states of the human body with respect to what is healthy and what is not; in order to preserve good health when is exists and restore it when is lacking*" supported the views of Rhazes. He stated that: *"We have to understand that the best and effective remedy for the treatment of patients should be through the improvement of the power of the human body in order to increase its immune system, which is based on the beauty of the surroundings and letting him listen to the best music and allow his best friends to be with him*".

It is now clear that the mind and the body interact, influence and regulate each other. The perception of stress can lead to production of 'stress hormones', as well as products of the immune system. These 'stress hormones' act in a feedback mechanism to regulate their own production and the production of certain immune products. These immune products act on the brain to modify behavior and ability to perceive and to respond to stressful challenges by inducing lethargy, fever and nausea (i.e. 'sickness behavior').

Based on recommendations of Rhazes and Avicenna, the Arab and Islamic medicine treats patients through a scheme starting with physiotherapy and diet; if this failed, drugs were used. Rhazes's treatment scheme started with diet therapy, he noted that "*If the physician is able to treat with foodstuffs, not medication, then he has succeeded. If, however, he must use medications, then it should be simple remedies and not compound ones*". Drugs were divided into two groups: simple and compound drugs. Physicians were aware of the interaction between drugs; thus, they used simple drugs first. If these failed, compound drugs, consisting of two or more compounds were used. If these conservative measures failed, surgery was undertaken (Figure 3) (Saad *et al.,* 2005, 2008(a), Said *et al.,* 2002 , Albitar *et al.,* 1974, Alturkmani *et al.,* 1930, Sena *et al.,* 1994, Syed, 2003 and Deuraseh, 2006).

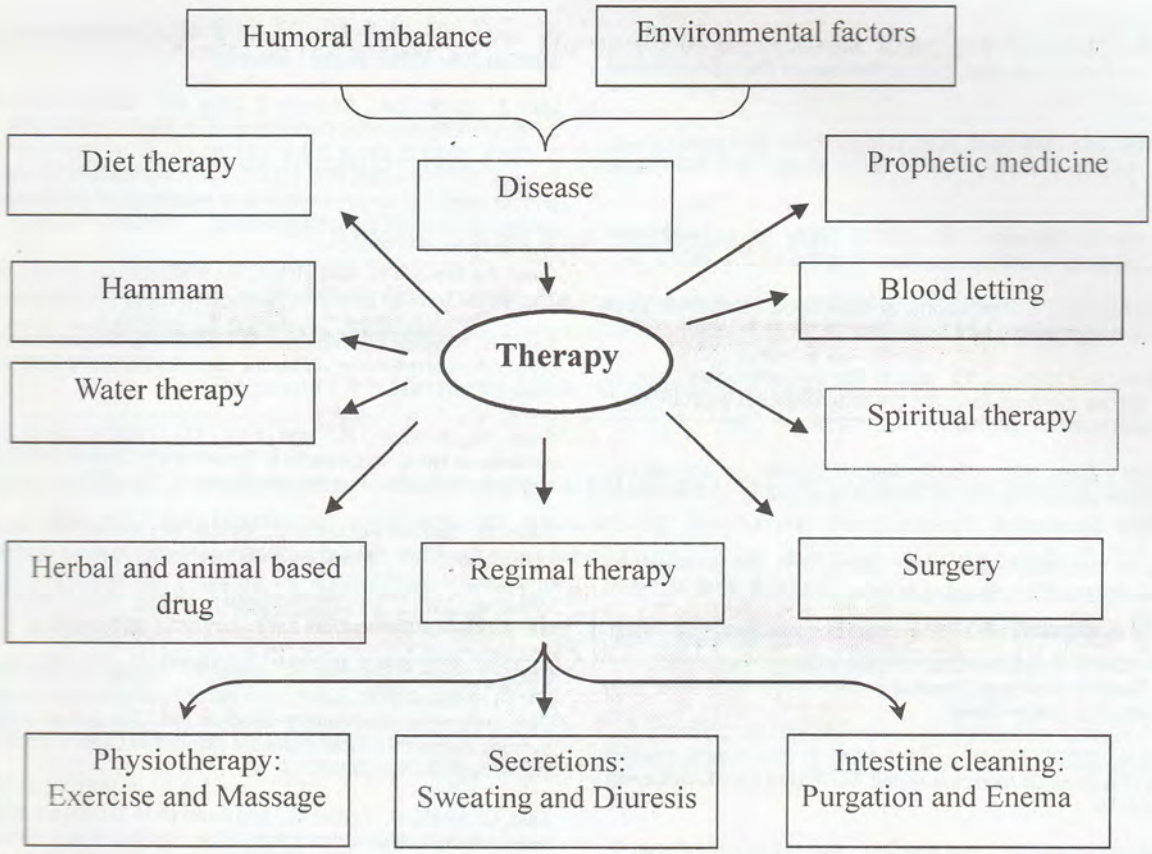


Figure 3. *Methods of therapy used in the Arab and Islamic medicine. The Greek and Roman humor theory or humouralism of the human body had a great influence on the development of the Greco-Arab medical system. Essentially, this theory held that the human body contains four basic fluids, called humors, which are in balance in healthy persons. Imbalances, e.g., an excess or deficit in one of four humors results in the development of a disease state. The humoral theory was adopted and further developed by Arab-Muslim physicians and became the most commonly held view of the human body among European physicians until the advent of modern medical research in the nineteenth century.*

Concluding remarks

Despite great progress in allopathic medicine, Arab-Islamic medicine has continued to be practiced within the Arab-Islamic world (Saad *et al.,* 2005, 2006 2008(a),2008(b), Said *et al.,* 2002, 2009). Cultural beliefs and practices often lead to self-care or home remedies in rural areas and consultation with traditional healers. Arab-Islamic therapies have been utilized by people who have faith in spiritual healers and herbalists. These people are the first choice for problems such as infertility, impotence, diabetes, obesity, epilepsy, psychosomatic troubles, and many other diseases. The modern use of Arab-Islamic herbal medicines has historical roots in Greco-Arab and Islamic medicine, which was developed in the golden age (seventh to fifteenth century) of the Islamic civilization and written in Arabic, the lingua franca of the Islamic civilization. This medical tradition has influenced the fates and fortunes of countless human beings. In addition to being fascinating in its own right, Arab-Islamic medicine is also important because of its influence on Europe where it formed the roots from which modern Western medicine arose. To be sure, the earlier Greco-Roman scholarly medical literature was the stem from which much Arab-Islamic medicine grew, just as, several centuries later, Arab-Islamic medicine was to be the core of late middle ages and early European medical education. As discussed above, however, Arab-Islamic medicine was not simply a continuation for Greek ideas but it was a venue for innovation and change. Medical innovations introduced by Arab and Muslim physicians included: discovery of the immune system, introduction of microbiological science, and the separation of pharmacological science from medicine. Owing to a statement (Hadith) by the Prophet Mohammad (PBUH), "*The one who sent down the disease sent down the remedy.*" and *"For every disease, God has given a cure."* every Muslim is encouraged to search for those remedies and use them with skill and compassion.

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REVIEW ARTICLE

T_H17 CELLS: NEW MEMBERS OF T HELPER (T_H) LYMPHOCYTE FAMILY

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ABSTRACT:

T helper cells (T_H) cells are special subpopulation of $CD4^+$ T cells which provide help to other cells of the immune system in mounting immune responses against specific pathogens by secreting their effector cytokines. The newly discovered T_H17 cells constitute an important third arm of T helper (T_H) cell family, have been the subject of recent attention and intense research for immunologist. Here we review, Th17 cell development, differentiation and it's role in host defense and autoimmunity.

Key words: *lymphocytes, T helper cells (T_H) Cells, T_H17 cells, Autoimmunity, interleukin (IL).*

INTRODUCTION

The hallmark of adaptive immunity in advanced vertebrates is the existence of lymphocytes, which induce and regulate immune responses. In 1986, Mosmann and Coffman introduced the concept of distinct types of helper T (T_H) lymphocytes, which was based on the type of cytokines that T cells produce when they are stimulated by pathogens in a specific cytokine mileu. Upon antigenic stimulation, naive T_H cells which are always $CD4^+$ T lymphocytes, differentiate into different subsets with distinct effector functions aimed at orchestrating and mobilizing other cell types to effectively clear invading pathogens. Initially they proposed existence of two distinct effector T_H subsets : type 1 helper T cells (T_H1 cells) and type 2 helper T cells (T_H2 cells). T_H1 cells produce interferon – ??(IFN-?) and mediate protection against intracellular pathogens, whereas Th2 cells produce interleukin-4 (IL-4), IL-13 and IL-25 and orchestrate the clearance of extracellular pathogens (Mosmann, and Coffman 1989). Recently this paradigm has been updated by the discovery of a third subset of T_H cells; which are, known as T_H17 cells, produce IL-17 and exhibit distinct effector functions (Yao *et al.*,1995). (fig: 1). While the function of this cell subtype is not completely elucidated, emerging data suggest that T_H17 cells may play an important role in host defense against those pathogens, which are not efficiently cleared by T_H1 and T_H2-type immunity (Kolls and Linden, 2004, Park *et al.*, 2005 and Ye *et al.*, 2001).

Origins of T_H17 cells:

The story begins when people found that $CD4^+T_H$ cells are producing cytokines that could not be classified according to the T_H1–T_H2 scheme. IL-17 was among these cytokines and the T cells that preferentially produce IL-17, could not produce T_H1 or T_H2 specific cytokines like IFN-??or IL-4 respectively, were named as T_H17 cells (Bettelli *et al.*, 2007 and Harrington *et al.*, 2005). Evidences for the existence of T_H17 cells were collected from several studies, which involves study of *Borrelia burgdorferi* infection in animal models. Stimulation with sonicates and synthetic antigens of the bacteria induced IL-17 production by T cells, independently of the production of T_H1 and T_H2 cytokines. The same pattern of IL-17 secretion was induced by the BCG strain of *Mycobacterium* bovis (Infante *et al.*, 2000).

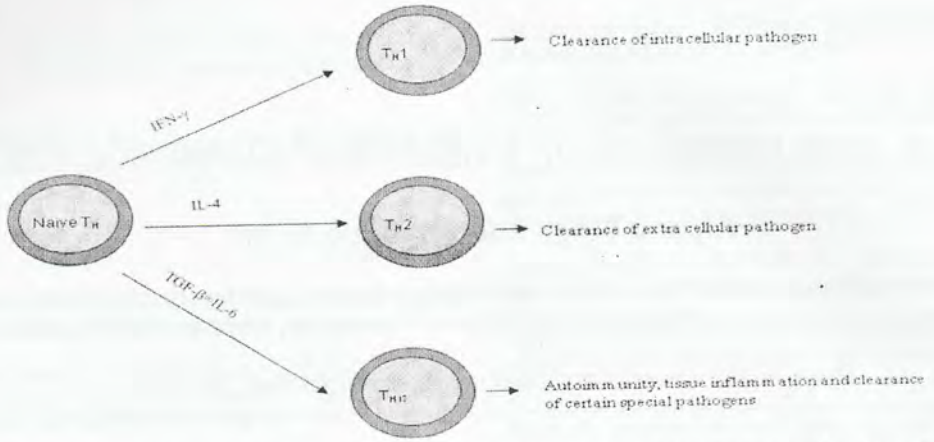


Fig 1: Depending on the cytokine milieu present in the peripheral immune compartment, naive $CD4^+$ T-Cells can be differentiated into various subsets of T helper cells (T_H1, T_H2 and recently discovered T_H17).

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Story begins when people found that $CD4^+T_H$ cells are producing cytokines that could not be classified according to the T_H1–T_H2 scheme. IL-17 was among these cytokines and the T cells that preferentially produce IL-17, could not produce T_H1 or T_H2 specific cytokines like IFN-□or IL-4 respectively, were named as T_H17 cells (Bettelli *et al.,* 2007 and Harrington *et al.,* 2005). Evidences for the existence of T_H17 cells were collected from several studies, which involves study of *Borrelia burgdorferi* infection in animal models. Stimulation with sonicates and synthetic antigens of the bacteria induced IL-17 production by T cells, independently of the production of T_H1 and T_H2 cytokines. The same pattern of IL-17 secretion was induced by the BCG strain of *Mycobacterium* bovis (Infante *et al.,* 2000). Further evidence that led to the discovery of this cell population came from studies in autoimmune murine models, namely rheumatoid arthritis (RA) and multiple sclerosis models (MS) (Sato *et al.,* 2006). The cumulative evidence about the existence of a distinct subset of T cells characterized by the secretion of large amounts of IL-17 has led to the proposal of a third lineage of T_H cells designated T_H17 cells (Park *et al.,* 2005). As with T_H1 and T_H2 cells, no single surface marker is specific for Th17 cells. However, co-expression of the chemokine receptors CCR4 and CCR6 (Acosta *et al.,* 2007) or expression of CCR2 in the absence of CCR5 (Kotenko *et al.,* 2001) appears to define human Th17 cells.

Differentiation and development of T_H17 cells:

The current concepts on the T_H17 cell differentiation have been drawn from murine models. Although there are major analogies have been observed in the development of T_H17 cells in humans and mice, some differences have also been observed. Unlike the murine T_H17 cells which are easily induced by synergistic action of TGF-□ and IL-6 cytokines (Veldhoen *et al.,* 2006 and McGeachy *et al.,* 2007), this combination is completely inefficient in generating human T_H17 cells. By the time of this study nothing was very sure about human T_H17 differentiation. But the discovery of IL-23 shed light on the differentiation route of this novel T-cell phenotype and now It has been well proved by several groups that IL-23 is fundamental for survival and for terminal differentiation of T_H17 cells (Langrish *et al.,* 2005, Murphy and Reiner 2002 and Veldhoen *et al.,* 2006), but its exact function in the induction T_H17 cell differentiation poses a major conceptual problem; partly due to the absence of the IL-23 receptor in the naive T cells, and due to its expression only in activated cells (Parham *et al.,* 2002).[]. Which surely indicate that IL-23 cytokine alone cannot differentiate highly purified naive T cells into T_H17 cells. Thus only previously activated or memory T cells become susceptible to the action of IL-23. This disability of IL-23 for differentiating naive T cells in T_H17 cells, raising the possibility that another cytokine(s) must be involved for the induction of human T_H17 differentiation.

Initially, TGF-β plus interleukin-6 were not considered to be differentiation factors for human T_H17 cells. But later on, it has been reported by several groups that in naive human T cells, combination of TGF-□ plus IL-6 or TGF plus IL-21 failed to induce the differentiation of human T_H17 cells (Acosta *et al.,* 2007a, Acosta *et al.,* 2007b, Mangan *et al.,* 2006 and Veldhoen *et al.,* 2006). Recent findings suggest that human T_H17 cells can be generated by a combination of TGF-□, IL-1□ and IL-6 (Acosta *et al.,* 2007a) or by synergestic action of TGF-□, IL-1□ and IL-23 (Wilson *et al.,* 2007). Three independent groups reported simultaneously that IL-21, is produced in overwhelming amounts by T_H17 cells could in combination with TGF-□ induce T_H17

differentiation (Korn *et al.*, 2007, Nurieva *et al.*, 2007 and Zhou *et al.*, 2007). Thus highlighting essential role of TGF-□ in T_H17 cell differentiation and also pointing towards a relevant function of IL-21 in amplifying the precursor frequency of differentiating T_H17 cells (Mangan *et al.*, 2006, Nurieva *et al.*, 2007, Veldhoen *et al.*, 2006 and Yang *et al.*, 2008). Now it has been well established by several studies that full differentiation of T_H17 cells requires three different steps: induction, amplification and stabilization. 1) The differentiation is initiated by the combined effect of TGF-□ and pro-inflammatory cytokines (IL-1β, IL-6, IL-23, and IL-21); 2) The amplification of T_H17 response is driven through the production of IL-21 by T_H17 cells; 3) the stabilization /maintenance of the T_H17 phenotype is achieved by IL-23 (Bettelli *et al*, 2007 and Zhou *et al.*, 2007).

T_H17-specific transcription factors:

Cytokines produced by cells of the innate immune system govern the differentiation of helper T cells. The cells of the innate immune system are the first line of defense against pathogens; their pattern-recognition receptors, which are not specific for any particular epitope, allow them to respond to a wide variety of microbial invaders by producing cytokines that activate T cells of the adaptive immune system. The differentiation of each helper T cell is initiated by signals from T cell receptor (TCR) and cytokine receptors present on naive T cells. These integrated signals then induce the expression of lineage-specific transcription factors that drive T_H cell differentiation. T_H1 and T_H2 differentiation are initiated by activation of T cells in the presence of IFN-□ or IL-4, respectively (Mosmann and Coffman, 1989 and Murphy and Reiner, 2002). Developing T_H subsets cross regulate expansion and functions of each other. While IL-4 antagonizes differentiation of T_H1 cells and IFN-γ inhibits the development of T_H2 cells, both cytokines antagonize the development of T_H17 cells (Fiorentino *et al.*, 1989, Mosmann and Coffman, 1989 and Murphy and Reiner, 2002).

Transcription factors T-bet, STAT1 and STAT4 specify T_H1 cell fate (Szabo *et al.*, 2000 and Takeda *et al.*, 2003), whereas GATA-3 and STAT6 govern T_H2 cell differentiation (Kaplan *et al.*, 1996, Murphy and Reiner, 2002, Takeda *et al.*, 1996 and Zheng and Flavell, 1997). For T_H17 cell differentiation, it has been shown that neither T_H1-cell-associated (STAT-1, STAT-4 and T bet) nor T_H2-cell- associated factors (STAT-6 and GATA-3) are required, further validating the argument that these cells are a distinct lineage of T_H cells (Harrington *et al.*, 2005). STAT3 has been reported to be a crucial component of T_H17 cells differentiation because conditional deletion of of STAT-3 in T-cell prevents T_H17 cell development (Yang *et al.*, 2007). Interestingly, it has been reported that Th17 differentiation factor (TGF-□ plus IL-6) is capable of inducing the expression of ROR-□T, which has been recognized as lineage-specific transcription factor required for T_H17 cell differentiation (Ivanov *et al.*, 2006 and Manel *et al.*, 2008). In addition to ROR-□T, ROR-□ has also been reported as an important transcription factor, for TH17 differentiation. More recently it has also been shown that T_H17 cell differentiation might be mediated by the combined effect of ROR-γT and ROR-α, both of which are expressed at high levels in differentiated T_H17 cells. Loss of any one of these transcription factors resulted in partial loss of T_H17 cytokines expression, and loss of both ROR-γt and ROR-α abrogated T_H17 differentiation (Ivanov *et al.*, 2006, Manel *et al.*, 2008 and Yang *et al.*, 2008).

T_H17 –specific effector cytokines:

Upon antigenic stimulation, naive $CD4^+$ T cells activate, expand and differentiate into different effector subsets termed T_H1, T_H2 and T_H17 and characterized by the production of distinct effector cytokines to mediate their functions. T_H1 cells produce interferon-γ (IFN-γ) and lymphotoxin (LT), and can mobilize the cellular arm of the immune system to combat intracellular pathogens. T_H2 cells secrete IL-4, IL-13, and IL-25, which are essential for the generation of appropriate class of antibodies and for the elimination of extracellular pathogens (Mosmann and Coffman, 1989 and Murphy and Reiner, 2002). T_H17 cells are characterized by the production of IL-17A (also called IL-17) and IL-17F and are thought to clear pathogens not effectively cleared by T_H1 and T_H2 cells (Harrington *et al.*, 2005 and Park *et al.*, 2005). It's name derived from IL-17 cytokine, as T_H17 cells produce large amount of IL-17 cytokine, and most of Th17-mediated effects are attributed to this cytokines (Bettelli *et al.*, 2006 and Kolls and Linden 2004). IL-17A and IL-17F have similar functions. Although there is often a coordinated expression of IL-17A and IL-17F in T_H17 cells, but it is now clear from recent evidences that T_H17 cells can express only IL-17A, IL-17F or an IL-17A-IL-17F heterodimer (Liang *et al.*, 2007). They induce the production of pro-inflammatory cytokines, chemokines and metalloproteinases from various tissues and cell types. As a result they recruit neutrophils to the site of infection (Ye *et al.*, 2001). In addition to IL-17A and IL-17F, T_H17 cells produce other effector cytokines, namely IL-21, IL-22, IL-6 and TNF-□ (Kolls and Linden, 2004 and Liang *et al.*, 2006). None of these cytokines (IL-21, IL-22, IL-6, TNF-□) are Th17 exclusive cytokines, but are preferentially expressed in T_H17 cells.

T_H17 cells in host defence and autoimmune disorders:

Being a new member of T_H family it's role in the pathogenesis of autoimmunity and several infections has not been completely elucidated and investigation. Primarily because the apparent limitations in the T_H1/T_H2 paradigm forced a reconsideration of the mechanisms of these disorders and, thereby, led to the understanding of the role of T_H17 subset. However, it is most likely that the evolutionary pressure for this specialized cell type likely came from its role in host defense. The impoance of the T_H17 subset in autoimmne diseases was first demonstrated in mice
deficient for the p19 chain of the IL-23. These mice had similar numbers of IFN-γ producing T cells than wild type mice but showed a dramatic decrease in IL-17-producing T cells and
were resistant to the development of experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (Becher *et al.,* 2002 and Cua *et al.,* 2003). Although much is now known regarding the biology of IL-17 in murine systems and there is compelling evidence for an important role of IL-17 in the etiopathogenesis of inflammatory and autoimmune disease condition in humans. The expression of IL-17 has been associated with numerous human autoimmune diseases, such as rheumatoid arthritis (RA) (Huang *et al.,* 2004), multiple sclerosis (MS) (Matusevicius *et al.,* 1995), systemic lupus erythematosus (SLE) (Wong *et al.,* 2008), inflammatory bowl disease (IBD) (Seiderer *et al.,* 2008) and psoriasis (Ma *et al.,* 2008).

The receptor for IL-17 is widely expressed on many cell types where it induces the expression of chemokines, pro-inflammatory cytokines, and colony-stimulating factors. These cytokines and chemokines in turn induce the recruitment of neutrophils and other myeloid cells to the site of infection, which is a critical feature of many infectious diseases (Ye *et al.,* 2001a). So far it is unclear which class of pathogen preferentially induces an effective T_H17 response because pathogens are as diverse as the gram positive, the gram negative bacteria, virus and fungi. It has been shown that IL-17 is critical for protection against gram-negative bacteria, like *Klebsiella pneumonia* (Ye et al., 2001b). In addition IL-17 also appears to play an important role in protection against *Mycobacterium tuberculosis* (Khader *et al.,* 2007). *Bordetella pertussis*-infected human dendritic cells produce high levels of IL-23, which results in high level of IL-17 production by T cells. Role for IL-17 in Lyme disease has also been reported. Both human and mouse Tcells produced enhanced amounts of IL-17 in response to *Borrellia burgdorferi* infection (Infante *et al.,* 2000). In addition, studies indicated that Th17 cells are also involved in antifungal defense both in mouse and in humans (Romani *et al.,* 2008 and Zelante *et al.,* 2008). Thus, it would appear that in contrast to Th1 and Th2 cells, which protect against intracellular bacteria and helminthes, a major function of T_H17 cells is to protect against extracellular bacteria and fungi (Aujla *et al.,* 2007a and Aujla et a., 2007b). But it's role in virus borne diseases like human immunodeficiency virus (HIV) mediated Acquired Immunodeficiency syndrome (AIDS) are not clear and still a topic of future investigation. Although Favre *et al.,*2009 found in simian immunodeficiency virus – infected rhesus macaques, T_H17 cells are markedly depleted. Significant loss of T_H17 cells from GI tract of HIV individual, was also observed by Brenchley *et al.,* 2006. Whereas another group have found that T_H17 cells promote persistent viral infection in a model of chronic virus infection by inducing anti apoptotic molecule and inhibiting T-cell cytotoxicity in IL-17 dependent manner (Hou *et al.,* 2009). Despite ongoing efforts in this direction T_H17 functional status remains controversial in viral diseases.

CONCLUSION

After two decades of T_H subsets classification, great progress has been made to understand the complexity of T_H differentiation pathways. During the last few years, the T_H1/T_H2 paradigm has been updated to include a third member of T_H effector cells called T_H17 cells. They appear at sites of inflammation with rapid kinetics and through the potent induction of chemokines, could bridge the gap between innate and adaptive immunity and attract other subsets of T helper cells to site of infection. Substantial progress has been made to understand the biology of T_H17 cells. Now it is very clear that TGF-β plus IL-6/IL-21/IL-1□induces the differentiation of naïve T cells into T_H17 pathways; IL-21 and IL-23 further amplifies and stabilizes Th17 generation, respectively. Loss of any of one of the members in the pathway (IL-6, IL-21 or IL-23) severely limits the T_H17 response. More likely, T_H17 development also depends on other T_H subsets as immune homeostasis is always maintained by their interplay.

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ISOLATION OF LP (a) FROM HUMAN SERUM LIPOPROTEINS AND ITS SIALIC ACID CONCENTRATION

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ABSTRACT:

Lp(a), a genetic variant of β-lipoproteins (LDL) was isolated from human serum and was characterized to study its behavior and sialic acid concentration. Like LDL, Lp (a) also contains significant amounts of sialic acid which can be digested completely by neuraminidase enzyme treatment for 24 hours at 37°C. Thus, Lp (a) samples treated with neuraminidase for 24 or 48 hours at 37°C showed negligible amounts of sialic acid. The electrophoretic mobility of these Lp (a) samples decreased in comparison to the native or untreated Lp (a) samples. Presence of high amounts of sialic acid in Lp (a) like that in LDL suggests that this carbohydrate moiety may play some role in these lipoproteins.

Key words: Sialic acid, human serum, lipoprotein.

INTRODUCTION

Lipoproteins are complexes of lipids and proteins having the solubility characteristics of proteins. Human serum lipoproteins can be isolated into a number of fractions depending on their electrophoretic mobility and their hydrated densities. These are high density lipoproteins (HDL or α-lipoproteins), low density lipoproteins (LDL or β-lipoproteins), very low density lipoproteins (VLDL or pre- β -lipoproteins) and chilomicrons (exogenous particles). In addition, another class of lipoproteins, designated as Lp (a), can be isolated from human serum. It represents genetic variant of β-lipoproteins and is composed of β -lipoproteins, varying amounts of adsorbed lipoproteins or albumin and the specific Lp (a) fraction. Lp (a) has apo B as the major protein constituent and the lipid moiety is indistinguishable from LDL (Kostner 1976 and Cazzolato *et al.,* 1983). The characteristic feature of Lp (a) is the presence of an additional apoprotein (a-protein) which is distinct from all other serum proteins and apoproteins. It is believed that Lp (a) is an additional risk factor for atherosclerosis and myocardial infarction independently from all other serum lipoproteins (Berg *et al,*1974 and Albers *et al.,* 1977). The later assumption is based on the finding that Lp (a) is synthesized differently from other apo B containing lipoproteins (Krempler *et al.,* 1980). Also, it has been shown that the catabolism of Lp (a) proceeds via the same routes as LDL since it is bound to the B/E receptor in cultured human fibroblasts (Krempler *et al.,* 1983). Further, the protein moiety of low density lipoproteins has been shown to contain 5-9 % carbohydrate consisting of various sugars including sialic acid (Schultze and Heide 1960, Ayrault – Jarrier,1961, Marshall and Kummerow, 1962, Swaminathan and Aladjem, 1974 and Kwiterovich *et al.,* 1974). Therefore, it was of interest to us to isolate Lp (a) from the human serum, study its behavior in terms of electrophoretic mobility and also to measure its contents of sialic acid.

MATERIALS AND METHODS

Isolation of Lp (a): Human serum from twenty different individuals was tested for Lp (a) positive nature against Lp (a) antibody horse no. XI by radial immunodiffusion using 1 % Latex agarose prepared in Rocket buffer. Two samples showing positive antigen-antibody precipitation reaction of 4-5mm diameter corresponding to approximately 60-70 µg/µl Lp (a) were used for the preparation of Lp (a).

The pooled serum (=380 ml) was centrifuged in Sorvall centrifuge at slow speed (3000 RPM) to free it from blood cells. The supernatant was brought to a density 1,055 with sodium chloride and was then ultra centrifuged at 50,000 RPM for 24 hours using Beckman quick seal tubes. After centrifugation, the tubes were sliced at the clear zone. The bottom fraction (=200 ml) was collected while the top fraction was discarded. The density of the bottom fraction was then brought to 1,125 with sodium chloride and it was ultra centrifuged again at 50,000 RPM for 24 hours. The tubes were sliced as before at the clear zone just below the cap and the top or upper Lp (a) rich fraction was collected with the help of

syringe (=38 ml). The Lp (a) rich fraction was then dialyzed against glycine buffer (0.9% Nacl + 0.05 M glycine + 0.1% EDTA and 0.1% Sodium acid, pH 8.2), with pressure to concentrate it from 38 ml to 5.2 ml. This concentrated Lp (a) sample was given to the column once the later was ready.

Preparation of the column: The chromatographic column (100x2.5 cm) was prepared by packing it with Biogel A5M. The column was washed for the first time with 50 ml of urea solution (7.5M urea + 1.5% Nacl + 0.05 M glycine, pH=8) and subsequently with glycine buffer (0.9% Nacl+ 0.05M glycine+ 0.1% EDTA+ 0.1% sodium acid). The pressure in the column was always adjusted to nearly 50 cm. Once the column was washed two to three times its volume with glycine buffer, it was ready for use.

The Lp (a) fraction was applied to the column across its walls at a time when buffer layer was nearly one mm over the gel in the column. When the sample moved into the gel, small amount of buffer was added over the gel layer and waited till this buffer also moved into the gel. Then filled the column with buffer and allowed the sample to move down through the whole length of the column. The outlet of the column was connected to the fraction collector which was adjusted to 20 drops/tube. All the fractions coinciding all the peaks on the chromatographic column were tested for Lp (a) positive nature by immunodiffusion and disc electrophoresis using 7% urea. Eleven fractions (Tube Nos. 32 to 41) constituting the first peak on the column showed Lp (a) positive reaction with Lp (a) antibody Horse no. XI. Therefore, these fractions were pooled together and were concentrated with pressure to nearly 4ml against glycine buffer (0.9% Nacl+0.05M glycine + 0.1% EDTA+ 0.1% Sodium acid, pH-8. 2). Once the sample was concentrated, it was dialyzed in the same buffer for two hours with vigorous shaking to mix the precipitate /contents of the sample thoroughly. The amount of lipoprotein in the prepared Lp (a) sample was measured at 280µ against 0.001N NaOH.

Incubation of Lp (a) with Neuraminidase:
The prepared Lp (a) was treated with known amounts of neuraminidase from *Clostridium perfringens* (Type IX, Sigma). For this, two samples of 250 µl each were kept at 37°C with 5 µl neuraminidase for 24 and 48 hours respectively. One sample of 250 µl was also kept at 37°C for 48 hours without neuraminidase to serve as control for the treated samples. After the neuraminidase treatment, the samples were tested for :-

1. Cellulose acetate electrophoresis,
2. SDS Electrophoresis with 3.5 and 5% gels, and
3. Concentration of Sialic acid.

The amount of N-acetyl neuraminic acid (Sialic acid) in Lp(a) fraction of serum lipoprotein with and without the neuraminidase treatment was determined by the thiobarbituric acid procedure of Warren, 1959. Protein determinations were done by the Lowry method (1951). Three samples of 250 µL each of Lp (a), pH-8.2, (referred as 2, 3 and 4) were used for these estimations. In sample numbers 3 and 4, 5µl neuraminidase (0.5 Units) was added separately with the help of micro-pipette and the contents after mixing thoroughly were kept in the oven at 37°C for 24 and 48 hours respectively. The sample 2 was also kept in the oven along with the samples 3 and 4 at 37°C for 48 hours but without neuraminidase to serve as control for the treated samples. In addition, another untreated Lp (a) control (sample 1) which was not kept in the oven was used to compare with sample 2. Following neuraminidase treatment, these samples were used for the estimation of sialic acid.

Lipid Electrophoresis: Another aliquot of 0.8 ml Lp (a) was treated with 16 µl (1.6 Units) neuraminidase (Type IX, same as before) at 37°C for 24 hours. The reaction mixture was then dialyzed against sodium bromide sodium of density 1,125 for 24 hours. The sample was ultra centrifuged for 24 hours at 45,000 RPM using 50.3 Rotar with sodium bromide solution of density 1,125. After centrifugation, the top fraction of the sample was collected with the help of syringe from the Beckman ultracentrifuge tube. Four aliquots of 10 µl each of this treated samples of Lp (a) were used for SDS electrophoresis while the remaining sample was dialyzed against glycine buffer (0.9% NaCl+O.05M glycine+1% EDTA+1% Na acid, pH-8.2) for overnight. This neuraminidase free de-sialated Lp (a) was used for lipid electrophoresis along with the native or untreated Lp (a) and the normal serum.

RESULTS AND DISCUSSION

Results from Cellulose acetate electrophoresis studies showed that neuraminidase treatment decreased the mobility of Lp (a) in comparison to untreated/control samples (Figure I). However, there was no difference in the mobility pattern of Lp (a) treated with neuraminidase for 24 and 48 hours respectively. Mobility pattern of Lp (a) kept in the oven at 37°C for 48 hours without neuraminidase to serve as control for the treated samples did not change and was similar to that of the native Lp(a) which was not kept in the oven (Figure I).

The results of lipid electrophoresis are shown in Figure II. These indicate that composition of Lp (a) resembles that of LDL as the electrophoretic band of Lp (a) sample corresponds with that of LDL band of the normal serum. However, the electrophoretic mobility of the

neuraminidase treated Lp (a) samples decreased in comparison to the untreated Lp (a) sample.

The amount of lipoprotein in the prepared Lp (a) sample was 11.6 mg/ml. The amount of sialic acid in various Lp (a) samples as calculated from the mean values obtained from the optical densities of two parallel samples and estimated according to the method of Warren (1959) were as follows:-

Sample 1: Native Lp (a) contained 84.56 µgm sialic acid/mg protein

Sample 2: Untreated control contained 84.47µgm sialic acid / mg protein

Samples 3 and 4: Neuraminidase treated Lp (a) for 24 and 48 hours respectively showed negligible amounts of sialic acid.

The amount of sialic acid estimated in two LDL samples was 9.77 and 10.76 µgm/mg protein.

The results obtained from Cellulose acetate electrophoresis (Figure I) and estimation of sialic acid indicate that most of the sialic acid from Lp (a) was removed with 24 hours neuraminidase treatment and 48 hours neuraminidase treatment did not cleave off sialic acid any further. A decrease in the mobility pattern of neuraminidase treated Lp (a) in comparison to native Lp (a) as seen in the cellulose acetate electrophoresis may be accounted for a decrease in the negative charge on Lp (a) due to the removal of negatively charged sialic acid. Treated Lp (a) with neuraminidase for 48 hours at 37°C.

The results of lipid electrophoresis as seen in Figure II indicate that composition of Lp (a) resembles that of LDL as the electrophoretic band of Lp (a) sample corresponds with that of LDL band of the normal serum. However, the electrophoretic mobility of the neuraminidase treated Lp (a) samples decreased in comparison to the untreated Lp (a) sample. This once again shows a decrease in the net negative charge on Lp (a) due to the removal of negatively charged sialic acid following the treatment with neuraminidase.

Estimation of sialic acid indicates that Lp (a) contains this in large amounts which gets digested completely with 24 hours treatment of neuraminidase and because of this digestion, neuraminidase treated Lp (a) for 24 and 48 hours respectively showed negligible amounts of sialic acid. Two LDL samples analyzed for the amount of sialic acid showed that these contained 9.77 and 10.76 µgm/mg protein sialic acid respectively thus suggesting resemblance of Lp (a) with LDL. These results confirm the studies which showed that the protein moiety of low density lipoproteins contain 5-9 % carbohydrate consisting of various sugars such as galactose, mannose, glucosamine and sialic acid (Schultze and Heide 1960, Ayrault – Jarrier, 1961, Marshall and Kummerow, 1962, Swaminathan and Aladjem, 1974 and Kwiterovich *et al.,* 1974). However, Swaminathan and Aladjem (1976) reported a marked variation in sialic acid values ranging from 6 to 17 µgm/mg protein in LDL. Further, our studies are in conformation with the results of Margolis and Langdon (Margolis and Langdon, 1966) who suggested that sialic acid residues could be removed from native LDL with sialidase treatment without affecting the lipid binding.

The presence of large amount of sialic acid in Lp (a) is not clear at present. A number of possible functions of carbohydrate moiety in glycoprotein have been proposed by many scientists. According to them, it may be involved in the secretion from the cell (Eylar, 1965), or in the regulation of their catabolism (Morell *et al.,* 1971). It has been shown that the removal of sialic acid from glycoproteins decreases their biological half-life (Morell *et al.,* 1971). However, similar studies using native and desialyzed iodide labeled LDL did not show significant differences in their respective rates of disappearance (Swaminathan, and Aladjem, 1974). Sialic acid may also play a role in membrane permeability (Gupta *et al.,* 1974). The presence of LDL in atherosclerosis plaques (Woolf and Pilkington, 1965 and Smith and Slater, 1970) has been demonstrated and also uptake of LDL by cultured fibroblasts has been reported by Brown and Goldstein (Brown, and Goldstein, 1974). These processes might involve the interaction of carbohydrate moiety of LDL with cell membranes. Since human LDL has been demonstrated to be taken up specifically and degraded by human aortic smooth muscle cells (Stein and Stein, 1975) and cultured fibroblasts (Goldstein and Brown, 1974(a)) and has been shown to regulate the content of free and esterified cholesterol in human fibroblasts (Goldstein and Brown, 1974(b)), the relative atherogenecity of LDL has been suggested to depend upon the carbohydrate moiety (Swaminathan and Aladjem, 1976). This may also be true for Lp (a) as it resembles with LDL in its composition and also contains large amounts of sialic acid.

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Figure Captions

Figure I: Results of Cellulose acetate electrophoresis-

1. Native/Untreated Lp (a), not kept in the oven;
2. Untreated Lp (a) kept in the oven for 48 hours at 37°C without the addition of neuraminidase;
3. Treated Lp (a) with neuraminidase for 24 hours at 37°C;
4. Treated Lp (a) with neuraminidase for 48 hours at 37°C.

Figure II: Results of Lipid electrophoresis-

1. Normal serum;
2. Native or Untreated Lp (a);
3. Treated Lp (a) with neuraminidase for 24 hours at 37°C.

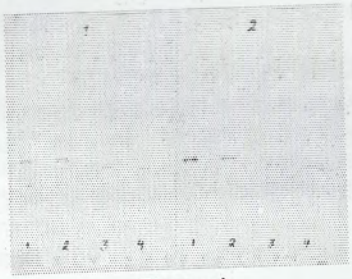


Figure I

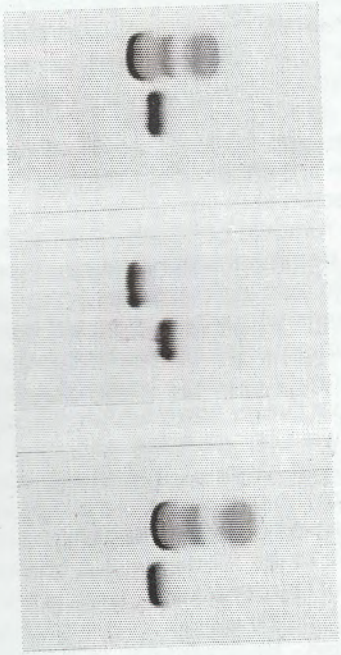


Figure II

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CELL SUSPENSION CULTURES OF *Tylophora indica* – ITS POTENTIAL AS A TOOL FOR PLANTLET REGENERATION

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ABSTRACT:

In vitro regeneration of Tylophora indica via suspension culture was established. Callus proliferation occurred on leaf explants cultured on Gamborg's medium (B_5) supplemented with 2, 4-D (2 mg/L) and BAP (0.5 mg/L). Suspension cultures were obtained by transferring and agitating callus clumps in liquid B_5 medium. Shoot bud differentiation was observed from cell aggregates on liquid B_5 medium containing BAP (4.0 mg/L) and multiple shoot formation was achieved on the same medium. Isolated shoots were rooted with high efficiency in B_5 medium (1/2 strength) containing IAA (1.0 mg/L). Regenerated plants were established in pots.

Key words: *Tylophora indica; Suspension cultures; Thidiazuron.*

INTRODUCTION

Plants have been used for medicinal purposes since ancient times. A number of medicinal plants synthesize extractable organic substances that are used as chemical sources of drugs and other medicines. *Tylophora indica* (Burm.f.) Merrill, previously called as *Tylophora asthematica*, a member of Asclepiadaceae is an important indigenous medicinal plant found in restricted localities in Indian sub continent. The roots have a sweetish taste turning acrid, aromatic odor and a brittle fracture. They possess stimulant, emetic, cathartic, expectorant, stomachic and diaphoretic properties and are used for the treatment of asthma (Shivpuri *et al.*,1968), bronchitis, whooping cough, dysentery, diarrhea and in rheumatic gouty pains (Kirtikar and Basu, 1991; Chopra *et al.*, 1996). The powdered leaves, stem, and root contain several alkaloids (Rao and Wilson, 1971) including tylophorine, tylophorinine , cryptopleurine, antofine and ficuseptine C which are pharmacologically active, and anticancer tylophorinidine has also been isolated from the roots of three-year old plant (Mulchandani *et al.*,1971).

Apparently due to non-availability of sufficient quality planting materials, commercial plantations of this important aromatic and medicinal species have not been widely attempted and presently only the wild population is exploited for extraction purposes. Due to overexploitation and lack of organized cultivation, the wild populations have declined fast. There are a number of constraints for the propagation and conservation through conventional methods like vegetative and seed propagation. The major ones are variations in edaphic and climatic factors, low percentage of seed set and seasonal dormancy. The propagation in its natural habitat is a rare phenomenon evidenced by close field observation. In an attempt of vegetative propagation, the stem cuttings of the plants failed to root with the application of different hormones. The above mentioned causes prompted us to find an alternate method of rapid micropropagation of this species. Clonal mass propagation can be widely applied to shorten the long sexual cycle and other problems like limited seed availability and problems of seed physiology.

So in view of medicinal importance, there is an urgent need to conserve this species *ex situ* through *in vitro* methods. There are few reports of micropropogation using various explants (Sharma and Chandel, 1992; Manjula *et al.*,2000; Chaudhuri *et al.*,2004; Faisal *et al.*,2007) however, the protocols for rapid plant regeneration and regeneration through cell suspensions are not yet available in *Tylophora indica*. Use of liquid medium (cell suspension) for *in vitro* regeneration has been considered as an ideal technology for mass production because it reduces

manual labour and easy to subculture Therefore, the present investigation was aimed to develop a suitable methodology for developing plants from cell suspension cultures of *Tylophora indica*.

MATERIALS AND METHODS

Plant material

Leaves collected from one year old mature plant of *Tylophora indica* (grown in medicinal plant garden of CCS HAU, Hisar)were washed with distilled water twice and then rinsed with 1% (v/v) detergent (Teepol) for 5 min, were surface sterilized with 0.1% (w/v) aqueous solution of $HgCl_2$ for 5 min followed by 4-5 rinses in sterilized ddH_2O. Leaf explants were cut into 8 x 20mm bits at different regions and used in further studies.

Media and culture conditions

The glassware was subjected to chromic and sulphuric acid mixture (1:3) for 24 hours and washed thoroughly with teepol (10%) detergent solution. It was then cleaned under running tap water, further rinsed with distilled water and oven dried. The vessels were decontaminated by autoclaving them at 15 lbs/in2 for 20 minutes followed by washing with detergent, tap water, distilled water, and finally oven dried. The basal medium used in the present study consisted of the salts and vitamins of Gamborg's (1968) media (B_5). Addition of plant growth regulators to medium was done with filter sterilization in aseptic conditions and unless it is specified, all media were fortified with 2% sucrose (w/v) and 0.8% agar (w/v;Qualizen, India). The pH of the medium was adjusted to 5.7 prior to the addition of agar. Molten medium (15-20 ml) was dispensed to glass tubes, flasks or bottles, plugged and autoclaved at 121°C for 15 min. All cultures were incubated at 25°C±2°C and under 16 h light/8 h dark and photoperiod with light intensity of 50μE m^{-2} s^{-2} provided by cool-white fluorescent lamps.

Callus induction

For callus formation, leaf segments were excised aseptically from one year old seedlings and were cultured on Gamborg's (B_5) medium facing the adaxial and abaxial side towards the medium. The B_5 medium supplemented with various concentrations and combinations of auxins [indole-3-acetic acid (IAA), -Napthalene acetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2, 4-D)] along with cytokinin[6-benzylaminopurine (BAP)] was used (Table-1). The different types of calli initiated and were subcultured to fresh medium after every 3 weeks of interval.

Suspension culture formation

Suspension cultures were obtained by transferring callus clumps to liquid B_5 medium of the same constituents as used for callus growth for several passages till freely suspended cells/small cell clusters were obtained. The flasks were agitated on a rotator shaker at 100 rpm.

Shootbud Initiation and Multiplication

Shoot bud differentiation was induced by cultivating freely suspended cells/ small cell clusters to liquid B_5 medium supplemented with different combinations and concentrations of cytokinins [6-benzylaminopurine (BAP) and Thidiazuron(TDZ)] and auxins [indole-3-acetic acid (IAA), -Napthalene acetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2, 4-D)] as shown in Table-2, for successive passages on the same medium. After induction, shoot buds were subcultured on the same medium but with agar, for multiple shoot formation. All cultures were subcultured after every 3 weeks. Data on percent shoot development, shoot number per explant and shoot length was recorded after 4 weeks of growth.

Rooting of excised shoots

For rooting, excised shoots (3-4cm long) were cultured on B_5 medium (¼, ½ and full strength) containing various auxins [indole-3-acetic acid (IAA), -Napthalene acetic acid (NAA), and Indole Butyric acid (IBA)] with different concenterations .

Root formation was visible from basal cut within 10 days of inoculation. Data were recorded on mean number of roots and length of roots after 4 weeks of transfer onto rooting media.

RESULTS AND DISCUSSION

Different media involving different combinations and concentrations of BAP (0.5 and 1.5 mg/L) along with 2, 4-D, IBA, NAA (2-4 mg/L) each, were tested for callus initiation from leaf segments (Table-1). Callus induction was observed on explants after 10-15 days of culture initiation. Out of 18 different types of media tested during the present investigation the best suited medium for callus induction was media containing 2, 4-D and BAP i. e. B_5 + 0.5 mg/L BAP + 2.0 mg/L 2, 4-D(Fig.1). However, medium containing IAA or NAA turned brown after 3-4 weeks and was incapable of differentiation.

Superiority of 2, 4-D for induction of regenerative callus has been reported earlier also, by Chanderasekhar *et al.*, 2006. 2, 4-D (2.0 mg/L) was found optimal for producing green and fast growing callus. At higher concentrations of 2, 4-D (3-4 mg/L), the amount of callus produced was more but the regeneration potential was low. It was observed that when lower concentrations of BAP (0.5 mg/L) was used along with 2, 4-D, the callus so produced was green and with high regeneration potential. While high concentrations of BAP (1.5 mg/L) were not so efficient. This suggests a critical

auxin/cytokinin ratio is required for callus induction and proliferation. Similar results were reported by Vasil and Vasil, (1982).

To establish suspension culture, method given by Smith and Street (1974) was followed. The B_5 medium was used as the basal media throughout the course of investigation which was reported to be best suited for suspension culture (Gamborg's *et al.*,1968). Response of different media for suspension culture formation was worked out. The callus proliferated on B_5 + 0.5 mg/L BAP + 2.0 mg/L 2, 4-D media was most friable and hence resulted in earlier and easier establishment of small clusters/ freely suspended cells in suspension culture as compared to other media.

Shoot bud differentiation from suspended cell aggregated was observed in liquid B_5 medium supplemented with different combinations and concentrations of plant growth regulators as shown in (Table 2) within 3 weeks of subculture with varying degree. After induction, shoot buds were subcultured on the same medium but with agar, for multiple shoot formation (Fig. 2). Significant shoot regeneration was obtained in 3 media i.e. B_5 + 8.8 ml TDZ, B_5 + 4.4 ml/L TDZ + 1. 5 mg/L 2, 4-D and B_5 + 4.0 mg/L BAP. Among these 3 combinations, B_5 + 4.0 mg/L BAP showed better response. Thidiazuron (TDZ), a substituted urea with cytokinin activity, was found to be less effective for shoot morphogenesis than BAP, alone. It induced significantly lower number of shoots but once shoot initials were formed, then TDZ treated explants turned green and grew more rapidly than BAP treated explants. On transferring these shoots to rooting medium the shoots previously grown on thidiazuron containing medium become taller and formed fewer roots over the plantlets grown on medium containing BAP.

Effect of TDZ on shoot organogenesis has also been reported by Fiolia *et al.*, 1990. The significant interaction was observed between auxin and cytokinin type. The percentage of shoots formed was high in medium containing TDZ along with 2,4-D as compared to medium containing BAP with 2, 4-D. The optimal concentration for organogenesis was found to be with 4.4 mg/L TDZ along with 1. 5 mg/L 2, 4-D. Use of a higher concentration of BAP (4.0 mg/L) along with 2, 4-D (1. 5 mg/L) resulted in a drastic decrease in the number of shoots/explants. This suggests that the cytokinin: auxin ratio is important for such response. The best response in terms of the number of shoots/explant was obtained using BAP (4.0 mg/L). Regeneration of shoots with BAP has also been reported earlier by Nema *et al.*, 2006.

Isolated shoots were cultured on ¼, ½ and full strengths of B_5 medium fortified with various concentrations of IBA, NAA and IAA individually for rooting (Table 3). Maximum number of roots per shoot was produced on IAA supplemented medium (Fig. 3) as compared to media supplemented with NAA and IBA. Reduction in B_5 salt strength to half was observed beneficial in enhancing rooting in all the three auxins supplemented media. Further reduction in salt strength of B_5 medium had inhibitory effects. Root growth was maximum on IAA supplemented media irrespective of salt strength or concentration of auxin.

Regenerated plantlets with well developed roots were taken out along with the medium from the vessels and rinsed to remove traces of agar, which might provide a substrate for microorganisms. Plants were transferred to half strength B_5 liquid medium before transfer to soil for hardening followed by transfer to sterile mixture of soil and soilrite (3:1; Fig. 4). Bavistin (0.5%) was sprayed once a week to avoid fungal contamination. Humidity was maintained by covering the plant with a beaker. The plantlets survived for 8-10 weeks after which they started drying. Only 20% plants survived after 8 weeks of pot transfer.

CONCLUSION

Use of cell suspension cultures for *in vitro* regeneration has been considered as an ideal technology for mass production because it reduces manual labour and easy to subculture. But the protocol for regeneration through cell suspensions is not yet available in *Tylophora indica*. In the present study, the complete plantlets were regenerated from cell suspension cultures of *Tylophora, indica*. Further, attempts are required to increase the survival percentage of regenerants on soil.

Table 1: Effects of B_5 and plant growth regulators on callus induction in *Tylophora indica*.

Cytokinin (mg/L)		Auxins (mg/L)		Response
BAP	0.5	NAA	2.0	+
	0.5		3.0	+ + +
	0.5		4.0	+
	1.5		2.0	+
	1.5		3.0	+
	1.5		4.0	-
	0.5	IAA	2.0	+
	0.5		3.0	+ +
	0.5		4.0	+ + +
	1.5		2.0	+
	1.5		3.0	+
	1.5		4.0	-
	0.5	2, 4 - D	2.0	+ + +
	0.5		3.0	+ +
	0.5		4.0	+ +
	1.5		2.0	+ +
	1.5		3.0	+
	1.5		4.0	+

(-) No callus; (+) Moderate callus; (++) Good callus and (+++) Profuse callus

Table 2: Effects of B_5 medium supplemented with different combinations and concentrations of plant growth regulators on shoot morphogenesis.

Cytokinin (mg/L)		Auxins (mg/L)		%Shoot developed *	Shoot number /explant *	Shoot length (cm) *
TDZ	4.4		0.0	57.96 ± 1.45	3.98 ± 0.17	3.21 ± 0.11
	8.8		0.0	74.53 ± 1.44	4.25 ± 0.12	6.52 ± 0.14
	4.4	NAA	1.0	61.86 ± 1.74	3.16 ± 0.11	5.00 ± 0.11
	8.8		1.0	52.95 ± 1.86	2.68 ± 0.12	4.36 ± 0.12
	4.4	2, 4 - D	1.5	72.61 ± 1.39	4.00 ± 0.11	5.43 ± 0.14
	8.8		1.5	67.45 ± 1.23	3.85 ± 0.14	5.56 ± 0.18
BAP	2.0		0.0	69.43 ± 1.86	3.05 ± 0.15	4.35 ± 0.12
	4.0		0.0	93.86 ± 1.78	4.48 ± 0.12	4.42 ± 0.15
	2.0	NAA	1.0	56.94 ± 1.56	3.98 ± 0.11	2.88 ± 0.12
	4.0		1.0	54.96 ± 1.94	3.90 ± 0.12	2.25 ± 0.11
	2.0	2, 4 - D	1.5	68.37 ± 1.26	2.69 ± 0.13	4.96 ± 0.14
	4.0		1.5	64.61 ± 1.20	3.05 ± 0.14	4.00 ± 0.14

***Data presented as the mean value ± standard error of shoot bud form from the leaf explant after 4 weeks of culture.**

Table 3: Effects of Auxins and salt concentration of B_5 medium on root differention in *Tylophora Indica* shoot regenerants

Auxins	B_5 Full		B_5 Half		B_5 One Fourth	
	No. of roots *	Root length (mm) *	No. of roots *	Root length (mm) *	No. of roots *	Root length (mm) *
IAA (1.0)	4.10 ± 1.0	2.60 ± 1.2	4.70 ± 1.0	24.10 ± 2.6	3.10 ± 1.2	4.60 ± 1.4
IAA (2.0)	3.09 ± 0.5	1.40 ± 0.2	3.60 ± 1.0	17.49 ± 2.4	1.90 ± 0.6	3.04 ± 0.8
NAA (1.0)	2.28 ± 0.6	2.00 ± 1.2	2.90 ± 1.3	16.22 ± 1.0	3.20 ± 1.0	4.16 ± 0.9
NAA (2.0)	1.90 ± 0.6	1.11 ± 0.6	2.30 ± 1.2	6.72 ± 0.8	1.90 ± 0.8	2.13 ± 1.1
IBA (1.0)	2.10 ± 1.3	2.25 ± 0.7	3.40 ± 1.2	4.24 ± 1.1	3.50 ± 1.6	2.20 ± 0.7
IBA (2.0)	1.80 ± 1.0	1.47 ± 0.8	2.10 ± 1.0	2.69 ± 0.5	1.90 ± 1.7	0.56 ± 0.3

*Data presented as the mean value ± standard error of root differention in shoot regenerants after 4 weeks of culture.

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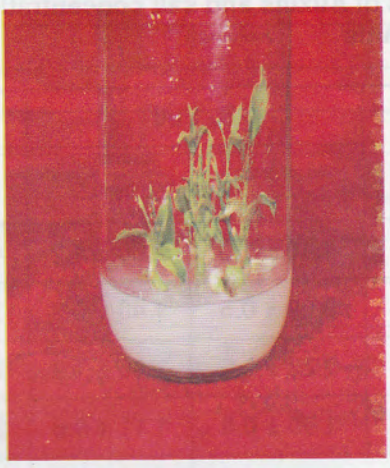
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(Figure – 1)



(Figure - 2)



(Figure – 3)



(Figure – 4)

Fig. 1-4 – Callus formation and plantlet regeneration from cell suspension cultures of *T. indica*.
(1) Callus formation from leaf explants on B_5 medium supplemented with 2, 4-D (2.0 mg/L) and BAP (0.5 mg/L).
(2) Shoot buds developing into multiple shoots on the B_5 medium containing BAP 4.0 mg/L).
(3) Induction of roots on 1/2 B_5 medium fortified with IAA (1.0 mg/L).
(4) Plantlet of *T. indica* transferred to pot containing soil and soilrite (3:1).

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ULTRASTRUCTURAL OBSERVATIONS ON THE EVENTS OF BmNPV INFECTION IN DZNU-Bm-1 (*Bombyx mori*) CELL LINE

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ABSTRACT:
Morphogenesis of Bombyx mori nucleopolyhedrovirus (BmNPV) was observed in the DZNU-Bm-1 cells. Aggregation of cells was evidenced immediately after the addition of virus inoculum in the cultures. The cytopathic effects such as loss of motility and hypertrophy of nuclei were prominent within 16-18 h post inoculation. Free enveloped nucleocapsids entered the host cell and appeared in the cytoplasm. The virogenic stroma formed in the nuclei by 24 h post inoculation. At 36-42 h post inoculation un enveloped virus rods were closely associated with virogenic stroma in the peristromal area and subsequently by 48 h several viral rods appeared in the cytoplasm traveling towards plasma membrane where most of them were budded off from the host cell acquiring the envelope of plasma membrane and releasing from cell surface as extra cellular virus (ECV). After 48 h many naked and enveloped virus particles appeared occluded in the developing occlusion bodies (OBs) and during the late period of infection ECV release was abrogated in favour of OBs formation.

Keywords: *Ultrastructure, Bombyx mori, DZNU-Bm-1, BmNPV infection, BmNPV morphogenesis.*

INTRODUCTION

Although the appearance of *Bombyx mori* nucleopolyhedrovirus (BmNPV) occlusion bodies (OBs) in the blood cells of infected silkworm was first described independently by Maestri (1856) and Cornalia (1856), only in the past 30 years a significant progress has been made in understanding the replication and molecular biology of baculoviruses in cell culture which is providing the basis to explore the nature of virus-host interaction including pathogenicity, host range, virulence and latency. Electron microscopic studies revealed that in the baculovirus-infected cells progeny nucleocapsids assemble in virus-induced subnuclear structure, called the virogenic stroma (reviewed by Williams and Faulkner, 1997). This stroma consisting of fibrillar electron-dense area and an electron-lucent intrastromal space where progeny nucleocapsids are formed (Summers, 1971; Harrap, 1972; Young *et al.,* 1993). Following maturation within the virogenic stroma, the nucleocapsids leave the stroma, with some of them acquiring their envelopes within the peristromal regions of the nucleus (Kawamoto *et al.,* 1977a, b; Fraser, 1986). This type of virion, called an occlusion-derived virus (ODV), is eventually encapsulated in an occlusion body within the nucleus after intranuclear envelopment (reviewed by Funk *et al.,* 1997). Baculoviruses can also produce another type of virion, termed a budded virus (BV). Nucleocapsids destined to become BVs are transported from the nucleus to the plasma membrane and obtain BV envelopes by budding through the plasma membrane instead of the intranuclear envelopment (reviewed by *Funk et al.,* 1997). Long back Raghow and Grace (1974) described the early stages of development of BmNPV in *B. mori* cultures but little is known about the morphogenesis of BmNPV in cultured cells. In this report we describe the ultrastructural studies on the events of BmNPV infection, in the highly susceptible indigenously developed *B. mori* larval ovarian cell line (DZNU-Bm-1) (Khurad *et al.,* 2006).

MATERIALS AND METHODS

Cell line

The cell line, DZNU-Bm-1 was maintained in MGM-448

medium supplemented with 10% FBS. No antibiotics were added to the medium for routine maintenance of cultures (Khurad et al., 2006). The cells were grown in 25 cm^2 polystyrene and glass tissue culture flasks and were incubated at 25 ± 1°C. The cells grew in suspension. They were sub cultured at an interval of 5 to 6 days.

Virus

BmNPV was obtained from diseased larvae of the silkworm, *B. mori* supplied by Centre for Sericulture and Biological Pest Management Research (CSBR), Ambavihar, Nagpur University, Nagpur. Each larva was inoculated individually via ingestion of 2-cm^2 piece of mulberry leaf coated with 10 µl suspension of OBs of BmNPV. The inoculated larvae that consumed the entire dosage were reared further on fresh mulberry leaves (Khurad *et al.*, 2004) .Turbid haemolymph of fifth instar larvae infected with BmNPV was collected through an incision on proleg. After centrifugation (1000g for10 min) the supernatant was diluted with equal volume of medium, passed through 0.45 µm membrane filter and used as an inoculum to infect the cultures. Budded virus of passage one was harvested and cell suspension was centrifuged at 1000g for 5 min at room temperature. The cell pellet was discarded and the virus containing supernatant was stored in the refrigerator at 4°C.

Virus infection

$TCID_{50}$ of passage one virus was calculated. Duplicate 25cm^2 cultures were seeded at 3x 10^5 cells /ml in MGM-448 medium and allowed to grow 1x10^6 cells /ml before being diluted to 5 x 10^5 cells/ml with 10 ml fresh medium. These cultures were infected at m. o. u. of 10 infectious units (IU). The infected cultures were maintained at 25°C. Samples were collected for transmission electron microscopy (TEM) processing at 4 hourly intervals post infection for the first 48 h. p. i. and subsequently every 24 h. p. i.

Light Microscopy

Routinely, healthy and infected cells were examined in culture flasks with a Zeiss inverted phase-contrast microscope. The criterion of BmNPV infection was presence of polyhedral inclusion bodies (PIBs) in a cell. Since the infected cells were heavily clumped, it was difficult to make differential counts of healthy and infected cells in a flask. To overcome this problem, a small number of cells was removed from the infected cultures and transferred to microscopic slides. The cells were then spread out by placing a cover glass over the slide. Differential counts of healthy and infected cells were made. At least 200 to 300 cells were counted from each flask. Percentage infection and number of OBs per cell were determined.

Electron Microscopy

The cells from infected cultures were harvested at different intervals post infection, pelleted and washed in 0.1 M phosphate buffer saline (PBS) with a pH of 7.2. They were fixed for 4 h at 4°C in modified Karnovsky fixative (David *et al.*, 1973) buffered with 0.1M Sodium Phosphate Buffer at pH 7.4. The pellet was then washed in fresh buffer, and post fixed for two hours in 1% Osmium Tetraoxide in the same buffer at 4°C. After several washes in 0.1M Phosphate Buffer, the cells were dehydrated in graded acetone solutions and embedded in CY 212 araldite. Ultrathin sections of 60-80 nm thickness were cut using an Ultracut E (Reichert Jung) microtome and the sections were stained in alcoholic uranyl acetate (10 min) and lead citrate (10 min). The grids were made and viewed under a transmission electron microscope (Morgagni 268 D) operated at 90 kV.

RESULTS AND DISCUSSION

A new cell line, DZNU-Bm-1 (Fig. 1) originated from larval ovarian tissue was found highly susceptible to BmNPV showing 92-94% infection and about 5.3-9.1 x 10^5 infected cells/ml yielding 1.0 x 10^7 to 1.65 x 10^7 OBs/ml (Khurad *et al.*, 2006). Susceptibility to infection is one aspect of virus replication that can vary between cell lines and differences in susceptibility of individual cell types exist in addition to species differences in susceptibility possibly due to the tissue types involved (Lynn, 1992, 2003).

Phase Contrast Microscopy

Addition of inoculum of BmNPV at m. o. i. of 10 to the healthy cells did not affect the integrity of the cells but within an hour the cells exhibited aggregation and formed both small and large clumps indicated the initiation of infection process. Subsequently at 16-18 hours post inoculation (h. p. i.), further signs of infection such as loss of motility and enlargement of cells due to nuclear hypertrophy could be seen in the cultures (Faulkner and Henderson, 1972; Vail *et al.*, 1973). Mitosis was also not common since cell division was apparently inhibited after infection (Vail *et al.*, 1973; Knudson and Tinsley, 1974). Faulkner and Henderson (1972) reported the appearance of small refractive polyhedra of TnNPV in few cells of *T. ni* cells within 20-24 h and between 48 and 72 h.p.i. 100% infection was achieved. Polyhedra production and maturation were usually completed by 72-96 h.p.i. In the present study at 36-42 h.p.i., small refractive occlusion bodies (OBs) appeared in the nuclei of aggregated cells (Fig. 2). The number of OBs/ nucleus was ranging between 10 and 90 depending on the cell size. By 66 h.p.i., mature OBs were prominently seen in the nuclei of infected cells. By 72 h.p.i. the cells started dissociating from the

aggregates and the infected cells loaded with OBs in the nuclei lysed thereafter releasing the OBs to the medium (Fig. 3) as reported earlier by Raghow and Grace (1974). By 96 h. p. i. over 92-94% cells in the culture were infected (Khurad *et al.*, 2006).

Ultrastructure of the Infected Cells

Virus Entry

The free nucleocapsids present in the inoculum were adsorbed to the surface of cells and attached to the plasma membrane within 1-2 h. p. i. The nucleocapsids engulfed in the invaginations of plasma membrane and subsequently bounded by a membrane that was presumably acquired from invaginated plasma membrane (Fig. 4). Thus, confirming the morphological observations of earlier workers with BmNPV (Raghow and Grace, 1974), SfNPV (Vaughn *et al.*, 1972) and AcNPV (Hirumi *et al.*, 1975). However, some data exist that the attachment of nucleocapsids to the cell membrane is receptor-mediated process (Horton and Burand, 1993) in which gp64 envelope fusion protein on the nucleocapsid membrane is the ligand for the receptor on the cell plasma membrane (Hefferon *et al.*, 1999). The virus envelope fuses with the resulting acidified endocytic vesicle releasing the nucleocapsid into the cell cytoplasm followed by its transportation to the nuclear membrane. The nucleocapsid was still enclosed within the vacuoles. The virus containing vacuole appeared only in the periphery of infected cells. Naked nucleocapsids were not seen entering the host cell surface. The ruptured vacuoles releasing the virus rods into the cytoplasmic matrix were appeared in the middle of cytoplasm (Fig. 5).

In the present study, although the traveling of nucleocapsid through cytoplasm within the phagocytic vesicle could be seen, the definite evidence for the entry of the whole nucleocapsid or portion of its content in the nucleus through nuclear pore was lacking. Raghow and Grace (1974) could see the attachment of non-enveloped nucleocapsids of BmNPV to the nuclear pores of *B. mori* cell cultures. They suggested however that BmNPV probably uncoated at the nuclear pore of cultured cells as the attachment of non-enveloped viral rods was evidenced at the nuclear pore. In contrast to this study, Hirumi *et al.* (1975) and Knudson and Tinsley (1974) studied the earlier events of virus-cell interactions with AcNPV and SfNPV in respective cell culture systems and in both instances, non-enveloped nucleocapsids were observed in cell nuclei within 3 h.p.i. Although Hirumi *et al.* (1975) reasoned that these particles probably represented inoculum virus, they were not able to determine the mode of AcNPV uncoating *in vitro*.

Formation of Virogenic Stroma

In most of the cells small aggregates of fine electron opaque granules appeared throughout the nucleus by 18 h. p. i. (Fig. 6). These structures are probably the precursors of virogenic stroma. About 24 h.p.i. the aggregates of numerous small particles increased in size and density. Nucleoplasmic matrices became considerably electron lucent 24 h.p.i., whereas the virogenic stroma increased in size and electron opacity and formed a mass in the nucleus. No distinct virus rods were detected in nuclei at this time. Granados (1980) reported that after the uncoating and entry of nucleocapsids at the nucleus through the nuclear pore, there is a characteristic eclipse and latent period in *in vivo*. *In vitro* one step growth curve studies with baculoviruses revealed that there is an eclipse and latent period of approximately 8-9 h and 10-12 h, respectively and this may vary depending on the virus-cell culture system (Knudson and Tinsley, 1974; Volkman *et al.*, 1976; Granados, 1978). In Bm-1 cells up to 16-18 h.p.i. progressive and characteristic changes like clumping of cells followed by enlargement of nuclei and displacement of chromatin along the nuclear envelope could be seen which may represent the eclipse period. The period between the end of the eclipse and the appearance of small refractive OBs i.e. 20-24 h may represent the latent period.

Formation of Viral Progeny

From 36-42 h.p.i. several unenveloped virus rods appeared and were closely associated with virogenic stroma. At this stage several viral rods were seen in the areas between the virogenic stroma and the nuclear envelope (peristromal area) (Fig. 7).

Virus Release

The majority of the virus rods were not occluded but were released from the nucleus into the cytoplasm. At *48 h.p.i several virus rods were observed in the* cytoplasm that appeared to be enveloped (Fig. 8). These enveloped virus rods traveled through the cytoplasmic matrix towards the plasma membrane (Fig. 9). They budded from the host cell surface, acquired the plasma membrane and subsequently were released from the host cell surface (Fig. 10). These extracellular non-occluded nucleocapsids may serve as inoculum for infection of other healthy cells in culture.

Occlusion Body Formation

A few developing OBs were seen in infected cells after 48 h.p.i., however early commencement of OBs formation i.e. 18-20 h.p.i. was reported by Knudson and Tinsley (1974), Hirumi *et al.* (1975), Granados (1976) and Volkman *et al.* (1976). Many naked and enveloped virus particles appeared around the OBs.

During the late period of infection ECV release was abrogated in favour of OBs formation as a result number of OBs were observed in the nuclei (Fig. 11). The shutdown of ECV production with the onset of OBs formation was demonstrated by Volkman *et al.* (1976). They suggested that budding of virus was completed before occlusion of virus within the nucleus. A large number of occluded nucleocapsids could be seen inside the developing OBs (Fig. 12). However, in the present study the budded virus could be seen even after the commencement of occlusion of virus within the nucleus.

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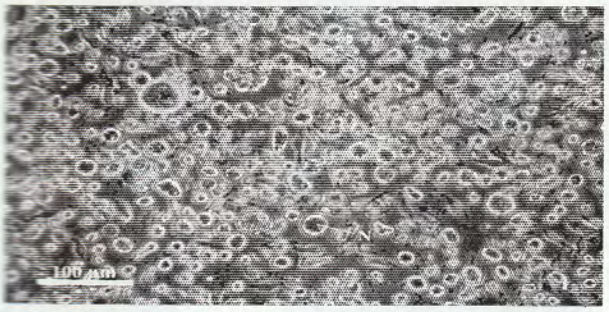
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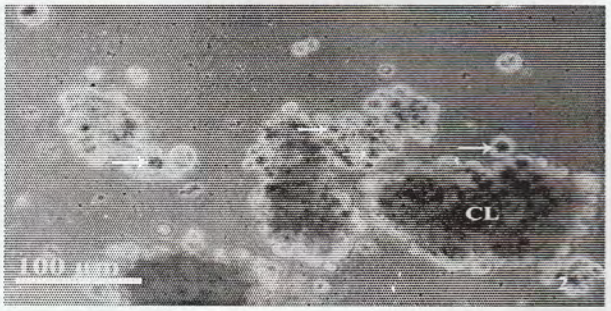
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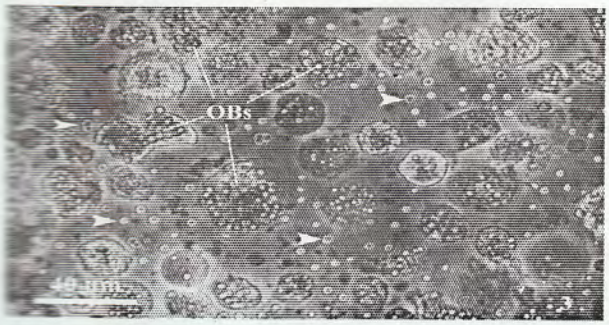
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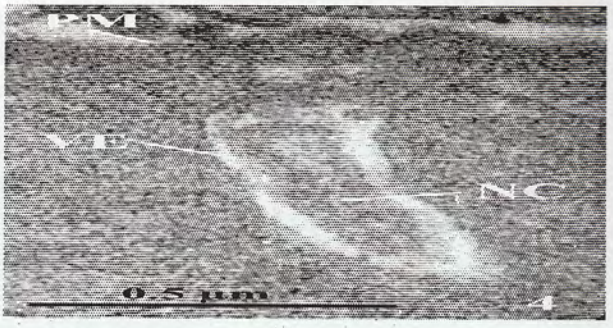
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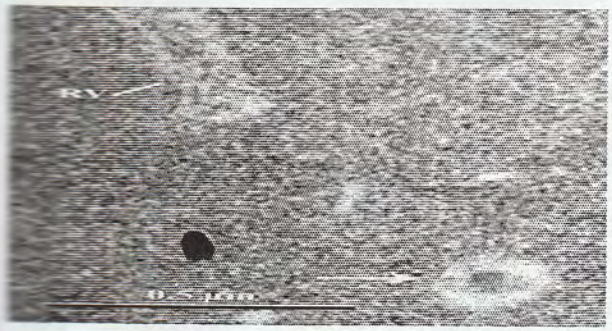
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OBs
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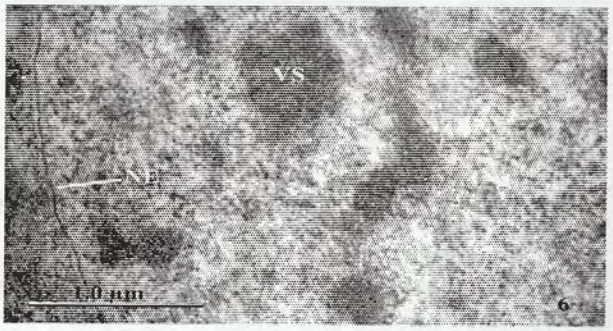
(3)


PM
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NC
0.5 μm
4

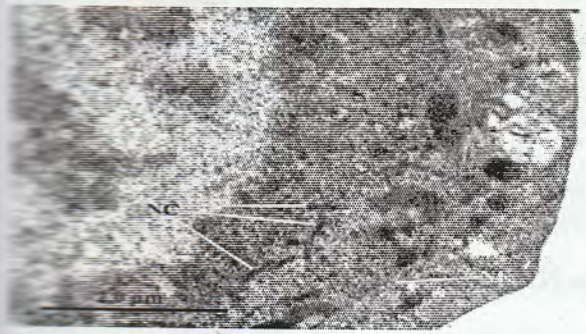
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RV
0.5 μm

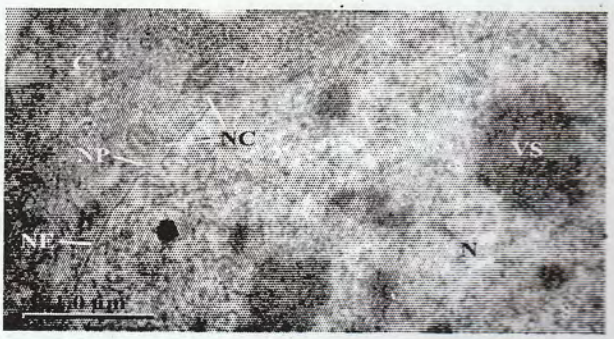
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VS
NE
1.0 μm
6

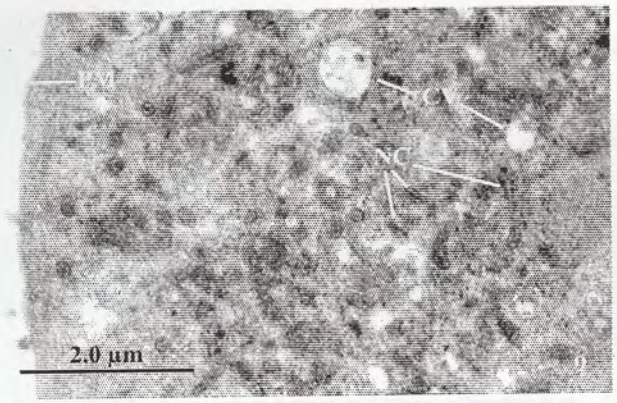
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NC

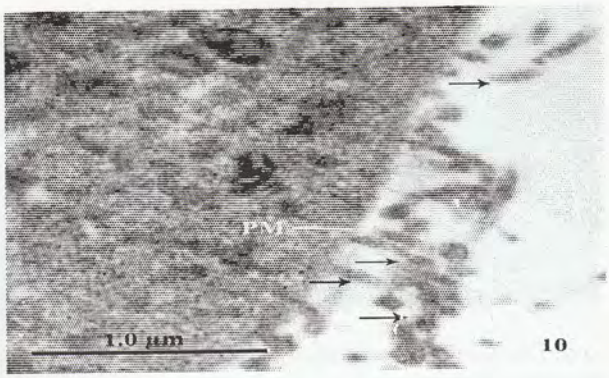
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NC
NP
VS
NE
N
1.0 μm

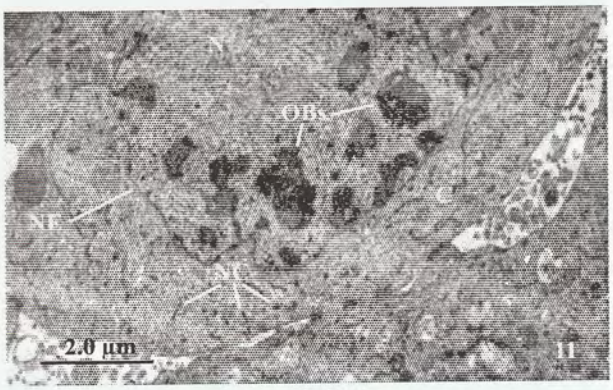
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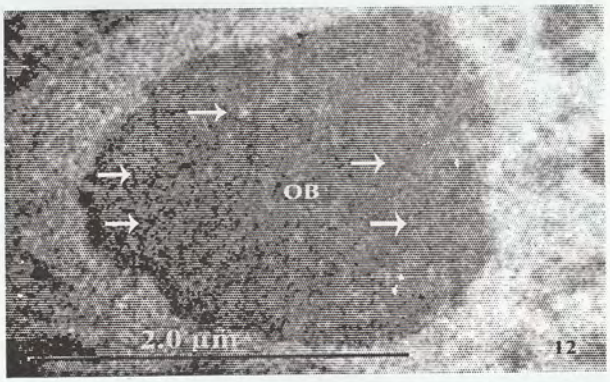
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(10)



(11)



(12)

Explanation of Figures

Fig. 1. Healthy freely suspended cells of DZNU-Bm-1 cell line showing large round, small round, spindle shaped and giant cells in the culture. N- nucleus (Phase contrast).

Fig. 2. Clumps of Bm-1 cells 36 h p. i. (CL). Note the appearance of occlusion bodies in the nuclei of BmNPV infected cells (arrows). Phase contrast.

Fig. 3. Bm-1 cells dissociated from the clumps with nuclei loaded with occlusion bodies (OBs) 72 h p. i. Note the lysis of OBs loaded cells and their release in the medium (arrow heads). Phase contrast.

Fig. 4. Nucleocapsid (NC) entered the host cell cytoplasm with viral envelope (VE) near the periphery of cell 2 h p. i.

Fig. 5. Ruptured empty viral envelop (RV) after release of the virus rods and the cross section of enveloped nucleocapsid (arrow) in the middle of cytoplasm.

Fig. 6. Portion of a nucleus of BmNPV infected cell showing the formation of virogenic stroma in the nucleus (VS) 18 h p. i. NE- nuclear envelop.

Fig. 7. Appearance of several unenveloped virus rods (NC) in the virogenic stroma (VS) and the nuclear envelope (NE) (peristromal area) 36 h p. i.

Fig. 8. Portion of a nucleus showing the virogenic stroma (VS) and the enveloped nucleocapsids (NC) in the cytoplasm (C) near the nuclear pore (NP) on the nuclear envelop (NE).

Fig. 9. Portion of a cytoplasm showing several enveloped nucleocapsids (NC) in the cytoplasm traveling towards the plasma membrane (PM). Note the presence of some cytoplasmic vacuoles (CV) in the cytoplasm.

Fig. 10. Budding of the nucleocapsids from the surface of cell (arrows) by acquiring plasma membrane (PM).

Fig. 11. Formation of occlusion bodies (OBs) inside the nucleus (N) near the nuclear envelope (NE). Note a few nucleocapsids (NC) traveling towards the cell surface.

Fig. 12. A single occlusion body (OB) showing a large number of occluded nucleocapsids (arrows).

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CLINICOCYTOPATHOLOGICAL STUDY OF GOITRE IN HIGH FLUORIDE AREAS OF BATHINDA DISTRICT PUNJAB

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ABSTRACT:
The prevalence of visible goiter in fluorosis was investigated in some fluorotic areas (water fluoride 7.75-10.25 mg/L) of Bathinda district, Punjab, India. The patients aged 30-42 were affected with dental fluorosis along with presence of visible goitre. Serum and urinary fluoride level were highly elevated. Biochemical analysis of thyroid hormone profile of these patients revealed the presence of hypo and hyper function of the thyroid gland. In case of patients affected with simple colloid goiter, decline in the T3 and T4 and marked elevation in TSH hormone concentration was observed. The clinical and biochemical evidence of thyrotoxicosis was observed in all other fluorotic patients. In case of colloid nodular goitre slight elevation in T3 and T4 with decrease in TSH was observed. In the remaining patients hormonal profile revealed high increase in T3 and T4 concentration and fall in TSH. The cytopathological examination of FNAC of these patients revealed presence of different types of visible goitres viz., colloid nodular goitre, thyroid colloid adenoma, cystic goitre, adenomatous goitre, simple nodular goitre and toxic goitre.

Key words: *Fluorosis, Goitre, Hyperthyroidism, Serum fluoride, T3, T4, TSH, Thyroid lesions*

INTRODUCTION

Fluoride containing minerals are widely distributed and leaching of fluoride from rocks is the major cause of high fluoride concentrations often found in groundwater in India (Srikanth *et al.*, 2008). The ingestion of water containing 1 ppm of fluoride during the time of tooth development may result in dental fluorosis in milder form (Jacksen *et al.*, 1995).

In addition to its well known deleterious dental effects, excess fluoride can exert toxic effects on many other tissues and organs, giving rise to broad array of symptoms and pathological changes (Waldbott, 1978; Zhan, 2006). Thyroid hormones play a crucial role in regulating development, differentiation and metabolism of almost every tissue in the human body (Koibuchi and Chin, 2000). The thyroid gland is one of the most sensitive organs in its histopathological and functional responses to excessive amount of fluoride (Shashi, 1986). Increase in dietary fluoride can result in thyroid enlargement (Mclaren, 1976), and reduced blood thyroxine and triiodothyronine (Elnour *et al.*, 2000).

The aim of present investigation was to study the thyroid lesions in patients affected with fluorosis, residing in some fluorotic areas of Bathinda district, Punjab, India.

MATERIAL AND METHODS

An epidemiological survey was conducted in some fluorotic areas of Bathinda district, Punjab, India to study prevalence of goitre. The patients suffering from fluorosis and having symptoms of thyroid disease with visible goitre were randomly selected. Goitres were graded according to the method of Perez *et al.* (1960) Permission to conduct the study was obtained from the Institutional Human Ethics Committee of the Punjabi University, Patiala.

The fluoride concentrations in drinking water, urine and serum were analyzed by ion selective electrode method (Hardwood, 1969).

Thyroid gland function test- The blood samples of these goitrous patients were analyzed for TSH, T3 and T4 hormone levels by using enzyme linked fluorescent assay (ELFA) with the VIDAS autoimmune analyzer.

Cytopathological examination

To study various types of thyroid lesions in fluorosis, the FNAC slides of all the patients were obtained from hospital and processed for cytopathological study. Smears were air-dried and stained using the May-Grunwald-Giemsa technique. Some smears were wet-fixed in 95% ethyl alcohol and stained using a modified Papanicolaou method (Goellner *et al.*, 1987). The thyroid lesions were confirmed by microscopic examination and were subsequently microphotographed.

RESULTS AND DISCUSSION

Analysis of water sample

The level of fluoride in the drinking water sample varied from 7.75 to 10.25 mg/L (mean 8.07±1.75 mg/L) in selected fluorotic areas of Bathinda district, India.

The fluoride contents in body fluids

The fluoride levels in serum and urine is shown in Table 1and Graph 1. The fluoride levels in urine as well as serum were higher in all patients affected with dental fluorosis and thyroid disorders in residential areas with high drinking water fluoride. The urinary fluoride concentration was found to be elevated and ranges from 2.75-4.85 mg/L and serum fluoride concentration was found to be 0.25- 0.35 mg/L (Table 1).

The clinical and hormonal profile examination of the fluorotic patients revealed presence of various types of visible goitre (Graph 2; Photographs 1-4; Plate I).

Clinicopathological findings

Colloid nodular goitre

Colloid nodular goiter was observed in a 30 years old female patient with the clinical and biochemical evidence of thyrotoxicosis. The serum T3 and T4 hormones showed elevation, while TSH was declined. The pathological changes involve presence of thick colloid seen in the form of porched earth.

The degenerated RBC's and macrophages were also seen. Karyopycnosis of follicular epithelial cells and reduction in cytoplasm was observed, this change may be due to high fluoride ingestion in drinking water by fluorotic patients (Figure 1; Plate II). Balloon shaped negatively stained degenerative area was visible. The epithelial cells were in the form of clumps, or acini of hypercellular area that were adherent to pink stroma with microfollicle formation (Figure 2; Plate II).

Thyroid colloid adenoma

Thyroid colloid adenoma was noted in a 32 years old female fluorotic patient with the hyperfunction of thyroid gland. The increase in concentration of T3 and T4 and decrease in TSH was observed. Areas of darkly stained thick and lightly stained thin colloid alongwith highly degenerated fibrous tissue were visible (Figure 3; Plate II). Clusters of pleomorphic hurthal cells were present. A large number of cells exhibited microbodies formation, while some showed flagella like projection. Some degenerative follicles in which follicular epithelium were almost lost were also visible (Figure 4; Plate II).

Cystic goiter

A 32 years old fluorotic patient suffering from cystic goiter demonstrated elevation in T3 and T4 and decrease in TSH concentration. The epithelial components were like isolated cell nuclei, even small clumps were also present. Numerous foamy macrophages were present with ingested debris in it (Figure 5; Plate II). Highly degenerated fibrous tissue and follicles with extensive decrease in colloid material was present. Trabacular and tubular pattern were evident (Figure 6; Plate II).

Adenomatous goitre

Hyperfunctioning of thyroid gland along with presence of adenomatous goiter was studied in a 35 years old female fluorotic patient. A well defined capsule of adenoma composed of follicles of various sizes, showing extensive decrease in colloid material was evident. The fibrous tissue exhibited compression in one region and degeneration in other region. There was some fibrous tissue which showed vacuolated small sized follicles in which small amount of colloid was present (Figure 7; Plate III). Highly degenerated fibrous tissue was present, and atrophy as well as hypertrophy had occurred in follicles. Some highly degenerated follicles showed complete loss of follicular epithelium. Some follicles were extensively hyperatrophied due to this connective tissue was being compressed (Figure 8; Plate III).

Simple colloid goitre

Simple colloid goiter was recorded in two female fluorotic patients. The biochemical assay demonstrated that TSH was highly elevated (115.4-119.0 µIU/ml). The amount of T3 and T4 was declined considerably. The colloid was thin membrane like with blue to purple in colour, patches of very thick colloid were observed. Some negatively stained vacuolated cells were visible (Figure 9; Plate III). The pathological analysis revealed that there was hyperplasia of parafollicular cells and endocytosis of colloid. Chain formation of follicular cells was observed. Bare lymphocytic like nuclei were also present (Figure 10; Plate III).

Toxic goitre

Toxic goiter was studied in male patient aged 42. Hormonal profile analysis revealed elevation in T3, T4 and decline in TSH. There was excessive cellularity of

parenchyma and increase in number of follicular cells. There was increase in the lymphoid tissue in interfollicular cell (Figure 11; Plate III). In some areas, large lymphoid follicles were formed. Various sized irregular and hypertrophid thyroid follicles were present (Figure 12; Plate III).

During the present study FNAC slides of fluorotic patients who had visible goiter has been studied. The cytopathological examination revealed four types of thyroid lesions viz. colloid, nodular, toxic and adenomatous goiter.

McLaren (1976) documented that fluoride can accumulate in the thyroid and causes structural and functional changes (Bobek *et al.* 1976; Shashi 1988; Trabelsi *et al.* 2001; Schuld 2005; Zhan 2006). In India, both iodine deficiency disorders and fluorosis are the two most prevalent endemic diseases which co-exist in certain regions in the country. Though goiter-an enlarged thyroid gland is commonly recognized as a visible sign of iodine deficiency, the health effects of iodine deficiency reach far beyond those of goiter (Susheela *et al.*, 2005). Goiter prevalence in a number of endemic areas has been shown to depend on factors other than iodine deficiency (Day and Powell-Jeckson, 1972). Several authors have reported an association between endemic goiter and fluoride exposure in human populations in India (Wilson, 1941; Siddiqui, 1960; Desai *et al.*, 1993); Nepal (Day and Powell, 1972); England (Murray *et al.*, 1948); South Africa (Steyn *et al.*, 1955; Jooste *et al.*, 1999); and Kenya (Obel, 1982).

During the present investigation pathological alterations involving presence of well defined capsule of adenoma composed of follicles of various sizes, showing extensive decrease in colloid material were observed. Highly degenerated fibrous tissue was present, and atrophy as well as hypertrophy had occurred in follicles. Some highly degenerated follicles showed complete loss of follicular epithelium. Many workers have reported such pathological changes as mild atrophy of the follicular epithelium (Ogilvie, 1953), distended endoplasmic reticulum in follicular cells (Sundstrom, 1971) and morphological changes suggesting hormonal hypofunction. Excess fluoride in drinking water was a risk factor of more rapid development of thyroid pathology (Bachinskii *et al.*, 1985). Shashi and Kaur (2008) recently have reported histopathological evidences of scalping of colloid, infiltration of chronic inflammatory cells, extensive fibrosis and formation of fibrous capsule, compressed glandular tissue and distorted follicles in various types of neoplastic and non-neoplastic thyroid lesions.

The fluorine content in the thyroid gland is reported to be second only to that of the aorta in non-bone tissues (Monsour and Kruger, 1985). Okayasu *et al.* (1985) observed hyperplastic nodules, consisting of thyroid parafollicular cells in all fluoride treated groups, of rats. Cytological changes seen in the thyroid gland of mice treated with NAF were characterized by follicular cell hyperplasia, an increase of follicular number and vascularity, and a decrease of colloid (Bouaziz *et al.*, 2005). It is also suggested the NAF depresses the endocytosis of colloid and thyroid secretion by inhibiting aerobic glycolysis in the follicular all (Williams *et al.*, 1972; Bouaziz *et al.*, 2005).

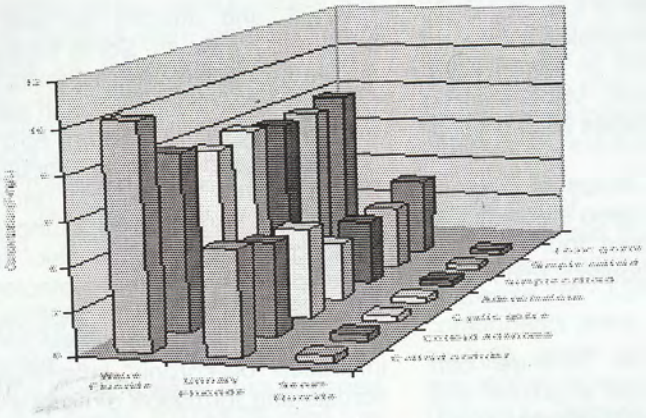
ACKNOWLEDGEMENT

We are grateful to Dr. D.S. Dhillon for providing FNAC of goitrous patients.

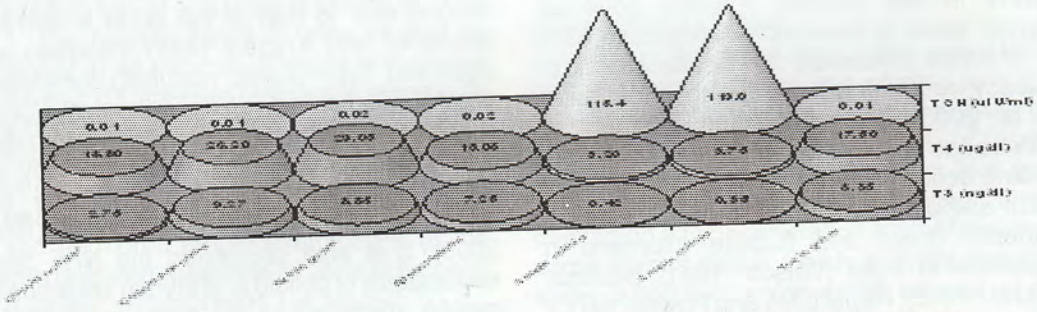
Table 1. Clinical characteristics of fluorotic patients affected with various types of goitres

Age / Sex	Type of goiter	Fluoride (mg/L)			Thyroid Function Test		
		Water	Urine	Serum	T3 ng/dL	T4 µg/dL	TSH µIU/ml
32/F	Colloid nodular	10.25	4.85	0.34	2.75	18.80	0.01
30/F	Colloid Adenoma	8.25	4.35	0.35	9.27	26.20	0.01
28/F	Cystic goitre	7.75	4.20	0.30	8.85	29.05	0.02
35/F	Adenomatous	8.05	2.75	0.30	7.25	15.08	0.02
40/F	Simple colloid	7.75	3.05	0.30	0.42	3.20	115.40
32/F	Simple colloid	7.75	3.02	0.28	0.35	3.75	119.00
42/M	Toxic goitre	8.05	3.75	0.25	8.35	17.50	0.01

Standard range of drinking water fluoride: <1 mg/L.
Fluoride in urine: normal upper limit 0.1 mg/L.
Fluoride in serum: normal upper limit: 0.02 mg/L
Standard range of T_3- 0.60-1.60 ng/ml
Standard range of T_4- 4.6-9.5µg/dl
Standard range of TSH- 0.25-5.00 µIU/ml



Graph 1. Fluoride levels in drinking water (mg/L) and body fluids of goitrous patients



Graph 2. Thyroid hormonal profile of fluorotic patients affected with various types of visible goitres.

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Explanation of Figures

Plate- II

Figure 1. Low- power view showing colloid nodular goiter in female patient of fluorosis. Macrophages and few scattered round to oval uniform looking follicular cells are visible. X100

Figure 2. Magnified view of above, showing ample colloid, RBC's and monolayered clusters of follicular cells. Follicular cells shows anisonucleosis. X400.

Figure. 3. Low- power view of thyroid colloid adenoma in a fluorotic female patient. Areas of darkly stained thick and lightly stained thin colloid are visible. X100

Figure 4. High- power view of above showing presence of pleomorphic hurthal cells. Some cells exhibit microbodies formation, while others have flagella like projections. X400.

Figure 5 Cystic goiter in a female fluorotic patient. The epithelial component in the form of isolated cell nuclei and macrophages with ingested debris init are seen. X400

Figure 6 Highly degenerated fibrous tissue and follicles devoid of colloid material in cystic goiter an fluoride intoxication. X400

Plate- III

Figure 7 Adenomatous goiter in a fluorotic female patient. Degenerated fibrous tissue along with vacuolated small sized follicles having small amount of colloid was visible. X100

Figure 8 FNAC of same female patient. Highly degenerated fibrous tissue with atrophied and hypertrophied follicles are in adenomatous goiter in fluorosis. X100.

Figure 9 Simple colloid goiter in fluorotic female patient aged 40. Patches of thick colloid with negatively stained vacuolated cells are visible. X100

Figure 10 High magnification view of above revealed hyperplasia of parafollicular cells and endocytosis of the colloid. Chain formation of follicular cells is also visible. X400

Figure 11 Toxic goiter in male fluorotic patient aged 42. There was excessive cellularity of parenchyma and hypertrophy of follicular cells. X100

Figure 12 Various sized irregular, atrophid and hypertrophid thyroid follicles in toxic goiter in fluorosis. X100.

PLATE-I



Clinical photographs (1-4) of the neck anterior view of female patients affected with visible goiter (Grade II) exposed to different concentrations of fluoride (mg/L) in drinking water.

1. 10.25 mg F^-/L ; 2. 8.25 mg F^-/L ; 3. 8.05 mg F^-/L ; 4. 7.75 mg F^-/L

PLATE-II

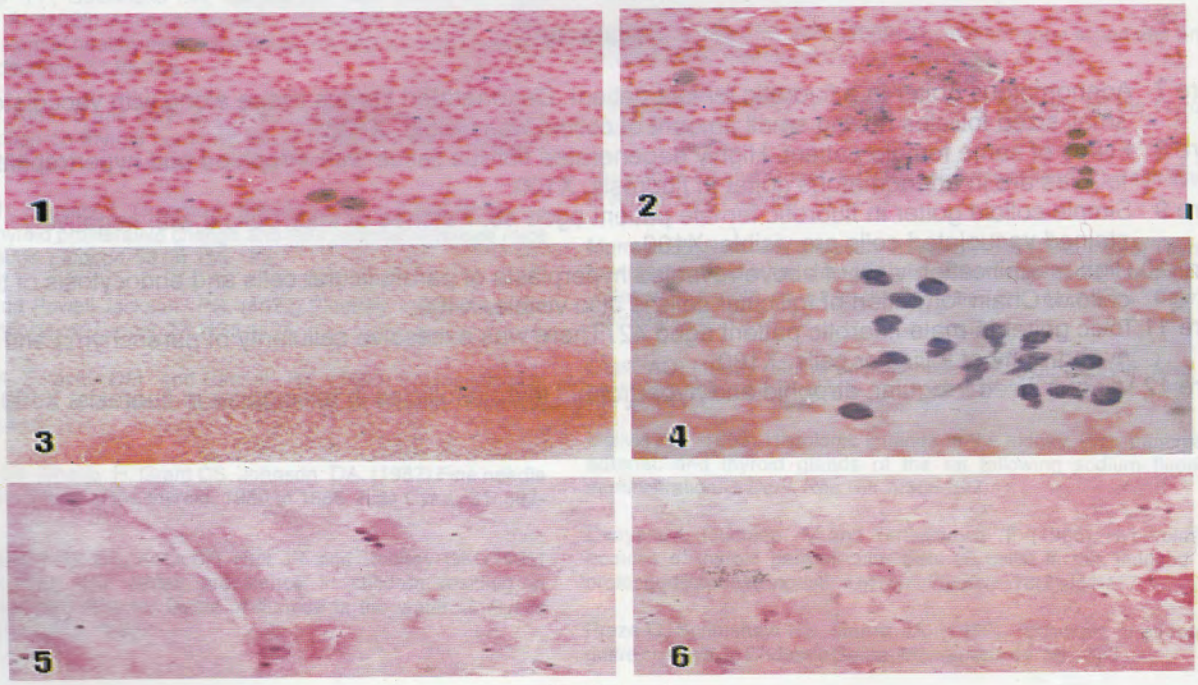
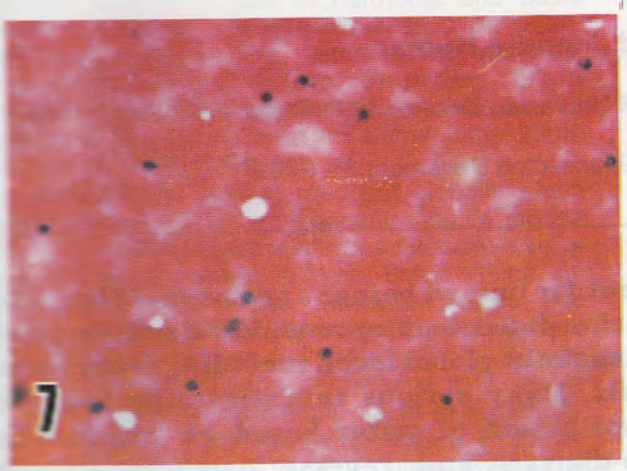
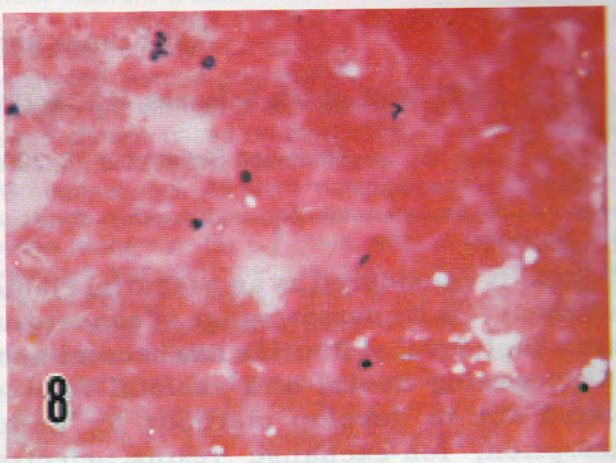
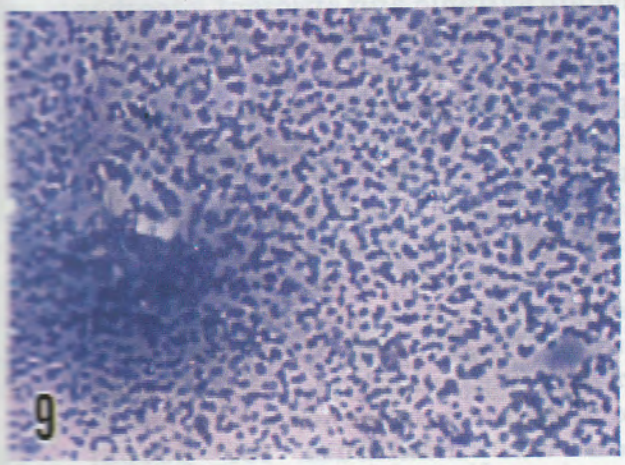
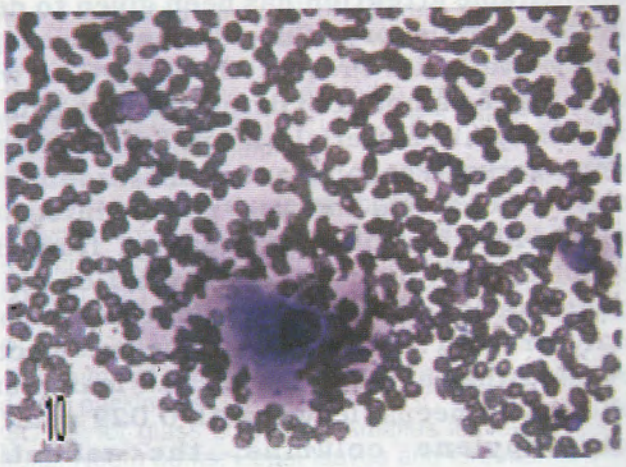


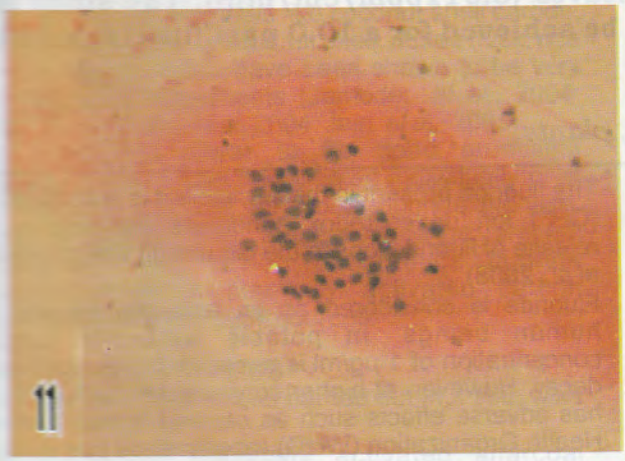
PLATE-III

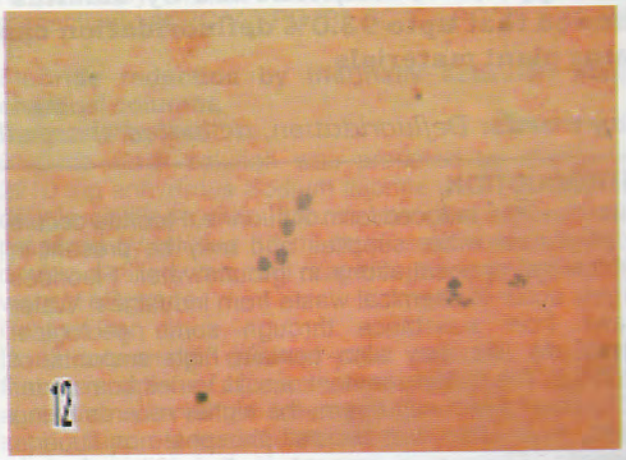












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DEFLUORIDATION OF WATER BY ADSORPTION ONTO THERMALLY ACTIVATED CHARCOAL OF PLANT MATERIAL SOURCE

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ABSTRACT:

Fluoride is considered as an essential element for human beings and the most important fact about it is its narrow range of concentration (0.5-1.5 ppm) considered safe for potable water. High level of fluoride has become a menace at global level, including many parts of India, causing dental and skeletal fluorosis. Many serious investigations have been carried out to develop methods for defluoridation of water based on precipitation, adsorption, ion-exchange resins, electro-dialysis and reverse osmosis. Recently, considerable attention has been given to develop suitable and efficient adsorbents using plant materials for defluoridation purpose, as they are cheaper, operatively simple and widely available. Present investigation was carried out with the major objective of studying the amount of defluoridation using thermally activated charcoal prepared from different plant materials. A total of 5 different plant materials (Amaltas leaves and fruits; Neem barks; Peepal leaves and Groundnut Shells) were used to prepare thermally activated charcoal and were packed into glass and polypropylene columns of known bed-height. All the columns prepared from different plant materials were screened for reduction of fluoride concentration in standard solution of 10 ppm fluoride concentration. Reduction in fluoride ion concentration was estimated using fluoride ion analyzer with fluoride ion-selective electrode. The results of present investigation revealed that amaltas leaves had maximum fluoride reduction of 0.042ppm/cm/min, followed by peepal leaves with 0.029 ppm/cm/min, when packed into glass column. In polypropylene columns, the reduction was maximum for peepal leaves (0.016ppm/cm/min) followed by amaltas leaves (0.011ppm/cm/min). The study also showed that upto 93.0% defluoridation can be achieved for a 10.0 ppm fluoride solution using plant materials.

Key words: *Defluoridation, activated charcoal, plants.*

INTRODUCTION

Fluoride is the reduced form of fluorine. Fluoride occurs in all natural water supplies and may be present in detrimental concentrations in groundwater. Fluorides largely occur in chemical waste from industries. Water drawn from substance through some geological formations like may also contain high amounts of fluorides. The fluoride content of soils varies from under 20 to several thousand ppm, the higher records being mostly from areas with bedded phosphate on fluoride deposits. Fluoride is present in the soil and rock formation in the form of fluor-apatite, fluorspar, amphiboles and micas weathering rock. Alkali contributes fluoride natural waters (Brewer, 1996; Elrashidi and Lindsay, 1986; Bishnoi and Arora, 2007). The fluoride present in these minerals is substituted by (OH^{-1}) ion under redox conditions resulting in the release of fluoride ions to the circulating waters (Kumar *et al.*, 2008).

Fluoride is considered as an essential element for human beings. In potable water, a fluoride concentration of 1 µg/ml is necessary to prevent tooth decay. However, at higher concentration (>2µg /ml), it has adverse effects such as causing fluorosis. World Health Organization (WHO) recommends that drinking water should not contain less than 0.5ppm or more than 1.5ppm of fluoride, though consideration of local conditions may require a more stringent threshold (Murlidharan *et al.*, 2002). Fluoride is a health affecting substance. Fluoride concentration in water above

1.5ppm has serious physiological implications resulting in dental and skeletal fluorosis. Contrary to it, a level lower than 0.5ppm results in hormonal and basic metabolism imbalance resulting in diseases like goitre, dental caries, osteoporosis, arthritis, hip fractures, cancer, infertility, thyroid disorder, brain damage,(Alley,1993; Berndt,1998), liver disorder and intestinal problem, etc (Ambika and Sumanlatha, 2005; Chandrasekar . *et al.*,2005)

Many studies have been carried out globally which comprehensively reflect high concentration of fluoride in ground and natural water resources. Globally, level of fluoride has been reported in India, Spain, China, Sri Lanka, Brazil, West Indies, Holland, Mexico and countries of North and South America (Tembhurkar and Dongre, 2006; Singh *et al.*,2004). In India, fluoride occurrence, far beyond the permissible limit, in top aquifer system is endemic in many places of Rajasthan, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Tamilnadu, Karnataka, Gujarat, Punjab, Harayana, Bihar, Jharkhand, West Bengal, Assam, and Kerala (Chandarsekar *et al.*,2005; Singh *et al.*,2004; Latha *et al.*,1998).

Extensive studies have been carried out to develop methods to mitigate the menace of high fluoride content in aquifer. Most of the methods for defluoridation of water are based on precipitation (use of alum or lime), adsorption (activated alumina, bone char, calcium hydroxide appetite bauxite), ion-exchange resin, electro-dialysis and reverse osmosis (Kumar. S., 2008; Weijesundara ., 2004; Srimurali and Karthikeyan , 2008).

Recently, considerable attention has been given to develop suitable and efficient adsorbent for defluoridation purpose as they are cheaper, operatively simple and widely available. Adsorption of activated carbon from roots of water hyacinth has been found efficient in reducing fluoride level from 15ppm to permissible range. Materials like coconut shell carbon, bone char, tea waste, groundnut shell, coffee husk, rice husk and nirmali seeds have been shown to be very effective fluoride adsorbents (Jamode *et al.*, 2004; Kumar , 2008; Singh . *et al*, 2004; Jain *et al.*, 2005).

Plant materials appear to be good promising candidates to be further investigated individually or in combination to mitigate the global fluoride menace. Therefore, looking into these facts, the present investigation was carried out reduce fluoride concentration using thermally activated carbon prepared from different plant sources.

MATERIAL AND METHODS

The whole investigation was carried out in a step wise manner –

(i)Preparation of thermally activated charcoal adsorbent columns.

(ii)Fluoride reduction by thermally activated plant charcoal columns.

Preparation of thermally activated charcoal adsorbent columns

Plant materials

All together 5 plant material samples were collected from different locations of Gwalior city (Table 1) and used for activated charcoal preparation to construct adsorbent columns for fluoride.

Activated charcoal preparation

For the preparation of activated charcoal, fresh leaves were chosen based on their crude fiber content from Peepal & Amaltas; Neem barks, Amaltas fruits and Groundnut shell. All plant materials were washed with water to remove dust and other impurities. They were dried in the sun for 5-7 days, after drying they were crushed manually/ mechanically. (This process saves the energy consumption than in hot air oven drying.) These dried and crushed plant materials were burnt in the thermal furnace at 400°C for 1 hr. to obtain thermally activated charcoal form of respective plant materials. The particles were filtered through a wire-mesh of pore size 0.4mm^2 to obtain a uniform particle size.

Column Preparation

All 5 activated charcoal of different plant materials taken were washed separately with distilled water and 1% formaldehyde solution repeatedly 8-10 times, to remove the colour and then dried in oven, maintained at temperature range of 120-140°C for a period of 12 hr. before packing of column.

For defluoridation study, columns (glass and polypropylene) were prepared by packing the thermally activated charcoal of plant materials into glass column (6.2 cm height and 0.76 cm width) and polypropylene column (PP) (12.5 cm height and 0.47 cm width). Bed height of different plant materials in glass column and PP column were as in Table 2.

All column of different samples were marked properly during packing as GC "A", GC "B", GC "C", GC "D", and GC "E" for glass column of Amaltas leaves, Amaltas fruits, Neem barks, Pepal leaves and Groundnut shell respectively while PP columns were marked, respectively, as PC "A", PC "B", PC "C", PC "D", and PC "E".

Fluoride reduction by thermally activated plant charcoal columns

Reagent Preparation

Fluoride stock solution was prepared by dissolving 2210 mg anhydrous Sodium fluoride (NaF) in 1000 ml double distilled water. Fluoride standard solution was prepared by diluting 10 ml stock solution to 1000 ml double distilled water. All reagents, used for the study, were of AR grade.

Column Run

In all columns of different plant materials, standard fluoride solution of 10 ppm concentration was drawn at a regular interval of time. Effluents were collected separately in a wash bottle (Tarson). All bottles were marked properly before collecting 5-10 ml of effluents of all different glass & polypropylene columns of plant materials.

Fluoride Estimation

Fluoride ion concentration, in different run samples, was measured by using fluoride ion analyzer (Eutech

Instrument) with fluoride ion-selective electrode PCD 6500 Eutech, USA. The detection limit of the instrument was 0.025-500 mg l^{-1}. The instrument was calibrated against standard fluoride solutions (1000 ppm), serially diluted in the range of 0 to 10 ppm by diluting appropriate volume. After calibration, fluoride ions were estimated in the samples by using a buffer called TISAB (Total Ionic strength Adjusting Buffer). Equal volume of buffer was added to the sample to adjust the pH and for providing free fluoride ion to the sample. Fluoride Ion-analyzer was used directly for fluoride ion concentration measurement of the different experimental samples (effluents).

RESULTS AND DISCUSSION

Fluoride reduction by thermally activated plant charcoal columns

Thermally activated charcoal of uniform pore size 0.4 mm^2 of five plant materials were packed in the column (Glass and Plastic, each) of measured bed height and were used for fluoride reduction study (Table 2) in duplicates. 10ppm of standard fluoride solution was passed from each column of different plant materials. The elutes were collected separately and were analyzed for fluoride concentration (in ppm) estimation. In glass columns, reduction of fluoride was maximum in GC 'D' (Peepal leaves) column of 9.38ppm *i.e.,* 93.80% while 8.46 ppm or 84.60% reduction was observed in GC 'A' (Amaltas leaves) column.

In Polypropylene columns maximum reduction was found to occur again in PC 'D' (Peepal) column of 6.57ppm *i.e.,* 65.70% followed by PC 'A' (Amaltas Leaves) column 4.98ppm or 49.80% (Table 3). The results are in good accordance with the previously reported findings (Murugan & Subramanium, 2006; Tembhurkar & Dongre, 2006). In rest of the columns the reduction of fluoride was not promising and ranged from 16% to 37% in Glass and Polypropylene columns.

Fluoride reduction per unit height and reduction per unit contact time

Fluoride reduction per unit height was observed maximum in (Peepal leaves) column GC 'D' 2.345 ppm per cm while the reduction per unit contact time was observed to be 0.117ppm per min. It was followed by (Amaltas leaves) column GC 'A', where the reduction per unit height was observed to be 2.287ppm per cm while reduction per unit contact time was estimated as 0.154 ppm per min (Table 4).

Similar trend was observed in Polypropylene columns with maximum reduction per unit height observed in (Peepal leaves) Column PC 'D' of 730ppm per cm and reduction per unit contact time of 0.146 ppm per min. Amaltas leaves Column PC 'A', provided a fluoride reduction of 0.553 ppm per cm (Reduction per unit height) and 0.100 ppm per min. (Reduction per unit contact time) (Table 4).

Fluoride reduction per unit height per unit contact time:

On analyzing the plant material on the basis of fluoride reduction per unit height per unit contact time column GC 'A' of Amaltas leaves had maximum reduction of 0.042 ppm per cm per min. followed by Peepal leaves column GC 'D' with the fluoride reduction of 0.029 ppm per cm per min (Figure 1). In polypropylene columns, the observations were similar to the glass column with Peepal leaves column PC 'D' having fluoride reduction of 0.016 per cm per min. while Amaltas leaves column PC 'A' gave 0.011 ppm fluoride reduction per cm per min (Figure 1).

The results of present investigation reveal that Amaltas leaves had maximum fluoride reduction of 0.042 ppm per cm per min. followed by Peepal leaves column with the fluoride reduction of 0.029 ppm per cm per min. when packed into glass columns. The results were almost similar when the plant materials were packed into polypropylene columns, in which Peepal leaves column had fluoride reduction of 0.016 per cm per min. while Amaltas leaves column gave 0.011 ppm fluoride reduction per cm per min. Also, the study shows that *upto 93.8% defluoridation can be achieved for a 10.0 ppm fluoride solution, using plant materials. The same* can be increased if the number of cyclic passage through the column can be increased or the contact time is increased, which needs to be investigated further.

Thus, the present study has given a strong evident support to the view that plant material can be used to develop efficient adsorbent for defluoridation purposes. Also, the investigation has provided a good platform for further studies to develop novel, efficient, cheaper and operatively simpler methods for defluoridation of water.

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Table 1: Plant material samples collected from different locations of Gwalior city

S. No.	Plant	Part of Plant taken for study	Collection point
1.	*Cassia fistula* (Amaltas)	Leaves	Jiwaji University Campus, Gwalior
2.	*Cassia fistula* (Amaltas)	Fruits	Jiwaji University Campus, Gwalior
3.	*Azadirachta indica* (Neem)	Barks	Jiwaji University Campus, Gwalior
4.	*Ficus religiosa* (Peepal)	Leaves	Jiwaji University Campus, Gwalior
5.	*Arachis hypogeal* (Groundnut)	Shell	Commercial vendor

Table 2: Bed height of Thermally Activated Charcoal Columns

Plants	Column Bed Height	
	Glass Column	Plastic Column
Amaltas leaves	3.7 cm	9.0 cm
Amaltas fruits	3.7 cm	9.0 cm
Neem barks	3.7 cm	9.5 cm
Pepal leaves	4.0 cm	9.0 cm
Groundnut shell	3.8 cm	9.2 cm

Table 3: Reduction of fluoride concentration

S.No.	Sample	Initial concentration (ppm)	Final concentration (ppm)	Reduction in Fluoride concentration (ppm)	% Reduction in Fluoride concentration
1.	GC 'A' (Amaltas leaves)	10.00	1.54	8.46	84.60
2.	PC 'A' (Amaltas leaves)	10.00	5.02	4.98	49.80
3.	GC 'B' (Amaltas fruits)	10.00	7.44	2.56	25.60
4.	PC 'B' (Amaltas fruit)	10.00	8.40	1.60	16.00
5.	GC 'C' (Neem barks)	10.00	6.40	3.60	36.00
6.	PC 'C' (Neem barks)	10.00	6.25	3.75	37.50
7.	GC 'D' (Peepal leaves)	10.00	0.62	9.38	93.80
8.	PC 'D' (Peepal leaves)	10.00	3.43	6.57	65.70
9.	GC 'E' (Groundnut shell)	10.00	8.37	1.63	16.30
10.	PC 'E' (Groundnut shell)	10.00	7.14	2.86	28.60

Table 4: Fluoride reduction per unit height and reduction per unit contact time

Column	Column Height (cm)	$[F^{-1}]$ ion Reduction (ppm)	$[F^{-1}]$ ion Reduction/ Height (ppm/cm)	Contact time (min)	$[F^{-1}]$ ion Reduction/contact time (ppm/min)
GC 'A'	3.7	8.46	2.287	55	0.154
GC 'B'	3.7	2.56	0.692	55	0.047
GC 'C'	3.7	3.60	0.973	90	0.040
GC 'D'	4.0	9.38	2.345	80	0.117
GC 'E'	3.8	1.63	0.429	60	0.027
PC 'A'	9.0	4.98	0.553	50	0.100
PC 'B'	9.1	1.60	0.176	55	0.029
PC 'C'	9.5	3.75	0.395	60	0.063
PC 'D'	9.0	6.57	0.730	45	0.146
PC 'E'	9.2	2.86	0.311	35	0.082

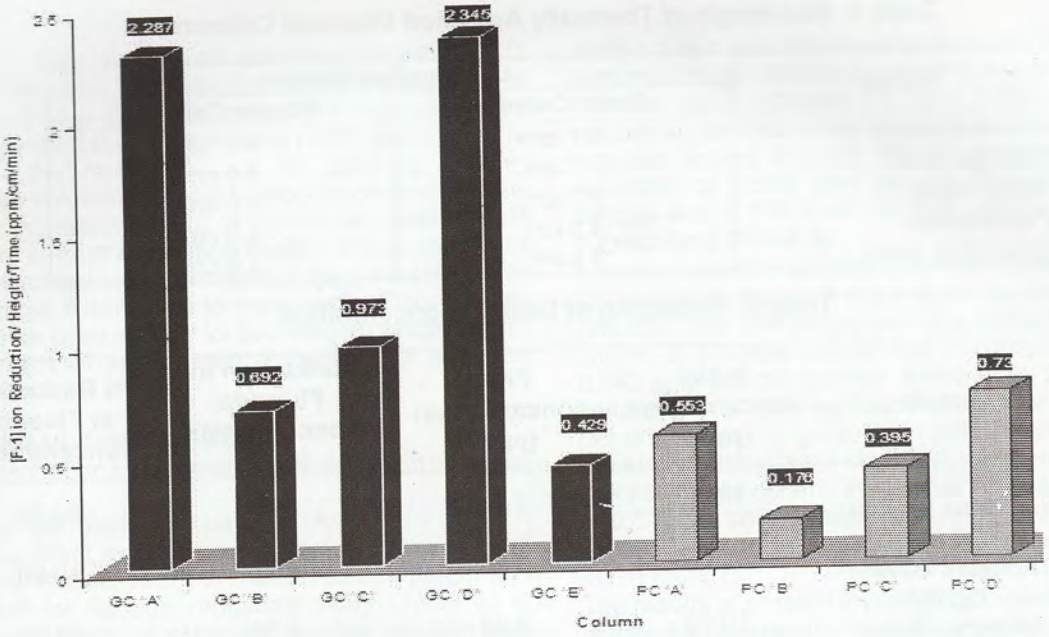


Figure 1: Fluoride reduction per unit height per unit contact time by Activated Plant Charcoal Columns

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IMPACT OF NEW HETEROCYCLIC COMPOUND ON FERTILITY IN MALE ALBINO RATS.

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ABSTRACT:

The present study was carried out on the effect of novel heterocyclic compound on reproductive function and fertility in male albino rats. The treatment with 3-Imino (2-Oxo-3,4-Dihydro -4 –Phenyl -5 -Carboethoxy - –Methyl -Pyrimidine -3yl)-Indole-2-Ones compound at the level of 20 mg/Kg body weight for 21 days did not bring any significant change in the body weight where as the weights of testis, epididymis, seminal vesicle and ventral prostates were decreased. The result showed a mild increase in sperm abnormalities, but in contrast to the same treatment caused a reduction in sperm count and motility in sperm collected from the cauda epididymis of treated rats. The fertility rate and GSI values were also decreased in treated rats.

Keywords: *Heterocyclic compound, Cauda epididymis, Sperm count, Motility, Sperm abnormality, Fertility, GSI.*

INTRODUCTION

Population explosion is a global burning problem, which affects the health and economy of world. There is an increasing need for new means of population control throughout the world. Our country ranks second after China population. The problem of rapid growth of population has been throwing a challenge to the entire human race. Fertility control is very essential for maintaining satisfactory standards in the developing countries. Thus the control of human fertility in the sense of its limitation is the most important and urgent. To control the population explosion, investigations are undertaken to discover an effective safe and low cost contraceptive methods.

Contraception is literally to prevent the conception (Winfindal and Gourley, 2000). The synthetic agents available today for fertility control produce severe side effects (Rice, 1971). Thus there is a need to replace these agents by non-toxic antifertility agents.

The development of new fertility regulating drugs from heterocyclic compounds is an attractive proposition because heterocyclics are widely utilized compounds in both pharmaceutical and agricultural fields (Lang and Li, 1984). In our previous work *3 -Imino (2 -Oxo-3,4 -Dihydro -4 –Phenyl -5 -Carboethoxy - –Methyl -Pyrimidine -3yl) -Indole-2 -Ones* compound has showed antifertility activity (Ajitha, 2009). Further same compound was also tried to assess whether it has any adverse effect on fertility in male albino rats. The new *heterocyclic compound developed in the Medicinal Chemistry Laboratories, University College of Pharmaceutical Sciences, Kakatiya University,* Warangal has been selected for the present study. This compound is prepared adopting the appropriate methods available in literature and is characterized by spectral data. The new compound possessing pyrimidine moiety because of structural similarities with nucleic acid bases exhibit various biological activities. Literature revealed that indole derivatives exhibit antifertility activity and aldose reductase inhibitory activity along with other biological activities. Keeping in view of biological significance of indole moiety and pyrimidine moiety present in the new compound, it is planed to study the effect of this novel heterocyclic compound on fertility activity adopting standard protocols available in literature.

MATERIAL AND METHODS

Wistar strain male albino rats weighing about 180-230 g were housed in polypropylene cages under controlled conditions (25°± 2°C and 12hr photoperiod) and were provided standard pellet food (Agro Corporation Pvt. Ltd., Bangalore, India) and tap water *ad libitum.* Experimental procedures were adopted as approved by the animal experimentation ethics committee and

maintained in accordance with the guidelines of the National Institute of Nutrition (NIN), Tarnaka, Hyderabad, India. The animals were used for the study after fifteen days of acclimatization.

Sixteen proven fertile male albino rats were divided into two groups (8 rats in each) and treated as follows.

Group I: Rats served as control and they were intraperitoneally administered with 0.3 ml of vehicle (1% sodium carboxy methyl cellulose) for 21 days.

Group II: Rats were intraperitoneally administered with novel heterocyclic compound at dose of 20 mg/kg/b.wt./day (suspended in 1% sodium carboxy methyl cellulose) for 21 days.

On the 22nd day, the rats were sacrificed and the testis and accessory reproductive organs i.e. epididymis, seminal vesicles and ventral prostate were removed cleared off fats, connective tissues and weighed on an electronic balance. The cauda epididymis was finely cut perfectly teased and the sperms were collected in 20ml of normal saline. Epididymal sperm counts were made using the haemocytometer and were expressed as millions/ml of suspension. One drop of normal saline was taken on to haemocytometer and the sperm count was recorded under high power microscope. Sperm motility of cauda epididymal spermatozoa were evaluated by the method of Prasad *et al.* (1972).

The sperm morphology test has been a commonly used sperm test for measuring spermatogenic damage induced by chemical agents. 2000 sperms per animal were analyzed for the abnormal shapes following the criteria described by Wyrobeck and Bruce, (1987). The fertility test was performed during last five days of the treatment. The male rats were cohabited with pro-estrous females in the ratio of 1:2 respectively. The presence of vaginal plug and sperm in the vaginal smear in the next morning were considered the index for positive mating. The mated females were separated and allowed to deliver after full term.

The weight of testis was recorded to calculate the gonadosomatic index (GSI). The body weight of the rats were taken before sacrificed and the male reproductive organs were removed and blotted for free of blood and mucus. The testis were weighed accurately using single pan *electrical* balance (Sartorias, West Germany). The gonado somatic indices were calculated by using the formula.

$$\text{GSI value} = \frac{\text{Weight of the testis}}{\text{Weight of the body}} \times 100$$

The body weight of each animal was recorded initially and then at the time of autopsy. Statistical analysis data were expressed as mean ± SEM and the significance of difference were analyzed by the student "t" test.

RESULTS AND DISCUSSION

As cytotoxic activity compound selected new heterocyclic compound investigated for its antifertility activity in male albino rats. As the acute intraperitoneal LD_{50} value of compound was 200mg/kg/b.wt./day determined, ten-fold lower ($1/10^{th}$) concentration was selected as sub lethal to study the effect of the compound (20 mg/kg/b.wt./day). It is random for the treatment to observe the effect of the compound because different phases of spermatogenesis are completed in 3 weeks of time.

The studied parameters help to learn more about the changes that took place in the reproductive organs and to identify the potential problems at an early stage itself. Hence an attempt has been made to study the change in sperm count, motility, and morphology and also the fertility rate to understand the impact of this compound on fertility in male albino rats. In addition to cytotoxic activity mild changes were also noticed in sperm count, motility, sperm morphology and fertility rate under the effect of this compound.

The data presented in table 1, revealed that the body weights of rats were not much altered after the treatment, indicating that the compound has no side effect and maintained normal metabolism. The result obtained by the effect of novel heterocyclic compound on testis weight, accessory sex glands weights and GSI value are shown in table 2. However a general reduction was observed in weights of testis (6.69%), epididymis (9.25%), ventral prostate (9.82%) and seminal vesicle (7.89%) in treated animals. The GSI value was also decreased (7.42%) in treated rats.

The reduction in weight of testis is indirectly indicative of the effect on spermatogenesis or related to loss of spermatozoa and spermatids which make up a substantial proportion of testicular volume by the same taken as a consequence of disruption of spermatogenesis (Gupta *et al.*, 2004 and Dobhal *et al.*, 2000). The reduction in weight of accessory sex glands and GSI indicates that the atrophy of glandular tissue and a reduction in secretary ability (Mathur and Malarvizhi, 1995). Reduction in the weight of accessory sex glands results in decreased number of sperms and effects fertility because the sialic acid which is rich in cauda epididymal fluid effects the maturation of sperms (Gupta *et al.*, 2007). The decreased weights of accessory sex glands also reflect an interference with androgen out put.

Sperm count, motility and morphology of control and treated rats are shown in table 3. The average number of sperms in control rats was 54.63 millions/ml while it was 41.47 millions/ml in treated rats. The mean sperm number was reduced 24.09% in treated rats. It was observed that the compound caused 22.02% reduction

sperm motility. Cytotoxic drugs depress spermatogenesis in mammals (Wyrobeck *et al.,* 1983) by causing death of developing sperm cells in the seminiferous tubules (Lu cc *et al.,* 1979). This results in the elimination of active cells of spermatogenesis and there by brings about a reduction in daily sperm production. Reduction in number of spermatogenic cells may be due to *reduction in* testicular weight and insufficient amount of testosterone (Nwanjo *et al.,* 2007). The sperm motility of treated rats showed that the sperms were sluggishly motile without any forward progression. The sperm motility has direct relationship to the fertility (David *et al.,* 1979). Immotile sperms can not penetrate the cervical mucus, thus fail to fertilize the ova (Amelar *et al.,* 1980).

From the data presented in table 3, it is seen that the sperm abnormalities were increased in treated rats. The abnormal sperms were increased in treated rats over control. The average numbers of abnormal sperms per 1ml of normal saline in treated rats were 56.28% while it was 43.72% in control group. The abnormalities of the epididymal sperm morphology observed were too long tail, folded, double head, balloon shaped head forms. The result indicates that the compound has effect on the sperm morphology. In several species abnormal sperms fail to reach the oviduct even if their proportion in the uterus is high (Krzanawska, 1974). Abnormal sperms can penetrate oocyte and develop normally (Burrel *et al.,* 1966). The abnormal spermatogenesis is caused by a shortage of androgen due to chemical action of compound.

Table 4 shows the reproductive impairment caused by the compound. During the present course of the study 50.00% negative fertility rate was observed after treatment. Normal proestrous females after mating with the males of proven fertile deliver an average 8-10 pups per female. The treated animals exhibited normal libido and mating behaviors, however in treated animals the fertility test gave negative fertility rate. The reduction in sperm number is associated with reduced fertility (Mathur *et al.,* 2005).

CONCLUSION

In this content an attempt has been made to assess the effect of novel heterocyclic compound on fertility in male albino rats. The results of the present study indicate that the compound caused a mild effect on the weights of testis and accessory sex glands with out significant changes in body weight. The reduction of weights of testes and accessory sex glands shows the cytotoxic effect of this compound. The administration of this compound caused a mild decrease in sperm count, motility, fertility rate and GSI value where as the same compound caused a mild increase in sperm abnormalities in treated rats. These observations suggest that the new heterocyclic compound is found to be toxic on male reproductive system.

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TABLE 1 : EFFECT OF NEW HETERO CYCLIC COMPOUND ON BODY WEIGHT OF MALE ALBINO RATS

Group		Average body weight (gm)	
		before treatment	after treatment
control	Mean	196.53	231.44
	SD	± 19.36	± 22.18
treated	Mean	187.83	225.78
	SD	± 20.71	± 35.62

All the values are mean ± SD (n=8).
SD -Standard Deviation.

TABLE 2: EFFECT OF NEW HETEROCYCLIC COMPOUND ON WEIGHT OF TESTIS, WEIGHTS OF ACCESSORY SEX GLANDS AND GSI VALUE

Group		Average organ weight mg/100g. b. wt.				GSI value
		testis	cauda epididymis	Ventral prostate	Seminal vesicle	
control	Mean SD	1239.35 ± 29.73	521.49 ± 21.04	387.18 ± 15.11	576.67 ± 44.27	1.239 ± 0.092
treated	Mean SD PC	1156.39 ± 31.40 (-6.69)	473.24 ± 26.19 (-9.25)	349.16 ± 21.33 (-9.82)	531.12 ± 18.25 (-7.89)	1.147 ± 0.19 (-7.42)

All the values are mean ± SD (n=8).
PC -Percent Change over control and SD -Standard Deviation.

TABLE 3 : EFFECT OF NEW HETERO CYCLIC COMPOUND ON SPERM COUNT, SPERM MOTILITY AND SPERM MORPHOLOGY IN MALE ALBINO RATS

Group		sperm count (millions/ml)	motility (%)	sperm morphology (%)	
				normal (N)	abnormal (AB)
Control	Mean SD	54.63 ± 21.27	74.83 ± 12.37	74.81 ± 09.85	25.19 ± 10.45
Treated	Mean SD PC	41.47 ± 17.19 (-24.09)	58.35 ± 21.86 (-22.02)	43.72 ± 11.95	56.28 ± 13.46

All the values are mean ± SD (n=8).
PC -Percent Change over control and SD -Standard Deviation.

TABLE 4 : EFFECT OF NEW HETERO CYCLIC COMPOUND ON FERTILITY IN MALE ALBINO RATS [n=8]

Group	No. of females cohabited with males 2 females/male	No. of females showed positive mating	fertility %
Control	16	15	93.75
Treated	16	08	50.00

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PROTECTION OF MICE HEPATOCYTES EXPOSED TO ARSENIC *IN-VIVO* BY *Scutia myrtina*

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ABSTRACT:

In the present study, Scutia myrtina was evaluated for its hepatoprotective effect in arsenic induced hepatocellular injury in rats. Hepatotoxicity was induced in male albino rats by intraperitonial injection of arsenic(0.5ml/kg body weight/2days). S. myrtina at a dose of 500mg/kg was administered orally for 15 days. The hepatoprotective effect of S. myrtina was evaluated by the assay of lipid peroxidation, hepatic marker enzymes namely Acid Phosphatase(ACP), Alkaline Phoshatase(ALP), Alanine Transaminase(ALT) and Aspartate Transaminase(AST) and enzymatic antioxidants including Catalase, Superoxide dismutase, Reduced glutathione and Glutathione peroxidase . The toxic effect of arsenic in S. myrtina treated group was controlled significantly by restoration of the levels of enzymic antioxidants and hepatic marker enzymes. The present study suggests that Scutia myrtina provides a good source of antioxidants that could offer potential protective effects against lipid oxidation and which could be exploited to make it a hepato protective agent against arsenic induced liver damages.

Key words: *Scutia myrtina, arsenic, hepatoprotective activity.*

INTRODUCTION

Liver is the organ regulating homeostasis in the body and is involved in almost all biochemical pathways related to growth, fight against disease, nutrient supply and energy provision (Ward and Daly, 1999). The impairment of the liver function is generally caused by xenobiotics, excessive exposure to various pharmacological and chemical agents, and protozoal or viral infections. Depending upon the severity of cellular injury, acute hepatitis can lead to chronic hepatitis, which is finally terminated to cirrhosis or malignant lesions in untreated cases. In case of deranged liver functions, the chemical composition of liver or its subcellular organelles are possibly altered. A slight alteration in hepatic structure and function may result in portal hypertension, ascities, jaundice, and increased bleeding, and causes multiple metabolic changes affecting other organs as well. Medical survey indicates exceptionally high occurrence of hepatic diseases and has become one of the most serious problem in the area of public health. Acute viral hepatitis is a diffuse inflammatory lesion of the liver usually accompanied by clinical and biochemical abnormalities and often caused by the well-characterized hepatitis virus. Acute hepatitis closely resembles viral hepatitis clinically, biochemically, and histologically. It can also be induced by chemicals or drugs. Arsenic and many of its compounds are especially potent poisons. Arsenic disrupts ATP production through several mechanisms.(Croal *et al.*, 2004)

Plants have been a model source of medicines as they are reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relive and treat many different human disorder.(Nelson, 2000).*Scutia myrtina* is widely available in south India, especially in kolli hills, Tamilnadu. It is commonly known as chimat (Hindi) a prickly shrub found throughout the hotter part of India, East Africa, Kenya, Tanzania and South Africa. The present study was aimed to test the inhibition of lipid peroxidation using rat liver homogenate and to assess the enzymatic antioxidant and hepatic marker enzymes activity in the plant of *Scutia myrtina*.

MATERIALS AND METHODS

ANIMALS

Wister strain of Male albino rats of six to eight weeks old (150-200g) were obtained from small animal breeding

center, Kerala Agricultural University, Trissur. Before the experiment, the clearance of the ethical committee for experimentation of animals was obtained. The animals were fed with standard diet from Hindustan Lever Ltd, Bangalore, India and water ad libitum.

EXPERIMENTAL SET UP

Four treatment groups were set up with 6 animals in each group. The treatment protocol is given below

Group I – control groups with normal diet

Group II- sodium arsenate treated groups.(0.5ml /kg body weight/2 days)

Group III- sodium arsenate followed by Scutia myrtina extract (oral administration 500 mg/kg body weight for the next 15 days)

Group IV- Control animals treated with Scutia myrtina extract (oral administration 500 mg/kg body weight for 15 days)

COLLECTION OF RAT TISSUE

After the end of the experimental period, the animals were sacrificed by cervical dislocation under mild Chloroform anesthesia. Liver was removed and washed with ice-cold saline.

PREPARATION OF TISSUE HOMOGENATE

A 10% homogenate of the washed liver tissues were prepared in 0.01M Tris –HCL buffer, pH 7.4. The homogenate was centrifuged at 12000g for 20 minutes in a refrigerated high speed centrifuge. The supernatant was used for the assay of enzymes.

PLANT MATERIALS AND EXTRACTION

The plant *Scutia myrtina* were collected from the kolli hills, Tamilnadu, India. The plant material was taxonomically identified by botanical survey of India, Coimbatore, Tamilnadu, India. The shade dried roots were powdered and passed through 10 mesh sieve. The coarse powder was soxhalated with 70% ethanol for nine hours [yield 5.7%]. Then the extracts were filtered and concentrated on rotary vacuum evaporator. Preliminary qualitative phytochemical screening of roots gives the positive test for Alkaloids, Flavanoids, Carbohydrates, Proteins, Glycosides and Phenols. The extract was resuspended in water and used for antioxidant activity, for hepatoprotective studies the same extract at the dose of 500 mg/kg body weight were received by the animals through oral gavage.

ESTIMATION OF BIOCHEMICAL PARAMETERS

The lipid peroxidation was measured according to the method of Okhawa *et al.*, (1979).Hepatic marker enzymes namely Acid Phosphatase(ACP), Alkaline Phoshatase, Alanine Transaminase(ALT) and Aspartate Transaminase(AST) were estimated according to the methods of Mitchell *et al* (1970),Bowers *et.al.* (1972), and IFCC method (1986) respectively. The enzymatic antioxidants including Catalase, Superoxide dismutase, Reduced glutathione and Glutathione peroxidase were estimated according to the methods of Luck (1974), Mishra *et al.* (1972), Moran *et al.* (1979) and Rotruck *et al.* (1973) respectively in the tissues of experimental rats. The present study was aimed to test the inhibition of lipid peroxidation and to assess the enzymatic antioxidant and Hepatic marker enzymes activity of the plant *Scutia myritina* using rat liver homogenate.

STATISTICAL ANALYSIS

All the values are represented as mean ± S.D. (n = 6). Data on biochemical investigations were analyzed using analysis of variance (ANOVA). A probability of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Table 1 showed the lipid peroxidation of control and experimental rats. Lipid peroxide was significantly increased in the group II animals during arsenic toxicity when compared to control(group I). Elevated levels of lipid peroxides were accompanied by altered antioxidant defense systems. It is molecular mechanism of cell injury leading to generation of peroxides and lipid peroxides which can be decomposed to yield a wild range of cytotoxin products, most of which are aldehyde (Ohkama *et al.*, 1979) *Scutia myrtina* extract inhibited lipid peroxidation significantly(group III). The extract could reduce the extent of toxin induced lipid peroxidation. Since the anti-oxidant and hepatoprotective activities of certain flavonoids from plant origin have already been established (Carlo *et al.*, 1999) we can speculate that these constituents may be responsible for the observed protective effects against lipid peroxidation. Phenolics are capable of scavenging peroxyl radicals (Oh *etal.*, 2004) we presume that these protective effects of the extracts are dependent on their phenolic composition.

ENZYMATIC ANTIOXIDANTS

Table 2 Illustrates the actitivities of enzymatic antioxidants in control and experimental rats. There was a significant reduction in the activities of enzymic antioxidants during arsenic toxicity (group II) when compared to control (group I). Treatment with *Scutia myrtina* extract decreased the Arsenic-induced Catalase, Superoxide dismutase, Reduced glutathione(GSH) and Glutathione peroxidase alterations(group III). GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals (Ko *et al.*, 1995). In the present study, a significant

decrease ($P<0.01$) in the liver GSH was observed. Increase in hepatic GSH level in *Scutia myrtina* treated mice could either be due to an effect on the *de novo* synthesis of GSH, its regeneration or both. As a consequence, hepatic GSH level could be sufficiently maintained to counteract the increased formation of free radicals (Ko *et al.*, 1995) Earlier reports demonstrated that herbs containing flavonoids, tannins and phenolic compounds were found to possess antioxidant property and attenuate cell death induced by oxidative stress which supported our present findings (Schroeter *et al.*, 2000 and Youdim *et al.*,2001)

Several workers have shown that phenolic compounds play a role as antioxidants through different mechanisms of action, such as: scavenging of free radicals, quenching of reactive oxygen species, inhibiting of oxidative enzymes chelating of transition metals or through interaction with biomembranes. These properties make those types of compounds good candidates as potential protectors against food oxidation and biological aging of tissues. Thus, phenolic compounds could be considered as natural antioxidants with potential applications in protecting the liver. (Liao and Yin , 2000)

Table 3 explains that the activities of Aspartate transaminase (AST), Alanine transaminase (ALT), acid phosphatase and Alkaline Phosphatase (ALP) were found significantly decreased ($P<0.01$) in liver tissue of Group II rabbits as compared to control(Group I). In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also found in mitochondria. In liver injury, the transport function of hepatocytes is disturbed resulting in the leakage of plasma membrane thereby causing increased enzyme level in serum. The elevated levels of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Rajesh and Latha, 2004)

The administration of plant extract to the toxic rats completely prevented the arsenic-induced perturbations in the activities of AST, ALT and ALP in liver tissue (Group III). Arsenic-derived hepatotoxic metabolites would have been trapped by the simultaneous administration of plant extract and may finally resulting in the restoration of the activities of transaminases and phosphatases to their normal level as suggested by Whitehouse *et al.*(1983) during the admistration of B6 in INH_induced toxicity.

Results from the study showed that *Scutia myrtina* aqueous extract could exert a beneficial action($p<0.01$) against liver damage induced by Arsenic. The preliminary phytochemical analysis of the extract showed the presence of flavonoids. Finally, the present study suggests that phenolic compounds and flavanoids of the *Scutia myrtina* provide a good source of antioxidants that could offer potential protective effects against lipid oxidation and which could be exploited to make a hepato protective effect against arsenic induced liver damage in rats.

TABLE-1

TBARS LEVEL(LIPID PEROXIDATION)

GROUPS	TBARS
Group I	1.4 ± 0.02a**
Group II	0.54 ± 0.02b**
Group III	1.1 ± 0.03cNS
Group IV	1.2 ± 0.03d NS

Values are expressed as a moles of MDA/g tissue
Values are mean ± SD (n = 6)
Group comparision : a- I vs II; b- I vs III; c- III vs I d- IV vs I
Statistical comparision : -p<0.05; -p<0.01; NS- not significant

TABLE-2

ENZYMATIC ANTIOXIDANT LEVEL

ENZYMIC ANTIOXIDANTS	Catalase	Superoxide dismutase	Reduced glutathione	Reduced glutathione
Group I	118 ±15a**	4.58 ±0.02a**	1.64± 0.01a**	1.03 ±0.001a**
Group II	77±08 b**	2.15 ±0.03b**	0.28± 0.01b**	0.05± 0.001b**
Group III	104 ±12c NS	3.90 ±0.05c NS	1.34± 0.01c	1.01± 0.001c NS
Group IV	113±10d NS	4.55±0.03d NS	1.62± 0.02 c	1.00±0.001d NS

Catalase was expressed as micromoles of hydrogen peroxide consumed/minute/mg protein
SOD was expressed as Units/mg protein/minute
Total reduced glutathione was expressed as microgram/ mg protein
Glutathione peroxidase was expressed as microgram of glutathione reduced/minute/ mg protein
Values are mean ± SD (n = 6)
Group comparision : a- I vs II; b- I vs III; c- III vs I d- IV vs I
Statistical comparision : -p<0.05; -p<0.01; NS- not significant

TABLE-3

HEPATIC MARKER ENZYME

HEPATIC MARKER ENZYMES	Acid Phosphatase	Alkaline Phoshatase	Alanine Transaminase	Aspartate Transaminase
Group I	1.16 ±0.02a**	4.45± 0.04a**	3.38 ±0.02a**	1.53 ±0.02a**
Group II	0.34 ±0.33b**	1.30± 0.02b**	1.05± 0.02b**	0.08 ±0.01b**
Group III	1.02 ±0.05c NS	3.37± 0.02c NS	3.28± 0.02c NS	1.20 ±0.02c NS
Group IV	1.07±0.04d NS	4.12±0.03d NS	3.12± 0.01d NS	1.45 ±0.01d NS

ACP and ALP were expressed as microgram of phenol liberated /minute/mg protein
AST and ALT were expressed as micromoles of pyruvate liberated/minute/mg protein
Values are mean ± SD (n = 6)
Group comparision : a- I vs II; b- I vs III; c- III vs I d- IV vs I
Statistical comparision : -p<0.05; -p<0.01; NS- not significant

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CHROMATE STABILISATION IN EXTRACELLULAR AND CELL FREE EXTRACTS OF PRESELECTED MIXED CULTURES.

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ABSTRACT:

The ability of the isolates to stabilize Cr(VI) was studied in preselected mixed cultures. Cell free extracts (CFE) could remove more than 75% of provided 45µg/ml Cr(VI).Further spiking reduced the uptake. Uptake was also poor in boiled portions. Initially poor uptake was noted with CFE which improved on addition of NADH. The NADH:CFE ratio of 1.25:1 allowed complete uptake of 125 µg/ml Cr(VI). Higher uptake was noted with CFE pregrown with chromate.Partially purified CFE followed a time course showing an increase in Cr(VI) uptake with time.

Key words: *Cr(VI) uptake; Consortium ; EPS; CFE; CFS; NADH.*

INTRODUCTION

EPS i.e. extracellular polymeric substances are mainly composed of microbial polysaccharides by definition, other polymeric substances may also be present, for example, proteins, nucleic acids and polymeric lipophilic compounds(Lawrence *et al.,* 2004). Siderophores are also capable of binding to metals with a high affinity. Chromate stabilization in cell free extracts is widely reported. The reduction was reportedly substantial in both CFE as well as immobilized intact cells of *Bacillus* sp ES 29 (Camargo *et al.,* 2004). Assays with crude cell-free extracts obtained from a locally isolated gram negative strain of *Brucella* sp. demonstrated that the hexavalent chromium reduction was mainly associated with the soluble fraction of the cell(Thacker *et al.,* 2007).A gram-negative chromate reducing bacteria , *Providencia* sp. could grow and reduce chromate to 100% at a concentration ranging from 100–300 mg/l (Thacker *et al.,* 2006). In cell-free extracts of chromium-resistant bacterium *Bacillus sphaericus* AND 303, the reductase activity was found to be solely localized in the soluble cell-fractions (S_{12}, S_{32}, and S_{150})(Pal *et al.,* 2005).The following study using mixed cultures was carried out to demonstrate the role of cell free extracts and cell free supernatants of such cultures in chromate uptake.

MATERIAL AND METHODS

Isolates tolerating a chromate concentration of 330 M l^{-1} were isolated from soil, electroplating effluents and sediments. Colonies that developed on King's B and nutrient agar (Hi media manual for microbiology and cell culture lab practice 2003) were further replica plated on chromate containing minimal agar (Khare *et al.,* 1997) as reduction could not be detected on either media. The plates were flooded with diphenyl carbazide reagent. A yellow halo confirmed chromate reduction, the diameter of which was proportional to the chromate reduced. (**Table 1**). Pure cultures were obtained from single colonies by standard microbiological techniques Kourtev *et al.,* 2006).Cultures were maintained on nutrient agar slants containing 1µg/ml dichromate and refrigerated. These selected cultures were repeatedly tested for chromate reducing ability using 1,5 diphenyl carbazide assay. (Horton *et al.,* 2006).

The isolates were further identified by Bergey's manual of systematic bacteriology (Krieg and Holt, 1984, Sneath *et al.,* 1986, Stanley *et al.,* 1986) using morphological and biochemical tests, G+C content etc. The consortium was raised by pooling equal amounts of culture suspension previously adjusted to A_{600} 0.5.

Role of extra cellular fractions produced in different media in presence and absence of chromate.

Exopolymer production was investigated in presence and absence of chromate in Glucose minimal medium (GMM), Sutherland Wilkinson's medium (Sutherland, 1972) Sucrose nutrient broth and Glucose mineral salts

medium (Sneath *et al.*,1986). Freshly harvested consortium (A_{600}=0.5) was inoculated with or without 10µg/ml Cr(VI). Cell free supernatants, (S1 and S2 fractions) were obtained. EPS was extracted from culture supernatants by alcohol precipitation in the proportion of 1:2. (Pfiffner *et al.*,1986) and refrigerated. The precipitate was air dried. Chromate uptake at 100µg/ml dichromate was monitored in phosphate buffer (pH 6.8). Cell free media was also precipitated using the same amount of alcohol and precipitate if any, was examined for chromate uptake. Total proteins were recorded by Lowry method using BSA as the standard protein.(Sutherland, 1972). Residual Cr(VI) was estimated after 24 h. using diphenyl carbazide reagent.(Pfiffner *et al.*,1986). Values reported are the averages of three determinations carried out in parallel.(Hernández et al.,1998).

Role of extracellular fractions in Cr(VI) reduction:
Protein fraction of the cell free supernatant responsible for chromate uptake would generally be inactivated by boiling. The supernatant was kept in boiling water bath for 10 min Chromate uptake was noted after 12h. at 50µg/ml using a supernatant : chromate ratio of 1:1. Thereafter respiking was done as the uptake was found to be significant. Residual Cr(VI) was estimated as before. **(Table 3).**

Role of crude and partially purified proteins of CFE in chromate uptake:
In order to determine whether chromate reduction occurred in cell free extracts (CFE), a consortium pellet was obtained. The wet weight of the pellet was 1.3g corresponding to total proteins of 1.70 mg/ml. Pellet was washed twice with Tris-HCl, resuspended in Tris-HCl buffer, and immersed in an ice bath. The disaggregation and puncturing of cells was done by alternate suction and release of the contents through a sterile 24 gauge injection needle-syringe(Nebe *et al.*, 1999), for 15 passages under cold conditions. Clear supernatant (ex1) obtained by centrifugation was used immediately for assaying chromate reduction . In a 1 ml reaction mixture , Tris-HCl was spiked with 125 g /ml of chromate. NADH (2mg/m)l in Tris-HCl was added at 200µg/ml and 1000µg/ml respectively. Chromate reduction was initiated by addition of cell free extracts to the reaction mixture in different ratios (as per addition table1) and incubated at 35°C for 3 h. Cr (VI) was analyzed as before.
Ten additional passages generated a dilute fraction (ex2). Extract free, NADH free controls were used to record initial Cr(VI). Chromate uptake in the absence of NADH using this extract was also recorded. Reduction in NADH concentration at 340nm was simultaneously monitored to indicate the utilization of NADH and the possibility of NADH acting as an electron donor in the reduction of Cr(VI) to Cr(III) along with CFE.

Addition table1: Preparation of the reaction mix.

Sr.no	Reference	Tris-HCl Buffer (ml)	Cr(VI) (ml)	Extract (ml)	NADH (ml)
1	Reduction by ex1	0.1	0.1	0.8	-----
2	Reduction by ex2	0.1	0.1	0.8	-----
3	Control	0.9	0.1	-----	----
4	Reduction by NADH	0.8	0.1	-----	0.1
5	Reduction by ex1 in presence of NADH	-----	0.1	0.8	0.1
6	Reduction by ex2 in presence of NADH	-----	0.1	0.8	0.1
7	Control	0.9	0.1	-----	
8	Reduction by NADH	0.4	0.1	-----	0.5
9	Control	0.9	0.1	-----	-----
10	Reduction by ex1 in presence of NADH	----	0.1	0.4	0.5
11	Control	-----	0.1	0.4	0.5
12	Reduction by ex2 in presence of NADH	-----	0.1	0.4	0.5
13	Control	------	0.1	0.4	0.5

Role of CFE from consortium raised at different chromate concentration was also investigated. Consortium was precultured with 15, 25 and 35µg/ml Cr(VI) and total proteins were recorded as before. Cell free extract was obtained as before. Chromate uptake was noted at 50µg/ml (2 h) for each of the CFE at a ratio (CFE: NADH) of 1.25:1 (addition table2) which was finalized after experimenting with different combinations of CFE and NADH. Boiled portions of the CFE was also tested.

Addition table2: CFE from consortium developed in presence of different concentrations of dichromate.

Sr.no	Reference	Tris-HCl Buffer	Cr(VI) 50µg/ml	Amount of CFE	NADH 2 mg/ml
1	Reduction by CFE(1) pregrown with 15µg Cr(VI)	--	0.1	0.5	0.4
2	Reduction by CFE(2) pregrown with 25µg Cr(VI)	--	0.1	0.5	0.4
3	Reduction by CFE(3) pregrown with 35µg Cr(VI)	--	0.1	0.5	0.4
4	Control	0.9	0.1	-	-
5	Reduction by NADH	0.5	0.1	-	0.4

Purification of catalytic protein fractions:

Partial purification of the CFE was done using a slightly modified protocol of Rajwade *et al.*(1999).Consortium was grown in nutrient broth with 1µg/ml dichromate , cells were harvested by centrifugation and resuspended in buffer. The buffers used were 20% sucrose in 20mM Tris -HCl buffer (pH 7.5) supplemented with a detergent (Tween 80, TritonX-100, SDS each at a concentration of 0.1%). Suspensions were incubated under refrigeration for 12h. , centrifuged (at 12,000xg for 20 min in an ice bath) to obtain cell free supernatants (S1fractions). The S1 fractions were subjected to ammonium sulphate precipitation (80% saturation) so as to partially purify the enzyme preparations.

Time course of chromate uptake (up to 4h.) was carried out at 10µg/ml Cr(VI) with the CFE (untreated and boiled fractions) after it was found that reduction was also possible in the boiled fractions of partially purified CFE. Enzymatic reactions follow a time course which will not be demonstrated by abrupt release of chelating agents because of boiling. CFE:NADH ratio was fixed at 0.8:1 after experimenting with different combinations. Cr(VI) estimation was carried out every hour for both the portions of CFE.

RESULTS AND DISCUSSIONS

Biochemical characterization of isolates:

Colony characteristics, morphology, Gram's nature, biochemical tests and G+C content (data not presented) were used in identification of the isolates using Bergey's manual of systematic Bacteriology. (Rajwade *et al.*, 2004).(**Table 1**).Some of the isolates included in this consortium have been implicated in chromate uptake such as *Corynebacterium, Bacillus* sp.(Campos *et al.*,1995), *Alkaligenes* sp.(Nies and Silver 1989).

Role of extra cellular fractions produced in different media in presence and absence of chromate.

Higher uptake was noted in whole cells and EPS pregrown with chromate in SNB and MSG media. EPS harvested from minimal media showed 30% uptake. (**Table 2**). It was reported (Priester *et al.*, 2006) that chromium exposure resulted in increased EPS production under sulfate reducing conditions. *Pseudomonas* sp provided high exopolymer production in a medium containing 2% glucose in 50 ml of ocean artificial seawater. (Decho and Moriarty 1990) Thus amount of EPS produced in different media at constant cell density and chromate concentrations is dependent on presence or absence of specific sugars. *Lactococcus lactis* produced more exopolysaccharide (EPS) on glucose than on fructose (Looijesteijn ***et al.,*** 1999). The EPS produced in SNB and GMM showed higher uptake than SW medium. Exopolymer production was increased in presence of sucrose. (**Table 2**). The reduction of chromate was also noted visually. A distinct yellow color of the chromate-containing media disappeared (Smith. and Gadd, 2000) and the solution either turned dull green or appeared colorless.

Role of extracellular fractions (CFS) in Cr(VI) reduction:

Chromate reduction occurred both extracellularly as indicated by uptake in CFS in the absence of NADH 100% chromate uptake occurred in the untreated fraction and almost 76% uptake was noted within 3h. Spiking with 50µg/ml dichromate reduced the uptake to 50%..(**Table 3**).

CFS may be mediating chromate binding by EPS or by use of specific (chromate reductases) or non specific enzymes such as the hydrogenases (Chardin *et al.*, 2003) liberated as a result of normal cell lysis.100% chromate uptake occurred in cell free supernatants of whole cells and 50% when respiked with chromate. Boiled fraction showed an uptake of 24%.This uptake may be due to sorption, precipitation , cell surface chelation. Since respiking reduced the uptake it indicates the non availability or saturation of binding sites of EPS due to chromate binding.

Role of crude and partially purified proteins of the CFE in chromate uptake:

If the ratio between NADH and extract was kept at 1.25:1 , the uptake was complete with both the extracts. (**Table 4**). The reduction in NADH concentration at 340 nm also indicated that it was being used as an electron donor. The CFE obtained in presence of 25µg/ml

chromate showed highest uptake.**(Table 5)**.
The role of NADH and NADPH as exclusive electron donors have been indicated earlier.(Campos *et al.*, 1995,Gruber and Jennette 1978). The aerobic activity is generally associated with a soluble protein fraction dependent on NADH as an electron donor (Horitsu *et al.*, 1987), or employed NADH for maximizing activity. (Ishibashi *et al.*,1990). Bae *et al.*(2005), observed that both NADH (0.4 mM) and NADPH (0.4 mM) were good electron donors, especially NADPH, which showed 61% of the activity compared to that of NADH.

Similar results were noted with CFE's catalyzing the reduction in presence or absence of NADH. 6.16 and 1.51 % chromate uptake occurred with CFE only. Almost 65-70% increase in uptake was observed in presence of 0.5 ml NADH in a CFE:NADH ratio of 0.8:1.Thus it may be said that the reduction by CFE was NADH dependent. It was noted by Pal *et al.*(2005) that the CFE obtained from *B.sphaericus* AND 303 showed accelerated chromate reductase activity in presence of additional electron donors, and an increase of 28% activity over control was recorded with 1.0 µM NADH.Chromate uptake was possible in CFS as well as in CFE's which increased with respect to time. (**Table 6).**

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Table1: Plate assay at 20µg/ml Cr(VI)

Culture	Diameter of the halo.(cm)
Bacillus brevis	2.8 ±0.3
Sporolactobacillus inulinus	1.9 ±0.5
Alkaligenes sp.	1.8 ±0.2
Micrococcus luteus	1.7 ±0.2
Bacillus laterosporus	2.2 ±0.3
Listeria sp.	2.0 ±0.1
Cellulomonas sp.	1.2 ±0.3
Bacillus subtilis	0.8 ±0.1
Curtobacterium citreum	1.2 ±0.3
Curtobacterium pusillum	0.7 ±0.1
Caryophanon sp.	0.8 ±0.1
Corynebacterium xerosis	0.8 ±0.2

Table 2: Chromate uptake (100 µg/ml) with consortium and EPS from different media.

Media	Pregrown with chromate		with out chromate	
	% uptake EPS	% uptake consortium	% uptake EPS	% uptake consortium
SW	42.86	71.43	100	100
SNB	71.43	57.15	22.23	11.12
MSG	70.84	67.15	34.55	18.19
GMM	32.2	80	30.10	73.12

Table 3: Estimation of EPS produced with and without chromate in GMM.

Cr(VI) µg/ml	Wet wt. of the pellet (g)	% Congo red uptake	Total proteins µg/ml	% uptake by EPS
3	0.010	48%	450	100
6	0.010	54%	362.5	100
9	0.010	54%	268.75	52.64
---	0.020	50%	512.5	68.43

Table 4: Chromate uptake in CFS.

Cell free supernatant	Initial Cr(VI) µg/ml	Final Cr(VI) µg/ml	% uptake 12h.
Untreated (I dose of chromate).	45.13	10.41	76.94
Untreated (spiked)	29.51	13.88	52.97
Boiled (I dose of chromate)	50.10	38.08	24
Boiled (spiked)	92.15	93.2	0

Table 5: Uptake at 125 µg/ml Cr(VI) at different ratios of the reaction mix.

Sr.no	Reference	Cr(VI) recorded (µg/ml).	% uptake.
1	Reduction by ex1	126.73	6.16
2	Reduction by ex2	133.01	1.51
3	Control	135.04	---
4	Reduction by NADH	130.20	3.59
5	Reduction by ex1+NADH(0.1ml) -- net reduction by ex1	119.79	11.3-3.59 = 7.71
6	Reduction by ex2 +NADH(0.1ml) net reduction by ex2.	125	7.44-3.59 = 3.85
7	Control	135.04	---
8	Reduction by NADH	100.69	23.79
9	Control	132.11	----
10	Reduction by ex1+NADH(0.5ml) net reduction by ex1	0 (3h.)	100-23.79 = 76.21
11	Control	132.1 (at 0h)	----
12	Reduction by ex2+NADH(0.5 ml) net reduction by ex2	120.05 (3h)	92.33-23.79 = 68.54
13	Control	130.02(at 0h)	----

Table 6: Uptake by CFE from consortia raised at different concentrations of dichromate.

Sr.no	Reference	Total proteins µg/ml	Cr(VI) 2h.	% uptake
1	Cr(VI) Reduction by CFE(1) (consortium pregrown with 15µg Cr(VI))	459.62	27.22	39.69-19.24 = 20.45
2	Reduction by CFE(2) pregrown with 25µg Cr(VI)	354.07	17.36	61.54-19.24 = 42.3
3	Reduction by CFE(3) pregrown with 35µg Cr(VI)	267.08	32.19	28.68-19.24 = 9.44
4	Control		45.13	---
5	Reduction in presence of NADH.		36.45	19.24
6	Reduction by boiled CFE(1)	----	31.25	30.76-19.24 = 11.52
7	Reduction by boiled CFE(2)	----	26.04	42.31-19.24 = 23.07
8	Reduction by boiled CFE(3)	------	34.72	23.07-19.24 = 3.83

Table 7: Time course for Cr (VI) uptake in partially purified fractions.

Sr.no	Reference	%uptake			
		1h.	2h.	3h.	4h.
1	Reduction by CFE(1)	0	40	50	80
2	Reduction by CFE(2)	0	20	40	80
3	Reduction by CFE(3)	20	40	40	40
5	Reduction by NADH	0	20	0	0
6	Reduction by boiled CFE(1)	0	20	0	0
7	Reduction by boiled CFE(2)	0	20	0	0
8	Reduction by boiled CFE(3)	0	20	0	0

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EFFECTIVITY OF THE DECOLORIZATION OF CRYSTAL VIOLET BY VARIOUS FUNGI, WITH SPECIAL REFERENCE TO THE WHITE ROT FUNGUS, *Trametes hirsuta*

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ABSTRACT:
Decolorizations of the dye, Crystal violet was investigated using the white rot fungus, Trametes hirsuta and other fungi like Aspergillus flavus, Aspergillus fumigatus, Penicillium sp and Fusarium oxysporium. Among the fungi used, Trametes hirsuta gave maximum decolorization for the dye, and among the other fungi Aspergillus niger and Aspergillus fumigatus gave maximum decolorization than the other fungi. The medium and growth conditions were optimized for the effective decolorization of the fungus, Trametes hirsuta. Decolorization of Crystal violet was optimum when maltose (89.3%) was used as the carbon source, yeast extract (81.6%) as the nitrogen source, at 30°C (88.5%) and at pH 4 (80.1%). The immobilized cells of Trametes hirsuta also showed decolorization for the dye, Crystal violet.

Key words: *Fungi, Decolorization, Crystal violet, Immobilized cells.*

INTRODUCTION

Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and/ or toxicity. The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995). Dye wastes and other effluents from textile processing can cause problems like foaming, color persistence, have abnormally high pH, temperature and heavy metal concentrations and variations in the hydraulic flow rates (Buckley, 1992). This alters the biochemical oxygen demand (BOD) and gives rivers intense colorations (Ajayi and Osibanjo, 1980). The use of these water resources is limited and ecosystem is affected.

Dyes are stable to light and oxidizing agents and are sometimes resistant to biooxidation and they contain chromophores. Many chemical dyes have been used increasingly in the textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to the natural dyes.

The release of dyes may therefore present an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain. Moreover, various dyes are also one of the major sources of heavy metals (Wagner, 1993) in water and soil and at times, they can be phototoxic (Raskin *et al.*, 1994) and due to their persistent nature cause a misbalancing in the ecosystem.

Currently various physical, chemical and biological treatment methods are used to remove color (Zhang *et al.*, 2003). Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater have not been widely applied in the textile industries (Robinson *et al.*, 2001). Biological dye removal techniques are based on microbial biotransformation of dyes. In the present study an attempt was made to degrade Crystal Violet using white rot fungus, *Trametes hirsuta* and the ability of other fungi like *Aspergillus niger, Aspergillus fumigatus, Penicillium sp* and *Fusarium oxysporum* to degrade the dye were also studied.

MATERIALS AND METHODS

Organisms used for the study:

The white rot fungi *Trametes hirsuta* (MTCC 1171) was procured from Institute of Microbial Type Culture Collection, Chandigarh, India to test for the dye degradation abilities. Other fungi used for the dye degradation include *Aspergillus flavus, Aspergillus*

fumigatus, Penicillium sp and Fusarium oxysporium.

Dye used:

The dye used for this study was Crystal Violet. Stock solutions of the dye was prepared with 100mg of dye in 100ml of sterile distilled water and filter sterilized and used for decolorization studies.

Studies on the decolorization of dye by the fungi on solid medium:

Malt Extract Agar (MEA) medium was prepared and sterilized. To each 15ml of the medium 5ml of sterile dye, Crystal Violet solution was added and poured into Petri dish separately. For each plate, a disc (5mm) of fungal mycelium was placed at the centre. Uninoculated plates served as a control. The plates were then incubated at 30°C and observed for visual disappearance of color.

Studies on the decolorization of dye by the fungi in liquid medium:

Decolorization Assay:

Decolorization studies were carried out in Malt Extract broth medium. Media was prepared, sterilized and supplemented with the dye, Crystal Violet solution. To this medium 5mm disc of fungal mycelium was inoculated. Following third day of inoculation, 2ml of culture media was taken, centrifuged and read at 580 nm using UV- Visible spectrophotometer. Medium without dye and inoculum was used as blank. Medium with dye but without inoculum was used as control. The rate of decolorization was estimated by the following formula:

$$\text{Rate of decolorization (\%)} = 100 - \frac{\text{Absorbance of treated dye solution}}{\text{Absorbance of control dye solution}} \times 100$$

Optimization of the medium and growth conditions for the effective decolorization of the dye by *Trametes hirsuta*:

Different parameters like temperature, pH, carbon source and nitrogen source of the medium were optimized for the decolorization assay. 350ml of Malt extract broth medium was prepared and sterilized. 25ml of the above said sterilized medium was dispensed into 100ml Erlenmeyer flasks. To this 25ml of stock dye, Crystal Violet solution was added separately. Three discs (5mm) of actively growing fungal mycelium were inoculated into each flask. To check for the optimum temperature for the effective decolorization the above said sterilized medium were incubated at temperature ranging from 25°C-40°C, for testing the optimum pH for the decolorization assay the pH of the medium was adjusted using 0.1N HCl and 0.1N NaOH and the pH ranging from 3-7 was used. To check for the carbon source for the effective decolorization, the medium was prepared with carbon sources like Glucose, Sucrose, Galactose, Maltose and Mannitol and for the effective decolorization of the dye the nitrogen source of the medium was also optimized, for which the medium was prepared using nitrogen components like Yeast extract, Ammonium chloride, Urea, and Peptone.

Efficacy of immobilized cells on the decolorization of dye:

Immobilized cells were prepared using sodium alginate. The fungal mycelium was immobilized for the decolorization studies. 150ml of Malt Extract broth medium was prepared and sterilized. 25ml of the sterilized broth was dispensed into 100 ml Erlenmeyer flasks. To this 25ml of stock dye solution, Crystal violet was added separately. Then using sterile spatula 10 beads (fungal beads) were added to the media and incubated at 30°C and rate of decolorization was studied using UV-Visible Spectrophotometer as said above. Medium with dye and beads alone served as control.

RESULTS AND DISCUSSION

Decolorization of dye on solid medium by *Trametes hirsuta* and other fungi:

The decolorization of the dye, Crystal Violet by the fungus *Trametes hirsuta* and the other fungi was performed on dye-agar plate. The decolorization ability of *Trametes hirsuta* was supported by many studies as reported by Abadulla *et al.* (2000).

The mycelial growth of the fungus is started from first day but decolorization started from third day. The decolorization rate was differing among the fungi used. *Trametes hirsuta* could effectively degrade the dye compared to the other fungi.

The solid-state decolorization assay was carried out among the other fungi like *Aspergillus niger, Aspergillus fumigatus, Penicillium sp*, and *Fusarium oxysporum*. *Aspergillus niger* and *Aspergillus fumigatus* showed maximum efficiency to decolorize the dye, Crystal violet. *Fusarium oxysporum and Penicillium sp.* also showed decolorization but was relatively low when compared to other fungi. Similar studies regarding Congo red and Crystal violet degradation by *Aspergillus niger, Aspergillus fumigatus, Penicillium sp, Fusarium oxysporum, Rhizopus and Cladosporidium sp.* had been observed by Poonkothai *et al.* (2009).

The solid-state decolorization assay of the present study revealed that high percentage of decolorization was observed with the white rot fungus. The decolorization started after the sufficient growth of mycelium. The results were similar to the reports of Sathiyamoorthi *et al.* (2006) in which the solid state decolorization of Blue CA, Black B133 and Corazol violet SR dyes were observed.

Decolorization of different dye in liquid medium by *Trametes hirsuta* and other fungi:

The efficiency of the fungi to decolorize the dye, crystal violet was studied. Maximum decolorization was observed with *Trametes hirsuta*. *Aspergillus niger* and *Aspergillus fumigatus* also showed moderate decolorization. The decolorization was very low in the case of *Penicillium sp* and *Fusarium oxysporium* (Fig: 1)

Optimization of medium and Growth Conditions for the Effective Decolorization by *Trametes hirsuta*:

As the decolorization was found to be maximum for the white rot fungus, *Trametes hirsuta* the medium and growth conditions were optimized for their effective decolorization. Decolorization rate was observed at different temperatures ranging from 25°C - 40°C at an interval of 5°C. Decolorization was optimum at 30°C (88.5%) (Fig: 2). Sharma *et al.*, (2009) reported that the optimum decolorization of Orange II dye by *Phanerochaete chrysosporium* on 5th day at 30°C.

The decolorization efficiency was also checked at various pH ranging from 3 - 7. The decolorization rate was maximum at pH 4 (80.1%) (Fig: 3). This was similar to the results obtained by Mohandass *et al.*, (2007) who observed maximum decolorization at pH 3 on *Aspergillus* sp. Decolorization study at different pH was also carried out by Rani *et al.*, (2005) who observed 96.4% decolorization after 8 days of incubation at a pH of 6.

The decolorization was maximum (89.3%) with maltose compared to other carbohydrates tested (Fig: 4). Mohandass *et al.*, (2007) reported glucose and mannitol as the best carbon source for the decolorization in which the decolorization was more than 95%. Sathiyamoorthi *et al.*, (2007) reported that effective color removal was observed with 2% glucose.

The effect of nitrogen sources such as Yeast extract, Peptone, Ammonium chloride and urea on decolorization showed maximum (81.6%) with Yeast extract, followed by Peptone (79.8%), Urea (75.1%) and Ammonium chloride (70%) (Fig: 5).

Decolorization of dye using immobilized cells of *Trametes hirsuta*:

The efficacy of the fungus, *Trametes hirsuta* for the decolorization of the dye was also observed by immobilizing the cells in sodium alginate to form beads. The decolorization was 44% for Crystal Violet (Table: 1). Similarly Pandey *et al.*, (2009) studied the biodegradation of texile dyes using the immobilized cells of microorganisms like *Phanerochaete chrysosporium* and *Pseudomonas fluorescens*. The results showed good decolorization of textile dyes.

The present study reveals that the White rot fungus *Trametes hirsuta* could effectively decolorize Crystal violet. The extent of decolorization differed from dye to dye. This may be due to the differerences in the components of the dye. Decolorization of dye using *Aspergillus niger, Aspergillus fumigatus, Penicillium sp, and Fusarium oxysporum* was also checked but the rate of decolorization was low than *Trametes hirsuta*. Thus the results prove that the test fungus, *Trametes hirsuta* is more efficient in the decolorization of the dye

and can be used for the treatment of dye industry effluents reaching the environment.

Fig: 1 Effect of decolorization of Crystal Violet by all the fungi used

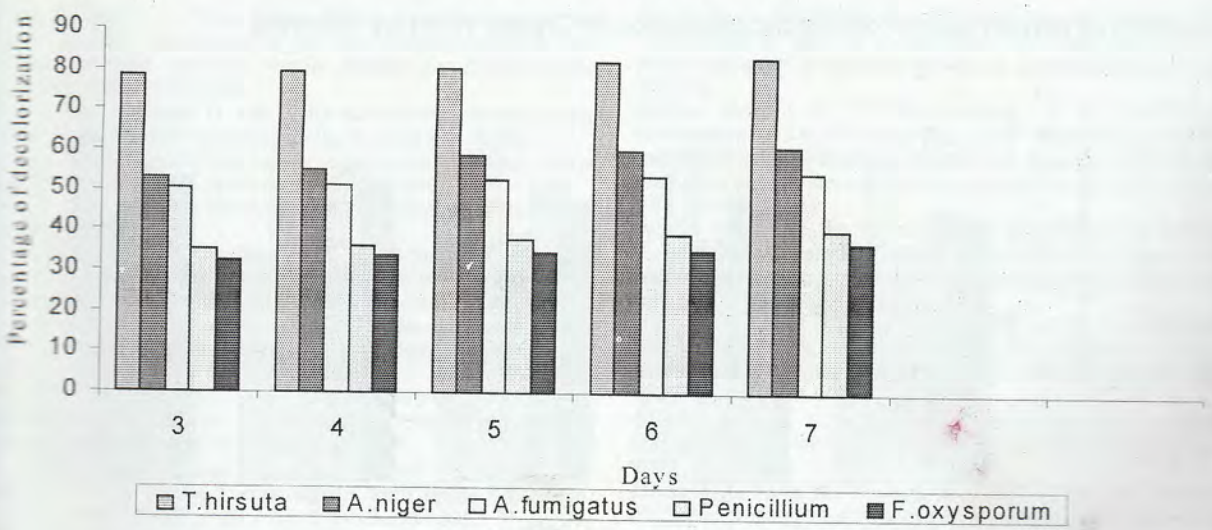


Fig: 2 Effect of temperature on the decolorization of Crystal Violet by *T. hirsuta*

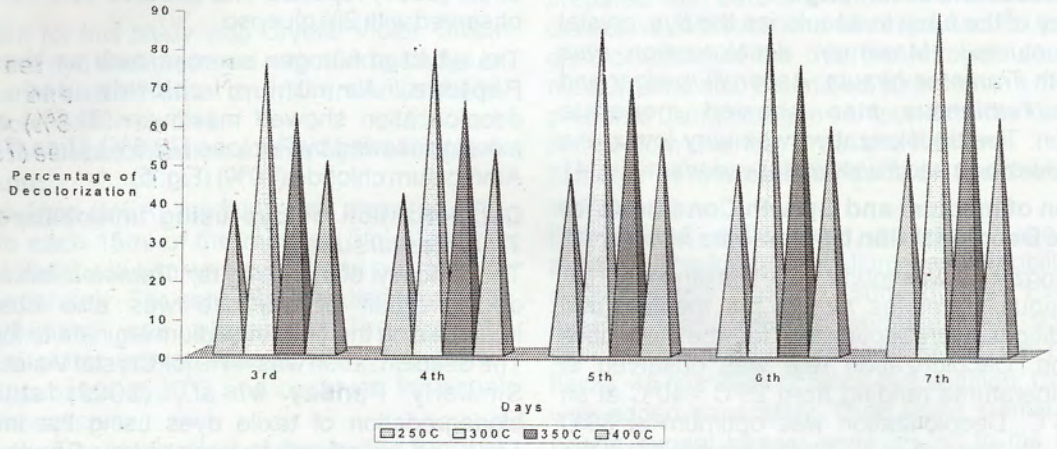


Fig: 3 Effect of pH on the decolorization of Crystal violet by *T.hirsuta*

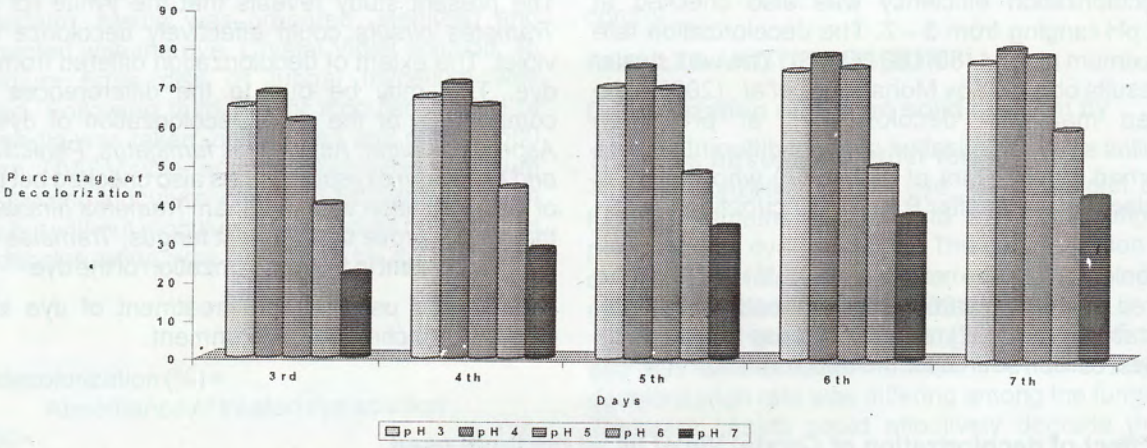


Fig: 4 Effect of carbon source on the decolorization of Crystal violet by *T.hirsuta*

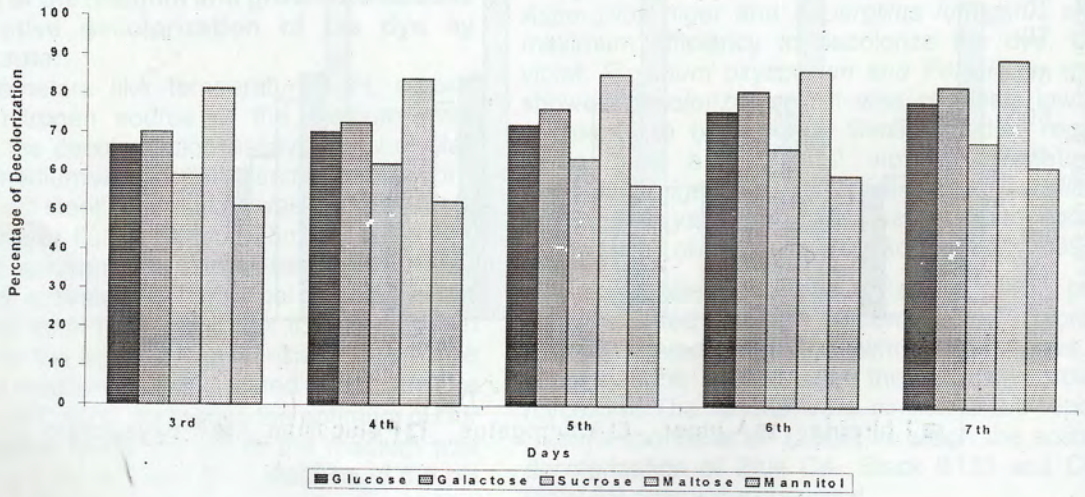


Fig: 5 Effect of nitrogen source on the decolorization of Crystal violet by *T. hirsuta*

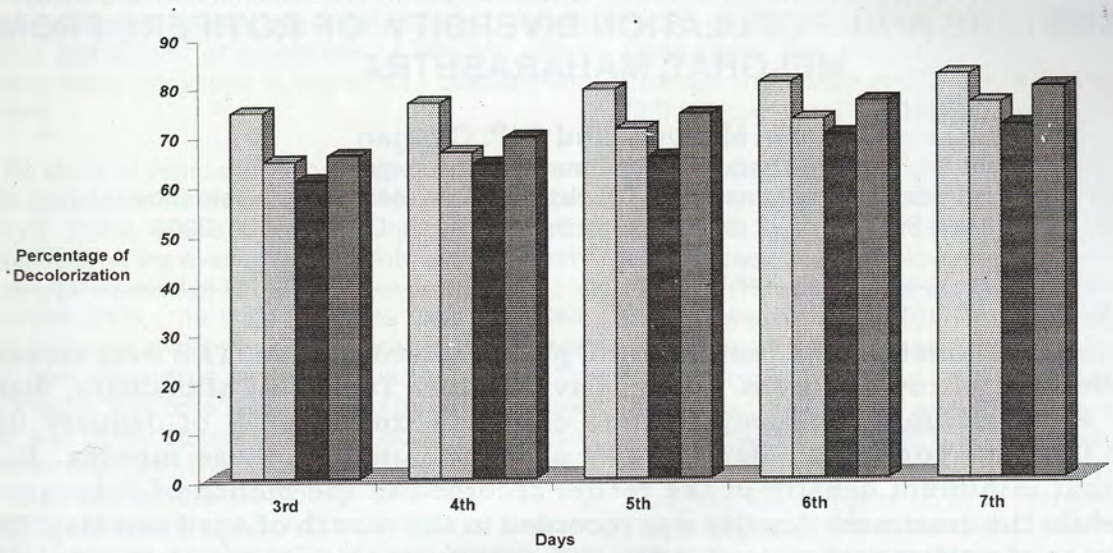


Table: 1 Decolorization of Crystal violet using immobilized cells of *Trametes hirsuta*

Days	Percentage of Decolorization (%)
3rd	36.4
4th	37.9
5th	40.8
6th	42.1
7th	44

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STUDIES ON INTER RELATIONSHIPS OF CERTAIN PHYSICO-CHEMICAL PARAMETERS AND POPULATION DIVERSITY OF ROTIFERS FROM MELGHAT, MAHARASHTRA.

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ABSTRACT:

Environmental parameters like temperature, pH, dissolved oxygen, TDS were measured during collection of rotifers from Paras Talav and Dev Talav in Chikhaldara, district Amravati, Maharashtra.. The samples were collected from month of January 08 to December 08. The population density was also measured in these months. It was observed that minimum density of the rotifer recorded in the month of February and October, while the maximum density was recorded in the month of April and May. There are abundance of rotifers were recorded The seasonal fluctuation in population density of rotifer is related to the physico-chemical factors and due to the organic pollution in the lake water by adding detergents to the water due to washing clothes in the lake water. In Paras talav various physico-chemical parameters determined that the fluctuation in water temperature, pH, dissolved oxygen (DO) and nutrients like sulphate, phosphate and nitrate were within the desirable limits. In dev talav, the contamination of water bodies might lead to a change in water that it is unsuitable for fish farming. Several physico-chemical or biological factors could acts as a barrier and adverse effect on the fish growth and reproduction. For that the regulation monitoring of physico-chemical and biological water quality parameters is essential to determine status of water body.

Key words: *Paras Talav, Dev Talav, Rotifer Population, Environmental Parameters.*

INTRODUCTION

In India agriculture is totally based on south-west monsoon and its consequent effects have made compulsion in utilization of the country's surface and ground water resources in the form of large number tanks, small resources besides the large multipurpose dams, wells, and diversion canals, to raise the gross irrigated areas. We constructed large multipurpose reservoir, the small irrigation reservoir, constructed on small intermittent water resources for serving to capture the surface run-off for its abstraction during seasonal irrigation demands. These projects have provide and revealed that an immense potential for fish husbandry, irrigation, drinking purpose. It is also possible through extensive techniques of aquaculture, and they would contribute significantly in the production of inland fisheries. It is managed with right scientific approaches. Good quality water is essential for living organisms. The quality of water can be known by studying its physico-chemical characteristics as well as zooplanktons , phytoplanktons and hydrophytes growing in it.because of vast population and negligence of human being the quality of water is deteriorated. The limnology play an important role in decision making process for problems like lake construction, pollution control, fish and aquaculture practices Muley and Gaikwad,(1999). A change in the water quality affects the biotic community of the aquatic ecosystem which ultimately reduces the primary productivity.

The aim of the present study was to assess the quality of water by studying its physico-chemical characteristics and rotifer diversity. Studies on physico-chemical characteristics and biological characteristics ao dam water in maharashtra are known through Narkhede (1996), Pawar and Pulle (2005), Gore and Pingale (2007), Kumawat *et.al.* (2007), Nandan *et.al.* (2007), Magar (2008).

MATERIALS AND METHODS

Melghat region situated between the latitude 21.15° N and 21.45°N and Longitudes 76.57°E and 77.33°E is one of the forest and hilly areas of India. It is situated in the mountain region of the Gavilgarh hills of Satpura from

Chikhaldara and Dharni tahsils of Amravati District of Maharashtra state. These parts of Satpura are known as Melghat and consist of succession of hills and valley showing many variations in aspect of gradient and altitudes.

For the study of various abiotic and biotic parameters, water samples were collected from the selected two lakes viz., Paras Talav and Dev Talav in Gavilgarh fort of Chikhaldara on the every month in between 7 to 8 am. regularly for a period of 12 months from January 2008 to December 2008. The water samples were collected, about 5 meters away from the shore and one-meter depth from the surface by using sieve net no. 40. One liter of water was collected in a wide mouth polythene bottle and tightly stoppered for subsequent Physico-chemical analysis. The care was taken to avoid air bubbling during the sampling and also during transporting of water samples from Kemmerer sampler to PVC Container. Another bottle of same capacity was similarly filled with surface water for biological analysis. Dissolved oxygen was estimated at the site. Plankton net (mesh size 65 µm) was used to filter 50 liters of surface water to obtain the net plankton concentration. All the samples were packed in a cane basket protecting them from intense sunlight and contamination was transported to the laboratory without any delay.

RESULTS AND DISCUSSION

The results of physico-chemical parameters with their mean and SE are given in Table 1 and Table 2 while the rotifer diversity is given in Table 3.

In the present study Paras lake was found to have maximum water temperature ranges of (32.3 °C) in the month of June and minimum (18.7 °C) in the month of December. The mean and SE (24.7 ± 1.34). Transparency value was higher in May (47) and lower (28) in November. The mean and SE (35.7 ± 1.77). pH value was higher (8.24) in April and lowest (6.79) in September. The mean and SE (7.4 ± 0.13). Turbidity was higher (49) in January and lower (23) in October. The mean and SE (40.1 ± 3.5). Total dissolved solids was higher(168) in November and lower (112) in October. The mean and SE (151 ± 5.51). Dissolved oxygen was higher (8.60) in December and lower (7.10) September. The mean and SE (7.65 ± 0.15). Free CO_2 found higher (4.90) in July and lower (0.60) in December. The mean and SE (2.55 ± 0.41). Total alkalinity was higher (261) in July and lower (21) in February. The mean and SE (83.3. ± 19.8). Total hardness found higher (273) in November and lower (72) in August. The mean and SE (193 ± 16.8). Calcium and magnesium hardness were found higher (87.10 & 58.14) in October and December while lower (20.10 & 18.12) in September and August, respectively. The mean and SE (49.43 ± 5.29 & 36.75 ± 4.37). Chloride was found higher (72.3) in February and lower (24.6) in August. The mean and SE (48.33 ± 4.72). Phosphate having maximum (0.046) in May and minimum (0.017) in March. Mean and SE (0.034 ± 0.002). the maximum sulphate (1.50) in September and minimum (0.80) in December, mean and SE (1.18 ± 0.06). Nitrate having higher (1.90) in june and lower (1.00) in November, mean and SE (1.39 ± 0.09). the maximum value of silicates (1.70) in the month of September while minimum (0.80) in November, mean and SE (1.30 ± 0.09).

Table 1: MONTHLY VARIATION AND MEAN ± S.E. ABIOTIC PARAMETERS OF WATER FROM PARAS LAKE FROM CHIKHALDARA DURING JAN – 2008 TO DEC – 2008.

Param / Month	WT	Tra	pH	Tur	TDS	DO	CO_2	TA	TH	CaH	MgH	Chl	Phos	Sul	Nit	Sil
Jan -08	19.6	32.0	7.10	49	162	7.30	0.90	65	165	35.20	24.10	68.10	0.023	1.00	1.20	0.90
Feb -08	21.2	33.0	7.25	48	164	7.10	1.30	21	270	58.10	54.16	72.30	0.022	1.10	1.40	1.10
Mar -08	23.6	41.0	7.53	46	165	7.30	2.10	29	245	46.00	56.20	58.10	0.017	1.30	1.60	1.50
Apr -08	28.7	45.0	8.24	41	152	7.60	2.60	129	176	48.00	31.56	38.00	0.045	1.40	1.80	1.70
May -08	31.1	47.0	7.62	37	160	7.50	3.40	131	182	52.00	32.75	35.00	0.046	1.20	1.70	1.50
Jun -08	32.3	41.0	7.34	34	158	8.20	4.60	123	156	47.00	28.40	37.00	0.029	1.10	1.90	1.60
Jul -08	29.2	31.0	6.82	33	163	8.00	4.90	261	189	65.00	24.36	51.00	0.035	1.20	1.00	1.10
Aug -08	26.1	32.0	7.23	27	145	8.60	2.80	25	72	21.60	18.12	24.60	0.041	1.30	1.10	1.30
Sep-08	24.6	33.0	6.79	25	114	7.10	3.20	36	175	20.10	35.42	26.80	0.045	1.50	1.30	1.70
Oct -08	22.2	35.0	7.25	23	112	7.20	3.50	48	165	87.10	21.55	43.70	0.043	1.40	1.60	1.50
Nov -08	20.1	28.0	8.10	65	168	7.40	0.80	67	273	54.90	56.28	61.20	0.039	0.90	1.00	0.90
Dec -08	18.7	31.0	7.86	51	158	8.60	0.60	65	256	58.20	58.14	64.20	0.027	0.80	1.10	0.80
Mean	24.7	35.7	7.42	40.1	151	7.65	2.55	83.3	193	49.43	36.75	48.33	0.034	1.18	1.39	1.30
SE	1.34	1.77	0.13	3.58	5.51	0.15	0.41	19.8	16.8	5.29	4.37	4.72	0.002	0.06	0.09	0.09

Table 2:MONTHLY VARIATION IN ABIOTIC PARAMETERS OF WATER FROM DEV LAKE FROM CHIKHALDARA DURING JAN – 2008 TO DEC – 2008.

Param / Month	WT	Tra	pH	Tur	TDS	DO	CO_2	TA	TH	CaH	MgH	Chl	Phos	Sul	Nit	Sil
Jan 08	19.4	31	7.07	54	163	7.50	1.10	132	271	37.6	59.36	82.5	0.023	0.6	0.7	1.7
Feb 08	21.1	33	7.09	45	166	7.30	1.20	125	265	62.30	54.30	83.4	0.016	0.9	0.9	1.7
Mar 08	23.4	39	7.60	41	167	7.10	2.10	124	230	61.2	39.5	81.2	0.031	1.2	0.8	1.5
Apr 08	28.5	37	7.71	37	153	7.05	2.50	136	187	45.2	32.1	70.2	0.019	1.1	0.5	1.5
May 08	30.9	41	7.52	31	145	7.20	3.20	125	169	64.3	29.6	71.3	0.018	1.6	0.4	1.6
Jun 08	32.2	49	6.67	29	158	7.40	3.60	111	210	62.5	32.56	65.0	0.028	1.4	0.8	1.1
Jul 08	29.1	42	6.17	22	162	6.70	2.50	96	196	74.3	29.47	52.3	0.032	1.1	0.9	1.2
Aug 08	25.9	37	6.17	18	165	8.20	2.80	67	185	25.6	38.45	41.2	0.031	0.9	1.1	1.2
Sep08	24.4	33	6.99	17	187	8.60	3.20	44	171	22.3	34.65	39.6	0.041	0.8	1.2	1.1
Oct 08	22.1	26	7.10	14	182	7.60	3.70	65	145	24.5	31.25	31.5	0.031	0.6	1.0	0.8
Nov 08	20.2	28	7.83	61	176	7.30	1.60	132	295	21.9	61.23	80.2	0.031	0.7	0.5	1.2
Dec 08	18.9	30	7.78	49	163	7.50	1.10	156	287	27.8	59.24	85.6	0.037	0.8	0.4	1.2
Mean	24.6	35.5	7.14	34.8	165.5	7.45	2.38	109	217	44.12	41.8	65.33	0.028	0.97	0.76	1.31
SE	1.32	1.9	0.16	4.47	3.37	0.14	0.27	9.85	14.6	5.69	3.69	5.58	0.002	0.09	0.07	0.08

Abbr : WT - Water temperature, Tra - Transparency, pH - pH, Cond - Conductivity, Tu - Turbidity, TDS - Total dissolved Solids, DO - Dissolved Oxygen, CO2 - Carbon Dioxide, TA - Total Alkalinity, TH - Total Hardness, Ca H - Calcium Hardness, Mg H - Magnesium Hardness, Chl - Chloride, Phos - Phosphate, Sul - Sulphate, Nit - Nitrate, Sil – Silicate.

Table 3:VARIATION IN THE ABUNDANCE OF ROTIFERS IN DIFFERENT LAKES OF CHIKHALDARA DURING JAN-2008 – DEC 2008.

Sr. no.	Name of Rotifers	Lakes	
		Paras Talav	Dev Talav
1	Anuraeropsis fissa	+	++
2	Ascomorpha sps.	-	+
3	Brachionus angularis	++	-
4	Brachionus bidentata	+	-
5	Brachionus calyciflorus v. hymani	-	+
6	Brachionus caudatus	+	++
7	Brachionus diversicornis	+	-
8	Brachionus durgae	+	-
9	Brachionus falcatus*	-	+
10	Brachionus forficula f. typicus urawensis	+	++
11	Brachionus quadridentatus	+	+
12	Brachionus patulus	+	+
13	Keratella cochlearis	+	-
14	Keratella valga	+	-
15	Lecane luna	-	+
16	Lepadella patella*	++	+
17	Monostyla closterocera*	++	++
18	Platyas quadricornis	-	+
19	Polyarthra vulgaris	++	-

(+) Denotes 1000 org. /l, (-) Denotes Absent, (*) Denotes Pollution Indicator Species

Taxonomic Study of Rotifers:

1. *Anuraeropsis fissa* Gosse,(1851)
Lorica with two plates, dorsal and ventral with lateral sulci. Dorsal plate arched and ventral plate flat. Cuadal extremity of the lorica truncated. Small in size.
Distribution : Cosmopolitan

2. *Ascomorpha ecaudis* Party, (1850)
Body sacciform and foot is absent. Apical field with few tufts of cilia. Trophi virgate type. Characteristic feeding on algae which thrive for some time in the stomach before being digested. Pelagic.
Distribution : Europe, India, Japan, China.

3. *Brachionus angularis* Gosse, (1851)
Lorica stippled, with two very small projections in occipital margin. Posterior spines absent. Small in size.
Distribution : Cosmopolitan.

4. *Brachionus bidentata*,Anderson (1889)
Lorica with dorsal, ventral and basal plates. Six occipital spines present, of which the laterals and medians are of same length. Lightly stippled. Forms with single lateral posterior spine were also present.
Distribution : America, Europe, Africa, China, Japan and India.

5. *Brachionus calyciflorus* var hymani,Dhanapathi (1974)
Lorica flexible, anterior dorsal margin with broad based stout spines with rounded tips. Saw-tooth like prominent short spiney extensions present at the base of median spines. Posterior lateral spines and short posterior spines flanking the foot opening, are present.
Distribution : India.

6. *Brachionus caudatus*, Barrios & Dadday (1894)
Lorica with foue occipital spines, the lateral slightly longer than the medians. Posterior spines long.
Distribution : Cosmopolitan.

7. *Brachionus diversicornis*, Daday (1883)
Lorica elongate with four occipital spines, of ehich the laterals longer than medians. Right posterior spine longer than the left. Foot long and toes with characteristic claws.
Distribution : Cosmopolitan

8. *Brachionus durgae*, Dhanapathi (1974)
Lorica smooth, nearly round, with dorsal and ventral plates. Anterior dorsal margin with six saw-tooth like spines of nearly equal length. Anterior dorsal margin with 'V' shaped sinus, ventral margin with projecions having truncated edges. Foot opening situated below the centre of the ventral plate, pear shaped and flanked by small anchor shaped projections. Foot sheath developed.
Distribution : India, Japan, Africa, S. America (Tropicopolitan).

9. *Brachionus falcatus*, Zacharias (1898)
Anterior dorsal margin with six unequal spines, the medians long and curved ventrally at the end. Posterior spines very long, bent inwards and in some forms almost touch each other at their tips. Variable species.
Distribution : Cosmopolitan.

10. *Brachionus forficula* f typicus-urawensis, Sudzuki (1955)
Lorica with four occipital spines. Posterior spines stippled and bowed inwards with characteristic knee-like swellings at the inner side. This differs from B. forficula var keralaensis Nayar & Nair 1969 by the presence of knee-like swellings for the posterior spines.
Distribution : India, Japan

11. *Brachionus quadridentatus*, Hermann (1783)
Lorica broader than long, with six occipital spines of which the median are long and curved outward. Two posterior spines present. Highly variable.
Distribution : Cosmopolitan

12. *Brachionus patulus* ,Muller (1786)
Occipital margin with six spines of which medians slightly longer than the outer. Ventral margin with four spines. Posterior lateral spines are longer than the median.
Distribution : Cosmopolitan

13. *Keratella cochlearis*,Gosse (1851)
Lorica with a strong median spine. Dorsum with characteristic median longitudinal line, with symmetrically arranged plaques on either side.
Distribution : Cosmopolitan.

14. *Keratella vulga*, Ehrenberg (1934)
Lorika broder at posterior margin. The posterior spines, one is long and another is short, divergent and unequal. Median occipital spines are little and longer than other.
Distribution : Cosmopolitan.

15. *Lecane luna* ,Muller (1776)
Lecanid of very common occurrence and variable. Lorica broadly oval with broad dorsal sinus. Second foot joint subsquare. Toes slender, parallel sided and ending in claws.
Distribution : Cosmopolitan.

16. *Lepadella patella*, Muller (1773)
Resembles L. ovalis but differes from it by convex dorsal plate and anterior and posterior margins of lorica.
Distribution : America, Canada, India.

17. *Lecane Monostyla closterocera*, Schmarda (1898)

Lorica sub-circular. Anterior dorsal and ventral margins coincedental making a broad 'V' shaped sinus. Posterior segment broad, semicircular and extends a little beyond the dorsal plate. Toe is slender, long and tapering into point.
Distribution : Cosmopolitan.

18. *Platyas quadricornis*, Ehrenberg (1832)
Lorica firm, stippled, compressed dorso-ventrally with regular pattern of facets. Occipital margin with two stout spines having truncated ends. Posterior spines equal in length.
Distribution : Cosmopolitan

19. *Polyarthra vulgaris*, Carlin(1943).
Body illoricate and little squarish. Four groups of major lateral paddles inserted dorsally and ventrally in the neck region. Each group with three paddles of equal length extending beyond the posterior end of the body. Accessory pair of ventral paddles present between ventral bundles. Vitellarium with 4-6 nuclei. Trophi modified virgate type. Distribution : India (Tropical)



1. *Anuraeropsis fissa*



2. Ascomorpha ecaudis



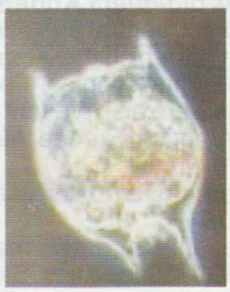
3. Brachionus angularis



4. Brachionus bidentata



5. Brachionus calyciflorus



6. Brachionus caudatus



7. Brachionus diversicornis



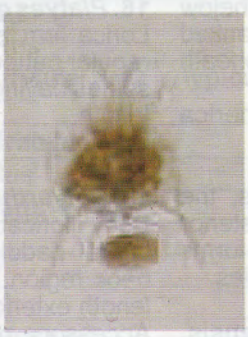
8. Brachionus durgae



9. Brachionus falcatus



10. Brachionus forficula



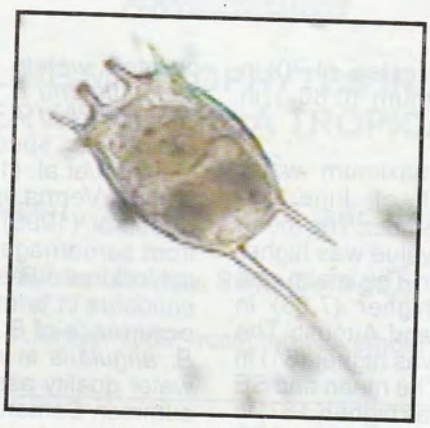
11. Brachionus quadridentatus



12. Brachionus patulus



13. Keratella cochlearis



14. Keratella vulga



15. Lecane luna



16. Lepadella patella



17. Lecane Monostyla closterocera



18. Platyas quadricornis



19. Polyarthra vulgaris

In the present study Paras lake was found to have maximum water temperature (32.3 °C) in the month of June and minimum (18.7 °C) in the month of December. The mean and SE (24.7 ± 1.34). Transparency value was higher in May (47) and lower (28) in November. The mean and SE (35.7 ± 1.77). pH value was higher (8.24) in April and lowest (6.79) in September. The mean and SE (7.4 ± 0.13). Turbidity was higher (49) in January and lower (23) in October. The mean and SE (40.1 ± 3.5). Total dissolved solids was higher(168) in November and lower (112) in October. The mean and SE (151 ± 5.51). Dissolved oxygen was higher (8.60) in December and lower (7.10) September. The mean and SE (7.65 ± 0.15). Free CO_2 found higher (4.90) in July and lower (0.60) in December. The mean and SE (2.55 ± 0.41). Total alkalinity was higher (261) in July and lower (21) in February. The mean and SE (83.3. ± 19.8). Total hardness found higher (273) in November and lower (72) in August. The mean and SE (193 ± 16.8). Calcium and magnesium hardness were found higher (87.10 & 58.14) in October and December while lower (20.10 & 18.12) in September and August, respectively. The mean and SE (49.43 ± 5.29 & 36.75 ± 4.37). Chloride was found higher (72.3) in February and lower (24.6) in August. The mean and SE (48.33 ± 4.72). Phosphate having maximum (0.046) in May and minimum (0.017) in March. Mean and SE (0.034 ± 0.002). the maximum sulphate (1.50) in September and minimum (0.80) in December, mean and SE (1.18 ± 0.06). Nitrate having higher (1.90) in june and lower (1.00) in November, mean and SE

(1.39 ± 0.09). the maximum value of silicates (1.70) in the month of September while minimum (0.80) in November, mean and SE (1.30 ± 0.09).

Similarly Dev lake was having maximum water temperature (32.2 °C) in the month of June and minimum (18.9 °C) in the month of December. The mean and SE (24.67 ± 1.32). Transparency value was higher in June (49) and lower (26) in October. The mean and SE (35.5 ± 1.90). pH value was higher (7.83) in November and lowest (6.17) in July and August. The mean and SE (7.14 ± 0.16). Turbidity was higher (61) in November and lower (14) in October. The mean and SE (34.83 ± 4.47). Total dissolved solids was higher(187) in September and lower (145) in may. The mean and SE (165.5 ± 3.37). Dissolved oxygen was higher (8.60) in September and lower (6.70) July. The mean and SE (7.45 ± 0.14). Free CO_2 found higher (3.70) in October and lower (1.10) in December. The mean and SE (2.38 ± 0.27). Total alkalinity was higher (156) in December and lower (44) in September. The mean and SE (109.4. ± 9.8). Total hardness found higher (295) in November and lower (145) in October. The mean and SE (217.5 ± 14.6). Calcium and magnesium hardness were found higher (74.3 & 61.23) in July and November while lower (21.9 & 29.47) in November and July, respectively. The mean and SE (44.12 ± 5.69 & 41.8 ± 3.69). Chloride was found higher (85.6) in December and lower (31.5) in October. The mean and SE (65.33 ± 5.58). Phosphate having maximum (0.041) in September and minimum (0.016) in February. Mean and SE (0.028 ± 0.002). The maximum Sulphate (1.60) in May and minimum (0.60) in January, mean and SE (0.97 ± 0.09). Nitrate having higher (1.20) in September and lower (0.4) in December, mean and SE (0.766 ± 0.07). The maximum value of silicates (1.70) in the month of January while minimum (0.80) in October, mean and SE (1.31 ± 0.08).

During Jan 08- Dec 08, rotifers were represented by 19 species of 8 genera, among rotifers; *Brachionus angularis & durgae* dominated the lakes Pollution indicator rotifers like *Monostyla, Lepadella,* and *Brachionus falcatus* were found. Paras Lake represented highest number of pollution indicator species. Among pollution indicator species *Lepadella* was abundant at both lakes. The lakes represented the highest number of the species of the genus *Brachionus* Ten species of this genus were recorded during the period of investigations. Similarly the genus *Keratella* with two species and remaining 7 genera were represented by one species each. The rotifers invariably constitute a dominant component of freshwater zooplankton and contribute significantly to their dynamics and production Sharma (1991). These organisms are regarded as valuable bioindicator to depict the trophic status of water quality.

Arora (1963) reported that species of Brachionus have a wide range of occurrence and are found from potable water to diluted sewage tanks. In much polluted water they occur in small number but abundant in moderately polluted waters. He recorded *B. calyciflorus, B. caudatus and B. quadridentatus* from heavily polluted Jumma lake. A number of studies have evaluated *Brachionus* species as indicator of eutrophication , Mahajan et.al. (1981), Takamura et.al.(1989), Sladeck (1983) , Verma and Dutta (1987). Chandrashekhar and Kodarkar (1995) described six species of *Brachionus* from saroornagar lake Hydrabad and reported that B. calyciflorus was most dominated followed *by B. caudatus* in term of seasonal occurrence and uniform occurrence of *B. forficula , B. durgae, B. bidentata and B. angularis* in monthly collections due to change in water quality associated with nutrient concentration in summer. Somani and Pejawar (2003) in lake Masunda Thane reported the dominance of this tolerant genera as an indication of onset of eutrofication in the system.

n the present observation *Lepadella patella, Monostyla* was dominant species followed by *B. falcatus, B. caudatus* . The occurrence of these species indicates the water of this lake is polluted. However further detail studies on seasonal variation in diversity and biomass would be helpful in evaluation and their bioindicator role in eutrophication.

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ROLE OF SHORELINE MACROPHYTES IN MANAGEMENT AND CONSERVATION OF Á TROPICAL LAKE

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ABSTRACT:

Shorelines of any wetland have many distinguishing features, the most notable of which are the presence of standing waters during the growing season, unique soil conditions and a great diversity of flora and fauna. Such transitional habitats are often ignored because of the different backgrounds of the lake and terrestrial ecologists. However these ecotones are important for species and processes that encounter them as boundaries. Fresh water bodies which have direct relevance to mankind for his basic needs, have been studied extensively by several workers in the last many decades. On the contrary, limnological studies of oligotrophic tropical lakes in relation to macrophytic diversity have been neglected, particularly in the sensitive transitional ecotones of shorelines. In the present study diversity of different macrophytes were studied in a large tropical man made lake of Bhopal called as Upper Lake, which has a water spread area of 30.72 sq.km. at FTL. Macrophyte abundance along the shoreline, which is an important link of trophic status has been studied to have an idea regarding their inter applications in the shoreline ecosystem for conservation and management of lakes. Macrophytes are extremely beneficial for nutrient mitigation since they are (1) capable of some direct uptake in the water (2) increase hydraulic retention time by decreasing water flow (3) provide soil oxygenation and (4) provide surface area for microbial biofilms. By determining specific plant retention of various nutrients, improved planning can be accomplished for best management practices and remediation techniques such as constructed wetlands or vegetated agricultural drainage ditche. In the present study 25 species of macrophytes belonging to various orders, such as Convolvulaceae, Ceratophyllacea, Hydrocharriaceae, Najadaceae, Potamogetonacea, Pontedriaceae and Onogracease have been documented along the four sampling stations of varied topography, spanning the entire shoreline of the lake. It was observed that these macrophytes induced multiple beneficial effects for the conservation of the lake, including sustaining water levels, nutrient recycling among various trophic levels, providing shelter and growth to about 83 species of insects belonging to various orders such as Hemiptera, Coleoptera, Odonata and Diptera and also causing significant denitrification as evident from changes in nutrients with reference to macrophyte biodiversity along the shorelines of Upper Lake. The paper presents a lake conservation and management model with reference to macrophyte abundance and interactions with emphasis on their use to attenuate Lake shoreline eutrophication.

Key words: *Shoreline macrophytes, water level fluctuations , attenuation of eutrophication, insect abundance.*

INTRODUCTION

Distribution of shoreline macrophytes, provide a holistic, ecosystem description that facilitates ecosystem concept and provides a tool for ecosystem analysis owing to the close relationship between insect and plants specially in aquatic ecosystem. It is a well recognized fact that the abundance of aquatic life is proportional to the length of the shoreline. This depends upon the concept that macrophytes grow more abundantly in shallow waters along the edges of the lake, which provide hiding places, materials for cases and cocoons, places to lay eggs, anchorage, food and oxygen to the rich aquatic biodiversity (Ervin, 2005).The macrophytes community structure in freshwater

shorelines is controlled by interactions between a wide range of environmental factors, including regional climate, hydrology, surface geology, the chemical oxidation and reduction potential, as well as the method of construction. Many of these factors change through time, both seasonally and on a yearly basis, as the shorelines mature. Unique adaptations have developed in shoreline plants, the macrophytes, to deal with the stresses that occur in systems in which the soil is inundated for part or all of the growing season. Shoreline macrophytes have developed adaptations to survive under the stress associated with soil saturation and limited oxygen supply .The ability of macrophytic species to adapt with environmental stressors is an important factor in regulating aquatic community zonation (Grace and Wetzel 1998 ,Cronk and Fennesy 2001).

Eutrophication caused by excess nutrients is a predominant water quality issue for many tropical as well as temperate water bodies, where nutrients are the fourth largest impairment of waterbodies, behind pathogens, metals and sediments. Non-point source runoff from agricultural fields may contain high levels of nutrients, particularly nitrogen (N), phosphorus (P), calcium (Ca) and chloride (Cl) which can be a major contributor to eutrophication of adjacent waters (Carpenter et al., 1998). In the present study several species of shoreline macrophytes of Upper Lake, a large man made water body in the tropical region have been found to be effective in removing nutrients from runoff waters. Thus the macrophytes have been found to remove excess nutrients, increase hydraulic retention time by decreasing water flow and providing
shelter and growth to about 83 species of insects, belonging to various insect orders. The present study's objective was to determine the role of shoreline macrophytes in conservation and management of a large tropical man made lake of Bhopal.

MATERIALS AND METHODS

The site Upper Lake selected for the work is situated in Bhopal, which is a vast man made Lake of Central India, having a water spread area of 30.72 sq.km. at FTL. The storage capacity is 101.6 million cu.m., the maximum and mean depth being 11.7 m and 6 m. respectively. Selection of the four sampling sites of the Upper Lake chiefly was done on the basis of abundance of macrophytes and consequent biomass available. sampling sites were designated as SI, SII, SIII and SIV. Monthly sampling from all these stations were made regularly for a period of 24 months from September 2003 to August 2005.

Water samples were collected in the noon hours between 10 a.m. to 12 p.m. and brought to laboratory for analysis. Excepting temperature and pH all parameters were analysed in the laboratory. The methods used for analysis were as described in standard methods for examination of water and waste waters 20th ed. American Public Health Association (APHA, 1998). To investigate the species richness and abundance of shoreline macrophytes, as well as their plant interactions, a belt transact method (Dicks *et al.*, 2002) was applied. The same four transects were used at the start to the end of the sampling season. Samples of shoreline macrophytes were collected from different types of habitat like lake margin and shallow water zones of study area. The macrophytes were brought to the laboratory in polythene bags and identified with the help of guidelines given by Needham and Needham (1962) and Haslam (1978).

RESULTS AND DISCUSSION

In the present study 25 species of macrophytes belonging to various orders such as Convolvulaceae, Ceratophyllacea, Hydrocharriaceae, Najadaceae, Potamogetonacea, Pontedriaceae and Onograceae have been recorded along four sampling stations viz SI, SII, SIII, SIV of the entire shorelines of Upper Lake. Maximum growth of several macrophytes i.e. Ceratophyllum demersum, Hydrilla verticillata, Najas minor, *Eichhornia crassipes, Aponogeton* and *Vallisnaria spiralis* were recorded during summer, followed by winter and rainy seasons.

Table - 1

Showing Physico chemical water quality measurements in different sampling stations of shorelines of Upper Lake in relation to macrophytic abundance

Physico - chemical parameters	Station I	Station II	Station III	Station IV
Temperature (^{0}C)	24.25	24.52	24.32	36.35
Light transparency (cm)	29.91	34.08	30.83	33.83
pH	8.3	7.9	8.2	8.2
Water level fluctuation (m)	6.6	3.7	4.0	3.5
D.O. (mg / l) 9.09	9.77	6.62	9.44	
Alkalinity (mg / l)12.18	14.14	19.95	15.08	
Chloride	20.13	14.14	19.95	15.08
Calcium hardness	31.33	16.12	26.29	15.41
Total hardness116.83	77.75	109.62	82.0	

On comparing the shoreline macrophytic density of Upper Lake at all the four sampling stations, it has been observed that station II and station IV had greater macrophyte abundance than the stations I and III. This may be attributed to the favourable weather conditions, balance nutrient levels, amount of dissolved oxygen, adequate light, temperature, pH and ofcourse less interference of human activities.

Role of macrophytes in sustaining water level fluctuation :

Water level fluctuations at different sampling stations of shorelines of Upper Lake were found to be seasonal and temporary which declined maximally upto 22 m. from its actual sampling point at station I in summer season. Whereas water level of Upper Lake increased during post-monsoon season by 8 m. at the same sampling station. During the entire period of study maximum water level fluctuation was observed at station I being 22 m where shoreline macrophytes were less abundant where as minimum fluctuations in water levels were observed at station II and IV just being 0.5 meters which was due to the dense and high macrophytic population. The Upper Lake was generally at their lowest levels in the summer months at all the sampling stations. In the summer, when the air above the lake was warm and moist and the lake was relatively warm, evaporation from the lake was greater, thus the water level declined to their seasonal lows. In rainy season, more water entered into lake, that leaving, this process caused water level rise.

Role of macrophytes in attenuation of nutrients via recycling among various trophic levels :

In the present study, maximum members of submerged macrophytes such as *Ceratophyllum demersum, Hydrilla verticillata, Najas minor, Potamogeton pectinatus* were dominantly present at shorelines of Upper Lake being in the range of 32 to 68 sps / m^2 whereas only one species of submerged macrophyte *Vallisnaria spiralis* was present sub-dominantly being in a range of 20 to 36 sps / m^2, thus these data clearly indicate that growth and inhibition of macrophytes in Upper Lake not only depend upon the individual capacity of the species to compete with other species but also on the interaction of other factors such as nutrient loading conditions of the lake, degree of stabilization and water retention and fluctuation periods .

In the present study, station II and station IV of Upper Lake showed minimum eutrophication where an inverse relationship of nutrient level and macrophytes was seen. The density of *Potamogeton pectinatus, Eichhornia crassipes* and *Leersia hexandra* was observed maximally at both the stations being 30 to 72 sps / m^2 in summer and rainy seasons. Despite excessive nutrient levels being present in these stations arising due to anthropogenic activities and agricultural runoff, the hardness of water was found to be considerably reduced at stations II and IV, being 77.7 mg / l and 82.0 mg / l respectively, due to the process of absorption as shown in Table 1.

Thus in the present study presence of the shoreline macrophytes particularly the dominant species such as *Leersia hexandra, Potamogeton pectinatus* and *Eichhornia crassipes* significantly decreased the water hardness of these sampling stations, indicating their high potential of natural ecobalancing (SI, SII, SIII, SIV).

Table – 2 showing Average of macrophytic abundance (per sq.) at different sampling stations of shorelines of Upper Lake

Types of Vegetations	Macrophytes	Family	SI	SII	SIII	SIV		
EMERGENT FORMS	***Leersia hexandra***	-	11.1	36.6	8.3	39.3		
	Jussiaea repens	Onograceae	22.6	30.0	15.3	32.6		
	Ipomoea aquatica	Convolvulaceae	28.8	49.3	14.3	38.6		
	Polygonum glabrum	Polygonaceae	13.3	17.3	11.3	28.0		
SUBMERGED FLOATING	***Hydrilla verticillata***	Hydrochariacea	15.3	18.3	4.3	13.3		
FORMS	***Ceratophyllum demersum***	Ceratophyllaceae		14	29.3	11.3	21.3	
	Najas minor	Najadaceae	23.3	47.3	20.3	37.3		
	Potamogeton pectinatus	Potamogetonaceae		18	56.6	12.0	55.3	
	Vallisnaria spiralis	Hydrochariaceae	19.2	30.6	13.3	51.3		
ROOTED WITH FLOATING	***Nelumbo nucifera***	Nymphaceae	-	9.0	-	-		
FORMS								
FREE FLOATING FORMS	***Azolla pinnata***	Salviniacea	5.3	12.6	-	11.3		
	Eichhornia crassipes	Pontedriaceae	25.3	66.6	13.3	46.6		
	Lemna minor		-	22.0	22.3	32		

Role of shoreline macrophytes in providing growth and shelter to several species of shoreline insects :
In the present study, it was seen that aquatic macrophytes such as *Hydrilla verticillata, Najas minor* and *Eichhornia crassipes* formed an important component of food web and played a vital role in the nutrient and oxygen balancing process and oxygen budget of the lake and also played a major role in insects protection and species emergence, as well quite significantly. In terms of population abundance, shoreline insects were seen maximum being 12 to 40 sps / m2 on littoral macrophytes such as *Eichhornia crassipes, Hydrilla verticillata, Potamogeton pectinatus, Vallisnaria spiralis* and *Ipomoea aquatica.* Higher abundance of various orders of insects like *Odonata, Hemiptera, Diptera* and Coleoptera out of which *Colymvetus fuscus, Dineutus indicus* and *Dineutus unidentatus* of order *Coleoptera* and *Dysdercus cingulatus* of order Hemiptera were seen on *Eichhornia crassipes* comparing to other aquatic vegetations of Upper Lake, because it was present during most part of the year and it had a capacity to absorb minerals, nutrients, heavy metals, organic substances etc. Similarly different species of order Orthoptera such as *Chrotogonus trachypterous, Chorthippus parallelus* and *Atractomorpha cranulata* showed significantly high abundance on *Leersia hexandra* being 20 to 52 sps/m2 These results suggest that loss of shoreline macrophytes may reduce the productivity of both aquatic ad terrestrial insects, possibly by adversely affecting the amount of allochthonous materials entering the shoreline and micro-climate conditions.
Shorelines are often defined by the relative influence of inflows of water, such as ground water, precipitation, and surface flow. The water level fluctuation of any water body is controlled by the water budget, as well as physical aspects of the surrounding environment, such as soil permeability, and most important the abundance of shoreline macrophytes, which directly modifies the physicochemical factors of the aquatic ecosystem such as soil makeup, redox potential and nutrient availability. In Lake habitats, temporal variations in flow play an important role in structuring macroinvertebrate communities. Particularly in large Lakes the inshore zone is affected by flow variations and drying up, both of which effect community and population structure. Shallow gravel banks of large lakes offer a wide range of periodically to permanently flooded areas. In such a gradient, ecological theory predicts that maximum species diversity is reached in sites with medium frequency and intensity of disturbance. In the present study it was observed that high growth of shoreline plants such as *Ipomoea aquatica, Potamogeton pectinatus, Leersia hexandra* and *Vallisnaria spiralis* affected the rate of water level fluctuation. Growth of these shoreline macrophytes was partly dependent on the weather and varied from month to month and year to year. In the summer season dense population abundance of macrophytes such as *Vallisnaria spiralis, Ceratophyllum demersum* and *Leersia hexandrn* reduced the flow of water on average by 2 percent. Therefore significant high population density helped to reduce water level fluctuation and also decreased the possibility of habitat loss for marginal insects. On the other hand several free floating macrophytes such as *Eichhornia crassipes, Azolla pinnata* and *Lemna minor*

were not found to be very effective in sustaining water level fluctuation. Fluctuated water picked up these macrophytes from shoreline areas and deposited them wherever the water got slowed down and were picked up again when the velocity of the water increased.

In the present investigation among the various sampling stations of Upper Lake, minimum nutrient level of water was recorded at station II and IV, which contained ecologically dense population and abundance of nutrient absorbent macrophytes such as *Leersia hexandra, Eichhornia crassipes* and *Potamogeton pectinatus.* These data clearly indicate that roots and storage organs of the several macrophytes like *Potamogeton pectinatus, Leersia hexandra* and *Eichhornia crassipes* acted as a long term nutrient sinks. According to Systma (1989) *Ludwigia peploids* has the capability of absorbing nutrients from water through its extensive adventitious root system and also played important role in denitrification. Recently several workers such as Bachand and Home (2000), Silvan *et al.*, (2004) and Deaver *et al.*, (2005) have also observed the absorption capacity of shoreline macrohytes. These workers have reported that aquatic plant community can be used in reduction of nutrients from agricultural runoff. The data of the present findings are extremely important for Lake management point of view, as through the weeds and macrophytes nutrient recycling and management in larger waterbody can be achieved economically and on a larger scale, keeping the aesthetic value of the lakes intact as well. An interesting part of the study was the observation that macrophytic population abundance significantly affected the survival and dominance of several insect communities such as *Ranatra varipes, Gerris lacustris, Colymbetus fuscus, Belostoma indicum* and *Dysdercus cingulatus* of the Upper Lake at various sampling stations. It was observed that several macrophytes confined themselves to the shallow euphotic zone of the lake, which provided significant shelter to a large number of insects. Similarly shoreline macrophytes provided temperature and microclimate control, habitat complexity and prey refuges coupled it with these increased abundance of food and nutrient input also. In the present study it has been observed that most of shoreline insects were specific to their host plants where as some insects species had variations in choosing their host plants. An interesting observation regarding variability of macrophytes was seen in present investigation that heteropteran insects such as *Nezara viridula, Dysdercus cingulatus* and *Reduvius personatus* survival rate was fount to be significantly higher when they were found to feed on different macrophytes like *Leersia hexandra* and *Aponogeton* as compared to those who fed on only specific macrophytes. Several earlier workers i.e. Berg (1950) and Chessman (1986) have also stressed upon plant insect interactions and reported that aquatic macrophytes affects directly to the insect fauna of any water body, which gave shelter, food and breeding space to them. Recently Romanuk and Levings (2003) has also suggested that shoreline insects were dependent on the presence and composition of shoreline macrophytes considerably. Insects directly or indirectly depend upon plant for their existence and are frequently attracted by the abundance of available food and by the shelter that different portions of the plants offer.

CONCLUSION -

Shoreline macrophytes induced multiple beneficial effects for the conservation of the lake, including sustaining water level, nutrient recycling among various trophic levels, providing shelter and growth to about 83 species of insects belonging to various orders such as Hemiptera, Coleoptera, Odonata and Diptera. Additional work is needed to clarify the mechanism of nutrient reduction as well as seasonal evaluations, and the possible effects of harvesting above ground biomass. Using individual species of plants and insects with regard to symbiotic relationships at the inter and intra community levels of large tropical water bodies which have a complex cascading trophic interactions.

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STUDIES ON POPULATION DYNAMICS OF CLADOCERANS AND COPEPODS IN LAKES OF CHIKHALDARA , MAHARASHTRA

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ABSTRACT:

Cladocerans and Copepods, the well recognized secondary consumers, form an important component of the zooplanktonic community which have adapted to diverse habitats. In the present study, 09 cladoceran species and 05 copepod species were recorded from different lakes of Chikhaldara in Amravati district of Maharashtra state. The communication provides an insight into the population dynamics of Copepods and Cladocerans inhabiting the lakes.

Key words: *Cladoceran and Copepod fauna, population dynamics, Chikhaldara lakes.*

INDRODUCTION

Zooplankton are abundant in the shallow areas of most water bodies and unlike phytoplankton are particularly distributed horizontally and vertically in an ecosystem. The zooplankton form an important group as they occupy an intermediate position in the food web, many of them feeding on algae and bacteria and in turn being eaten up on by fishes. They also indicate the tropic status of a water body, their abundance increases in eutrophic water. They are also sensitive to pollution and many species are recognized as indicators of pollution.Zooplankton, an important index of secondary production and a natural source of food for higher organisms, including fishes in an aquatic medium, constitutes potentially functional and dynamics community in an aquatic ecosystem. Generally the species included in zooplankton belong to Protozoa, Rotifera, Cladocera, Copepoda and Ostracoda which plays a key role in transferring energy from one trophic level to other in the aquatic habitats. Besides, they are also used as biological indicators of trophic status of a water body. Though their pattern of distribution, periodicity, abundance and growth in different aquatic habitats have been a cynosure for great many researchers across the globe for more than five to six decades, yet only few studies have been made from sub-Himalayan region of North India Chakrabarty *et al.,*(1959); Nasar and Munshi, (1975); Nasar, (1975, 1977); Jyoti and Sehgal,(1979); Sehgal, (1980); Joshi, (1996); Gupta, (2002) and Kour, (2002).

MATERIALS AND METHODS

The present study was conducted for a period of one year from January2008 to December 2008. Plankton samples were collected every month from sakkar Talav, Macchi Talav, Paras Talav and Dev Talav during 7 to 9 am in the morning. The four study stations were established in the littoral zone along the pond circumference. Each sample was collected by filtering 50 L of water through plankton net. Filtrate was collected in 20ml plastic bottle and 4% formalin was added to preserve the samle for further studies in the laboratory. Plankton species belonging to two groups, Cladocera and Copepoda, were identified with the help of standard literature Edmondson,(1959); Pennak, (1968); Adoni, (1985) and Muruganetal,(1998).

RESULTS AND DISCUSSION

Cladocerans in Chikhaldara lakes were represented by 09 species viz., *Simocephalus, Ceriodaphnia, Daphnia, Diphanosoma, Leydigia, Chydorus, Moinodaphnia, Alona rectangula, Macrothrix* and copepoda comprises of 5 species viz., *Eurytemora affinis, Centropages typicus, Pseudodiaptomus forbesi, Eucyclops speratus, Mesocyclops.*

The table 1 and 2 reveal the variations in the abundance of cladocerans and copepods in different lakes of Chikhaldara during the study period. Both Cladocerans and Copepods acquired two distinct population peaks. Cladocerans acquired peaks during January, May and June (Table 3. Fig. 1), whereas Copepods during January,March ,April, May and June. In earlier studies such bimodal peaks in different aquatic habitat have also been reported by Smith *et al,*(1979), Nayak(1986) and Gochait and Nayak (1990).

During the present study highest population densities of Cladocerans and Copepods were found in the month of May and June (Table 3, Fig.1). The reported population maxima could de attributed to easy availability of food and favourable physico-chemical conditions. The increase in the population of these plankton groups may also be attributed to the net increase in the number of one particular species rather than a single genus.

During the study Cladocerans were represented by 9 species. The population abundance of *Leydigia and Moinodaphnia* showed their dominance over all the cladocerans and their maximum number were reported at Sakkar Talav and Macchi Talav. Similarly *Moinodaphnia (M. macleayii)* were recorded to be the highest at all lakes exhibited by the rich biota, among all the cladocerans species. *Simocephalus, Alona, Macrothrix and Chydorus* were densely populated at all lakes.

Similarly Copepods observed from Lakes of Chikhaldara were represented by 5 different species *Eurytemora and Pseudodiaptomus* dominated the lakes. Lake wise abundance of copepods showed major population at Sakkar and Macchi Talav. Population represented by lakes as Macchi Talav >Sakkar Talav > Paras Talav > Dev Talav Though the abundance of us *Ectocyclops (E. speratus), Centropages (C. typicus)* and *Mesocyclops was* comparatively low.

In case of cladocerans the increase in population density is mainly on account of increased numerical count of *Leydigia , Moinodaphnia* and *Daphnia sp.,* whereas in the case of copepods, the increase is due to the *Pseudodiaptomus forbesi* and number of *Eurytemora affinis* in the collection. Similar finding have also been made in earlier studies by Welch (1952), Hutchinson (1967), Sehgal (1980) and Dalpatia (1998).

During monsoon and early winter seasons a decrease in the population density of both cladocerans and copepods was recorded (Table 3 & 4). This may be attributed to decrease in the availability of food. During monsoon turbidity caused by agricultural and surface run-off and soil erosion affect severely the production of zooplankton.

In winters the number of cladocerans and copepods started increasing due to luxuriant growth of phytoplankton in the pond, which may be due to favourable environmental conditions. Gochait (1991) suggested that the seasonal abundance of cladoceran population is closely linked with favourable condition i.e. food and temperature.

A perusal of (Table 3 & 4 and Fig 1 & 2) also reveals that the total zooplankton too exhibit bimodal growth thereby resulting in two peaks; the first recorded during January and the second in May and June.

Presence of healthy Cladocerans and Copepods in Chikhaldara lakes also suggests that lakes can very well meet the needs for culturing species of food fishes that depend on plankton for their nutritional requirement.

Table : 1. VARIATION IN THE ABUNDANCE OF CLADOCERANS AT DIFFERENT LAKES OF CHIKHALDARA DURING JAN-2008 – DEC 2008.

Sr.No.	Cladocerans	Lakes			
		Sakkar Talav	Macchi Talav	Paras Talav	Dev Talav
01	Simocephalus	+	+	+	-
02	Ceriodaphnia*	+	++	+	+
03	Daphnia	++	+	++	+
04	Diphanosoma	+	++	+	+
05	Leydigia*	++	++	++	+
06	Chydorus	+	+	+	+
07	Moinodaphnia*	++	++	++	+
08	Alona rectangula	+	+	+	+
09	Macrothrix	-	+	+	+

(+) Denotes 1000 org. /l, (-) Denotes Absent , (*) Denotes Pollution Indicator Species.

Table : 2. VARIATION IN THE ABUNDANCE OF COPEPODES AT DIFFERENT LAKES OF CHIKHALDARA DURING JAN-2008 – DEC 2008.

Sr.No.	Copepodes	Lakes			
		Sakkar Talav	Macchi Talav	Paras Talav	Dev Talav
01	Eurytemora affinis	+++	+++	++	++
02	Centropages typicus	++	+++	++	+
03	Pseudodiaptomus forbesi	+++	+++	+++	++
04	Eucyclops speratus	++	++	+	+
05	Mesocyclops	++	++	++	++

(+) Denotes 1000 org. /l, (-) Denotes Absent , (*) Denotes Pollution Indicator Species.

Table : 3. MONTHLY VARIATION OF CLADOCERANS (ORG. / L) AT LAKES OF CHIKHALDARA DURING JAN 2008 – DEC 2008

Months	Sakkar Talav	Macchi Talav	Paras Talav	Dev Talav	Total
Jan	1240	1560	1200	650	**4650**
Feb	960	1120	1150	720	**3950**
Mar	870	1240	1210	680	**4000**
Apr	560	1360	1360	860	**4140**
May	1280	1480	1490	970	**5220**
Jun	1410	1460	1520	950	**5340**
Jul	560	780	580	450	**2370**
Aug	670	720	670	490	**2550**
Sep	680	730	810	510	**2730**
Oct	760	750	560	630	**2700**
Nov	850	1150	920	380	**3300**
Dec	790	1240	950	750	**3730**
Total	**10630**	**13590**	**12420**	**8040**	**44680**

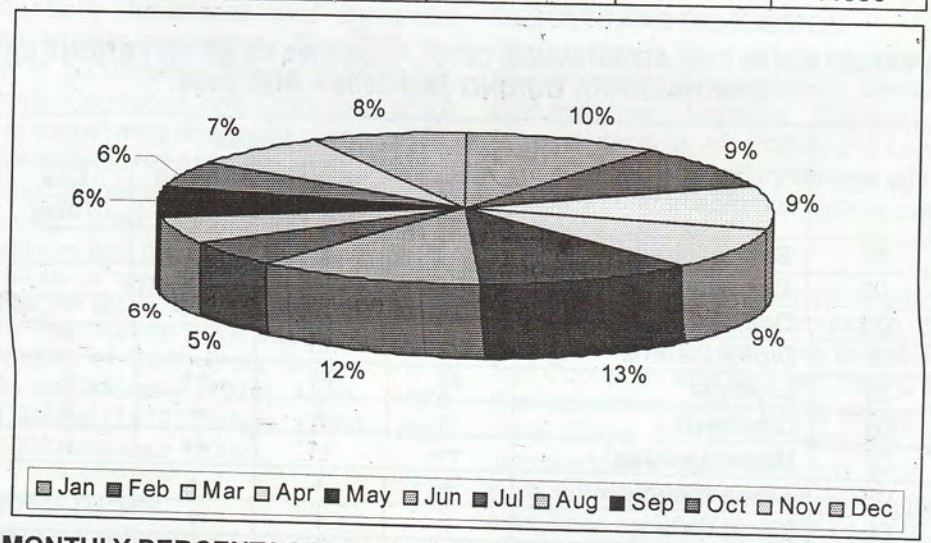


Fig. 1 MONTHLY PERCENTAGE DISTRIBUTION OF TOTAL CLADOCERANS AT LAKES OF CHIKHALDARA DURING JAN 2008 – DEC 2008

Table : 4. MONTHLY VARIATION OF COPEPODES (ORG. / L) AT LAKES OF CHIKHALDARA DURING JAN 2008 – DEC 2008

Months	Sakkar Talav	Macchi Talav	Paras Talav	Dev Talav	Total
Jan	1360	1120	960	820	4260
Feb	1280	1250	650	650	3830
Mar	1410	1350	980	460	4200
Apr	950	1480	1250	510	4190
May	1320	1560	1310	970	5160
Jun	1840	1280	1280	850	5250
Jul	510	640	370	620	2140
Aug	630	750	480	460	2320
Sep	580	790	510	820	2700
Oct	540	810	570	630	2550
Nov	970	920	720	760	3370
Dec	1240	1240	830	470	3780
Total	12630	13190	9910	8020	43750

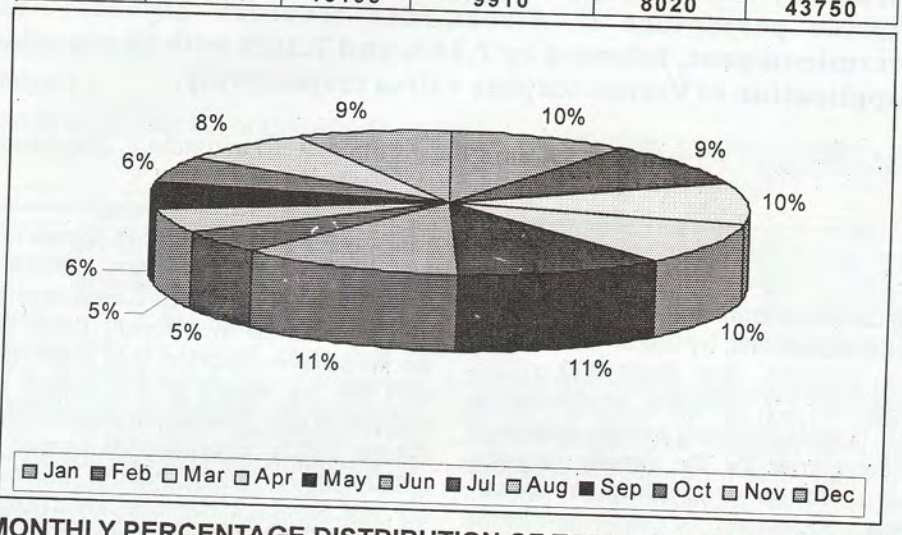


Fig. 2. MONTHLY PERCENTAGE DISTRIBUTION OF TOTAL COPEPODS AT LAKES OF CHIKHALDARA DURING JAN 2008 – DEC 2008

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COMPARATIVE EFFICIENCY OF BIO-FERTILIZER AND CHEMICAL FERTILIZER ON STEVIOSIDE CONTENT OF MEDICINAL PLANT *Stevia rebaudiana* BERTONI

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ABSTRACT:

The present study was done to evaluate the comparative effects of agro-techniques on stevioside percentage in Stevia, where a field experiment was conducted at Sanwaria Herbal and Musali Farm Misrod, Bhopal (MP), India, by applying a chemical fertilizer, urea and bio-fertilizer, Vermicompost singly and in combination. The results showed that the stevioside percentage in Stevia, was recorded highest 7.52% with the application of vermicompost, followed by 7.14% and 7.10% with the applications of urea and combined application of Vermicompost + Urea respectively.

Key words: *Medicinial Plant, Stevia, Bio-fertilizer, Stevioside, Bio-Sweetener, Crop-Diversification.*

INTRODUCTION

Diabetes, a leading cause of death in many countries, is becoming a truly global epidemic by 2025. The World Health Organization predicts that about 300 million people will be affected by diabetes, worldwide. A natural herb Stevia, is fast becoming a major source of high potency bio-sweetener for the rapidly growing market for natural foods, replacing chemical sweeteners. Stevia, botanically known as *Stevia rebaudiana*, is a South American plant, basically from Paraguay and Brazil, belonging to the family Compositae. Dr. M.S. Bertoni officially discovered the sweet herb stevia in early 20th Century (1905). The first reports of commercial cultivation in Paraguay were in 1964 (Katayama and Sumida, 1976). Sweetener property and its minimal side effects focused on the importance of Stevia, mainly due to the presence of complex mixture of natural sweet diterpene glycosides viz stevioside and rebaudiosides, which are 300-350 times and 450 times sweeter than sugar respectively (Zhang and Kutowy, 1999).

The dry weight composition of Stevia is given as protein (6.2%), lipids (5.6%), total carbohydrates (52.8%), Stevioside 15% (Geuns, 1998) and 42% water soluble substances (Kinghorw, 1992). Literature survey reveals Stevia's versatile applications in treatment of diabetes, obesity, weight loss, dental health, high blood pressure, oral health, carbohydrate cravings, skin toning and healing, tobacco and alcohol cravings and antihyperglycemic effects of stevioside in type 2 diabetic subjects (Gregersen, 2004). Stevia is now successfully grown in Paraguay, Mexico, Central America, Japan, China, Malaysia, South Korea and its cultivation has recently started in different parts of India. Several workers have reported that stevioside content varies with the dry weight of the leaves depending on the cultivation and growing conditions (Nepovim, 1998 and Geuns 2003). International demand is only for high quality Stevia leaves having minimum 9% stevioside content. Hence it is necessary to improve the existing agrotechniques for increasing the stevioside content of Stevia.The global market size and business of medicinal plant materials including Stevia and health care products based on these herbs comes to around 62 billion US $ and is likely to cross the 1 trillion mark by 2020 and 5 trillion by 2050 (Patra and Khanuja, 2005) .These targets can only be achieved by improving agro-practice with latest techniques. Influence of fertilizer levels on growth, yield and uptake of Stevia has been reported by Chalapathi *et al.*, (1999), but no such comparative study of agro-techniques so far has been reported till date.

The present study was conducted with the objective of comparative influence of bio-fertilizer Vermicompost and chemical fertilizer urea, separately and in combination on Stevia plant to achieve maximum stevioside percentage towards the development of scientific agro-techniques for Stevia cultivation.

MATERIALS AND METHODS

A field experiment was conducted during Kharif season (Jun-Aug) 2008 at Sanwaria Herbal and Musali Farm,

Misrod, Bhopal (M.P.) India. The location of the experimental site was 23'16" N latitudes and 77'36" E longitudes and 499 m above the sea level. The experimental soil was vertisole having pH 6.9, electrical conductivity .41mmhos.cm (Piper 1966 and Jackson 1973), organic carbon .45% (Volumetric Walkle Method), available N .45 Kg ,ha (Volumetric Walkle Method), available p 17.4 Kg .ha (Olsen Method) and available K 468 Kg .ha (Flame Photometer Method). Four raised beds A,B,C,D (height 15 cm, width 60cm, distance between two rows 40 cm) were formed in a field. Experiment was done on 500 plants (125 plants in one row with a distance of 23 cm between two plants). Treatments T_1, T_2, T_3, T_4 were given to Beds A,B,C,D respectively. T1 = Absolute control ,no application of fertliizers ; T2 = Application of N at 40kg ha as Urea: T3 = Application in combination of N at 50kg .ha as Urea and application of N 50 qtl. ha as vermicompost; T4= Application of N at 50 qtl. ha as vermicompost. Before planting, four soil samples S_{IA}, S_{IB}, S_{IC}, S_{ID} were extracted from A, B, C, D Beds respectively and tested for N and K and pH. A variety of Stevia S.R.B. = 512 as a test plant were planted in A, B, C, D.

The plants were allowed to grow for a period of 90 days. After 90 days, Plant samples and also soil samples were extracted from Beds A,B,C,D respectively. Plant samples were analysed for stevioside percentage by HPLC method and soil samples were analysed for N.K. and pH by following the methods as mentioned earlier.

RESULTS AND DISCUSSION

Plant Sample Analysis : -

Table1. Effect of mode of application of Chemical fertilizer and Bio-fertilizer on the change in stevioside percentage in plant treatment

Treatments	T_1	T_2	T_3	T_4
Plant Samples	P_{1A}	P_{1B}	P_{1C}	P_{1D}
Stevioside %	5.29%	7.14%	7.10%	7.52%

From the data of table 1, it is concluded that stevioside percentage got significantly increased over that of stevioside percentage present in the control treatment T1 (5.29%). The magnitude of changes in stevioside percentage however, varied with treatments. The highest recorded stevioside was 7.52% in treatment T_4 i. e. application of bio-fertilizer, this was followed by 7.14 % (T_2 treatment) and 7.10% (T_3) with the application of urea and combined applications of vermicompost + urea respectively.

The stevioside percentage was recorded highest with the application of bio-fertilizer as a sole might be due to greater utilization of available Nitrogen by plants in presence of Vermicompost. Stevia plants respond well to fertilizers with lower nitrogen content. Nitrogen application is a must for the production of dry matter. Stevioside percentage is low with the application of chemical fertilizer as sole and in combination with bio fertilizer might be due to dilution effect of N absorption by the plants.

Fertilizer requirements are moderate for stevia, partially due to its adaptation to poor quality soils. Fertilizer trials show yield reduction at high rates of fertilizers (Geonadi 1985, Karnienko 1995, Kang. 1979, Lee, 1980). Most organic fertilizers would work well since they release nitrogen slowly. Since the feeder roots of stevia plants tend to be very close to the surface, it is a good idea to add compost for extra nutrients if the soil is sandy. The recommended dose of fertilizer is 110:45:45 NPK.

A good compost usually satisfies nutrient requirements. Mark Langan of Mulberry Creek Herb Farm recommended low nitrogen or organic fertilizers as excess nitrogen promoted rank growth with poor flavour. For maximum nutrient availability it was suggested to mix organic fertilizers into the soil a few months before planting the compost. For Poor Fertility Soils (Oddone 1997) of Guarani Botanicals, Inc., has recommends incorporating 6 to 7 pounds of cattle manure per square yard.

Shadchina and Dmitriev (1995) have found a close relationship between chlorophyll in leaves and accumulation of total Nitrogen dry matter of the whole plant as chlorophyll content is found to be an informative parameter for the estimation of nitrogen uptake from the soil. Chalpathi *et al.* (1999), reported that the application of 60:30:45 NPK kg. ha produced higher dry leaf yield and recorded higher nutrient uptake by Stevia plant. Even though theoretical nutrient usage of Stevia might be 105kg N, 23kg P, 180Kg K per hectare based upon nutrient removal through harvesting, maximum rates of NPK 40-20-30 for India are suggested (Chalapathi *etal*, 1997). Higher rates with split dressings, particularly for nitrogen, are also suggested (Colombus 1997, Oddone 1999).There appears to be no unusual requirements for minor elements and nutrient deficiency symptoms are typical

of other crops (Sheu 1987,Utumi 1999, Zhao 1985). There can be variable interactions between some nutrients at different availabilities (Filho and Malavolta 1997).

When using chemical fertilizers, a low nitrogen formula such as 6-24-24 is recommended in a split application during planting time and again in mid summer (Columbus, 1997). Use of Bio-fertilizers have shown positive interaction with an average dose of applied N in several field crops, with an average response equivalent to 15-20 kg. N. ha (Biswas, 2001). With increasing levels of N application up to 75kg N. ha. the contents of N application up to 75kg N. ha, the content N, P and K increase slightly. (Sharma and Tandon,1992) reported that among major nutrients high yielding varieties of medicinal plants have been found highly responsive to nitrogen fertilization.

It is concluded that in the present scientific approach, the stevioside percentage in Stevia was found to increase over that of absolute control, with the application of bio-fertilizer and chemical fertilizer applied alone and in combination. Vermicompost improved the soil aeration which promoted rapid plant growth. The soil's water retention capacity also improved as vermicompost contains absorbent organic matter. It is suggested that vermicompost is an excellent soil enhancer for the luxurious growth of plants and its application will be quite superior as compared to either its combination with inorganic fertilizer or as sole application of the chemical fertilizer.

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CYTOCHEMICAL ANALYSIS OF GASTROCNEMIUS MUSCLE OF FLUORIDE INTOXICATED ALBINO RAT

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ABSTRACT:

In the present study, cytochemical localization of glycogen in gastrocnemius muscles of rat exposed to different levels of sodium fluoride (NaF) for one month have been investigated. An elevated level of glycogen in the sarcoplasm and sarcolemma of hypertrophied and atrophied muscle fibres was observed in rats treated with 30 and 45 mg NaF/kg bw/day for two weeks. In rats exposed to 75 mg NaF/kg bw/day for two weeks, the intense localization of glycogen in disintegrated muscle fibres and perimysium as well as endomysium was seen. The muscle fibres of the animals treated with 30 mg NaF showed moderate staining reaction for glycogen as compared to control, while formation of angulated muscle fibres having abnormal sarcolemmal rim of staining was observed in rats dosed with with 45 mg NaF for one month. The sarcoplasm contained moderate amount of glycogen. The severely fluorosed muscle fibres exhibited unstained necrotic areas in angular muscle fibres with erosion of sarcolemma in rats treated with 75 mg NaF/kg bw/ day for one month. A very intense increase in glycogen concentration occured in sarcolemma and sarcoplasm of these muscle fibres.

Key words: *Albino rat, Gastrocnemius muscle, Glycogen, Sodium fluoride.*

INTRODUCTION

Fluoride even as a trace element can be found in varying accumulative quantities overtime through out the body. The specific roles that it plays are diverse. Prolonged ingestion of fluoride through drinking water, particular at high concentrations, leads to structural and biochemical alteratations, occurs due to fluorification of soft tissues (Shashi, 2003; Shashi *et al.*, 2007). Fluoride enhances the permeability of sarcolemma, and membrane of the muscle cell. In rabbit microscopic studies revealed that all components of muscle fibres including the actin and myosin are affected by fluoride (Kaul and Susheela, 1974; Shashi, 1989). The present study was undertaken to study cytochemical localization of glycogen in gastrocnemius muscles during fluoride intoxication.

MATERIALS AND METHODS

Sprague Dawley albino rats (100-300 g) were fed on pellet diet obtained from Hindustan Lever Ltd. Mumbai, India and water was supplied *ad libitum*. The rats were acclimatized for one week prior to the start of experiment. All the methods and protocols were approved by Ethical and Animal Use Committee of Punjabi University, Patiala, India.

Rats were injected with 30, 45, and 75 mg NaF / kg bw/day subcutaneously for two weeks and one month and control group was given only deionized double distilled water.The gastrocnemius muscles from various sites were excised, blotted free of blood and processed for histochemical localization of glycogen. Glycogenosis in gastrocnemius muscles was studied by using Best carmine's staining method (Best, 1955). The sections were studied under microscope and subsequently microphotographed.

RESULTS AND DISCUSSION

In control, the Type-I, intermediate and Type-II muscle fibers were well defined. The perimysial and endomysial connective tissue sheaths, peripheral, intermediate, and central region of both types of fibres exhibited the normal distribution of glycogen (Fig. 1).

In the animals treated with 30 mg NaF/kg bw/day for two weeks, the hypertrophied (Type-II) and atrophied (Type-I) muscle fibres exhibited increase in the concentration of glycogen in sarcolemma, and

sarcoplasm as compared to control. The intensity of glycogen was more towards the marginal regions. The hypertrophied (Type-II) muscle fibres have slightly intense distribution of glycogen as compared to atrophied (Type-I) fibres (Fig. 2).

All types of muscle fibres showed varying degrees of fusion. The atrophied (Type-I) and hypertrophied (Type-II) muscle fibres have moderate increase in intensity of glycogen as compared to rats treated with 30 mg NaF. The connective tissue sheaths and muscle fibres revealed increase in glycogen in rats exposed to 45 mg NaF/kg bw/ day for two weeks as compared to control. The central, peripheral, and intermediate regions of all types of fibres showed uniform distribution of glycogen (Fig. 3).

In rats exposed to 75 mg NaF/kg bw/day for two weeks, Type-II fibres became larger in size and have more splitting and degeneration than Type-I. The hypertrophied muscle fibres showed necrotic areas with intense localization of glycogen as compared to other fibres (Fig. 4). The intensity of glycogen has been more in the disintegration muscle fibres, connective tissue sheaths, and necrotic areas (Fig. 5).

In test rats treated with 30 mg NaF/kg bw/ day for one month, the perimysium and endomysium revealed intense color reaction. Type-II fibres showed invagination and infolded loops of the sarcolemma which have been deeply stained (Fig. 6). The microscopic examination exhibited distortion in both types of fibres but was more marked in Type-II. The majority of the fibres revealed moderate staining reaction for glycogen. The Type-II muscle fibres became angulated, split with sarcoplasmic masses, showing week staining pattern for glycogen (Fig. 7).

The gastrocnemius muscles of rat dosed with 45 mg NaF/kg bw/ day for one month, the hypertrophied (Type-II) and atrophied (Type-I) muscle fibre gets fused. There was formation of angulated muscle fibres. These muscle fibres showed an abnormal staining pattern and were having subsarcolemmal rim of staining. The sarcoplasm depicted moderate color reaction with Best's carmine for glycogen (Fig. 8).

In rats treated with 75 mg NaF/kg bw/ day for one month, the severely fluorosed muscle fibres showed unstained necrotic areas in angular muscle fibres with erosion of sarcolemma. A very intense increase in glycogen content occured in sarcolemma and sarcoplasm of these muscle fibres (Fig. 9).

In the present study, fluoride treatment for two weeks caused an enhancement in the level of glycogen in gastrocnemius muscle in fluoridated rats. The intense localization of glycogen in disintegrated muscle fibres and perimysium as well as endomysium was observed. The accumulation of glycogen probably resulted from its decreased utilization. The increase in fluoride level in muscle affects the synaptic transmission due to altered acetylcholine release or metabolism, cause change in muscle contraction which accounts for glycogen accumulation (Chinoy and Memon, 2001). The significant enhanced level of glycogen with a concomitant decrease in phosphorylase enzyme activity, could alter the contraction of quick contracting white muscle fibres (Chinoy *et al.*, 2004).

During present investigation, moderate quantities of glycogen in gastrocnemius muscle was seen in animals, which received one month fluoride treatment. There was formation of angulated muscle fibers having abnormal sarcolemmal rim of staining. These muscle fibres showed an abnormal staining pattern and were having subsarcolemmal rim of staining. The inhibition of succinate dehydrogenase, an oxidative enzyme involved in the kreb's cycle has been reported to be decreased in the gastrocnemius muscle of fluoride treated mice. This decrease affects conversion of succinate to fumerate and might cause a block in the Kreb's cycle (Vani and Reddy, 2000). Skeletal muscle is sensitive to fluoride- induced muscle cell damage in chronic fluoride toxicity resulting in functional changes with muscle ache and weakness, and alterations to the levels of succinate dehydrogenase (Li *et al.*, 1989). The toxicity of fluoride is due to the toxicity of the fluoride ion, a direct cellular poison that binds calcium and interfere with the activity of proteolytic and glycolytic enzymes. Fluoride inhibits oxygen consumption, blood clotting, diminishes erythrocytic glycolysis (McIvor, 1990) and causes depletion in mucosubstances (Shashi *et al.*, 2008).

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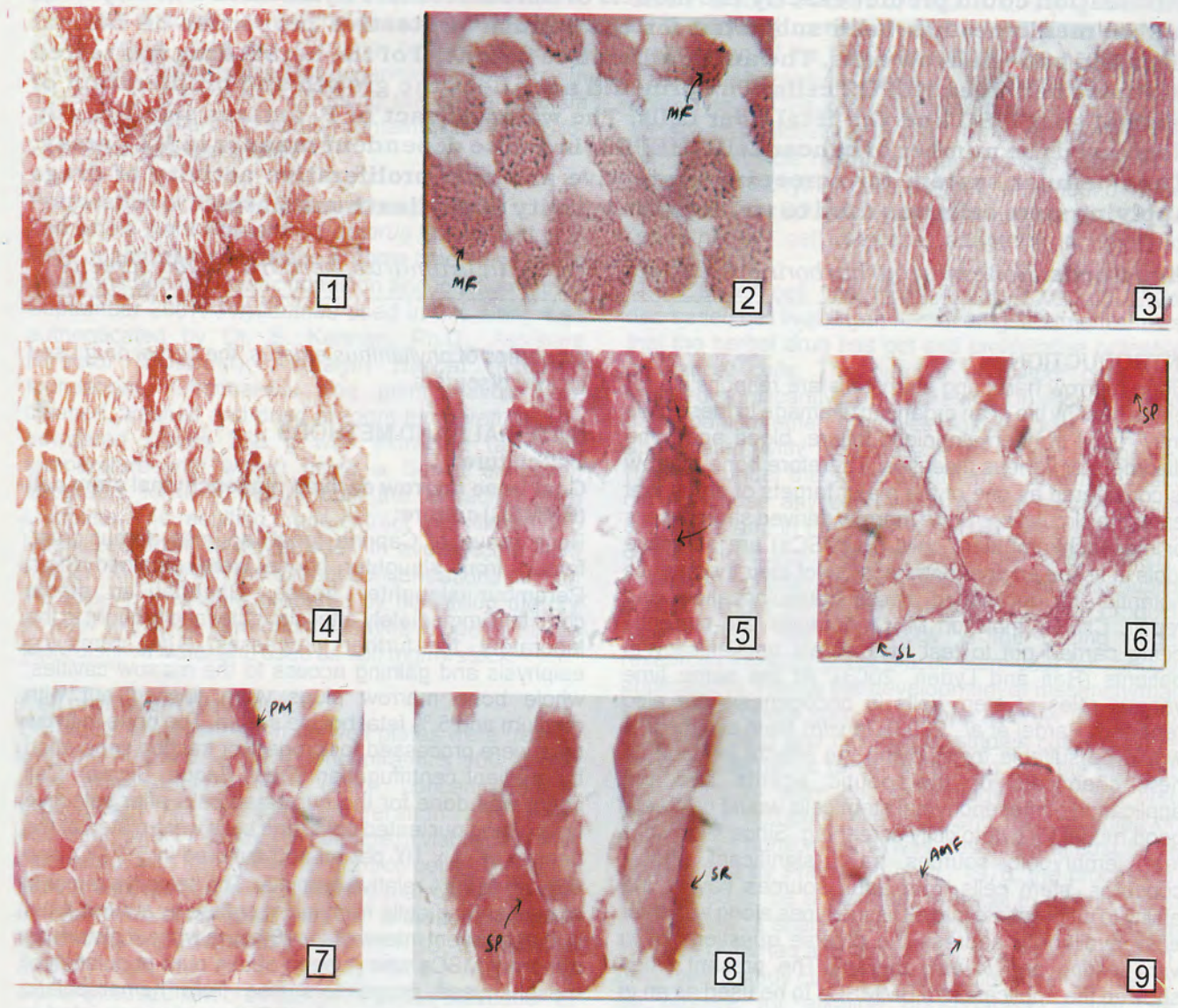
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Photomicrographs of the Best's caramine stained transverse sections of gastrocnemius muscle of rat treated with NaF for 15 and 30 days, showing distribution of glycogen. Fig. 1. Control (X 100); Fig. 2. Experimental rat treated with 30 mg NaF/Kg bw/day for 15 days (X 400); Fig. 3. Experimental rat treated with 45 mg NaF/Kg bw/day for 15 days (X 400); Fig. 4. Experimental rat treated with 75 mg NaF/Kg bw/day for 15 days ((X 100) ; Fig. 5. Experimental rat treated with 75 mg NaF/Kg bw/day for 15 days ((X 400) ; Fig. 6. Experimental rat treated with 30 mg NaF/Kg bw/day for 30 days (X 400).

Photomicrographs of the Best's caramine stained transverse sections of gastrocnemius muscle of rat treated with NaF for 15 and 30 days, showing distribution of glycogen. Fig. 7. Experimental rat treated with 30 mg NaF/Kg bw/day for 30 days (X 400) ; Fig. 8. Experimental rat treated with 45 mg NaF/Kg bw/ day for 30 days (X 400) ; Fig. 9. Experimental rat treated with 75 mg NaF/Kg bw/ day for 30 days (X 400).



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BONE MARROW DERIVED MESENCHYMAL STEM - CELL BIO - SCREENS FOR ANALYZING THE PROPERTIES OF *Phyllanthus amarus*

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ABSTRACT:

In vitro assays have been used to test the drugs before clinical recommendations. In vitro stem cell models would be suitable for studying the effects induced by new chemicals or therapeutic agents and the application of mesenchymal stem cells would be a very good model for cytotoxicity screening as bone marrow derived MSC on mitogenic stimulation could predict exactly the models of fibrosarcoma. Phyllanthus a widely used herbal medicine, has been subjected for the cellular protective properties on normal liver and bone marrow cells. The antiproliferative potential of the herbal drug was tested in tumor cell lines HEPG2 cells. The drug did not affect the growth and proliferation of normal goat MSC and the fetal liver cells. The water extract of P. amarus significantly decreased the number of cancer cells (HEPG2) in a dose dependent manner in MTT assay. Thus cellular system for screening protective and anti proliferative activity of drugs applying stem cells was used to validate the activity of phyllanthus amarus.

Key words: *Bone marrow harboring stem cells, Phyllanthus amarus, herbal drug, bioscreens*

INTRODUCTION

Bone marrow harboring stem cells are responsible for entire healthy life of an organism. Damage to these cells may result in haematopoietic failure, blood and bone disorders or various diseases. Therefore bone marrow is considered as one of the major targets of drugs that enter the circulation. Bone marrow derived stromal cells or mesenchyaml stem cells (BMMSCs) are attractive tools in tissue engineering because of their therapeutic potential for promoting tissue vascularization and repair. Clinical trials on MSC therapies are currently being carried out to test their safety and efficacy in patients (Rafi and Lyden, 2003). At the same time various roles of stem cells in oncogenesis are also reported (Pardel *et al.*, 2003). *In vitro* stem cell models would be suitable for studying the effects induced by new chemicals or therapeutic agents and the application of mesenchymal stem cells would be a very good model for cytotoxicity screening. Since stem cells from embryonic sources have significant ethical concerns, stem cells from adult sources having the ability to differentiate into all tissue types along with their easy availability address the diverse questions of *in vitro* testing and *in vivo* therapy The present study showed the application of BMMSCs to be used as an *in vitro* bio screen to analyse the cellular protective properties of *phyllanthus amarus*, the herbal drug used for liver disorders.

MATERIALS AND METHODS

Cell culture:

Goat bone marrow derived mesenchymal stem cell (BMMSC) culture:

Bone tissue of Caprine (goat) sources (femur crust, femur) from slaughter house were obtained (from Perambur slaughter house, Chennai) in sterile condition immediately after slaughter and brought to the laboratory for further processes. After removing epiphysis and gaining access to the marrow cavities, whole bone marrow plugs were flushed out with medium and 5 % fetal bovine serum. The bone marrow cells were processed for progenitor fraction separation by gradient centrifugation (Ficoll Paque, Sigma). Cell count was done for the nucleated cells after selective lysis of non nucleated cells, and cells were plated at the density of 1×10^6 per ml and cultured in 5% CO_2 and 95% air at 99% relative humidity. The selective removal of suspending cells from initial culture in around 4 hrs and at frequent intervals for 48 hours without disturbing adherent MSCs was done to obtain pure population of mesenchyaml progenitors free from hematopoietic cells. Colony forming units (CFU) were appreciated in

around 7-10 days and the cells attained confluency in around 21 days of initial culture. These cells were trypsinized and replated with a split ratio of 1:3. The third passage cells were used for analysis. The cells were stained with May - Grunwald Giemsa stains and analyzed using light microscope (Nikon, Japan).

The mesenchymal progenitor cells were confirmed by morphology, cell surface marker analysis (negative selection for CD 14 and CD 45 cell surface antigens by immunofluorescence and histochemical analysis for endogenous alkaline phosphatase activity (ALP Kit, Sigma).The cancer cell line used in this study was HePG2, a human liver cell line obtained from National Institute for Cell Sciences, Pune and were maintained. (Minimum Essential Medium (MEM) with Earle's BSS adjusted to contain 1.5g/L sodium bicarbonate, 0.1 mM non – essential amino acids, and 1.0 mM sodium pyruvate, with 10% fetal calf serum). Ovine embryonic liver cells cultured as per the protocols of Freshney, (2006) were used for studying cellular protective principles. The cells used for experiments were growth arrested at sub-confluence using the same medium with restricted serum condition containing 0.1% FBS for 24 hrs prior to experiments.

Preparation of *Phyllanthus amarus* extract:

The whole plant *Phyllanthus amarus* was collected in large quantities for extraction at same time, to minimize variations due to climatic condition and location during September 2007. *Phyllanthus* used in this study was authenticated by Dr. S. Kannan, Ph.D., Assistant Director (Botanist), Asthagiri Herbal research foundations, Chennai-59. The plant leaves were cleaned, washed and dried at room temperature. The powdered leaves 250 g was extracted (at room temperature with water) using a Soxlet extraction apparatus for 8-10 hours until the extraction was complete. The resulting crude extract was filtered and lyophilized down to dry powder. In average the yield was 27.10 % (w/w). The extract concentrations used in the experiments were prepared by dissolving the dry powder extracted from original leaves in one ml sterile triple distilled water and used as 100 mg/ml stock.

Cell proliferation studies:

BMMSCs were seeded in 96 well plates were treated with the herbal drug from concentrations 100 to 600 g/ml from stock solution (Each concentration in triplicates). The cellular proliferation has been assessed by MTT assay. Percentage of viable cells was calculated with reference to control cells. The drug activity was studied in normal bone marrow MSCs and in fetal liver cells. Cells with or without *P. amarus* treatments were washed once with PBS, followed by adding 0.5 ml of 0.05 mg/ml 3-[4,5]-2, 5 diphenyltetrazolium bromide (MTT). After incubation at 37°C for 4 hrs, medium was removed and the colored precipitate formed by the cleavage of MTT in living cells was solubilized in 0.5 ml DMSO. The cell survival and background was determined by absorbance at 570 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Initial approaches to enrich primary MSC cultures utilized spatial separation of hematopoietic cells from marrow stromal cells within liquid suspension culture systems resulted in typical spindle morphological cells, adhering to the culture surface. The initial pure population (3-5 days) culture were glistening round cells starts adhering to the plastic surface and changing morphology to flattened spindle cellular phenotypes tend to form colonies (Colony Forming Units-CFUs). These cells assume confluent fibroblastic morphology cells in around 21 days of initial culture (Fig 1A). The CFU forming potential is an indication of the proliferation of the MSCs in culture. The number and size of the colonies found to be increasing during culture days. Cultured cells were positive for alkaline phosphatase activity (Fig 1B). The pure population of confluent spindle morphological cells was analyzed to be of mesenchymal cells as they were found to be negative for CD 45 (hematopoietic) and CD 14 (endothelial) cell surface antigens in immuno fluorescence.

The Cancer cell line HEPG2 was found to show decreased cell livability in herbal drug treatment shows that the herbal drug has got anti proliferative potential on cancer cells. The number of cancer cells was reduced significantly by herbal extract in dose and time dependant manner. Complete loss of cells could be appreciated with high dosage or longer exposure. However under the same treatment *P. amarus* extract did not cause any cell loss in normal mesenchymal stem cells and in the ovine fetal liver cells, showed its cellular protective property on normal cells (Table1). There are societal issues as well as increasing public concern about the use of animals for testing product safety. Stem cell technology is the cutting edge research offering highest plastic stem cells for various applications. Hence the development of mesenchymal stem cell models from adult bone marrow for *in vitro* drug screening and cytotoxicity studies could provide the best alternative to animal testing with the efficient application of stem cell technology. Since the first isolation of fibroblast like cells and their colonies of MSCs from bone marrow was described almost for a decade ago by Frienstein *et al.*, 1976, the process is considered to be a standard protocol to isolate bone marrow MSCs shared similar morphology and cultural features (Aldehaq and Mao,2004), the cells obtained in the present study colony forming units were reported to be positive for alkaline phosphatase activity, as

reported earlier (Mangalgowri, 2003; Mrugala *et al.*, 2008).

The supplementation of different concentration herbal extract had an inhibitory activity on the growth of HEPG2 cells and reduced stimulation index (S.I) was observed in drug supplemented cells. As stated by Simoes *et al.*, (1999) assessment of cytotoxicity is clearly an important part of the evaluation of potential antiproliferative activity in a compound. The cytotoxicity evaluation *in vitro* has been done by cell viability assays, such as the uptake of dye by dead cells after breakdown of the cellular permeability barrier or by mitochondrial function (MTT assay). These endpoints have been established for many years and in many cell types (Vlietinck *et al.*, 1997; Eisenbrand *et al.*, 2002). The assay has been used to test various concentrations of a drug, which affects the end point response by 50 % (EC 50) and is widely used to rank the test compound. In the present study culture system has been used to analyze both the cellular protective properties and anti proliferative potential of *Phyllanthus amarus*. This preliminary report also demonstrated the antiproliferative activity of herbal extract of *Phyllanthus amarus* and supports the dietary use of *Phyllanthus amarus* as a supplement for cancer prophylaxis. The results indicated that the drug has cellular protective properties on liver and bone marrow cells.

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Table 1 Effect of different concentrations of Cyclosporin A on the proliferation of spleen cells

S. No.	Concentration of Drug extract in µg / ml	Mean SI ± SE (MTT ASSAY)	F value
control	0	$0.601^{c} \pm 0.38$	18.69 **
MSC	50	$0.729^{b} \pm 0.31$	
MSC	100	$0.601^{c} \pm 0.38$	
Fetal liver cells	50	$0.838^{d} \pm 0.28$	
Fetal liver cells	50	$0.938^{d} \pm 0.28$	
HEPG2 CELLS	50	$0.495^{e} \pm 0.35$	
HEPG2 CELLS	100	0.328± 0.95	
HEPG2 CELLS	200	0.238± 0.55	

Mean values with different superscripts differ significantly ($P < 0.01$).

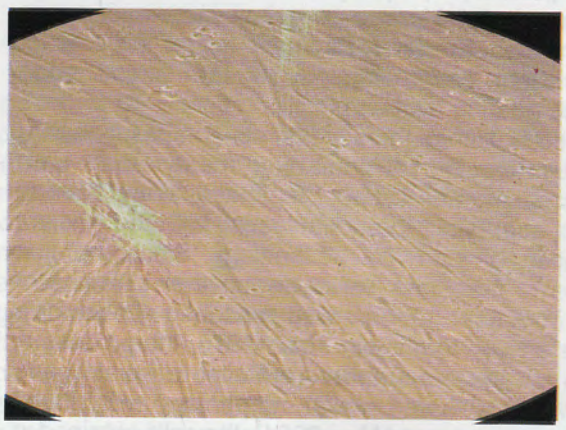


Fig1(A)Showing cells having confluent fibroblastic morphology.

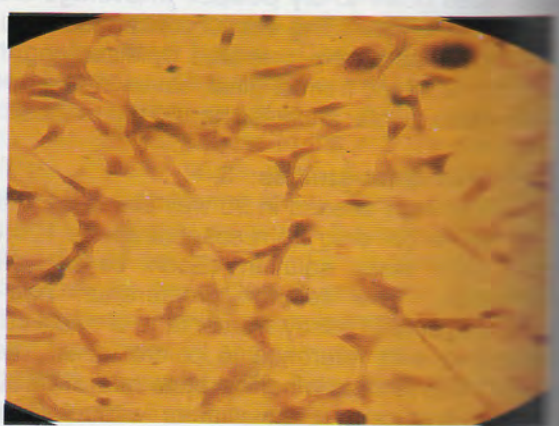


Fig1(B)Showing cells having positive alkaline phosphatase activity.

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TROPHIC STATUS OF AMBADI DAM IN RELATION TO PHYSICO-CHEMICAL CHARACTERISTICS OF ITS WATER

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ABSTRACT:

Dams and rivers are the lifeline of the rural peoples, for their drinking purpose, irrigation, fisheries and other agricultural activities. In the present study, an attempt has been made to record the trophic status of the Ambadi Dam. During the study period various correlations were recorded for different parameters. The dam shows average gross primary production of 396.08 gC/l/day and net primary production of 119.90 gC/l/day. The values of dissolved oxygen ranged from 5.30 to 6.60 mg/l, while Carbon dioxide in the dam water ranged from 1.00 to 8.00 mg/l. organic matter present in the sediments originated from the runoff matter and in associated with anthropogenic activities is the source of nutrients of dam water. Average concentration of nitrates and phosphates were recorded 0.13 mg/l and 14.72 mg/l respectively. Dam water shows well alkaline pH. Total dissolved solids in water of dam showed peak value of 1040.00 mg/l. microbial population in the soil sediments and in water, with abundance of phytoplankton showed the mesotrophic nature of Ambadi dam.

Key words: *Trophic status of Dam, Phosphates, Nitrates, Microbes,*

INTRODUCTION

The total environment is a complex entity, of which water is an essential component for the survival of living beings. In aquatic environment life is largely governed by physicochemical characteristics and their stability, which intern have enabled biota to develop many adaptations that improve sustained productivity and regulate the metabolism in water body. Trophic status of an ecosystem depends upon rate of energy flow which may be assessed by estimating primary production. The most characteristic criterion to assess the trophic structure of a water body still remains to be primary productivity as the planktons form basic link of food chain for all aquatic animals, thus playing a key role in its stability, (Zafar, 1964 and Hart and Zabbey, 2005).

Productivity means living substance producing capacity of a water body, lake and ponds are having self sustaining ecosystems. Living components of any ecosystem consists of producers and consumers, phytoplankton and zooplanktons depend on each other and they form a link of a food chain. The productivity of a ecosystem depends upon the available standing crops, rate of removal and rate of production. The gross primary productivity (GPP) is a rate of photosynthesis and includes the organic matter in the respiration during ;sthe measurement period. The net primary productivity (NPP) is the rate of storage of organic matter in plant tissues in excess of the respiratory utilization by the producers during the period of measurement, (Hart and Zabbey, 2005).
Meena *et al.*, 2007).

MATERIALS AND METHODS

Ambadi irrigation dam is located at 770 – 05' – 45" North and 210 – 12' – 15" East, 14 Kms, away from the Akot, district Akola (M.S.), in central India. The dam is situated in Satpuda hill ranges and 246 meters above the sea level. The dam is mostly utilized by the locality for irrigation, fisheries, water consumption by cattle's and some human activities are also observed on the bank of dam, such as bathing, swimming, cattle washing, cloth washing, vehicle washing etc. During rainy season the surface runoff water carries decaying organic matter with eroded soil from the catchments area, in to the dam water. The frequency of visiting the cattle's and human activities on the bank of dam increases during the summer days.

The water samples were collected by Mayer's sampler, monthly by various depths. Five sampling stations I, II, III, IV and V were selected for the sampling of water. Station I is the inlet water area of the dam. Station II is characterized by the frequency of human domestic activities. At station III the localities perform fishing activities, station IV is the center of dam and station V is the part of dam where human as well as animal activities both are seen. Various physical and chemical parameters were estimated by the standard methods of APHA, 1985.

RESULTS AND DISCUSSION

S.No.	PARAMETERS	RANGES	AVERAGE
1	Ambient temperature (0C)	21.6 to 33.6	26.35
2	Water temperature (0C)	20.02 to 31.34	24.17
3	Turbidity (NTU)	21.90 to 65.80	40.31
4	Total dissolved solids (mg/l)	40.00 to 1040	315.40
5	Hydrogen ion concentration	6.18 to 7.90	7.20
6	Dissolved oxygen (mg/l)	5.30 to 9.60	8.01
7	Free carbon dioxide (mg/l)	0.00 to 8.00	2.86
8	Total alkalinity (mg/l)	24.00 to 244.00	92.11
9	Total hardness (mg/l)	68.00 to 234.00	159.66
10	Chlorides (mg/l)	8.99 to 36.03	18.33
11	Nitrates (mg/l)	0.19 to 7.00	0.88
12	Phosphates (mg/l)	0.02 to 0.31	0.13
13	Sulphates (mg/l)	4.50 to 40.00	14.72
14	GPP (gC/l/day)	112.50 to 806.25	396.08
15	NPP (gC/l/day)	7.50 to 652.50	119.90
16	Faecal coliform (org/100 ml)	100 to 2410	415.00
17	Soil microbes (org./gm)	100 to 4100	1388.86
18	Water microbes (org/100ml)	110 to 3200	614.43
19	Total phytoplanktons (unit #)	140 to 5110	1586.71
20	Total zooplanktons (unit/l)	140 to 2770	918.71

The data harvested during the study period indicates the marked variation in different water quality characteristics, (Hart and Zabbey, 2005). Temperature is the controlling factor for quality and quantity of planktonic flora and fauna and also for some water quality parameters. Temperature of dam water showed variation from 20.020Cto 31.340C. however, temperature showed positive correlation with gross primary production and negative relationship with that of dissolved oxygen (Khali and Mac'Donald, 1975). The values of turbidity ranged from 21.90 NTU to 65.80NTU. statistical data indicates the negative relationship of turbidity with gross and net primary production and dissolved oxygen, chlorides and total hardness contents of dam water. Present findings corroborate with that of Forel, (1982) Ambadi dam water showed well alkaline nature and has been found to have average pH of 7.20. Increase of hydrogen ion concentration may be due to phytoplanktonic biomass with inorganic deposits at the bottom of dam . the data during the study period showed negative correlation of pH with nitrates, phosphates and net primary production, (Verma and Shaura, 1970). The solubility and availability of nutrients affects the oxygen content of water and therefore the productivity of aquatic ecosystem.

Dissolved oxygen content of dam water varied between 5.30 to 9.60 mg/l. the lower values of dissolved oxygen is sthe sigh of organic pollution in the dam water, (Nair *et al.*, 1988). However, the dissolved oxygen in dam water showed positive correlation with gross and net primary production of the dam.

Toia! alkalinity is mainly contributed by bicarbonates present in the water. Dissociation of bicarbonates and rate of consumption of carbonate and bicarbonate ions by the aquatic flora, imparts the alkalinity to the dam water. In dam water alkalinity ranged from 24.00 mg/l to

244.00 mg/l. it shows significant correlation with sulphate contents of water and positive relation ship with gross and net primary production, (Davis, 1955). Organic wastes of animals contains high chloride contents than the pollutants originated from the human domestic activities. Chloride concentration in dam water varied from 8.99 mg/l to 36.03 mg/l, (Pawar *et al.*, 2001). Total dissolved solids showed the peak values of 1040 mg/l, that elevates the density of water and influences the osmoregulatory processes of the aquatic fauna. It shows positive relationship with alkalinity, pH and hardness of water.

The salts present in dam water and the organic and inortganic pollutants are the sources of phosphate concentration. In absence of less concentration of phosphates in water sediments acts as a limiting factor for the phytoplanktons, where as the increased concentration causes eutrophication, (Meena *et al.*, 2007). Average phosphate concentration recorded in ambadi dam is 0.13 mg/l. Nitrogen contents in water ranged minimum 0.19 mg/l to 7.00 mg/l. the main source of nitrogen in the dam is the organic wastes originated from runoff water, animal wastes, human domestic activities on the bank of dam. Nitrogen content in dam water showed positive correlation with that of net primary production, (Munawar, 1970).

Organic pollutants contributes the sulphate contents of the water, hence the dam water is found to be rich in sulphates contents and having averagte value of 14.72 mg/l. during the study period sulphate contents showed positive correlation with net primary production.

The overall gross primary production in Amvadi dam ranged from 112.50 gC/l/day to 806 gC/l/day with average value of 396.08 gC/l/day. The peak values of GPP observed in the month of November at station – I, in the month of December at station – II, in the month of February at station – III and during January at station –IV and in May at station –V. The lower ranges were observed in the months of December, June and July, September and June at ssampling stations I,II,III,IV and V repectively. NPP of dam ranged from 37.50 to 300 gC/l/day at station I, 26.25 to 652.50 gC/l/day at station II, 30.00 to 378.75 gC/l/day at station III, 7.50 to 270 gC/l/day at station IV, and 30.00 to 300.00 gC/l/day at station V. the present trophic production in dam water is significant and shows moderate eutrophication rate. Hunter (1970) has attempted to catagorise the standing water body in to Ultra oligotrophic, Oligotrophic, Mesotrophic, Eutrophic and Hypertrophic, having mean primary production range of < 50,50-250, 300-1000, 1000-10000 and >10000 respectively. As per this classification the present water body may designated as mesotrophic water body.

During the present study it is evident that the organic wastes originated from anthropogenic activities as well as from the surface runoff in the dam water during summer and rains respectively, increase the nutrient load to the dam water and sediments. These nutrients are least available to the aquatic flora for its growth and development during the low light intensity and low temperature of winter months. However in summer n months, the increased water temperature of enhances the rate of microbial degradation organic matter and release of nutrients from dam sediments in to the water. Excessive amount of nutrients and more light intensity, which, intern increase the primary productivity of the dam. The results obtained in the present study are in corroboration with the findings of Meena, 2007). It is also evident from the present investigation that, the Ambadi dam has a good primary productivity and can be used as a resource for the development of fisheries and other aqua cultural practices for the betterment of locality, as has been shown by several workers like Ali (2000) using biomass management practices for improved aquaculture production

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BIOSCIENCE BIOTECHNOLOGY RESEARCH COMMUNICATIONS

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BLOOD SUGAR AND LIPID PROFILE CHANGES DURING PREGNANCY

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ABSTRACT:

High Lipid profile level is a major factor behind atherosclerosis which may lead to unconsciousness. To avoid such problems during pregnancy study was done to investigate the effect of pregnancy on blood sugar and lipid profile changes during all three trimesters. Blood sugar and lipid profile changes were measured in 35 cases during each trimester of pregnancy and in control group of 30 cases of non pregnant women. The study proves that blood sugar level in pregnant woman is within normal range but it is towards lower side. Lipid profile, total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol were found to increase from trimester to trimester while high density lipoprotein cholesterol decreased as compared to control group. The results reveal metabolic changes in blood sugar and lipid profile of pregnant women of Akola region.

Key words: *Blood Sugar, Lipid Profile, Pregnancy.*

INTRODUCTION

Pregnancy is a new sequence of events after fertilization of ovum and is accompanied by changes in maternal lipoprotein and sugar metabolism which may serve for satisfying the fetuses nutritional demands. (Landazuri *et al.,* 2006). Lipid profile changes during pregnancy are a result of physiological adaptation to the state of pregnancy. There are increases in the blood concentrations of cholesterol, triglycerides, low density lipoprotein, VLDL cholesterol and decreases in high density lipoprotein cholesterol. (Husain *et al.,* 2006) The signs of hypoglycemia in pregnancy do not vary much from normal symptoms namely shaking profuse sweating, nervous and anxiety attacks, intense hunger, dizziness, extreme headache, change in vision and sudden irritability. In pregnant woman's blood sugar level is within the normal range but it is towards the lower side, because their bodies have increased energy requirements which are supplied by stored glucose much faster than a normal person. The main objective of this study was to investigate whether the sugar and lipid profiles during pregnancy differ from non-pregnant women of similar reproductive life. The study is based upon the effect of pregnancy on serum lipid profile and sugar metabolism so that steps may be minimized to solve cardiovascular, hypoglycemia and other complications, which may help to promote and preserve women's reproductive health.

MATERIALS AND METHODS

A total number of 35 cases of 1st, 2nd and 3rd trimester of pregnancy were participated as study group in the age between 24-35 yrs. 30 cases of non-pregnant women matched on reproductive age were considered as control group.

Fasting blood samples were collected from all subjects and biochemical analysis of serum was carried out for estimation of total cholesterol, HDL cholesterol, Triglycerides, LDL cholesterol and VLDL cholesterol and blood sugar by using kit method in the department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola. In addition to this post meal blood samples were also collected for the estimation of blood sugar level. Following kits were used for the estimation of

1. For blood sugar GOD-POD method (Autospan kit)
2. For Lipid profile :

Total cholesterol –CHOD-POD method (Life chem.kit)

HDL cholesterol –CHOD-POD method (Life chem.kit)

Triglyceride –GPO/POD method (Liquizyme kit)

RESULTS AND DISCUSSION:

The 35 cases with 1st, 2nd and 3rd trimester of pregnancy and 30 cases matched on reproductive age were taken in the present study. Data were expressed as mean in tables. All the results were expressed in mg/dl.

Table: Mean of total cholesterol, HDL, triglyceride, LDL, VLDL, blood sugar fasting and post meal levels in pregnant women aged between 24-35years in the first, second and third trimester against control group of same age.

Tests used	control group	1st trimester	2nd trimester	3rd trimester	Normal values
Total cholesterol	158.2	176.7	249.1	255	130-250
HDL cholesterol	46.8	40.6	35.8	29.6	36-60
Triglyceride	102.9	148.6	171.4	198.8	60-170
LDL cholesterol	91.4	104.5	185.9	191.7	< 130
VLDL cholesterol	19.8	28.9	34.2	39.7	2-38
BSL fasting	95.1	74.6	73.1	73	65-110
BSL post meal	103	85.3	81.2	83	< 120

Unit:mg/dl

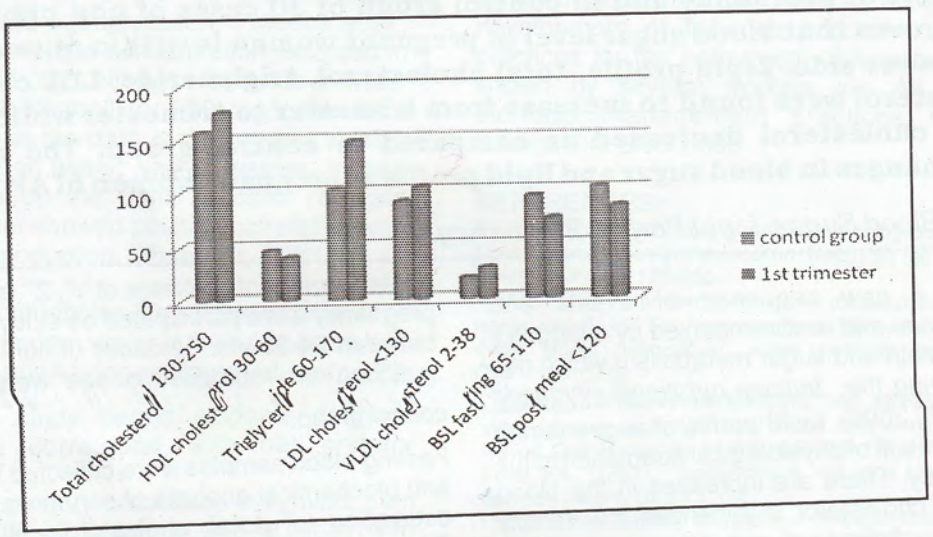


Figure 1 :

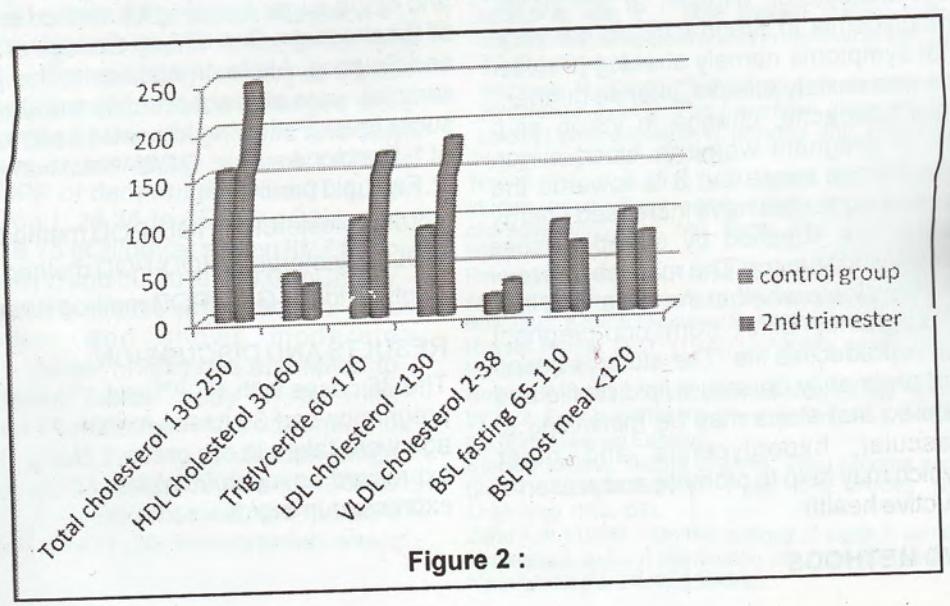


Figure 2 :

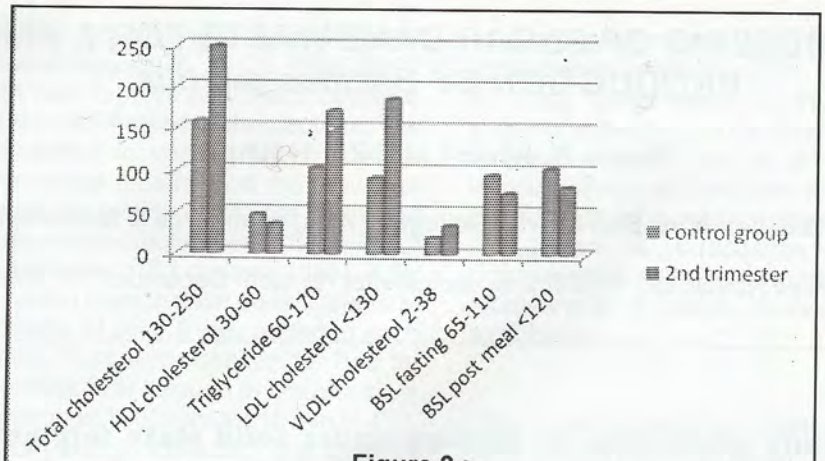


Figure 2 :

The above figures show that mean value of total cholesterol, HDL, LDL, VLDL,and Triglyceride were found to be 158.2 mg/dl, 46.8 mg/ds, 91.4 mg/dl, 19.8 mg/dl, 102.9 mg/dl respectively and blood sugar fasting and post meal were found to be 95.1 mg/dl & 103.0 mg/dl respectively.

The findings of the present study showed that there is progressive increase in concentration of total cholesterol, triglyceride, LDL, VLDL with slight decrease in HDL cholesterol during the course of 2nd trimester of the pregnancy, there was significant increase and decrease during the course of 3rd trimester of pregnancy as compared to the controls.

It is also shown that total cholesterol was about 11.6%, 58% and 61% in 1st, 2nd and 3rd trimesters of pregnancy respectively. Triglyceride showed 44%, 66% and 98% in 1st, 2nd and 3rd trimester respectively LDL cholesterol showed 14%, 85% and 95% in 1st, 2nd and 3rd trimester respectively. VLDL cholesterol showed 45%, 72% and 90% in 1st, 2nd and 3rd trimester respectively. HDL cholesterol showed about 86%, 76% and 63% in 1st, 2nd and 3rd trimester respectively. Besides this BSL fasting showed 78%, 76% and 75% and BSL post meal showed 80%, 78% 76% in 1st, 2nd and 3rd trimester respectively.

On the analyses of about 300 pregnant women during 1st, 2nd and 3rd trimester all aged 35 years it was concluded that an altered lipid profile during trimester, increased the risk of atherosclerosis, restricted fetal growth and risk of uncomplicated pregnancy. (Harvey and Champ, 1994 and Takahashi *et al.,* 2008).

It is difficult to explain now pregnancy hormones increased the serum total cholesterol level. Estrogen seems to be responsible for most of the alternation in lipoprotein metabolism during pregnancy. Progesteron has been to shown to oppose the action estrogens on lipoprotein metabolism leading to increased concentration of LDL cholesterol and decreased concentration of HDL cholesterol. (Lippy, 1998 and Husain *et al.,* 2006).

Besides this, above study shows that blood sugar level during pregnancy tends towards the lower side of the normal range, there may be chance of decrease blood sugar level below normal range and may create many problems such as hypoglycemia. Hypoglycemia can occur as a side effect of diabetes mellitus, as a condition itself (reactive or fasting), or through the other illness that disrupt the body's natural insulin production and management. The findings of the present study prove that energy requirements increase during pregnancy that may lead to hypoglycemia in pregnant women. Lipid profile also gets disturb during pregnancy that may result into heart attack, generally gynecologists do not refer to check lipid profile during pregnancy, to overcome critical problem of hypoglycemia and heart during pregnancy Gynecologists should suggest to do sugar and lipid profile at proper stage of pregnancy as well as advicse them for suitable intake of diet.

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BIO-PROCESSING OF SUGAR CANE WASTE FOR A AMYLASE PRODUCTION BY *Bacillus subtilis.*

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ABSTRACT:

In the present study production of amylase under solid state fermentation (SSF) by Bacillus subtilis utilizing sugar cane waste as carbon source has been investigated. Enzyme production parameter was optimized by varying different carbon source concentration, incubation period, pH, temperature and nitrogen source. 20 gms of sugar cane waste in the inoculums media yielded maximum enzyme production.Maximum enzyme production was also observed after 72 hours which decreased with further incubation. Optimum pH and temperature of the culture medium for enzyme production was 7 and 35°C respectively. Nitrogen source such as urea, peptone and casein were added to the media however supplementation with chickpea peel (as nitrogen source) enhanced the amylase production optimally.

Key words: *a-amylase, Bacillus subtilis, Sugar cane waste, Chickpea peel.*

INTRODUCTION

Alpha amylase is a hydrolytic enzyme and in recent years interest in its microbial production has increased due to wide spread use in food, textile, baking and detergent industries (Asghar *et al.*,2000, 2002). Besides its use in saccharification and liquefaction of starch, the enzyme is also use for the warp sizing of the textile fibers, clarification of haze formed in beer and fruit juices and for the pretreatment of the animal feed to improve the digestibility (Stedt, 1988). A growing new area of the application of α-amylase is in the field of laundry and dish washing detergents (Van der Maarel *et.al*, 2002). Asia has the largest production of sugar cane as it is good source of carbon. This paper reports the optimization of fermentation parameters for α-amylase production by *Bacillus subtilis* through SSF of sugar cane waste.

MATERIALS AND METHODS

Pure culture of *Bacillus subtilis* was obtained from the department of microbiology of Shri Shivaji College, Akola, which was maintained at nutrient agar starch at pH 7 and 35°C temperature, in the present investigation sugar cane waste was used as a carbon source and it was obtained from the markets of Akola City. After this it was crushed into powder form with the help of grinder.

The spores of the *Bacillus subtilis* were transferred aseptically to a 500 ml conical flask containing 100 ml of the presterilised inoculums medium containing g/100 ml, glucose 2, yeast 0.3, peptone 0.5, NaCl 1.5, Na2HPO4 2H2O 1.1,NaH2PO2 2H2O 0.61, KCl 0.3, MgSO4 7H2o 0.01, in laminar air flow. The flasks were then kept it in shaker at 37° C for 24 hr. All the treatments were done in duplicate. The pH of the sugar cane waste was adjusted to 7 with the help of HCl/NaOH and sterilized it in autoclave for 15 min at 121°C. After cooling; inoculums were added into the flask in the laminar flow with the help of sterilized measuring cylinder. The flasks were then incubated for 24 hour at 35° C in incubator. The SSF media flasks were gently shaken after every 12 hour for uniform mixing of Carbon source, and microorganism. The growth medium of sugar cane waste was fermented with *Bacillus subtilis* for the optimization of different parameters for the α–amylase production. Growth medium containing 10 gm of sugar cane waste were incubated for (24-96 h) at pH 7 and 35° C temperature. Conical flasks containing different carbon source level (5-25 gm) were inoculated in (25 ml.) and incubated for 72 h at pH 7 and 35° C temperature. pH of 10 gm of sugar cane waste was adjusted at different levels- 4, 5, 6, 7 and 8 before inoculation and incubation for 72 h. Sugar cane wastes

(10 gm) were inoculated (25 ml) and incubated at pH 7 under different temperatures viz 25, 30, 35, 40° C for 72 h. The SSF medium was supplemented with chickpea peel (as a nitrogen source) instead of the peptone. The chickpea peel was added in varying concentrations (0.05 – 0.25) to investigate its effect on the growth of *B.subtilis* and enzyme production under pre-optimized conditions. α-amylase was extracted from SSF medium by a simple method (Ramesh & Lonsane 1989). After specific incubation period in each case equal volume of sodium phosphate buffer of pH 6.9 was added to each experimental flask the flask were shaken for half an hour and the material was filter through the muslin cloth the filtrate was centrifuge at 1000 rpm for 10 min at 4°C. The supernatant was carefully collected and used as crude enzyme extract. α-amylase activity was determined by the spectrophotometric method as described in standard texts where the mixture containing enzyme extract, starch as substrate and DNS as a coupling reagent, as well as reducing agent was taken and it was reduced into 3, amino 5 nitrosalisylic acid as its colour changed from yellow to orange. Absorbance was estimated at 540 nm.

RESULTS AND DISCUSSION

Different fermentation parameters were optimized for the α-amylase production by conducting a series of experiment and the results have been discussed as under. Duplicate flask containing 10 gm of sugar cane waste autoclaved and incubated for 24, 48, 72 and 96 h the maximum activity of α-amylase (0.24 mg/ml maltose) was found after 72 hour of SSF at pH 7 and 35° C temperature. Similarly Bajpai *et.al.*, (1992) had produced α-amylase by *B. licheniformis* 44 MG 82-G, using glucose as a carbon source and optimum enzyme activity of culture. Medium was recorded after 96 h. Fermentation media containing 5,10, 15, 20 and 25 gm of sugar cane waste were sterilized, inoculated and incubated for at pH 7 and 35° C. It was observed 20 gm of sugar cane waste in the fermentation medium yielding maximum (0.5 mg/ml maltose), (fig. 1) α-amylase after 72 hour at pH 7 and 35° C. A further increase does not increased the enzyme concentration because 25 ml inoculum was added in each flask and increased in substrate level. Krishna and Chandrasekaran (1996) reported maximum α-amylase producing by *B. subtilis* utilizing 10 gm of banana stalk as substrate.

Data showing the effect of pH on α-amylase production by *B. subtilis* in SSF of sugar cane waste are presented in fig. 2. The maximum activity of α-amylase (0.25 mg/ml maltose) was observed in the fermentation medium adjust at pH 7. At pH 4, activity was law due to more acid but as the pH increase to 7 tne enzyme induction increases our results are similar to those of Terai (1973), who reported 6.8 as optimum pH for the production of α-amylase by *B. subtilis. Bacillus subtilis* showed maximum activity of α-amylase (0.25 mg/ml maltose) the sugar cane waste medium (10 gm) when incubated at 35° C for 72 h at pH 7. A decrease or increase in temperature caused decrease in the enzyme production by *B. subtilis* fig. 3. The optimum temperature observed for α-amylase production from Banana stalk using *B.subtilis* was also 35° C as reported by the Krishna & Chandrasekaran (1996).

The growth medium was supplemented with chickpea peel (as a nitrogen source) instead of peptone. The chickpea peel was added in varying concentration (0.05 – 0.25 gm in 25 ml). The result showing maximum activity of α-amylase (0.48 mg/ml maltose) was observed in the fermentation medium containing 0.25 gm chickpea peel (table-1).

Table – 1: Effect of varying concentrations of Nitrogen Source on α-amylase production by *Bacillus subtilis*

Sr.No.	Nitrogen source in gm (chickpea peel)	α-amylase activity in term of product mg/ml.
1	0.05	0.20
2	0.10	0.30
3	0.15	0.34
4	0.20	0.45
5	0.25	0.48

Fig. 1

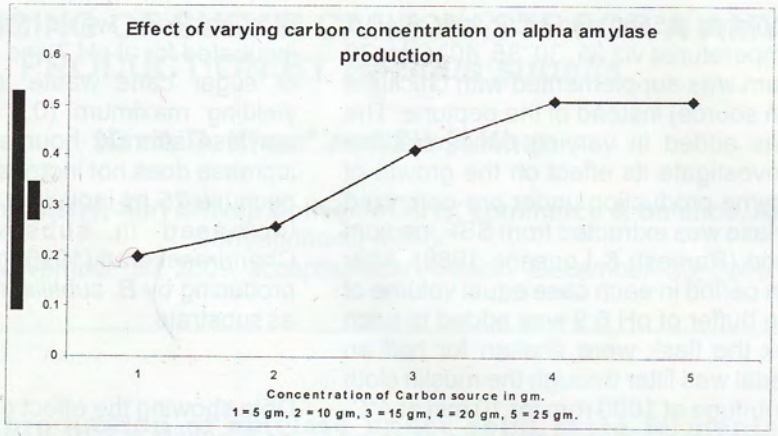


Fig. 2

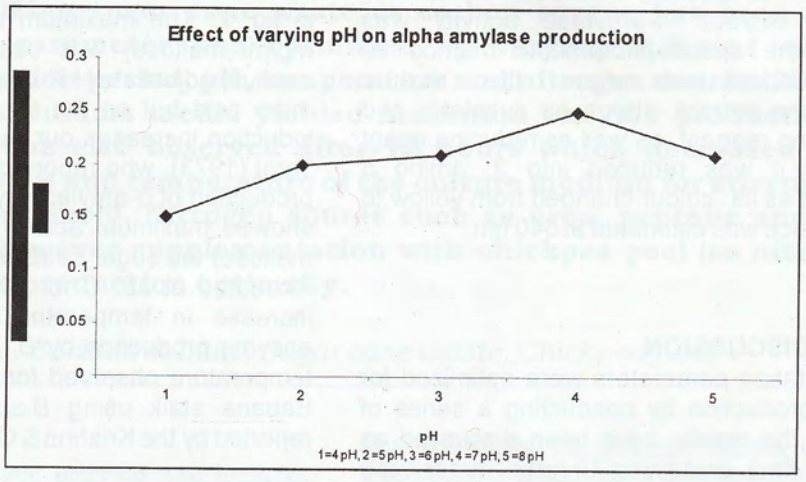
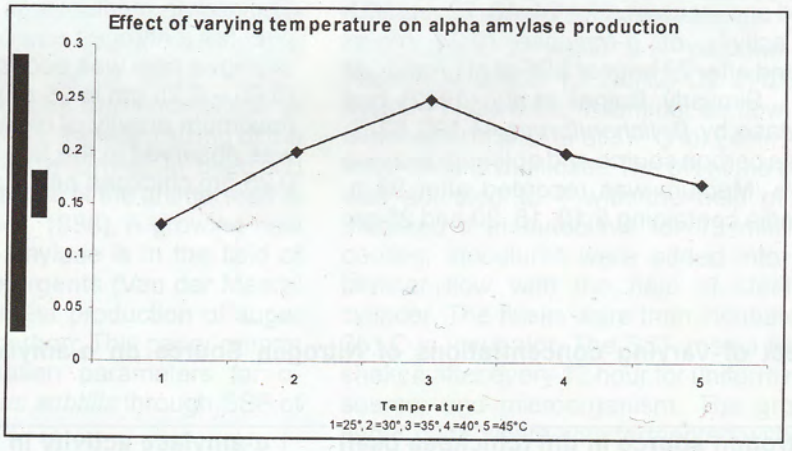


Fig. 3



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IMPACT OF AGRICULTURE ON THE WATER QUALITY OF RIVER WAINGANGA OF BHANDARA REGION.

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ABSTRACT:

Along with the other sources of organic and inorganic pollution, the non-monsoon river basin agriculture in Wainganga river is deteriorating the precious water source. The direct mixing of decaying biological manures is polluting the water to a great extent. During the study period of two years, the weekly sampling of physico-chemical parameters showed significant depletion of oxygen levels beyond the permissible limits. Dissolved oxygen and carbon dioxide ranged between 5.23 mg/l to 3.45 mg/l and 8.38 mg/l to 22.9 mg/l respectively. Temperature of water ranged between 31.9 mg/l to 34.5 mg/l whereas conductivity and BOD ranged from 397 μ mho/cm to 567 μ mho/cm and 21.1 mg/l to 63.1 mg/l respectively. The paper discusses some aspects of agricultural waste and its impact on river ecology.

Key words: *River basin pollution by agriculture activities*

INTRODUCTION

Water has many uses and since everybody uses it, a study of water from different aspects becomes important. Although the percentage of utilization of water resources for purposes other than irrigation is low at present, this is expected to rise appreciably in the future with increasing industrialization and power generation. The conservation and efficient utilization of available water resources need maximum emphasis. Since, the volume of wastes and wastewaters have increased considerably, there is a need to give serious consideration while making decisions, (Rogers, 1991).

Nature plays major role to lower the water pollution but the process is very slow (Pande and Sharma, 1999). The potential of pollution contributed by runoff waters originating from rural communities, cattle farming and agricultural drainage should not be underestimated. The non-monsoon agricultural practices inside the river basin in Wainganga river is the concern of serious threat, as the direct mixing of fertilizers and manures from the crop fields pollutes the water.

In the present situation the plantation of crop fields of water melon, muskmelon and other vegetables in the middle of river basin, is deteriorating the river water continuously during the summer season. However, comparatively less flow of river during the post winter and summer seasons, fails to stabilize the pollutants, as the quantity of pollutants becomes more.

MATERIAL AND METHODS:

The river water is continuously polluted with the decaying cow dung used to manure the watermelon and muskmelon plantation in side the river basin. The organic matter is continuously flows in the main stream of river water. The farmers convey the small streams through their basin farms, which runs through farm and carry the organic matter in the main stream, however, input of domestic sewage also contributes the nutrients in the river. Though the activities are limited at any one specific spot, the pollution caused does not remain confined to the spot, but contaminates whole stretch of river. In view of prepared agricultural farms in side the river basin, four stations are selected. Station S1 is located far away from the localities of the town and considered to be pollution free, while station S2,S3 and S4 receives the organic manure in river water. The analysis of all parameters were performed using the standard methods given by NEERI (1986).

RESULTS AND DISCUSSION:

Naturally the temp of river water changes with seasonal change in atmospheric temperature, however it increases from up stream station S1 to down stream station S4, with load of increasing organic matter, Mahadevan and Krishnaswamy (1983) have quoted the range of temperature 27°C to 34.5°C for polluted stations. The decaying cow dung manures from agricultural farms located in the river basin mixes the

plenty of organic matter in the main stream of river. The intensive microbial activities during summer and their enhanced rate of decomposition processes increase the temperature at down stream stations S2, S3 and S4.

Less flow of river during summer offers more concentration to the suspended particles in the water. In association with the pollutants from basin farms, the other secondary sources increase the suspended particulate matter in the river water and intern turbidity.
It is evident from present study that values of TDS gradually increases from the month of February to May, with decrease of water flow in river. Carbonates and bicarbonates of sodium, calcium, potassium and chlorides from organic pollutants enhance the values of dissolved solids in water, Narayan and Chauhan (2000) have recorded similar findings.

Increased enzymatic activities of micro fauna during hot days and increased rate of biodegradation activities essentially enhance the chlorides in the river water. Viet and Bhargava (1989) have recorded parallel findings in Saigon river. Lowered rate of photosynthetic activities of aquatic fauna in turbid water, reduces the assimilation of CO2 in river water may be the cause of increased concentration in the water, (Hymavathi *et al.* (1999). However the lowered rate of bicarbonate assimilation by plants in water may be responsible to deflect the alkalinity and hydrogen ion concentration values in river water. The extent of turbidity in water may derive the values of Carbon dioxide, pH and Alkalinity in river water.

From the present investigation it is concluded that dissolved oxygen varies in the seasons. Minimum concentration of dissolved oxygen occurs during summer. It is attributed to increase temperature of river water which increases the rate of microbial activities and their oxygen consumption , (Thomas *et al.*, 2001). The rich supply of organic matter is conveyed in the main stream of river water, however it increases the requirement of oxygen for stabilizing the biodegradable organic matter in the river. In addition to it the less flow of water again offers the more concentration to the organic pollutants and increases the BOD values.

The depletion of O2 is occurred at the down stream station below the permissible limits (4.0 mg/l), during the summer days, with increase in temperature of river water. During the present two year study period of river water, it is concluded that the river basin agriculture practices deteriorates the quality of river water during summer season. Increased values of temperature, biochemical oxygen demand and carbon dioxide may causes the depletion of oxygen in river water below 4.0 mg/l. increased agricultural practices in the river basin all over the study area harms the river ecosystem and intern affects the riverine fisheries. Mostly it even deteriorates the potable water quality of Wainganga..

Table -1
Physico-chemical status of Wainganga river water, during the year 2000

Parameters	Parameters	Stations			
		S1	S2	S3	S4
Temperature	Temperature	31.9± 1.2	32.7± 1.5	33.1± 2.5	33.5± 1.3
Conductivity	Conductivity	415±21	590±04	614±14	667±10
Turbidity	Turbidity	13.7± 2.6	17.5± 3.1	23.3± 3.0	28.0± 3.1
TDS	TDS	291±20	297±08	508±06	553±08
Chlorides	Chlorides	31.4± 1.7	35.5± 1.9	36.3± 2.1	45.9± 1.5
CO2	CO2	9.14± 0.9	14.4± 0.9	16.2± 1.4	22.9± 0.6
pH	pH	7.44± 0.14	7.49± 0.17	7.60± 0.15	7.79± 0.26
Alkalinity	Alkalinity	40.46 ±2.7	43.95 ±1.5	46.39 ±1.6	52.66 ±3.3
DO	DO	5.23± 0.34	4.90± 0.54	3.95± 0.61	3.63± 0.48
BOD	BOD	21.0± 0.66	45.4± 3.1	52.4± 4.2	59.4± 2.8

Table -2
Physico-chemical status of Wainganga river water, during the year 2001

Parameters	Stations			
	S1	S2	S3	S4
Temperature	33.5±0.32	34.2±1.7	34.07±2.5	34.7±2.1
Conductivity	397±18	528±14	578±15	653±19
Turbidity	13.3±4.6	21.8±5.1	24.8±4.9	31.3±4.09
TDS	294±07	312±07	523±09	607±06
Chlorides	30.6±2.1	36.8±2.1	38.9±2.4	46.1±1.8
CO2	8.38±1.2	10.8±0.86	15.0±1.5	21.1±0.81
pH	7.55±0.08	7.63±0.15	7.80±0.11	7.89±0.16
Alkalinity	43.59±2.4	47.98±2.2	54.43±4.9	58.10±5.2
DO	4.85±0.28	4.40±0.36	4.33±0.34	3.45±0.64
BOD	22.3±1.7	44.5±3.6	52.6±2.32	63.1±1.6

Figure - (A). Physico-chemical status of Wainganga river during the year 2000 & 2001.

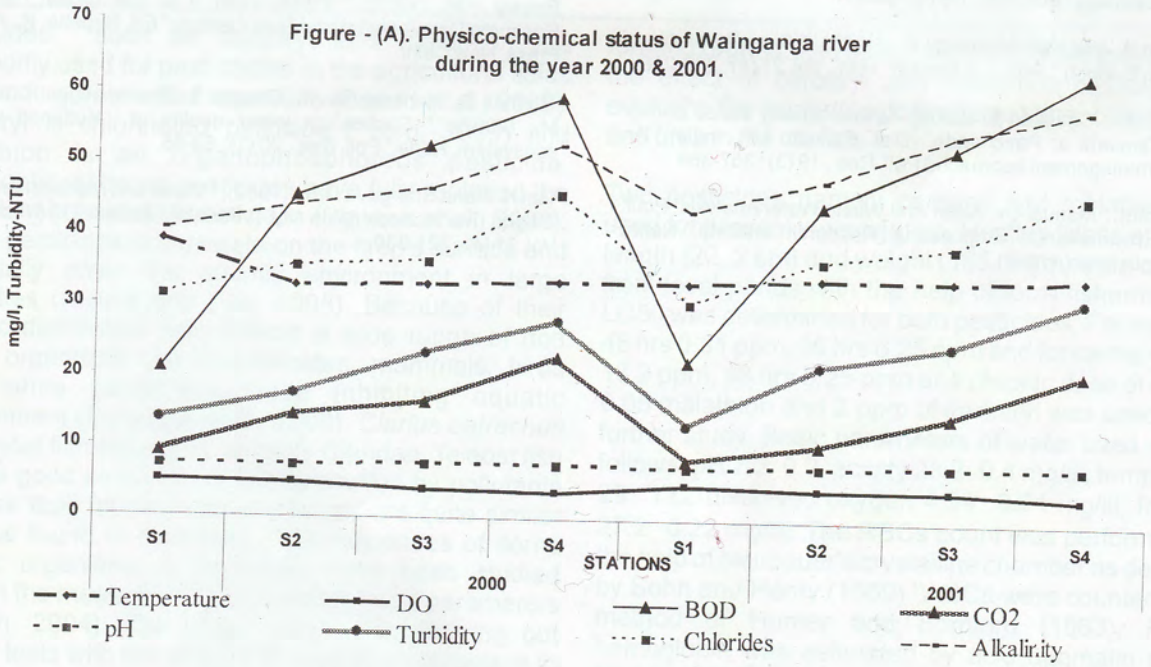
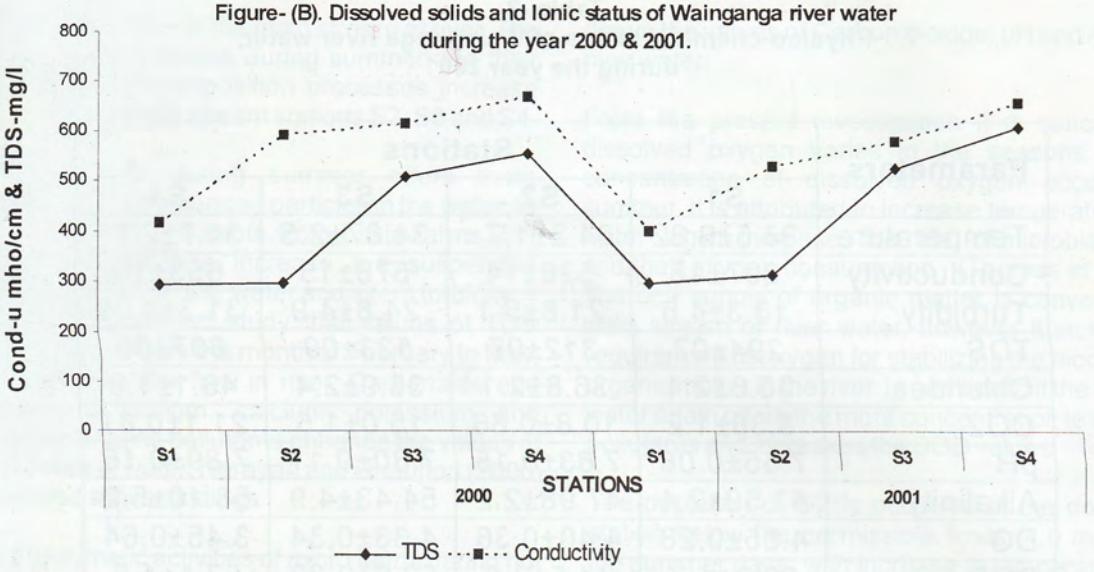


Figure- (B). Dissolved solids and Ionic status of Wainganga river water during the year 2000 & 2001.



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Short Communication

IMPACT OF CARBARYL AND MALATION ON HEAMATOLOGY AND NITROGEN METABOLISM OF *Clarius batrachus* (Linn.)

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ABSTRACT:

The aim of the study was to assess the effect of carbaryl and malathion on hematological indices and nitrogen metabolism of the fish, Clarius batrachus. The fish was exposed to 48 hrs, 96 hrs and 45 days of carbaryl exposure of 13.24 ppm, 5.25 ppm and 2.0 ppm respectively. Similarly it was exposed to malathion 0.31 ppm, 0.25 ppm and 0.05 ppm for 48 hrs, 96 hrs and 45 days exposure respectively. The pesticides induced anemia (as RBCs level decreased) and also there was a decrease in immunological response (decrease in WBCs). The serum protein and urea levels also got affected due to the effect of these pesticides.

Key words: *Clarius, hematology, serum-urea, blood-protein.*

Pesticides such as carbaryl and malathion are commonly used for pest control in the agricultural fields surrounding freshwater reservoirs (Saeed *et al., 2005*). Carbaryl is chlorinated pesticide (Rao, 1999) and malathion is an organophosphorous pesticide. Organophosphorous pesticides have fully replaced the persistent chlorinated pesticides (Banaee *et al.,* 2008). These pesticides accumulate on the crop's surface and eventually enter the aquatic environment in large quantities (Kuivila and Foe, 1995). Because of their aquatic distribution they affects a wide range of non target organisms like invertebrates, mammals, birds and fishes especially those inhibiting aquatic environment (Burkepile *et al.,* 2000). *Clarius batrachus* is a teleost fish belonging to family Claridae. Teleost fish may be good indicators of contamination by pollutants because their biochemical responses are quite similar to those found in mammals. The responses of some aquatic organisms to pollutants have been studied through the measurement of hematological parameters (Begum, 2004). The major reason for carrying out toxicity tests with fish and other aquatic organisms is to determine which concentrations of substances are harmful to the organisms and which have no apparent effect (Banaee *et al.,* 2008). Haematological and clinical parameters can be detected rapidly and hence can be used for prediction and diagnosis of pesticide toxicity. Alternations in these parameters show toxic stress in the treated animals especially on blood and blood forming organs. The study was performed to investigate the effect of carbaryl and malathion pesticides and evaluate the hematological alternations, blood protein and urea in the cat fish.

Two pesticides namely carbaryl and malathion were used for present investigation. Healthy fishes of uniform length (22 2 cm) and weight (155 5 gm) were collected from local ponds with the help of local fishermen. The LC50 was determined for both pesticides; For malathion 48 hrs 0.31 ppm, 96 hrs 0.25 ppm and for carbaryl 48 hrs 13.2 ppm, 96 hrs 5.25 ppm and chronic dose of 45 days 0.05 malathion and 2 ppm of carbaryl was used for the further study. Basic parameters of water used were as follows, pH 7.2 0.2, acidity 28.2 0.4 mg/lit, temperature 29 1°C, dissolved oxygen 4.54 0.21 mg/lit, free CO_2 27.2 0.22 mg/lit. The RBCs count was performed with the help of Neubauer's crystalline chamber as described by Sohn and Henry (1969). WBCs were counted by the method of Hunter and Bomford (1963). Percent hemoglobin was estimated by acid haematin method (Sahli, 1962). Also the total serum protein was estimated by Biurette method (Layne 1957) and urea content was recorded by method of Natelson (1971).

The haemoglobin content, WBCs and RBCs count values of the fishes exposed to carbaryl and malathions for 48 hrs, 96 hrs and chronic (45 days) dose were

recorded (Table no. 1) with respect to control group of fishes. A significant decrease was observed in values of Hb, WBCs and RBCs.

This decrease might be due to the destruction of blood cells. Similar studies were recorded by Bhaktvatsalam (1999) in *Anabas testidineus*. There was a decrease in erythrocyte count as well as haemoglobin content in *Channa punctatus* (Anees, 1978) in *Cyprinus carpio* (Svoboda *et al.,* 2001) and *Clarius batrachus* (Patnaik and Patra 2006). In each case the fishes were exposed to pesticides. The WBCs values also decreased may means dysfunction in haematological tissues or certain infectious diseases (Banaee *et al.,* 2008). The elevation in the serum protein levels under impact of carbaryl and malathion are represented in table no. 2. There is an increase in the total protein and albumin but decrease in the globulin as compared to control fishes. Decrease in RBCs results in decreased level of protein content and thus confirms anemic condition of *Clarius batrachus*. Similar study has been performed by Rao (1999) and Prasad *et al.* (2002) in *Channa punctatus* and *Channa marulius* respectively.

Urea levels are more in tissues like liver, muscle and gills literature reveals that this levels of urea in all the three tissues get elevated when exposed to pesticides such as carbaryl, phenthovate and carbaryl + penthoate (Rao, 1999) suggesting significant role of liver in nitrogen metabolism. But in the present investigation decreased serum urea levels were recorded of the experimental fishes exposed to carbaryl and malathion (Table no. 2).

These results depicts that nitrogen metabolism has been affected due to the exposure to pesticides. From these investigations, we conclude that there is a significant decrease in haemoglobin content, RBCs and WBCs count. The decrease in RBCs and WBCs count can be reason for anaemic condition in *Clarius batrachus* and an indication of weak immune status of the fish which can get infected easily to various infections. Further studies revealed a decrease in serum urea level and low protein content due to exposure to pesticides.

Table No. 1: Blood parameters of control, carbaryl and malathion exposed fish for 48 hrs, 96 hrs and 45 days.

S. N.	Blood Parameters	Control	48 hrs		96 hrs		45 day	
			Carbaryl	Malathion	Carbaryl	Malathion	Carbaryl	Malathion
1	Hemoglobin (Hb/gm %)	8.22 ± 0.324	7.83 ± 0.274	6.79 ± 0.192	7.23 ± 0.214	6.61 ± 0.156	7.10 ± 0.052	6.50 ± 0.089
2	RBCs / cmm (in millions)	2.92 ± 0.213	2.36 ± 0.133	1.89 ± 0.207	2.64 ± 0.139	2.32 ± 0.112	2.89 ± 0.144	2.46 ± 0.135
3	WBCs / cmm (in thousands)	2.62 ± 0.071	2.18 ± 0.089	2.04 ± 0.084	2.01 ± 0.092	1.89 ± 0.079	1.66 ± 0.035	1.47 ± 0.041

Table No. 2: Level of blood proteins & urea in control, carbaryl and malathion exposed fish for 48 hrs, 96 hrs and 45 days.

S. N.	Blood Contents	Control	48 hrs		96 hrs		45 day	
			Carbaryl	Malathion	Carbaryl	Malathion	Carbaryl	Malathion
1	Total Protein (mg / ml)	9.1 ±0.071	9.2 ±0.091	9.4 ±0.037	9.3 ±0.081	9.6 ±0.072	10.8 ±0.039	10.7 ±0.041
2	Albumin (mg / ml)	7.1 ±0.091	8.3 ±0.027	7.3 ±0.039	8.0 ±0.037	7.5 ±0.053	8.7 ±0.032	8.6 ±0.023
3	Globulin (mg / ml)	2.0 ±0.041	1.9 ±0.031	2.1 ±0.072	1.3 ±0.052	2.1 ±0.052	2.1 ±0.012	2.1 ±0.023
4.	*Serum Urea Content (mg/dl)*	*30.50* ±0.25	*18.40* ±0.19	*17.25* ±0.15	*17.33* ±0.17	*16.50* ±0.25	*16.33* ±0.30	*14.50* ±0.12

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Short Communication

AVIAN EMBRYONIC CARDIOMYOCYTES - *IN VITRO* MODEL FOR DRUG TESTING

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Received September 9, 2009; accepted November 10, 2009.

ABSTRACT:
Embryonic cells offer the advantages of promising and innovative alternatives for obtaining large number of candidate cells early efficacy and higher toxicity screening. The hepatocytes and cardiomyocytes from embryonic origin cultured in vitro can be used routinely for screening new compounds for hepatotoxicity and cardiotoxicity, the two leading causes of failure in preclinical development of new therapeutic drugs. In birds and mammals, cardiomyocytes terminate mitotic activity in the neonatal period and regeneration of cardiac muscle does not occur after myocardial injury in adult hearts. The presence of similar morphologies during the development of the chick and the human hearts of the embryo has been demonstrated by descriptive and anatomical studies. Hence an in vitro culture model has been established in this study with protocols for the derivation of cardiomyocytes from embryonic heart tissue as a system suitable for drug testing. A two stage culture protocol was followed to obtain the cardiomyocytes alone.

Key words: *Avian Embryonic Cardiomyocytes - In vitro Model.*

The embryo derived cells offers the advantage of testing drugs and chemicals where *in vivo* approach has inherent limitations. Differentiation is the process whereby cells acquire new morphological and functional characteristics (Alvarez *et al.,* 2003). Embryonic cells offer the advantages of promising and innovative alternatives for obtaining large number of candidate cells early efficacy and higher toxicity screening. The hepatocytes and cardiomyocytes from embryonic origin cultured *in vitro* can be used routinely for screening new compounds for hepatotoxicity and cardiotoxicity, the two leading causes of failure in preclinical development of new therapeutic drugs. In birds and mammals, cardiomyocytes terminate mitotic activity in the neonatal period and regeneration of cardiac muscle does not occur after myocardial injury in adult hearts. The presence of similar morphologies during the development of the chick and the human hearts of the embryo has been demonstrated by descriptive and anatomical studies (Bird *et al.*,2003). Though several anomalies are different, many cardiovascular malformations found in chick embryo are similar to those in humans. Therefore the information obtained by means of chick embryo could be extrapolated for evaluating the results obtained from the studies in the human embryos. Heart tissue of embryonic origin could be an appropriate model for studies on teratology, development and are candidate cells for testing drugs. Hence the *in vitro* culture conditions for the chick embryonic heart tissues was standardized in this present study and cultural characteristics were studied.

White leghorn embryos of 10-11 day old are used for collecting heart tissue. The heart tissue was carefully dissected and washed in washed in Hanks' balanced salt solution, and again rinsed y in M 199-medium with antibiotics. Then, the heart tissues were minced well using scissor and forceps and digested using 0.25% Trypsin EDTA solution (Freshney 1994). The cells were added with FBS to stop activity and centrifuged.

The cell pellet was seeded into a 25 cm^2 culture flask in - MEM medium supplemented with 20 percent fetal calf serum. The suspension cells after 2 hours of primary culture were removed and cultured separately in CO_2 incubator with 5 percent CO_2 (Renke *et al.*, 1996). The suspension cell pellet was then plated on to a plate pre-coated with 0.2% gelatin and after two hours non-adherent cells were separated and adherent cells were propagated.

The cells were then followed up for the cellular changes, cellular morphologies and attachment properties under inverted and Phase contrast microscope (Nikon) with fluorescent attachment.

Cell viability determination

The cells in culture were analyzed for the proportion of living, or viable, cells. This is determined by assessment of membrane permeability, under the assumption that a cell with a permeable membrane has suffered severe, irreversible damage.

Trypan blue exclusion

This is a rapid test for gross damage which is conveniently combined with determining cell number. A small aliquot of cell suspension was mixed with an equal volume of 0.4% trypan blue (Sigma) solution. Within 1-5 min, the suspension was introduced into a hemocytometer chamber. Non-viable cells appear blue, with the nucleus staining particularly darker. The viable (unstained) cells and the total number of cells were counted. The number of viable cells is expressed as a percentage of the total.

Estimation of viability by dye uptake:

The percentage of live cells during different days of culturing was done periodically using fluorescent dye method. A cell suspension at high concentration, $1x10^6$ cells per ml was prepared in RPMI -1640 medium without phenol red. Fluorescent dye mixture was added at a proportion of 1: 10 to give a final concentration of 1 g/ml of diacetyl fluorescein and 50 g/ml of propidium iodide. The cells were incubated at 37°C for 10 minutes. Drop of the cells was placed on a clean microscopic slides, a cover slip was added and examined the green fluorescing live cells under fluorescence microsopy (Nikon, Japan). The percentage of positive cells to the total number of cells at different time interval was calculated.

The cells were cultured first as primary heart tissue culture and the supernatant suspension cells after 24 hrs were cultured separately. In the primary culture, cells were adherent cells and showed fibroblast cell morphology in 12 to 16 hrs whereas the suspension cells were elongated and took a longer time for attachment (24 - 48 hrs). These cells showed spontaneous pulsing and beating was observed in cell clumps. The cells were predominantly elongated, cylindrical and uniform with glistening surface. First three days culture showed many unattached cells, while 6th, 7th day cultures showed more flattened cells towards poles, indicating their attachment. After 8th day vacuolation appears in the cells. The live and dead cells were observed by fluorescent diacetate (FDA) and propidium iodide (PI) staining on 3rd, 6th and 9th days of culture and the fluorescent staining revealed 83 and 17 percent, 68 and 32 percent and 23 and 77 percent of live and dead cells respectively in these culture days.

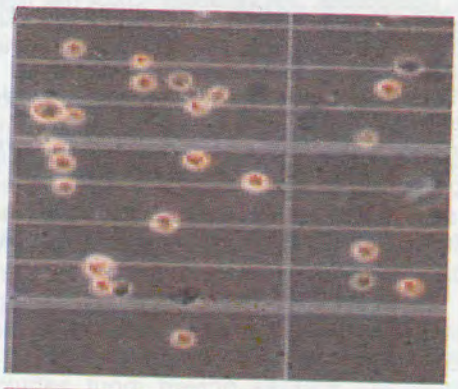


Fig A

Live cells



Fig B

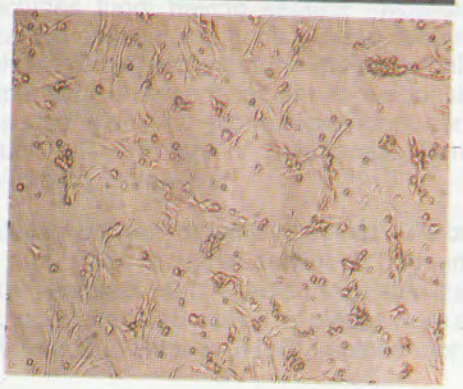


Fig C

Dead cells

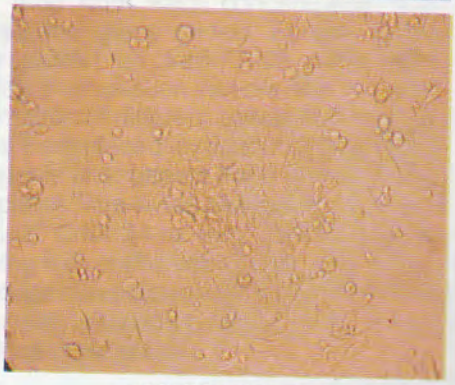


Fig D

EXPLANATION OF FIGURES.....

EXPLANATION OF FIGURES

Fig A. Trypan blue dye exclusion showing live and dead cells, microscopy 200 x.
Fig B: Fluorescent dyes FDA, PI staining live and dead cells respectively.200x.
Fig C. Cardiomyocytes showing changes in morphology 5-7 days culture 100x
Fig D. Monolayer of cardiomyocytes 100x, showing glistening elongated oblong cells and multinucleated cell mass shown beating in culture.

Embryonic heart tissue from chick embryos could easily be disaggregated and viable primary cultured cells were obtained. The second stage supernatant culture cells could be identified as cardiomyocytes by the specific pulsation in these cells which was not observed in the first stage culture cells. The cardiomyocyte cells were elongated, oblong and the cytoplasmic details were not clear. After three days adherence was seen in these cells with varied pulsation, increased pulse frequency could be appreciated in few cells and certain cells became quiescent after rapid pulsing. These finding could be correlated to the findings of Kunysz *et al.* (1995) that the quiescent cells observed in embryonic chick heart cells in culture was due to overdrive suppression and post drive hyper polarization phenomenon which were dependent on stimulation of an electrogenic sodium-potassium pump.

The two stage culture protocols followed could be appreciably useful to obtain pure cardiomyocyte culture as the non pulsing cells were separated. The beating cell clumps simulate a myocardial tissue mass would be much useful in the transplantation studies. The increasing percentage of dead cells as culture days increases could be correlated to the appearance of vacuolation in cell, followed by breakage of vacuoles and cell membrane shrinkage, causing a leakage of the cell contents, eventually resulting in cell death. Also the death of beating cell mass would be related to the lack of blood flow dynamics and a three dimentional mileu required for the cells as *in vivo*. These findings observed in this report would be applicable for drugs, which can diffuse to the cardiomyocytes and can be tested with ease than in an *in vivo* condition. The striking potential benefits of *in vitro* cultured embryonic cells, when applied to drug toxicity testing, result from their unique properties compared to the adult cells (Davila *et al.* 2004). These cells can be routinely applied to screen new chemical entities for cardiotoxicity, the leading cause of failure in preclinical development of new therapeutic drugs.

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Short Communication

ANTIFUNGAL ACTIVITY OF *Pseudomonas fluorescens* AGAINST PHYTOPATHOGENIC FUNGUS *Alternaria solani*

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ABSTRACT:

Antifungal activity of Pseudomonas fluorescens was tested against the plant pathogen, Alternaria solani in vitro. Different concentrations (500, 1000, 1500, 2000 and 2500 µg/ml) of Pseudomonas fluorescens were used. The results indicated that Pseudomonas fluorescens presented a most significant value against the phytopathogenic fungus. Inhibition of the fungus started from 1500 µg/ml and stopped at 2500 µg/ml. The presented data exhibit the antifungal activity of P. fluorescens and indicate the possibility of using P. fluorescens as a biological control agent for plant pathogenic fungi such as A. solani. However, this requires further screening of a large number of Pseudomonas strains from different regions of India to control various phytopathogenic fungi which can work as a good source of biological control.

Key words: *- Pseudomonas fluorescens, Alternaria solani.*

Biological control really developed as an academic discipline during the 1970s and is now a mature science supported in both the public and private sector (Baker, 1987). Fungicides used in the crop field develop danger to not only humans, animals, water but also for ecosystem. Products synthesized biologically, have been demonstrated to be not only specifically effective on the target organisms but also inherently biodegradable. The genus Pseudomonas has been heterogeneous since Migula first named it in 1894. He designated and described the species associated with the genus in 1895. Pseudomonas is gram-negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of the rhizosphere of various crop plants, and have broad spectrum antagonistic activity against plant pathogens.

The Genus Alternaria consists of several saprophytic species. It belongs to sub-division Deutromycotina which includes fungi having septate mycelium and reproduce by asexual methods. These fungus lack sexual phase or the perfect stage therefore called as fungi imperfectii. Alternaria solani is responsible to cause disease in several economically important crop plants such as members of Solanaceae family for example Tomato, Potato etc.

Diseased plant parts of tomato and potato were brought from the crop fields in sterile polythene bags into the laboratory. The plant parts were surface sterilized and cut into small pieces (2-5 mm). The pieces were dipped into 0.1% mercuric chloride ($HgCl_2$) solution for about one minute. These pieces were transferred to Petri plates containing sterile distilled water to free them from the chemicals and saprophytic microorganisms if any followed by washing. The rinsed pieces (2-4) were placed at equidistance in a single solidified potato dextrose agar (PDA) plates in aseptic condition by using sterile forceps. The plates were incubated at 25°C and examined daily for the growth of the fungus. After the incubation period of four days the fungal culture were examined under the microscope and identified by using standard literature. A. solani was identified under the microscope and screened to develop pure culture. Stock solution (2500 µg/ml) of the P. fluorescens was prepared by dissolving 5 ml of the culture in 1 ml of distilled water. Required concentrations (500, 1000, 1500, 2000 and 2500 µg/ml) was prepared from each stock solution by diluting with distilled water.

One drop from each concentration was placed on grease-free glass slides. Fungal spores (200-300) were picked up from 7-10 days old culture with sterilized inoculation needle and mixed in solution of the fraction of different concentrations separately. The slides were placed in moist chambers made by placing two sterile filter papers each on the lid and base of the petriplates. The slides with spores were then incubated at 25±2° C for 24 hr. Germination was observed after staining with cotton blue prepared in lactophenol under binocular microscope. Spores mixed in sterile distilled water only served as control. The experiments were conducted in triplicate.

Table No: - 1. Effect of *Pseudomonas fluorescens* against *Alternaria solani*

Sr. No.	Fungus	Concentration µg/ml						Co efficient of Variation CV.
		Spores inhibition %						
		Control	500	1000	1500	2000	2500	
1.	*Alternaria Solani*	44.25	70.50	75.00	78.35	85.42	98.30	0.21
		44.25	69.00	75.25	78.50	86.00	98.50	0.21
		44.25	70.00	75.60	78.25	86.00	99.00	0.21

P. fluorescens was screened for its antifungal activity against *A. solani*. Table 1 presents the different concentrations (500, 1000, 1500, 2000 and 2500 µg/ml). Maximum inhibition of spore germination of phytopathogenic fungus starts from 1500 µg/ml and fungus growth stops completely at 2500 µg/ml. These data revealed that *Pseudomonas* exhibited significant antifungal activity. Percentage of spore inhibition increased with the increase in the concentration of microbe and thus exhibiting concentration dependent activity. The biological control of soil-borne pathogens with antagonistic bacteria, particularly *Pseudomonas* spp. belonging to plant growth promoting *Rhizobacteria*, has received prominent attention because of the dual role of these bacteria in plant-growth promotion and disease control (Zehnder *et al.,* 2001) . Experiments *in vitro* and *in vivo* by (Rozsnyay *et al.,* 1992) showed that some strains of *P. fluorescens*, some epiphytic bacteria and some fungi inhibited canker and dieback diseases of apricot. In earlier studies (Nandakumar *et al.,* 2001a, 2001b) a talc-based formulation of the *P. fluorescens* strains also used here reduced sheath-blight incidence under field conditions in different zones of Tamil Nadu. Some species of *Pseudomonas* can also produce levels of HCN that are toxic to certain pathogenic fungi (David & O'Gara 1994). These characteristics make *Pseudomonas* species good candidates for used as seed inoculant and root dips for biological control of soil-borne plant pathogen. The presented data exhibit the antifungal activity of *P. fluorescens* and indicate the possibility of using *P. fluorescens* as a biological control agent for plant pathogenic fungi such as *A. solani*. However, this requires further screening of a large number of *Pseudomonas* strains from different regions of India to control various phytopathogenic fungi which can work as a good source of biological control.

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Short Communication

ALTERATIONS IN ELECTROLYTE COMPOSITION IN VARIOUS TISSUES OF *Rasbora daniconius* EXPOSED TO SODIUM FLUORIDE

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ABSTRACT:

The fish, Rasbora daniconius were exposed to 200 mg/l sodium fluoride to study its effect on ionic contents of various tissues. Significant to highly significant depletion was noted in tissue ionic content of R. daniconius. The depleted Na+ and Ca^{++} in tissue like gills and bones is of concern as calcium and sodium ions are very important in cell functions. The exposure to sodium fluoride made the fishes lethargic due to disturbances in ionic concentrations which may affect the fish production.

Key words: *Tissue Electrolyte Composition Rasbora daniconius Exposed to sodium fluoride.*

Human activities results in substantial fluoride input to the aquatic environment. Exposure of living organisms to abnormal levels of fluoride may result in an alteration of the organism's biochemistry and electrolyte compositions. Although fluoride leads to significant environmental concentrations only in the vicinity of the sources, low level of fluoride pollution appears to be more wide spread. Growth (1975) stated that freshwater organisms are evolved in an environment, almost fluoride free, thus they are not well equipped to tolerate fluoride levels contaminated in polluted streams. Carpenter (1969) stated that slight increase in fluoride in fresh water becomes toxic to the organisms. However, the survey of literature indicates that much of the work is carried out on human beings and less attention has been paid on the effect of fluoride on freshwater fishes and hence an attempt has been made to know any alterations in electrolyte composition in various tissues of the fish, *Rasbora daniconius* exposed to sublethal dose of sodium fluoride. The fresh water fish, *Rasbora daniconius* were collected from Wadali reservoir, Amravati. They were disinfected in 1% $KMNO_4$ solution to avoid infection and then maintained in glass aquaria for two weeks. Chlorine free aged tap water was used in both control as well as experimental aquaria. The sodium fluoride (NaF) was used as toxicant. That was obtained from Qualigens Fine Chemicals, Mumbai. The experimental fish, *Rasbora daniconius* was treated with 200 mg/l of sodium fluoride for 30 days. After 30 days of exposure of the fish were dissected and tissues like bone, gills, liver, kidney and muscles were removed to estimate electrolytes (Na^+ and K^+) by using flame photometer and bone calcium (Trinder, 1962). The tissue homogenate was prepared in 0.25M Sucrose solution. The homogenate was centrifuged at 4000 rpm for 10 minutes. The supernatant was used for the estimation of sodium by using Na^+ filter and potassium using K^+ filter. Dried samples of bones were used for determination of calcium. Ash was prepared in silica crucibles by heating in furnaces at 700°C for 6 hours. 5 mg of ash dissolved in 10 ml of TRIS-HCl buffer (pH 7.4). During dissolution solution was kept slightly warm. Thereafter, calcium level was determined by the method of Trinder (1960). In all the tissues of the fish, Rasbora daniconius exposed to 200 mg NaF/l. All the tissues under investigation exhibited significant depletion in all the ions. In gill significant depletion was observed in potassium and calcium content. The percent decrease was 38.30 and 25.56 (Table 1). However, in liver no significant ($P<0.01$) increase was noted except for sodium ions.

After 30 days of exposure to NaF, the electrolytes showed significant decline in kidney. The sodium ions showed 16.67% and phosphorus 39.31% depletion. The decrease was significant at $P<0.01$. Similar decrease in the muscle was noted, the percent decrease in sodium ion was 24.85 and in calcium 17.81 (Table 1).The phosphorus (inorganic) ions in kidney of experimental fishes were also found to be depleted significantly ($P<0.01$). However, in rest of the tissues under study the depletion in inorganic phosphorus ions was significant.

Table 1: Electrolyte alterations in the fish, *Rasbora daniconius* exposed to – NaF for 30 days.

Cont./Expt.	Electrolytes			
	Sodium (µ mol/kg)	Potassium (µ mol/kg)	Calcium (mg/gm)	Phosphorus (mg/gm)
Control	121 ± 2.32	12.35 ± 0.17	8.66 ± 0.62	0.867 ± 0.01
Experimental	85* ± 1.07	7.62** ± 0.51	6.36** ± 0.12	0.832 ± 0.01
	(29.56)	(38.30)	(26.56)	(4.04)
Control	122 ± 2.05	12.26 ± 0.24	6.68 ± 0.13	0.660 ± 0.01
Experimental	82* ± 1.64	10.12 ± 0.20	6.84 ± 0.14	0.620 ± 0.01
	(32.79)	(17.46)	(2.39)	(6.07)
Control	60 ± 1.18	7.22 ± 0.14	6.26 ± 0.17	0.318 ± 0.01
Experimental	50* ± 0.61	6.54 ± 0.13	7.64 ± 0.15	0.193** ± 0.003
	(16.67)	(9.41)	(9.68)	(39.31)
Control	157 ± 3.11	27 ± 0.46	7.41 ± 0.14	8.23 ± 0.16
Experimental	118* ± 2.36	24.53 ± 0.44	6.09* ± 0.13	7.66 ± 0.15
	(24.85)	(9.15)	(17.81)	(6.93)

All values are mean ± SE of five replicate.
*P<0.01, NS – Not significant.
Figures in parenthesis indicate percent change over control.

The decreased electrolytes in all the tissue under investigation may be due to the secretion of ion regulating. Hormones like cortisol and prolactin (McKim *et al.*, 1970; Skidmore, 1970). It is also a result of the physiological adjustment of the fish to the changed ionic concentration in surrounding water to maintain the osmoregulation. The present results are well in agreement with that of Fu *et al.* (1989). Flik *et al.* (1985) also reported the increase in the permeability of the gill epithelium leading to massive efflux of calcium ions and to increased osmotic influx of water in the fish, *Orechromis mosambicus* exposed to polluted water. Further the cells of corpuscles of Stannius are the presumptive hypocalcaemia producing cells (Urasa and Wendelaar Bonga, 1999). In the present investigation, the experimental fish showed excessive secretion of mucus over the gills which might have reduced membrane permeability to ions and water resulting into decreased ionic contents not only in gill but also in all the tissue under study. Thus, exposure of the fishes to sodium fluoride alters the ionic uptake by gills and ultimately also results in significant depletion in bone calcium making the fish lethargic leading to stunted growth and ultimately affecting the fish production.

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Short Communication

EFFECT OF *Terminalia arjuna* AS DIETARY SUPPLEMENT IN THE FORM OF TEA ON THE SERUM LIPID PROFILES OF PATIENTS SUFFERING FROM HYPERCHOLESTEREMIA

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ABSTRACT:

The present study was designed to investigate the cardio protective role of Terminalia arjuna, if used as dietary supplement, routinely. Ten volunteer patients were chosen of different age & sex. Their Total Serum Cholesterol, HDLc, Serum Triglycerides, VLDLc, LDLc were estimated before treatment and at the end of the 12-week trial. In this group i.e patients treated with formulation named, Vindhya Herbal Arjun Tea was found to be significantly helpful in lowering the above-mentioned parameters. Thus the present study conforms with the previous studies regarding the efficacy of Terminalia arjuna in reducing the cholesterol level in the patients of hypercholesteremia.

Key words: *Terminalia arjuna, Hypercholesteremia*

The bark of *Terminalia arjuna*, a deciduous tree of Combretaceae family, has been widely used in Indian System of Medicine in cardiac ailments (Nadkarni, 1976; Vaidya, 1994). In recent times, both experimental and clinical studies have claimed that the dried bark powder of *Terminalia arjuna* has significant beneficial effects in ischemic heart disease (IHD). Moreover, an antioxidant effect of the *Terminalia* species has also been documented in in-vitro conditions (Karthikeyan *et al.*,2003; Gauthaman *et al.*,2003; Gautham *et al.*,1997). Animal studies suggest *Terminalia* might reduce blood lipids. Rabbits made hyperlipidemic by feeding them an atherogenic diet were given an oral Terminalia extract. Animals given Terminalia had a significant, dose-related decrease in total- and LDL-cholesterol, compared to placebo ($p < 0.01$). However, the amounts used (100 mg/kg and 500 mg/kg body weight) were very large, and it remains to be seen if these significant changes be simulated in humans when taken in relatively smaller oral doses (Ram *et al.*, 1997). In a similar study of rats fed cholesterol (25 mg/kg body weight) alone or along with Terminalia bark powder (100 mg/kg) for 30 days, Terminalia feeding caused a smaller increase in blood lipids and an increase in HDL cholesterol compared to the cholesterol-only group. The researchers felt inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid excretion, and stimulation of receptormediated catabolism of LDL cholesterol caused Terminalia's lipid-lowering effects (Khanna *et al.*, 1996).

With the above views in mind a study was designed to evaluate the efficacy of of *Terminalia arjuna* if used routinely as dietary supplement in the form of Vindhya Herbal Arjun Tea, an Ayurvedic herbal preparation. Vindhya Herbal Arjun Tea contains several useful plant ingredients like *Terminalia arjuna* (Arjun) 70%, *Cinnamomum zeylanicum* (Dalchini) 10%, *Syzygium aromaticum* (Laung) 5% and *Elattaria cardamomum* (Elaichi) 15%.

Ten volunteers who were suffering from hypercholesteremia and taking medication from a local Govt. Ayurvedic Health Clinic (Sanjeevni Ayurved Kendra, Bhopal) were chosen at random. Patients, who were diabetic and taking medications that affect lipid metabolism or blood pressure, were excluded from the study.

Total cholesterol and triglycerides in the serum estimation were carried out in Private Pathology (Jaya Diagnostic Centre, Bhopal) testing centre following standard laboratory methods. Fractions of lipoproteins such as LDLc, VLDLc and HDL cholesterol were estimated by using the methods of Friedwald (1972) and Lopez Virella (1977). After clinical evaluation and basal estimations, subjects received Vindhya Herbal Arjun Tea in the dose of 1/2 tea spoon decoction with milk in the form of tea, three times a day. The study was continued for a period of 12 weeks. The final serum lipid measurements were done after the end of 12 weeks.

The initial serum cholesterol and triglycerides were significantly higher in all the subjects. Eight out of ten subjects showed significant lowering of Total Serum Cholesterol, HDLc, Serum Triglycerides, VLDLc, LDLc levels after 12 weeks of treatment (Table-1). This reduction was noticed at the end of 8 weeks and remained so at the end of 12 weeks.

Table-1: Changes in lipid profiles following therapy in patients

S.No	Parameters	Initial Level mg/dl (Average, n=10)	At 12 Weeks mg/dl (Average, n=10)	Ref. Range mg/dl
1.	Total serum cholesterol (mg%)	350.2	212.6	150-240
2.	HDL cholesterol (mg%)	41.3	46.3	30-70
3.	Serum Triglycerides (mg%)	309.4	148.4	70-170
4.	VLDL cholesterol (mg%)	62.2	33.6	5-35
5.	LDL cholesterol (mg%)	246.4	104.8	Upto 110
6.	LDLc:HDLc ratio	5.96:1	2.26:1	Normal 3:1 High risk 5:1

In the present study, Vindhya Herbal Arjun Tea not only showed lowering in the levels of Total Serum Cholesterol, Serum Triglycerides, VLDLc, LDLc to a significant levels but also enhanced the level of cardioprotective HDLc. The consequent decrease in the ratio of LDLc to HDLc indicates a lowering of risk of cardiovascular diseases. Therefore the present study highlights the cardioprotective bioactivity of *Terminalia arjuna* given in the form of tea as a dietary supplement. The effect of use of *Terminalia arjuna* on a routine basis is still not known, therefore, another study with a larger sample size should be initiated to evaluate its efficacy and safety in a larger population is needed.

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Short Communication

IN VITRO PROPAGATION OF *CARICA PAPAYA* L. cv. RED LADY FROM SHOOT APEX AND NODE EXPLANTS

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ABSTRACT:

***Carica papaya* L. cv. Red Lady is tolerant to papaya ring spot virus. Thus in the present study multiple shoots were obtained from shoot apex and node explants of two month old seedlings regenerated *invitro* of *Carica papaya* L. cv. RedLady. The shoot apex and node explants were inoculated on MS medium fortified with BAP, NAA, adenine sulphate and coconut milk in various combinations. IBA dip treatment given to the cut end of the shoots produced healthy roots. The combination of soil: vermiculite: cowdung (1: 1: 1) were used as substratum for the hardening of the plantlets.**

Key words: *Carica papaya L., Papaya ring spot virus (PRSV), multiple shoots.*

Carica papaya L. is a dicotyledonous, polygamous, diploid species and it belongs to the family Caricaceae. It has three sex forms male, female and hermaphrodite (Ming *et al.,* 2001). Papaya is an important fruit crop, popular for its easy cultivation, rapid growth, quick economic returns, adaptation to diverse soils and climates (Campbell, 1984). However, viral diseases have reduced the effective crop life (Pernezny and Litz, 1999).

Papaya ringspot virus (PRSV) causes the disease papaya ringspot. The disease causes a wide range of damage, losses in yield and control on a commercial scale is very difficult. The symptoms on fruit consist of circles and C-shaped markings that are dark green, which become gray and crusty in texture. Plants infected by PRSV show mosaic and cholorosis of leaf lamina, water-soaked oily streaks on the petiole and upper part of the trunk and distortion of young leaves (Gonsalves, 1998). *Carica papaya* L. cv. Red Lady-786 (Taiwan) is an early, vigorous and productive papaya that is tolerant to papaya ring spot virus. Plants begin to bear fruit at 60-80 cm height and have over 30-100 fruits per plant. Flesh is red, thick, fruit weight is between 1.5 and 2 kilos. Seeds are easily available but highly priced. Since *Carica papaya* L. cv. Red Lady is tolerant to papaya ring spot virus it was selected for *in vitro* propagation from shoot apex and node *via* multiple shoots.

Shoot apex and node explants of two-month-old seedlings of *Carica papaya* L. cv. Red Lady were regenerated on MS medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.8% agar, fortified with BAP (0.5-5.0 mg/l), NAA (0.5-5.0 mg/l) and adenine sulphate (lower range of 0.5-5.0 and higher range of 10-100 mg/l) used singly or in various combinations supplemented with (5-15%) coconut milk. The pH of the culture media was adjusted to 5.8 prior to autoclaving at 121°C for 20 minutes under 15 pounds/square inch pressure. All cultures were maintained at 25 ± 2° C and 18 h photoperiod with 5000 lux. For rooting cut ends of the regenerated multiple shoots were excised and dipped for 10 to 30 seconds in various concentrations of NAA, IAA and IBA (250-3000 mg/l) and then inoculated on MS medium. The combination of soil: vermiculite: cowdung (1: 1: 1) were used as substratum for the hardening of the plantlets. For each treatment, 10 cultures were raised and every experiment was repeated thrice and the quantitative results were tabulated.

Shoot apex and node explants of *Carica papaya* L. cv. RedLady cultured on MS medium fortified with BAP, adenine sulphate and NAA used singly or in various combinations did not produce any shoots or elicited poor response. However the shoot apex and nodal explants cultured on MS medium fortified with BAP, NAA and adenine sulphate and coconut milk in various combinations produced multiple shoots within 6-8 weeks. The best response was elicited by MS medium fortified with BAP (2 mg/l), adenine sulphate (1.0 mg/l), NAA (0.5 mg/l) and 10% coconut milk with 1.91 shoot

buds per shoot apex and 3.84 shoot buds per node explants (Table 1).

Regenerated shoots were separated and the base was dipped in various concentrations of NAA, IAA and IBA for 30 seconds then inoculated on solid MS medium either failed to produce any roots or showed profuse callus formation. However cut ends of the regenerated multiple shoots excised and dipped for 30 seconds in IBA (2500 mg/l) and then inoculated on MS medium showed 62.6 % rooting in 18.3 days with 52% hardening success in soil: vermiculite: cowdung (1: 1: 1) used as substratum for the hardening of the plantlets in green house (Table 2).

Table 1: Effect of MS medium fortified BAP, AS, NAA and CM on the number of shoot buds initiated on shoot apex and nodal explants of seedling regenerated *in vitro* in *Carica papaya* L. cv. RedLady.

MS medium BAP + AS + NAA (mg/l)	Coconut milk (%)	Shoot buds / explants	
		Shoot apex	Node
2.0+0.5+0.05	10	1.59±0.12	1.57±0.18
2.0+0.5+0.1	10	1.33±0.08	1.30±0.10
2.0+0.5+0.5	10	1.38±0.14	1.37±0.11
2.0+0.5+1.0	10	1.23±0.16	1.19±0.11
2.0+1.0+0.05	10	1.76±0.08	1.67±0.18
2.0+1.0+0.1	10	1.67±0.21	1.65±0.20
2.0+1.0+0.5	10	1.91±0.17	3.84±0.15
2.0+1.0+1.0	10	1.22±0.10	1.17±0.10

Values are mean of three sets of determinants. Each set containing 10 explants. BAP - 6-Benzyl aminopurine; AS - Adenine sulphate; NAA – Naphthalene aceticacid; CM-Coconut milk.

Table 2: Rooting and hardening of the multiple shoots in *Carica papaya* L. cv. RedLady

Base of the shoot dipped in IBA (2500 mg/l) for 30 seconds, inoculated on MS basal medium	Rooting (%)	62.6±2.08
	Time taken (days)	18.3±0.57
Substratum - Soil: vermiculite: cow-dung (1:1:1) in green house in 4 weeks	Hardening success (%)	52.0±1.73

Values are mean of three sets of determinants. Each set containing 10 explants.

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Short Communication

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NITROCHALCONES

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ABSTRACT:

Six different chalcones I(a) – I(f) were synthesized by standard method from 2-hydroxy-3-nitro-5-methyl-acetophenone and tested for antibacterial activity against Staphylococcus aureus, Escherichia coli, Proteus vulgaris, and antifungal activity against Candida guillermondii, Candida albicans, Candida tropiculis and Candida crusei. Six nitrochalcones $I_{(a)}$ to $I_{(f)}$ were tested for antibacterial activity which showed good antibacterial activity while other shows moderate antibacterial activity. P. vulgaris exhibited no activity. Compound $I_{(a)}$ to $I_{(f)}$ showed good antifungal activity against Candida tropiculis and Candida crusei whereas $I_{(a)}$ to $I_{(d)}$ demonstrated moderate antifungal activity.

Key words: *Antibacterial antifungal activity of nitrochalcones.*

It is well known that chalcones are synthesized by condensation of (2-hydroxy-acetophenone) with aromatic aldehyde in presence of acidic (Clasen, 1881) and basic media (Chaparede, 1881). Chalcones show amoebicidal (Sanfi *et al.*,1983), antimicrobial activity. Chalcones are important intermediate in the synthesis of flavanones, flavanols, benzal coumaranones; in the biosynthesis of flavanone and anthocyanine. Condensation of 2-hydroxy-acetophenones with aromatic aldehydes in presence of acidic (Kostanencki, 1896) or basic (Sanfi *et al.*, 1984) condensing agent gives chalcones, flavanones or mixture of these. The first known example of chalcone in nature is carthamin, a red pigment from the flower of *Carthamus tinetoria* (safflower). It was first obtained by as red needles with green iridescence using pyridine as solvent (Kametaka & Perkin, 1910). Chalcones and their derivatives are reported which have antimicrobial (Mokale *et al.*, 2004), analgesic (Viama *et al.*, 2003), antimalarial (Liu, *et al.*, 2001), antiviral (Onyilagna *et al.*, 1997), antitubercular (Siva Kumar *et al.*, 2007), anti-inflammatory (Hsieh et al., 2000), cardiovascular (Marmo, . *et al.*, 1973), anticancer (Ahluwalia, . *et al.*, 1987), antiviral (Ninomiya, *et al.*, 1990). Chalcones are effective in pharmaceutical and medicinal applications (Ebenezer, *et al.*, 1995). Here synthesized six nitrochalcones $I_{(a)}$ to $I_{(f)}$ by condensation of various aromatical aldehydes with 2-hydroxy-3-nitro-5-methyl acetophenone. The structure of the synthesized nitrochalcones compound has been supported by IR, NMR spectral data.

Table1: showing the various compounds having antibacterial activity

Sr. No.	Name of compounds
1.	2'-hydroxy-3-nitro-5-methyl-chalcone $I_{(a)}$
2.	2'-hydroxy-3-nitro-5-methyl-4-methoxy chalcone $I_{(b)}$
3.	2'-hydroxy-3-nitro-5-methyl-2-furfuryl chalcone $I_{(c)}$
4.	2'-hydroxy-3-nitro-5-methyl (benzoyl)-4-phenyl-1,3-butadiene $I_{(d)}$
5.	2'-hydroxy-3-nitro-5-methyl-3-nitro chalcone $I_{(e)}$
6.	2'-hydroxy-3-nitro-5-methyl-2-hydroxy chalcone $I_{(f)}$

Table 2: Antibacterial activity of chalcone $I_{(a)}$ to $I_{(f)}$.

S.N.	Compound No.	M. F.	Zone of inhibition (in mm)			
			S. aureus	*E.coli*	*P. vulgaris*	*S. subilis*
1.	$I_{(a)}$	$C_{16}H_{13}NO_4$	16	18	--	16
2.	$I_{(b)}$	$C_{17}H_{15}NO_5$	12	19	--	4
3.	$I_{(c)}$	$C_{14}H_{11}NO_5$	14	22	--	20
4.	$I_{(d)}$	$C_{18}H_{15}NO_4$	15	21	16	12
5.	$I_{(e)}$	$C_{16}H_{12}N_2O_6$	10	20	--	8
6.	$I_{(f)}$	$C_{16}H_{13}NO_5$	--	--	--	8
7.	Chloramphenicol	--	24	25	27	22

Table 3: Antifungal activity for compounds $I_{(a)}$ – $I_{(f)}$.

S. N.	Compound No.	M. F.	Zone of inhibition (in mm)			
			Ca. guillermondii	*Ca. albicans*	*Ca. tropicalis*	*Ca. Crusel*
1.	$I_{(a)}$	$C_{16}H_{13}NO_4$	12	16	17	18
2.	$I_{(b)}$	$C_{17}H_{15}NO_5$	--	14	10	9
3.	$I_{(c)}$	$C_{14}H_{11}NO_5$	10	18	8	--
4.	$I_{(d)}$	$C_{18}H_{15}NO_4$	--	10	16	20
5.	$I_{(e)}$	$C_{16}H_{12}N_2O_6$	--	--	16	21
6.	$I_{(f)}$	$C_{16}H_{13}NO_5$	--	--	17	19

The products have been screened for their antibacterial activity against bacterial species *Staphylococcus aureus*, *Escherichia coli* & *Proteus vulgaris*. The antibacterial activity was carried out using cup-plate method (Kavanagh, 1963) by measuring the zones of inhibition in mm. each well (size 8 mm) was located with 0.5 ml of 1000 µg/ml test compound solution in DMSO solvent. Chloramphenicol was used as a standard drug for comparison. DMSO was run as a control and the result were recorded at the end of an incubation period 24 ± 2 hours. The results of antibacterial activity are shown in table 2.

The antifungal activity was carried out using saboura and dextrose. Agar (with chloramphenicol) medium. The zones of inhibition were recorded at the end of an incubation period of 48 hours. Organism used for antifungal activity included the pathogenic yeast species *Candida guillermondii, Candida albicans*, *Candida tropiculis* and *Candida crusei*. The results of antifungal activity are shown in table 3.

All chalcones exhibited good inhibitory activity against *E.coli* with highest antibacterial spectrum for compounds $I_{(c)}$ and $I_{(e)}$ comparatively all chalcones were near about effective like chloramphenicol towards *E.coli*. Only $I_{(d)}$ was effective against *Proteus vulgaris* were as $I_{(a)}$ and $I_{(d)}$ have good activity against *S. aureus* although they were less effective than chloramphenicol . Compound$_{(a)}$, $I_{(d)}$ and $I_{(e)}$ showed strong antifungal action against *Candida crusei* and moderate antifungal action against *Candida tropicalis*. Compound I(c) was effective against *Candida albicans*.

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Short Communication

TWO NEW SPECIES OF *THAXTERIELLA* PETR. FROM MELGHAT

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ABSTRACT:
This paper deals with two new species of Thaxteriella from Melghat district Amravati. All species were collected from different localities with aerial distance of approximately 15km.

Keywards : *Thaxteriella, bitunicate asci, ascospores septate.*

During mycological surveys, fungi were collected on different hosts, on examination, the collection was found to be new species of genus *Thaxteriella* Petr.
The fungi under investigation are caulicolus in nature. The specimens were wrapped in butter paper and bagged in separate envelops, with proper indexing. By taking hand sections, semi permanent microscopic slides were prepared by using cotton blue as stain, fine sections of the material were studied with the help of relevant keys and relevant literature (Barnett and Hunter 1972, Ainsworth *et al* 1973, Bilgrami *et al* 1979, Bilgrami *et al* 1991, Sarbhoy *et al* 1996, Jamaluddin *et al* 2004). The specimens were deposited in Agharkar Myclological Herbarium, Pune (AMH).

1. Thaxteriella melghatiella sp. nov. (Pl. 1)(Etymology : After locality - Melghat)Perithecia brunnea, globosa, immerse, magnit 198 - 362.5 x 306 - 351.9□*m*; asci, cylindracei, bitunicati, stipitatae, magnit 76-228.0 x 11.4-15.2 □*m*; ascospora oblongata, fusiformia pleuriseptata, olivaceo - brunnea, magnit 49.4-152.0x3.8-11.4 □*m* paraphysoidia hyaline et filiformia.

Perithecia brown, globose, immersed in subiculum, composed of thick walled, dark hypha which formed thick mat on the surface of the host, measure 198 - 362.5 x 306 - *351.9*□*m*; wall of th ascocarp composed of polygonal cells; asci cylindrical bitunicate with long flexuous stipe, measur 76 - 228.0 x 11.4 - 15.2□*m*, ascospores elongate, fusiform, multiseptate, with tapering ends, hyaline measure 49.4 - 152.0 x 3.8 -11. □*m*, paraphysoids hyaline and filiform.

Matrix : Found on dead stem of *Dendrocalamus stictuis Neer.* (Fam : Poaceae) legit D.V.H. at Semado (Melghat) on 10/04/99 AMH No. 8436.

2. *Thaxteriella satpudella* sp. nov. (Plate - 1)
(Etym :- After the mountain range - Satpuda)

Perithecia brunnea, globosa, immersa, magnit 350-425 x 330-335□*m*; asci cylindricei, bitunicati, stipitatae, magnit 105-120x5.7□*m*; ascospora oblongata, fusiformia, 35-40-2-3 □*m*; paraphysoidia hyalina et filiformia.

Perithecia brown, globose, immersed in a subiculum composed of thick walled dark hyphae which formed a thick mat on the surface of the host, measured 350-425 x 300- 325□*m*, wall of ascocarp composed of polygonal cells; asci cylindrical, bitunicate with long flexuous stipe measured 105-120 x 5-7 □*m*; ascospres elongate, fusiform, 10-15 septate with tapering ends, greenish-brown, measured 35-40 x 2-3 □*m*; paraphysoids hyaline and filiform.

Matrix : Found on dead stem of *Dendrocalamus stictus* Nees (Fam Poaceae) legit D.V.H. at Semadoh (Melghat) on 10/04/99. AMH NO. 8436

Table 1 : Comparative statement of species of *Thaxteriella*

Sr. No	Species	Perithecia	Asci	Ascospores	Reference
1	*T. indica* Dharne & Muller	306 - *476 ?m* in diam	247 - 285 x 22.8 -32.8 *?m*	60.8 - 91.2 x 9.5 - 15.2 *?m*	Narendra D.V. (1979
2	*T.silentvalliensis* Rao & Manivarghese	440 - 500 x 430 - *5l6? m*	150 - 235 x 25.5 -30.5 *?m*	60 - 87.5 x 11 - 12 *?m*	Rao & Manivarghesi (1979)
3	*T. eugeniae* Subhedar & Rao	230 - 350 *?m* in diam	140 - 180 x 16 -24 *?m*	44 - 60 x 8 - 10 *?m*	Subhedar & Rao (19
4	*T. lignic/a* Teng	180 - 280 *?m* in diam	95 - 130 x 14 -16 *?m*	30 - 48 x 5 -6.5 *?m*	Teng S.C. (1996)
5	*T melghatiella* sp. nov.	198-362.5x306-351.9 *?m*	76 - 228.0 x 11.4 -15.2 *?m*	49.4- 152.0x3.8-11.4/zm	under study
6	*T. satpudella* sp. nov.	350 - 425 x 330 - 335 *?m*	105- 120 x 5.7 *?m*	35 *-40x2-3 ?m*	under study

The comparison indicated that asci and ascospores in *T.melghatiella* are significantly larger and *in T.satpudella* are significantly small (vide table No. 1). Keeping in view, distinctness in morphology" of specimens under study, are treated as new species.

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Plate - 1



Fig (A)



Fig (B)

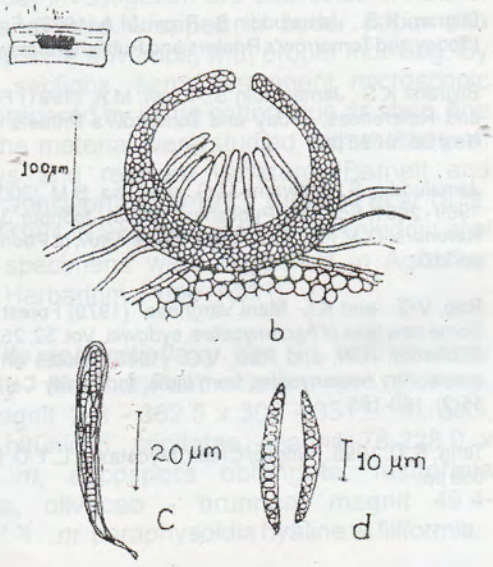


Fig. 1

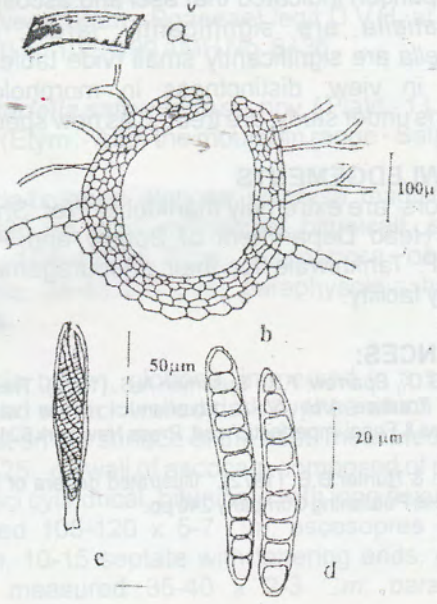


Fig. 2

***Thaxterella melghatiella* sp. nov. Fig (A) Dehisced perithecium,**

Fig. (B) *Bitunicate asci* with ascospore liberating (arrow.)

Fig. 1 *Thaxteriella melghatiella* sp. nov. (a) habit (b) perithecium (c) ascus (d) ascospore

Fig. 2 *Thaxteriella satpudella* sp. nov. (a) habit (b) perithecium (c) ascus (d) ascospore

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BIOSCIENCE BIOTECHNOLOGY RESEARCH COMMUNICATIONS

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Short Communication

Ecology of Pedhi River, District Amravati (Maharashtra)

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ABSTRACT:

Water is essential element for life on earth but due to rapid growth of population, urbanization and industrialization causes heavy and varied pollution, which directly or indirectly affects the living components. The contaminated water induces many health hazards causing many diseases. An estimated 80% children from villages suffer from waterborne diseases which is due to contamination of river waters. In this context, the present investigation on physico-chemical parameters of Pedhi river was carried out to observe the extent of pollution as the river is fed by open Amba Nullah sewerage of a metro city, Amravati.

Key words: *Pedhi river, physcio-chemical parameter.*

In recent years due to the population growth and heavy urbanization rapid degradation of lakes and rivers has occurred (Kodarkar,1995, Bhave and Borse, 2001 and Boddey *et al.*, 2007). Pedhi river receives the sewerage through Amba nullah of metro city Amravti,which is highly polluted by faecal matter.Interestingly the water from Amba nullah is largly used for irrigation purposes. If properly managed the water can be used for irrigation as well as for fisheries as reported by several earlier workers (Ali, 2000 and Pista, 2000)

Pedhi river was selected for the present study which is 14 km away from Amravati city and receives sewerage in it. The geographical position of the river lies 20°,56'00"N and 77°,38'00"E.

Four sampling stations were selected for investigation.

Station –A-Amba nullah water
Station-B-At junction.(nullah & river)
Station-C-down stream.
Station-D-Up stream.

Physico-chemical characteristics were analyzed on monthly basis during 2008-09.Physical and chemical parameters like water temperature ,pH, turbidity and dissolved oxygen were analyzed using water analysis kit "Systronic Make" at the sampling site, while total alkalinity, total hardness, chloride and nitrates were examined in the laboratory using standard methods prescribed by APHA(1996) and Trivedi and Goel(1988)

It was found that the water temperature of all the station remain unfluctuated and was in the range from 21.1°c minimum to 25.1°c maximum. Water pH showed acidic nature to slightly alkaline. At Station-A & Station-C turbidity was higher. The D.O was in the range from 6.48 mg/L to 8.29mg/L.The river water is alkaline in nature Chlorine content of water found below the permissible limit similarly nitrate content of river was identical at all the stations with little fluctuation.(Table 1).

In the present investigation examined water temperature from all the four stations were not much fluctuated, however minimum water temparature recorded during winter months it might be due to the shorter photo period. Kannan and Jog (1980)also observed decline in water temperature during winter and an increase in temperature during summer months.

The acidic pH observed mostly at all sampling sites could be correlated with finding of Bobdey *et al.* (2007) they observed neutral pH even to suspended inorganic,organic decay matter which is hazardous to fish death. Sustainable life of water organisms highly depends upon oxygen present in water in dissolved state,ulteration may cause respiration of animals, plants and bacteria, photosynthesis by green vegetation and also causing depletion habitation of flora and fauna(Piska,2000).

Seasonal range and mean for all the observed parameters are shown in table-1

Table 1: MEAN ±SE AND SEASONAL RANGE OF VARIOUS PHYSICO-CHEMICAL PARAMETERS FROM DIFFERENT STATIONS OF PEDHI RIVER.

Sr. No.	Parameters	Station			
		A	B	C	D
01	Water Temperature°C	(21.4-25.1) 23.22 ±0.373	(21.3-24.1) 23.06 ±0.373	(21.4-24.1) 22.83 ±0.318	(21.1-24.6) 22.93 ±0.369
02	pH	(6.1-7.4) 6.46±0.13	(6.02-7.2) 6.46±0.107	(6.29-7.31) 7.03±0.1	(6.14-7.58) 6.96±0.159
03	Turbidity(NTU)	(44.11-53.72) 49.09±1.084	(46.22-52.55) 48.82±0.746	(43.89-54.78) 49.22±1.304	(42.67-53.17) 49.28±1.11
04	Dissolved Oxygen(mg/l)	(6.78-8.27) 7.42±0.144	(6.59-8.21) 7.32±0.164	(6.46-8.21) 7.63±0.226	(6.69-8.29) 7.69±0.172
05	Total Alkalinity(mg/l)	(140-338) 191.11±22.33	(132-268) 183.11±13.58	(142-304) 187.11±17.67	(136-268_ 183.11±15.17
06	Total Hardness(mg/l)	(60-140) 91.11±9.2	(62-132) 94.00±8.192	(60-144) 94.67±9.27	(62-136) 87.33±8.02
07	Calcium Hardness(mg/l)	(28.6-58.9) 37.56±3.16	(31.1-8) 39.23±2.97	(26.9-47.9) 35.29±2.25	(26-136) 33.87±2
08	Magnesium Hardness(mg/l)	(5.4-19.7) 13.01±1.77	(5.9-22.2) 13.60±1.8	(6.2-23.4) 14.44±1.95	(6.5-26.2) 14.11±2.06
09	Chloride(mg/l)	(13.01-145.1) 78.96±16.29	(18.01-119.1 78.63±14.2	(020.02-115.1) 67.06±12.76	(21.02-111.1) 59.95±10.3
10	Nitrate(mg/l)	(0.61-0.78) 0.70±0.018	(0.66-0.80) 0.71±0.019	(0.64-0.80) 0.72±0.021	(0.62-0.79) 0.70±0.021

(Figures in parenthesis are minimum and maximum ranges.)

In the present study no definite trend was noted, however D.O was found more during winter months which can be attributed to some pattern as observed by Singhai *et al.* (1990), Bhave and Borse (2001). Total alkalinity was found to be mainly contributed by carbonates in the water.

Alkaline water is significant for biological activities and water of Pedhi river is hard which may be due to continuous accumulation of salts. The higher values can be attributed to influx of domestic waste through adjoining Amba nullah. Sakhare and Survase (2001) reported higher values in Papnas wetland in Tuljapur town. Chloride content in water might be the effect of accumulation of organic matter of animal origin.In the present study random variation in nitrates was observed and no definite pattern was seen; however, higher values of nitrate were recorded during winter months which agrees with values observed by Singhai *et al.* (1990).

Looking towards the state of water quality of Pedhi river which receives domestic sewerage from corporate Amravati city the water can be used for irrigation but will be unhygienic for human health.

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SHORT COMMUNICATION

IDENTIFICATION AND QUANTIFICATION OF THYMOQUINONE FROM THE SEEDS OF *Nigella sativa* LINN.

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ABSTRACT:

Nigella sativa, a grassy plant commonly found in India is widely used for its therapeutic purpose. High Performance Liquid Chromatography (HPLC) of the aqueous water extract of seeds of *Nigella sativa* revealed the presence of the active ingredient, thymoquinone in significant quantity.

Key words: *Seeds Nigella sativa, HPLC.*

Nigella sative,(Black cumin) locally known as Kalaunji is a grassy plant with green to blue colored flowers and small black seeds that grows in temperate and cold climates. The plant is widely grown in different parts of the world and is an annual herb cultivated in India. As an oriental spice, *Nigella sativa* has long been used as a natural medicine for treatment of many acute as well as chronic conditions. Its seeds are believed to have carminative, stimulatory and diaphoretic properties (Chopra *et al.,* 1956, El-Alfy *et al.,* 1975 and Hailat *etal.*,1995). The seeds of *N. sativa* have been subjected to a range of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as antibacterial (Ferdous *et al.*, 1992, Hanafy and Hatem, 1991, Rathee *et al.*, 1982), antitumor (David *et al.*, 1998), anti-inflammatory(Houghton *et al.*, 1995, Mutabagani and El-Mahdy, 1997), CNS depressant and analgesic (Khanna *et al.*, 1993), hypoglycemic (Al-Hader *et al.*, 1993), smooth muscles relaxant (Aqel, 1993, Aqel, 1995, Aqel and Shaheen, 1996) cytotoxic and immunostimulant (Swamy and Tan, 2000). The therapeutic effect of *Nigella sativa* oil on patients with allergic diseases(allergic rhinitis, bronchial asthma, atopic eczema) have been reported(Kalus *et al.*,2003).In addition , Labib 2005 in a recent review has summarized the therapeutic properties of *Nigella sativa* L. Seed and emphasized the potent immunomodulatory effects of this plant. The health enhancing potential of black cumin has been attributed to the active ingredients that are mainly concentrated in fixed and essential oil (Ramadan, 2007). This plant has been a great focus of research for centuries and has been extensively studied for its chemical constituents and biological activities. From the oil of its seeds (Nigellidine), sterols like cholesterol, campesterol, stigmasterol and ß-sitosterol have been identified (Salama, 1973 and Rehman *et al.,*1995).In the present study we report the presence of thymoquinone in the aqueous water extract of the plant *Nigella sativa*.

The seeds were purchased from the local market and identified in the Botany department of Saifia College of Science, Bhopal. The seeds were dried at room temperature in the absence of sunlight. The macerated aqueous extract was prepared as follows: one hundred grams of *Nigella sativa* seeds were grinded, added to 250 ml distilled water and was kept at room temperature for 48 hours while shaken intermittently. The solution was filtered, centrifuged (2000xg, 20°C), lyophilized in freeze dryer and stored at -20 °C. The lyophilized plant extract was analysed at the Minor Forest Produce Processing and Research Centre (MFP PARC), Bhopal by HPLC. The HPLC analysis was performed on a Perkin Elmer instrument, column used was RP-18 with particle size of 5µm. Compositions of solvent mixtures and flow-rates were adjusted as necessary to maximize separations. All the chemicals used are of HPLC grade.

The lyophilized extract of *Nigella sativa* seeds gave a brownish dried extract as 2.136 gram of the extract was obtained after lyophilization. Thymoquinone is one of the most promising active ingredients of *Nigella sativa*. The separation of water extracts of *Nigella sativa* (seed) under optimized conditions is shown in Fig. 1. Thymoquinone (Sigma-Aldrich) was chosen as marker compound for standardization purpose. The separation of thymoquinone was achieved on a RP-18 column using water:methanol:2-propanol (50:45:5%v/v) at a flow rate of 1ml/min. Thymoquinone was monitored by UV detection at 254 nm. Using a reversed-phase HPLC system, the amount of thymoquinone in lyophilized *Nigella sativa* extracts was quantified and was 0.0356%. Ghosheh *et al.* (1999) carried out High Performance Liquid Chromatography (HPLC) analysis of the oil of *Nigella sativa* using the isocratic mobile phase of water-methanol-2-propanol (50:45:5%v/v) which revealed that the concentration of different constituents in the oil including thymoquinone.

Thymoquinone is more concentrated in black cumin essential

oil ranging from 18-24% (Burits and Bucar, 2000) whereas extraction of fixed oil by means of water retains meager quantity as in case of present investigation. Some organic extracts of black cumin seed also reported to possess appreciable amounts of thymoquinone. In one such study, Singh *et al.* (2005) estimated thymoquinone contents of 11.8% in acetone extract seed. Moreover, thymoquinone belongs to class of compounds known as terpenoids; most members are volatile in nature and their heating losses ranged from 20-50% (Bendahou *et al.*, 2008) that could also be a possible reason for lower quantity of thymoquinone in extract and the solubility in water too. The purpose of our study is to show that significant concentration of thymoquinone in the aqueous water extract of *Nigella sativa* exists which can be used for various therapeutic purposes

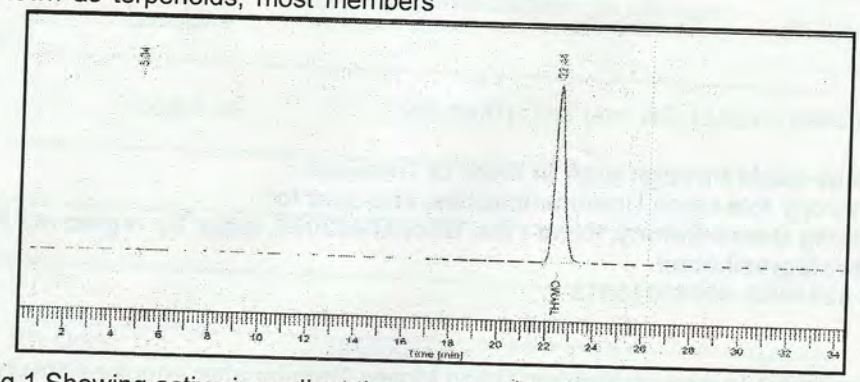


Fig 1 Showing active ingredient thymoquinone from water extract of *N. sativa*

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