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RESEARCH ARTICLE

Effect of Fluoridated versus Non Fluoridated Homeopathic Dentifrice on Enamel Micro Hardness: an *In vitro* Study

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ABSTRACT

Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain. The remineralization produced by saliva is less and also a slow process, therefore remineralizing agents are required.The aim of the study was to check the remineralizing efficacy of commercially available homeopathic dentifrice on enamel micro hardness of primary teeth and to compare the remineralizing potential of commercially available fluoridated dentifrice and non-fluoridated homeopathic dentifrice.A total of 20 teeth were sectioned into equal parts with a diamond disc.

The 40 sections obtained were then evaluated under the Vickers microhardness indenter for baseline microhardness of enamel. The 40 Sections coated with a nail varnish leaving a window of 1 mm were subjected to demineralisation for 72 hours.The sections were then again evaluated under the Vickers microhardness indenter and the hardness after the demineralisation noted. The 40 sections were divided into 2 groups: Group 1 – Kidodent(child formula fluoride dentifrice), Group 2 – Fresh gel(non fluoridated homeopathic dentifrice) and subjected to remineralisation respectively for 7 days. The specimens were again evaluated under the vickers microhardness indenter for the remineralisation values.

The remineralising values were significantly higher in Kidodent group than Fresh gel.Based on the results obtained from the present study, the child formula dentifrices containing NaF have the ability to remineralize the initial carious lesions in the primary teeth. There is need for more studies with different

analytical techniques to study the remineralisation potential of the homeopathic dentifrice and compare them with the other remineralizing agents.

Keywords: Fluoridated, Homeopathic non-fluoridated, Enamel, Micro hardness

INTRODUCTION

Dental caries is the infectious microbial disease resulting in dissolution and destruction of the tooth.Early childhood caries, is the presence of 1 or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger[1]. It is the most common oral disease of children. It can rapidly develop and causes several health problems in children.

White spot lesion, the earliest clinical sign of dental caries, is characterized by enamel demineralization of the subsurface, with increasing porosity due to removal of minerals into the outer surface.Remineralization is the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain[2].Throughout the dental caries process beneath the enamel, it is subjected to repeated demineralization and remineralization, cycles of unknown intensity and duration.The primary teeth are more susceptible to caries development because of lower mineral and higher organic content of enamel[3].

Fluoride-containing toothpaste was introduced in industrialized countries during the late 1960s and is today the most common vehicle delivering fluoride to the oral cavity[4].Fluoride dentifrices are the most widely used products that deliver topical fluoride to the oral environment. The cariostatic effect of fluoride is primarily due to its ability to decrease the rate of demineralization by forming Fluor hydroxyapatite and enhancing the remineralisation of incipient carious lesions[5].

Fluoride can enhance the process of remineralization when used properly. However, repeated ingestion of fluoride can result in chronic fluoride toxicity, the most common manifestation of which is dental fluorosis[6].The analysis of these issues, especially fluorosis, shows that new therapeutic approaches must be tested. Therefore, an appropriate nonfluorideanticaries agent is the demand of time.Homeopathy is the second largest system of medicine in the today's world recognized by the World Health Organization. It is an emerging field of dental medicine that is useful in management of conditions affecting orofacial structures[7].

Various homeopathic medicines have been also used systemically such as CalcareaPhosphorica (calc-p), CalcareaFluorica (calc-f) for the treatment of various dental problems as they contain mineral salts that they play role in the mineralization of teeth and bone[8].The leaves of plantago major contain mucilage, tannin and silic acid. An extract of them has antibacterial properties, anti-plaque and anti-inflammatory activity[9].Plantago tincture is a remedy that most beneficial when rubbed onto or around a tooth or teeth that are sensitive to hot or cold, or applied into cavity[10].Kreosotum is useful for premature decay of milk teeth and rapidly occurring decay in primary teeth[11].

MATERIALS AND METHODS

Armamentarium

The following armamentarium was used for the study:

- Stainless Steel Moulds, Cold setting Resin and Hardener and Extracted Teeth were used for the preparation of the enamel blocks.
- Dentifrices - Non Fluoridated homeopathic dentifrice (group 1), Sodium Monoflorophosphate (group 2)
- 1% Citric Acid maintained at 3.3 pH (Demineralization Medium)
- Self-Prepared Artificial Saliva (Remineralisation Medium)
- Distilled Water (Rinsing Enamel Blocks)
- Vickers Micro indenter

Selection of Teeth

Ten caries free primary teeth (either exfoliated or extracted) were selected. Carious, hypoplasticdiscolored teeth and teeth with cracked areas and white spots were excluded. Teeth were stored in 10% formalin.

Sample Preparation

The teeth were decoronated at cemento-enamel junction.Then sectioned mesio-distally into two halves using a high speed diamond disc. The samples were equally divided into two groups. The samples were mounted in moulds filled with self-cure acrylic resin and polished. A Vickers micro hardness indenter was used to evaluate the baseline micro hardness under 50 g loads was applied to the surface for 5 seconds.

Demineralization

Teeth were then dried and coated with an acid resistant nail varnish, leaving a rectangular window 4 cm × 3 mm wide for demineralization, on the buccal or lingual surface. They were then completely immersed in 20ml demineralizing solution for 96 h to produce artificial carious lesions. This was in accordance to Ten Cate and Duijsters pH cycle.Demineralizing solution was prepared using the following chemicals: 2.2 mM calcium chloride, 2.2 mM potassium hydrogen orthophosphate, unstirred solution of 0.05 M acetic acid and 1 M potassium hydroxide pH at 4-5. 1050 ml of distilled water was taken in a beaker and 2.2 g of calcium chloride was added to it. To this, 2.2 g potassium hydrogen orthophosphate, 3 g of acetic acid and 56 g of potassium hydroxide was added. Thiswas in accordance the demineralizing solution used in the pH cycle used by Ten Cate and Duijsters.Following demineralization, surface micro hardness measurements were made using the VH indenter.

7 days dentifrice treatment

Enamel blocks were immersed into the freshly prepared dentifrice slurries (5 gmofdentifrice + 10 ml of artificial saliva) for 2 minutes. The daily cycling regimen comprised of 3 x 1 min acid challenges and 2 x 2 min treatment periods. Rinsing with Distilled water and replacing into artificial saliva and SMH was re-measured after 7 days.

Statistical Analysis

- All the statistical results were calculated according to SPSS software.
- The statistical analysis was done by using Independent sample T test.
- P value ≤ 0.05 was considered as significant

RESULTS AND DISCUSSION

Ten sections in group A were treated with kidodent (contains 500 ppm NaF) and ten sections in group B were treated with fresh gel (homeopathic non fluoridated dentifrice). At baseline, mean value in group A was 306.60 and in group B 305.93. The mean difference was 0.67.After demineralization, there was decrease in the values in both the groups. In group A, the mean value was 214.89 and in group B, the mean value was 217.01. The mean difference between group A and group B was -2.12. After remineralization, there was significant increase in values in group A than group B. The mean value was 233.29 in group A and 217.90 in group B. The mean difference between group A and group B was 15.39($P<0.001$)

In the presence of the fluoride, the hardness of remineralized samples increased significantly in comparison to the enamel hardness in the other non-fluoridated homeopathic group. Surface micro hardness is a physical property which assesses the effect of chemical and physical agents on hard tissues of 0teeth. It is an appropriate test for enamel due to its fine microstructure, non-homogenous and brittle nature. Micro hardness indentation provides a relatively simple, rapid and non-destructive method in demineralization and remineralization studies.

VHN was adopted as the basis for investigation over Knoop'sbecause the square shape of indent obtained in VHN is more accurate to measure. Extracted or naturally exfoliated primary incisors were used for lesion formation. So that there will not be any changes in the process of demineralization.Single-section model, had the advantage that a single section was fully evaluated prior and after the exposure period. Thus, any change was only due to exposure of the experimental solutions[12].

The concept of *in vitro* pH cycling was first proposed by Ten Cate and Duijsters in 1982, in experiments where they exposed artificial carious lesions in enamel to a combination of remineralizing and demineralizing solutions. These experiments were designed to stimulate the dynamic variations in mineral saturation and pH associated with the natural carious process. As fluoride dentifrices have a dose – response relationship; this is important because dentifrices for children usually contain between 250 and 500 ppmfluoride, in order to reduce the risk of fluorosis[13].

GoelPankajet al[14] suggested that kreosotum is useful for premature decay of milk teeth.Modesto A et al[15] suggested that extracts of calendula officinalis has anti-microbial activity which can be useful in caries prevention.KalpanaBansal et al[8] evaluated the effectiveness of homeopathic CalcareaFluorica (calc-f) tablets as remineralizing agents on artificial carious lesions using scanning electron microscope (SEM) and surface microhardness (SMH) testing. They concluded that the calc-f tablets can be used as safe and cost effective remineralizing agent but there is need for more studies with different analytical techniques to study the remineralization potential of the homeopathic remedies and compare them with the other remineralizin agents such as fluoride and CPP-ACP.

Almeida et al[16] evaluated the effects of homeopathic medicines on teeth of rats that were fed on cariogenic diet. They proposed the hypothesis for the presence of deposits on the surface of the teeth of rats that were treated with these medicines viz.; calc-p, nat-f, kreos, calc-f, and 0.05% NaF. They also stated that the deposits are due to the fact that these medicinal substances are more related to the physiopathology of caries (pathogenesis). The exaggerated medicinal stimulation that is, daily administration of medicine for 35 days resulted in a form of aggravation represented by the deposit.

CONCLUSION

Based on the results obtained from the present study, the child formula dentifrices containing NaF have the ability to remineralize the initial carious lesions in the primary teeth.There is need for more studies with different analytical techniques to study the remineralization potential of the homeopathic dentifrice and compare them with the other remineralizing agents.

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Table 1: Enamel hardness values (VHN) of at baseline, after demineralized and remineralized enamel samples in Group I and Group II

	Group	N	Mean	Mean Difference	P Value
Baseline	Kidodent	10	306.60	0.67	0.887
	Fresh gel	10	305.93		
After demineralization	Kidodent	10	214.89	-2.12	0.415
	Fresh gel	10	217.01		
After dentifrice application	Kidodent	10	233.29	15.39	<0.001
	Fresh gel	10	217.90		

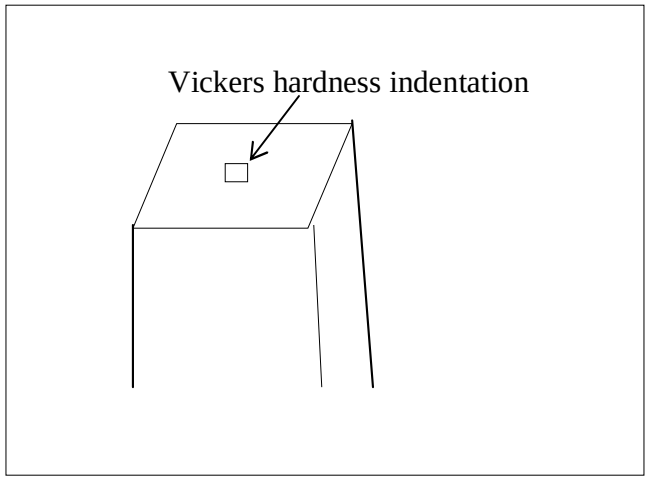

Figure 1

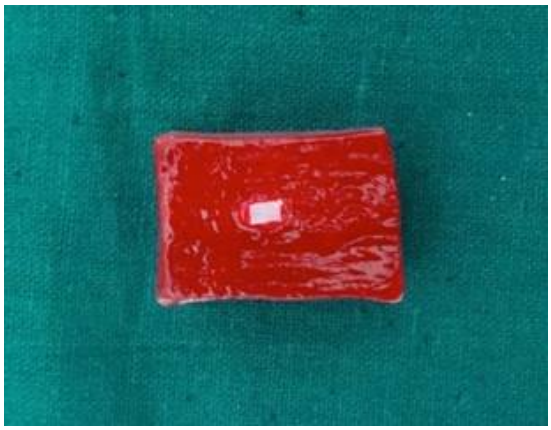

Figure 2.Readings Obtained on Enamel Block using Vickers Micro-indenter

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REVIEW ARTICLE

Effects of Chemical Pesticides

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ABSTRACT

Chemical insecticides have been used for a long time. Their use has been recorded in the epic odyssey (about 1000 B.C.). The era of modern chemical pesticides began with the discovery of organophosphates in 1939 by Paul Muller.Ever since many different classes of chemical insecticides have been developed. In this review I have studied some of the widely used classes of chemical insecticides like pyrethroids, organophosphates, organochlorines, carbamates, neonicotinoids, formamidines and phenylpyrazoles. I have studied about their mechanism of action and their harmful effects on human bodies. I have also tried to find what effects they have on the non-target organisms like birds. This review shows the role of neonicotinoids in the decrease of honey bee populations.Pyrethroids which are considered safer than most of the chemicals insecticides are very toxic to fish and aquatic organisms. Organophosphates are known to be toxic to children. They also affect birds. Organochlorines have wide ranging environmental effects like biomagnification. They are also considered to be carcinogenic. Carbamates are also highly toxic with their effects similar to that of organophosphates. However many countries including are taking steps to regulate the use of chemical insecticides.

Keywords : Insecticides, Pesticides, Pyrethroids, Populations, Organophosphates.

INTRODUCTION

Insecticides are particular class pesticides that are used to kill insects. Insecticides and other pesticides are some of the most important chemicals used for the well-being of mankind. They are indispensable in maintaining the high levels of health, nutrition and quality surrounding. In agricultural production, pesticides are a regular component of most systems, and their development has given rise to entirely new ways of growing crops. The energy and soil conserving approaches of modern agricultural would not have been possible without them. One of the first records of insecticides is found written in the Ebers Papyrus, written about 1500 B.C., which lists the preparations to expel

fleas from the house. The oldest available record is Homer's mention (about 1000 B.C.) that Odysseus burned sulphur ". . . to purge the hall and the house and the court" (Odyssey XXII, 493–494).There are records showing that by 900 A.D. the Chinese were using arsenic sulphides to control garden insects. In 1669, the earliest known record of arsenic as an insecticide in the Western world mentioned its use with honey as an ant bait. Use of tobacco as contact insecticide for plant lice was mentioned later in the same century.

The period between 1935 and 1950 was characterized by the development of major classes of pesticides, particularly insecticides. Müller (1939) found that DDT (dichlorodiphenyltrichloroethane), which had been first synthesized in 1874, acted as a poison on flies, mosquitoes, and other insects. DDT was commercialized in 1942 and was used extensively and successfully for the control of typhus epidemics and particularly of malaria. Together with DDT, other chlorinated hydrocarbon insecticides were developed. In the early 1940s, scientists in England and France recognized the gamma isomer of hexachlorocyclohexane, commonly known as lindane, which had been first synthesized in 1825 by Faraday, as a highly potent insecticide (Ecobichon, 1992). Starting in the mid-1940s several other chlorinated insecticides were commercialized, including chlordane, heptachlor, aldrin, and dieldrin.The organophosphorus insecticides were first synthesized in Germany in the late 1930s. Gerhard Schrader, a chemist at the I. G. Farben Industrie in Germany, is considered the "father" of organophosphorus insecticides. The first one, tetraethyl pyrophosphate (TEPP), was brought to the market in 1944. Several thousand molecules were synthesized, and one was eventually introduced into the agricultural market under the trade name parathion, to become one of the most widely employed insecticides in this class. During those years, compounds of much greater toxicity than parathion, such as sarin were synthesized as potential chemical warfare agents. The mechanism of action of organophosphates, inhibition of acetyl cholinesterase, was soon discovered, primarily by knowledge of the effects and mechanism of action of physostigmine. This alkaloid had been isolated in 1864 from Calabar beans, the seeds of Physostigma venenosum, a perennial plant in tropical West Africa (Casida, 1964).

Insecticides enter the soil via spray drift during foliage treatment, wash-off from treated foliage, release from granulates or from treated seeds in soil. Some insecticides are applied directly into soil to control pests and plant diseases presented in soil. Persistence of these insecticides in soil can vary from few hours to many years in case of OC insecticides. Despite OC insecticides being banned or restricted in many countries, they are still detected in soils (Shegunova *et al.*,2007; Toan *et al.*,2007; Li *et al.*,2008; Hildebrandt *et al.*,2009; Jiang *et al.*,2009; Ferencz and Balog 2010). Soil microorganisms play a key role in maintaining the mineral balance of the soil. Inhibition of species, which provide key process, can have a significant impact on function of whole terrestrial ecosystem. A few studies show that some organochlorine insecticides suppress symbiotic nitrogen fixation resulting in lower crop yields. These explain the trend in the past 40 years toward stagnant crop yields despite record high use of insecticides and synthetic fertilizers worldwide (Fox *et al.*,2007; Potera, 2007). A recent review of insecticides effects on earthworms showed on negative effects on growth and reproduction by many insecticides (Shahla and D'Souza, 2010). Decline of wild bees have been reported after repeated application of insecticide (Brittain *et al.*,2010). Insecticides can get into water via drift during insecticide spraying, by runoff from treated area, leaching through the soil. Insecticides can be applied directly onto water surface e.g. for control of mosquitoes. Insecticides usually occurred in mixture of multiple compounds, even if individual insecticide were detected bellow limits. This potentially can lead to underestimation of toxicity when assessments are based on individual compounds. Insecticide contamination results in significant changes of structure of lotic communities. Neurotoxic insecticides exhibited the strongest drift-initiating effects on stream-dwelling insects and crustaceans. Decline of farmland bird species has been reported over several past decades due to the increased application of insecticides.

Types of insecticides

Several other classes of insecticides (pyrethroids, neonicotinoids, N-phenylpyrazoles) have also been developed in the past few decades. However, insecticides do have some serious disadvantages. Insecticide resistance, pest

resurgence and pest replacement can occur due to frequent use of these compounds. These chemicals are also toxic to humans and can cause damage and even death if applied improperly.

Pyrethroids

Pyrethroids are one of the fastest developing groups of modern insecticides. They are replacing the older insecticides because of their ease in application and their effectiveness. The term pyrethroids comes from the words "Pyrethrum" and "oid" meaning something that resembles something else. In other words it means pesticides that resemble pyrethrum.

Chemistry of pyrethroids

Pyrethroids alter the normal functioning of insect nerves by modifying the kinetics of voltage sensitive sodium channels, which mediate the transient increase in sodium permeability of the nerve membrane that lead to an action potential (Soderlund *et al.,*2002). All pyrethroid insecticides contain an acid moiety, a central ester bond and an alcohol moiety (Fig 1). The acid moiety contains two chiral carbons, thus pyrethroids typically exist as stereoisomeric compounds (trans and cis). Some pyrethroids also have a chiral carbon on the alcohol moiety, allowing for a total of eight different stereoenantiomers. These chemical considerations are relevant, as pyrethroids' effects on the sodium channels, their insecticidal activity, and their mammalian toxicity are stereospecific. The cis isomers are generally more toxic than the corresponding trans isomers (Casida *et al.,*1983). The acute oral mammalian toxicity of pyrethroids is generally low. Values of LD_{50} range from 100mg/kg (Deltamethrin) to 10000 mg/kg (Phenothrin). To underline the relevance of stereospecificity, the LD_{50} trans-resmethrin is 8000 mg/kg but that of the cis-resmethrin is 100 mg/kg (Casida *et al.,*1983).

MODE OF ACTION

The low mammalian toxicity of pyrethroids is confirmed by the fact that despite the widespread use of pyrethroids there are relatively few reports of human poisonings (Bradberry *et al.,*2005). Most deaths however occurred due to accidental or intentional exposure to pyrethroids. Like the 45 year old man who died three hours after eating beans and cheese prepared by using 10% cypermethrin solution instead of oil (Poulos *et. al.,*1982). The dermal toxicity of pyrethroids is seen to be lower because of limited absorption trough skin. Upon absorption, pyrethroids are very rapidly metabolized through two biotransformation routes:

- hydrolysis of the ester linkage, which is catalysed by hepatic and plasma carboxylesterase
- oxidation of the alcohol moiety by cytochrome P450 (Miyamoto, 1976; Soderlund and Casida, 1977).

These initial reactions are followed by further oxidation, hydrolysis and conjugation with sulphate or glucuronide. The relative importance of the hydrolytic or oxidative biotransformation varies from compound to compound, and from isomer to isomer for each pyrethroids. For example, the trans isomer of permethrin is more susceptible to hydrolysis than the cis isomer (Soderlund and Casida, 1977; Ross *et al.,*2006). Type II pyrethroids are less susceptible to hydrolysis. For example deltamethrin which is type II pyrethroid containing a cyano group (Fig. 1), and present solely as the cis isomer is more extensively metabolized by hepatic cytochromes P450, particularly CYP1A2 and CYP1A1 (V_{max}/K_m = 34.9) than by carboxylesterase (V_{max}/K_m = 11.5) (Anand *et al.,*2006). Though it has been suggested that oxidative metabolism may lead to in certain cases, to bioactivation of certain pyrethroids (Dayal *et al.,*2003; Ray and Fry, 2006), the current line of evidence would suggest that hydrolytic and oxidative metabolism achieve detoxification of the parent, active compound (Soderlund *et al.,*2002). Inhibition of cytochrome P450 by piperonyl butoxide indeed increases pyrethroid toxicity, and so does inhibition of carboxylesterase (Casida *et al.,* 1983). Piperonyl butoxide is added to most pyrethroid formulations as a synergist. Inhibition of carboylesterase may be of significance, if unauthorized pyrethroid/organophosphate mixtures are utilized (Ray and Forshaw, 2000). In fact, several organophosphates inhibit carboxylesterase activity, and may thus be expected to potentiate pyrethroid toxicity (Choi *et al.,*2004).

The mode of action of pyrethroids in mammals is the same as in insects, disruption of the voltage-gated sodium channels (Narahashi, 1996). Pyrethroids bind to the alpha subunit of the sodium channels and slow the activation (opening), as well as the rate of inactivation (closing) of the sodium channel, leading to a stable hyperexcitable state. Sodium channels then open at more hyperpolarized potentials, and are held open longer, allowing more sodium to cross and depolarize the neuronal membrane (Shafer *et al.,* 2005). In general, type II compound delay the inactivation of sodium channels substantially (>10 ms) than do type I compounds (<10 ms) (Ray and Fry, 2006). Type I compound prolong channel opening only long enough to cause repetitive firing of action potential (repetitive discharge), analogous to DDT (Vijevberg *et al.,* 1982); type II compound hold the channels open for such long periods that the membrane potential ultimately becomes depolarized to the point at which generation of action potential is not possible (depolarization-dependent block) (Shafer *et al.,* 2005). The difference in the time of opening of sodium channels are believed to be at the basis of the difference of the differences observed between the T and CS syndromes (Ray and Fry, 2006). The higher of insects to pyrethroid toxicity, compared to mammals, is believed to result from a combination of high sensitivity of insect sodium channels, lower body temperature, and slower biotransformation (Ray and Fry, 2006). Type II pyrethroids, but not type I compounds, also bind to, and inhibit GABA-gated chloride channels (Lawrence and Casida, 1983), at higher concentrations than those sufficient to affect sodium channels. This effect is believed to contribute to the seizures that accompany severe type II pyrethroid poisoning. However, drugs that enhance GABAergic transmissions like diazepam have modest effects towards deltamethrin-induced choreoatetosis or seizures. Type II pyrethroids, such as deltamethrin, also inhibit at low concentration (10^{-10}M) voltage dependent chloride channels (Forshaw *et al.,* 1993). Because chemicals that open these chloride channels, like ivernectin and pentobarbital, antagonize pyrethroid-induced choreoatetosis and salivation, inhibition of maxi voltage-dependent chloride channels by pyrethroids may contribute to the type II poisoning syndrome (Forshaw *et al.,*2000) (Table 1).

EFFECT ON NON-TARGET ORGANISMS AND ITS TOXICITY

Pyrethroid insecticides are a potent group of chemicals used to control insect pests in agricultural and aquatic systems. The mode of entry of pyrethroids into aquatic habitats is both direct and indirect. Direct sources include purposeful applications of pyrethroids in vector control as well as agricultural and silvicultural pest control programs. Indirect means through which water bodies could be contaminated with pyrethroid residues include spray drift, runoff and erosion.

Young mammals are more prone to the acute toxicity of certain pyrethroids, such as deltamethrin and cypermethrin (Sheets, 2000), most likely because of a lesser capacity for metabolic detoxification (Anand *et al.,* 2006); however, only minor age-related differences were found for other compounds (Sheets, 2000). Some studies have suggested that certain pyrethroids may cause developmental neurotoxicity, but current evidence has been judged inadequate (Shafer *et al.,* 2005). Furthermore, levels of background pyrethroid exposure (presumably through residues in the diet) in children have been found to be of orders of magnitude lower than the corresponding acceptable daily intake (Heudorf *et al.,* 2004). Also, the use of deltamethrin-impregnated bed nets does not appear to pose any health risk to children and neonates (Barlow *et al.,* 2001), while substantially reducing infant mortality from malaria (Alonso *et al.,* 1991).Upon occupational exposure, the primary adverse effect resulting from dermal contact with pyrethroids is paresthesia (Flannigan *et al.,* 1985; He *et al.,* 1989). Symptoms include continuous tingling or pricking or, when more severe, burning. The condition reverses in about 24 hours, and topical application of Vitamin E has been shown to be an effective treatment. Paresthesia is presumably due to abnormal pyrethroid-induced repetitive activity in skin nerve terminals (Ray and Fry, 2006). Chronic studies with pyrethroids indicate that at high dose levels they cause slight liver enlargement often accompanied by some histopathological changes. There is little evidence of teratogenicity and mutagenicity (Miyamoto, 1976; Ray, 1991). An increased rate of lymphoma incidence in rodents has been reported for deltamethrin, but the effect was not dose-dependent (Cabral *et al.,* 1990). There is no compelling evidence that pyrethroids may acts as endocrine disruptors (Kim *et al.,* 2004).

Pyrethroids are particularly toxic to fish but not to birds (Miyamoto, 1976). Aquatic insects are also susceptible to pyrethroids. Their extreme toxicity of pyrethroids to aquatic organisms hinders the use of pyrethroids in agriculture (Coats *et al.,* 1989; Mugni *et al.,* 2013). Aquatic insects are highly sensitive to insecticide poisoning even at extremely low concentrations (Coats *et al.,* 1989; Anderson, 1982, 1989; Mian and Mulla, 1992). The pyrethroids are very toxic to fish in fact they are more toxic than organophosphorus and carbamate insecticides. The high acute toxicity of pyrethroids to aquatic invertebrates and fish limit their use in areas near aquatic habitats. This has raised concerns over the registration of new pyrethroids by the United States Environmental Protection Agency. Picket (a permethrin product) was found to cause a significant drop in larval densities and emergence of adult midges, *Chironomus riparius* (Diptera: Chironomidae) in ponds treated >10mg/L. Older larvae survived to emergence, but younger larvae did not (Conrad *et al.,*1999). Conrad *et al.,* (1999) observed that emergence of adult midges from treated ponds resumed within four weeks and that emergence levels were comparable to that in the control pond within two months. The recovery of the midges may be due to the short life cycle of midges and the reduction of permethrin toxicity within the pond ecosystems as a result of rapid degradation or reduction of bioavailability from the water column. Similarly permethrin application to lakes, streams and ponds affected the insect population of the ephemeroptrans and the odonates for only a brief period with recovery occurring a few weeks to a few months after treatment (Mian and Mulla, 1992). Based on intrinsic sensitivity, biological traits, mode of action, members of Ephemeroptra, Plecoptera, Trichoptera and Odonata were potentially most vulnerable to pyrethroids in aquatic insects (Rico and van den Brink, 2015).The greater sensitivity of aquatic insects to pyrethroids may not directly relate to greater uptake or deficiencies in metabolism, as some aquatic insects are known to have well developed detoxification enzyme systems (Siegfried and Young, 1993). Rather, the greater sensitivity of aquatic insects may be caused by greater sensitivity of physiological sites in aquatic insects. The mode of action of pyrethroids is believed to involve the disruption of the axonal transmission of nerve impulses as a result of altering ion permeability (Clark and Matsumura, 1982). Since freshwater aquatic organisms live in an extremely dilute environment, the processes involved in maintaining ionic balance and osmoregulation are critical to maintenance of homeostasis (Schmidt-Nielsen, 1997). Maintenance of high cellular concentration of ions against a concentration gradient is regulated by active transport. An insect's ability to maintain ionic balance in water may be affected by exposure to pyrethroids, thereby making them more susceptible. Such effects have also been reported for fish, in which species exposure to pyrethroids disrupts respiratory surfaces and ion regulation (Bradbury and Coats, 1989; Dyer *et al.,* 1989; Symonik *et al.,* 1989). Such effects may generally contribute to the higher sensitivity of aquatic insects and other aquatic organisms.

Exposure of mature male salmon to low levels of water-borne cypermethrin has been shown to inhibit their ability to detect and respond to the female salmon pheromone PGF_{2alpha}. The levels of plasma sex hormones were significantly reduced in the presence of the pyrethroids as a result of the impaired olfactory detection of the priming pheromone. Several studies have also indicated that a number of generic pesticides have a similar toxic effect on pheromone mediated endocrine function in Salmon (Moore and Warring, 1996; Waring and Moore, 1997; Moore and Warring, 1998). However, unlike atrazine (Moore and Warring, 1998), there are no evidences that exposure to cypermethrin influenced the either the metabolism of steroids or their accumulation within the bile. In addition, there appeared to be no direct impact of cypermethrin upon the testes and in this respect the toxic mode of action of cypermethrin on the reproductive endocrine function of the male salmon was similar to carbofuran (Warring and Moore, 1997). Exposure of salmon eggs and milt to cypermethrin also reduced the level of fertilisation, suggesting a further toxic impact of the pesticide on salmon reproduction. Therefore even if the olfactory priming response was not significantly inhibited by cypermethrin the pesticide could have a deleterious impact on populations as a result of decreased fertilisation. Short-term exposure to the pyrethroid esfenvalerate also resulted in a reduction in fecundity of the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) and the failure of eggs to hatch (Barry *et al.,* 1995). Studies on this particular species demonstrated that fertilized eggs were not sensitive to the pyrethroid (Barry *et al.,* 1995), suggesting that the toxic effect occurred prior to fertilization. Reduced spawning and hatching of offspring has been seen to occur in bluegills (*Lepomis macrochirus*) exposed to esfenvalerate (Tanner and Knuth, 1996). It was also recorded that the exposure to pyrethroid caused a delay in spawning in this species. The delay in spawning may have been resulted from the disruption of the pheromone based synchronization of the spawning

between the sexes. Cyclomethrin clearly reduced the ability of the olfactory system to detect the priming pheromone. It has also been suggested that exposure to the pesticide probably acted directly on the sodium channels, inhibiting nervous transmission within the olfactory system and resulting in the male salmons' inability to detect and respond to the pheromone.

Pyrethroids have also been shown to inhibit ATPases associated with active transport (Gray and Soderlund, 1985), and may affect ion movement and osmoregulation. Two types of Ca^{2+} stimulated ATPase activities have been recognized. One is Ca^{2+}-Mg^{2+}-ATPase which is temperature and ageing sensitive and requires the simultaneous presence of Mg^{2+} and K^{+} for optimal Ca^{2+} stimulation. The second is Ca-ATPase, which is comparatively temperature insensitive, stable and resistant to aging, and requires the presence of Na^{+} and K^{+} but not Mg^{2+} (Matsumura and Clarke, 1980). The function of Ca^{2+}-Mg^{2+}-ATPase is in the sarcoplasmic reticulum of muscle, where it functions to pump Ca^{2+} against a concentration gradient from the intracellular medium into the lumen of the reticulum. Studies with brain synaptosomes (Blaustein *et al.,* 1978) found ATP-utilizing systems to be active in the endoplasmic reticulum of the presynaptic region, transporting intracellular Ca^{2+} into this reticulum (McGraw *et al.,* 1980). This process is believed to help these cells regulate intracellular free Ca^{2+} levels, leading to an increase in neurotransmitter release as happens in heptachlor epoxide poisoning (Yamaguchi *et al.,* 1980).

Organophosphates

Organophosphates include Malathion, parathion, methyl parathion and diazinon. These compounds are well-known neurotoxins that exhibit their effects by the inhibition of acetylcholinesterases. They are also known to suppress the immune system.

CHEMISTRY OF ORGANOPHOSPHATES

The chemistry of OPs has been thoroughly investigated (Chambers *et al.,* 2001). The general structure of an OP insecticide.Where X is the leaving group (Fig. 2), that is displaced when OP phosphorylates acetylcholinesterases (AChE), and is the most sensitive to hydrolysis; R_1 and R_2 are most commonly alkoxy groups (i.e. OCH_3 or OC_2H_5), though other chemical substitutes are also possible; either an oxygen or a sulphur can also be present with a double bond. Based on chemical differences, OPs can be divided into several different types, which include Phosphates, phosphorothioates, phosporamidates, phosphonates etc. (Fig 3). Most of the pesticides are phosphorotiorates which need to be bioactivated in vivo to their oxygen analogues to exert their toxic action, but some like dichlorvos have P=O bonds. Most OPs used as insecticides have two methoxy or ethoxy side chains.

MECHANISM OF ACTION

The complex array of reactions involved in the biotransformation of OPs in target and nontarget species has been extensively studied (Tang *et al.,* 2006). For the compound that contain a sulphur bound to the phosphorus , a metabolic bioactivation is necessary for their biological activity to be manifested, as only the compounds with a P=O moiety are effective inhibitors of AChE. This bioactivation consists in an oxidative desulphuration mediated, mostly but not exclusively in the liver, by cytochrome P450 enzymes, and leading to the formation of an "oxon", or oxygen analog of the parent insecticide. Multiple CYPs have been shown to activate organphosphorothioates to their oxons, with different substrate specifities. For example, diazinon is activated by CYP3A4/5 and CYP2C8, and chloropyrifos by CYP2B6 (Kappers *et al.,* 2001; Tang *et al.,* 2001; Mutch *et al.,* 2003). In addition to oxidative desulphuration, other reactions can activate OPs (Costa, 1988).

The interaction of OPs with AChE has been studied in much detail. OPs with a P=O moiety phosphorylate a hydroxyl group on serine in the active site of the enzyme, thus impeding its action on the physiological substrate. The first reaction leads to the formation of a Michaelis complex, while a subsequent reaction leads to the formation of the

phosphorylated AChE. Rates of these two reactions, that are usually very rapid, indicate the affinity of the enzyme for a given OP. The bond between the phosphorus atom and the esteratic site of the enzyme is much more stable than the bond between the carbonyl carbons of acetate (in acetylcholine) at the same enzyme site. While breaking of the carbon-enzyme bond is complete in a few microseconds, breaking of the phosphorus-enzyme bond can take from a few hours to several days, depending on the chemical structure of the OP (Fig 4). Phosphorylated AChE is hydrolysed by water at a very slow rate, and the rate of spontaneous reactivation depends on the chemical nature of the R substituents. Reactivation decreases in the order: dimethoxy> diethoxy>> diisopropoxy (Gallo and Lawryk, 1991). Whereas water is a weak nucleophilic agent, certain hydroxylamine derivatives, known as oximes, can facilitate dephosphorylation of AChE, and are utilized in the therapy of OP complex has aged. poisoning.

Aging consists of the loss of one of the two alkyl (R) groups by nonenzymatic hydrolysis, and the rate of aging depends on the nature of the alkyl group. When phosphorylated AChE has aged, the enzyme can be considered to be irreversibly inhibited, and the only means of replacing its activity is through synthesis of new enzymes.

NON TARGET EFFECTS AND TOXICITY OF ORGANOPHOSPHATES

Although organophosphates are less persistent than organochlorines in the environment, OPs have high acute toxicity, with oral LD_{50} values in rat often below 50 mg/kg, though for some widely used compounds like chloropyrifos and diazinon, toxicity is somewhat lower due to effective detoxification. One exception is Malathion, which has oral LD_{50} in rat of >1 g/kg, due, as said, to rapid detoxification by CarE. For several OPs acute dermal toxicity is also high, with some exceptions like azinphosmethyl and Malathion (Murphy et al., 1986). The primary target for organophosphates is AchE, a B-esterase whose physiological role is that of hydrolysing acetylcholine, a major neurotransmitter in the central and peripheral nervous systems. Acetylcholine released from cholinergic nerve terminals is disposed of solely through hydrolysis of AChE. In fact, in contrast to other neurotransmitters like norepinephrine, it is choline the product of acetylcholine hydrolysis by AChE that is taken up by the presynaptic terminal. Hence, inhibition of AChE by OPs causes accumulation of acetylcholine at cholinergic receptors of the muscarinic and nicotinic type. As these receptors are localized in most of the body, a “cholinergic syndrome” ensues, which includes increased sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhoea, tremors, muscle twitching, and various central nervous system effects. When death occurs, this is believed to be due to respiratory failure due to inhibition of respiratory centres in the brain stem, bronchoconstriction and increased bronchial secretion and flaccid paralysis of respiratory muscles (Gallo and Lawryk, 1991; Lotti, 2000, 2001). The time interval between exposure and onset of symptoms varies with the route and degree of exposure, and chemical nature of the OP. The time interval between exposure and onset of symptoms varies with the route and degree of exposure, and the chemical nature of the OP. While respiratory failure is a hallmark of severe OP poisoning, mild poisoning may display no clear-cut signs and symptoms (Lotti, 2001). Therefore diagnosis is made through symptom recognition; miosis is observed most often, followed by gastrointestinal symptoms (nausea, vomiting, abdominal pain) and hypersalivation.

A few OPs may cause another type of toxicity, known as organophosphate-induced delayed polyneuropathy (OPIDP). Signs and symptoms include tingling of the hands and feet, followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities, and ataxia (Lotti, 1992; Ehrich and Jortner, 2001; Lotti and Moretto, 2005). These may occur 2-3 weeks after a single exposure, when signs of both the acute cholinergic and the intermediate syndromes have subsided. OPIDP can be classified as a distal sensorimotor axonopathy. Neuropathological studies in experimental OPIDP have evidenced that the primary lesion is a bilateral degerative change in distal levels of axons and their terminals, primarily affecting larger/ longer myelinated central and peripheral nerve fibres, leading to breakdown of neuritic segments and myelin sheaths (Ehrich and Jortner, 2001). OPIDP is not related to AChE inhibition. One of the compounds involved in several epidemics of this neuropathy, including the Ginger-Jake paralysis in the 1930s in the United States is tri-ortho-cresyl phosphate (TOCP), which is a very poor AChE inhibitor. Studies (Johnson, 1982; Johnson and Glynn, 2001; Lotti,

1992) have identified the target for OPIDP as an esterase, present in the nerve tissues as well as other tissues like lymphocytes. For OPIDP to be initiated, phosphorylation and subsequent aging of at least 70% of NTE (neuropathy target esterase) is necessary, and this two-step process occurs within hours of poisoning. Though reductions in axonal transport have been found to precede over the clinical signs, the exact chain of events occurring between phosphorylation and aging of NTE and axonal degeneration remain obscure. Recent studies have shown that NTE has a 41% identity with the Swiss Cheese Protein (SWS) in the neurons of *Drosophila*. Studies in genetically modified mice have indicated that NTE is required for normal vasculogenesis and placental development, and that absence of brain NTE results in neuronal degeneration and loss of endoplasmic reticulum in various brain areas (Moser *et al.,* 2004; Akossoglou *et al.,* 2004). Interestingly, in *Drosophila* SWS mutants, extensive vacuolation is seen in the brain (Kretzschmar *et al.,* 1997). NTE also appears to play a role in membrane lipid metabolism, and may be involved in intra-neuronal membrane trafficking and lipid homeostasis (Zaccheo *et al.,* 2004; Glynn, 2006).

The acute exposure to high doses of OPs may result in long-lasting adverse health effects (particularly in the CNS) in animals as well as humans (Sanchez-Santed *et al.,* 2004; Rosenstock *et al.,* 1991). More controversial is the possibility that low exposure to OPs, at dose that produce no cholinergic signs, may lead to long-term adverse health effects, particularly in the central and peripheral nervous systems. Chronic exposure of animals to OPs, at doses that significantly inhibit AChE, but may not be associated with clinical signs, results in the development of tolerance to their cholinergic effects (which is mediated, at least in part, by down-regulation of cholinergic receptors), and has been associated with neurobehavioral abnormalities, particularly at the cognitive level (Costa *et al.,* 1982; Prendergast *et al.,* 1998). Evidence describing long-term neuropsychological or neuropsychiatric alterations in humans upon low exposure is inconclusive (Daniell *et al.,* 1992; Jamal and Julu, 2002). OPs as a class are not considered to be mutagenic. In the past decade, a report from the National Academy of Sciences has highlighted the potential higher exposure of children to pesticides (NRC, 1993), and FQPA indicates that in the risk assessment process, an additional who are more presumed to be more sensitive to the effects of toxicants (FQPA, 1996). This increased sensitivity does not appear to be due to intrinsic differences in AChE, but rather to lower detoxification abilities of young animals. In recent years accumulating evidence suggests perinatal exposure to OPs may cause developmental neurotoxicity. Studies in rodents indicate that OPs can affect various cellular processes like DNA replication, neuronal survival and neurite outgrowth. They may also affect the non-cholinergic pathways like serotogenic synaptic functions, the adenylate cyclase system, and cause various behavioural abnormalities (Song *et al.,* 1997; Dam *et al.,* 1998; Jett *et al.,* 2001; Aldridge *et al.,* 2003; Ricceri *et al.,* 2003; Garcia *et al.,* 2005). Such effects are often seen at dose levels that produced no cholinergic signs of toxicity.

Non-target wildlife are frequently exposed to OP pesticides. Birds appear to be much more sensitive to OPs than mammals (Hill, 1995). Birds have lower hepatic microsomal mono-oxygenase (HMO) and A-esterase activities than mammals which may reduce their ability to metabolize the OPs (Brealey *et al.,* 1980; Walker, 1980). In birds and mammals, the preoptic area/anterior hypothalamus (POAH) is the primary centre for control of body temperature (Gordon, 1993). The POAH integrates information from peripheral and deep body thermal receptors, compares this information to a "temperature set point," and signals the appropriate thermoregulatory effectors (Gordon, 1993). The set point is defined as the value of a regulated variable which a healthy organism tends to stabilize by the processes of regulation (IUPS, 1987). In birds and mammals, acute "sublethal" exposure to OPs commonly results in pronounced, but short-lived, hypothermia (Grue *et al.,* 1991; Gordon, 1994). These effects appear to be mediated primarily through thermoregulatory processes within the central nervous system (CNS) because peripherally acting cholinergic antagonists do not block OP- and -induced hypothermia (Gordon, 1994). Hypothermia caused by these compounds result from excitation of cholinergic synapses in the anterior hypothalamus and decreases in metabolism in the liver (Meeter *et al.,* 1971). OP induced reductions in body temperatures in birds and mammals are frequently associated with decreases in brain AChE activity of more than 50 percent and the return of body temperatures to normal is rapid (usually occurring within 24 hours). Hypothermia was associated with a threshold of 53 percent of normal brain AChE activity in adult (8 months old) American Kestrels *(Falco sparverius)* given a single oral dose of the OP methyl parathion and subjected to cold for 10 hr. Body temperatures approached normal in 10 hour post-dose.

Toxicity was enhanced at -5°C with 60 percent mortality in kestrels given 2.25 mg per kg body weight, a sublethal dose at thermo-neutral temperature (Rattnerand Franson, 1983). The toxicity of the OP parathion increased up to 2-two fold in 8-10 week-old Japanese Quail *(Coturnix japonica)* when birds were exposed to acute or chronic heat (37°Cfor 10 days after treatment). It has been found that in birds the interaction between low temperatures and pesticide toxicity is not the result of a temperature induced increase in activity of the toxicant, but rather, a pesticide-induced decrease in the ability to withstand the cold as a result of impairment of thermoregulation (Martin and Solomon, 1991).Reductions in food consumption are frequently associated with intoxication. Consumption of clean feed by Common Grackles *(Quiscalus quiscula)* given an oral dose of the OP dicrotophos was reduced 76 percent. Similarly, mortality in Weaver Birds *(Quelea queled)* given small epidermal applications of the OP fenthion was attributed to anorexia and subsequent starvation (Pope and Ward, 1972). Brain AChE in birds that survived was inhibited 59-67 percent after 3 days on clean feed (Grue, 1982). Similar thresholds have been reported by Bennett (1989) for Northern Bobwhite. Studies in which birds or mammals have been given a choice between OP contaminated diets and uncontaminated food suggest that they can maintain normal levels of food consumption if they can discriminate among the diets (Bennett, 1994). Birds can detect the presence of OPs and in their diets at levels below those that produce signs of toxicity. Above a threshold, birds consume proportionally less contaminated diet (Bennett, 1989). In addition, exposure to ChE-inhibitors may interfere with an animal's ability to discriminate among diets. Bussiere *et al.,* (1989) reported that Northern Bobwhite chicks given an oral dose of methyl parathion and then given a choice of diet treated with the OP or clean feed actually preferred the contaminated diet. Reductions in body weights of adult and young following "sublethal" exposure to ChE-inhibitors can be severe. Losses of up to 40% have been reported in adult birds during subacute dietary exposures to some OPs (Grue, 1982). Weight losses are considerably lower in adult birds following a single dose. Adult male and female European Starlings that survived a single dose of the OP dicrotophos lost up to an average of 14 percent of their initial body weights 24 hr. post dose. In contrast, weight losses of up to 31 percent were reported in 5-day-old European Starlings alive after a single oral dose of the same OP (Grue and Shipley, 1984). Losses in 15-day-old starlings were intermediate (26%) (Grue and Shipley, 1984). Intoxication in the nestlings was characterized by a sharp reduction in begging for food. Studies have examined the effects of OP-induced weight losses on fledging weight, time to fledging, and post fledging survival. Four-day-old White-throated Sparrows *(Zonotrichia albicollis)* given an oral dose of the OP fenitrothion suffered reductions in body weight and fledged at lower weights (Pearce and Busby, 1980). Sparrows given a single dose of the OP at 3 and 5 days of age suffered reductions in weight and also fledged at lower (Pearce and Busby, 1980). Similarly, nestling European Starlings given daily sublethal doses of the OP famphur fledged at lower weights (Powell and Gray, 1980). Conditioned aversions to contaminated foods may actually protect wildlife within treated habitats from more chronic poisoning (Bennett, 1994). Other direct toxic effects of ChE-inhibitors may also reduce or enhance the ability of exposed wildlife to forage effectively. In addition to lethargy and gastrointestinal disturbances, sublethal exposure to AChE inhibitors has been shown to impair vision, learning and memory, and alter endogenous rhythms (Grue *et al.,* 1991; Boyes *et al.,* 1994). In addition to thermoregulation and food consumption, the hypothalamus serves as the control centre for reproduction in birds and mammals. The majority of the studies conducted on the reproductive effects of these chemicals on wildlife have been conducted on birds. Embryotoxicity and teratogenicity associated with exposure to OPs. These compounds can alter levels of reproductive hormones primarily through their effects on synapses within the central nervous system (Fry, 1995). Many species of wildlife (particularly birds) begin and end the reproductive process with migration to and from their breeding grounds. Studies (Bingman *et al.,* 1984, 1988; Sherry and Vaccarino, 1989) indicate that the hippocampal complex is critical to the acquisition of spatial reference memory (homing and retrieval of food catches). Adult Whitethroated Sparrows given 256 ppm of the OP acephate in their diet for 14 days did not orient themselves properly for migration during the 6 days post exposure (Vyas *et al.,* 1995). Acephate altered memory of the migratory route and wintering ground in the adults. Sublethal exposure to ChE inhibitors has also been shown to alter sexual behaviour. Song in birds is believed to be under cholinergic control (Ryan and Arnold, 1981) and decreases in song production by wild birds within treated habitats have been reported. Grue and Shipley (1984) observed a 50 percent decrease in singing and displaying by captive male European Starlings within 4 hr. after being given 2.5 mg dicrotophos per kg of body weight. The elaboration of aggressive behaviour also appears to be under cholinergic control (Reis, 1975) and

increased aggression has been associated with sublethal exposure to ChE inhibitors (Grue *et al.,* 1991). McEwen and Brown (1966) reported an unusual amount of intensive challenging and fighting among wild Sharp-tailed Grouse *(Typanuchus phasianellus)* on breeding grounds 24 hr. after some of the birds were given a sublethal dose of the OP Malathion; enzyme inhibition was not measured. Pine Voles *(Microtus pinetorum)* fed azinphos-methyl, an OP, for 5 days were less aggressive during the subsequent 10 days they were on clean feed (Durda *et al.,* 1989). Reductions in egg laying associated with OP-induced decreases in food consumption and changes in levels of reproductive hormones are the most dramatic reproductive effects observed to date in birds following exposure to these compounds. Reductions in food consumption alone accounted for reductions in egg laying by Northern Bobwhite given OP methamidophos in their diet for 15 days (Stromborg, 1986). The highest dietary concentrations were associated with 68 percent decrease in food consumption and in some cases, total cessation of egg production and death. Intake of 400 ppm of methyl parathion in the diet of male and female mallards for 8 days resulted in a 50 percent reduction in the number of eggs laid with effects seen after 2 days on the diet (Bennett *et al.,* 1991). Altered gonadotropin secretion may account for the observed reductions in egg laying by the OP-dosed quail. Short-term food deprivation such as that caused by OP-induced anorexia could significantly reduce reproductive success in small mammals. Female European Starlings that were given a single oral dose of dicrotophos on day 6 of incubation spent less time on their eggs whereas males spent more time; hatching success was not affected. Females (with young) given the same dosage made fewer sorties to the nest within 48 hr. post dose and their nestlings lost weight relative to controls (Grue *et al.,* 1982). Meyers *et al.,* (1990) gave incubating Redwinged Blackbirds *(Agelaius phoeniceus),* a species in which only females are involved in parental care, a single oral dose of methyl parathion. OP-dosed red-wings spent less time incubating their eggs during the first 2 hr. after dosing, but hatching and fledging success and overwinter survival were not affected.

Organochlorines

Organochlorines are the oldest major insecticides, having been the first widely used synthetic organic insecticides. All insecticides of this group contain chlorine, hydrogen and carbon. Occasionally these insecticides also have oxygen and sulphur. Although very effective, the use of chlorinated hydrocarbon in the United States is negligible because of the environmental and human safety concerns. The organochlorine insecticides include the chlorinated ethane derivatives, such as DDT and its analogues; the cyclodienes, such as chlordane, aldrin, dieldrin, heptachlor, endrin, and toxaphene; the hexachlorocyclohexanes, such as lindane; and the caged structures mirex and chlordecone.

Mechanism of action

DDT interferes with the sodium ion channels in the axonal membrane by mechanism similar to that of Type I pyrethroids (Vijevberg *et al.,* 1982). DDT has little or no effect on the resting potential or the rising phase and peak amplitude of the action potential. However, it greatly prolongs the depolarizing afterpotential of the action potential, and this produces a period of increased neuronal excitability immediately after the spike phase. This, in turn, enhances the probability of repetitive firing, and the insurgence of a “train” of action potentials. The principal effect of DDT is to slow down the closing of sodium channels once they have opened, while having little or no effect on closed gates. In addition to this effect on sodium channels, DDT also affects ATPases. Inhibition of a Ca^{2+}- ATPase may be involved in the effects of DDT. DDT exposure alters the levels of some neurotransmitters such as acetylcholine, norepinephrine, and serotonin, (Woolley, 1982). Hexachlorocyclohexanes like lindane have moderate to high acute oral toxicity. These compounds are readily absorbed through the skin. They primarily affect the central nervous system. They interfere with the gamma-aminobutyric acid (GABA) - mediated neurotransmission. Hexachlorocyclohexanes bind specifically on the chloride channel, thereby blocking its opening and thus antagonizing the inhibitory action of GABA (Cole and Casida, 1986; Eldefrawi and Eldefrawi, 1987; Narahashi, 1996). Chlordecone induces hepatic drug metabolizing enzymes, and causes hepatosplenomegaly in rats and humans. It is not mutagenic, but can induce liver tumours in rodents (Smith, 1991). Chlordecone also causes reproductive toxicity in animals, likely by mimicking the effects of excessive oestrogens.

Toxicity and non-target effects

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) is probably the most famous insecticide. Its use has largely been banned in the United States and other countries. The primary reason for this ban is the stability and lipophilicity of the chemical. In humans DDT is upon absorption is distributed in all the tissues. The highest concentration of DDT is in the fat tissues. In humans DDT metabolization is extremely slow with DDE, DDD, DDA being the primary metabolites (Smith, 2001). DDE is stored in the adipose tissue. Excretion occurs through bile, urine and milk. Exposure to high doses of DDT can cause motor unrest, abnormal susceptibility to fear and external stimuli like light, touch, sound. This is followed by the development of tremors, tonic-clonic convulsions. Death may even occur after 24-72 hours due to respiratory failure.

One of the major concerns about the use of DDT is that the invertebrates that consumed the vegetation with DDT residues stored the DDT in their body fats. These invertebrates were fed upon by higher level consumers. In the process of eating and being eaten DDT reaches the top consumers. DDT accumulates at higher concentrations in the bodies of the top consumers. This phenomenon is called biomagnification. This has adverse effects on the health on the top predators. DDT also causes thinning of egg shells and reproductive failures in birds. DDT is highly toxic to aquatic animals and affects their heart and brain (W.H.O., 1979).

Acute exposure to hexachlorocyclohexanes and cyclodiene affects the nervous system in human. This may lead to vomiting. The insecticides also affect the cardiovascular and musculoskeletal systems. Chronic exposure may lead to a decrease in the numbers of red and white blood cells. These also affect the immune system, liver and kidney. Even in small quantities their contamination may lead to headache, nausea, dizziness, tremors and muscular weakness. They enter the atmosphere and precipitates on the ground during rainfall. They are highly toxic to wild animals and birds as like DDT they are lipophilic.

The primary manifestation of chlordecone toxicity is the presence of tremors, which are observed in animals as well as in humans (Guzelian, 1982). Mirex and chlordecone can stay in soil, water and sediments for years. Mirex is converted to photomirex which is even more poisonous than mirex. Aquatic animals are exposed to the water contaminated with mirex and chlordecone. These substances then accumulate in the body. Humans get exposed to mirex and chlordecone by being exposed to food particularly fish from the contaminated areas. Mirex can be harmful to the eyes, intestines, liver, kidneys and thyroid. Short term exposure to mirex can cause trembling, tiredness and weakness. Chlordecone is known to be a hepatotoxic substance.

Organochlorines are known as potent endocrine disruptors. An isomer of DDT can act as an agonist at oestrogen receptors. DDE, a metabolite of DDT inhibits androgen binding to androgen receptors. Chlordecone also has estrogenic properties (Guzelian, 1982). Other organochlorines like dieldrin, endosulphan, toxaphene, lindane and beta isomer of hexachlorocyclohexane has weak estrogenic properties. Chlordecone has also been shown to affect the male reproductive system (Guzelian, 1982; Kavlock, 2001). They may induce trans-generational defects in spermatogenic capacity and sperm viability.

Carbamates

The carbamates are broad-spectrum insecticides that are widely used in agriculture. They were developed by the Geigy Corporation in 1951. Its effects and persistence is similar to the organophosphates. Carbamates present different degrees of oral toxicity, ranging from moderate to low toxicity like in Carbaryl and extremely high toxicity like in aldicarb. Dermal toxicity is lower, but skin penetration is increased by organic solvents and emulsifiers present in the formulations (Ecobichon, 2001).

Chemistry of carbamates

Carbamate insecticides are derived from carbamic acid containing –OCONOCON group. The different types of carbamates included are (Fig 5):

- Heterocyclic carbamates like isolan which have a heterocylic group
- Phenyl carbamates like carbaryl, carbofuran and arprocarb which have a phenyl group
- Oxime carbamates like aldicarb and oxamyl which contain an imino group

Mechanism of action

Carbamates are susceptible to a variety of enzyme catalysed biotransformation reactions, and the principal pathways involve oxidation and hydrolysis (Fukuto, 1972; Tang *et al.,* 2006). For the most part, the metabolites are devoid of biological activity, but there are some exceptions. Two metabolites of aldicarb, a sulphoxide and a sulphone, are better acetylcholinesterases than their parent compounds (Risher *et al.,* 1987). The mechanism of toxicity of carbamates is similar to that of the organophosphates. They both inhibit AChE. However there is a significant difference with the organophosphate in that the carbamates inhibit the AChE reversibly. The reversible inhibition occurs because of the rapid reactivation of the carbamylated in the presence of water. The carbamylated AChE does not undergo the aging reactions.

Toxicity and non-target effects

The signs of carbamate poisoning are the same as observed following intoxication with OPs and include miosis, urination, diarrhoea, salivation, muscle fasciculation and CNS effects. However differing from the organophosphates, acute intoxication caused by carbamates is generally resolved within a few hours. Maximal inhibition is achieved very rapidly, as carbamates are direct AChE inhibitors and do not require metabolic bio activation. The enzyme activity returns to control levels after a short time. There are several cases of human poisoning associated with exposure to various carbamates, in particular Carbaryl (Cranmer, 1986) and propoxur (Hayes, 1982). Most cases, however, involved aldicarb. This compound, which has a very high acute toxicity, is also highly water soluble. Though, because of this characteristic, it is not registered for use on any fruit or vegetable having high water content, its illegal use in hydroponically grown cucumbers, and in watermelons has led to outbreaks of poisoning (Goes *et al.,* 1980; Goldman *et al.,* 1990). Contamination of drinking water has also been reported (Zaki *et al.,* 1982). Carbamates can inhibit NTE, but because carbamylated NTE cannot age, they are thought to be unable to initiate OPIDP. Additionally, when given before a neuropathic organophosphate, carbamates offer protection against OPIDP (Johnson and Lawerys, 1969), but when given after, they can promote OPIDP (Lotti, 2002). A few case reports indicate that exposure to very high dosages of methylcarbamates like carbaryl, carbofuran, may result in a peripheral polyneuropathy similar to OPIDP (Dickoff *et al.,* 1987; Yang *et al.,* 2000). This implies that aging is not required for OPIDP to develop, or, carbamates may have amplified a pre-existing subclinical neuropathy. Development of tolerance to some carbamates (propoxur, carbaryl) upon repeated exposure has been observed, and this appears to be due to an induction of microsomal enzymes (Costa *et al.,* 1981). As a class, methylcarbamates are not mutagenic, and there is also no evidence of carcinogenicity. Embryotoxicity are observed only at maternally toxic doses (Baron, 1991). Limited evidence suggests that carbamates (e.g., aldicarb) may be more acutely toxic to young animals than to adults (Moser, 1999), possibly because of lower detoxification. Conclusive evidence concerning the mutagenic potential of aldicarb is not available; two studies suggest that this insecticide might have mutagenic potential. In the first study, the effects of aldicarb on human lymphocytes in vitro was investigated in the presence of an exogenous metabolic activation system by means of an analysis of sister chromatid exchange (SCE) and mitotic delay. In this study, cyclophosphamide (CP) was used as a positive control to compare the chromosomal effects of aldicarb with a known genotoxic agent. Data obtained from this study revealed that aldicarb, as well as CP, induced a significant increase of SCE values, leading the authors to suggest that metabolic activation of aldicarb seems to

occur in human blood cultures. In the second study, chromosomal aberrations in male albino rats were investigated. The animals received IP injections of 0.00121, 0.00666, or 0.0121 mg/kg body weight of aldicarb in a 1:1 acetone and water vehicle. Controls received only the acetone: water solution. Subacute (1 injection per day for 5 days) and acute (sacrifice 6, 24, or 48 hr. following a single injection) phases of the experiment were conducted. Bone marrow cells of rat femurs were analysed, and increases in both structural and numerical aberrations were observed in all animals treated with aldicarb. Significant structural chromosomal aberrations were observed in the form of chromatid breaks and gaps, centric fusions, centromeric attenuation, and end to end association, suggestive of chromosomal damage occurring during the G1 stage of the cell cycle. The numerical aberrations were primarily due to polyploidy and endomitosis, the latter indicating a specific effect of the insecticide on the mitotic spindle, and leading to concomitant increase in the mitotic index at all stages. The highest increase in numerical aberrations was observed following subacute treatment with the maximum tolerated dose. The authors suggested that aldicarb may have a cumulative effect and may be a clastogenic agent in rats (Sharma *et al.,* 2010).

Non-target effects of winter methiocarb application on summer-active carabids were infrequent, inconsistent and much more difficult to interpret. Reductions in some years in the activity of *Agonum dorsale,* and *Agonum muelleri**,*** appear to be a consequence of winter pellet applications although the mechanism for such effects is far from clear. Direct toxicity is virtually impossible since *A. dorsal* leaves cereal fields to overwinter in field boundaries (Coombes and Sotherton, 1986) and only reappeared on the study site ***5-6*** months after application. Any methiocarb effect must, therefore, be an indirect one and most plausibly, may be a behavioural influence reflecting in some way prey availability. Griffiths, Wratten and Vickerman (1985) have shown that *A. dorsale* actually moves less over the ground when actively feeding on aphid prey. In 1989/90, low catches of *A. dorsale* on plots with very high aphid densities probably reflect such altered foraging behaviour and explain the apparently inconsistent response of this species to methiocarb use. The activity of *A. dorsale* in nonaphid years and that of other summer-active species (*A. muelleri, Loricera pilicornis**),*** which also showed significant positive or negative summer effects, illustrate how pitfall catches may reveal rather subtle pesticide influences on predator populations which do not necessarily indicate real changes in abundance but which require more detailed investigation and careful interpretation (Luff, 1987). The influence of winter-applied methiocarb slug pellets on species active at the time of use is, however, clear and unequivocal. Such pellets applied in winter are highly attractive and toxic to a relatively non-dispersive carabid community of probably very opportunistic and catholic feeding habits. The long-term environmental harm of this non-target effect appears to be relatively slight, however, since in all but one case, that of *Bembidion obtusum**,*** the species involved seem capable of full recovery by the following cropping season. den Boer 1981,1985 has suggested that carabids as a group are subject to continual local extinctions particularly so in disturbed agro-ecosystems and that long-term survival of such populations depends largely on their dispersal abilities within a heterogeneous composite environment leading to a ‘spreading of risk’ of extinction. Luff (1987) emphasises this point in his discussion of carabid population regulation and in the current study there seems ample evidence for this view in a ‘worst case’ situation involving a baited pesticide and relatively immobile winter-active species. The real long-term threat to such populations seems, therefore, not to be from specific agricultural practices, even when repeated locally on an annual basis, but from the geographic scale of intensive arable farming and range of detrimental practices (Potts and Vickerman, 1974) which extend the localised nature of such eliminations and deplete population reservoirs from which recolonisation can take place. From an agronomic viewpoint, localised elimination of beneficial predatory populations may still be significant even if they are temporary in nature. Kendall, Smith, Chinn and Wiltshire (1986) on the basis of limited observations in one trial suggested a possible link between methiocarb pellet application and increased incidence of Barley Yellow Dwarf (BYDV) in barley. Results from the current study show a severe reduction of predatory carabid activity on methiocarb treated areas during the critical phase of aphid introduction and spread of virus within the crop. However, carabids are only a relatively minor part, of the polyphagous predator fauna known to feed on aphids at least during the summer months (Sunderland *et al.,* 1987).

Neonicotinoids

Neonicotinoids are relatively new class of synthetic insecticides. This class is prominently represented by the compound imidacloprid. Imidacloprid is a systemic and contact poison with primary effects on piercing-sucking insects such as aphids, leafhoppers, thrips and whiteflies. It is also effective against termites, soil insects and some beetle species. They upset the central nervous system by causing irreversible blockage of postsynaptic nicotinergic acetylcholine receptors. These insecticides have relatively low toxicity to mammals. By various chemical modifications of nicotine and other nAChRs agonists, new classes of insecticides have been developed that contain a nitromethylene, nitroimine or cyanoimine group, and are referred to as neonicotinoids. One of the first compounds synthesized was nithiazine, a nitromethylenyl heterocyclic compound highly toxic toward insects but with low mammalian toxicity. Nithiazine was not developed commercially because of its photo-instability. Further structure-activity studies led to the development of imidacloprid, nitenpyram, acetamiprid, and other neonicotinoids compounds (Matsuda *et al.,* 2001).

Mechanism of Action

The insecticidal activity of neonicotinoids is attributed to activation of nAChRs (nicotinic acetylcholine receptor).They are used primarily for crop protection as systemic insecticides, but are also effective against fleas in cats and dogs (Schenker *et al.,* 2003).

Toxicity and non-target effects

The mammalian toxicity of neonicotinoids is due to the agonist action and binding affinity at the nAChRs. Acute oral toxicity (LD50) in rats ranges from180 to >2000 mg/kg, while dermal toxicity is much lower, likely because of the low lipophilicity (Tomizawa and Casida, 2005). Signs and symptoms of toxicity are attributable to stimulation of nAChRs. Some neonicotinoids (imidocloprid, thiacloprid) are particularly toxic to birds, others (thiacloprid) to fish. Most neonicotinoids are neither mutagenic nor teratogenic. Neonicotinoids undergo limited biotransformation in mammals, involving mostly cytochrome P450-mediated oxidative reactions (Sheets, 2001; Tomizawa and Casida, 2005).

Neonicotinoids account for 10–15% of the total insecticide market, and their use is increasing faster than other insecticides (Matsuda *et al.,* 2001; Tomizawa and Casida, 2005). The main reason for their success lies in their selectivity profile, which is largely attributable to their specificity toward insect but not to mammalian nAChRs. The nAChRs consists of diverse subtypes assembled in combination from ten $\alpha$and four β, γ, δ, ε subunits. The most abundant subtypes in the vertebrate nervous system are $\alpha4\beta2$ and $\alpha7$, which are insensitive and sensitive, respectively, to α-bungarotoxin. In insects, neonicotinoids have been shown to bind to at least three pharmacologically distinct nAChRs (Sheets, 2001; Matsuda *et al.,* 2001). Structural features of neonicotinoids that contribute to their selective actions at insect nAChRs have been described (Nakayama and Sukekawa, 1998; Matsuda *et al.,* 2001; Tomizawa and Casida, 2005).

Neonicotinoids show high acute toxicity to honeybees. For mass-dying of bees in spring near corn fields during sowing of neonicotinoid-treated seeds, there now is a one to one proven causal link. Imidacloprid, thiamethoxam and clothianidin have been shown to rapidly induce flight muscle paralysis in honeybees exposed to guttation drops containing these substances, resulting in the cessation of wing movements (Girolami *et al.,* 2009). Imidacloprid further impairs the mobility of bees, as reflected by decreases in running and walking and increases in the time that exposed bees remain stationary (Medrzycki *et al.,* 2003). Neonicotinoids may act on larval development with consequences for the adult stage. Adult honeybees exposed to imidacloprid during the larval stage exhibit

impairment of olfactory associative behaviour (Yang *et al.,* 2012). This could be due to altered neural development. Impairments in mushroom body development in the bee brain and the walking behaviour of honeybee workers have been observed in individuals exposed to imidacloprid during the larval period (Tomé *et al.,* 2012). Optimal function of the honeybee nervous system is critical to individual and colony functioning (Desneux *et al.,* 2007; Thompson and Maus 2007). There is evidence that sublethal exposure of imidacloprid can affect learning, memory and orientation in honeybees. Learning and memory represent fundamental functions involved in the interaction of individuals with their environment and are critical in enabling bees to respond to the requirements of the colony throughout their life. Imidacloprid impairs learning and olfactory performance via both acute and chronic exposure pathways, and summer bees appear more sensitive than winter bees (Decourtye *et al.,* 2003). Accurate navigation is essential for efficient foraging and, hence, for colony health and survival. Neonicotinoids may impair navigation in different ways. Sublethal exposure of honeybees to clothianidin and imidacloprid elicits a decrease in foraging activity and induces longer foraging flights (Schneider *et al.,* 2012). Exposure to imidacloprid, clothianidin can lead to reductions in the proportion of active bees in the hive and, furthermore, initiate behaviours that can reduce the efficiency of foraging flights. Neonicotinoids such as thiacloprid, thiamethoxam and imidacloprid decrease brood production, larval eclosion, colony growth rate and the number of queens reared in bumblebees (Tasei *et al.,* 2000; Mommaerts et al. 2010; Whitehorn *et al.,* 2012). Imidacloprid can act synergistically with the pathogen *Nosema* spp. By increasing *Nosema*-induced mortality (Alaux *et al.,* 2010). It affects social immunity and so increases the number of *Nosema* spores in the guts of bees from imidacloprid-exposed colonies exposed in cage studies (Pettis *et al.,* 2012). Sequential exposure to *Nosema ceranae* can sensitize bees to thiacloprid by eliciting potentiation that leads to high mortality rates, a feature shared with fipronil (Vidau *et al.,* 2011; Aufauvre *et al.,* 2012). Adult honeybees reared in brood combs containing high levels of pesticide residues exhibit higher levels of infection by N. ceranae and higher levels of Nosema spores (Wu *et al.,* 2012). Di Prisco *et al.,* (2013) demonstrated that clothianidin and imidacloprid negatively modulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB*,* a protein involved in DNA transcription) immune signalling in insects and adversely affects honeybee antiviral defences controlled by this transcription factor.

In the case of butterflies or moths that inhabit adjacent to areas where pesticides are applied via aerial spraying, indirect effects of drift from spraying may pose risks both during and after applications (Sinha *et al.,* 1990). In the 1980s for example, helicopter application of pesticides in vineyards of the Mosel Valley in Germany nearly led to the extinction of an isolated population of the Apollo butterfly (*Parnassius apollo*) which was restricted to adjacent rocky slopes (Kinkler *et al.,* 1987; Richarz *et al.,* 1989; Schmidt1997). In Northern Italy, butterfly communities in natural grasslands have suffered drastic declines downwind of intensively sprayed orchards, leading to the disappearance of all but the most generalist species (Tarmann 2009). Furthermore, spray applications of pesticides may alter soil quality (Freemark and Boutin 1995) and thereby indirectly affect the larvae and pupae of moth species residing in the upper layers of the soil surface during the spring. Several species of hoverfly and parasitoid wasps attack agricultural pests, but also subsidise their diet with nectar. Therefore, these insects can be affected by neonicotinoids, which are translocated into the nectar and pollen of treated crop plants (Stapel *et al.,* 2000; Krischik *et al.,* 2007). Parasitoid wasps such as *Gonatocerus ashmeadi* can come into contact with neonicotinoids when emerging from the eggs of its host. One such host, the glassy-winged sharpshooter (*Homalodisca itripennis*), a common agricultural pest of many different crops, lays its eggs on the underside of leaves, beneath the epidermal layer. If eggs are laid on neonicotinoid treated plants, G. ashmeadi nymphs may be exposed to toxins when they emerge from the egg and chew through the leaf to get to the surface (Byrne and Toscano 2007). For the leafcutter ant *Acromyrmex subterraneus subterraneus*, Galvanho *et al.,* (2013) found that sublethal doses of imidacloprid reduced grooming behaviour. Grooming behaviour in this ant is a defence against pathogenic fungi like *Beauveria*. Barbieri *et al.,* (2013) recently discovered that interactions between different ant species may be negatively affected using sublethal doses of neonicotinoids. In interspecific interactions, individuals of a native ant species (*Monomorium antarcticum*) lowered their aggression towards an invasive ant species *(Linepithema humile*) although survival was not affected. Exposed individuals of *L. humile* displayed an increase in aggression with the outcome that the probability of survival was reduced. When exposed to turf plots treated with imidacloprid, the carabid beetle *Harpalus pennsylvanicus* displayed a range of neurotoxic problems

including paralysis, impaired walking and excessive grooming Coccinellids predators are well known for their ability to control common pests, both in agricultural and domestic environments. In soil treatments of imidacloprid, reduced mobility and delayed reproduction have been found in pollen feeding species such as *Coleomegilla maculata* (Smith and Krischick 1999), whilst egg production and oviposition periods of the Mealy bug destroyer (*Cryptolaemus montrouzieri*) (Khani *et al.*,2012) and *Hippodamia undecimnotata* (Papachristos and Milonas 2008) were significantly reduced. Szczepaniec *et al.,* (2013) discovered that the application of neonicotinoids supressed expression of plant defence genes when applied to cotton and tomato plants.

Earthworms are vitally important members of the soil fauna. Their activities improve soil structure by increasing porosity and aeration, facilitating the formation of aggregates and reducing compaction (Edwards and Bohlen 1996; Mostert et al. 2000). Soil fertility is enhanced by earthworm effects on biogeochemical cycling (Coleman and Ingham 1988; Bartlett *et al.,* 2010). Neonicotinoid and other systemic insecticides can pose a risk of harm to earthworm survival and behaviour. The same neural pathways that allow neonicotinoids to act against invertebrate pests are also present in earthworms (Volkov *et al.,* 2007). Thus, when neonicotinoids are applied for the protection of agricultural and horticultural crops, earthworms can be exposed by direct contact with the applied granules or seeds, or with contaminated soil or water. Their feeding activities may result in ingestion of contaminated soil and organic particles (Wang *et al.,* 2012).

Formamidines

Formamidines are a group of insecticides that gained acceptance in the 1970s mainly for use against organophosphate and/or carbamate resistant pests. Formamidines like chlordimeform [(N'-(4-chloro-o-tolyl)- N, N-dimethylformamidine] or amitraz [N'- 2,4- (dimethyl-phenyl)- N- N ((2,4- dimthylphenyl) imino) methyl- N-methanimidamide] are used in agriculture and in veterinary as insecticides or acaricides (Hollingworth, 1976).

Chemistry and mode of action

The structure of formamidine is closely related to the neurotransmitter norepinephrine. In invertebrates, these compounds exert their toxicity by activating an octopamine- dependent adenylate cyclase (Nathanson, 1985). In mammals, symptoms of formamidines poisoning are sympathomimetic in nature (Beeman and Matsumura, 1973). There is structural similarity between the insect octopamine receptors and mammalian alpha2- adrenergic receptors.

TOXICITY AND NON-TARGET EFFECTS

Chlordimeform, a formamidine has been classified as a probable human carcinogen (Group 2A) by IARC in 1990. Several cases of amitraz, another formamidine, have been reported particularly in Turkey (Yaramis *et.al,* 2000; Casken *et.al,* 2003; Elinav *et al.,* 2005; Proudfoot, 2003). Signs and symptoms of poisoning mimicked that of alpha2-adrenergic receptor agonists such as clonidine, and included nausea, hypotension, hypreglycemia, bradychardia and miosis. Formamidines decrease hepatic non-protein sulphydryls. They affect the levels of hepatic GSH, possibly through interactions with hepatic alpha 2- adrenoreceptors. They also have stimulative or depressive effects on the central nervous system. They lead to raise in plasma glucose levels and suppress insulin release from the pancreas. Hypothermia (decrease in body temperature) occurs due to the inhibitory effect of formamidines especially amitraz on prostaglandin E2 synthesis (Shitole, Kulkarni, Sathe, Rahate, 2010).

Phenylpyrazoles

Phenylpyrazoles are a new class of insecticides with a single chemical, fipronil. Fipronil acts as a potent blocker of GABA- regulated chloride channels, but binds to a site different from the picrotoxin binding site used by

organochlorine insecticides. It also has a much higher specificity for insect receptors over mammalian receptors (Hainzl *et al.,* 1998). It is effective against insects that are resistant to pyrethroid, cyclodiene, organophosphorus and/or carbamate insecticides. However, they still exhibit toxic or other undesirable side effect on non-target organisms (Huijbregts *et al.,* 2005). The extensive or inappropriate use of these compounds could cause soil and water pollution, thus harm to human health.

TOXICITY AND NON-TARGET EFFECT

Fipronil by a photochemical reaction is converted to a disulfinyl photodegradate. This photoproduct has high neurotoxicity. Although this is not a metabolite in mammals; it does have a high affinity towards the insect GABA system. This contributes to fipronil's selective toxicity toward insect (Casida *et al.,* 1996). Fipronil is toxic to mammals via ingestion (U.S. EPA, 1996). Fipronil is highly toxic on an acute oral basis and highly toxic on a sub-acute dietary basis to upland game birds like Mallard duck (U.S. EPA, 1996; Bobe *et al.,* 1997).

Fipronil is considered to be highly toxic to rainbow trout and very highly toxic to bluegill sunfish with an LC_{50} of 0.246 ppm and 0.083 ppm. The sulfonite metabolite is more toxic to the rainbow trout and bluegill sunfish than the parent compound. Fipronil was shown to affect the growth of *Daphnia.* It is highly toxic to termites and has severe and long-lasting negative impacts on termite populations. It thus poses a long-term risk to nutrient cycling and soil fertility where termites are beneficial key species in these ecological processes. Its toxicity to termites also increases the risk to the ecology of habitats in which termites are a dominant group, due to their importance as a food source to many higher animals. This risk has been demonstrated in Madagascar, where two endemic species of lizard and an endemic mammal decline in abundance because of their food chain link to termites. Fipronil is highly toxic to bees (LD= 0.004 mg/bee). It is also toxic to lizards where LD for *Acanthodactylus dumerili* (Lacteridae) is 30 microgram/gram body weight. Fipronil is also toxic to gallinaceous bird like Northern bobwhite quail. Fipronil is a relatively new insecticide that has not been in use for long enough to evaluate the risk it may pose to human health.

CONCLUSION

Due to the various harmful effects of insecticides various countries have promulgated laws to control the use of insecticides. A few examples of such laws are:

- Agricultural chemicals regulation law in Japan legislated in 1948 and then amended in 2007
- Chemical usage (Agriculture and Veterinary) Control Regulation in 1999 in Australia
- Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
- Federal Insecticide, Fungicide, and Rodenticide Act, 1947 in the United States of America
- The Insecticides Act, 1968 in India
- The Control of Pesticides Regulation, 1986 in the United Kingdom
- Regulations on Pesticide Administration in China promulgated by Decree No. 326 of the State Council of the People's Republic of China on November 29, 2001
- Pest Control Products Act, 2002 in Canada
- Hazardous Substance and New Organism Act, 1996 in New Zealand
- Agrochemicals Control Act, 1957 amended in 2009, South Korea
- Control of Vectors and Pesticides Act, 1998 in Singapore
- About safe handling of pesticides and agrochemicals, 1997, Russian Federation

Many new generation pesticides have lower toxicity to non-target organisms and to humans. Newer agricultural practices like organic farming have less harmful effects on the environment. Uses of crop varieties that are resistant to insect attacks require less insecticide applications. At the same time use of pesticides of biological origin are used increasingly thereby leading to lesser use of harmful chemicals. In addition the Stockholm convention on Persistent Organic Pollutants, adopted on 22nd May, 2001 in Stockholm, Sweden (came into force on the 17th May, 2004) has sought to decrease the use of chemical insecticides like chlordecone, lindane, Endosulfan.

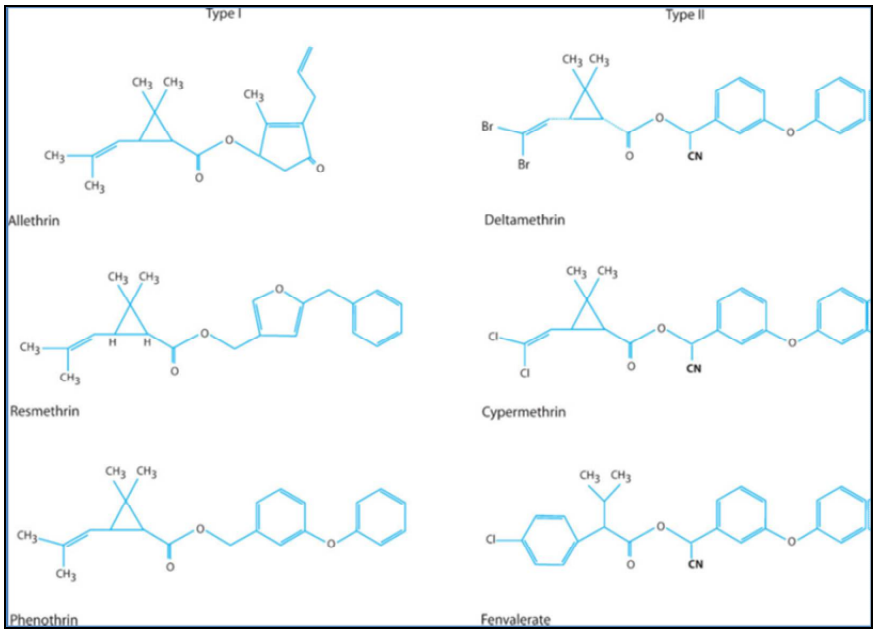

Figure 1.Structures of Type I and Type II pyrethroid insecticides. All Type II pyrethroids contain a cyano (CN) group.

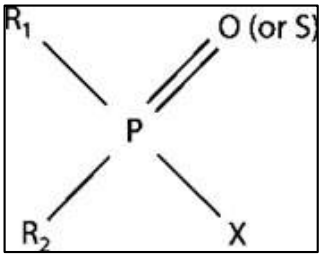

Figure 2.General structure of an organophosphate.

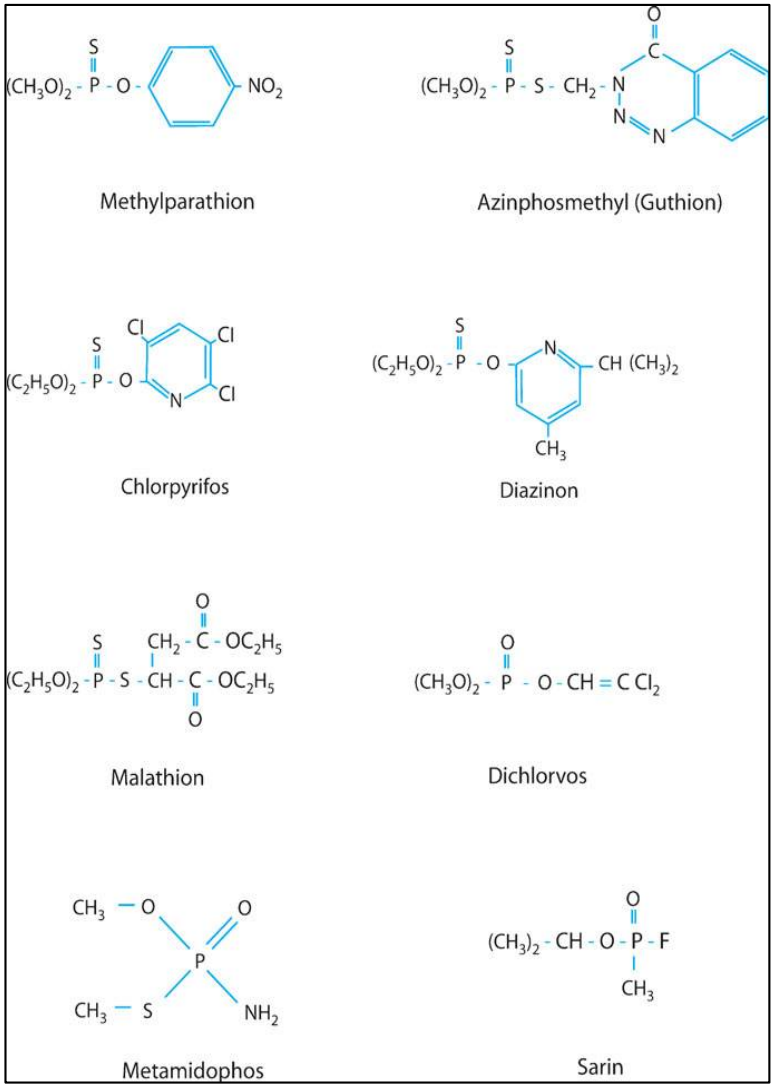

Figure 3.Structure of different types of OP insecticides and nerve gas Sarin.

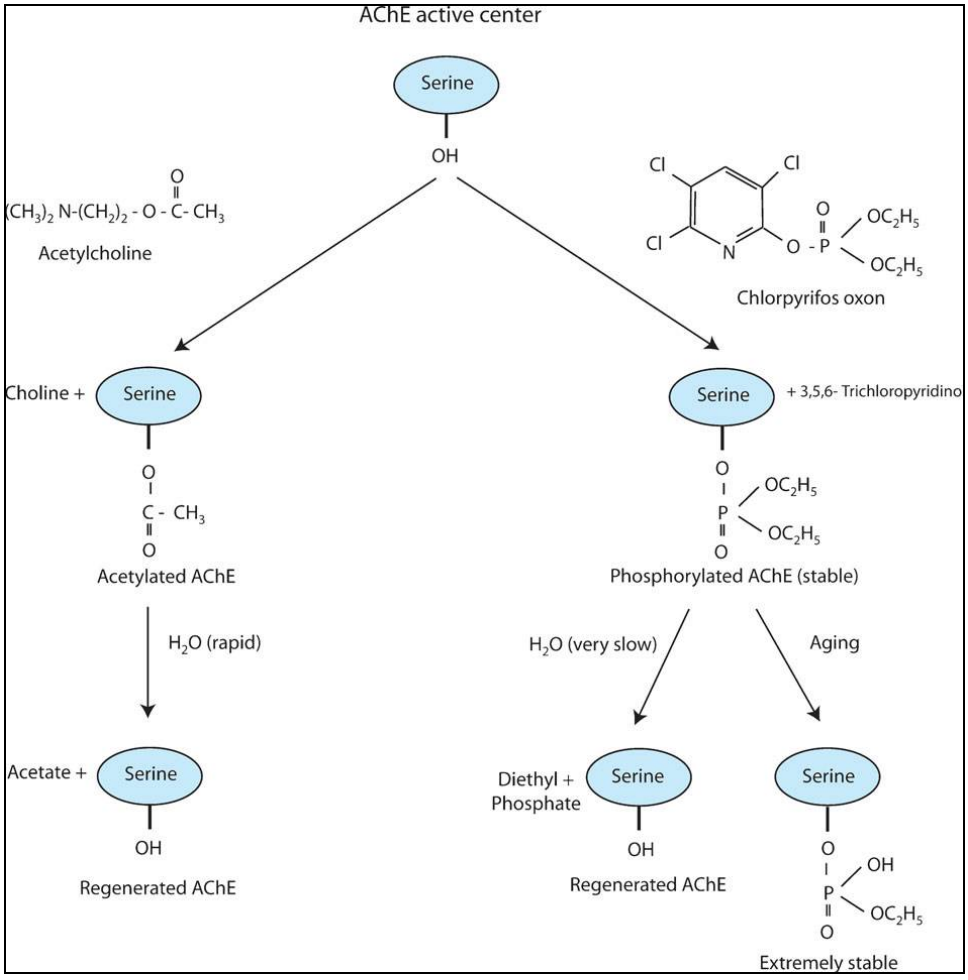

Figure 4.scheme of hydrolysis of acetylcholine by acetylcholinesterases and reaction of chloropyrifos with AChE.

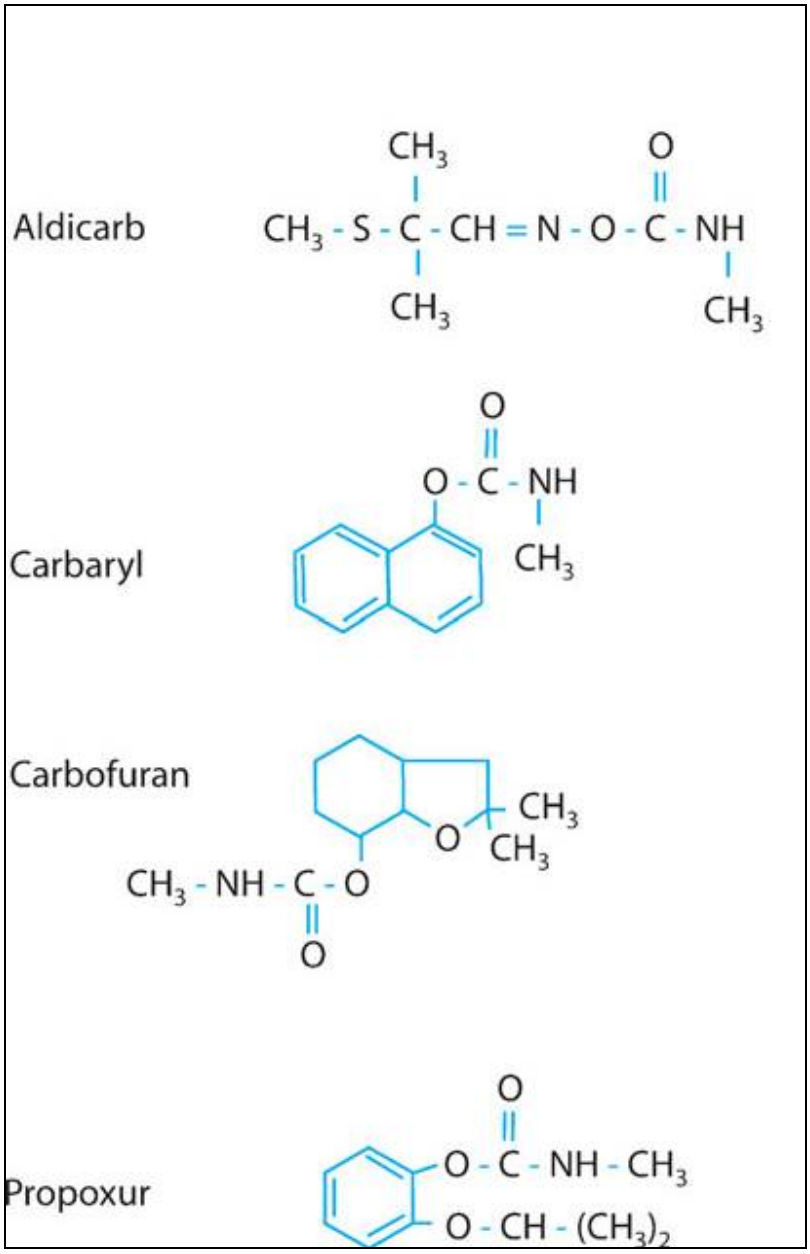

Figure 5 Different types of carbamates

Table 1. Classification of pyrethroids based upon toxic effects in rat (Verschoyle and Aldridge, 1980)

Syndrome	Signs and symptoms	Examples
Type I (T syndrome)	Aggressive sparring, Increased sensitivity to external stimuli, Whole body tremors, Prostration	Allethrin, Bioallethrin, Resmethrin, Phenothrin
Type 2 (CS syndrome)	Pawing and burrowing, Profuse salivation, Coarse Tremor, Choreoatetosis, Clonic seizures	Deltamethrin, Fenvalerate, Cypermethrin, Cyhalothrin

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RESEARCH ARTICLE

Study the Effect of Pressure on the Mechanical Properties of CdS Nanocrystals using Density Functional Theory

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ABSTRACT

First principles density functional calculations were performed to study the electronic structure, elastic and mechanical properties of CdS.The calculations are carried out within the generalized gradient approximation (GGA) with the effect the pressure on the properties with range (0 $\pm$ 50)GPa for 8 , 16, 54 and 64 atom. Ground state properties such as lattice constant (a_0), bulk modulus (B) are calculated. The elastic constants (C_{11}, C_{12} and C_{44}) and mechanical properties such as Poisson's ratio (σ), Young's modulus (E), shear modulus (G_H), anisotropic factor (A) are also calculated. The result shows that the CdS is ductile according to the analysis of (B/G_H) and Cauchy pressure (C_{12} - C_{44}).

Keywords: Ab-initio method, DFT , mechanical properties , pressure.

INTRODUCTION

The size-dependent properties of semiconductor nanocrystals have attracted considerable interest from physicists and chemists both because of the scientific questions they raise and because of their potential technological applications [1]. As the investigation of size-dependent properties progresses, experimental techniques for nanocrystal synthesis and analysis continue to improve [2]. At the same time, theoretical methods for computing nanocrystal properties develop increasing accuracy.

Past theoretical studies of electronic and structural properties have included effective mass calculations, tight binding calculations, band-structure discretization, and other approaches [3,4]. Such varied schemes have been suggested

because semiconductor nanocrystals are in a difficult size regime; they are generally too big for molecular techniques but too small for a bulk computation that ignores the nanocrystal surface.

Materials modeling and simulations have attracted great attention in the last decade because of the substantial growth in the processing speed of computers and progressive algorithms [5]. Density functional theory based calculations are extensively used in the study of various physical and chemical properties of solids with great accuracy. One of these properties of solids is elastic compliance constants or simply elastic constants (ECs). Elastic constants is a response function to the external forces and are of significant importance in the materials properties [6-9].The elastic constant are important parameter that describe the response to an applied macroscopic stress and especially important as they are related to various solid state phenomena. The elastic properties of materials under pressure provide better understanding of some basic physical aspects such as inter atomic forces, elasticity, mechanical features, phase transitions and so forth. Nonetheless, elastic property measurements under pressure are usually challenging, and the lack of experimental data can be compensated by computational methods [10].

CdS is the inorganic compound , and CdS nanostructure have generated great interest due to their unique size depend chemical and physical properties . It is a direct band gap semiconductor (gap 2.42 eV) [11]. As well as this obvious property others properties result. CdS used in manufacturing of photoresistors (light dependent resistors) sensitive to visible and near infrared light. CdS was also one of the first semiconductor materials to be used for thin-film transistors (TFTs)[12] . CdS is important material for the development of various modern technologies of solid state devices and has been attracted for research and application [12] .Cadmium sulfide has, like zinc sulfide, two crystal forms; the more stable hexagonal wurtzite and cubic zinc- blende structure.

The present work will be involved in calculating mechanical properties of CdS nanocrystals as the size and shape of these nanocrystals change. We were used density functional theory at the generalized-gradient approximation level (Perdew, Burke, and Ernzerhof PBE approximation) coupled with large unit cell method (LUC- DFT) to simulate the structure of CdS which is a well developed theory that had been applied repeatedly for the nanocrystals structure [13-15].

Theory

The large unit cell method (LUC) which can be used to simulate systems of periodical symmetry such as the bulk or surfaces of ordinary crystals. The LUC is adopted for the simulation of the electronic structure of nanocrystals in conjunction with the k = 0 approximation (k is the reciprocal wave vector). This approximation is used to drop sums of contributions from other points in k space except the origin [16-18].

This is translated in nanocrystals structure by saying that we have a limited translational symmetry in the inside “core” of the nanocrystal only. Since the inside core of the nanocrystal has a well-defined 3D symmetry structure such as in the present zinc-blende structure, we optimize the structure by optimizing the lattice constant of the inner core only. This method can be used to simulate nanocrystals surfaces by adopting the k = 0 approximation but with more elaborate optimization procedure. Density functional theory at the generalized gradient approximation level coupled with large unit cell method (LUC-DFT) is used to simulate the electronic structure of zinc-blende cadmium sulfide nanocrystals. Four LUC cores are considered in the present work 8, 16, 54, and 64 atoms [19].

The bulk modulus is calculated by Cohen empirical formula [20]:

$$B_o = \frac{(1970 - 200I)}{d^{3.5}} \text{.....(1)}$$

Where I is the ionicity factor which equals 0, 1 and 2 for IV, III-V and II-V groups respectively [20], *d* is the inter-atomic distance [21].

$$d = \frac{\sqrt{3}}{4} a_o \text{.....(2)}$$

Where a_0 lattice constant at zero temperature (t) and pressure (P)

And speed of sound equal to :

$$v_o = \sqrt{\frac{B_o}{\rho}} \text{ .....(3)}$$

Where v_o the speed of sound , ρ density equal to 4.85 g/cm^3 to Boron Nitride [22] .

The lattice constant (a) at any pressure :

$$a = a_o \exp(-P/3B_o) \text{.....(4)}$$

According to (Kumar and Sastry) [23] the plasmon energy (*Ep*) is defined as:

$$Ep = \left(\frac{d}{15.3}\right)^{-3/2} \text{ .....(5)}$$

The elastic moduli require knowledge of the derivative of the energy as a function of the lattice strain. It is well known that a cubic system has only three independent elastic constants namely C_{11}, C_{12} and C_{44}. Hence, a set of three equations is needed to determine all the constants. The first equation involves calculation of bulk modulus (B), which is related to the elastic constants as: [24]

$$B = \frac{1}{3}(C_{11} + 2C_{12}) \text{.....(6)}$$

The second step involves volume-conservative tetragonal strain given by the following tensor:

$$\begin{bmatrix} \delta & 0 & 0 \\ 0 & \delta & 0 \\ 0 & 0 & \frac{1}{(1+\delta)^2} - 1 \end{bmatrix} \text{.....(7)}$$

where $\delta = (1+e)^{-1/3}-1$ with e as strain tensor. Application of this strain has an effect on the total energy from its unstrained value as follows:

$$E(\delta) = E(0) + 3(C_{11} - C_{12}) + V_0\delta^2 + O(\delta^3) \quad \text{.....(8)}$$

where V_0 is the volume of the unit cell.

Finally, for the last type of deformation, we use in the volume-conserving rhombohedra strain tensor given by:

$$\frac{\delta}{3}\begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \quad \text{.....(9)}$$

which transforms the total energy to

$$E(\delta) = E(0) + \frac{1}{6}(C_{11} + 2C_{12} + C_{44}) + V_0\delta^2 + O(\delta^3) \quad \text{.....(10)}$$

The transverse and longitudinal sound velocities respectively obtained by using the elastic constants as follows:[25,26]

$$v_t = \sqrt{\frac{\left[C_{11} + \frac{2}{5}(2C_{44} + C_{12} - C_{11})\right]}{\rho}} \quad \text{.....(11)}$$

$$v_l = \sqrt{\frac{\left[C_{44} - \frac{1}{5}(2C_{44} + C_{12} - C_{11})\right]}{\rho}} \quad \text{.....(12)}$$

where C_{11}, C_{12} and C_{44} are second order elastic constants and ρ is mass density per unit volume.

RESULTS AND DISSCUSSIONS

The values of three independent elastic constants (C_{11}, C_{12} and C_{44}) for cubic lattice were calculated by applying a proper strain to the equilibrium structure from different directions [27–30]. These values are included in Table 1. All these values are positive and satisfy the generalized criteria [31] for mechanically stable crystals: $(C_{11}-C_{12})> 0$, and $(C_{11}+2C_{12})> 0$, $C_{44}> 0$. These conditions also lead to a restriction on the value of the bulk modulus B, which is required to be between C_{11} and C_{12}, i.e., $C_{12} < B < C_{11}$.

Polycrystalline elastic constants are more attractive in technological characterizations of materials, to obtain such quantities ; we have used the Reuss assumption to estimate the bulk modulus *B*, shear modulus *G*, Young modulus *Y*, Poisson ratio σ , to the following relations [32]:

$$Gv = \frac{C_{11} - C_{12} + 3C_{44}}{5}$$

$$G_R = \frac{5C_{44}(C_{11} - C_{12})}{4C_{44} + 3(C_{11} - C_{12})} \quad \text{.....(13)}$$

$$G = (G_V + G_R)/2$$

Also the Young's modulus and Poisson ratio can be expressed in terms of Bulk modulus and shear modulus given by :

$$Y = \frac{9GB}{G + 3B} \quad \text{.....(14)}$$

$$\sigma = \frac{3B - 2G}{6B + 2G} \quad \text{.....(15)}$$

The Young's modulus, also known as the tensile modulus *Y*, is defined as the ratio between stress and strain and used to provide a measure of stiffness, i.e., the larger the value of *Y*, the stiffer the material. .

Another important parameter is the elastic anisotropic factor (A) , which gives a measure of the anisotropy of the elastic were velocity in a crystal and is given as :

$$A = \frac{2C_{44}}{C_{11} - C_{12}} \quad \text{.....(16)}$$

Furthermore, Kleinman parameter (ξ), and Lames' coefficients (μ and λ) are also calculated for the lanthanum mono chalcogenide using the following relations [33-35]:

$$\xi = \frac{C_{11} + 8C_{12}}{7C_{11} + 2C_{12}} \quad \text{.....(17)}$$

$$\mu = \frac{Y}{2(1+\sigma)} \quad \text{.....(18)}$$

$$\lambda = \frac{Y\sigma}{(1+\sigma)(1-2\sigma)} \quad \text{.....(19)}$$

The Kleinman parameter (ξ) for cubic materials describes the relative ease of bond bending to the bond stretching. Minimizing bond bending leads to ξ = 0 and minimizing bond stretching leads to ξ = 1 **[36]** .

All these parameters listed in Table (I).The shear modulus *G* represents the resistance to plastic deformation while *B* represents their resistant to fracture [37]. If *G*/*B*<0.5 the material will behave in a ductile manner, and vice versa, if *G*/*B*>0.5 the material demonstrates brittleness. In our case, we have found that G/B ratio is 0.36, classifying this material as ductile.

Cauchy pressure has been suggested that it could be used to describe the angular character of atomic bonding in metals and compounds [38].If Cauchy pressure is negative, then the material is non-metalic with directional bonding, but if Cauchy pressure is positive , then the material is expected to be metallic. The CdS has a positive Cauchy pressure which indicates its ductile nature.Bulk modulus of BN nanocrystal as a function of number of atoms in the core is shown in Fig. (1). This Figure shows that the bulk modulus increases with the number of atoms in the core. This relation can be simply attributed to the fact that the lattice constant for the core part of the crystal decrease with increasing number of atoms, the latter equation means that the decrease in lattice constant entails a decrease in interatomic distance (*d*) which leads to increasing in the bulk modulus according to equation (1). And this relation shows in Fig.(2) for speed of sound with number of core atoms, while the speed of sound depends on the atoms arrangement [39].

The compression stress increases the density and decreases volume due to the shrinkage of orbital as shown in Fig. (3), it is can be seen the ratio V/Vo decreases smoothly with increase the pressure, however , the change in distance is getting smaller is due to the mutual repulsion of the atoms, with leads to the difficulty of compression of the crystal under high pressure [40].Figure (4) shows the variation of plasmon energy with compression . Kornyushin has showed that the plasma affects the charge distribution and so the sound wave [41], On the other hand, the phonon frequency affects the effective charge [42], which depends upon the charge distribution and determines the plasma frequency [43]. the vibrational energy depends upon the behavior of the total energy which increases with stresses due to repulsion and attraction forces [44]. On the other hand the speed of sound as in fig.(5) which also represents the average of phonon velocity [45] .

Figure (6) displays a further comparison for the pressure behavior of the bulk modulus in diamond for the considered pressure scale. The bulk modulus depends upon the density directly, and the young modulus which depends upon the bulk modulus [46], and vice verse for the tensile stress. From the common physical equation of the bulk modulus ($B = \Delta P/\Delta V$), one can expect an increment for *B*, because of its direct proportion to applied pressure. Thus, the bulk modulus of diamond dramatically increases in Fig. 6 . The Young modulus (*E*) is the resistance to uniaxial tensions and gives the stiffness degree, i.e., the higher the value of *E*, the stiffer is the material [47]. Like the bulk modulus, the shear and Young moduli of diamond also point out the same behavior under pressure as in Fig. 7. The Poisson ratio (σ)is the ratio between the transverse strain and longitudinal strain in the elastic loading direction. It delivers detailed knowledge about the bonding character of solids. In general, the Poisson ratio values are of about 0.1 for covalent materials, 0.25 for ionic materials and change between 0.28 and 0.42 for metals [47] . As in Fig. 8, the Poisson ratio of CdS at 0 GPa is 0.36, which reflects the covalent bonding nature of diamond.

CONCLUSION

In summary, the mechanical properties of CdS have been investigated using density functional theory within the generalized gradient approximation under variation of pressure . The main results can be summarized as follows:
(i) Increases the number of core atoms increase the bulk modulus and speed of sound.
(ii) The variation of volume decrease with increase the pressure but the speed of sound , bulk and young modulus , Poisson ratio are increases with increase the pressure .
(iii) It has found that G/B ratio is 0.36, classifying this material as ductile.

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Table 1 : Calculated values of elastic constant and mechanical properties for CdS

Elastic constant (commat. 2011)[36]	**G**	**G/B**	**A**	ξ	λ	μ	v_t	v_l	**Cauchy pressure C_{12}-C_{44}**
C_{11}=67.6 C_{12}= 46.3 C_{44}=29.5	**19.61**	**0.36**	**2.76**	**0.77**	**50.61**	**19.68**	**4130**	**2130**	**16.8**

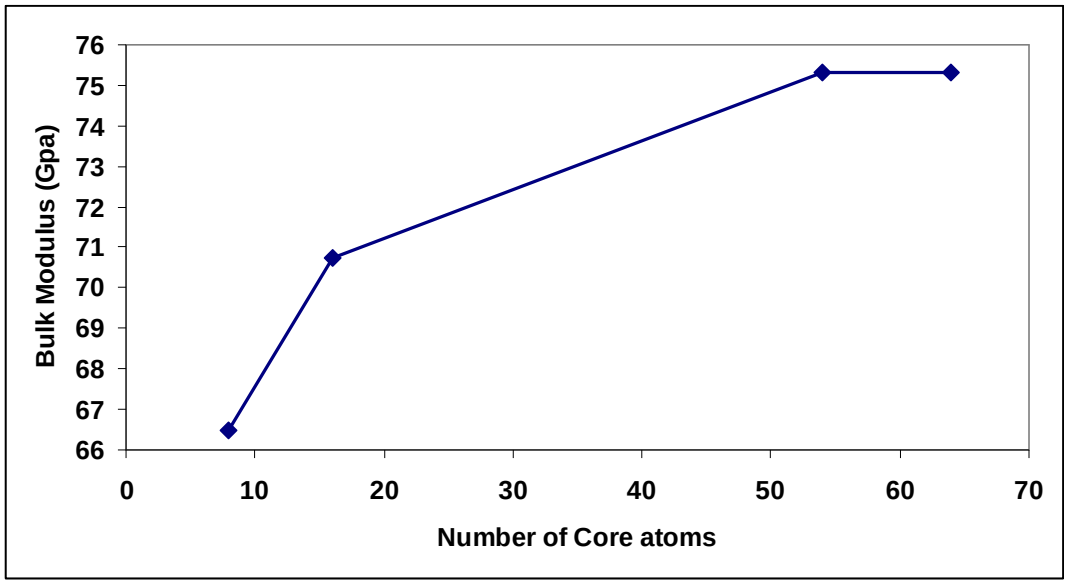

Fig.1 : Bulk modulus as a function of the number of core atoms for CdS nanocrystals.

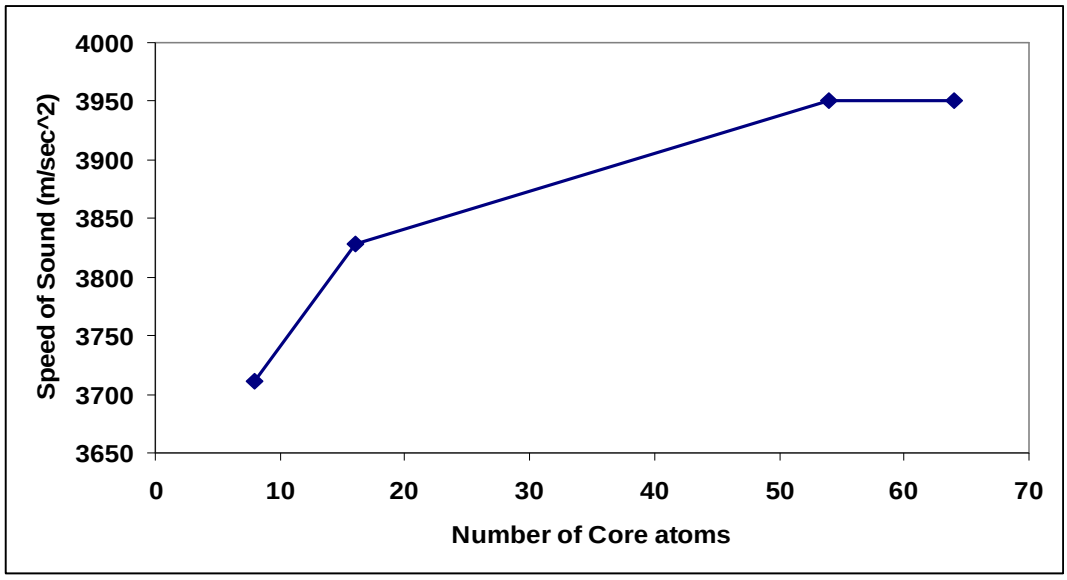

Figure 2: Speed of sound as a function of the number of core atoms for CdS.

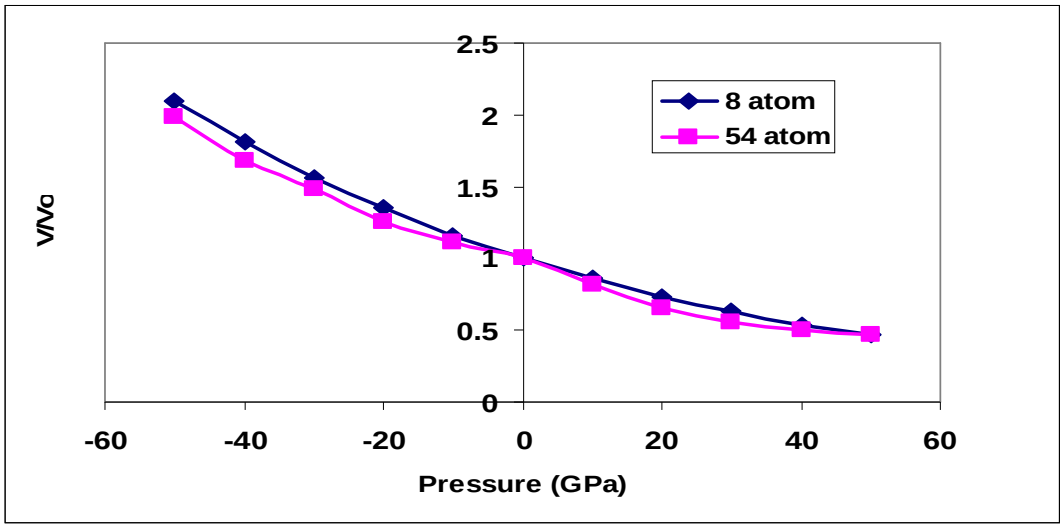

Figure 3 : The variation of volume with pressure.

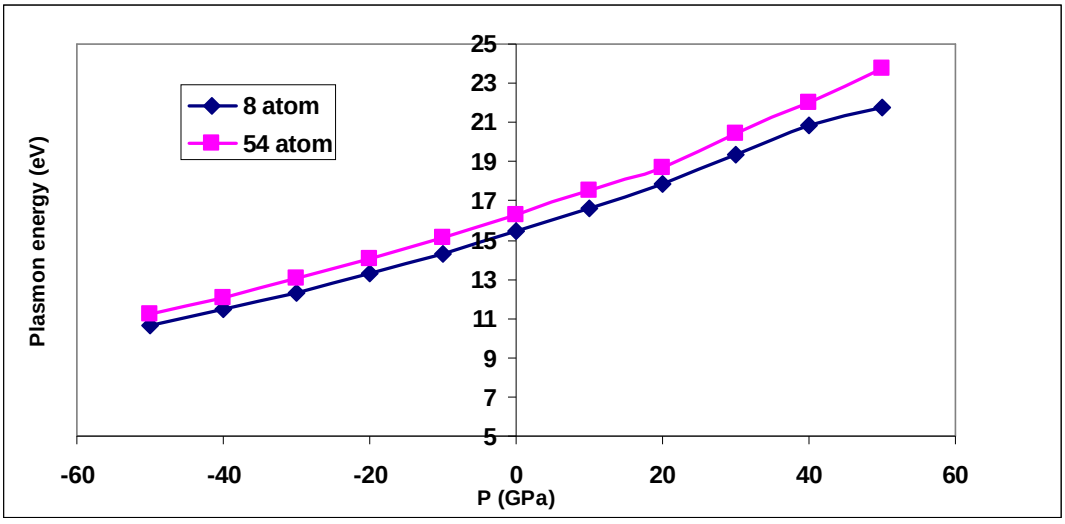

Figure 4: The variation of Plasmon energy with pressure.

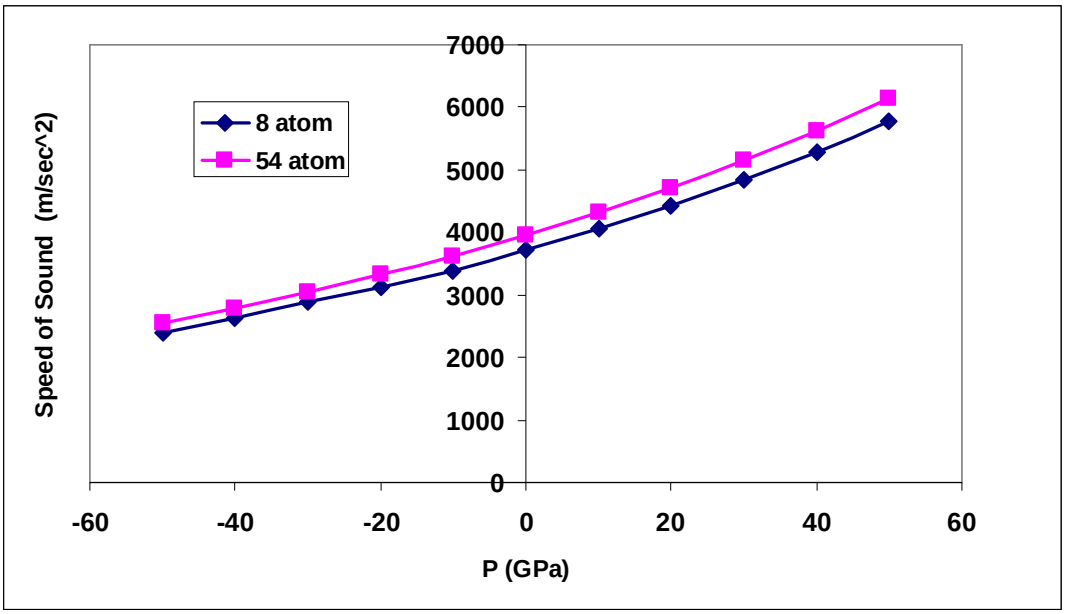

Figure 5 : The variation of Speed of sound with pressure

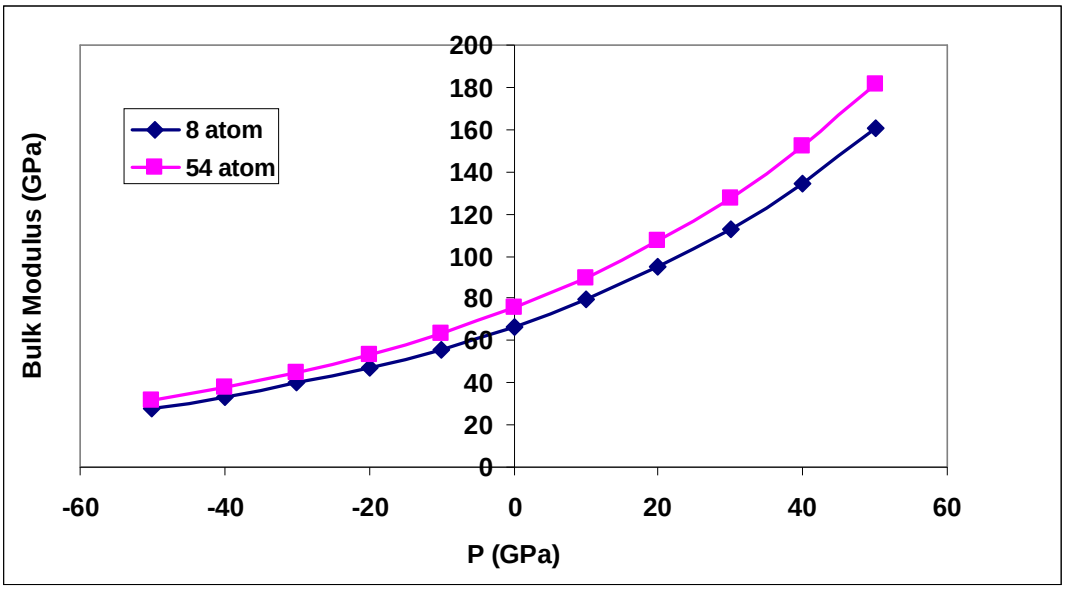

Figure 6 : The variation of Bulk modulus with pressure.

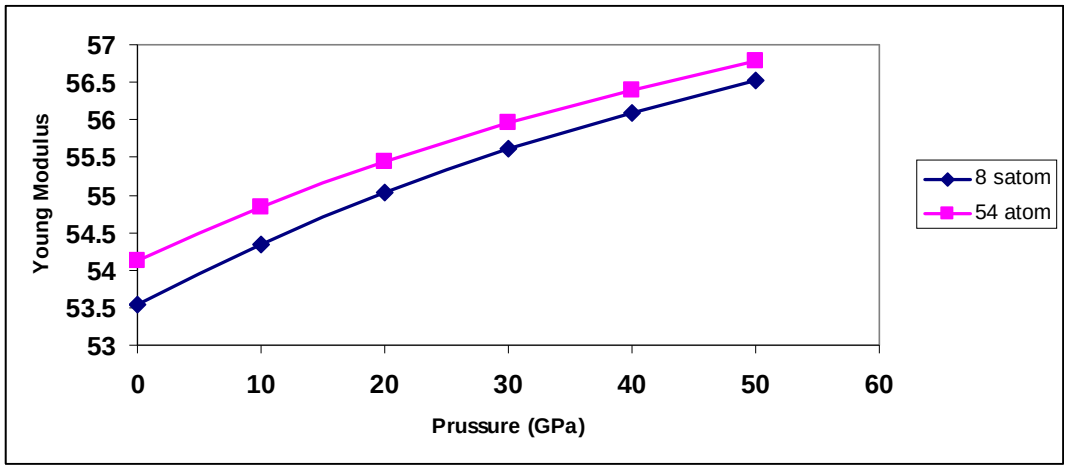

Figure 7: The variation of Young modulus with pressure.

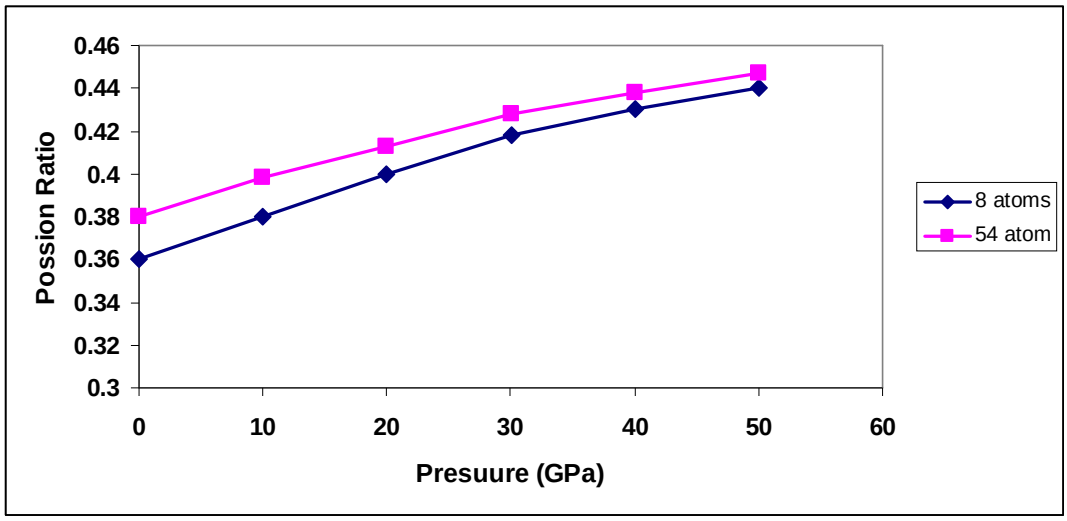

Figure 8 : The variation of Poisson ratio with pressure.

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RESEARCH ARTICLE

Cytotoxic Effect of Ethanolic Extract of *Aloe vera* on Vero Cell Lines

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ABSTRACT

The plant *Aloe vera* is extensively used in Ayurvedic, Homeopathic and Allopathic streams of medicines, and neutraceuticals and has great demand in global market. The present study was carried out to extrapolate the cytotoxic effect of ethanolic extract of Aloe vera on vero cell lines. Vero cells treated with ethanolic extract of Aloe vera in multifarious doses for 24, 48 and 72 hours resulted in dose and time dependent cell death as indicated by MTT assay and flow cytometric analysis. Furthermore, confirmation of cell death was done by measuring loss of mitochondrial trans-membrane potential and apoptosis staining. Results of the study proved a significant cytotoxic activity of ethanolic extract of Aloe vera on vero cells which may have predominant translational application for treating cancer.

Keywords : *Aloe vera,* cytometric analysis, cytotoxic effect, Homeopathic,Allopathic.

INTRODUCTION

Naturally derived products have a central place in the ethno-medical practice. Search for anti-cancer properties in the medicinal plants increased in the recent years (1).Interestingly, ethanolic extracts of many medicinal plants have anti-tumor and anti-viral properties and may have potential applications in the allopathic medicine (2-3).*Aloe vera*as whole plant, leaves or its extracts used in the treatment of various disease conditions (e.g., minor burns, skin wounds and irritation, constipation, cough, diabetes, cancer, headache, arthritis and immune deficiencies). The active ingredients hidden in its succulent leave have the power to soothe human life and health in a myriad ways and believed to have immuno-modulator, anti-viral and anti-neoplastic properties (4,5& 6). The present study was designed to investigate cytotoxic/anti-proliferativeproperties of *Aloe vera* extracts in vero cells.

MATERIALS AND METHODS

Collection and Preparation of Ethanolic extract of *Aloe vera*

The *Aloe vera* plant was collected from defense research laboratory, Pithoragarh Uttaranchal, bulk amount of leaves was washed under running water and wiped with muslin cloth and Leaves chopped with sharp knife in to small pieces of size 1-2 cm thick and 4-5 cm long and The dried under hot circulating air at 60ºc for 24-48 hours.Grinding of the dried chopped leaves was done in grinding mill having stainless steel blades, powder was filled in air tight container and stored in cool and dry place. The ethanol extract was prepared by usingdried powder in mixing with absolute ethanol in an incubator cum shaker at 37°C, with gentle swirling at 120 rpm/min, initially for 48 hours then for 24 hours each for 3 times. The supernatant was collected and double filtered and then filtrate was centrifuged at 5,000 rpm for 10 min. and filtrate was spread in a large glass plate and kept inside incubator equipped with fan at 50°C for 24 hours. The semi dried gel was collected and lyophilized. The final yield (g/10 g powder) of the lyophilized ethanolic extracts was 0.85 grams.

Cell culture

Vero cells were cultured in 75 cm^2 flasks containing 30 mL of culture medium (GMEM, Sigma, USA) with 10% new born calf serum (Sigma, USA), penicillin 100 IU/mL, streptomycin 100µ/mL. Flasks incubated at 37ºC with 5% CO_2 and 95% relative humidity until cells reached confluence. Later, cells were harvested and sub-cultured in 96-well plate.

MTT assay

For MTT assay selected doses used were 50µg/ml, 100µg/ml, 200µg/ml, 500 µg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml of aloe extracts with the time intervals of 48hr, 48hr and 72 hr. Approximately, 20 µl of MTT stock (Sigma, USA) (4 mg/mL in PBS) was added to each well after incubation for 4 hr. at 37ºC with 5% CO_2 and 95% relative humidity. The absorbance of was measures using computerized ELISA (Micro Scan) reader at 570 nm.

Staining and flow cytometry of cells

After treatment with ethanolic extract, cells were harvested by centrifugation (5,000 rpm for 5 minutes), washed twice with ice cold PBS (pH 7.4) and fixed in ice cold 70% ethanol at -20ºC over night. Fixed cells were re-suspended in one milliliter of binding buffer and 500 µl of cell suspension were then incubated with 10 µl of propidium iodide (PI, Sigma Aldrich) for 10 minutes at room temperature in the dark. Another set of re-suspended cells were treated with 10 µl of 3,3′-dihexyloxacarbocyanine iodide (DiOC6, 40 mM, Invitrogen) stain for the determination of mitochondrial trans-membrane potential (delta psi). The cell cycle and loss of mitochondrial trans-membrane potential were evaluated using flow cytometry (BD FACScan, Becton Dickinson, USA).

Fluorescent microscopy

Nuclear changes and apoptotic body formation were visualized by Acridine orange/Ethidium bromide (AO/EB) staining. Briefly cells were cultured in 6-well plates on cover slips for 24 hours then the extracts were added and incubated for 48 hours,media removed and cover slips were washed with PBS thrice and then stained with AO/EB (both 100 µg in 1 mL PBS) for 5 min. After staining, cover slips were washed with PBS thrice. Cells were fixed with 4% para-formaldehyde for 20 min at room temperature and nuclear morphology was observed under the fluorescent microscope.

RESULTS AND DISCUSSION

Spectral analysis of Ethanolic extract of *Aloe vera*

The wide use of aloe plant as therapeutics accompanied by an upsurge of both clinical and chemical research which is reaching more closely towards the active ingredients and their biological activity. Presently, the emphasis is changing towards definition of active constituent or constituents so they can be used accurately in the formulations (7). Therefore, it is mandatory to investigate the quality of aloe species extracts by spectral analysis for their recorded variability in curative properties.Spectral analysis of water and ethanol extract (1mg/ml) was carried with the range of OD from 190nm to 600nm. (Table 1and Figure 1). The chemical fractionation study of aloe barbadensis confirmed the presence of anthraquinone aloin, aloe emodin, polysccharides, sugars, enzymes and organic acids (Obata, 1993; Coats *et al.,* 1996).

MTT dye reduction test

With the MTT assay there was less cell cytotoxicity observed at low doses and at less time interval, with the increased dose and time interval toxicity of the extract also increased.Ethanol extract caused 0.8-1.9% growth inhibition at 50 µg/ml,1.2-2.3% growth inhibition at 100µg/ml, 2.1-3.2% growth inhibition at 200 µg/ml, 7-12% growth inhibition at 500 µg/ml, 10-18.5% growth inhibition at 1mg/ml, 11.5-20% growth inhibition at 2mg/ml, 20-40% growth inhibition at 5mg/ml, 28-56% growth inhibition at 10g/ml, 34-74% growth inhibition at 20mg/ml and 60-86% growth inhibition at 40mg/ml. (Table 2, Figure 2). It was observed that at doses from 50µg/ml to 2mg/ml maximum cytotoxicity at 72hr of incubation observed was 20% for ethanol extracts. Similarly Aloe-emodin- and emodin caused the cell death in a dose- and time-dependent manner of lung carcinoma cells (8).

Fluorescent microscopy

Effect of the extracts for the detection of typical morphological changes characteristic to the apoptotic cells i.e. cell pyknosis, chromatin condensation and nuclear fragmentation was studied by staining the extract treated cells with the fluorescent dyes acridine orange and ethidium bromide. There were no much significant changes were observed between treated and untreated cells. (Figure 3)

Flow cytometry –PI staining

Flow cytometry is a sensitive and quantitative assay for analysis of apoptotic population and has been widely used (9,10 & 11). Flow cytometric profiles of PI-stained cells indicating amount of DNA degradation after treating cells with ethanol extracts at doses 5mg/ml and 10mg/ml for 48hr. In untreated control 4.66% cells showed PI- fluorescence ethanol treated cells 7.3% at 5mg/ml, and 13.60% at 10mg/ml cells exhibited fluorescence. (Figure 4 A, B and C).Aloe emodin a hydroxyanthraquinone, isolated from aloe plant, induced apoptosis in promyelocytic leukemia HL-60 cells which is confirmed by both DNA fragmentation study and flow cytometry (Chen *et al.,* 2004).

Flow cytometry- $DiOC_6$ dye staining

Effect of extracts for cause of loss of mitochondrial transmembrane potential ($\Delta\Psi m$) which is an early indicator of cells undergoing apoptosis was studied using $DiOC_6$ dye. The cells treated with extract at 5mg/ml for 48hr were stained with the $DiOC_6$ dye and analyzed through flow cytometry. Where 5.41% of control cells, 12.25% of water extract treated and 14.64% ethanol extract treated cells showed loss of transmembrane potential (Figure 5). A study comparing the *in vitro* responses of carcinoma cells, immortalized and non-tumorigenic epithelial S-G cells, and

normal fibroblasts, to green and black tea extracts, noted that carcinoma and immortalized cells are more sensitive, in terms of growth inhibition, and apoptosis induction, than normal cells (12)

CONCLUSION

From the current study it can be concluded that the aloe extracts, even though showed significant cytotoxicity particularly at higher doses needs to be further investigated regarding its effect on normal cells in conjunction with tumor cells.

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Table 1: Spectral analysis of Aloe ethanolic extract

Wavelength (nm)	Absorbance for Ethanolic extract
190	2.0
200	3.2
270	2.4
300	2.6
360	2.05

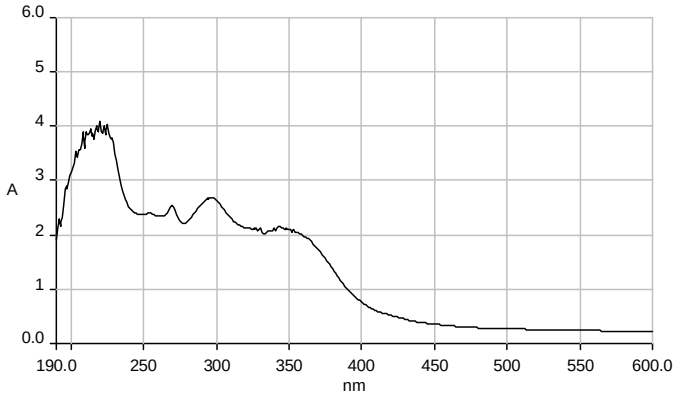
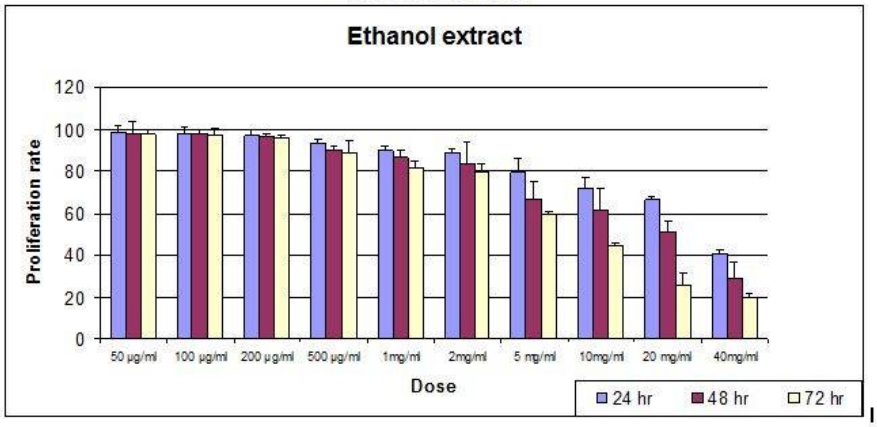


Figure 1: Graphical representation of spectral analysis of Ethanolic extract of Aloe vera

Table 2: Effect of ethanol extract at different doses and time intervals revealed in MTT dye reduction test

Sl.No	Dose	Rate of proliferation		
		24 hr	48 hr	72 hr
1	50 µg/ml	0.992 ± 0.036	0.988 ± 0.037	0.981 ± 0.023
2	100 µg/ml	0.988 ± 0.023	0.981 ± 0.019	0.977 ± 0.034
3	200 µg/ml	0.979 ± 0.028	0.972 ± 0.018	0.968 ± 0.012
4	500 µg/ml	0.931 ± 0.031	0.899 ± 0.023	0.885 ± 0.035
5	1mg/ml	0.900 ± 0.023	0.868 ± 0.031	0.815 ± 0.034
6	2mg/ml	0.885 ± 0.023	0.837 ± 0.02	0.792 ± 0.023
7	5 mg/ml	0.797 ± 0.032	0.670 ± 0.031	0.598 ± 0.012
8	10mg/ml	0.717 ± 0.031	0.614 ± 0.02	0.443 ± 0.029
9	20 mg/ml	0.661 ± 0.018	0.507 ± 0.032	0.260 ± 0.036
10	40mg/ml	0.406 ± 0.019	0.290 ± 0.029	0.138 ± 0.021

Figure 2: Effect of ethanol extract at different doses and time intervals revealed in MTT dye reduction test



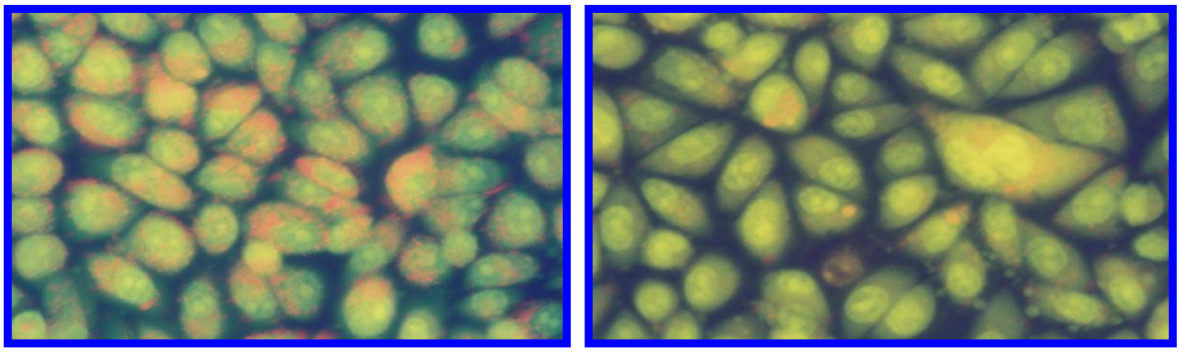


Figure 3: Vero cells stained with AO/EB, observed under fluorescent microscope

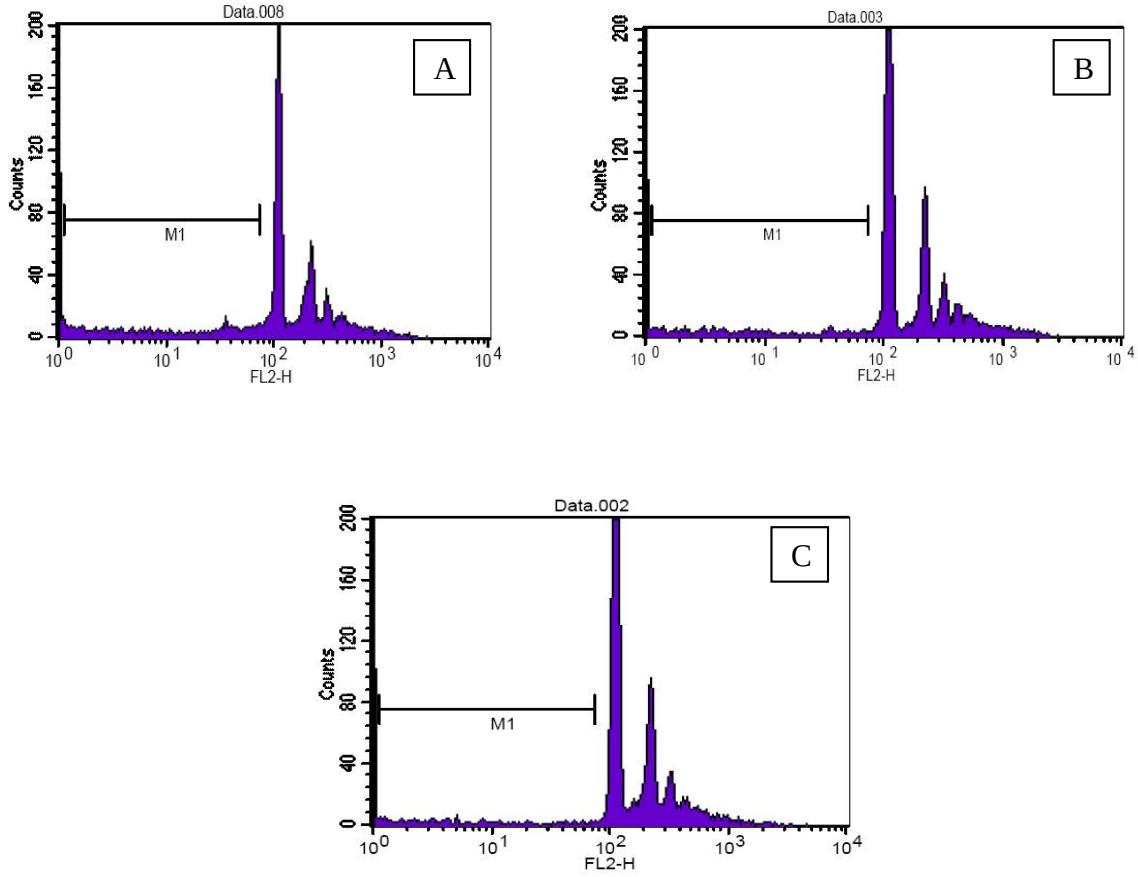


Figure 4: Flow cytometric analysis of vero cells exposed for 48 hours.

A – Ethanolic extract @ 5 mg/ml, B- Ethanolic extract @ 10 mg/ml ,C – Untreated group

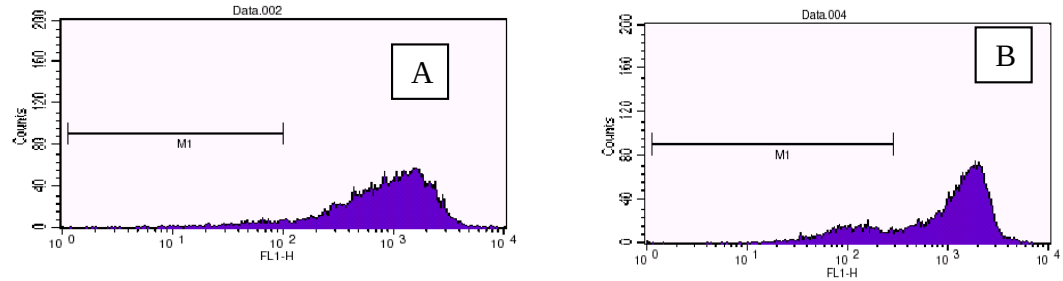


Figure 5: Flow cytometric analysis of vero cells stained with DiOC6 dye.

A – Control , B- Ethanolic extract

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RESEARCH ARTICLE

Haemato-biochemical and Physiological Indicators of Eutocia and Dystocia Cases in Crossbred Cows

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ABSTRACT

The present study reports the haemato- biochemical and physiological parameters under normal birth and dystocia in 40 clinical cases in crossbred cows subjected to caesarean section. From this study, it is concluded that in order to obtain high fetal and dam survival and also to reduce the loss, cows with dystocia should be presented without undue delay. Haemato- biochemical evaluation revealed no significant variation in RBC, WBC, hemoglobin and PCV, neutrophil, lymphocyte, calcium and total protein levels of eutocia and dystocia cows. However, significantly lower eosinophil, monocyte count and higher glucose levels were observed in dystocia.

Keywords: Crossbred cow, dystocia, caesarean, dam survival rate.

INTRODUCTION

India ranks second position in cattle and goats population, whereas first in buffalo population in the world. Livestock plays an important role in the Indian economy and provides livelihood to two-third of rural community. About 20.5 million people depend upon livestock for their livelihood which contributes 16% to the total income of small farm households as against an average of 14% for all rural households and 8.8% employment to the population. India is having a mega livestock sector which contributes 4.11% GDP and 25.6% of total Agriculture GDP [1]. As per 19th Livestock census, 2012, India's livestock sector is one of the largest in the world with a total of 512 million, holding of 11.6% of worlds total livestock population which consists buffaloes (57.83%), cattle (15.06%), sheep (7.14%), goats (17.93%), camel (2.18%), equine (1.3%), pigs (1.2%), chickens (4.72%) and ducks (1.94%). Population of exotic and crossbred cattle registered a significant increase of 20.18% while the indigenous cattle decreased by 8.94%[2].

Dystocia also called as difficult birth or obstructed labour has been a long-standing problem in dairy cows, occurring in 3 to 25% of cattle pregnancies. It is associated with numerous factors such as pelvic area of the cow, birth weight of the calf, age of dam, twin pregnancy, presentable disposition, gestation length and body condition of the cow at calving[3]. Dystocia can lead to increased incidence of retained fetal membranes, uterine infections, reduction of milk production, failure to conceive and survival of calves[4]. The reported dystocia rates in dairy cattle internationally are generally <5%, apart from those in the United States, where they are higher. Phenotypic dystocia trends are generally increasing internationally and this trend has been partially attributed to the introduction of cross bred genes. Achievement of heifer rearing targets prior to both service and calving and appropriate periparturient management decisions are prerequisites for controlling dystocia in dairy cattle[5].

The process of parturition, though physiological, is a stressful act for cows and their calves and abnormal parturition further exacerbates the stress[6] for both the dam and the fetus even in uncomplicated cases[7]. So the present study was designed to understand variations in haemato-biochemical and physiological parameter in dystocia and eutocia cases presented to Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bangalore.

MATERIALS AND METHODS

The study was conducted on forty crossbred cows maintained by the farmers in and around Bangalore city, Karnataka, India with a history of calving difficulty presented to the Department of Veterinary Gynecology and obstetrics, Veterinary College, Bangalore, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU) during April 2015 to May 2016.

Immediately after presentation, complete history regarding the obstetrical clinical status of the cows was obtained. The clinical status of each animal was noted down and detailed reproductive tract examination was conducted to know the cause of dystocia as well as to record any abnormalities of vulva (viz. edema, bruising and necrosis), presence of vaginal discharge and presentation of extremities of fetuses outside the vulva were also recorded. Body temperature, respiratory rate, pulse rate and heart rate were recorded for all cows.The age of the dam at the time of its presentation with the complaint of dystocia was obtained from each case-record. The gestational period at the time of presentation of the obstetrical case was obtained to analyze the relationship between the duration of pregnancy and the incidence of dystocia[8].

Caesarean section was performed with Epidural and line infiltration of local anesthetic (2 % lignocaine hydrochloride). Surgical intervention was made via left ventrolateral site. After removal of the fetus, loose fetal membranes and evacuation of the fluid, the uterus was sutured in two layers using chromic catgut (no. 1) in an inversion (Lambert) pattern. Abdominal wall was closed. Intravenous fluid (Ringers Lactate) therapy was instituted perioperatively. Analgesics and antibiotics were administered preoperatively on the day of operation and same drugs were used for another 4 to 6 days respectively. Antiseptic dressing of the incision line was continued twice daily from day of operation up to 5 days till suture removal. All the animals were discharged on the day of surgery. Postoperative care was assigned to the local field veterinarian. The progress of the cases was ascertained regularly from the owner's on telephone every alternate day till suture removal. The suture was removed on 10th postoperative day. Survival of dams and post-partum complications if any, were also recorded.

Blood collection and processing

Blood samples were collected from all the cows suffering from dystocia before Caesarean section. Blood samples were also collected from thirty one eutocia cows within fifteen minutes after parturition, which served as control. About 20 ml of blood was collected in two parts, one with and other without anticoagulant, from each animal aseptically in clean, sterilized test tubes by jugular vein puncture method. The blood samples containing

anticoagulant were used for estimating hematological parameters including red blood cells (RBC) count, packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC) count and differential leukocyte counts. Serum was separated from blood samples without anticoagulant and stored at -20°C until analysed for calcium, glucose and total protein levels. The blood serum calcium and total proteins were estimated by spectrometric methods using commercial kits as per the instructions of the manufacturer.

Statistical analysis

To establish the temporal relationship between the haematological and physiological parameters were compared by Univariate Chi-square test as per the method described by Steel *et al* [9]. The values are expressed in Mean± Standard error (M ± SE) for the concentrations of various hematological and biochemical parameters for eutocia and dystocia cows. The data were analyzed statistically using Analysis of Variance, Tukey test was applied for multiple means comparison, where necessary. For all test the value of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Dystocia and Eutocia on Physiological Attributes

Rectal temperature

In the present study, the mean rectal temperature was significantly higher ($P<0.05$) in dystocia than in eutocia cows, respectively (Table 01). Previous studies reported that dystocia cows showed a non-significant increase in rectal temperature[10-12]. The increased temperature in dystocia cows may be discounted for excitement and stress due to dystocia.

Respiratory Rate (per minute)

The study revealed that effect of dystocia on respiratory rate (per minute) of cows was not significant (Table 01). However, dystocia showed a marginal increase in respiratory rate than eutocia cows. These findings are supported by the reports of Seyrek-Intas *et al* [11] who also observed no significant variations in the respiratory rate among eutocia and dystocia cows.

Heart Rate (beats per minute)

The study revealed that effect of dystocia on heart rate (per minute) of cows was not significant (Table 01). However, dystocia showed a marginal increase in respiratory rate than eutocia cows. However, these findings are at variance with those reports of Rodrigues *et al*[13], who observed tachycardia immediately after calving in both eutocia and dystocia cows.

Pulse Rate (minute)

The pulse rate of both eutocia and dystocia cows showed no- significant difference (Table 01). However, Seyrek-Intas *et al*. (2013), Derar and Abdel-Rahman (2012) and Mohammad and Abdel-Rahman (2013) reported a significant increase in pulse in dystocia cows.

In cows, parturition was accompanied with some physiological disturbances that were more prominent during dystocia than eutocia as a result of some changes in the animal's body to meet the more stressful situation of dystocia [14-16].

Effect of Dystocia and Eutocia on Hematological Attributes

Alterations in hematological and biochemical indices occur in cattle around the time of parturition [17]. Severe stress caused by dystocia may lead to neuroendocrine and metabolic disturbances in cows [18]. Dystocia when protracted is accompanied by serious physiological, haematological and biochemical derangements in various body system of animals[19]. The hematological constituents of eutocia and dystocia cows were shown in the Table02**.**

Total erythrocyte count

No significant difference in total erythrocyte count was observed among eutocia and dystocia cows**.** These findings are in line with those reported by previous workers[20-24]. These observations suggest that the stress of dystocia has negligible influence on RBC values in cows.

Hemoglobin concentration (Hb)

The concentration ofhemoglobin did not differ between the dystocia and normally calving cows. The findings of the present study are well supported by the earlier reports[23-26] who have also observed non- significant variations in the hemoglobin concentrations among eutocia and dystocia affected buffaloes and cows. Further, it has been observed that the hemoglobin levels tend to change during pregnancy and at parturition due to the stress[27]. This finding suggests that stress of dystocia may not significantly affect the hemoglobin levels. In the light of the observations made in the present study, as well as the observations of Junid and Krad (1987).

Packed cell volume (PCV)

The packed cell volume counts did not vary significantly between normal parturient cows and cows with dystocia. These finding are in congruence with the previous reports of Prabhakaran *et al*. (2006), Ali *et al.* (2011) and Hakim (2012) who also observed non-significant variations in PCV values between normal calving and dystocia affected buffaloes, but in contradict with the observations of Yıldız *et al*. (2011) who reported significantly higher PCV values in normal calving cows as compared to dystocia affected cows. Yuksel *et al*. (2011) and Kaur and Singh (1993) who observed significantly lower hematocrit concentration in buffaloes with uterine torsion and dystocia[21,28,29]. The decrease in PCV values in dystocia affected animals was possibly attributed to release of antidiuretic hormone as a result of stress, anorexia and toxemia[30].

White Blood Cells count (%)

No significant variations in the WBC count of eutocia and dystocia cows was observed. Similar findings have also been reported earlier by Ali *et al*. (2011) Yıldız *et al*. (2011) and Hakim (2012) in buffaloes and cows with dystocia as compared to the normal calving animals. In contrast to the present findings, a significant increase in the WBC count was observed in uterine torsion cowsand buffaloes and in buffaloes with dystocia[21, 31,32]. Yuksel *et al*. (2011) recorded significantly lower WBC counts in cows after relieving dystocia.

Neutrophils count (%)

The mean neutrophil count did not vary significantly between dystocia affected and normal calving cows. These findings gain support from the report of Ali *et al*. (2011) who reported no significant variations in neutrophils count among buffaloes with uterine torsion and eutocia buffaloes. Significant increase in the neutrophil counts was attributed due to increased level of cortisol because of stress[32]. However, neutrophilia has also been reported during excitement, exercise, adrenaline and ACTH release[31]. It has been previously reported that the estradiol 17β concentrations was found to be significantly lower in buffaloes with dystocia[23].

Lymphocytes counts (%)

No significant variations in lymphocytes counts of dystocia affected and normally calving cows was recorded. These observations, are well supported by the previous reports of non- significant variations in lymphocytes count in buffaloes[23,32] and between normal parturition cows and cows with dystocia[24]. However, lymphocytes count was found to increase significantly following relieving dystocia in cows[28].

Eosinophil count (%)

The eosinophil count was found to be significantly higher in eutocia cows as compared to cows with dystocia and indicated that eosinophil counts are influenced by stress of dystocia cows. These findings contradicts the previous reports in normal calving and dystocia buffaloes[29] and in normal parturition and cows with dystocia[24,33] as well as among cows before and after relieving dystocia [28]. These findings contradict the observations of Kaur and Singh (1993) and Ali *et al*. (2011) observed no significant variations in eosinophil counts in buffaloes with uterine torsion. Although, significantly lower eosinophil counts was observed in the presently studied dystocia cows, however, eosinophil counts for eutocia and dystocia cows recorded in the present study were within the normal ranges[20,24,32].

Monocytes count (%)

The monocytes count was significantly higher in eutocia cows than those cows with dystocia. The previous investigations have reported that the monocytes count did not vary significantly between normal parturition cows and cows with dystocia[24,33]. However, significantly decrease in cows with dystocia was also reported earlier [28] and these observations are in contrast to the present findings. However, monocyte counts for eutocia and dystocia cows were within the normal ranges. Increase number of the monocyte count was observed in cows with long standing uterine infection[34].

Effect of Dystocia and Eutocia on Bio-Chemical Parameters

Serum Total protein: Theconcentration of total protein showed no significant variations between eutocia and dystocia cows (Table 02). Similarly, Sathya *et al.* (2005) reported marginally lower levels in buffaloes suffering from dystocia, as compared to the normally calving buffaloes[35]. Thus, present findings corroborate with those of Sathya *et al.* (2005). While Singh *et al*. (2009) found that the plasma total protein concentration did not vary before and after reliving dystocia in buffaloes[36]. Total plasma protein in dystocia affected cows showed marginally lower values as compared to normally calving cows. Lower level of plasma protein may be due to stress of dystocia and increased utilization of protein due to starvation[19].

Serum calcium concentration

The mean calcium concentration was non-significantly lower in dystocia affected cows as compared to normal parturient cows. The findings of the present study corroborates with the previous reports of Sevcik *et al*. (1980) who also reported that the blood serum calcium concentrations did not differ between normal cows and those with dystocia[37]. In yet another study, Yokus *et al*. (2010) noticed non-significant difference in serum calcium levels of cows with dystocia due to absolute birth weight, twin pregnancy and presentation disposition as compared to the cows with normal parturition[38]. Estrogens have been found to depress serum calcium levels while simultaneously increasing serum phosphorus levels (Kaneko and Cornelius, 1970). Insufficient production of oestrone sulphate and delayed regression of the corpora lutea were suggested to be associated with dystocia in dairy cows[39]. Further, it has also been observed that the serum estradiol-17β concentrations were found to be lower in cows and buffaloes affected with dystocia compared to the normal calving cows[23,39].

Blood glucose

Significantly higher blood glucose level was observed in cows with dystocia as compared eutocia cows in the present study (Table 02). Dystocia is the major stressful event, resulting in high rate of maternal and fetal deaths[40]. Severe dystocia is demonstrated to increase serum glucose concentrations in cows as compared to normal parturition (Nakao and Grunert, 1990), suggesting that dystocia is more stressful for cows than normal calving[41]. Civelek *et al*. (2008) was of the opinion that elevated plasma glucose levels are likely to be induced by dystocia-related stress to meet the increased energy demand[42]. This might be due to higher glucocorticoid level at the time of calving which induced glycogenolysis, gluconeogenesis and decreased peripheral utilization of glucose [43,44]. The study reported by Amer, *et al*. (2008) observed that the blood glucose levels were higher in buffaloes suffering from dystocia reflecting a higher degree of stress [32]. It was also observed that blood glucose level increased following various obstetrical procedures. Obstetrical manipulations have been found to be stressful in the past [17]. The elevated blood glucose level was due to elevated cortisol and catecholamine, which increased following various obstetrical procedures as a response to stress [45,46]

CONCLUSION

In conclusion, the present study revealed that the stress of dystocia in cows did not significantly ($P<0.05$) alter haematological attributes except for eosinophil and monocyte count. The calcium concentrations decreased in dystocia affected cows. The possible reason for decreased serum calcium might be due to increased parathyroid hormone and decrease in estradiol 17β. The endocrine imbalance might be subscribed for these variations. A significant increase in blood glucose levels of dystocia affected cows is discounted for stress associated increase in cortisol and lowered insulin levels. In conclusion, dystocia has a profound stress full effect on cows and especially is associated with metabolic dysfunction as evidenced by marked increase blood glucose concentrations. The body temperature and respiratory rate was significantly higher in cows with dystocia as compared to eutocia cows. But the pulse, respiratory and heart rate showed no significant alterations in dystocia affected cows as compared to eutocia.

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Table 1.Physiological Parameters of Eutocia and Dystocia Cows

Parameter	Eutocia	Dystocia
Rectal Temperature (°F)	101.10±0.17^{A}	102.30 ±0.24^{B}
Respiratory rate per min	25.06±0.47^{A}	28.65 ±1.33^{A}
Heart rate (bpm)	73.00±1.40^{A}	76.00±2.04^{A}
Pulse rate per min	58.72±1.28^{A}	59.43±2.06^{A}

Note: Means bearing different superscript values vary significantly (P<0.05)

Table 2: Haemato-Biochemical Parameters of Eutocia and Dystocia Cases in Crossbreed Cows

Parameter	Eutocia	Dystocia
RBC (10^6/µl)	6.12 ±0.24^{A}	6.44 ±0.24^{A}
Hb (g/dl)	9.07±0.23^{A}	9.35±0.36^{A}
PCV (%)	32.40 ±1.66 ^{A}	32.60±1.42^{A}
TLC (10^3/µl)	17.70±1.65^{A}	15.30 ±2.22^{A}
Neutrophils (%)	58.10 ± 2.53^{A}	60.10 ±3.35^{A}
Lymphocytes (%)	39.70± 3.16^{A}	39.40±2.83^{A}
Eosinophil (%)	4.06± 0.374^{A}	2.00 ±0.16^{B}
Monocyte (%)	3.94 ± 0.52^{A}	0.55 ± 0.12^{B}
Blood glucose (mg/dL)	65.70±3.24^{A}	74.10±3.89^{B}
Calcium (mg/dL)	7.68±0.38^{A}	6.99±0.34^{A}
Total Protein (mg/dL)	6.77 ±0.19^{A}	6.30±0.17^{A}

Note: Means bearing different superscript values vary significantly (P<0.05)

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RESEARCH ARTICLE

Immunosuppressive Principles from *Carthamus tenuis* Growing in Egypt

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ABSTRACT

From the aerial parts of *carthamus tenuis*,caffeic acid 9-*O*-glucoside (1), quercetin (2), luteolin (3), chrysoeriol (4), quercetin-7-*O*-β-glucoside (5), azaleatin (6), proline (7) and choline (8) were isolated by several chromatographic techniques and identified by comparing their spectral data (UV, ^{1}HNMR, ^{13}CNMR and MS) with that reported in literatures. Microhaemagglutination test was used to evaluate the immunosuppressive effect of several plant extracts and isolated compounds. The Immunosuppressive property of choline at doses 2.5- 20 mg/kg b.wt was higher than that of standard prednisolone (10 mg/kg b.wt.); quercetin at dose 20 mg/kg b.wt showed immunosuppressive effect similar to that of prednisolone (10 mg/kg b.wt.).Caffeic acid 9-*O*-glucoside andluteolin decreased anti-SRBC titer in comparison with control groups.*carthamus tenuis*was proved to have a potent immunosuppressive effect which can be further investigated for the development of potent immunosuppressive drugs.

Keywords: *Carthamus*, Immunosuppressive, Phenolic Compounds, Flavonoids.

INTRODUCTION

The genus *Carthamus* from the Asteraceae family comprises 16 recognized species;it is native to Europe, North Africa and parts of Asia(Vilatersana*et al*., 2000). Some*Carthamus* species are considered to be immunosuppressive and anticoagulant (Leung and Foster, 1996)and have long been used in traditional Chinese medicine for treatment of

rheumatoid arthritis (Hsu, 1979). The flower of *C. tinctorius*is an important medicinal material in prescriptions used for cardiovascular, cerebrovascular and gynecological diseases (Jinous and Nastaran, 2013). Several *Carthamus* species were proved to contain polyunsaturated fatty acid linoleic acid and monounsaturated oleic acid with small amounts of stearic acid (Knowles and Ashri, 1995).More than 200 compounds have been isolated from *Carthamus* species and the commonly known ones are flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids and polysaccharides (Zhou*et al*., 2009).*Carthamus tenuis*(Boiss. & C.I.Blanche) Bornm. is native to the Mediterranean, it is one of the Egyptian *Carthamus* species, which grows in the delta region of Egypt (Tackholm, 1974).Although *Carthamus* species were used traditionally as immunosuppressive, nothing was found in the available literature to scientifically assess this use. So, in the present study, immunologically guided separation and identification of the main active components of *Carthamus tenuis* on humoral immune responses in mice is followed. In addition to, evaluation of immunosuppressive effect of the isolated compounds.

MATERIALS AND METHODS

Plant material

Flowering aerial parts of *Carthamus tenuis*(Boiss. & C.I.Blanche) Bornm. (Asteraceae) was collected from Tanta region, Al-Gharbia, Egypt, during June 2014. The plant was kindly authenticated by Marey A. PhD, Professor of plant taxonomy, Faculty of Science, Al-Azhar University, Cairo, Egypt. A voucher specimen has been deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

General procedure

Evaporation of solvents was done at 40 ^{o}C under reduced pressure, using a Buchi rotary evaporator, Model 011; ultraviolet absorption spectra were obtained using a Hewlett–Packard HP845 UV–Vis spectrometer; the ultraviolet lamp used in visualizing TLC plates and PC was a Mineralight device, multiband UV, 254/366 nm, obtained from UVP, Inc., San Gabriel CA; melting points were determined on a Mettler FP 80 Central Processor supplied with a Mettler FP 81 MBC Cell Apparatus, and were uncorrected; ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5 and DMSO-d_6 on a Bruker Avance DRX500 instrument (Bruker Biospin GmbH, Rheinstetten, Germany) at 500 MHz for protons and 125 MHz for carbons using the residual solvent signal as an internal standard; mass spectra were obtained by LC-MS (API Quattro micro) equipped with direct probe and a Z-spray electrospray ion source (Micromass, Quattro Micro ™; Waters, Milford, MA).

Quercetin, Quercetin-7-*O*-β-glucoside, luteolin, chrysoerioland azaleatin were purchased from Fluka (Buchs, Switzerland). Caffeic acid 9-*O*-glucoside, proline and choline were obtained from Sigma ChemicalCo. (St. Louis, Mo, USA). HPLC-grade acetonitrile was obtained fromMerck (Darmstadt, Germany).Aluminium chloride ($AlCl_3$) sodium acetate (NaOAc)and boric acid (H_3BO_3)were purchased from SigmaChemicalCo. (St. Louis, Mo, USA). All laboratory chemicals used in this study were of reagentgrade.The classical shift reagents were prepared according to theliterature (Ducrey *et al*., 1995).

Antigen; Sheep red blood cells (SRBC) suspension was collected in Alsever's solution and was washed three times with pyrogen free sterile normal saline. Cells count was adjusted to 5×10^{9} cells/ml and was used for sensitization.

Extraction and isolation

Dried and powdered plant material (400 g) was percolated with methanol atroom temperature.The methanol extract was concentrated under low pressure and temperature (43.6 g, fraction A). The marc was extracted again with 80% followed by 50% methanol-water(v/v) and the extracts were evaporated at low temperature under vacuum to give 4.5

g (fraction B) and 15.7 g (fraction C), respectively. Fraction A was extracted with petroleum ether followed with chloroformand ethyl acetate, solvents were evaporated in vacuum under low temperature to yield 3.2 g (fraction A1),7.2 g (fraction A2), 8.5 g (fraction A3),respectively and 20.4 g of residue (fraction A4).

A part of fraction A4 (10 g)was applied to Sephadex LH-20 column (50 cm x 2.5cm, 100 g) and eluted with methanol. Thirty fractions (200 mL) werecollected and evaporated to dryness in vacuum. Fractions (5–12, 430 mg, A4-1), (17-25, 346 mg, A4-2)were combined, accordingto their TLC similarities, and purified by semi-preparativeHPLC (C_{18} reversed phase column) using 15%water:acetonitrile as eluent, yielding compounds 1-3 from fraction A4-1 and compounds 4- 6 from fraction A4-2(73, 230, 70, 62, 55, 72 mg), respectively.

Five hundred mg of each of fractions B and C were separately subjected toPC using Butanol: acetic acid: water (4:1:5, v:v:v) as eluent to obtain two fractions (B1, 135 mgand C1, 147 mg) which gave yellow and purple spots with Dragendorff's reagent, respectively.100 mg of each of fractions B1and C1, werechromatographed separately on sephadex LH20 (10 cm x 1 cm, 1 g) using methanol: water50:50 as eluent. Compound 7 (66 mg) and compound 8 (54 mg) were isolated from fractions B1 and C1, respectively.

Compound 1: Caffeic acid 9-*O*-glucoside ($C_{15}H_{18}O_9$), UV λ_{max} nm in MeOH: 331, 299.EIMS: m/z %: 180(52), 179(62), 163(100), 136(80), 121(20); ^{1}H-NMR (DMSO-d_6): δ 7.47 (1H, *d*, *J*= 15.2 Hz, H 7), 7.10 (1H, *d*, *J* = 1.7 Hz, H 2), 6.86 (1H, *dd*, *J* = 8.2, 1.7 Hz, H 6), 6.75 (1H, *d*, *J* = 8.2 Hz, H5), 6.24 (1H, *d*,*J* = 15.2 Hz, H 8), 5.30 (1H, *d*, *J* = 6.8 Hz, Glu H1'), 3.44-5.35 (6H, *m*, sugar protons); ^{13}C-NMR (DMSO-d_6): δ 170.3 (C-9), 150.4 (C-4), 149.65 (C-3), 146.6 (C-7), 128.2 (C-1), 125.4 (C-6), 117.8 (C-8), 117.8 (C-5), 115.4 (C-2) and sugar: 60.9 (C-6'), 69.4 (C-4'), 79.6 (C-3'), 74.6 (C-2', 5'), and 102.9 (C-1').

Compound 2: Quercetin ($C_{15}H_{10}O_7$),yellow crystals, m.p. 316- 317 ^{o}C. EIMS: m/z %: 302(100), 273(10),153(10), 137(18); UV λ_{Max} nm in MeOH: 370,298(sh), 256; NaOMe 423, 302(sh), 260 (dec);$AlCl_3$ 446, 292(sh), 270; $AlCl_3$/HCl 424, 360,290(sh), 270; NaOAc 360, 292(sh) ,270; NaOAc/H_3BO_3 380, 299(sh), 260.

Compound 3: Luteolin ($C_{15}H_{10}O_6$), yellow crystals, m.p. 282- 285 ^{o}C. EIMS: m/z %: 286 (100), 152 (10) 137 (15); UV λ_{max} nm in MeOH: 346, 258; NaOMe 405, 267;$AlCl_3$ 420, 320(sh), 265; $AlCl_3$/HCl 392, 375,274; NaOAc 402, 360, 266; NaOAc/H_3BO_3 370, 257.

Compound 4: Chrysoeriol (3′-methoxy luteolin, $C_{16}H_{12}O_6$), EIMS: m/z %:300(17), 286(70), 151(18), 153(30), 148(10),134(20). UV λ_{max} nm in MeOH: 355, 268;NaOMe 405, 266; $AlCl_3$ 403, 360(sh), 293(sh), 270, 260(sh); $AlCl_3$/HCl 403, 358, 293(sh), 275,257(sh); NaOAc 366, 320(sh), 270; NaOAc/H_3BO_3 423(sh), 372, 292(sh), 263.

Compound 5: Quercetin-7-*O*-β-glucoside ($C_{21}H_{22}O_{12}$),yellow powder, m.p. 244- 247 ^{o}C, EIMS: m/z %: 316(100), 168(6), 167(25), 137(34). UV λ_{max} nm in MeOH:377, 267, 260; NaOMe 457, 366, 290, 240(sh); $AlCl_3$440, 332, 275, 260(sh); $AlCl_3$/HCl 426, 360, 300(sh), 270; NaOAc 427, 375, 285; NaOAc/$H_3BO_3$384, 289(sh),260. Enzymatic (β-glucosidase) and 1% HCl hydrolysis gave quercetin and glucose.

Compound 6: Azaleatin (3,7,3′,4′-tetrahydroxy-5-methoxyflavone, $C_{16}H_{12}O_7$), yellow amorphous powder, UV λ_{max} nm in MeOH:370, 274(sh), 260; $AlCl_3$ 432, 274; NaOAc 370, 278(sh), 265; NaOAc/$H_3BO_3$379, 260. (DMSO d_6): δ 7.77 (1H, *d*, J= 2.5, H-2′), 7.75 (1H, *dd*, J= 2.6 and 9, H-6′), 6.93 (1H, *d*, J= 9, H-5′), 6.83 (1H, *d*, J= 2.5, H-8), 6.44(1H, *d*, J= 2.5, H-6), 3.80 (3H,*s*, CH_3O).

Compound 7: Proline ($C_5H_9NO_2$),transparent crystals, m.p. 205- 208 ^{o}C.EIMS:m/z%: 115(8), 87(8), 71(5), 70(100), 68(17). ^{1}H-NMR (Pyridine d_5): δ3.70 (1H, *dd*, *J* = 6.4, *J* = 8.7 Hz, H1), 2.05 (1H, *m*,H 2), 1.86 (1H, *m*, H 2'), 1.77 (2H, *m*, H3, 3'),3.21 (1H, *m*, H 4), 3.06 (1H, *m*, H 4').

Compound 8: Choline ($C_5H_{14}NO^+$),white crystals, m.p. 305- 306°C.EIMS:m/z%: 104(100), 88(18), 59(5), 45(5), ^{1}H-NMR (DMSO d_6): δ 3.80 (2H, *m*, , H1), 3.40 (2H, *m*, H 2), 3.16 (9H, *s*, CH_3); ^{13}C-NMR (DMSO d_6): δ55.15 (C-1), 67.26 (C-2), 53.12 ($3CH_3$).

Animals

Swiss albino female mice (20- 25 g) were obtained from animal facility, Al-Azhar University, Cairo, Egypt. The animals were kept in standard cages and maintained under standard laboratory conditions (humidity 55 ± 5%, temperature 25 ± 2°C with 12 h light/ 12 h dark cycle) with free access to diet pellets (Al-Nasr, Abou-Zaabal, Cairo, Egypt) and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, Al-Azhar University, Cairo, Egypt, and all applicable institutional guidelines for the care and use of animals were followed.

Microhaemagglutination test

Groups of animals, each of 6 mice, were treated by intraperitoneal injection (i.p.) with different doses (31.25, 62.5, 125 and 250 mg /kg) of each fraction of A, B, C, A1, A2, A3, A4, A4-1, A4-2, B1, C1 and isolated compounds (1-8) at different doses (2.5, 5, 10, 20 mg/ kg).

Control groups received i.p. injection of 0.5 ml of normal saline. The test and control groups were treated for 6 consecutive days. On the 8th day, all mice were injected by200 µl of 5×10^9 SRBC/ml (i.p.). After 6 days, blood samples were collected from the retro-orbital plexus of individual animals of all groups, serums were separated and Hemagglutinating Antibody titer (HA) was determined by the micro-titer plate method (Rezaeipoor*et al*., 1999 andSaeidnia*et al*., 2004), 96 wells (12×8) bottomed titter plate was used. Prednisolone was used, as a positive control, in doses 5 and 10 mg.

Statistical analyses

The results were subjected to statistical analysis using ANOVA, differences with P< 0.05 between experimental groups wereconsidered statistically significant.

RESULTS AND DISCUSSION

Different fractions of *C. tenuis*(fractions A, A1, A2, A3, A4, B and C) were evaluatedfor immunosuppressive effect using microhaemagglutination test. FractionsA4, B and C showeda significant decrease in the anti-SRBC titer at all the tested doses (31.25- 250 mg/kg b.wt.), fraction A4 showed the highest activity (222.3, 105.3, 104.2 and 100.0 at doses 31.25, 62.5, 125 and 250 mg/kg b.wt, respectively) as presented in Table 1.The immunological guided fractionation led toisolation of compounds 1-6fromfraction A4 byusing sephadex LH-20 column chromatography followed by purification on semi preparativeRP-HPLC. These isolated compoundswere identified as caffeic acid 9-*O*-glucoside (1),quercetin (2), luteolin (3), chrysoeriol (4), quercetin-7-*O*-β-glucoside (5) and azaleatin (6)by a comparison of their spectral data (UV,^{1}HNMR,^{13}C-NMR,and MS) with thosereported in literatures (Agrawal, 1989; Harborn, 1993; Markham, 1982;Saeidnia *et al*., 2005; Gohari *et al*., 2003; Saeidnia *etal*., 2009 and Vladimir, 2015). Furtherpurification of fractions B and C using PC followed by sephadex LH-20 column chromatography led in isolationof compounds 7and 8 from fractions B and C, respectively. The compounds ontreatment with Dragendorff's reagent gave yellowand purple spots, respectively which are specificreactions of quaternary amino acid derivatives ofbetaine type. The ^{1}H and ^{13}C-NMR spectral dataof compounds(7) and (8) in pyridine-*d5* and DMSO-*d6*, respectively showed good agreement with those reported inreferences as proline (7) and choline (8) (Mehlfuhrer *et al*., 1997 & Saeidnia *et al*., 2004). Immunosuppressive activity of isolated compoundswas tested usingabove mentioned method.

Caffeic acid 9-*O*- glucoside (1), quercetin (2), luteolin(3)and choline (8)showed asignificant decrease in the anti- SRBC titer ofmice compared to control group at all the tested doses (2.5- 20 mg/kg b.wt.). Choline showed the highest activity(573.3, 462.3, 460.5 and 455.7 at doses of 2.5, 5, 10 and 20 mg/kg b.wt, respectively) followed by quercetin(840.4, 720.7, 630.0 and 630.0at doses of 2.5, 5, 10 and 20 mg/kg b.wt, respectively). Chrysoeriol (4), quercetin 7-*O*-β-glucoside (5),azaleatin (6)andproline (7), showed nosignificant activity at any tested dose (Table 2).Choline, whichwas determined in 50% methanol (fraction C) showed a significant inhibitory effect on antibodyproduction (Saeidnia *et al*., 2004). It seems that choline which is more active than prednisolone as astandard immusuppressive drug (10 mg/kg) isresponsible for the immunosuppressive effect offraction C. Fraction B showed the least immunosuppressive effect(2217.4, 1320 .0, 1117.3 and 1105.6 at doses 31.25, 62.5, 125 and 250 mg/kg b.wt),fraction B needs further investigation of their immunosuppressive constituents as the only detected compound in it was proline which showed no significant immunosuppressive activity.

Caffeic acid 9-*O*- glucoside (1), quercetin (2) and luteolin (3)were isolated from fraction A4 which showed the highest activity.Caffeic acid-9-*O*-glucoside isolatedfrom A4 fraction showed inhibitory activitycomparable to Prednisolone, It is one of the phenolicglycosides which are classified into phenethylalcohol or phenyl propanoid glycosides.Jionosides A1 and B1 isolated from *Rehmanniaglutinosa* are two samples of this class which haveshown *in vivo* immunosuppressive activityattributed to the phenethyl alcohol moiety of themolecule (Sasaki *et al*., 1989).Quercetin showed immunosuppressive effect nearly similar to that of standard prednisolone (10 mg/kg). A review of literature revealed that quercetinprevented the UV-induced suppression of thecontact hypersensitivity and reduced percentageof CD8+ cells in spleen and lymph nodes (Steerenberg*et al*., 1997).Also it has been shown that quercetin inhibitsboth *in vitro* generation and effecter function ofalloantigen specific cytotoxic T lymphocytes (Schwartz and Middleton, 1984). In addition to, it was proved that quercetin has immunosuppressive effect on dendritic cell activation and function (Huang*etal*., 2010). Luteolin which showed the least immunosuppressive effect among A4 isolated compounds (3863.4, 3566.6, 3538.6 and 3520.0 at doses of 2.5, 5, 10 and 20 mg/kg b.wt, respectively) was reported to have an inhibitory effect on lipopolysaccharide (LPS)/interferon γ (IFN-γ)-induced NO and proinflammatory cytokine production in rat primary microglia and BV-2 microglial cells(Tsung *et al*., 2011).

CONCLUSION

In conclusion, this study proves the presence of phenethyl alcohol glycoside, flavonoid aglycones and glycosides in addition to quaternary nitrogen compounds in different extracts of *C. tenuis* which showed immunosuppressive effect comparable to standard prednisolone and suggest further study of the plant extracts to isolate other minor immunosuppressive compounds.

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Table 1: Effect of fractions from aerial parts of *C. tenuis* on hemagglutinating antibody titer in mice immunized with SRBC

[a]Antibody titer Mean ± SD					Group	Dose (mg/kg b.wt.)	[a]Antibody titer Mean ± SD
Group (Extract/fraction)	Dose (mg/kg b.wt.)						
	31.25	62.5	125	250			
Fraction A (methanol)	8430.3 ± 238.1	8400± 246.3	8380± 237.2	8380± 238.4	Normal	-	90.0 ± 15.2
Fraction A1 (pet. ether)	8593.7 ± 261.4	8457± 304.2	8448± 310.4	8256± 238.3	[b]Control (saline)	-	8650.21 ± 223.6
Fraction A2 (chloroform)	8640.5 ± 258.4	7620± 238.1	7510± 223.5	7500± 245.6	prednisolone	5	2300.0* ± 53.3
Fraction A3 (ethyl acetate)	8486.1 ± 214.4	8200± 239.5	8120± 240.0	8120± 243.4	prednisolone	10	620.0* ± 14.8
Fraction A4 (Residue)	222.3* ± 12.6	105.3*± 9.6	104.2*± 11.2	100.0*± 7.3			
Fraction B (80%)	2217.4* ± 88.3	1320 .0*± 53.5	1117.3*± 43.5	1105.6*± 44.2			
Fraction C (50%)	1670.2*± 74.3	630.5*± 27.4	320.0*± 8.4	320.0* ± 9.3			

[a]P< 0.05 , [b]Control group (mice received saline instead of extract) showed significant increase in antibody titer compared to normal group (mice did not receive either SRBC or extract).
*Significant decrease in the anti-SRBC titer compared with control group

Table 2: Effect of isolated compounds from aerial parts of *C. tenuis* on hemagglutinating antibody titer in mice immunized with SRBC

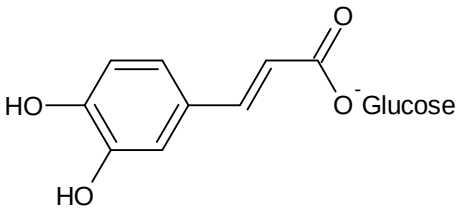
[a]Antibody titer Mean ± SD					Group	Dose (mg/kg b.wt.)	[a]Antibody titer Mean ± SD
Group (Isolated compound)	Dose (mg/kg b.wt.)						
	2.5	5	10	20			
Caffeic acid 9-O-glucoside (1)	1452.5* ± 327.5	1363.0*± 212.7	1250.5* ± 206.4	1250.5* ± 200.5	Normal	-	90.0 ± 15.2
Quercetin (2)	840.4* ± 32.6	720.7*± 25.3	630.0* ± 19.4	630.0* ± 19.5	[b]Control (saline)	-	8650.21 ± 223.6
Luteolin (3)	3863.4* ± 113.7	3566.6*± 104.6	3538.6*± 112.7	3520.0* ± 115.3	Prednisolone	5	2300.0* ± 53.3
Chrysoeriol (4)	7546.7 ± 247.6	7530± 238.4	7500± 236.9	7487± 276.0	Prednisolone	10	620.0* ± 14.8
Quercetin 7-O-glucoside (5)	6430.0 ± 148.3	6400.0± 137.2	6248.5± 125.6	6250.3± 137.4			
Azaleatin (6)	5428.0 ± 315.5	5176± 245.7	4760± 235.5	4629± 230.4			

Proline (7)	6530.3 ± 84.5	6484.2± 74.8	6496.4± 85.3	6520.4± 95.5			
Choline (8)	573.3* ± 15.5	462.3* ± 13.6	460.5* ± 13.7	455.7* ± 16.2			

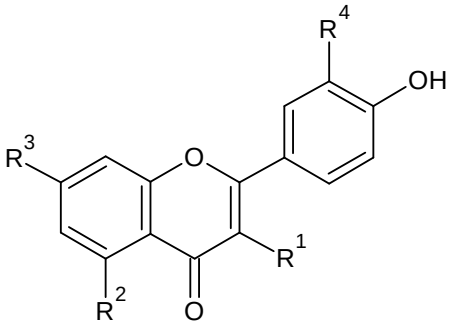
[a]P< 0.05

[b]Control group (mice received saline instead of extract) showed significant increase in antibody titer compared to normal group (mice did not receive either SRBC or extract).

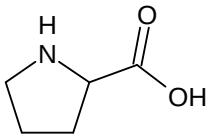
*Significant decrease in the anti-SRBC titer compared with control group



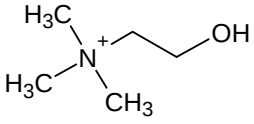
Compound (1)



Compound	R^1	R^2	R^3	R^4
(2)	OH	OH	OH	OH
(3)	H	H	OH	OH
(4)	H	OH	OH	OCH_3
(5)	OH	OH	O-glucose	OH
(6)	OH	OCH_3	OH	OH



Compound (7)



Compound (8)

Figure 1: Structure of isolated compounds

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REVIEW ARTICLE

Proniosomes: A Novel Approach to Drug Delivery System

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ABSTRACT

Novel drug delivery systems like vesicular systems can increase the bioavailability of encapsulated drug and provide therapeutic efficacy in a controlled manner for a prolonged period of time.Proniosomes are dry formulation of water-soluble carrier particles that are coated with non-ionic surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. Proniosomes can avoid many problemsassociated with aqueous noisome dispersions andphysical stability.It prolongs the availability of the drug in systemic circulation and decreases toxicity.This review covers all the aspects of proniosomes including structure, composition, methods of preparation, characterizations, and application.

Keywords : Proniosomes, Non-Ionic surfactant, Niosomes, Bioavailability

INTRODUCTION

Nanotechnology is considered as an emerging technology for the 21st century. The term “Nano” is derived from Greek word meaning “dwarf”. Nanotechnology is defined as the science carried out in the nanoscale (10-9 meters). Nanotechnology are used for disease diagnostics, monitoring and treatment. Nanodevices are 100 to 10000 times smaller than human cells. They are similar in size to large biological molecules such as enzymes and receptors. So this technology can be used to treat various diseases like cancer, lung diseases, heart diseases, colon diseases [1,2,3].
Nanoparticulate systems are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The goal of nanotechnology is to obtain systems with optimized drug loading, release properties, long shelf-life and low toxicity [4].

Application of nanotechnology

- Nanodevice
- Nanofabrics
- Naobiotechnology
- Nanodevices
- Cosmetics
- Defences & security
- Bio engineering
- Energy
- Medicine & drugs

Proniosomes

Proniosomes are dry formulations of water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation inhot aqueous media within minutes[5].It can carry both hydrophilic drugs and hydrophobic drugs.Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation.It minimize problems of vesicular systems such as aggregation, fusion and leakage of drug and provide additional convenience in transportation, distribution, storage and dosing[6].

Advantages of proniosomes over other conventional dosage forms

- Non-toxicity, biocompactable, biodegradable
- Non-immunogenic as it is non-ionic in nature
- Greater stability
- Easy handling and storage
- Improved bioavailability, reduced side effects
- Both hydrophilic and hydrophobic drugs can be encapsulated
- Shows controlled and sustained release of drugs due to depot formation[7]

Structure of proniosomes

Proniosomes are microscopic bilayer lamellar structure. They combine a non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class of cholesterol followed by hydration in the aqueous media. The surfactant molecule direct themselves such that the hydrophilic end of the non-ionic surfactant orient outward, while the hydrophobic end are in the opposite direction to form the bilayer. In proniosomes, the bilayer are made up of non-ionic surface active agent. On the basis of method of preparation proniosomes are unilamellar or multilamellar[8].

Types of Proniosomes

1) Dry granular proniosomes
- Sorbital based proniosomes
- Maltodextrin based proniosomes

2) Liquid crystalline proniosomes

Sorbitol based proniosomes

Sorbitol based proniosomes are dry formulation that contains sorbitol as a carrier. They are usually made by spraying the surfactant mixture prepared in organic solvent on to the sorbitol powder, then the solvent is evaporated. It is useful in case where the active ingredient is susceptible to hydrolysis. The advantage of Sorbitol based proniosomes isuniformsize distribution. The disadvantage of this proniosome is that the residual sorbitol decreases the entrapment efficiency to less than one-half of that observed without sorbitol.It is prepared by slow spraying method[9, 10].

Maltodextrin based proniosomes

It is prepared by fast slurry method. Proniosomes of high surface to carrier ratio can be prepared. The method is very simple. Since maltodextrin morphology is preserved, hollow blown maltodextrin particles can be used for significant gain in surface area. The higher surface area results in thinner surface coating, which makes the rehydration process efficient. They can be used for delivering of hydrophobic and amphiphilic drug molecule [8,9,10].

Liquid crystalline proniosomes

When the surfactant molecule are kept in contact with water, there are three ways through which lipophilic chains of surfactant can be changed into a disordered, liquid state called lyotropic liquid crystalline state. These three ways are

- Increasing temperature at kraft point (Tc),
- Addition of solvent which dissolve lipids,
- Use of both temperature and solvent.

Advantages:-
1) Stability
2) High entrapment efficiency
3) As a penetration enhancer
4) Easy to scale up as no lengthy process is involved; moreover it avoids the use ofpharmaceutically unacceptable additives.[9,10,11]

Components of Proniosomes

The essential components of the delivery system are as follows:

Surfactants

Surfactants are the surface active agent usually organic compounds that are amphiphilic in nature. They can be also used as solubilizers, wetting agents, emulsifiers and permeability enhancers. Commonlyused non-ionic amphiphiles are alkyl ethers, alkyl esters, alkyl amides and esters of fatty acids. Three factors to be considered to select a proper surfactant:
(i)HLB value:Surfactant having HLB number in between 4 and 8 is a good for vesicle formation.Entrapment efficiency decreases as the HLB value decreases from 8.6 to 1.7.The encapsulation efficiency of Tween is relatively low when compared to span. Because of the larger size of vesicles and less lipophilic nature of tween, span is used to increases the lipophilicity of the drug.
(ii)Critical packing parameter(CCP):On the basis of the CPP of a surfactant, the type of vesicle, which it will form, can bepredicted. The chain length and size of the hydrophilichead group of the non-ionic surfactant affect theentrapment

efficiency of the drug. Non-ionic surfactantswith stearyl (C18) chains show higher entrapment efficiency than those with lauryl (C12) chains.
(iii)Phase transition temperature: It plays a major role in the degree of entrapment. As the transition temperature of surfactants increase, entrapment efficiency increases and permeability decreases. Spans with highest phase transition temperature provide the highest entrapment for the drug and vice versa [12].

Carrier material

The carrier used in the proniosomes preparation permits the flexibility in the ratio of surfactant and other ingredientsused. It also increases the surface area and thus efficient loading. The carriers should be safe and non-toxic, free flowing, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Commonly used carriers are Sorbitol, Mannitol, Glucose, Lactose, Sucrose stearate.Commonly usedcarrier is maltodextrin. It has minimal solubility in organic solvent. It forms particles by simply adding surfactant in organic solvent. They are used as carrier for spray-drying of active substances [13].

Membrane stabilizers

Cholesterol and lecithin are commonly used as membrane stabilizer. Steroids are maincomponents of cell membrane and their presence in membrane leads changes to bilayer stability, fluidity andpermeability. Cholesterol is a steroid of natural origin used as membrane additive. It prevents aggregation by the incorporation of molecules that stabilize the system against theformation of aggregate by electrostatic effects. It leads transition from thegel state to liquid phase in niosomes system. Cholesterol increases or decreases the percentageencapsulation efficiency depending on either the type of the surfactant or its concentration. Theamount of cholesterol to be added depends on the HLB value of the surfactants. It was found that above a certainlevel of cholesterol, entrapment efficiency decreased possibly due to a decrease in volume diameter[14].

Solvent and aqueous phase

Alcohol used in proniosomes has a major role on vesicle size and drug permeation rate. Vesicles formed from different alcohols are of different size and they follow the order:
Ethanol > Propanol > Butanol > Isopropanol.
Ethanol has greater solubility in water hence leads to formation of highest size of vesicles instead of Isopropanol which forms smallest size of vesicle due to branched chain present[14].

Drug

Drug should have following characteristics:[15].
1. Low aqueous solubility of drugs.
2. High dosage frequency of drugs.
3. Short half-life.
4. Controlled drug delivery suitable drugs.
5. Higher adverse drug reaction drugs.

Hydration medium

For the preparation of proniosomes derived niosomes,Phosphate buffer are commonly used as the hydration medium.The pH of hydration mediumis determined by the solubility of drug being encapsulated. The temperature of hydrationmediumalso plays a major role in governing the self-assembly of non-ionic surfactant into vesicles and affects their shape and size. In case of preparation of proniosomal gel, the hydrating temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system [16].

Formation of niosomes from proniosomes

The niosomes can be prepared from the proniosomes by hydrating the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant [16].

$T > Tm$

Where,

T = Temperature

Tm = Mean phase transition temperature

METHOD OF PREPARATION OF PRONIOSOMES

Proniosomes are prepared by following methods.

a. Slurry method

Proniosomes can be formulated by addition of the carrier and the entire surfactant solution in a round bottom flask which is fitted to rotary flash evaporator and a dry and free flowing powder is produced when the vacuum is applied. Finally, the formulation should be stored in tightly closed container. The time required for proniosomes production is independent of the ratio of surfactant solution to carrier material and appears to be stable.The proniosomal powder formed is stored at 4°C. Maltodextrin is mainly used as carrier in this method.

Advantages

a) Polysaccharide like maltodextrin is easily soluble in water and it is used as carrier material in formulation; they were easily coated by simply adding surfactant in organic solvent to dry maltodextrin.

b) Due to uniform coating on the carrier it protect the active ingredient and the surfactants from hydrolysis and oxidation.

c) The higher surface area leads to thinner surfactant coating which makes the rehydration process easy.

Disadvantages

a) Time consuming method and equipment with vacuum and nitrogen gas are used.

b) The thin film allows only for a predetermined lot sizes so material often wasted [6,17].

b. Coacervation phase separation method

Proniosomal gels can be formulated by this method which consists of surfactant, lipid and drug in a glass vial along with small amount of alcohol in it. The mixture is heated in a water bath at 60-70^{0}C for 5minutes until the surfactant mixture dissolvescompletely. Then the little aqueous phase is added to a vial and heateduntil a clear solution is formed which is then converted into proniosomal gel on cooling. After hydration of proniosomes, they are converted to niosomes.

Advantages

a) Less time consuming and simple method

b) mainly used for gel preparation

c) Small quantities or small dose formulation can be formulated on lab scale[6,17].

c. Slow spray coating method

In this method, the surfactant is incorporatedinto an organic solvent and sprayed onto carrier and then the solvent is evaporated. This process is continued until the desired surfactant loading is achieved, because the carrier is soluble in the organic solvent. As the carrier is dissolved, hydration of this coating enables the formation of multilamellar vesicles. These niosomes have uniform size distribution.

Advantages

Simple method used for hydrophobic drug

Disadvantages

1.Time consuming method

2. Equipment with vacuum and nitrogen gas are used.

3. The thin film approach allows only for a predetermined lot sizes so material often wasted so minute quantities or small dose batch can be tedious one [6,17,18].

Stability studies on proniosomes

Stability studies are performed by storing prepared proniosomes at various temperature conditions like refrigeration (2-8°C), room temperature (25°±0.5°C) and elevated temperature (45°C±-0.5°C) for a period of one to three months. Drug content and variation in the average vesicle diameter were periodically checked. ICH guidelines recommend that stability studies for dry proniosomes powder for reconstitution should be studied for accelerated stability at 75% relative humidity as per international climatic zones and climatic condition[5].

Research works published on proniosome

Lamivudine loaded maltodextrin based proniosomes(2016) were prepared by slurry method using different non-ionic surfactants (Span 40, Span 60, Tween 60) in various concentrations keeping the concentration of drug, cholesterol, maltodextrin as constant. This study suggests that Lamivudine proniosomal formulation can provide sustained action of the entrapped drug that can decrease the side effects associated with frequent administration of the drug and potentiate the therapeutic efficacy of the drug[22].Candesartan cilexetil loaded proniosomes (2015) were prepared by slurry method. Proniosome formulations increase the oral bioavailability of lipophilic drugs that cannot be formulated due to their low solubility and limited oral bioavailability[23].Proniosomal powders of acemetacin (2014) were prepared by slurry method using maltodextrin as carrier. Proniosomal powders were compressed into tablets by direct compression method. The dissolution of proniosomal tablet indicated a lower drug release percentage compared to powdered proniosomes and acemetacin plain tablets[24].Nateglinide provesicles (2014) were prepared by a slurry method using the non-ionic surfactant, Span 60 and cholesterol as vesicle forming agents and maltodextrin as carrier. Nateglinide provesicles will give higher bioavailability than pure nateglinide and provide a more effective treatment for type II diabetics[25].Megesterol proniosomes (2012) were prepared by slurry method. Proniosomal drug products have shown improved oral bioavailability[26].Methotrexate entrapped Proniosomes (2012) were prepared by slurry method using cholesterol, Span 80 and maltodextrin. Studies concluded that prepared proniosomes are stable and have prolonged activity[27].Norfloxacin loaded maltodextrin based proniosomes (2012) were prepared by slurry method with different surfactant to cholesterol ratio. Proniosomes formulation in refrigerator condition showed greater stability for 90 days when compared with reconstituted niosomes [28].Proniosomes of curcumin (2011) were formulated by encapsulation of the drug in a mixture of Span 80, cholesterol and diethyl ether by ether injection method, and then converted to transdermal drug delivery system (TDDS). Studies concluded that the proniosomes are very stable and have sustained release for curcumin[29].Ibuprofen loaded maltodextrin (2010) based proniosome were formulated by slurry method with different surfactant to carrier ratio. Proniosome formulation has showed appropriate stability for 60 days by storing the formulations at different conditions[30].Aceclofenac proniosomes(2008) was prepared, characterized and optimizedusing central composite design and carry out stability studies. Based on central composite design, 16 batches of proniosomes were formulated by slurry method and evaluated for the percentage drug entrapment (PDE) and mean volume diameter (MVD). The PDE and MVD (dependent variables) and the converted values of independent variables were subjected to multiple regressions to produce a second order polynomial equation. The polynomial equations and contour plots were developed using central composite design permit us to prepare

proniosomes with optimum responses[31].Transdermal drug delivery system of captopril (2007) was formulated by coacervation-phase separation method in which drug is encapsulated in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters,cholesterol,lecithin. Prepared proniosomes have prolonged delivery for captopril and good stability characteristics[32].

CONCLUSION

Proniosomes are promising drug carriers for the future with better stability. They can incorporate amphiphilic drugs. It avoids the drawbacks of niosomes like aggregration, fusion, leaking etc.They improve the stability of the entrapped drugduring delivery. They do not require special conditions for handling, protection, storage. In future,definitely they will provide better treatment than other conventional dosage forms.

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Table 1: List of Surfactants used in the Formulation of Proniosomes [12]

NON-IONIC AMPHIPHILES	EXAMPLES
Alkyl ether and alkyl glyceryl ethers	Polyoxyethylene 4 lauryl ether(Brij 30)
Polyoxyethylene cetyl ethers	Brij 52,56,58
Polyoxyethylene stearyl ethers	Brij 72,76
Sorbitan fatty acid esters	Span 20,40,60,80
Polyoxyethylene sorbitan fatty acid esters	Tween 20,40,60,80

Table 2: Characterization of Proniosomes [6]

SL.No	Characterization	Instrument /Technique Used
1.	Angle of repose	Funnel method
2.	Vesicle size and morphology	Scanning electron microscopy (SEM), transmission electron microscopy(TEM), Optical microscopy
3.	Zeta potential	Zeta meter
4.	Drug entrapment	Ultracentrifugation
5.	Drug content	UV spectrophotometer
6.	*In vitro* drug release	Franz diffusion cell

Table 3: Applications of Proniosomes

SL.No	Formulation	Drug	Use	References
1	Proniosomal transdermal drug delivery system	Captopril	Antihypertensive	32
2	Proniosomal transdermal drug delivery system	Carvedilol	Antihypertensive	19
3	Proniosomal transdermal drug delivery system	Curcumin	Antibacterial	29
4	Proniosomal tablet	Acemetacin	NSAID	24
5	Proniosomal gel	Ketorolac	NSAID	5
6	Proniosomal gel	Flurbiprofen	NSAID	5
7	Proniosomal gel	Metformin	Anti diabetic	20
8	Transdermal patch	Levonorgestrel	Contraceptive agent	21

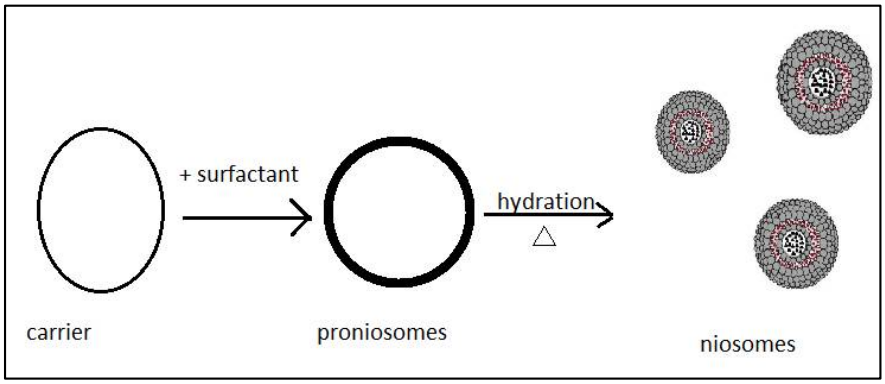

Figure 1: Formation of niosomes from proniosomes

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REVIEW ARTICLE

An Overview on Herbal Remedies of Pediculosis

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ABSTRACT

Pediculosis caused by *Pediculushumanus capitis*is common in the school aged children. This may cause pruritus, utricaria, sleeplessness and pyoderma. The itching sensation due to the infestation makes restlessness and irritation. Though a number of synthetic pediculocidal agents are available in the market, they could not only mark an end to this, but also by their usage users suffers from several adverse reactions and develop resistance and cross – resistance. Hence the safest and effective alternative is to return back to nature and plants. The herbal remedies show excellent activity rather than the synthetic ones. And the cure is without gifting the adverse effects.

Keywords : Pediculosis, Pediculicidal agents,utricaria, pyoderma

INTRODUCTION

Head lice are hematophagous ecto parasite .The Infestation of lice is known as Pediculosis. Head louse is *Pediculus humanus capitis De Geer* of the order Phthiraptera and family Peliculidae [1].They infest mankind all over the world. They mostly affect the school going children of 3 -11 years. They are transmitted directly by host to host contact and indirectly through the combs, pillowcases, bed sheets, blankets and hats [2],[3]. Infestation may be symptomatic or asymptomatic. In symptomatic cases, the itching is found in a high rates among the patients which may be caused either by the bite of lice on the skin or the irritative allergic reaction caused by the deposition of saliva on the scalp.[5] It may also cause skin irritation, pruritus, utricaria, restlessness, sleep loss, bacterial infection like impetigo and pyoderma[6].The substances which are used to treat the lice are known as Pediculicide. The common treatment to control pediculosis is the use of chemicals that include neurotoxic synthetic insecticides like lindane, malathion, pyrethrin and so on.[7] The use of these insecticide are increasing day by day. The usage of synthetic pediculicide

results in several adverse reactions. Besides this, multiple treatment, it is safe and biodegradable. Across the world in folk medicine, different medicinal plants are used against head lice [8]. Many plant extracts of*Artemisia annua, Azardiracta indica, Curcuma longa, Lawsonia inermis, Melia azedarach, Syzgium aromaticum* and essential oils have been utilized to eradicate lice and nits [9, 10].

Head lice, (*Pediculus humanus capitis)* are wingless ectoparasites that live on human head by feeding on human blood. The head louse lay oval shaped eggs which are known as nits that has size range of 0.3–0.8 mm. The lay eggs behind the neck or ears. The female holds the nits to the hair, about 1–3 cm from the scalp with the help of strong water-proof cement. A day they lay up to 10 nits. In its life span is about 30 to 40 days and they lay about 300 nits in their life time[12]. Pediculosis has also been reported worldwide, in about 1.6%-87%. However, this variation may be observed due to several factors such as eradication methods, number ofdirect head contacts, diagnostic techniques,headlice policy in school (no-nit policy), development of pesticide resistance, and knowledge regarding head lice. Pediculosis is mostly observed in densely populated places. It may be due to lack of adequate cleanliness and hygiene[13].

The life cycle of head lice has 3 stages such as egg, nymph, adult. Egg- Eggs are laid by the female adult lice and these eggs are glued to the base of the shaft. To attach the eggs the female adult secreate glue – like substance on to the shaft which hardens and later form nit sheath that cover shaft and nit together but except operculum through which it respires. Nits takes 1 week (6-9days) to hatch[14,15]. Nymph – Egg hatches to release nymph.The size is similar to a pin head. It matures after 3 moults. It becomes adult after 7 – 10 days after hatchingThe 1st and 2ndmoults are immobile but 3rd and adult is mobile which is transmitted the most[16].

Adult – It is about 2- 4 mm similar to sesame seed and has got 6 legs with claws for each of them. Its colour may be grayish – white or black. Adult male usually dies after copulation. The females are larger than males. It moves with a speed of 23cm/min. The Life span: It lives upto 30 days on head and lays 10 eggs per day. And it could live for about 36 hours away from host without feeding on human blood. It feeds on blood for 4 to 5 times per day[17].

Synthetic Pediculicides

In these days there are so many synthetic pediculicides available in the market among them the commonly used pediculicide is permethrin 1%.

- Permethrin 1% - In 1986 introduced in market and available by prescription but later in1990 available as OTC product as “Crème rinse” (Nix). It is a synthetic pyrethroidwith less mammalian toxicity. Besides the side effects observed such as pruritus, edema and erythema it is also reported to develop resistance.[18]
- Malathion 0.5%- It is cholinesterase inhibitor. It was withdrawn from market due to prolonged application time, flammability and odor and reintroduced in 1999(Ovide). It has high ovicidal action but risk of development of resistance is reported.[19][20]
- Benzyl alcohol 5%- It is approved by FDA in 2009(Ulesfia). The common adverse effects observed are pruritus, erythema, pyoderma and ocular irritation. It is available by prescription and not recommended for neonates.[21]
- Ivermectin 0.5% – It is an anthelmintic agent. It was approved by FDA in 2012 (Sklice). It is available by the prescription. The adverse effects observed are skin or eye irritation, burning or dryness and erythema.[22]
- Spinosad 0.9% suspension – it is approved by FDA (Natroba). Used in children older than 6 years and available by prescription. Adverse reactions are application site erythema and irritation and ocular erythema[23].

Herbal Remedies

Many of the essential oils and the plant extract are known to possess potential anti-lice activity without development of resistance.

Essential Oils

Essential oils are well known for their insecticidal and repellent activity. Plant essential oils have been used as medicines, fragrances and insect repellents. They consistvolatile, low molecular weightterpenoids. Many modern pediculicidesseems to be a failure because of their low efficacy on nits, whereas essential oil constituents promise to have good ovicidal activity[18].The mode of action of the essential oil and compounds was likely by vapor action by respiratory system blocking[24].

Tea tree oil

Tea tree oil is derived from plant *Melaleuca alternifolia*. 1 % concentration of the oil has potential anti- lice activity and neridol , a most effective oil has ovicidal activity. When these oils were mixed together, in ratio 1:2, that is at less concentration the total lice killedwithin 30 min and the complete inhibition of nymphs and high concentration produced about complete mortality after 20 minutes [25].

Melaleuca oil and lavender oil,eucalyptus oil and lemon tea tree oil

These oils were evaluated for their ovicidal effects and compared among themselves..Ovicidal efficacy is varied for each oils.The suffocation pediculicide was found to have best ovicidal efficacyand the melaleuca oil and lavender oil was found to be the moderateand eucalyptus oil and lemontea tree oil pediculicide was found to have minimal efficacy[26].

Argentinian essential oils

About twenty five plants of different families were collected from Argentina. Four of these plants, *Aloysia citriodora, Baccharis salicifolia and Chenopodium ambrosioides* and *Sature japarvifolia* were found to be effective.The most effective essential oils were *Cinnamomum porphyrium*(studied earlier), followed by *Aloysia citriodora and Myrcianthes pseudomato.* Therefore these essential oils could be incorporated into pediculicide formulations once proper formulation and toxicological tests are performed [27].

Coconut oil, anise oil and ylang- ylang oil

A natural remedy Chick- chack was prepared which contain three oils such as coconut oil, anise oil, ylang – ylang oil. An anotherspray formulation was also prepared containing permethrin, malathion, piperonyl butoxide, isododecane and propellant gas. Hence it showed better anti – lice activity with no side effects [28].

Plant Extracts

Many of plant extracts and plant-derived compounds have been screened for toxicity to treat head lice infestation

Datura innoxia* and *Cyclea peltata

Datura innoxia belongs to the family Solanaceae and *Cyclea peltata* belongs to the family Menispermaceae. These plants exhibits potent pediculicidal activity. The former was fund to be more effective than the latter. These plants hence tends to replace the synthetic pediculicidal agents[29].

***Dichrostachys cinerea*:** *Dichrostachys cinerea* Wight &Arn belonging to the family Mimosaceae. Both ethanolic and aqueous extracts were prepared. Among this the ethanolic extract showed better mortality in 90 minutes. The ethanolic extract was mixed with carrier oil like Coconut oil, Castor oil and Gingelly oil. And the extract with coconut oil showed a reduced mortality time ie, to 60 minutes and showed better anti –lice activity[30].

***Pongamia pinnata* Linnaeus**: belongs to the family Fabaceae. Different extracts of the leaves were taken and tested for the pediculicidal activity. Among them, the petroleum ether extract was found to have excellent anti –lice activity followed by the chloroform and methanol extracts. Water showed a minimal anti –lice activity[31].

***Acorus calamus* Linn.*, Phyllanthus emblica* Linn., and *Zanthoxylum limonella* Alston:** Shampoos were formulated by using these native plants of Thailand and its efficacy were compared with carbaryl shampoo (Hafif shampoo, 0.6 %w/v), malathion shampoo (A-Lice shampoo, 1.0 %w/v), and commercial shampoos (Babi Mild Natural' N Mild and Johnson's baby shampoo).And among these the most effective pediculicide wasZ. limonella shampoo, followed by A. calamus shampoo, P. emblica shampoo, carbaryl shampoo, malathion shampoo, and commercial shampoo, respectively[32].

Azadiracta indica: Commercial shampoo based on seed extract of *Azadirachta indica* (Wash-Away Louse) was compared with permethrin 1%. This shampoo was found to be highly effective *in vitro* against head lice[33].

***Vitex negundo*:** belong to the family Verbenaceae. The aqueous extract of the leaves were taken and can be used as an effective, reducing agent; which along withTiCl4 solution synthesize TiO2 Nanoparticles. The biological reduction of metal oxide develops, a nontoxic, and environmentally acceptable metal oxide nanoparticles. The synthesized nanoparticles are hydrophilic in nature; disperse uniformly in water, highly stable and effective pediculicidal activity [34].

Home Remedies

Some of the home remedies that can help to remove lice are:

- Coconut oil and olive oil - by combining these oils kill adult lice.
- Aloe Vera gel - this gel is used to remove nits.
- Lemon juice- lemon juice helps to kill both lice and nits.
- Raw unfiltered apple cider vinegar –kills the lice, nymphs and nits.
- Herbal hair packs – grind some of the herbs into powder and make a mud mask and apply on head and leave for some time. Then wash it off using apple cider vinegar.[37]

CONCLUSION

Pediculosis remains as a problem among children. The synthetic over the counter topical pediculicidal agents are available in markets everywhere. These agents could definitely give a relief but are less effective than first introduced. On the other hand; herbal remedies such as the essential oils and plant extracts not only eradicate lice but also doesn't cause adverse reactions and resistance. Hence the safest and best recommended approach to get devoid of head lice is the natural way than the synthetic way.

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Figure 1: a) adult lice **b) nit** [11]

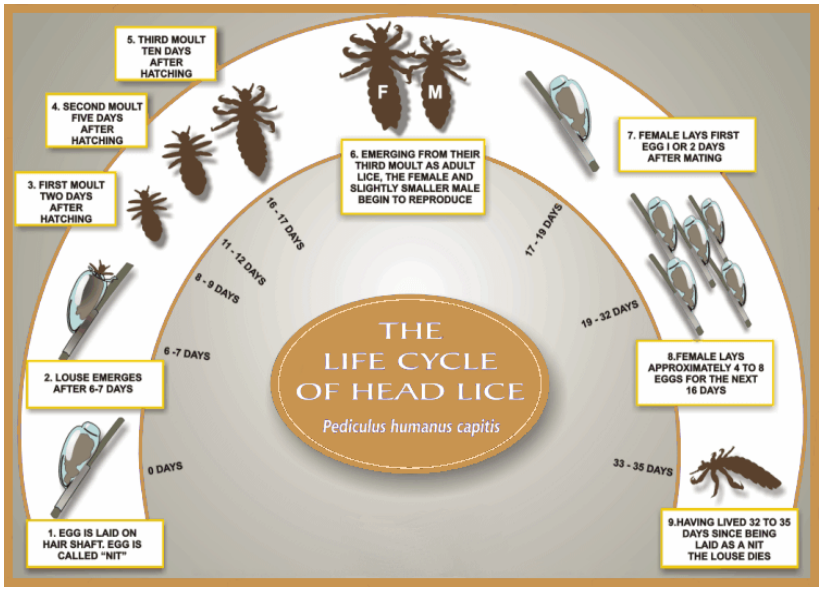

Figure 2: Life cycle of head lice

Table 1: Common and Scientific Name of Medicinal Herbs

COMMON NAME	SCIENTIFIC NAME
Citronella Grass	*Cymbopogon nardus*
Quassia	*Quassia amara*
Balsam of Peru	*Myroxylon balsamum*
Lavender	*Lavandula angustifolia*
Pawpaw	*Asimin atriloba*
Rosemary	*Rosmarinus officinalis*
Great Morinda	*Morinda citrifolia*
Sugar Apple	*Annona squamosa)*
Labrador tea	*Rhododendron groenlandicum*
Pasqueflower	*Pulsatilla patens*

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RESEARCH ARTICLE

Incidence of Hepatic Disorders in Dogs: A Prospective Study

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ABSTRACT

The present study was conducted on 110 dogs of different breeds, gender and age (2.5 months to 15 years) presented at veterinary college hospital Bengaluru. The dogs were presented with vague clinical signs and suspected to be suffering from liver disorders. The occurrence of hepatic diseases in this study was more in Labradors (28%) followed by breeds like non-descript Pomeranian and German shepherd. The difference in the breeds could be attributed to hereditary mechanism. The gender wise occurrence of hepatic disorder was more in males (62.72%) than in females (37.27%). This could be due to higher proportion of males in the population and their popularity. Age wise, it was found that the median age of occurrence of hepatic disease was 7.00 years. The highest incidence was recorded in the group of dogs aged between 5.1 to 10 years (41.86%) followed by other age group. Thus the results obtained in the present study indicate that the incidence of hepatic disorder in dog is 0.75 per cent (110/14639).

Keywords : Prospective study, hepatic diseases, breeds, gender and age.

INTRODUCTION

Liver disease is a common finding in dogs and the clinical signs may be variable, making it a difficult disease to diagnose just based on clinical symptoms and hence difficult to treat. Liver disease is the fifth leading cause of death in dogs, and it's estimated that three percent of all diseases in dogs are connected to the liver. Liver disease can affect many body functions and in turn the liver can be affected by many other organs and systems of the body (Routhuizen, 2010). Liver disorders can occur from direct damage to the liver by toxins or other infectious agents as

well as metabolic, immune mediated or neoplastic conditions (Cornelius, 1997).Sumathi (2012) reported that the incidence of canine liver disease was found to be 0.15 per cent of dogs in the study population (66540). Out of which, parenchymal disorders were 73 per cent, biliary 18 per cent and neoplastic 9 per cent. Prevalence of liver diseases varies with the age, breed, sex and feeding habits of the pet.

Pets with liver disease can present with array of clinical conditions, from severely ill to asymptomatic (Puja *et al.* 2010). Some vague signs can be depression, weight loss, anorexia, vomiting, lethargy, small body stature and poor or unkempt hair coat (Varshney and Hoque, 2002; Bunch, 2003). Besides these clinical signs, clients may notice alcoholic feces or other abnormal fecal coloration. More specific signs of liver disease include icterus, ascites, hepatomegaly, microhepatica, coagulopathies and hepatic encephalopathy. Polyuria and polydipsia can also be observed.

MATERIALS AND METHODS

In the present research work, an endeavour was made to know the incidence of hepatic disorders in dogs. Dogs presented/referred to Veterinary College Hospital, Bengaluru with clinical signs suggestive of liver disorders such as anorexia, inappetance, lethargy, anaemia, vomition, diarrhoea, icterus, abdominal pain, weight loss, ascites, nervous signs, bleeding tendency, poor hair coat, polyuria and polydipsia were identified while selecting the cases and subjected for thorough history collection and haematological, biochemical and ultrasonographic examination.Information regarding age, breed, gender and food habits were collected using a questionnaire.

RESULTS AND DISCUSSION

The present study was conducted on 110 dogs of different breeds, gender and age (2.5 months to 15 years) presented at veterinary college hospital Bengaluru. The dogs were presented with vague clinical signs and suspected to be suffering from liver disorders.Hepatic disease is one of the commonly encountered clinical entity in small animal veterinary practice which requires an early appropriate diagnosis for effective therapy as it is concern for both clinician and owner. Therefore the present study was undertaken to evaluate various diagnostic techniques and tests for the early diagnosis of liver disorders and to evaluate various treatment approaches in dogs.

Breed wise occurrence of hepatic disorders– The prospective study conducted on occurrence was more in Labrador (30.90%) followed by ND (17.27%), German shepherd (14.5%), Pomeranian (10%), Golden Retriever (9.09%), Crossbreed (2.73%), Dachshund (1.82%), Dalmatian (1.82%), Doberman (1.82%), Cocker spaniel (1.82%), Great Dane (1.82%), Pug (1.82%), Grey hound (0.91%), Boxer (0.91%) Spitz (0.91%) Rottweiler (0.91%) and Belgian Shepherd (0.91%).The details are shown in the Table no. 1 and Figure no. 1.

Gender wise occurrence of hepatic disorderswas 62.72% in males and 37.27 % in females. The details are shown in the Table no. 1 and Figure no. 2.Age wise occurrence of hepatic disorders in prospective study of the median age of occurrence of hepatic disease was 7.00 years. The highest incidence 41.86% (46/110) was recorded in 5.1 to 10 years group, followed by 23.63 % (26/110) in 1.1 to 5 yrs. group, 21.81% (24/110) in 10.1 to 15 yrs. age group and 12.72% (14/110) in up to 1 year age group. The details were shown in the Table no. 3 and Figure no. 3.

The results obtained in this study were similar to the reports of many researchscholars. Sumathi (2012) reported higher incidence in non-descript (26 %) followed bySpitz (20%), Labrador (14%), German shepherd (12%) and Doberman (7%). Dixit *et al.*(2010) opined that Pomeranians were more predisposed (37%) for both hepatic and extrahepatic disease followed by nondescript (20.71%), German shepherd (19.28%),Dobermann (7.14 %), Spitz (7.14%) and Golden retriever (4.28%).Similar findings were reported by Anderson and Sevelius, (1991) who reported ahigh incidence of chronic liver disease and cirrhosis in Labrador retrievers. A highincidence of copper-associated hepatitis was reported in Labrador retriever (Haffman*et al.*,2006; Shai *et al.*, 2007). Pooja*et al.*, (2010) recorded more

hepatic disease incidence inLabrador and Pomerian compared to other breeds.The scientific reason for higher incidence of hepatic disorders in Labradors couldbe attributed to hereditary mechanism (Hoffmann, 2006). Contrastingly, Vijayakumar *et al.,* (2003) reported that Doberman and German shepherd were significantly overrepresented (31.69 and 26.74 respectively) among various breeds of dogs affected withhepatic diseases. Variation in the present findings compared to the above authors'observations could be due to the difference in proportion and popularity of various breedsin different geographical areas.

Gender wise occurrence of hepatic disorders - In this research work, as per theprospective study, the occurrence was more in males (62.72%) than in females (37.27%)**.**Age wise occurrence of hepatic disorders **-** In the present study, it was found that the median age of occurrence of hepaticdisease was 7.00 years. The highest incidence was recorded in the group of dogs agedbetween 5.1 to 10 years (41.86%), followed by 1.1 to 5 yrs. age group (23.63%), 10.1 to 15yrs. age group (21.81%) and less than 1 year age group (12.72%).The findings on the average age of dogs most susceptible for hepatic disordersobtained in this study fall well within the age group reported by Rutgers and Haywood(1998) who stated 4 to 7 years age group as most susceptible group to chronic activehepatitis and Sumathi (2012) who stated 4 to 8 yrs. age group as most susceptible group toliver disorders. Similarly Poldervart*et al.,* (2009) and Anderson and Sevelius, (1991) reported the median age of dogs with the diagnosis of hepatic diseases was 7.7 years and5.9 years, respectively. In the present study also the median age of dogs with hepaticdisorders was found to be 7 years.

CONCLUSION

The results obtained in the present study indicate that the incidence of hepaticdisorder in dog is 0.75 per cent (110/14639). The occurrence of hepatic diseases in thisstudy was more in Labradors (28%) followed by breeds like non-descript, Pomeranian andGerman shepherd. The difference in the breeds could be attributed to hereditarymechanism. The gender wise occurrence of hepatic disorder was more in males (62.72%)than in females (37.27%). This could be due to higher proportion of males in thepopulation and their popularity. Age wise, it was found that the median age of occurrenceof hepatic disease was 7.00 years. The highest incidence was recorded in the group of dogsaged between 5.1 to 10 years (41.86%) followed by other age group.

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Table no 1: Breed wise and gender wise occurrence of Hepatic disorder (Prospective study n=110)

Breed	Male	Female	Total	Percentage
Belgian shepherd	1	0	1	0.91
Rottweiler	1	0	1	0.91
Spitz	1	0	1	0.91
Boxer	0	1	1	0.91
Grey Hound	0	1	1	0.91
Pug	1	1	2	1.82
Cocker spaniel	1	1	2	1.82
Great Dane	1	1	2	1.82
Doberman	1	1	2	1.82
Dalmatian	1	1	2	1.82
Dachshund	2	0	2	1.82
Cross breed	3	0	3	2.73
Golden retriever	3	7	10	9.09
Pomeranian	6	5	11	10.00
German shepherd	11	5	16	14.55
Nondescript	13	6	19	17.27
Labrador	23	11	34	30.91
Total	69	41	110	100.00

Table no 2 - Age wise distribution of dogs with hepatic disorders (Prospective study n=110).

Age(yrs.)	No. of Dogs affected	Percentage
up to 1	15	13.64
>1 to 5	26	23.64
>5 to 10	46	41.81
>10 to 15	23	20.90

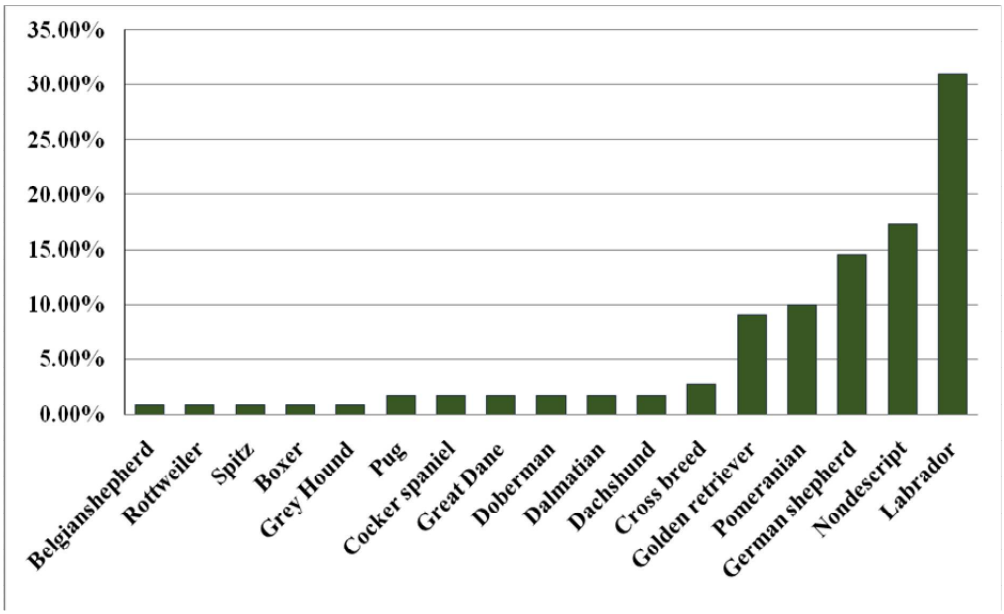

Fig. no. 1 - Breed wise and gender wise occurrence of Hepatic disorders (Prospective study n=110)

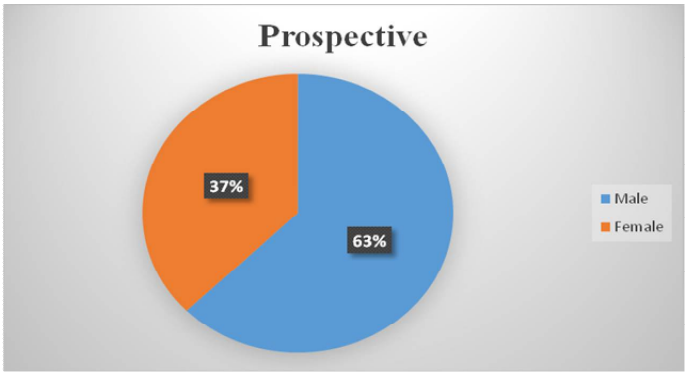

Fig. no. 2 .Gender wise occurrence of hepatic disorders (Prospective)

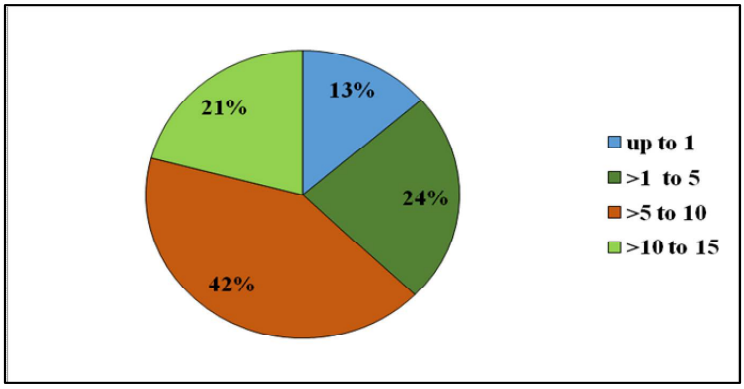

Fig. no. 3.Age wise distribution of dogs with hepatic disorders (Prospective study n=110).

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RESEARCH ARTICLE

Rapid Method of Screening for Assessing Brown Planthopper (*Nilaparvata lugens* (Stal)) Resistance in Nagina22 Mutant Lines in Rice (*Oryza sativa* L.)

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ABSTRACT

Rice production is highly affected by biotic and abiotic stresses. Biotic stresses, especially insect pest causes more damage to rice crop. Brown planthopper (BPH) is one of the devasting pest causes hopperburn and transmit viral diseases to rice. In concern, breeding for resistance to BPH was initiated earlier. Screening for resistance to BPH results in finding of many resistant entries. Historically the use of mutagenesis in breeding has involved forward genetic screens and the selection of individual mutants with improved traits and their incorporation into breeding programmes. In the present studies, Ethyl Methane Sulphonate induced mutant lines of Nagina22 (N22) was used to identify resistant to BPH. Considering the number of mutant lines to be screened, an attempt was made to evolve a method of screening over the existing methods using protrays. Among 900 mutant lines screened N22-M10- C-SG-2341 shows resistance to BPH when N22 shows hopper burn. Most of the mutant lines showed score of 7.0 – 9.0 resembling wild N22. This identified line has to be validated using other screening methods for further usage in breeding programme.

Keywords : Rice, Brown planthopper, Mutant lines and Screening methods

INTRODUCTION

Rice (*Oryza sativa* L.) remains as the staple food for over half the people in the world for a longer period of time than any other crop since it was domesticated between 8,000 to 10,000 years ago (Greenland, 1997). Each year, an additional 50 million rice consumers are added to the world population (Zeigler, 2009). Biotic stresses cause greater concern for the stability of rice production in many countries. For instance, more than 100 species of insects are known to infest the rice crop, causing an average yield loss of 37 per cent in tropical Asia (Sogawa, 1982). Among

them, the brown planthopper (BPH) *Nilaparvata lugens*, (Stal) is an important insect pest causing serious threat to rice cultivation. Heavy infestation by BPH causes drying of leaves and wilting of tillers, a phenomenon called 'hopper burn'. In addition, BPH causes indirect damage by acting as vectors of rice viruses (Anjaneyalu, 1986). The study of plant resistance has been conducted along many fronts with varying levels of coordination. Based on this, the growing of insect resistant rice varieties was considered as a major tactic in the integrated control of rice insect pests. Screening rice accessions for BPH resistance was initiated in 1967 at IRRI. Large scale screening of rice accessions for BPH resistance resulted in the identification of accessions with varying levels of resistance.

One approach in identifying new sources of plant resistance is induced mutagenesis. Mutants are valuable resources for genetic variations in crop improvement. Many rice mutants with resistance to insect pests and pathogens have been isolated (Liu *et al*., 2004). Induced mutagenesis provides an opportunity to identify the plant resistance to insects by using comparative screening methods on mutants and wild-type plants. Furthermore, a combination of mutational analysis along with reverse genetics strategies can identify plant defense genes against pests and diseases (Bouchez and Hofte, 1998). Historically the use of mutagenesis in breeding has involved forward genetic screens and the selection of individual mutants with improved traits and their incorporation into breeding programmes. The novel genetic variations obtained from either spontaneous or induced mutants using physical or chemical mutagens can be exploited in crop genetics and their application in functional genomics and molecular breeding (Krishnan *et al*., 2009, Jiang and Ramachandran, 2010).

With the advancement of DNA sequencing methods at present sequencing of crops became easier. It is now possible to find the mutation responsible for mutant phenotype of our interest. MutMap is one of the approaches using whole-genome sequencing (Abe *et al*., 2012). MutMap, a method that allows rapid identification of causal nucleotide changes of rice mutants by whole genome resequencing of pooled DNA of mutant F_2 progeny derived from crosses made between candidate mutants and the parental line.

The inheritance of many biological traits is explored based on the simple phenotyping methods to reach the outcome of the established genetic models. In the case of identifying phenotypes for insect resistance in plants is based on the three important components of resistance *viz*., antixenosis, antibiosis and tolerance established by Painter (1951). As the most important insect pests of rice, BPH demanded the attention of entomologists and breeders to develop easy and reliable screening techniques to screen a large number of germplasm and breeding materials to develop cultivars with improved resistance to BPH (Heinrichs *et al.,* 1985). All the screening programmes for BPH, across the rice growing countries were based on the exclusive usage of Standard Seed-box Screening Test (SSST). Velusamy *et al*., 1986 evolved Modified Seed Box Screening Test (MSST) to assess the level of resistant in rice cultivars. Besides these screening methods Kadirvel *et al*., 2007 used new screening technique, days to wilt to map QTLs associated with BPH resistant in rice. All these screening methods required more time, space and resources to screen large number of breeding materials. Based on above constrains, in the present study an attempt has been made to use protray as a base for screening mutant lines of Nagina22 (N22) for assessing the levels of resistance to BPH over the existing phenotypic screening methods.

MATERIALS AND METHODS

Advanced Ethyl Methane Sulphonate (EMS) induced mutant lines of rice variety N22 available at Paddy Breeding Station (PBS), Coimbatore was used for screening BPH resistance. Based on MSST result, N22 shows susceptible reaction with damage score of 7.0 for BPH resistance. As N22 shows susceptible reaction, search for gain of function for BPH resistance in EMS induced mutant lines of N22 was done.

Insects were mass reared on the susceptible rice line (host) Taichung Native 1 (TN1) following the method of Heinrichs *et al*. (1985). Initial BPH population was collected from the rice field at PBS, Coimbatore. The collected

insects were reared and maintained in 45 days old host plants in separate culture room which was protected with wire mesh. Adult male and female insects in ratio of 1:1 were let for ovipostion in 45 days old plants covered with milar cage, twelve to fifteen days after release. First instar nymphs were emerged out which were uniform in nature and will be of more effective for screening.

Around 900 advanced mutant lines of N22 (M_3, M_5, M_{10} and M_{14}) were used for screening under glasshouse condition for BPH resistance. Instead of screening using MSST, protray with fifty holes were used for rapid screening (Figure 1). Pre-germinated seeds of each mutant line (at least 15 to 20 seeds per entry) were sown in three replications. On seventh day after sowing, the protrays were transferred to plastic trays containing 5 cm depth of water. The seedlings were infested with second instar nymphs on 12th day after sowing. After infestation, the protrays were covered with wire mesh wooden cages in order to protect the released nymphs from other predators and also prevent the escape of nymphs. Each hole can accommodate 15-20 seeds. In each protray, two checks (TN1 and PTB33) and N22 wild type were sown along with the test N22 mutant lines. Damage rating of the test lines was done on individual plant basis when 90 per cent of the plants either in the susceptible check row (TN1) in the seed-box were died using Standard Evaluation System for Rice (SES) scale (IRRI, 1996) (Table 1). Along with it the mutants which shows resistance equal to PTB33 (resistant check) also noted and proceeded forward for other screening methods for confirmation.

RESULT AND DISCUSSION

As N22 showed susceptible reaction to BPH, search for mutants with gain of function was done. Mutants were designated as gain of resistance to BPH based on their damage rating in relation to N22. Among 900 mutant lines, 24 lines were with the damage score of 4.0- 5.0, 315 mutant lines were with damage score of 6.0-7.0 and rest of the 560 mutant were with damage score of 9.0 respectively (Figure 2). One putative mutant N22-M10- C-SG-2341 with altered resistance to BPH was identified from the preliminary screen of 900 mutant lines (Figure 2) which is same as PTB33 (resistant check). This line remains green when all the other mutant lines and N22 were dried out with damage score of 3.0. Other than this mutant line rest of the mutant lines showed reaction same as that of wild type (N22). Mutant lines with damage score of 4.0 – 5.0 were further validated compared with MSST screening method. In MSST screening, N22-M10- C-SG-2341 and 24 moderately resistant mutant lines showed the damage score varying from 5.0 to 7.0 respectively (Figure 3). N22-M10-C-S-2341 showed the damage score of 5.0 in MSST screening method. Rest of the 24 mutant lines were with damage score ranging from 5.0 to 7.0 respectively.

Understanding the phenotypes is more important before embarking on its inheritance pattern to identify the gene or genes controlling the trait. In this study, though 25 mutant lines were obtained in the preliminary screening using protray, the same result was not obtained with MSST method of screening. This difference may be due to lack of insect movement or escape of the test lines from the insect infestation in protray method of screening. Moreover this screening method is like going back to standard seed box screening test using bulk seedling. This method didn't guarantee the insect movement as of MSST.

Protray screening method can be used as preliminary screening method when there is large number of accessions are to be screened in short period of time and with limited resources. Whatever the screening method, it needs to provide detailed information regarding the phenotype. Among the various methods used for phenotyping to assess the levels of resistance to BPH to map the genes/QTL, SSST was used by majority of the scientific community because of the easiness over the other methods. MSST was used by recent groups of scientists and the rest of the methods were mostly by the individual groups. Velusamy and Heinrichs (1986) demonstrated that MSST provides a better separation levels of resistance compared to the SSST and hence proposed MSST for selecting genotypes with quantitative resistance to planthoppers. The MSST provides a method of identifying field resistance under greenhouse conditions and is expected to be a useful tool in the breeding of high yielding rice cultivars which have

durable resistance to BPH. Both SSST and MSST are based on choice test where insects have the choice to select its host (Horgan, 2009).

The SSST and MSST have been extremely successful in identifying resistant phenotypes and leading to the large number of resistant rice varieties now available. However, in most modern varieties, BPH resistance is predominantly feeding-related. Novel resistance mechanisms may be required to improve durability, and this will require new screening methods. Although the MSST is an improvement on the SSST, because of its higher cost and longer turnover time, it is seldom used in bulk screening. Further manipulative experimentation is required to clearly determine the mechanisms behind resistance in protray method of screening with some fine tune in procedure.

Sangha *et al.*, 2008 used IR64 mutants for dissecting insect and disease defense pathways and for conducted functional analysis on the various genes involved in this process. Currently, several molecular and genomic tools are available to differentiate mutants from wild type plants. These tools can be used on mutants to identify the genes involved in resistance to BPH. A comparative analysis of up or down regulated genes will help to identify defense related genes and understand pathways activated in rice resistance against BPH. This information would be useful to generate rice germplasm having durable resistance to BPH.

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Table1. Scale based on Standard Evaluation System for Rice (SES)

Grade	Criteria
0	No damage
1	Very slight damage
3	First and Second leaves of most of the plants partially turns yellowing
5	Pronounced yellowing and stunting or about half the plants wilted or dead
7	More than half of the plants dead
9	All plants dead

Figure 1. Protray setup for assessing resistance to BPH across mutant lines of Nagina22

Figure 2. a. Mutant lines showing hopper burn.b.N22-M10- C-SG-2341 mutant line showing resistance to BPH when all other mutant lines dried after BPH infestation

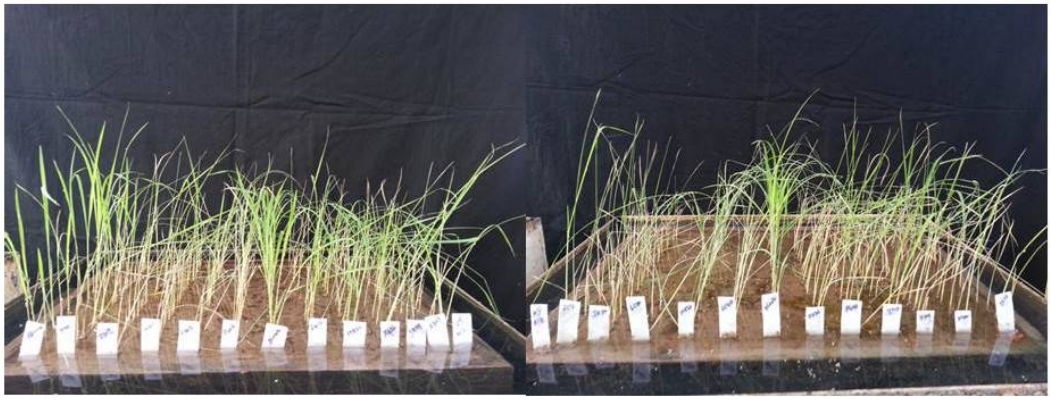

Figure 3. Screening of moderately resistant mutant lines in MSST method

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RESEARCH ARTICLE

Effect of 900 MHZ Cell Phone Radiation for Different Time Intervals on Sperm Parameters of Male Wistar Albino Rats

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ABSTRACT

With the increased use of cell phones, there may be possible hazardous effects of electromagnetic radiation on humans and their offspring. The recent increase in the use of cell phones worldwide has created a fresh impetus on the development of natural disaster, which may lead to cancer or birth defects. Our objective is to investigate the effect of 900 MHz cell phone radiation for different time intervals on sperm parameters of male wistar albino rats. The rats were divided into 5 groups, in which group A served as a control group and the remaining 4 groups *viz.* groups B, C, D and E (n=14) were exposed to 900MHz RF-EMF radiation generated by a mobile phone at a specific time of the day (during the light period) for 30, 60, 90 and 120 min/day over a period of one month. There was a significant decrease in testis and accessory organ weight, sperm count and testosterone, and increase in sperm abnormalities in all the mobile phone radiation groups compared with the controls. Mobile phone radiation affects sperm parameters by inducing a decrease in testis weight, accessory organ weight, sperm count and viability, and an increase in defective sperm as well as vital hormones such as serum testosterone.

Keywords : cell phones, sperm parameters, testosterone, vital hormones.

INTRODUCTION

Currently, in modern society, humans are exposed to a number of electromagnetic fields (EMFs) that are generated by televisions (TV), personal computers (PC), radios and mobile phones. The radio frequency (RF) EMFs that are generated from mobile phones, cordless phones and broadcasting towers have frequencies of hundreds of MHz. A frequency range of about 900 to 2,200 Megahertz (MHz) of radiofrequency radiation emanates from mobile phones

(Usikalu *et al.,* 2012). Due to the use of mobile phones, the most common health problems that occur are impairment of short-term memory, headaches, brain tumors, other cancers, sleep disturbance, depression, tiredness, neuroendocrine functions, miscarriages and impairment of semen quality (Sage, 2000). Rats exposed to mobile phone radiation of 900 MHz for 2 months showed changes in body weight and histological changes in brain tissue (Usikalu *et al.,* 2012). Greater usage of mobile phones can possibly result in hazardous effects of electromagnetic radiation on humans as well as their offspring. Innovations in cell phone technology in recent decades have led to effects on male fertility. Usage of cellular phones is associated with alterations in various body systems including the central nervous system, the cardiovascular system and the male reproductive system (Makker, 2009). In addition, usage of cell phones is associated with decreased concentration, fatigue and headache (Oftedal *et al.,* 2000).

Dasdag *et al.,* (1999) reported that rats exposed to cell phone radiation showed a decrease in seminiferous tubule diameter. A study of exposure to cell phone RF-EMW for the first time showed that it can cause oxidative stress in ejaculated human semen, resulting in a significant increase in the reactive oxygen species (ROS) level and decrease in the ROS-TAC score (reactive oxygen species-total antioxidant capacity) (Agarwal *et al.,* 2008). Reproductive organs such as the testis, when exposed to cellular phone radiation as a result of constantly carrying a mobile phone, may cause dysfunction and particularly a decrease in sperm development and production, thus affecting fertility in men (Yan *et al.,* 2007; Agarwal *et al.,* 2009; Salama *et al.,* 2010; Gutschi *et al.,* 2011; Meo, *et al.,* 2010; Kesari, *et al.,* 2010, & 2011), and also significantly higher incidences of sperm cell death and abnormal clumping of sperm cells (Yan *et al.,* 2007). Long-term mobile phone radiation exposure causes hypospermatogenesis and maturation arrest in the spermatozoa (Meo *et al.,* 2011). Testosterone and FSH levels are altered due to mobile phone exposure, which in turn affects reproductive functions (Sarookhani *et al.,* 2011). Very few studies exist on the effect of cell phone radiation for different time intervals on sperm parameters of rats, our aim is to investigate the effect of 900 MHz cell phone radiation for different time intervals on sperm parameters of male Wistar albino rats.

MATERIALS AND METHODS

Ethical statement

All procedures were approved by the institutional animal care and use committee of the University of Mysore, Mysore, India. Experiments were conducted according to CPCSEA guidelines for the ethical treatment of animal guidelines. A total of sixty two two-month-old male Wistar albino rats weighing 140-160 grams were selected for the experiment. Animals were housed in cages in a similar environment and were fed with normal laboratory chow and water ad libitum. They were maintained under a controlled temperature of 22-24°C.

Experimental protocol

The rats were divided into 5 groups. Group A (n=6) served as a control group and the remaining 4 groups *viz.* groups B, C, D and E (n=14) were exposed to 900MHz RF-EMF radiation generated by a mobile phone at a specific time of the day (during the light period) over a period of 1 month. Rats in group B were exposed to mobile phone radiation for 30 min/day, group C for 60 min/day, group D for 90 min/day and group E for 120 min/day. In this experiment, handsets of global system for communication (GSM) mobile phones of the same brand and model were used. A mobile phone was placed inside the cage and a call was placed using another mobile phone; it was also ensured that the mobile phone inside the cage was powdered on, with the call accepting (answering) mode, and the rats were in close proximity to the mobile phone.

Gravimetry

The weights of the testis and accessory organs were calculated for 100mg body weight of an animal using the following formula: weight of the organ (mg) divided by body weight (g) and multiplied by 100.

Sperm parameters

The animals from all groups were autopsied after the completion of treatment and cauda epididymis was collected and rinsed in 1ml of saline. The suspension was filtered through a nylon mesh into an Eppendorff tube. To the filtrate, one drop of 1% eosin was added and keep for 30 min to stain the sperm (Garner and Hafeze, 1993).

A few drops of the prepared suspension were spread on a clean glass slide and sperm morphology defects, *viz.* normal, curved flagellum, flagellum with ansa, bent at the cephalocaudal region, amorphous and hookless flagella were observed in the smear of the sperm suspension according to the procedure of Narayana *et al.,* (2002) and Vijayalaxmi and D'Souza (2004). A total of 1000 spermatozoa were screened/cauda epididymis and the number of spermatozoa with a defective morphology was recorded in randomly selected areas of the smear and expressed as percentage of abnormal spermatozoa.

Hormone Assay

Serum concentrations of testosterone, LH, FSH and Estradiol were determined using commercially available ELISA Kits (DRG instruments, Germany) according to the instructions of the manufacturers.

Statistical Analysis

Data were described as Mean ±Standard Error of Mean (SEM); the student t-test was used and a p-value <0.05 was considered significant.

RESULTS

Reproductive organ weights

In the present study, the animals, when exposed to 900MHz radio frequency electromagnetic waves (RF-EMW) from a mobile phone, showed a significant decrease in testis weight ($p<0.05$) on 90 minutes/ day exposure in the 30-day group compared with the control group, and there was a significant decrease in prostate weight ($p<0.05$) on 90 minutes/day exposure ($p<0.05$) and 2hrs/day exposure in the 30-day group compared with the control group. There was a significant ($p< 0.05$) increase in seminal vesicle weight in the 2hrs/ day exposure group compared with the control group (Table -1)

Total Sperm count and defective sperm morphology

The result showed that, when rats were exposed to 900 MHz (RF-EMW),the total sperm count significantly decreased ($p<0.05$) in all the exposure groups and there was a significant increase in the percentage of abnormal sperm in all the exposure groups compared with the control group (Table - 2). In the present study, different sperm abnormalities were found in the treated groups, viz. coiled with microcephali, flagellum with ansa, double headed, etc. There was a significant increase ($p<0.05$) in sperm abnormalities of up to 74.10 % in the treated group over a period of one month as shown in Table -3.

Hormone Assay

The present study observed alterations in the levels of serum testosterone, LH, FSH and Estradiol when Wistar rats were exposed to 900 MHz for different durations of mobile phone radiation compared with their age-matched controls. Serum testosterone, LH, FSH and Estradiol levels were significantly lower ($p<0.05$) in Wistar albino rats that were exposed to mobile phone radiation for 120 minutes daily over a period of one month compared with their age-matched controls. However, there was no significant difference between the control group and the group exposed to mobile phone radiation for 30 minutes/ day for over a period of one month compared with their age-matched controls (Table - 4).

DISCUSSION

The male reproductive system is a highly sensitive biological system that requires the integration of intrinsic and extrinsic factors for proper functioning. The generated electrical currents may alter the hormonal milieu and testicular microenvironment that are necessary for sperm production. Additionally, sperm are electrically active cells and exposure to cell phone electromagnetic waves and currents may affect their motility, morphology and even their count. The present study aimed to investigate the effect of 900 MHz cell phone radiation for different time intervals on sperm parameters of male Wistar albino rats. In this study, with cell phone radiation exposure for different time intervals over one month, rats showed a decrease in testis organ weight and accessory organ weight compared with the age-matched controls. Mobile phone radiation groups studied for different time intervals for one month showed a significant decrease in the total sperm count and an increase in abnormal spermatozoa compared with the age-matched control group. Serum concentrations of testosterone, FSH, LH and estradiol were significantly lower in rats exposed to a long duration of radiation of 120 min/ day for one month compared with the controls.

The testis depends mainly on surface conduction rather than blood flow for temperature control; this represents an important target for the thermal effect of RF-EMW (Dasdag, 1999). Because the testis is a superficial organ, it may absorb more EMW energy than other organs. It is well known that the temperature of the testicular within the scrotum is 1- 2 °C lower than the core body temperature and if it exceeds this temperature, spermatogenesis may be affected (Kandeel and Swerdloff, 1988; Jung and Schill, 2000; Agarwal *et al.,* 2008). Exposure of the testis and secondary sex organs to RF-EMW exerts a detrimental effect on spermatozoa. The exact mechanisms by which RF-EMW may affect the spermatozoa have not yet been clarified. Some authors have reported that acute EMW exposure can have a direct effect on seminiferous tubular epithelium through an increase in testicular temperature (Saunders and Kowalczuk, 1981; Kowalczuk, 1983). They exposed mice to 2.45 GHz (30 w/kg), 1.7 GHz (50 mw/cm2) and 2.45 GHz (44 w/kg), respectively, and observed altered histology of seminiferous tubular epithelium and frenzied semen parameters such as sperm count and sperm morphology. In the present study, with cell phone radiation exposure for different time intervals of 30, 60, 90 and 120 min/ day for one month, rats showed a decrease in testis organ weight and accessory organ weight compared with the age-matched controls. RF-EMW causes a decrease in both testis and prostate weight perhaps due to the thermal effect of mobile phones (which generates heat when it is in the radial mode) on these tissues and may be due to the effect of oxidative stress in the testis and prostate, causing atrophy in their tissues, because of an increase in the amount of radio frequency energy emitted from the mobile phone absorbed into these tissues.

Wdowiak *et al.,* (2007) carried out a retrospective study on 304 men, noting that there was a significant decrease in the percentage of forward progressive motile sperm, correlated with the frequency of cell phone handling. It was concluded that RF-EMW emitted from cell phones may increase oxidative stress in human spermatozoa, leading to decreased motility and viability characteristics (Agarwal, 2009). Fejes *et al.,* (2005) reported, in an observational study, a significant decrease in sperm count related to cell phone handling frequency. This study analyzed 231 men over a 13-month period and showed that among heavy users of cell phones, sperm counts were 30% lower than those in

men who did not use a cell phone (Fejes *et al.,* 2005). Moreover, in an animal study that exposed rats to cell phone RF-EMW for 2 h/day for 35 days at 0.9 SAR, a decreased mean value of the total sperm count (31.14 ± 13.6 *vs.* 61.33 ± 3.68) and an increased mean percentage of apoptotic cells (13.15 ± 1.26 *vs.* 5.93 ± 1.64 %) were observed (Kesari *et al.,* 2010). Furthermore, Salama *et al.,* (2010) carried out a study on rabbits exposed to mobile phone radiation (GSM mode, 800 MHz, standby status). RF-EMW exposure of 8 hours/day led to a significant decrease in the sperm count after 8 weeks of exposure and a decrease in motility after 10 weeks of exposure. Investigation of several authors showed that prolonged exposure of microwave results in decreased sperm count, sperm cell morphology, weight of testis and epididymis (Akdag *et al.,* 2003; Dasdag *et al.,* 2003). Mobile phones might have a negative effect on sperm motility characteristics with prolonged usage and it can be concluded that this could be a consequence of accumulation of radiation effects, whereas standby communication signals did not affect the sperm parameters significantly (Fejes *et al.,* 2005). Deepinder's study (2007) showed that the use of cell phones adversely affects the quality of semen, resulting in a decrease in the total sperm count, sperm viability, motility and morphology. Exposure to 915MHz frequency/ 1 hr for 7 days a week over 2 weeks did not affect sperm count, motility and morphology in adult rats (Pisl *et al.,* 2010). Erogul *et al.,* (2006) reported that there was a significant decrease in rapid progressive motility, an increase in slow progressive motility and an increase in the percentage of immotile sperm when neat semen of a human volunteer was exposed to cell phone radiation for 5 min. Use of GSM phones for 6 hours/day for a period of 5 days led to a decrease in the rapid progressive motility of sperm cells (Davoudi *et al., 2002*). In the present study, we found a significant decrease in the total sperm count and sperm viability with an increase in the duration of exposure to 900MHz cell phone radiation for 30, 60, 90 and 120 minutes over 30 compared with the age-matched controls. The reduction in the total sperm count might be due to the effect of RF-EMW exposure, which leads to infertility.

Rats, after exposure to 2 hours of 2450 MHz continuous and pulsed RF radiation, showed a dose-dependent increase in DNA single- and double-strand breaks (Lai and Singh, 1996). Aitken *et al.,* (2005) suggested that, after mice were exposed to RF EMW, 900 MHz, for 12 hours a day for 7 days, a genotoxic effect may have occurred on epididymal spermatozoa, which damaged the mitochondrial and nuclear genome in epididymal spermatozoa. In the present study, our results showed a significant increase in defective sperm morphology as the duration of time intervals increased at 30, 60, 90 and 120 minutes over a period of 30 days of 900MHz cell phone radiation exposure compared with the age-matched controls. The increase in the percentage of types of abnormality occurred because of damage of sperm due to radiation exposure.

Testosterone is a primary male sex hormone that is secreted by Leydig cells of the testis. Exposure to mobile phone radiation for a long duration of 60 min/ day for 3 months leads to a significant reduction in serum testosterone levels, which can affect reproductive and general health (Meo *et al.,* 2010). Recently, Kesari and Behari (2010) reported that exposure to microwave at 2.45 GHz and 0.11 W/kg of SAR for 35 days resulted in increased apoptosis in Leydig cells of the testis. Leydig cells are cells that are most susceptible to EMFR and injury to these cells may affect spermatogenesis (Wang *et al.,* 2003). In twenty one healthy men, on exposure to 900 MHz RF radiation emitted from a cell phone (2 h/day x 5 days/week x 1 month), no effect was found on the gonadotropin concentrations of anterior pituitary hormones FSH and LH (De Seze *et al.,* 1998). In this study, a decrease in serum levels of testosterone, FSH, LH and Estradiol concentrations was found at all the time intervals studied and there was a significant decrease in the group with radiation exposure for 120 min/ day over 30 days compared with their age-matched control. The reduced testosterone level might be because of the radiation effect, which causes damage to the Leydig cells, which in turn leads to reduced testosterone secretion thus affecting spermatogenesis.

CONCLUSION

Exposure to mobile phone radiation for different time intervals over a one-month period affects reproductive morphology by inducing a decrease in sperm count and viability, and an increase in defective sperm as well as vital

hormones such as serum testosterone. Long-term exposure to mobile phone radiation leads to a reduction in serum testosterone levels. Testosterone is a primary sex hormone and any alterations in normal levels may have an adverse impact on reproductive and general health.

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Table 1 Effects of 900 MHz RF-EMW radiation from a mobile phone on reproductive and accessory organ weights of male Wister albino rats

Parameters/ Groups	Testis (mg/100g BW)	Epididymis (mg/100g BW)	Prostate (mg/100g BW)	Seminal vesicle (mg/100g BW)
Control	384.3 ± 10.73	133.2 ± 2.577	426.5 ± 20.54	136.0 ± 3.347
30 minutes/day exposure for 30 days	334.2 ± 9.297	124.0 ± 1.612	411.0 ± 3.225	160.6 ± 7.222
1h/ day exposure for 30 days	304.8 ± 10.24	119.4 ± 1.990	389.8 ± 3.089	177.2 ± 0.860
90 minutes/day exposure for 30 days	296.6 ± 0.509	105.0 ± 1.871	374.6 ± 1.913	177.2 ± 1.685
2h/ day exposure for 30 days	343.0 ± 2.025	111.6 ± 1.435	364.8 ± 2.871	186.8 ± 2.818
F-test	*	*	*	*
S.E(m) ±	7.885	1.937	9.528	3.870
S.E(d) ±	11.15	2.739	13.47	5.473
C.D. at 5%	23.42	5.754	28.31	11.50
C.V. (%)	5.301	3.651	5.416	5.164

± Standard Error (SE) values * Significant at P = 0.05

Table 2 Effects of 900 MHz RF-EMW radiation from a mobile phone on sperm parameters in male Wister albino rats

Parameters/ Groups	Sperm Count x 10^6 / ml	Live sperm %	Abnormal Sperm %
Control	1,676.8 ± 8.521	123.4 ± 1.249	1.560 ± 0.216
30 minutes/day exposure for 30 days	1,500.6 ± 20.27	106.2 ± 0.860	20.22 ± 0.729
1h/ day exposure for 30 days	1,376.0 ± 6.221	105.4 ± 3.108	24.44 ± 0.957
90 minutes/day exposure for 30 days	1,340.8 ± 7.308	90.00 ± 2.757	27.00 ± 0.491
2h/ day exposure for 30 days	1,376.4 ± 4.532	112.2 ± 3.583	30.18 ± 0.708
F-test	*	*	*

S.E(m) ±	10.92	2.546	0.669
S.E(d) ±	15.44	3.60	0.946
C.D. at 5%	32.43	7.562	1.987
C.V. (%)	1.679	5.298	7.231

± Standard Error (SE) values * Significant at P = 0.05

Table 3 Effect of 900MHz RF-EMW radiation from a mobile phone on sperm morphological parameters in Wister albino rats

Parameters/ Groups	Normal	Curved flagellum	Flagellum with ansa	Bent at cephalocaudal region	Amorphous
Control	75.68 ± 0.817	8.740 ± 0.225	7.460 ± 0.117	2.546 ± 0.123	0.292 ± 0.031
30 minutes/day exposure for 30 days	43.34 ± 0.583	6.620 ± 0.183	8.780 ± 0.188	3.840 ± 0.103	0.376 ± 0.014
1h/ day exposure for 30 days	40.18 ± 0.393	5.520 ± 0.139	9.400 ± 0.141	5.860 ± 0.319	2.300 ± 0.230
90 minutes/day exposure for 30 days	37.40 ± 0.748	4.480 ± 0.097	12.72 ± 1.050	8.120 ± 0.235	3.740 ± 0.244
2h/ day exposure for 30 days	27.52 ± 0.497	2.560 ± 0.093	13.32 ± 0.162	14.08 ± 0.437	5.320 ± 0.177
F-test	*	*	*	*	*
S.E(m) ±	0.627	0.156	0.489	0.274	0.170
S.E(d) ±	0.887	0.220	0.692	0.387	0.241
C.D. at 5%	1.864	0.463	1.454	0.813	0.506
C.V. (%)	3.130	6.240	10.59	8.878	15.84

Continuation,

Parameters/ Groups	Multiple abnormality	Hook less flagella	Total sperm abnormality
Control	1.354 ± 0.018	-	25.93 ± 0.256
30 minutes/day exposure for 30 days	1.516 ± 0.053	0.960 ± 0.075	38.52 ± 0.723
1h/ day exposure for 30 days	3.320 ± 0.227	0.900 ± 0.045	45.58 ± 1.399
90 minutes/day exposure for 30 days	4.400 ± 0.158	1.060 ± 0.060	57.38 ± 0.575
2h/ day exposure for 30 days	5.480 ± 0.198	2.108 ± 0.218	74.10 ± 1.217
F-test	*	*	*
S.E(m) ±	0.154	0.108	0.933
S.E(d) ±	0.218	0.153	1.320
C.D. at 5%	0.458	0.322	2.773
C.V. (%)	10.73	24.09	4.321

±: Standard Error (SE) values *: Significant at P = 0.05

Table 5 Effects of 900MHz RF-EMW radiation from a mobile phone on serum concentration of hormone levels in male Wister albino rats

Parameters/ Groups	Testosterone (ng/ml)	LH (1U/l)	FSH (1U/l)	Estradiol (Pg/ml)
Control	6.164 ± 0.049	3.848 ± 0.169	4.104 ± 0.079	3.936 ± 0.034
30 minutes/day exposure for 30 days	5.220 ± 0.139	3.116 ± 0.033	3.718 ± 0.181	3.178 ± 0.057
1h/ day exposure for 30 days	4.140 ± 0.038	2.968 ± 0.023	2.574 ± 0.190	2.904 ± 0.061
90 minutes/day exposure for 30 days	4.140 ± 0.196	2.980 ± 0.034	2.042 ± 0.036	2.300 ± 0.052
2h/ day exposure for 30 days	3.760 ± 0.103	2.640 ± 0.065	2.020 ± 0.030	1.984 ± 0.007
F-test	*	*	*	*
S.E(m) ±	0.120	0.084	0.125	0.047
S.E(d) ±	0.170	0.119	0.176	0.066
C.D. at 5%	0.358	0.250	0.370	0.138
C.V. (%)	5.746	6.054	9.636	3.644

± : Standard Error (SE) values *: Significant at P = 0.05

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RESEARCH ARTICLE

Screening, Characterization and Antimicrobial Activity of Marine Actinomycete *Streptomyces* sp. Isolated from Kattumavadi Region of Palk Bay, Southeast Coast of India

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ABSTRACT

In this study the potency of marine actinomycetes to produce antimicrobial substances has been identified in 10 strains isolated from Kattumavadi region of Palk Bay, Southeast coast of India. Antibacterial activity of all 10 isolated actinomycete strains were assessed by cross streak method against 21 bacterial pathogens. Among 10 isolates tested, K8 isolate was found to be potential antibacterial compound producers which showed the maximum activity against all pathogens. The morphological and physiological characterization of K8 isolate was studied and also confirmed by molecular characterization and it was identified as *Streptomyces* sp.

Keywords : Palk Bay, Marine Actinomycetes, Antibacterial activity, *Streptomyces* sp, Bacterial pathogens

INTRODUCTION

Oceans account for more than 70% of the earth's surface and the microorganisms growing in marine environments are metabolically and physiologically diverse from terrestrial organism (Takizawa *et al*., 1993). The marine derived antibiotics are more efficient at fighting microbial infections than the terrestrial bacteria have not developed any resistance against them (Donia and Humann, 2003). Among the microorganisms, Streptomycetes produce a wide variety of secondary metabolites possessing antibacterial, antifungal, antiviral, antitumor, immunosuppressive,

antihypertensive and antihypercholesterolemic properties. They have indispensible role in mineralization of all complex organic as well as inorganic matter. Streptomycetes are important decomposers of plant and animal remains and recalcitrant compounds in the soil. They produce a large repertoire of enzymes for performing degradation activities (Anderson and Wellington, 2001).

Evolution of novel disease, toxicity of currently used compounds and emergence of drug resistant pathogens which cause life threatening infection and risk undermining the viability of healthcare systems, especially in immunodeficient patients revealed the need for new and novel antibiotics (Hakvag *et al*., 2008). The prevalence of antimicrobial resistance among pathogens is increasing at an alarming rate worldwide (Singer *et al*., 2003). The present study reveal the efficacy of marine actinomycete *Streptomyces* sp. isolated from sediment samples of Palk Bay region, southeast coast of India.

MATERIALS AND METHODS

Collection and pre-treatment of samples

The sediment samples were collected from Kattumavadi coastal region located along the Palk Bay, Tamil Nadu, India. From each location, 50g of sample was collected at 50 to 100 cm depth from the surface. These samples were taken and placed in small pre-labelled plastic bags and tightly sealed. The samples were air-dried for 6 days. The air dried and sieved samples were kept at 55°C for 60 min. in a glass container for pre-treatment (Sweetline *et al.,* 2012). The pre-treated sediments samples were used for the isolation of actinomycetes.

Isolation of Actinomycetes

After pre-treatment, the sediments samples were taken and subjected to serial dilution (up to 10^{-6} dilution) by adding 5 gram of soil sample in 50 mL of distilled water. About 1.0 ml of diluted sample was placed on Actinomycete isolation agar by spread plate technique and incubated at 28ºC for 7-10 days. After incubation, the powdery colonies were sub cultured on ISP2 medium mixed with seawater/starch casein agar supplemented with antibiotics, cycloheximide (25 g/ml) and nalidixic acid (25 g/ml) (Himedia, Mumbai, India).

Identification of Actinomycetes

Actinomycetes were recognized by their characteristic tough leathery colonies, branched vegetative mycelia, presence of aerial mycelia and spore formation. Because of these criteria, only colonies with well developed and branched hyphae were included in this study.

Morphological and cultural characterization

Morphological characteristics of the most potent strain K8 grown on ISP2 medium at 27°C for 7 days and was examined under light microscope. The cultural characteristics were studied in accordance with the guidelines established by the International *Streptomyces* Project (Shirling and Gottlieb, 1969). Microscopic characterization was done by cover slip culture method (Kawato and Shinobu, 1959). The mycelium structure, colour and arrangement of conidiospore and arthrospore on the mycelium were observed under light microscope. The utilization of different carbon and nitrogen sources were analyzed (Pridham *et al*., 1948).

Determination of Antimicrobial Activity

Test microorganisms

Totally 21 pathogens such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisiae, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecalis, Proteus vulgaris, Bacillus cereus, Vibrio cholarae, Vibrio parahaemolyticus, Aeromonas hydrophylla, Vibrio* sp., *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus luteus, Enterobacter aerogens, Salmonella typhi* and *Shigella flexneri* were used to evaluate the antimicrobial activity of selected Actinomycete strain.

Screening of antibiotics producing Actinomycetes

The screenings of antibiotic producing actinomycetes were done by primary screening and secondary screening as follows:

Primary screening

Screening of Actinomycetes was done by the antimicrobial activity, primarily studied by cross streak method against 21human pathogenic bacteria, the 23 isolated actinomycetes strains were streaked as parallel line on Muller Hinton plates. The plates were incubated at 28±2°C for 7-14 days. After observing a good ribbon like growth of the Actinomycetes on the Petri plates the single colonies of two days old test microorganisms were cross streaked perpendicular to actinomycetes strains on the test plates. Positive control plates for the test were prepared by streaking only the test microorganisms on the agar plates without the Actinomycetes. After cross streak, the plates were incubated for 48 hours at 37±2°C. After the incubation period, the inhibition of the test microorganism's growth was observed. Based on the results of antagonistic activity the strains were selected for further study.

Secondary screening

Based on the results of preliminary screening, potential strain (K8) was selected depending upon their activity against the pathogens. Fermentation for production of antibiotic and subsequent extraction of the antibiotics was done as described by Murrey *et al*. (1995). The K8 strain was cultivated in 1000 ml ISP2 broth. The flasks were then incubated on a rotary shaker at 120 rpm at 28±2°C for 7-9 days. After 7 days, actinomycetes cultures were filtered using 0.2 micrometer filter. Antimicrobial compound was recovered from the filtrate by solvent extraction with ethyl acetate. Ethyl acetate was added to the filtrate in the ratio of 1:1 (v/v) and incubated in shaker for 1hr for complete extraction. The ethyl acetate phase that contains an antibiotic agent was separated from the aqueous phase. It was evaporated to dryness in water bath at 80-90°C. The dried precipitate was dissolved in minimum amount of ethyl acetate and was used to determine the antimicrobial activity. Secondary screening was done by well-diffusion method on Muller Hinton Agar plates. Crude extract of antimicrobial compound which was produced by ethyl acetate extraction was used. A 100 µl of compound was placed into the plates that were previously seeded with different pathogenic bacteria. The plates were incubated at 30-37 °C for 48hrs and examined for zones of inhibition.

Characterization of Actinomycetes

Morphological characteristics

Morphological characteristics such as aerial hyphae, spore mass, spore surface, colour of aerial, substrate mycelia and soluble pigments production were conducted by growing the organism on ISP2- media according to Bergey's manual of determinative bacteriology (2000).

Biochemical characterization

Actinomycetes isolates were characterized using Melanin reaction, H_2S production, Tyrosinase reaction, Starch hydrolysis, Gelatin hydrolysis, Casein hydrolysis, Nitrate reduction, Milk coagulation and peptonisation according to International *Streptomycetes* Project (Nonomura, 1974).

Physiological and cultural characterization

The physiological and cultural characterization of actinomycetes was estimated by assessing their ability to grow at various ranges of temperature (5-50^{o}C), different ranges of pH (5-12), different concentrations of NaCl (5-30 %), different carbon and nitrogen sources and different culture mediums according to Vengadesh Prabhu *et al.* (2011).

Molecular characterization

Isolation of DNA from actinomycetes was done according to Wilson (1987). In brief, the DNA of the selected K8 Actinomycete strain was isolated from cells grown in yeast malt extract broth (YMB) with 0.2% of glycine (Yamada and Komagata, 1970). The isolated DNA was amplified by PCR. DNA sequences were initially aligned using CLUSTAL X (Thompson *et al.,* 1994), and default gap and extension penalties were used followed by manual editing using SeqApp 1.9 (Gilbert, 1994). A Blast search was performed on GenBank for each sequence and the matching homologous sequences were retained for subsequent alignment. Alignments were adjusted and Neighbour Joining method of phylogenetic analyses were implemented using mega version 5 (Kumar *et al.,* 2001). The secondary structure of selected Actinomycete strain K8 was predicted using the bioinformatics tools available online (www.genebee.msu.su/services/rna2-reduced.html).

RESULTS

Totally 23 strains were isolated from marine sediment samples collected from different locations at Kattumavadi coastal region located along the Palk Bay, India. Isolation of actinomycetes made by serial dilution method, the powdered colonies were observed on the plates on 7th day. Among the 23 isolates, 10 morphologically different cultures were selected for further study and the strains were named as K1 to K10. Ten morphologically different colonies have different types of colours like white, ash white, brownish white, greenish yellow and yellow. Some of the selected strains can able to produce melanoid pigment, while there was no soluble production on all the strains and the nature of the growth was differing from one strain to another. Among the 10 strains, K1, K4 and K8 isolates were grew well in nature. Among the 10 strains, K8 strain showed the maximum activity against all the 21 pathogenic strains tested. Based on the activity K8 strain was selected for the secondary screening and showed potential against 7 pathogens. The K8 strain was characterized and identified by microscopical, biochemical and physiological observations. Identification of the isolates revealed that all isolates belong to the genus *Streptomyces*. The isolated marine actinomycetes were screened for their antimicrobial activity against the human bacterial pathogens.

Molecular characterization of isolated Actinomycete

The molecular characterization of isolated Actinobacterial K8 strain was evaluated by isolation of genomic DNA and PCR amplification of 16S rRNA gene. The genomic DNA and amplified products were separated in agarose gel. The 16S rRNA gene of selected actinomycete K8 strain was partially sequenced using actinobacteria specific 16S rRNA sequence primers and the K8 strain was 100% blast similarity with *Streptomyces* sp. The sequence of K8 strain was submitted to the Genbank (NCBI) with the accession number KR871398.

The Neighbor-joining tree of the K8 strain and representative species of the suborder *Streptomyces* sp. was constructed based on nearly complete 16S rRNA gene sequences. Numbers at the nodes indicate the levels of bootstrap support based on 1000 resampled data sets.

DISCUSSION

Marine organisms have produced enormous antibiotics of diverse chemical structures (Molinski, 2004). Among the marine organisms, marine Actinomycetes account for >45% of all bioactive metabolites discovered in nature (Berdy, 2005). The isolated Actinomycetes were identified based on the colony morphology and Gram staining (Holt *et al*., 1994). Based on the colony morphology and Gram staining results, we have identified the Actinomycete by the presence of powdered colonies on the surface of agar plate. The isolated Actinomycete showed colony formation, vegetative and aerial mycelium, structure of sporophores and spores as reported earlier by Waksman (1957). In the present study, the colour of aerial mycelium was considered for the grouping and identification of actinomycetes as agreed by Pridham and Tresner (1974). Spore morphology is considered as one of the important characteristic features in actinomycetes identification and it varies among the genus and species (Ramesh and Mathivanan, 2009). Rajan and Kannabiran (2013) described that the aerial mycelium have white colour, aerial spore mass colour was white grey and the colour of the colony was white hence reported as *Streptomyces* sp. These results were support our findings which conclude isolated K8 colony as *Streptomyces* sp. Poosarla *et al*. (2013) studied the biochemical characterization of S*treptomyces* sp. which support the present study.

Physiological properties are very significant for the identification of actinomycetes at genus level but not useful for the identification at species level. However, they can be used at least as markers by which an individual strain can be recognized. Different physiological characteristics are influencing the growth rate of the actinomycetes (Chakraborthy *et al.,* 1995; Shimizu *et al.,* 2000). The present results were similar to Moncheva *et al.* (2002), Sahin and Ugar (2003) and Oskay *et al.* (2004) who stated that the actinomycetes grew better at 10-50 ppt salinity. Poosarla *et al*. (2013) described that the optimal pH for actinomycetes was ranged between pH 5 and 9 and optimal temperature for actimycetes was 20-40^{o}C. The pH between 6.5 and 10 and temperature between 25^{o}C and 45^{o}C was also found optimum by Prasantha Kumari (2013). These results were similar to our present findings.

An approach has been developed to solve taxonomic problems associated with actinomycetes is the study of nucleic acids. The 16S rRNA sequence analysis has become an important tool in bacterial identification, since it provides information about the phylogenetic relationship of the species (Brenner *et al.,* 2001). In the present study variation in the 16S rRNA sequences for the selected actinomycetes was documented. The phylogenetic analysis of partial 16S rRNA sequences of selected actinomycetes showed 100% similarities to the existing their own related species. Dhanasekaran *et al.* (2005) have studied the isolation and identification of marine actinomycete *Streptomycetes* sp. The presently identified actinomycetes gene was 99% similar to the existing *Streptomycetes* sp. which is strongly agreed with the previous study. Vijayakumar (2006) has isolated and reported same *Streptomycetes* sp. Nandini *et al.* (2012) have studied the isolation and molecular characterization of secondary metabolite producing *Streptomyces* sp. Kothagorla and Tamanam (2013) have isolated and identified the biologically active strain of *Streptomyces* sp. from mangrove soil of Visakhapatnam region. These earlier findings are coinciding with our present study.

CONCLUSION

The present investigation revealed that the Kattumavadi coastal sediment samples are rich in actinomycetes diversity. Further the study concluded that the *Streptomyces* sp. isolated from Kattumavadi coastal region is considered to be a potential candidate for antibacterial compound production. Further investigations are needed in order to determine the active metabolites of this isolate.

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Table 1. Actinomycetes strains isolated from Kattumavadi region of Palk Bay, Southern India

S.No	Isolates	Aerial mycelium	Riverside pigment	Soluble pigment	Melanoid pigment	Nature of growth
1	K1	White	Pale yellow	-	+	+++
2	K2	Ash white	Pale yellow	-	+	++
3	K3	White	Pale yellow	-	+	++
4	K4	Ash white	Yellow	-	-	+++
5	K5	Brownish white	Brown	-	+	++
6	K6	Ash white	Yellow	-	-	++
7	K7	White	Golden yellow	-	-	+
8	K8	White	Golden yellow	-	-	+++
9	K9	Greenish yellow	Greenish yellow	-	-	+
10	K10	White	Yellow	-	+	++

Table 2. Primary screening of selected Actinomycetes strains against 21pathogens

Strains	List of pathogens																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
K1	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-
K2	+	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-
K3	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
K4	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-		-	-	+	+	-
K5	+	+	+	+	+	+	-	-	+	-	+		-	-	-	+	+	-	-	+	+
K6	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
K7	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
K8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
K9	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
K10	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

Table 3. Microscopic, biochemical and physiological characterization of selected Actinomycete (K8) strain

Reaction	Results	Reaction	Results	Reaction	Results
Microscopic characterization		Temperature 5	-	Nacl (%) 5%	+
Motility	No	10	-	10%	+
Gram staining	+	15	+	15%	+
Acid fast staining	-	20	+	20%	+
Biochemical test		25	+	25%	+
Indole production	-	30	+	30%	-
Methyl Red Test	+	35	+	35%	-
Voges Proskauer	-	40	+	40%	-
Citrate utilization	+	45	-	Carbon sources Starch	+
H 2 S production	-	50	-	Dextrose	+
Nitrate reduction	+	pH		Fructose	+
Urease Test	+	5	+	Maltose	+
Catalase Test	+	6	+	Mannitol	+
Oxidase Test	+	7	+	Nitrogen sources L-Cysteine	+
Melanin production	-	8	+	L-Valine	-
Starch hydrolysis	+	9	+	L-Phenylalanine	+
Haemolysis	+	10	+	L-Histidine	+
Triple sugar iron	+	11	-	KNO_3	+

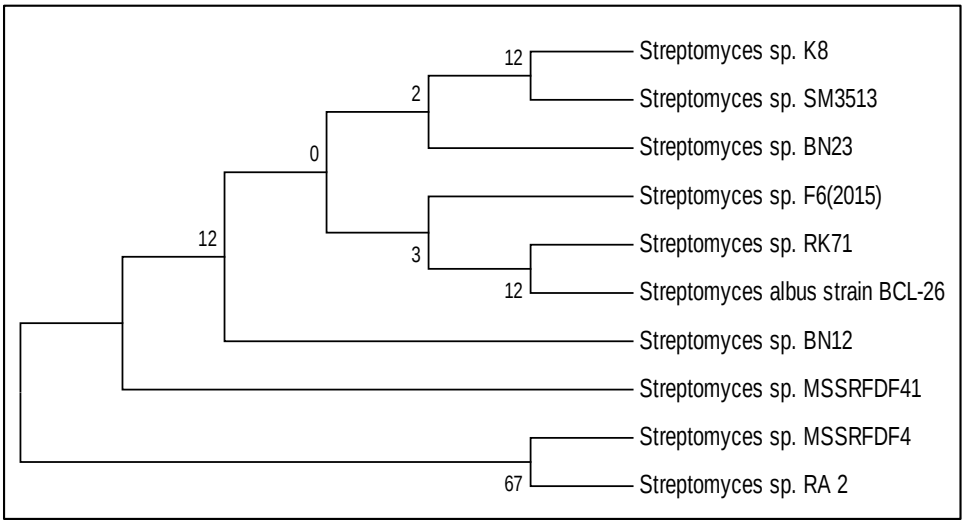

Fig.1.Phylogenetic analysis of Marine Actinomycete *Streptomyces* sp. (K 8)

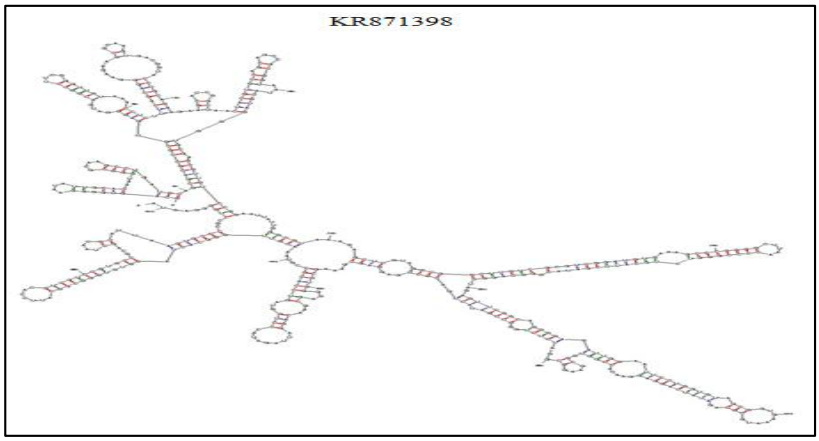

Fig. 2.Secondary structure predicted by 16s rDNA of Marine Actinomycete *Streptomyces* sp. (K 8)

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RESEARCH ARTICLE

Development of a Measurement's Tool to Evaluate the Support Staff Perspectives about University's Organizational ClimateDimensions

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ABSTRACT

The point of views of most support staff members as technical, managerial, labors, services and mobility workers were rarely considered in research. They support all types of task force tasks and provide entertainment of elites. This study focused on the support staff perspectives about university's organizational climate dimensions, by developing modern measurement scale based on systematic review of previous studies and instruments of data collection. The study developed a strong evaluation tool and modified the most effective practices on employees' performance. Findings showed that this tool helped to identify workers' demands to enhance their abilities and excel experiences by remarking direct and effective requirements to develop the working environment and support homogeneity climate between employees.

Keywords : Measurement, Tool, Evaluate, Support Staff, University, Organizational Climate, Kingdom of Saudi Arabia.

INTRODUCTION

The procedures of evaluating the quality of services that offer successful and elaborate products are different 1. Institutions are seeking to obtain certain international accreditation in the field of service quality; for example:

universities that are seeking for the academic accreditation have to pass the stages of the objective assessment to find out how to apply it based on a number of assessment's standards related to this accreditation **2.** Meanwhile, tests – as one of the evaluation tools- are considered certified tools to the standard of evaluating the products while measuring the standard of transmitted knowledge to students in parallel with the level of jobs held by the university for the purpose of promotion, which measure the accumulated experience of its employees. At the same time, the accreditation of a particular standard is not necessarily suited to all seeking institutions to accreditation, assessment, or measurement 3. It is not required if this accreditation is successful or not, but rather its ability to enhance the ability of institutions to achieve accreditation ultimately 1. Thus, it is required to have a flexible and graceful schedule to the tasks related to accreditation during the institutional organization stage4. In that sense, universities have to improve the skills of human resources, and achieve job satisfaction, whether they are administrative staff or 'support staff'5**.**

While reviewing many of the previous studies, it showed that the issue of support staff are not addressed in a serious and deep way, but most of it recommended the need to o shed light on the this type of staff due to its importance in supporting the process of implementing the rest of staffs' jobs at universities, as well as improving the overall process of implementing the University's procedures. In addition, these jobs contribute to the support of all the related works to teaching, scientific research and community service processes and strengthening its development process, which improves the mental image of the university and eases pressure on universities to promote their role in society 6, where this category has become one of the marginalized jobs and its entitlements are also unrecognized 7. In spite of its important role in the accreditation process in various institutions, but the reason for its neglect lies in the university's acquiescence to the requirements of the academic category and researchers without regard to the needs of these jobs 8. A number of universities have sought to deal with them under other terms such as; professional employees, non-academic-specialists, administrative staff or technicians 9, 10, 11, 12, 13, 14, 15**.** According to 16 , the non-academic staff is considered one of the pivotal components of the modern higher education stages. In the same context, 17 deemed universities would be unable to work without the help of those employees who provide support to the rest through what they are doing to manage the various daily tasks. Due to the importance of the support staff in the universities, it has become evident the need to focus on them and make their voices heard to decision-makers and not to neglect or marginalize them through identifying their needs after taking their views and perceptions on the organizational climate at the university and its organization, as well as their assessment of the components of this environment. It is expected by revieUwing their views to identify the strengths and weaknesses enhancing their self-confidence and self-esteem, which contributes to raising the functional competence to support other functions/jobs and to achieve the strategic objectives of the organization, where the organizational climate is a multidimensional concept 18, 16, and 19.

5, 20, 21 and 22 showed the role of researchers in evaluating the organizational climate of the university through the development of its concept based on the views of academics and specialists by considering a set of concepts that measure a number of themes such as; performance and expertise within specific criteria. Here comes the need to develop a specific measure for the organizational climate at the university as a basis for an organizational assessment21. Multiple studies have indicated that the organizational climate is considered a framework for discussion on how to go toward the development, enhancement and preservation of opportunities that promote the growth of the organization in general. It should be noted here that the concept of 'point of view' or 'perception' is a way to limit ideas and understand needs through a person's perception of things related to his job in order to give the classification that characterize the functional environment23**.** Solicit opinions process, views and its discussion by the staff form a pivotal tool in determining the level of staff's commitment to work missions related to the dimensions of the organizational climate and the ways of its implement as valued used in measuring the extent of belonging and loyalty to the career. In certain cases, these values are lacked by employees who acquiesce to leading circumstances that marginalize and deprive them of their rights, as well as promoting their careers status, especially the university, which considers them a marginalized part because of the priority of the academic jobs of teaching and scientific research, which calls for the development of an assessment of the views of the support staff and support tool in an

effort to promote the march of the university in the deployment of its vision and to achieve its objectives. From this point, the study sought to conduct an exploratory survey of the views of support staff, through the development of the measurement model of their views named: **Support Staff Perspective Scale) (SSPS)**. Results were analyzed using the **Confirmatory Factor Analysis (CFA)** to assess the validity of the dimensions of the underlying elements.

The Organizational Climate

The Definition of the university's organizational climate

The university's climate such as the performance, behaviors, and values of individuals and groups at the University is considered one of the pivotal dimensions in the process of organizing the academic work, which sets the level of the full capacity of the individual and group 22, which can be determined by their perceptions about the job situation from the standpoint of the university's organizational climate. For the purposes of the present study, experiences and perspectives of individuals and groups in other universities were analyzed through a number of studies, where 3 sees that in order to make the university's environment conducive to knowledge, so university has to provide an academic atmosphere that prepares opportunities for college students to work together for optimal utilization of education and the acquisition of knowledge, where they have the freedom of expression, civilian and gallantry handle, self-esteem, equal opportunities, and the preservation of the member and the group's interest. The "American Association of Colleges" and the universities of (AAC & U) in 1995 suggested that universities have to work on the task of the configuration of a comprehensive and knowledgeable frameworks and settings to all the contributors to the university organizational climate and to respect their views 24. To have a university that serves the knowledge-based society, it is important to create a university's organizational climate that is based on the values of fairness and justice. Meanwhile, the concepts of the environment, culture, and climate are interactively employed among many researchers 25 and 21, it is possible to differentiate between them and find out their differences. Therefore, we must acknowledge that in spite of the terms and concepts thread and its role in the comprehensiveness of thinking about the dimensions of the organization, however, it remains different and varied in use and application. It seems that there is a weakness in achieving a global agreement on developing a certain definition and perception for the organizational climate 25 and 21**.** In this study, the organizational climate is seen as an assessment of the individual's perceptions about a particular functional system 21. The climate is evaluated through the perceptions and beliefs of the respondents 16 and expectations 22. Researchers here have acknowledged that the decision-makers are asking for information on staff engagement in those organizational climates, and it is an interaction and sharing process at the individual level, practices, and orientated-culture for the strategies of decision-making process 26. In conclusion, we have to shed light on the way to change the organizational climate of the university **18**.

Evaluating the university's organizational climate

Measuring the organizational climate at the present time has become the focus of a growing interest among the various universities 22**.** The use of multiple assessments of the functional climate at the university has shifted from reaction in nature to become a proactive organizational practice that is designed to estimate and deal with important issues in the university21. Moreover, studies have linked the results of the organizational climate of the university evaluation to the important educational outcomes only 18. Then the optimal use of these measurements came through the measures undertaken by policymakers to enhance their awareness of areas of work and to build on the achievements in pursuit of perfection and excellence 21. Scholars have created a broad basis in their theoretical review for the organizational climate during the sixties and seventies due to the widening spread of this concept 26. In spite that their theoretical framework offered a large knowledge base for the study of organizational climate, but it was not the focus of attention in the broader organizational climate of the universities as is the case in the organizational behavior of the institutions26. Meanwhile, steps have been taken in recent years to evaluate the organizational climate, but it is confined within the integrated development of the responsibility of the institutions,

which requires taking into account the plan, evaluation and focus on the factors and various specifications that affect the evaluation process 5, 27 and 21. To prepare a proper analysis of data in respect of professionals in any university, they have to be prepared to understand many complex functional issues 27. In addition, 26 asserted that the university's climate is more comprehensive and it is difficult to understand it as it is challenging to researchers in establishing an accurate perception and to measure tightly its dimensions. On the other hand, 3 believes that the mere difficulty of determining factors mean that we have to turn a blind eye to this important dimension. Academics and professionals assess university's climate to gain a deep and acceptable understanding in the light of their perception of various dimensions. 20 , 21 and 22 see that to enhance the process of change in universities 8. Moreover, the evaluation process provides multiple ways to identify the differences between the organizational components at the university 3. Immediately after identifying these differences, decisions required can be taken then for the development of innovative strategies and to improve learning environments that support the objectives of the university. Before going further in this regard, it is necessary to know the dimensions of the organizational level of the university to determine the orientations of stakeholders and decision-makers about their vision that they want the university. 28 and 18 has shown that the university is made up of a collection of multi-faceted social systems formed by interactions between individuals and their dealings, structural planning, organizational objectives and principles, cultural background, as well as huge social and historical considerations. The academic climate effects on the success of the students and their academic achievement, as well as staff and support staff and their professional success, in addition to their social welfare. Previous researches show that different groups depict the academic climate in a different way and that their perceptions may affect the results of work and knowledge negatively 19. So far, we have not found any evaluation to the perception of the organizational climate of the university by support staff, and there were not available means to verify the accuracy of the validity of the last measurements orientated for academics mostly that can be used in creating a measuring model adapted to modern technology used to develop a measuring tool such as the one developed by 29 and 30.

METHODOLOGY

First Step 1: The Qualitative Research: Focus groups

1. The first phase of developing a measuring tool for the organizational climate of the university (The Northern Border) was represented in the process of collecting data of the selected sample, which is made up of working employees groups in support jobs, professionals and technicians. In addition, they were divided into three main groups of qualified and trained staff in their work field.
2. The second phase of the process of asking questions for discussion on these groups to see different perspectives on the university's organizational climate to get to the best proposals included acceptance and reluctance on the questions asked and was classified according to different axes and dimensions including the lack of clarity of meaning, challenges and concerns about university's climate and the related proposals to enhance it. This method has been implemented without repeating the answers by selecting (12) employees in the three groups of the sample selected.
3. The third phase selected Notes were taken and discussions were recorded to achieve a higher degree of constancy and consistency in research themes through the questions asked, where this way contributed to the review of the responses for verification to ensure not to lose any important information and to explore the collected data to determine the mechanism of the qualitative analysis of the data, the result has concluded into determining (64) influential elements in the areas that represent the organizational dimensions of the university's climate.

Second Step 2: The Quantitative Research

The Tools of Survey and Inquiry

The results of the answers to the questions posed for discussion have concluded into designing a study's tool, which was offered to (9) of specialized academics to examine this tool with reviewing the orientated draft of the study to the employees in support jobs, this method was used as a mechanism to validate questions and its proper usability of measuring while asking it, and this has based on the concept of measuring of the organizational climate as defined by 31.

Data Collection

The researchers collected data from a sample selected through personal interview and conducting questionnaire for personal answers and the students from the Faculty of Business Administration participated in it and they have been trained for this purpose through many interviews, which are characterized by objectivity and impartiality, where the questionnaire consisted of information explaining the goal of this survey and the methods of the interview without specifying the respondent's name, where the percentages of the responses have reached to 80%; 70% of it is a complete survey, in addition to (140) individual non-duplicate interview that have been collected from those who are working in support jobs for the three groups.

Data Analysis Method

Controlling and changing samples' method was used, and sample's size was relatively small for interviews and it has reached to (140) interviews. This method facilitates the mechanism to deal with biometric small-sized samples 32. This method fits the heterogeneous and small samples to avoid the lack of impartiality, or the appearance of different swing values with high relative differences between the relatively small samples, as well as it gives stability and strength when it is used on a variety and different or unfamiliar data largely, it also facilitates the standard errors exceeding process due to the lack of the collected data that causes contraction of the robustness of the method 32.

RESULTS

The Exploratory Factor Analysis for the scale

An **Exploratory Factor Analysis (EFA)** has been conducted by using **SPSS** to determine the dimensions of support jobs' employees' perception for the concept of the university's climate 30 by examining the communication models between the (64) elements that adopted questions. Meanwhile, a series of tests were conducted to confine the specific criteria for its components according to 28. The elements with the values that are less than (0.5) were excluded and the model was re-formulated within (6) dimensions contain (28) items as a whole. Moreover, all the appendices of the items were recorded at the Stability Level that is higher than (0.7) that gives it a credibility of the response according to 33, which leads to acceptable internal consistency level. The six derivative dimensions according to the results are: University's policies and legislations, as well as services, Wages and incentives, the administrative supervision, Relations in the work environment, Opportunities for improvement and development, the level of satisfaction and commitment

The Confirmatory Factor Analysis (CFA)

To examine the measurement's tool that has been developed to assess the dimensions of the organizational climate of the University, the **Confirmatory Factor Analysis (CFA)** was conducted on the (28) elements in the newly created

model, which was developed based on the scale of 29. *Figure (1)* shows the measurement model for the level of communication between the dimensions of the (6) elements and the extent of combination within domains, in addition its results were proportional to the values, where it has reached to (Chi-square (64) = 240.987, p = 0.000; GFI = 0.961; CFI = 0.969; RMR = 0.059; RMSEA = .06), and after modification the actual measurement model became relatively satisfactory.

Deviation and Reliability

Based on the above analysis of the results, all indicators lead to a dimension of one measurement in which each element in it corresponds to one construct unit only29. **Table No. 1** shows that the reliability coefficient of ***Cronbach's alpha's*** value ranged between (0.70 - 0.90), and thus the validity of the tool has been verified, where all the values of that the reliability coefficient of ***Cronbach's alpha*** have exceeded (0.7). For the compound reliability, see **Table (2)** that indicates its acceptable and satisfactory values and lead to the adoption of the results of the study's answers in the analysis process according to 33.

The validity of rapprochement and excellence

To verify the validity of rapprochement between dimensions, the correlation of indicators has been verified effectively by using the method of (PLS) for the controlling and changing sample, the results showed that each element of the assessment scale of (SSPS) that corresponds to a related dimension as it is shown in Table (2) that the value of the **Confirmatory Factor Analysis (CFA)** for all indicators exceeded the value of (0.85) and all of it are positive values associated with the value of (t-value) which ranged between (31.789) to (269.998). Based on these results, the validity of the rapprochement between all the indicators has been achieved, and moreover the validity of excellence through the **Confirmatory Factor Analysis (CFA)** has been assessed, and which proved the correlation between the six dimensions of measure and its integration into one static measurement dimension. The researchers also verify that the uncorrelated elements are not actually correlated according to the methodology of 29 where the primary model showed that all items are basically freed. After that, the modifications made to the model showed that all indications lead to one an independent unit, and then the models were analyzed. Through conducting the test of **Chi-square** to identify the differences the primary freed model and the modified developed model by researchers, it showed that each indicator in the modified model gave a high quality value that is less than the correspondent one in the original model largely and by about (5%). Based on this analysis, results showed the presence of the validity of the value of excellence in the modified model of measurement because the probability values in the integration of all procedures have achieved satisfactory values that correspond to the same elements that are less than (0.05%).

The Confirmatory Factors Analysis (CFA) – Class II

Until the verification of the validity of performance and the quality of its design to serve the purpose of this study, the **Confirmatory Factor Analysis (CFA)** – Class II was conducted 34. *Figure (2)* was designed on the basis of the results that emerged from the analysis process, where the views of workers in support jobs have recorded positive and correlated values in the second phase of the **Confirmatory Factor Analysis (CFA)**, which enhanced the correlation between the related six dimensions in the organizational climate of the university from their point of view.

The Validity of Nomological

To examine the validity of Nomological to verify the diversity of concepts, the consequences of the analysis, and the variability of evaluation through the usability of the difference in the level of operations related to the operational mechanisms of the analysis and its stability or volatility according to the different situation of analysis **35**. The level of recognizing the support staff in support jobs for the six dimensions was conducted through the network of

Nomological including the adverse productive behaviors, where previous studies concluded that the existence of a positive relationship to make staff recognize the organizational climate is related to the positive behaviors such as: the innovative behavior or the organizational citizenship behavior 27 and 36, where the majority of staff tends to accept and be convinced by the climate of their organization, where they see that the organizational climate significantly affects their career behaviors 37. Completely unlike, the negative impact of the uncorrelated work environments on career behaviors and often caused counterproductive in the organization's performance 36. The functional erroneous actions of the organization's climate are considered the cause of the destruction of the organizational objectives and pose a danger to it and adversely affect its work38. Based on the above assumption, the researchers presumably have put that: The realization that the employees in support jobs of the organizational climate of the university adversely effect on the opposite behaviors in the uncorrelated work environment as shown in the Figure 3. To test this hypothesis, the scale of 39 is used to measure the reverse results of career behaviors as in Figure (3), and with four values ranging from "I Strongly Disagree" and "I Strongly Agree", the items included: "'Neglect to follow the instructions of your line manager", "The dispersion of your work environment", "Come to work late without prior authorization", and "Offering little effort to perform your job", where the credibility value of the reliability coefficient (Cronbach's alpha= 0.81). It has been shown that the link between recognizing support staff according to Figure (3) toward the university and the reverse behaviors of large negative values (value = -2.648, and the result of t-value = -207.954). It should be noted here that the results of recent analysis verify the validity of the test Nomological about the perspective of workers in support jobs towards the dimensions of the organizational climate of the University.

DISCUSSION

It has been found through the study that the developed multi-dimensional model about the perception of those who are working in support jobs towards the organizational climate in the university (SSPS)- which was the reason for the breadth of the field of literary discussion in various studies and researches, but it has been limited in its conceptual framework - provides a new form of the organizational climate through its modern definition, the methods of measurement, developing a various conceptual framework, and a mean of measurable assessment, where the related dimensions axes to the common services have recorded the highest value in the scaling factors. This finding is consistent with the potential of this important factor on the practical nature of the staff as a variable, which plays a role in promoting the process of change, performance and functional behavior, and it is also considered a distinct factor from the rest factors due to its high level of compatibility with the statistical analysis of the values of the responses to adverse behaviors. The developed mean of measurement (SSPS) with its six dimensions and (28) elements is characterized with its success as a proposed model in the self-assessment by those who are working in support jobs toward the organizational climate in the University since it succeeded in analyzing the reliability's values and alpha's coefficient, the level of correlation between elements, the values of dimensions in the primary model (the prototype) and its counterparts in the modified one, and the high value of the effect in all variables in spite of the small sample (140) respondents at one of the Saudi universities that applies one career policy and legislation, in addition to the success of tests to verify the results, whether in surveys or exploratory stages, 15, as well as in the stages of the **Confirmatory Factors Analysis (CFA)**. Thus, the test of values relating to the factor of common services has led to considering it as a measurement tool to the process of support the realization of staff towards the organizational climate of the university that is useful for decision-makers who are trying to create a functional environment and who are seeking for creating a functional environment that is also satisfactory and appropriate to the academic work environment.

CONCLUSION

The results of the study showed the possibility of extending the framework of procedures related to organizational climate at the university, enhance the working conditions measures, constructing the knowledge, the accumulation of

experiences, and developing jobs through the involvement of employees in support jobs in the process of developing the university's vision and evaluating the levels of organization in it through their perception survey on the issues affecting it and related to the level of commitment and satisfaction in job environments so as to provide effective possibilities for the completion of the organizational statistics and the university's work 27. University's efficiency in directing and developing policies and strategies, building fruitful relationships and partnerships between the various sectors of the organizational climate of the university 3, in addition to get acquainted with the attitudes, behaviors and orientations of the members of the university's community19, and recognizing the areas of convergence and contradiction between groups 40. On the other hand, 41 has presented a literary elaboration showing the impact of the exploitation of the institutions to the results of investigations on the organizational climate optimally to enable them to look to the future by enhancing functional practices, where he showed that the professionals at the university have to offer the available improvement opportunities for the decision-makers in the university who have to focus on the needs of employees in these opportunities and improve their work environment and maintain the hierarchy of the priority needs of the workers at the university. Moreover, it doesn't show only that results contribute only to the identification of needs, but also it is a way to understand how the university can make progress in the process of achieving its goals. 27 indicated the importance of the university in improving the various functions that reflect positively on academic careers, courses and methods of education and scientific researches. The study concludes into the need to adopt dimension related to the provision of services as part of the measurement and improvement not just as a "symbolic" tool, as well as the need to repeat the evaluation process using (SSPS) and its development during the time march of the university and enhancing it with the emerging proposals depending on the variety of support jobs and breadth of staff's sample, in addition to widening the fieldwork with enlarging the university's size through buildings and students. This conclusion supports many studies and researches as the importance of attitudes, perceptions, behaviors and beliefs are obvious in resolving issues related to the organizational climate process 40, 25 and 15, in addition to using these information to make changes 27. As discussed earlier, the climate assessment of individuals' perceptions, as well as the familiarity within a particular environment 25, 26, 18 and 19**.** The development of a method for measuring and evaluating these indicators helps university's administrators, academics and researchers to envision a better organizational climate in the university20, in addition to providing a deep understanding of the convergences and divergences between data in different universities and to make proposals to improve the academic climate, as well as to evaluate the effectiveness of these proposals40. However, there are some restrictions affecting the process of Generalization of the results because of the freedom of the self-selection of the respondents to participate in the study, which led to the small size of the sample since it was neutral selection Uwith high values. Cases like this show such results when the psychological factor of the respondent affects his participation in the study.

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Table 1: Factor loadings for the underlying dimensions of Support Staff Perspective toward university

	F1 University's policies and legislations, and University services	F2 Wages and incentives	F3 Administrative support and supervision	F4 Relations in the work environment	F5 Opportunities for improvement and	F6 The level of satisfaction and commitment
Eigenvalue	4.383	2.209	2.7201	1.897	3.161	2.219
Cumulative % variance	321.789	40.289	56.019	61.479	71.056	79.103
Cronbach alpha	0.70	0.82	0.89	0.90	0.78	0.71

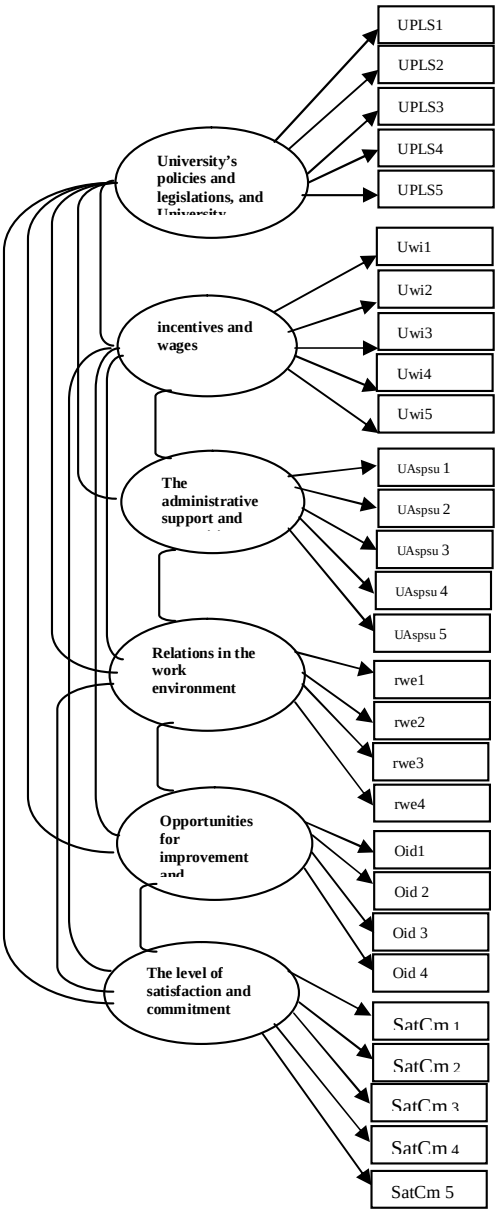

Figure 1: The measurement model

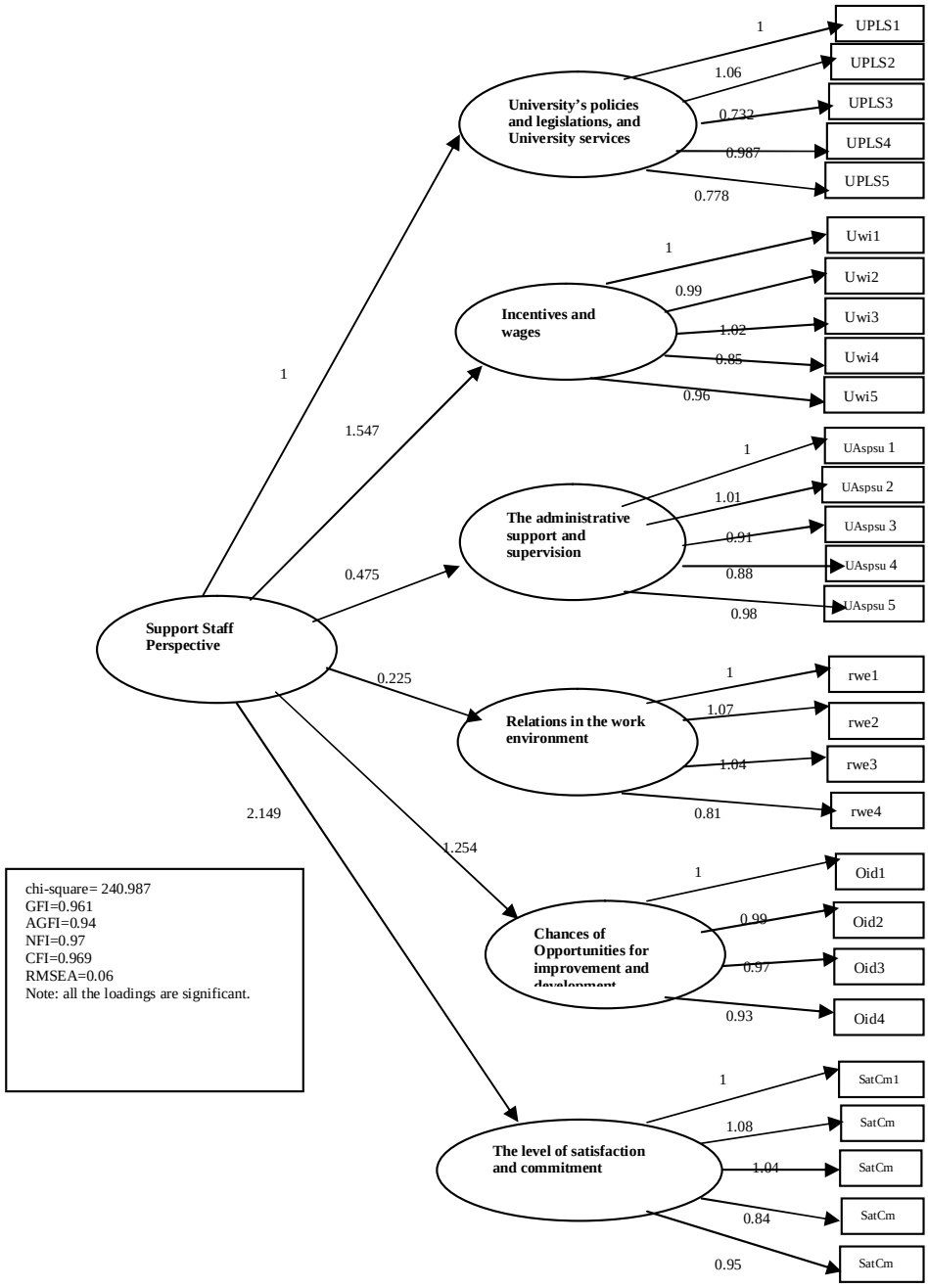

Figure 2: A second-order Confirmatory Factor Analysis

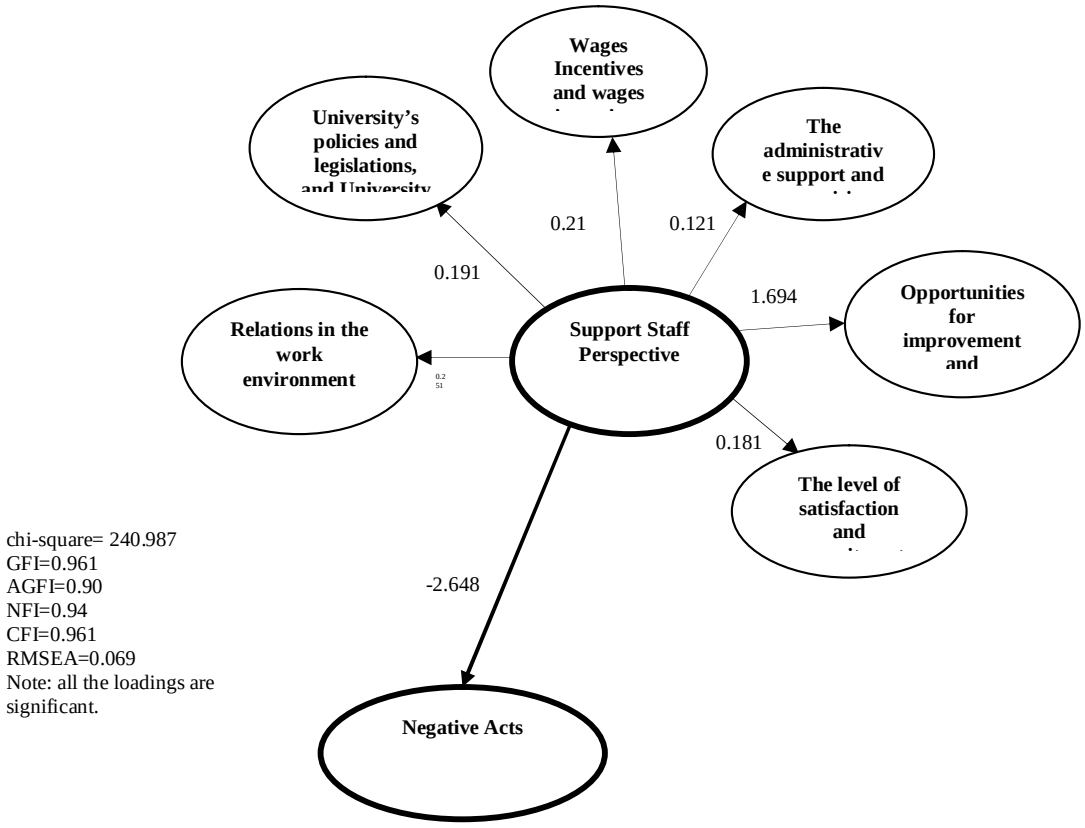

Figure 3: The effect of Support Staff Perspective on Negative Acts

Table 2: Properties of the confirmatory factor analysis for Support Staff Perspective

Items	Loading	t-statistics	Composite reliability
University's policies and legislations, and University services (*Fact1*)			0.809
UPLS1	1		
UPLS2	0.81	136.985	
UPLS3	0.91	35.988	
UPLS4	0.996	89.258	
UPLS5	0.951	91.0198	
Incentives and wages (*Fact2*)			0.879
Uwi1	1		
Uwi2	1.01	35.286	
Uwi3	0.989	34.002	
Uwi4	0.9101	33.955	
Uwi5	0.989	80.976	
The administrative support and supervision (*Fact3*)			0.79
UAspsu 1	1		
UAspsu2	0.872	29.830	
UAspsu3	0.909	30.958	
UAspsu4	0.838	12.237	
UAspsu5	0.885	10.919	
Relations in the work environment (*Fact4*)			0.749
RwE1	1	73.158	
RwE2	0.979	125.014	
RwpE3	0.799	74.314	
RwE4	0.88	95.255	
Opportunities for improvement and development (*Fact5*)			0.769
Oid 1	1		
Oid 2	0.89	86.358	
Oid 3	0.989	19.358	
Oid 4	0.823	92.354	
The level of satisfaction and commitment (*Fact6*)			0.819
SatCm1	1		
SatCm 2	0.786	97.2	
SatCm 3	0.78	88.89	
SatCm 4	0.879	96.36	
SatCm5	0.90	77.12	

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RESEARCH ARTICLE

A Study on Optimum Design Periods of Wastewater Treatment System

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ABSTRACT

An initial task for the designer of wastewater treatment works is the selection of a time period for which the works are intended to serve. Selection of this deign period establishes the boundary conditions i.e. population to be served, quantity of waste water to be treated, degree of treatment required, funds required to finance the project, and the revenues to be generated. This makes it to proceed in the usual manner with the design of particular unit treatment process. In this chapter an attempt has been made to present briefly research overview regarding the concept of stage design period as suggested by the different research workers over the past three decades. Some local wastewater treatment plants data were also collected and evaluated with a review to meet the objective of the study.

Keywords : Wastewater Treatment Plant, Cost Analysis, Optimum Periods, Water Economics

INTRODUCTION

With the present population of over 192,826,502 according to 2016 census from non-official sources but authorized, Pakistan ranks as the seventh most populous country in the world. Movement of population from rural to urban areas and corresponding rise in the living standards has boosted the process of industrialization however this change has occurred without proper planning and careful anticipation. Cities are becoming bigger and disposal (UN-Water 2014).

Shortage of technical skill and high capital investment has primarily handicapped the industries have been reluctant in constructing treatment plants due to high initial costs that could cut their profit and lack of government restrictions. Sanitation agencies in big cities have, by and large, relied on insufficient preliminary treatment or direct disposal due to high cost of treatment where as municipal agencies in small cities and towns have not bothered about wastewater treatment (Fionn 2016).

In addition, Pakistan today faces an inexorable momentum of population growth. The population growth rate estimated to be as 2.69%according to 1998 census is much higher as compared to the other major developing countries of the world. In order to ensure that in view of the high population growth rates, financial constrains, bank mark up and the high initial investment, the wastewater treatment facilities in stages and taking into account the assimilative capacity of the recipient water bodies (Mulkerrins et al. 2003).The economy of wastewater treatment depends upon the climatic situation of any country for example technical operation of waste water treatment system and cheaper treatment system of wastewater needs the optimum environmental condition such as temperature, humidity etc.

For the analysis of financing wastewater treatment works over time, we must consider the dynamic factors involved in planning for future growth. A solution for this problem will indicate the most advantageous staging for the construction capacity of wastewater treatment works over the time period consideration. Staging will dependent on such dynamic factors as population growth, quantity of wastewater to be treated, concentration of the waste, availability and cost of borrowing monies, and opportunities to make investments in such time increment (Pasztor et al. 2008).A model for this problem must necessarily contain statements, which reflect the requirement of the situation. These requirements or constraints should indicate for each period, the funds required, the level of treatment, the quantity of waste water to be treated, and founds available.An optimum solution to the problem will indicate the capacity or size of treatment plant to be constructed in each time period; the total funds available for the program in each time period; the funds borrowed in each period; the treatment paths to be considered for each increment of capacity; the per capita service charge for each period and a schedule for investment of funds in each period (Roeleveld & van 2002).

In the Present Study, It is investigated the Feasibility of Design in Wastewater Treatment System under Local Conditions.

METHODOLOGY

It describes the procedure adopted for the development of cost models and calculates the economy of scale factors to be used for calculations of design periods for wastewater treatment facilities.
To develop cost models, assumptions are mostly same as given by Manne Model described in literature survey (Sangsawat et al. 2010). Two different courses of action for the determination of design periods are considered. These options were as follows:

I. By use of cost data of existing wastewater treatment plants located in various cities of the country.
II. By sizing of proposed wastewater treatment systems.

Waste stabilization ponds and Aerated lagoon systems were considered for this study. Other treatment systems such as Trickling Filter, Activated Sludge Process etc were not considered due to non-availability of technical data in Pakistan presently.

(I) By use of cost data of existing wastewater treatment plants

Cost data of existing wastewater treatment plants from different cities of the country will be collected, and will be used to develop the Cost Model. The pertinent design criteria and implementation cost of these plants are described

in literature survey .In Pakistan there are few domestic sewage treatment plants in operation. For our study, the data of treatment plants having same treatment system were required.

(ii) Proposed Waste Stabilization Ponds

System comprising f Anaerobic Ponds followed by series of Facultative and Maturations Ponds of different capacities will be designed to meet the National Environmental Quality standards, (NEQS) with a view to generate costly data to be used to develop Cost Models. Sixteen proposed wastewater treatment plants based on waste stabilization pond technology are sized for different populations ranging from 25,000 to 1,000,000. Wastewater flows for these treatments plants were calculated by consideration 200 M^3 of water demand (Sin & Vanrolleghem 2006).

RESULTS AND DISCUSSIONS

The development of a mathematical model for optimal design periods for wastewater treatment system for a given population growth rate is the best option for the study the treatment methods using in wastewater treatment system as well as optimum methods. It is also useful for the development for the accurate pollutant discharge results according to the environmental standards of wastewater treatment system.Studying about the specific microorganism which is used in microbiological treatment of wastewater.

Design Period

Historically environmental technologists has been aware of the importance of judicious selection of the design period, but little or no assistance is offered to the designer in making a wise decision in this matter except in terms of generalizations. Fair and Geyer suggest that because of the nature of work involved, wastewater disposal systems at the time of their construction are made large enough to satisfy the need of the community for a reasonable number of years in future with out requiring important condition or changes.

These suggestions for evaluation of a design period revolve about the notion of economical functional utility. The designer is required to plane a system, which will meet the operational and functional requirements of the community, viz., design a plant , which will produce the require degree of treatment. Simultaneously he has the responsibility for producing this system at the least cost to the community. The technologist's implicit charge therefore is to produce a system, which will offer the desired function at a minimum Cost (Svensktvatten 2015). Lynn et al. were the pioneer to develop linear programming, and employed to search for that combination of unit processes that would remove a given amount of BOD most economically .he applied analysis techniques to stage development in waste water systems. He described, financing the development of a waste treatment system over time required, for each time increment, consideration of population growth, treatment requirement, availability and cost of borrowed funds, and other investment opportunities.Steel, indicates that economy in design is related to its length of life, first cost, ease and cost of increasing capacity and the possibility of obsolescence.

Economic and financial analysis of wastewater treatment system

It is generally agreed that economic and financial analysis are a fundamental part of science & technological analysis and are, in fact, so closely associated that from a practical point of view analysis with out this consideration is meaningless. However economic are not an integral part of analysis but if included at all, are separate a price tagging review of alternative engineering plans. If economics is to play the important role that it should cost and efficiency as well as technical consideration should be an inherent part of design. It has been with this approach in mind that a system analysis of the cost and efficacies of municipal sewage treatment is being attempted, by directly relating the cost and efficiency of individual units. It is theoretically possible to develop the most economical; design of a

treatment plant for given conditions of quantity, strength, land costs, power cost, labour cost and effluent requirement, etc. this does not infer plant design by computer; while from an economic point of view there is only one solution to a given problem. There are many practical considerations other than cost, which influence the final design decision. While these consideration many outweigh the purely economic factor, there is nevertheless an important need to be able to eliminate as many variables from the analysis as possible (WHO 2011).

Cost and Sewerage Plant Efficiency

A first attempt to directly relate costs and efficiencies in the applied environmental field was the study of economics of sewage treatment by Sehroepfer based on observations and statistics gathered on a nation-wide basis in U.S.A. he analyzed both construction and operation costs of treatment in terms of Suspended Solids and BOD reductions. The study carried out before the development of high rate tricking filters and the acceptance of nation-wide standard of operating efficiency, showed widespread variation in both cost and efficiencies. Its use as a design tool was implied but not seriously recommended.

In the early 1960 the Harvard Water Recourse Group investigated the problem of selecting the design capacity for a wastewater treatment plant. A preliminary mathematically model was developed which would select an optimum plant size given, a population growth rate, a benefit function describing the value of the availability of treated water, a linear cost capacity relationship for wastewater, an interest rate and an economic time horizon. In the above study, optimality was defined as the maximum of the net benefits, that is the benefits derived from availability of treated water as reflected by per capita consumption less the investment required for construction of the facility (WSP 2010). In short, this study also presents a detailed literature survey regarding modeling of design period by different Environmental Engineers: Fair and Geyer (1954), Steel (1960), Harvarad water resources groups (1963), Lynnet al (1964), and importance of economic and financial analysis of wastewater treatment systems has also been discussed (Romanski et al. 1997).

Research work on modeling of wastewater treatment facilities over time, has been discussed. The modeling of stage design period by different research workers: Walte.R.Lynne`s (1964), "Wastewater treatment facilities over time Model, Manne Model (1961) and Thomas (1969) Time Capacity Expansion Model has been reviewed (Romanski et al. 1997).

CONCLUSION

As well as, Pakistan is a developing country and has not municipal wastewater treatment system as needed (Only one percent facility available in Pakistan), so it requires skill to check the operation and establishment of municipal wastewater treatment systems.The decrease in initial sage will help the industries and municipalities to develop their wastewater treatment systems. The development in waste water treatment system in line with the available existing assimilative capacities also help in improving the overall environmental condition of the natural receiving water bodies.The study will be used to develop the optimum periods for wastewater treatment system. By using the concept of design of wastewater treatment system in stages, initial construction cost of the treatment system will be decreased. In addition to that municipal wastewater treatment plants should working for long time as 7 to 10 years if research work conduct on it.Determination of this initial design capacity calls for the exercise of skill in the interpretation of social economic trends, as well as the use of sound judgment in the analysis of past experience for the purpose of predicting future requirement. They also suggest that for the selection of a design period, consideration must be given to following factors:(a) ease of extension, (b) location, (c) useful life of components, obsolescence, wear and tear, (d) interest rate for borrowed funds, (e) inflationary trends during the life of bonds and, (f) performance of the works prior to maturity.

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RESEARCH ARTICLE

Influence of Humic Acid on Soil Properties, *Azotobacter* population and Carrot Growth under Malathion Pesticide Treatments

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ABSTRACT

Humic acid significantly enhances the overall soil fertility and lowering the adverse effects of contaminants toxicity, pH, salinity, etc. Based on these properties this study was conductedat Khartoum-Sudan during summer months of2014 and 2015to evaluate the influence of humic acid on soil properties, *Azotobacter* population and Carrot growth under Malathion pesticide treatments. The electrical conductivity (EC), pH, total N (TN), available P (AP) and total K (TK), the number of *Azotobacterspp* colonies, carrot height, fresh weight and the leaves number were analyzed. In this study, two doses of HA (10 and 20 kg of HA ha^{-1}) and Malathion (0.880 L ha^{-1} as recommended dose and 1.76 L ha^{-1} as high dose) were used. The results indicated that the soil EC and pH were decreasedwhile,the soil total N, available P and total K were increased ($p< 0.05$) with humic acid applications mainly 20 kg of HA ha^{-1} under Malathion recommended dose. Further, the *Azotobacterspp* population carrot height, fresh weight, and the leaves number recorded significance maximum values with humic acid applications under Malathion recommended dose. On the contrary, Malathion high dose had adverse effect on the soil, *Azotobacter* and Carrot growth. Moreover, Malathion high dose with humic acid applications significantly

enhanced the soil properties, *Azotobacter* and Carrot growth compared to Malathion high dose without an addition of humic acid.

Keywords : *Azotobacter*; Electrical conductivity; Humic acid; Nitrogen

INTRODUCTION

Recently pesticides are considered as the most occurring pollutants in the environment. A great concern was a raised about the impacts of pesticides on human health and the environment. While they can be very useful in protecting plants, most of the pesticides are hazardous by nature. In their report [1] revealed that pesticides could improve the productivity of crops through their role in controlling pests, but this goal requires a sound management and authorized pesticides application. In Khartoum state, Sudan, 52% of tested farmers use Malathion pesticide for their vegetables [2]. Furthermore, farmers often apply pesticides in high doses and very frequently this, in fact, due to the farmers do not understand the instructions on the chemical containers because they have written in languages different from their native language [3]. Several studies reported that using of higher doses of Malathion has a negative influence on crops growth and thus soil properties [4,5,6]. According to [7] excessive use of Malathion normally result in adverse effect on membrane stability index (MSI), chlorophyll stability index (CSI), and relative water Content (RWC). One of the possible solution to minimize the harmful effect of pesticides, using of humic substances (HS) due to their effect in increasing the speed of the photolysis process through a photosensitizing impact [8]. HS are natural complexing ligands widely spread in nature. And also a fragment of humus-soil organic matter rising from the microbiological, physical and chemical transformation (humification) of biomolecules, and HS can be separated into three constituents: fulvic acids (FAs), humic acids (HAs) and humin [9]. HS are associate to create a balance in the soil nutrients availability, good aeration and stability of the soil pH. Also, HS increase water retention, reducing salt concentrations [10,11,12]. Humic acids reduce the salts levels in the soil through chelating these salts and reduce absorption of some toxic elements [13,14,15,16,17]. Humic substances mediate degradation or inactivation of toxic substances. Moreover, Soil HS function to either inactivation or assist in the degradation of toxic materials for example nicotine, aflatoxins, antibiotics and most organic pesticides [18]. According to [19], humic acids stimulate the activity of microorganisms responsible for the breakdown of toxic elements and humic compounds may control the high solubility of Aluminum in acid soils, dissolved Al can reach a certain concentration level, which is toxic to plants and microorganisms. The aim of this study was to investigate the effect of humic acid on carrot growth and some soil properties under Malathion pesticide treatments.

MATERIALS AND METHODS

Experimental Site

A field experiment was conducted on Carrot at the Wadi Soba - Sharq Elneel -Khartoum (50 km east) –Sudan. The climatic conditions in this area are semi- arid with low rain fall and the average of temperature about 30^0C. Chemical and physical properties of the soil under investigation are shown in Table 1.The Carrot was grown during the crop season in the area spanning from April to July (2014 and 2015) in randomized block design.

Treatments

Hum acid (HA) was applied in powder form to the crop rows after seed emergence as control (H0: 0 kg of HA ha^{-1}) and with two different levels of HA (HA1 = 10 kg of HA ha^{-1} and HA2 = 20 kg of HA ha^{-1}). The Malathion doses were sprayed after 15 days from plant growth as; 0.880 L ha^{-1} (Recommended dose) and 1.76 L ha^{-1} (High dose) according to [20]. The treatments of HA with Malathion pesticide were as follow:

1.Control (0.0 Malathion and 0.0 Humic Acid (HA)). 2. Malathion Recommended dose 0.880 L ha^{-1} (R.D+ HA 0) 3. Malathion High dose 1.76 L ha^{-1} (H.D+ HA 0). 4. Malathion Recommended dose 0.880 L ha^{-1} with 10 kg of HA ha^{-1} (R.D+ HA 1). 5. Malathion Recommended dose 0.880 L/ha with 20 kg of HA ha^{-1} (R.D+ HA 2). 6. High dose of Malathion 1.76 L/ha with 10 kg of HA ha^{-1} (H.D+ HA 1). 7. High dose of Malathion 1.76 L ha^{-1} with 20 kg of HA ha^{-1} (H.D+ HA 2).

Soil and plant parameters

The soil samples were collected from the depth of (0–30 cm) all soil samples were mixed thoroughly and then air-dried. After that passed through sieve (2-mm). The soil Electrical Conductivity (EC) and pH were measured by the method that described by [21] and the pipette method, based on the work of [22] was used to measure particle size distribution texture. The Kjeldahl method was used to evaluate the total N level according to [23]. The sodium bicarbonate extraction method was used to measure the available P (AP) according to the Olsen Method [24], while the total K (TK) was determined with ammonium acetate solution, based on the method described by [21].The morphological characteristics (the number of leaves, plant height (cm) and fresh weight (g) at the stage of harvesting were measured according to [25].

Isolation of *Azotobacterspp*.

Using sterile tools seven soil samples from the depth of (0 - 15 cm) were collected (one soil sample from each treatment) after carrot harvesting according to [26]. According to the sodium benzoate medium method that described by [27], *Azotobacterspp*. were isolated, after suspension made (1 g of soil to 10 ml distilled water). Some identification tests including, citrate test, indole test, urease test, methyl red test, catalase test and *Azotobacter* cysts were carried out using a method that described by [26].

Statistical analysis

The data acquired were employed to Analysis of Variance (ANOVA) using MaxStat 3.06 statistical package. Means for significant treatments separated using Duncan's multiple range test ($p<0.05$), according to [28]. Further, an interactions of some soil properties with carrot growth and *Azotobacter* population were done using GraphPad Prism 6.

RESULTS AND DISCUSSION

Effect of humic applications on soil pH and EC under Malathion treatments

The average of soil pH was 8.1, 7.6 and 7.4 for control, R.D+HA0 and H.D+HA0 respectively. While 7.2, 6.5, 6.3 and 6.3 for R.D+ HA1, R.D+ HA2, H.D+ HA1 and H.D+ HA2 respectively, as seen in Table 2. The pH values were significantly decreased compared with the control. And the lower value recorded by R.D+ HA2 (6.5), H.D+ HA1 (6.3) and H.D+ HA2 (6.3), similar findings are observed by[20] they reported that Malathion treatments decreased soil from pH 6.8 to 6.4. Further, [10] indicated that humic acid could reduce soil pH due to their polar and hydrophobic feature. The EC was recorded an average of 1.9, 2.1, and 2.5 dS/cm for control, R.D+E0 and H.D+E0 respectively whereas 1.5, 1.3, 2.3 and 2.1 dS/cm for R.D+ HA1, R.D+ HA2, H.D+ HA1 and H.D+ HA2 respectively, as shown in Table 2. On analysis, the soil EC increased with Malathion treatments concurring with the results of [20], while humic acid minimized the effect of Malathion on soil EC and humic acid with recommended dose of Malathion registered a lower EC values. These findings agree with the explanation of [10], they reported that the structural feature of HS consists of both polar and hydrophobic environments in the same molecule. As a result, HS can fix both polar and

hydrophobic xenobiotic organic compounds, and mineral ions. Moreover, HS changed their toxicity and bioaccumulation.

Effect of humic applications on soil total N, available P and total K under Malathion treatments

The average of TN was 0.08, 0.06 and 0.03% for control, R.D+HA0, and H.D+HA0 respectively. While 0.07, 0.15, 0.05 and 0.05% for R.D+ HA1, R.D+ HA2, H.D+ HA1 and H.D+ HA2 respectively, as seen in Table 2. The remarkably higher values ($p<0.05$) recorded by R.D+ HA2 (0.15%), can be linked to soil organic matter, it acts as an important source for providing N for crop growth. [29] reported that organic amendments increase soil organic matter and thus enhance soil nutrients. And significantly lower values found in H.D+HA0 (0.03%) these percentages concurred closely with those of [20]. The AP showed an average of 5.3, 3.3 and 2.4 Mg kg^{-1} for control, R.D+HA0 and H.D+HA0 respectively and 3.7, 3.9, 2.8 and 3 Mg kg^{-1} for R.D+ HA1, R.D+ HA2, H.D+ HA1 and H.D+ HA2 respectively, as evident in Table 2. AP showed statistically decrease in all treatments when compared to control. These results agreed closely with those of [20]. The average of TK was 141.1, 139.1 and 133.6 Mg kg^{-1} for control, R.D+HA0 and H.D+HA0 respectively. While 137.5, 138.8, 138.2 and 135.8 Mg kg^{-1} for R.D+ HA1, R.D+ HA2, H.D+ HA1 and H.D+ HA2 respectively, as evident in Table 2. The remarkably higher values ($p<0.05$) found in control (141.1 Mg kg^{-1}) and significantly lower values acquired by H.D+HA0 (133.6 Mg kg^{-1}). Normally, it appears that addition of humic acid has a positive effect on maintenance of soil K, this results supported by reports of [10].

Effect of humic applications on *Azotobacter* spp under Malathion treatments

The average of the *Azotobacterspp* colonies remained almost equal in R.D+ HA1 ($70.6x10^4$) and R.D+ HA2 ($71.6x10^4$) as well as control ($73.6x10^4$), this results can be associated with beneficial effect of humic acid on soil microbes [30]. The R.D+HA0 ($69x10^4$), H.D+HA0 ($16x10^4$), H.D+ HA1 ($14.9x10^4$) and H.D+ HA2 ($14x10^4$), as seen in Fig. 1a, registered statistically lower values. Moreover, the results indicated that there was no significance difference between control ($51 x10^6$), R.D+HA0 ($51 x10^6$) and R.D+ HA2 ($51 x10^6$). Further, the lower values recorded by H.D+HA0 ($2x10^6$), R.D+ HA1 ($40 x10^6$), H.D+ HA1 ($4x10^6$) and H.D+ HA2 ($5x10^6$), as evident in Fig. 1b. Meanwhile, the average value of R.D+ HA1 ($26x10^8$) showed statistically higher values. A lower values recorded by control ($20x10^8$), R.D+HA0 ($11x10^8$), H.D+HA0 ($1x10^8$), R.D+ HA2($24x10^8$), H.D+ HA1 ($0.4x10^8$) and H.D+ HA2 ($0.7x10^8$), as seen in Fig. 1c. From results, It was observed that the high dose of Malathion had a harmful effect on the *Azotobacter* population, closely concurred with findings of [31,32]. Furthermore, the application of humic acid enhanced the population of *Azotobacter* and minimized the harmful impact of Malathion on *Azotobacter* numbers, this agreed with an explanation of [10]. Malathion has a direct effect on *Azotobacter* growth by causing toxicity to these bacteria and indirect impact through raising the soil EC. A negative correlation (r^2 = -0.56, -0.52 and -0.81 for 10^4, 10^6 and 10^8 respectively) was observed between the soil EC and *Azotobacter* spp population as seen in Fig. 2. These findings agree with the records of [33] who reported that correlation increasing soil salinity leads to decrease *Azotobacter* population.

Effect of humic applications on Carrot Growth under Malathion treatments

The average of the plant height recorded a remarkable increase in R.D+ HA2 (65.45 cm) compared with control (53.2 cm), R.D+HA0 (57.39 cm), H.D+HA0 (24.2 cm), R.D+ HA1 (63.55 cm), H.D+ HA1 (40 cm) and H.D+ HA2 (39.1 cm). While plant fresh weight registered statistically increase by R.D+ HA1 (53.05 g) and R.D+ HA2 (54.85 g) compared with control (41.7 g), R.D+HA0 (51.25 g), H.D+HA0 (15.85 g), H.D+ HA1 (19.85 g) and H.D+ HA2 (21 g). Moreover, a remarkable increase in plant leave number was noticed in R.D+ HA2 (28.5). And statistically lower values recorded with control (23.22), R.D+HA0 (25.18), H.D+HA0 (13.81), R.D+ HA1 (26.75), H.D+HA1 (19.48), H.D+ HA2 (19.65), as seen in Table 3. From analysis; the carrot height, fresh weight and leave number were decreased under the Malathion high dose, this concurred closely with findings of [4,5]. Furthermore, using of humic acid enhanced the carrot growth, and minimized harmful effect of Malathion high dose, this agreed with reports of [10,30]. A negative

correlation (r^2 = -0. 8, r^2 = -0.68 and r^2= -0.81) was observed between the soil EC and carrot height, fresh weight and leave number respectively, as seen in Fig. 3a, b and c. These results agree with the findings of [34] they reported that the carrot yield decreased with the increasing of salt concentrations particularly NaCl. Moreover, a positive correlation (r^2 = 0.57, r^2 = 0.5 and r^2 = 0.62) was observed between the soil N and carrot height, dry weight and leave number respectively, as evident in Fig. 4a, b and c. These results can be associated with the role of N as essential nutrients for plant growth. According to [35] they stated that carrot growth was increased with increasing N. Also Carrot height, fresh weight, and leave number indicated a positive correlation (r^2= 0.4, r^2= 0.36 and r^2 = 0.35, respectively) with available soil P, as seen in Fig. 4d, e and f. This relationship can be attributed to the ability of phosphorus to improve early plant growth. According to [36] they observed that a higher carrot growth when treated with organic P fertilizer.

CONCLUSION

From this study, it can be concluded that the soil EC and pH values reduced with applications of humic acid, On the contrary, the soil total N, available P and total K are increased ($p< 0.05$) with humic acid applications. The recommended dose (R.D) with humic acid (10 and 20 kg ha^{-1}) proved to be the best in increasing the total N, available P and total K under Malathion treatments. The use of excessive doses of Malathion pesticide has shown adverse effects on Carrot growth. Amending soil with humic acid enhances the carrot fresh weight, height and number of leaves through the reducing harmful effects of Malathion and increase the availability of soil nutrients. Among the tested treatments, the recommended dose (R.D) with humic acid (10 and 20 kg ha^{-1}) showed to be the best in enhancing the carrot growth under Malathion treatments. *Azotobacterspp* population is negatively affected by high dose of Malathion while the recommended dose has a slight effect on *Azotobacterspp*. On the contrary, the recommended dose (R.D) with humic acid (10 and 20 kg ha^{-1}) proved to be the best in increasing the number *Azotobacterspp* population in the soil.

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Table 1.Chemical and physical soil properties of investigated area

Soil depth Cm	pH	$CaCo_3$ %	Clay %	Silt %	Sand %	Texture	CEC Cmol/100g	ECe dS/m
0-30	8.5	9	35	20	45	Sandy clay loam	35	1.8

ECe: Electrical Conductivity of saturated soil extract.

Table 2. Effect of humic acid on selected soil properties under Malathion treatments

Treatments	ECe dS/m			pH			N %			P Mg kg-1			K Mg kg-1		
	2014	2015	Av.	2014	2015	Av.	2014	2015	Av.	2014	2015	Av.	2014	2015	Av.
Control	1.8^{b}	1.9^{b}	1.9^{a}	8.5^{a}	8.1^{a}	8.3^{a}	0.08^{b}	0.07^{b}	0.08^{b}	5.5^{a}	5.0^{a}	5.3^{a}	142.1^{a}	140.0^{a}	141.1^{a}
R.D+HA0	1.9^{b}	2.2^{b}	2.1^{a}	7.7^{b}	7.6^{b}	7.7^{b}	0.07^{b}	0.05^{bc}	0.06^{c}	3.5^{b}	3.2^{b}	3.3^{b}	139.9^{b}	138.2^{b}	139.1^{b}
H.D+HA0	2.2^{a}	2.8^{a}	2.5^{a}	7.6^{b}	7.4^{b}	7.5^{b}	0.02^{c}	0.03^{c}	0.03^{d}	2.7^{b}	2.1^{c}	2.4^{c}	133.9^{c}	133.2^{c}	133.6c
R.D+ HA1	1.41^{c}	1.49^{c}	1.5^{b}	7.2^{c}	7.2^{b}	7.2^{bc}	0.07^{b}	0.06^{b}	0.07^{bc}	3.8^{b}	3.5^{b}	3.7^{b}	138^{b}	136.9^{b}	137.5^{b}
R.D+ HA2	1.33^{d}	1.35^{c}	1.3^{c}	6.8^{d}	6.5^{c}	6.7^{c}	0.16^{a}	0.14^{a}	0.15^{a}	4^{b}	3.8^{b}	$3.^{9b}$	139.1^{b}	138.5^{b}	138.8^{b}
H.D+ HA1	2.1^{a}	2.4^{ab}	2.3^{a}	6.6^{d}	6.3^{c}	6.5^{c}	0.06^{b}	0.04^{bc}	0.05^{c}	3^{b}	2.5^{bc}	2.8^{bc}	138.3^{b}	138.0^{b}	138.2^{b}
H.D+ HA2	1.9^{b}	2.2^{b}	2.1^{a}	6.5^{d}	6.3^{c}	6.4^{c}	0.07^{b}	0.03^{c}	0.05^{c}	3.2^{b}	2.7^{bc}	3^{bc}	136.1^{bc}	135.4^{bc}	135.8^{bc}

Average (Av) values with different superscript letters in the same column differ significantly ($p<0.05$).

Table 3.Effect of humic acid on carrot height, dry weight and leave number under Malathion treatments

Treatments	Plant parameters								
	Plant height (cm)			Fresh weight (g)			Number of plant leaves		
	2014	2015	Av.	2014	2015	Av.	2014	2015	Av.
Control	53.0^{d}	53.3^{c}	53.2^{d}	42.1^{b}	41.3^{c}	41.7^{c}	23.33^{bc}	23.1^{b}	23.22^{c}
R.D+HA0	58.57^{c}	56.2^{b}	57.39^{c}	50.5^{b}	52^{b}	51.25^{b}	25.66^{b}	24.7^{b}	25.18^{b}
H.D+HA0	27.51^{f}	20.9^{c}	24.2^{f}	16.6^{e}	15.1^{e}	15.85^{e}	14.72^{d}	12.9^{d}	13.81^{e}
R.D+ HA1	63.1^{b}	64^{a}	63.55^{b}	52.2^{a}	53.9^{b}	53.05^{a}	26.3^{b}	27.2^{a}	26.75^{b}
R.D+ HA2	65.8^{a}	65.1^{a}	65.45^{a}	52.4^{a}	57.3^{a}	54.85^{a}	28.2^{a}	28.8^{a}	28.5^{a}
H.D+ HA1	41.12^{e}	38.8^{d}	40^{e}	20.9^{d}	18.8^{de}	19.85^{d}	19.6^{c}	19.1^{c}	19.48^{d}
H.D+ HA2	40.2^{e}	37.9^{d}	39.1^{e}	21.4^{c}	20.6^{d}	21^{d}	20^{c}	19.3^{c}	19.65^{d}

Average (Av) values with different superscript letters in the same column differ significantly ($p<0.05$).

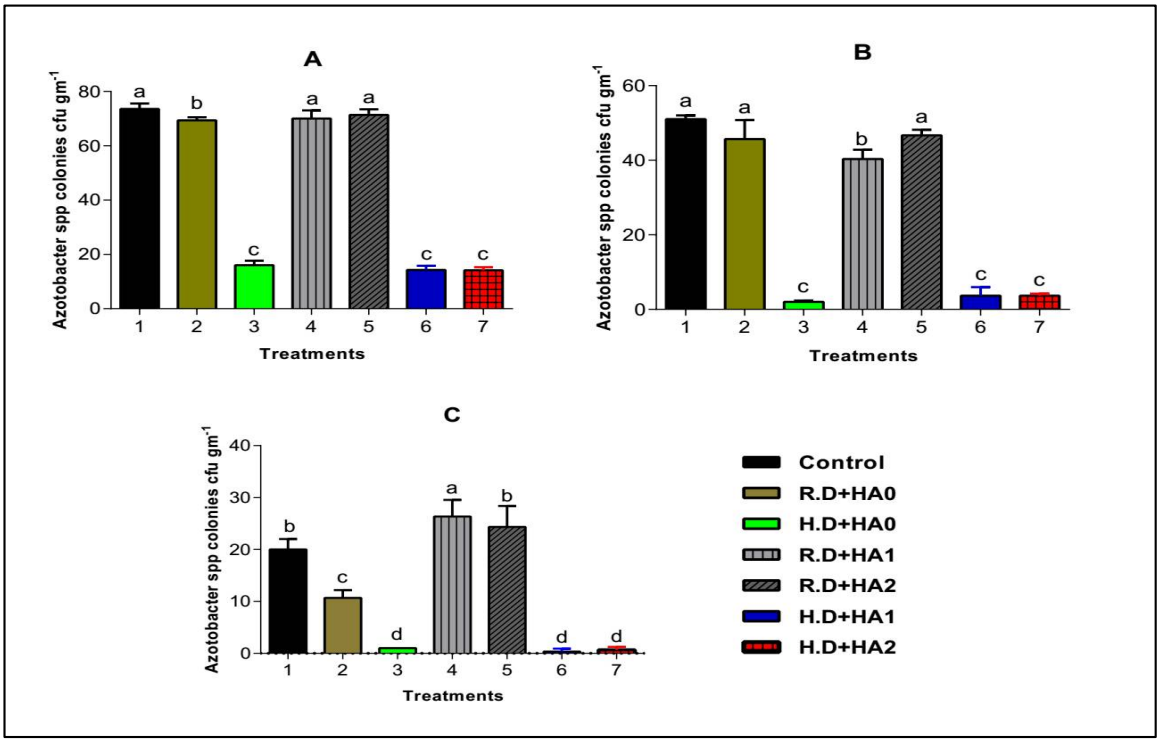

Fig. 2. Variations in Azotobacter population among different serial dilutions (a) 10^{4}, (b) 10^{6} and (c) 10^{8} under humic acid and Malathion treatments.

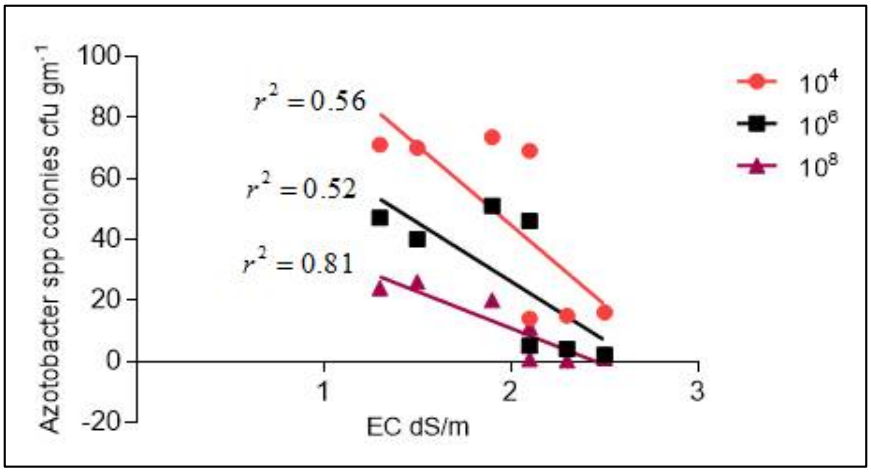

Fig. 3 Correlation coefficient of soil EC with Azotobacter population

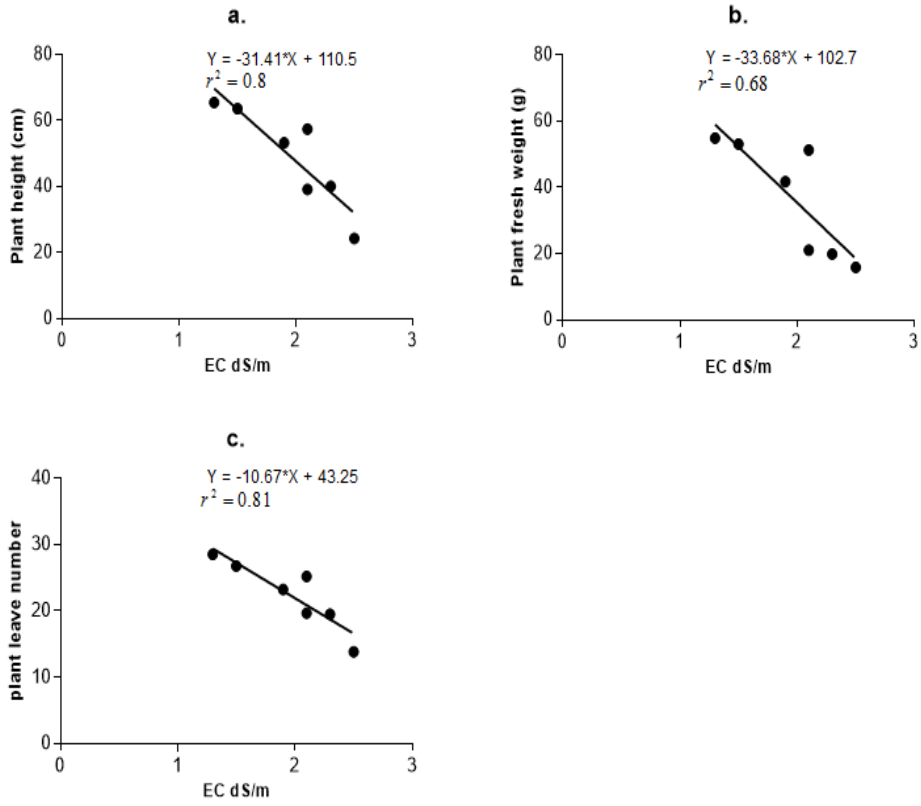

Fig. 3 Correlation coefficient of soil EC with (a) carrot height, (b) fresh weight and (c) leave number

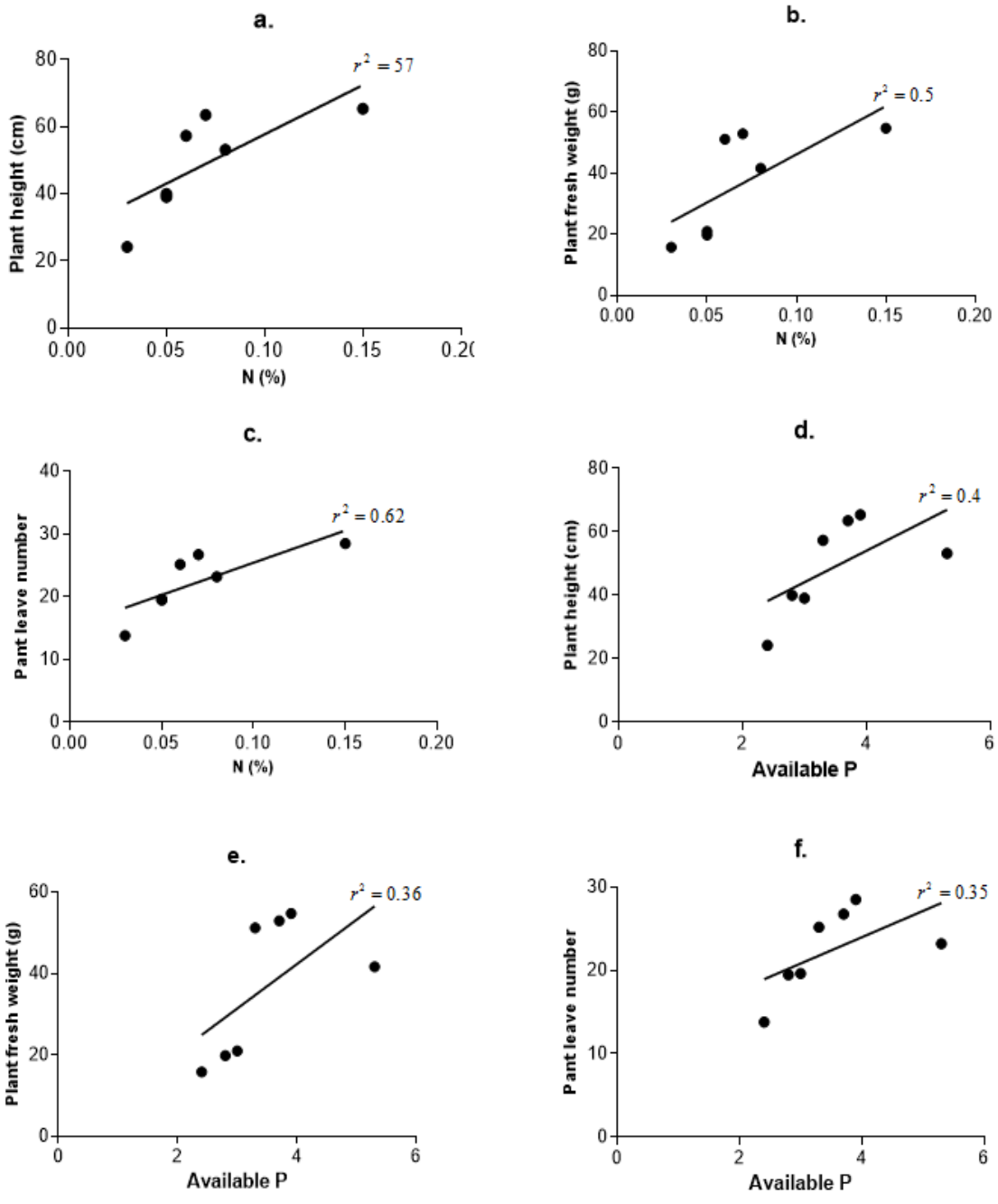

Fig. 4. Correlation coefficient of soil total N with (a) carrot height, (b) dry weight (c) leave number and soil available P with (d) carrot height, (e) fresh weight (f) leave number.

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RESEARCH ARTICLE

Present and Future Prospective of Drinking Water Management

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ABSTRACT

Water resources management is a big and hot issue of all over the world population but it depend on the need of high treatment and management cost. Cost effective drinking water treatment methods (coagulation, boiling and Chlorination) developed for humans and animals (cows and buffaloes) was given in very low cost as from Rs.0.15 to Rs. 0.75 as shown in previous studies. In this study for better health and safe drinking, it is an affordable drinking water treatment cost for common people in present and future. Contaminated canal water for drinking purpose needs dose 3mg/l of chlorine for complete disinfection without residues and 1.5 mg/l dose of chlorine is required for treating the drinking water of storage tanks without any toxicity causing by chlorine residues as mentioned in research papers.

Keywords : Water Resources, Treatment Cost, Toxins, Pollutants, Disinfections

INTRODUCTION

Almost the world's population now presently facing the deficiencies of potable water with better quality, with using of correct and applicable technology or water purifications above methods for domestic water is a wonder full solution of these problems with very low prices. Increased efforts to adopt the advanced technology and methods for making potable water as free from microbes and their released toxins chemicals for all domestic purposes including the storing in house or in farms for animals drinking and also can also be national and international level used the water treatment methods in this research as removing the organic material in forms of toxins with coagulation

process as Aluminum sulphate, ferric chloride and boiling (dosing and time details is mentioned in previous section), disinfection method involves chlorination with specific dose as described different in the section of management plane (Badar *et al.*, 2016).

Hygiene information is very important for better utilization of safe water drinking. Moreover, the procedure involved for drinking water management system and how to store at the domestic level, it is need to increase knowledge of individual and community about the awareness of water hygiene and public health. The awareness of this type is very useful to achieve and support to the final objective of research about covered and piped potable water for the World's population, then it will help to reduce water borne disease like diarrhoea and cholera in the our community (Batool *et al.*, 2016).

Coagulation experiments conducted to know actual effective and improved dose used to optimize for coagulation process for maximum removal of toxins in form of organic matter from drinking water. Selected Aluminum sulphate coagulation dosing (10mg-27mg) for treating the contaminated drinking water, this dose does not causes of toxicity to human and animal health as the Aluminum residues present in drinking water supplies (Zia *et al.*, 2016).
The objective of the present study is the assessment of safe drinking water quality in present situation and needs for future panning.

DATA ANALYSIS

Data analysis give the different field studies about management and treatment of drinking water and make it more economical and physible for populations. According to previous research much possibilities are available under different method of treatment needs suggest a strong analysis for present and future plan to adopt for minimize the drinking water risks. Previous researches on drinking water have clear vision on making the planning and policy for sustaining the water resources management (Khokhar *et al.*, 2016).

Secondary data as collect the random blood sampling from different places (animals use for meat and milk) was with the frequency of samples (116). All samples were collected by syringe in sterilized blood vessel used as container and blood sample 5 ml collected by volume and actual capacity of container was 5 ml. The temperature of the day when collect the samples was 16 ^{0}C. Serums of samples were collected after mechanical centrifugation of the samples blood, and start the analysis of clinical chemistries of blood samples. Similar way that drinking water samples are tested as same parameters as toxins and pollutants for similarity showing the pollutants in blood samples (Moghira *et al.*, 2016).

RESULTS AND DISCUSSION

Toxic effects are also appeared on animals and human population by taking the medical lab tests of blood samples for knowing the performance of liver and kidneys. From tests values, it is clearly observed that liver and kidneys have effected in same ways of both infected human and animals due to contaminated water and food taking (Badar *et al.*, 2016).In present study, it is investigate the toxins in drinking water samples from microbe's activities, very harmful health effect on humans and animals and especially their liver functions disturb badly, for this purpose draw the blood samples of both humans and animals for LFTs medical lab test. Liver abscesses in cattle and buffalos have a major economic impact on the beef and milk processing business for the reason that of liver problem can shrunk body size and animal performance (Batool *et al.*, 2016).

Inside the liver, enzymatic activity have been raised up, this is may be due to synthesis of enzymes, their low levels indicate that the enzymatic inhibition due to liver injury without specific regeneration. Among liver enzymes, amylase GOT, GPT and ALT were elevated in the samples of animals blood, it was showing acute liver damage

(hepatitis), while in samples of animal's blood, all these enzymes were inhibited showing hypocondition or dysenzymia (Moghira *et al.*, 2016).

Small and taste of Water is normalized by using adsorption process with granular activated carbon and achieved effective 98.6% removes the organic carbon in form of toxins. The results show that coagulation techniques is very useful and cheaper for removing organic matter as compare to other techniques like as filtrations or electrolysis. Overall quality of drinking water can be maintained by monitoring microbes and their toxins and possible their reductions by adopting the methods like boiling, coagulation and chlorination (Ahsan *et al.*, 2016).Presently, very less people have known almost existence of other bacteria like C. Botulinum, algae like cyanobacteria pathogens in water body and their metabolites mean toxins under different environmental conditions that are most important understandings for active control on water borne diseases. The microbial contamination sources from human and animals is better understandings to control water borne diseases which is still a great risk for public health (Badar *et al.*, 2016).

In addition to establish the link between waterborne pathogens and toxins that is an important task for chemists and microbiologist, deliver further advanced visualization about drinking water quality checks.

CONCLUSIONS

Further introduce new and cost effective chemical compounds are needed for water treatment and make it mineralized water quality. A company or organization should be lunched for public awareness on waterborne diseases effects on general public health and its treatments. Besides that studies should also be done from mathematical and Statistical modeling angles.

This study further highlights the actual causes of water pollution in rural areas of Sheikhupura district:

- Lack of complete data on water and environment.
- Absence of civic control measures.
- No National water Quality Standards.
- Unawareness to the general public regarding environmental impact.
- Role of Government monitoring agencies not at the required pace.
- Role of city policy makers/managements has not taken up this issue as future health hazards of the habitants of the Sheikhupura District.
- Analysis of water quality trend indicates escalation of the pollution over the years because of population growth and increased Agriculture use.
- The results of the study provide significant value bases for decision makers to carry out effective air pollution control and environmental management plans.

RECOMMENDATIONS

Keeping in view the quality of drinking water of rural area under study following recommendations has been made. The drinking water of rural area under study should be boiled before drinking it.

- This reported case of waterborne pathogens and their toxins in drinking water is alarming. Government should fulfill its basic complacence of providing safe drinking water and awareness to community.
- Contaminated canal water for drinking purpose needs dose 3mg/l of chlorine for complete disinfection without residues.
- 1.5 mg/l dose of chlorine is required for treating the drinking water of storage tanks without any toxicity causing by chlorine residues.
- Florid element should be added 5 ppm in drinking water quality standards by WHO and drinking water authority of countries because it is necessary for dental normal growth.

- The quality of drinking water related to pathogenic microbes and their toxins should be listed in the drinking water guideline established by WHO and Pakistan
- Toxins limits that should be included in WHO and Pakistan's standards of drinking water quality and it must be applied these standards all over world including Pakistan.
- The general cleanliness and hygiene of water at main storage reservoirs may be maintained at regular basis and must be established rules by the district and federal governments.
- Canal water should be treated and disinfected by adding the chlorine dose (3 mg/l) before using for drinking and domestic purposes for the general public.
- When decisions are made on water-use, the local municipality should involve suitable professional disciplines, especially environmentalists, doctors and town planners for the health protection of general public.

FUTURE WORK

The areas of environmental degradation in Sheikhupura district that have needed more on following next issues as include:

- Medical treatment of water born toxins diseases
- Effects on Soil & Agriculture Pollution due to contaminated water
- Cumulative water quality Pollution Model
- Impact, assessment, evaluation and modeling of Traffic Pollution
- Model for Water Quality
- Model for Sewage, Sludge Treatment.

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RESEARCH ARTICLE

Evaluation Teacher's Competence toward Knowledge- Based Society Transformation

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ABSTRACT

Countries seeking to diversify their economy by investing in knowledge, this study focused on teacher's competence towards Knowledge- Based Society Transformation (KBST), using technology to enhance traditional learning methods and teaching techniques, to develop knowledge, scientific research, manipulating training courses and administrative support toward (KBST). Selected sample was (n = 6269) teachers, while the random sample was (5% of n = 315) teachers of Saudi public schools in the Northern Border Region. For study purposes, a statistical questionnaire was designed as a study tool for collecting data. Findings showed that there are no differences at the level of significance ($\alpha = 0.05$), between study factors: gender, computer skills, experience, teaching phase, level of training or manipulating of specialized courses and learning techniques towards (KBST). The results showed a great consistency, where experience has a strong effect on promoting teachers to evolve technology and computer skills in education, and designing modern learning techniques. Meanwhile, educational planning plays significant role in supporting knowledge generation and scientific research. Results verified a strong correlation between administrative support and competence level of the teacher in applying principles of the (KBST). Moreover, based on results; recommendations were derived.

Keywords : Teacher, Knowledge, Knowledge-based Society, Learning Methods, Training, Administration Support, Saudi Arabia.

INTRODUCTION

Almost the world's population now presently facing the deficiencies of potable water with better quality, with using of correct and applicable technology or water purifications above methods for domestic water is a wonder full solution of these problems with very low prices. Increased efforts to adopt the advanced technology and methods for making potable water as free from microbes and their released toxins chemicals for all domestic purposes including the storing in house or in farms for animals drinking and also can also be national and international level used the water treatment methods in this research as removing the organic material in forms of toxins with coagulation process as Aluminum sulphate, ferric chloride and boiling (dosing and time details is mentioned in previous section), disinfection method involves chlorination with specific dose as described different in the section of management plane (Badar *et al.*, 2016).

Teacher is important part of economic based on the Ninth Development Plan in all sectors of the Kingdom of Saudi Arabia[1], within the teaching and learning plan based on a number of themes that define the teacher's ability to support the process of transformation and development of capabilities and skills of the students, who are being counted on to lead the march of comprehensive development in the sectors of the state in the future. As the pillars of educational systems are based on the curriculum, the teacher, the student and the rules and regulations in addition to the infrastructure, the importance of teacher's role has become noticeable, in the ttransformation to a knowledge-based society. The most prominent question in the study that is: How to measure the competency of the teacher in the education sector in terms of practice and use of technology among "**Traditional Methods**" toward Knowledge-Based Society Transformation (KBST).

Literature Review

[2], Shanmugam [3] and Stehr [2] explained in their studies the knowledge reflects the understanding that man has contemporary or old issues cumulatively over the years. Also, [4] has indicated the human ability to acquire knowledge lies in the development of solutions to fit every problem separately and participatory with others. Knowledge society- based on a study conducted by [5] **-** consists of main elements: ***Knowledge Transformation;*** it is related to the teacher, ***Knowledge Resettlement*** throughout traditional teaching methods using technology, and ***Training Process and Rehabilitation*** of teachers. According to [6], KBST based on the linking of artificial intelligence techniques, programming, and the use of computers in a way enables man to understand human knowledge by making it a simple virtual learning method. [7] conceptualized the fact of education decline lies in the followed traditional methods and not moving to the stage of diversification in the construction of knowledge, as well as not linking it to the cumulative knowledge of the students with the requirements of the economic development of society. Nevo, McClean [8] explained where it attributed the importance of reforming knowledge by students and teachers based on governing legislation, curricula, infrastructure, strengthening the status of the teacher through the processes of training and development, the use of creativity and innovation methods, motivating students, innovation and diversity in teaching methods in order to serve the construction of knowledge in the community to be able to shift towards a knowledge society in light of the requirements of the economy **[9].** Theories and world contemporary experiences and practices confirm on different drivers of economic growth today than it was in the past, where economy has become more dependent on its growth on a factor to know more than ever the hosts of the history of mankind, and give the attention of economic policy knowledge; in both the innovation and its investment in all sectors, the new role of technology, entrepreneurship, education, continuous learning, upgrading the skills of the workforce, and transition to the management from the hierarchical structures to the horizontal structures together to take advantage of electronic networks of transactions and the more efficient communication [10], [1]. In a study conducted by the Emirates Center for Studies and Research (2005) concluded that the training of human cadres in various sectors through human capital development and encouraging creativity and innovation, as well as enhancing infrastructure capabilities[11]. Muller [12] addressed factors in the dissemination of knowledge, through the analysis and formulation of the curriculum, as well as the development of a responsible approach to education policy. On the

other hand, [13] and [14] showed in their studies an essential and accurate analysis of the relationship between knowledge and application of economic factors, and its impact on the main mechanisms that facilitate the flow of knowledge process, where the responsibility was on the skills and abilities of the teacher in the dissemination of knowledge through modern teaching methods to create generations that are aware of the serious nature of the economy and its importance. While [15] through his study discuss how to bridge the education gap, which is a shock to the children, he showed the teacher's role in supporting the ability of schools to improve reading conditions of the students, as well as the ability of the teacher in viewing the content of school curricula by simplifying its most complex level to acceptable basics to be understood and read. Universities that graduate teachers represent the "storage unit" of the different mechanisms of knowledge transformation, where it supports innovation depending on what has been presented in the "Conference of Knowledge Transformation 2012" [16]. [17] evaluated the performance level for (25) educational competence, in their the study that focused on educational leaders, supervisors, and teachers, they concluded into the importance of active learning environment and the principle of partnership between the teacher and the learner to develop Knowledge transformation. On the other hand, **[18]** concluded the importance of teacher's training in order to access knowledge society and linking teachers' training, development of skills based on knowledge society demands, as well as the importance of the link between the types of training courses and the level taught by the teacher, whether elementary, middle school or high school, because of the different patterns and abilities of the students to gain knowledge from them through the stages of study. [19] showed through his program the importance of the traditional methods of education and the contents of the curriculum toward (KBST) by using technology and communications that linked to the requirements of labour market[20]. AL Khatib and Abdul Haq [21] found out that low level of students in general, before and after the educational development process affected by weak level of teachers, regarding to educational skills and abilities they own in the process of KBST among students, in addition to curricula, and the inability of the teacher to use stimulating educational methods in classrooms. Meanwhile, Joudah [22] showed the development of education to build a KB economy, the importance of the development of educational institutions, evaluation of curricula and its development, and the level of spending on education in order to access to the knowledge-based economy. [23] focused on the weakness of the practical side used the theoretical side in training courses and workshops that teacher receives in strengthening the ability to apply the principles of the knowledge economy, the found an existence of a gap between knowledge and practical abilities, lack of practice, and their use of traditional methods of education for motivation and brainstorming in teaching, and the lack of using technology in the traditional educational skills. [24] in his book entitled **"With the Teacher"**, which based on the results of statistics of polls in a number of countries in the world within the capacity for education and self-confidence as refereed in [25], [26], the role of educational leadership, the level of education and rehabilitation, the impact on students, in addition to a number of themes that the pillars of education based on the student, the curriculum and learning environment require new educational practices in the classroom through the development of teaching strategies, he also stressed on the teacher's performance process is directly influenced by the process of teacher's preparation and developing his skills that require achieving employment stability, to enhance the development of teaching methods, and counseling programs.

Meanwhile, in a study conducted by [27] they evaluated the specialization subject, teaching methods and strategies, the nature of the learner and his development, educational technology, measurement and evaluation, educational administration and management of classroom based on the study of [28] to evaluation teacher's competence in his possession of skills, courses, teaching experiences, and use of technology in training. The study has concluded into the need to innovate new training patterns by adopting modern training programs for teachers in the schools in order to facilitate the transformation process from traditional education to e-learning in teacher training using modern technological techniques and designing educational courses attractively and academically using multimedia programs.

METHODOLOGY

Hypotheses of the study

This study seeks to address the following hypothesis:

There are statistically significant differences at ($\alpha = 0.05$) between the means of the teachers perspectives about using traditional educational methods in terms of (the use of technology, learning methods, knowledge development and scientific research, training level, the application level, administrative support), and the associated requirements of Knowledge- Based Society Transformation (KBST) that are attributed to the variables of: (gender, the degree, years of experiences, computer literacy skills, number of training courses that have been obtained through study and work, and the phase taught by the teacher).

Study's Instrument

Study's instrument was prepared after reviewing previous studies, including the subject of the study, the research adopted questionnaires prepared in this area for a number of researchers, where the paragraphs of the questionnaire have been reworked in line with study's community and the dominant culture.

Statistical Analysis and Results

Data shown in **Table (1)** indicates that the number of teachers in the Northern Border region reached to 6269; where 3062 of them are male teachers by (49%) and 3207 of them are female teachers by (51%).

Study's Sample Analysis

Due to the heterogeneity of the study's sample (the research community) and its distribution to the three stages of education, after its division at the level of teachers (male/female), the study relied on sample's random approach represented in the Random systematic representative where teachers (male/female) were represented at the three stages of education. See **Table (2)**.

Instrument Validation and Reliability

To verify the validity of the content of study's tool, the questionnaire has been shown initially for ten (10) specialized arbitrators, where some observations modified. The reliability of study's tool verified by calculating the **Cronbach's alpha** where the value reached to (**0.913**) by more than the percentage of (0.7%) for the study's big/huge sample relatively, which resulted in the success of the reliability of study's tool test and the adoption of statistical analysis to reach to the results.

Descriptive Statistics

This part addresses the descriptive statistical indicators of the variable of (teacher's competence in Knowledge- Based Society Transformation (KBST) in accordance with the statistical differences between teachers depending on each of the arithmetic mean, standard deviation, and the value of *T* used by the study to examine the significance of differences.

First: Studying the differences between male and female teachers according to the variable of (teacher's competence in Knowledge- Based Society Transformation (KBST). Findings shown in **Table (4)** showed that the values of ***T*** have reached to (1.130) and it is **insignificant** value at any probabilistic level and it emphasises on the lack of differences between male and female teachers in study's sample who are in the Northern Border Region with respect to the variable of teacher's competence in Knowledge- Based Society Transformation (KBST).

Second: Studying the differences between male and female teachers distributed on over the three academic levels according to the variable of Knowledge- Based Society Transformation (KBST)**.** According to results shown in **Table (5)**, findings suggest that the values of *T* has reached to (1.246) in the Primary Stage, (1.153) in the Middle Stage, and (1.028) in the Secondary Stage respectively and all of them are **insignificant** values at any probabilistic level.

These findings are consistent with the result described in the previous paragraph and described in **Table (6)** that demonstrates the absence of differences between (male/female) teachers and those who represent the Northern Border Region with respect to the variable of teacher's competences in Knowledge- Based Society Transformation (KBST).

Third: Studying the differences between the three academic levels of education according to the variable of Knowledge- Based Society Transformation (KBST). The findings shown in **Table (7)** indicate that all of ***T*** values insignificant at any probabilistic level.The results shown in Table (8) indicate the extent of the statistical consistency with the results of the previous two items shown in Tables (6) and (7) that prove the homogeneity between the result of the lack of differences between male and female teachers within the study sample.

Regression Analysis

Regression analysis between independent variables and the dependent variable of teacher's competences in Knowledge- Based Society Transformation (KBST) represented in its three domains. The study aimed to identify the influence imposed by correlation relations to variation in the first domain (Traditional learning methods using Technology) of the dependent variable by using Multiple Linear Regression by identifying the value of each ***(F)*** value and its significance level, linear regression coefficient, the value of *T* and its significance level, in addition to identifying the value of the coefficient of determination (**R^2**), which reflects the relative contribution of each independent variable in the interpretation of variation in the dependent variable.

Regression analysis between independent variables and the first domain (learning methods)

Findings Shown findings in **Table (9)** suggest that there is only three significant variables in the stepwise multiple regression which is; teaching experience, educational degree and administrative support, while the three other variables were excluded as it was **insignificant** in its interpretation of the variation in the dependent variable of (learning methods) represented the first domain, while the value of ***F*** refers to the **significance** of **regression** function; whereas its value has reached to (10.179), which is **significant** value at the probabilistic level of 0.01.

Findings showed a unique contribution of the variable of teaching experience in the interpretation of differences in **Traditional learning methods** (the first domain) using technology has reached solely to almost 24%. These results suggest the effect of the evolution of the academic stage at the level of technology's use, and finally the variable of the administrative support came to prove the importance of the role of the Ministry of Education in the formulation of plans for training and development of human resources, in addition to motivating (male/female) teachers to participate more effectively in the organization and management of educational activities inside and outside schools.
Regression analysis between independent variables and the second domain (Using Technology): as shown in Table (10) suggest that there is only three significant variables in the Multiple Linear Regression which is; computer

skills, the level of training and skills of application, while the three other variables were excluded as it was **insignificant** in its interpretation of the variation in the dependent variable of (using technology) represented the second domain, while the value of *F* refers to the significance of regression function; whereas its value has reached to (14.408), which is **significant** value at the probabilistic level of 0.01.

The findings show that the overall coefficient of determination for the regression function equals (0.441) and this means that the unique contribution for the variables of computer skills, the level of training and skills of application together have reached to (44%) almost throughout the variation in using technology (second domain). The variable of skills application came to confirm the consistency of the relationship between computer skills and the training level, as well as to emphasize on the importance of skills application for (male/female) teachers as an essential input for the development of all returns and educational outputs.

Regression analysis between independent variables and the third domain (development of knowledge and scientific research): as shown in **Table (11)** suggest that there is only three significant variables in the Multiple Linear Regression which is; computer skills, the level of training and skills of application, while the three other variables were excluded as it was insignificant in its interpretation of the variation in the dependent variable of (the development of knowledge and scientific research) represented the third domain, while the value of *F* refers to the significance of regression function; whereas its value has reached to (12.117), which is significant value at the probabilistic level of 0.01.

The shown findings in **Table (11)** indicate that the overall coefficient of determination for the regression function equals (0.456) and this means that the unique contribution for the variables of the administrative support, application skills and training level together have reached to (46%) almost in the development of knowledge and scientific research (third domain). This refers to the importance of these variables in the development of knowledge and scientific research as one of teacher's competences axes in the Knowledge- Based Society Transformation (KBST), and perhaps these results are associated with the role of the Ministry of Education in guiding training plans and educational supervision to improve the integration of teachers in all the processes that encouraging them to submit their proposals and scientific researches, as well as linking their university studies to the priorities and needs of the ministry with the purpose of developing the level of teacher's competences in the Knowledge- Based Society Transformation (KBST)[19], [11] and [29]. Moreover, the results emphasise on the importance of the variable of application skills to confirm the significance of the relationship between administrative support, application skills, and the training level, considering that the teachers' application skills as one of the main elements of activating experiences and knowledge bases, and turning them to field applications inside classrooms. Finally the variable of training level of (male/female) teachers came to ensure the development of courses and training programs in proportion to their knowledge needs and career experiences with emphasis on the associated factors with both of planning and organization in terms of activating the role of teachers in the development of knowledge and scientific research[30].

CONCLUSION

The study has concluded into a set of analytical results related to the role of teachers in the Knowledge- Based Society Transformation (KBST), where the variables of teaching experiences, educational degree and administrative support were associated together with traditional learning methods (the first domain), while the variables of computer skills, training level, and applications skills were associated with the use of technology (the second domain), and finally the variables of the administrative support, application skills, and training level were associated together with the development of knowledge and scientific research (the third domain)[24], [23] and [31]. In the light of these results, a **"Conceptual Framework"** has been formulated that aims to lay/develop the analytical underpinnings and operational mechanisms enriched by the plans and programs that are placed on each of the near-term and long-term,

as well as the training courses for the development of the role of teachers (male/female) in the Northern Border Region in the Knowledge- Based Society Transformation (KBST)[32].

Extra Recommendations

Based on above results and conclusions study suggested the following recommendations:

The first axis: The role of learning methods in raising the level of teacher's competences towards a knowledge society:

1.First axe: (A) Strategic Plans

Axis	Strategic Plans
The role of learning methods in raising the level of teacher's competences towards a knowledge society	A field plan to build a database on the level of the Northern Border Region, which includes all of the educational experiences, disciplines and years of teaching experience[33].
	A participatory program to integrate teachers (male/female) who are academic degrees holders (MA and PhD) in all the activities of planning and development departments in the Department of Education[29].
	An operational plan for the study of the specializations required by the area's schools according to the foundations of scarcity, specialization and directing/guiding teachers to abide by it.
	A plan to develop the curriculum to include training courses for the knowledge society.
	Special programs linking the process of learning methods to the process of achieving the goals and principles of the knowledge society.
	A plan to motivate talented (male/female) teachers in training sessions related to the curriculum of the Knowledge- Based Society Transformation (KBST).
	Scientific programs to motivate (male/female) teachers to produce field and scientific research that focus on the knowledge society.

2.First axe: (B) Training Programs

Axis	Training Programs
The role of training in raising the level of teacher's competences towards a knowledge society	Developing a training plan for the staff in universities in various specializations to train them on modern software related to the knowledge economy and knowledge-based education[34].
	Adding semester curriculums during the study period related to the concepts of knowledge economy, economic projects, innovation and entrepreneurship.
	Developing an integrated training plan between university study and labor market to train students on the projects related to knowledge economy and knowledge economy- orientated education[35].
	Developing teachers' training programs to use technology in the classroom teaching process and the preparation of lessons and practical application.
	Supporting the researches and studies of teachers that involve students in its implementing to raise the knowledge level of them in the process of research

	preparation, studies, analysis of the results and solving problems within the framework of the knowledge economy[36].
	Developing mechanisms to give teachers career and financial rewards in recognition of their distinguishing in the field of preparing research and studies, as well as the development of proposals on the improvement of learning methods and building knowledge and scientific research.
	Creating a database of patents, innovation, entrepreneurship and scientific research for talented teachers and students, as well as investing their energies in national developmental projects during the period of work and in the future development plans within the perspective of the knowledge economy[37].

The second axis: (A) -: the role of the use of technology in raising the level of teacher's competences towards a knowledge society:

Axis	The use of technology
The role of the use of technology in raising the level of teacher's competences towards a knowledge society	A special training plan using computers in the Knowledge Society applications as a part of the field training exercises/practices for teachers.
	A technology plan to support all schools in the laboratories of computers and interactive halls specialized in the application of Knowledge- Based Society Transformation (KBST)[38].
	A development program aims to define the foundations of the knowledge economy issues for both students and teachers.
	A plan to create a high council to the Knowledge- Based Society Transformation (KBST) at the level of the Ministry of Education that oversees the integration of the principles of a knowledge society in the curriculum.
	A program to develop ways to motivate teachers to build and generate the principles of Knowledge- Based Society Transformation (KBST)
	A plan to link the use of technology in schools to training courses to be set up for (male/female) teachers[30].
	Applications programs with students to use multi-media and computer about the knowledge economy- based education

The second axis: (B) -: the role of the development of knowledge and scientific research in raising the level of teacher's competences towards a knowledge society:

Axis	Supporting knowledge and scientific research
The role of the development of knowledge and scientific research in raising the level of teacher's competences towards a	A ministerial plan to study the experiences of developed countries in the field of development of knowledge and scientific research and analysis of cognitive and economic aspects[38].
	Scientific programs to motivate teachers to study the experiences of the field of knowledge development and how to use them within the Northern Border Region
	An operational plan for the study of the foundations of the development of knowledge and its standards of evaluation, as well as studying its impacts and

knowledge society	outputs on the student, the school and the community.
	A plan to develop the curriculum to include the foundations of scientific research (theoretical and practical curricula) simplistically to commensurate with students' capabilities [39].
	Special programs with the participation of students with teachers in field and scientific researches regarding the circumstances of their schools and local community[29].
	A plan to support the role of teachers to contribute to the field contribution in all scientific researches that are supervised by the Ministry of Education.
	Developing scientific programs for the development of teachers' capacities in the field of development of knowledge and scientific research in the Knowledge-Based Society Transformation (KBST)[5].

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Appendices

The following is the definitions of the study's terms:

- **Teacher (male/female):** He/ She is the human element who runs the educational process within the classroom through the presentation of the curriculum by using modern educational techniques and means to enable him/her to transfer knowledge to the future learner – the students- [40].
- **Education System:** It is a set of legislation and regulations, human elements, students, infrastructure, curricula and courses, and activities that interact with its local environment in one pot in order to create the relationship between the teacher and the learner [41].
- **Knowledge:** It is the reflection of the skills and experience gained in humans, which can be relied upon to solve problems and help the community in the development and the development process [42], [43] and [30].
- **Knowledge Economy:** It is the economy that is based on the accumulated knowledge of mankind and associated with various forms of financial and commercial activity in the State[44].
- **Knowledge Economy-based Education:** It is the linking process of educational systems in the State to its economy activity requirements in it [32].
- **Knowledge Economy – Orientated Education:** It is a set of the developed educational systems' elements so as to be able to keep up with the requirements of the knowledge economy[45].
- **Knowledge- Based Society Transformation (KBST):** It is the process integration of the elements of the educational systems based on the knowledge economy and the elements of knowledge-based economy to reach a society where everyone has equal opportunities to achieve better living standards[46], [9], [35].

Table 1. Overall Community Sample

Level	Number of Teachers (Male)	Percent	Number of Teachers (Female)	Percent	Total
Primary	1761	53%	1553	47%	3314
Middle	721	46%	831	54%	1552
Secondary	580	41%	823	59%	1403
Total	**3062**	**49%**	**3207**	**51%**	**6269**

Table 2. Study Sample

Level	Number of Teachers (Male)	Percent	Number of Teachers (Female)	Percent	Total
Primary	88	53%	78	47%	166
Middle	37	46%	42	54%	79
Secondary	29	41%	41	59%	70
Total	**154**	**49%**	**161**	**51%**	**315**

Table 3. Alpha – Cronobach of Dependent Variable and Sub-domains

Variable	Alpha Coronbach	F- value	Level of α
First Domain: Learning Methods	0,863	7.934*	**0.05**
2nd Domain: Using ICT	0.927	14.831**	**0.01**
3rd Domain: Knowledge Development and Scientific Research	0.902	11.086**	**0.01**
(Independent Variable): Teacher Competency toward Transformation to Knowledge-based Society	0.913	12.273**	**0.01**

Table 4. Analyzing differences between (M/F) Teachers based on independent Variable - Teacher Competency toward Transformation to Knowledge-based Society

Mean		Standard Did.		T value	A – value
Male	85.39	Male	5.69	1.130	0.269
Female	85.06	Female	5.76		

Table 5. Statistical Factor Description of (Mean) and Standard Deviation for (M/F) Teachers among Teaching Levels based on Independent Variable

Level	Mean		Standard Div	
	Male	Female	Male	Female
Primary	85.37	85.25	5.18	5.11
Middle	85.65	85.41	5.42	5.71
Secondary	86.14	85.79	5.26	5.37

Table 6. Differences of (T value) between (M/F) Teachers among Teaching Levels based on Independent Variable - Teacher Competency toward Transformation to Knowledge-based Society

Differences Between M and F Teachers	T – value	α – value
Primary	1.246	0.198
Middle	1.153	0.204
Secondary	1.028	0.283

Table 7. Statistical Factor Description of (Mean) and Standard Deviation for Overall sample of Teachers among Teaching Levels based on Independent Variable

Level	Mean	Standard Div
Primary	85.29	5.14
Middle	85.54	5.57
Secondary	85.79	5.32

Table 8. Differences between Teaching Levels based on Independent Variable - Teacher Competency toward Transformation to Knowledge-based Society

Differences Between M and F Teachers	T – value	α – value
Primary - Middle	1.156	0.104
Primary - Secondary	1.318	0.217
Middle - Secondary	1.179	0.263

Table 9. Multiple Regression series of the first domain (Learning Methods)

Analysis Variables	Partial Regression Factor	T - value	R^2 – Coefficient	
			Partial	Cumulative
Teaching Experience	1.028	7.955**	0.242	0.242
Educational Degree	0.625	5.850**	0.146	0.388
Administrative Support	0.101	2.259*	0.032	0.420
F value	10.179**			

Table 10. Multiple Regression series of the Second domain (Using Technology (ICT))

Analysis Variables	Partial Regression Factor	T - value	R^2 – Coefficient	
			Partial	Cumulative
Computer Skills	1.622	9.923**	0.330	0.330
Training Level	0.439	7.768**	0.092	0.422
Application Skills	0.114	3.329*	0.019	0.441
F value	14.408 **			

Table 11. Multiple Regression series of the Third domain (Knowledge Development & Scientific Research)

Analysis Variables	Partial Regression Factor	T - value	R^2 – Coefficient	
			Partial	Cumulative
Administrative Support	1.409	9.874**	0.291	0.291
Application Skills	0.628	7.519**	0.097	0.388
Training Level	0.135	3.786*	0.068	0.456
F value	12.117**			

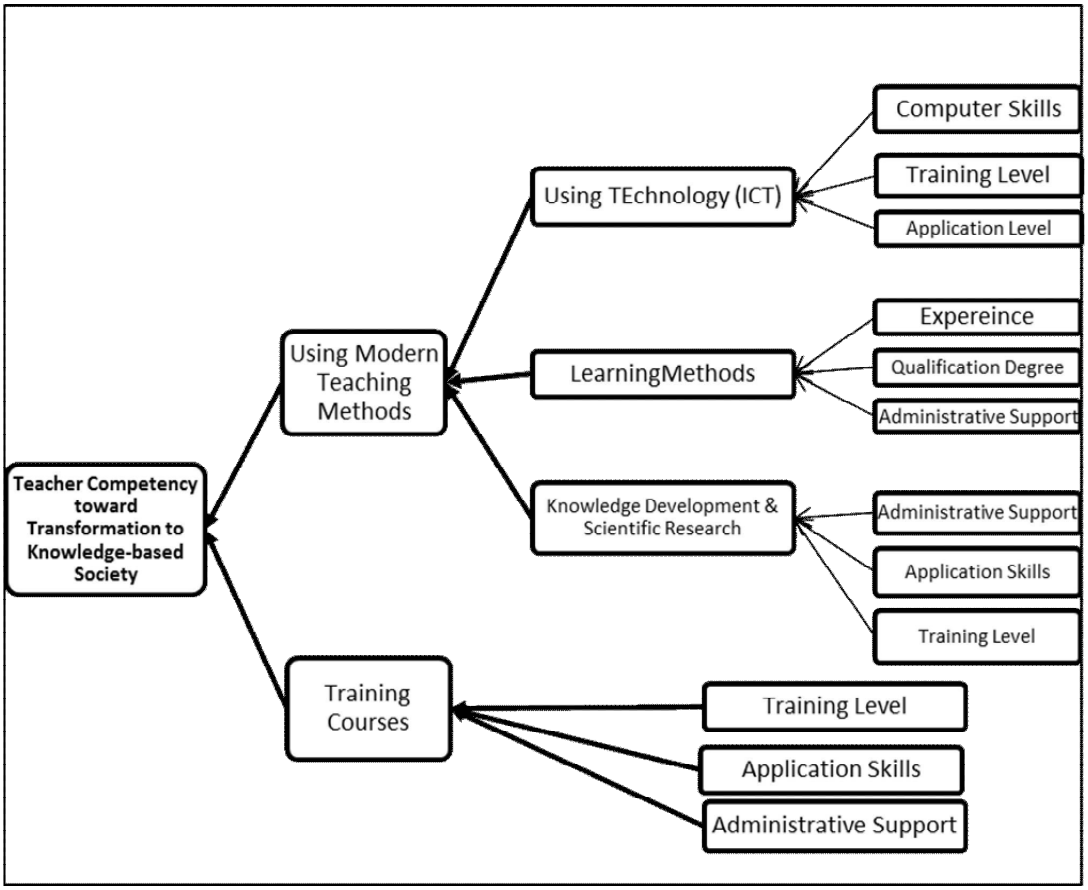

Figure1.The Conceptual Framework

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RESEARCH ARTICLE

Safety Evaluation of Levofloxacin on Haemato-biochemical Parameters Following Repeated Oral Administration in Dual Purpose Chicken

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ABSTRACT

The present study is to study the safety evaluation of levofloxacin following repeated oral administration in dual purpose chicken. Levofloxacin is a third generation fluoroquinolone with broad spectrum nature and is a levo isomer of ofloxacin It's spectrum of activity includes most strains of gram positive and gram negative anaerobic bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal tract, skin and soft tissue infections. It has an excellent broad-spectrum activity against *Mycoplasma* and *Chlamydia* organisms in veterinary medicine. The experimental birds (35 day old) were randomly allotted into three groups (n=30), Group I birds served as control (Distilled water), Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg and 20 mg/kg body weight respectively directly into the crop using a thin plastic tube attached to a syringe for 28 days. There was a significant increase ($p<0.05$) in AST and ALT serum enzyme values in Groups III on day 21 and 28 in the

experimental birds as compared to control group. Suggestive of producing the toxic effect which were supported by the gross and histopathological observation in liver samples.

Keywords : Levofloxacin, AST, ALT, Dual Purpose chicken

INTRODUCTION

Fluoroquinolone are important group of antimicrobial drugs used in veterinary medicine. They have broad-spectrum activity against bacteria, mycoplasma and rickettsia (Brown, 1996). Fluoroquinolone are very potent antimicrobials and effective against a wide range of pathogenic organisms and are well distributed in the body after administration. They exhibit excellent oral bioavailability, extensive tissue penetration, low protein binding and long elimination half-life (Brunton *et al*., 2005). The present study is to study the safety evaluation of levofloxacin following repeated oral administration in dual purpose chicken. Levofloxacin, a third-generation fluoroquinolone, is the S-isomer of ofloxacin and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria (North *et al.,* 1998). It also has more pronounced bactericidal activity particularly against organisms such as *Pseudomonas*, *Enterobacteriaceae* and *Klebsiella* spp (Klesel *et al.,* 1995). The bactericidal effect of levofloxacin is achieved through reversible binding to DNA gyrase and subsequent inhibition of bacterial DNA replication and transcription (Fu *et al.,* 1992). The levofloxacin distributes well to target body tissues, fluids and its uptake makes it suitable for use against intracellular pathogens. However, it penetrates poorly in to central nervous system (Langtry and Lamb, 1998). The levofloxacin acts by a concentration-dependent killing mechanism, whereby the optimal effect is attained by administration of high doses over a short period of time (Drusano *et al*., 1993) followed by a relatively prolonged post antibiotic effect (Aliabadi and Lees, 2001).

MATERIALS AND METHODS

Experimental animals

The study was conducted in 30 to 35 days old (n= 30) healthy dual purpose chicken Indian Rock-3(IR-3), a strain of White Plymouth Rock developed by Karnataka Veterinary Animal and Fisheries Sciences University, Bidar . The study was performed at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru. The birds were kept under observation for one week prior to commencement of experiment and subjected to clinical examination in order to exclude the possibility of disease. The birds were provided antibiotic-free standard broiler ration for fourteen days. The animal house was maintained at room temperature (25±2^{0}C) and at 45 to 65 per cent relative humidity. Food and water were supplied *ad libitum* and standard managemental practices were followed to keep the birds free from stress. The prior approval of the Institutional animal Ethics Committee (IAEC)was obtained before the commencement of the experiment(LPM/IAEC/181/2014, Date: 10/01/2014).

Drugs and Chemicals

Levofloxacin hemihydrate Injection and oral solution 10% (Meriflox®, Vetoquinol India Animal Health Private Limited, Mumbai, India) were used for the study. The Levofloxacin from Vetoquinol, India Animal Health Private Limited, Mumbai and diagnostic kits from ERBA Mannheim (Transasia Biomedicals Ltd, HP) used for safety evaluation of levofloxacin following repeated oral administration in dual purpose chicken**.**

The experimental birds (35 day old) were randomly allotted into three groups (n=30),Group I birds served as control (Distilled water), Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20 mg/kg bw respectively directly into the crop using a thin plastic tube attached to a syringe for 28 days. The food was withheld for 12 h before oral dosing but not water and water was provided *ad libitum* before the drug administration. The selection of the dosage based on, levofloxacin at 10 mg/kg bw considered as therapeutic dosage in the poultry birds. Therefore 20 mg/kg of levofloxacin was selected as high dose based on the therapeutic dosage of levofloxacin to see the any adverse effect with respect to serum biochemical analysis.

Serum biochemical analysis

The serum samples used for the determination of biochemical parameterson day 0, 7, 14, 21 and 28by using clinical biochemical analyzer - Microlab 300 (Vitalab Scientific, Netherlands). The serum biochemical parameters were estimated using commercially available diagnostic kits from ERBA Mannheim (Transasia Biomedicals Ltd, HP) by following the manufacturer instructions furnished in the leaflet supplied along with the diagnostic kit.

1. Aspartate aminotransferase (AST)
2. Alanineaminotransferase (ALT)

Statistical analysis

The data were analyzed by using one-way ANOVA. The mean values and standard error of the different groups were compared by Duncan's multiple range test using Statistical Package for Social Sciences (SPSS16, 2010). Data were considered as significant from one another when $P \leq 0.05$.

RESULTS AND DISCUSSION

Serum aspartate aminotransferase (AST)

The mean AST values for levofloxacin in Group I (Control), Group II (10 mg/kg bw) and Group III (20 mg/kg bw) of experimental birds were measured at weekly interval and have been summarized in Table.2 and graphically represented in Fig.1.

The mean serum AST (U/L) values were 168.40±0.60, 214.24±0.98, 224.30±0.78,219.58±0.90228.86±0.85U/L for Group II and 172.32±0.65, 218.64±0.80, 230.64±0.64 250.65±0.90,267.80±0.68U/L for Group III and170.40±0.88,210.34±0.20, 216.44±0.13, 218.97±0.64, 220.64±0.82U/L for control group on day 0, 7,14, 21 and 28 respectively.

There was a significant increase ($p<0.05$) in AST values in Groups III on day 21 and 28 in the experimental birds as compared to control group.There was no significant increase ($P> 0.05$) in AST values in Groups II on day 0,7,14, 21, 28 and in Groups III on day 0,7, 14 as compared to control group throughout the experiment.

Serum alanine aminotransferase (ALT)

The mean ALT values for levofloxacin in Group I (Control), Group II (10 mg/kg bw) and Group III (20 mg/kg bw) for experimental birds were measured at weekly interval and have been summarized in Table.3 and graphically represented in Fig.2.

The mean serum ALT values were 10.20±0.24,12.48±0.42, 12.64±0.18,13.02±0.90, 13.42±0.40U/L for Group II and 10.70±0.78, 13.25±0.80, 14.20±0.64, 16.56±0.62,17.24±0.92U/L for group III and 9.90±0.27, 12.92±0.52, 12.10±0.47,12.26±0.28, 12.48±0.73U/L for the control group on day 0,7,14, 21 and 28 respectively.There was a significant increase (P<0.05) in ALT values in Groups III on day 21 and 28 in the experimental birds as compared to control group.There was no significant increase (P> 0.05) in ALT values in Groups II on day 0,7,14, 21, 28 and in Groups III on day 0, 7,14 as compared to control group throughout the experiment.The biochemical parameters (AST and ALT) were estimated from serum samples obtained on day 0,7,14,21 and 28 of experiment period after administration of levofloxacin in dual purpose chicken.

Aspartate aminotransferase (AST)

In the present study, a significant increase (P<0.05) in AST activity in Groups III of the experimental birds on day 21 and 28 as compared to control group.This finding is supported with Oda *et al*. (2014) who reported that a significant increase in serum AST value on first and four weeks after administration of levofloxacin hydrochloride at the dose of 82 mg /kg bw through oral route once daily for four weeks in rabbits.Elkholy *et al. (*2009) studied an increased in serum AST activities following repeated oral administration of enrofloxacin at 10 mg /kg bw once daily for five consecutive days in laying hens. Fatai *et al*. (2013) reported an increase in AST activity after the administration of ciprofloxacin in rats for a period of five days.

Alanine aminotransferase (ALT)

In the present study, a significant increase(P<0.05) in ALT concentration in Group III of the experimental birds on day 21 and28 as compared to control group.The present finding is in agreement with findings of Elkholy *et al. (*2009) reported an increase in ALP activities following repeated oral administration enrofloxacin at 10 mg /kg bw once daily for five days in laying hens.Oda *et al*. (2014) reported that a significant increase(P<0.05) in serum ALT activity on first and four weeks after administration of levofloxacin hydrochloride at 82 mg /kg bw through oral route once daily for four weeks in rabbits. An increased in the ALT activity after the administration of ciprofloxacin in rats for period of five days was reported by Fatai *et al*.,(2013).The degeneration of hepatocytes and subsequent leakage of enzymes were the reasons attributed for increase in the levels for ALT and AST serum enzymes (Leeson *et al.,* 1995).The degeneration of skeletal muscles and increase in the osteoblastic activity lead to an increase in the ALP activity (Falconer and King, 1970). Histological observations such as degenerative and inflammatory changes, vacuolar degeneration of hepatocytes in liver and other organs of the present study uphold the alteration of the serum enzyme values in Group III experimental birds administered with 20 mg/kg bw of levofloxacin in dual purpose chicken.

CONCLUSION

The safety evaluation of levofloxacin following the repeated oral administration was conducted in dual purpose chicken. The birds were divided into three experimental groups. Group I (Control), Group II and Group III experimental birds were administered with 10 mg/kg bw (therapeutic dose) and 20 mg/kg bw (high dose) for a period of 28 days. The estimation of serum biochemical parameters (AST, ALT) were conducted. There was a significant increase in AST and ALT liver specific enzymes in the Group III administered with high dose of levofloxacin at 20 mg/kg bw for 28 days suggestive of producing the toxic effect which were supported by the gross and histopathological observation in liver samples.

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Table. 1. Experimental design for safety evaluation of the levofloxacin

Sl. No	Groups	Dose (oral route)
1	Group - I (Control) (n = 10)	Distilled water
2	Group – II (n = 10)	10 mg/kg bw, Therapeutic dose
3	Group – III (n = 10)	20 mg/kg bw, High dose

Table.2. Effect of levofloxacin on Aspartate aminotransferase activity (U/L) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	170.40±0.88	168.40±0.60[a]	172.32±0.65[a]
7	210.34±0.20	214.24±0.90[a]	218.64±0.80[a]
14	216.44±0.13	224.30±0.78[a]	230.64±0.64[a]
21	218.97±0.64	219.58±0.90[a]	250.65±0.90[b]
28	220.64±0.82	228.86±0.85[a]	267.80±0.68[b]

Values are mean ± SE n= 6 a:Nonsignificant(p>0.05) b: Significant (p<0.05)

Table.3.Effect of levofloxacin on Alanine aminotransferase activity (U/L) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	9.90±0.27	10.20±0.24[a]	10.70±0.78
7	12.92±0.52	12.48±0.42[a]	13.25±0.80[a]
14	12.10±0.47	12.64±0.18[a]	14.20±0.64[a]
21	12.26±0.28	13.02±0.90[a]	16.56±0.62[b]
28	12.48±0.73	13.42±0.40[a]	17.24±0.92[b]

Values are mean ± SE n= 6 a:Nonsignificant(p>0.05) b: Significant (p<0.05)

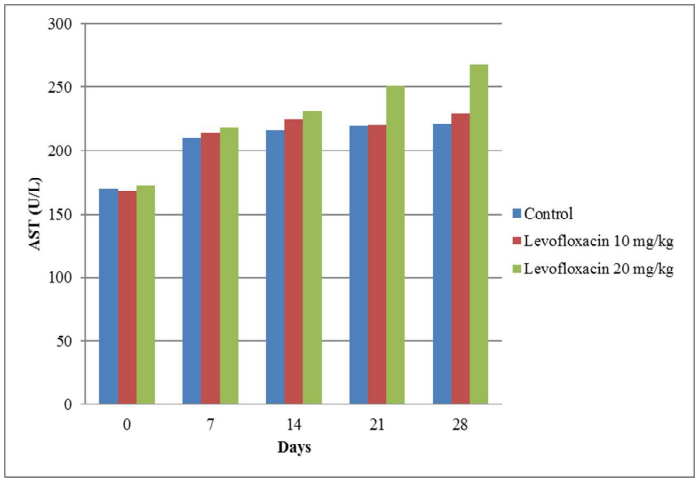

Fig.1.Effect of levofloxacin on Aspartate aminotransferase activity (U/L)in dual purpose chicken

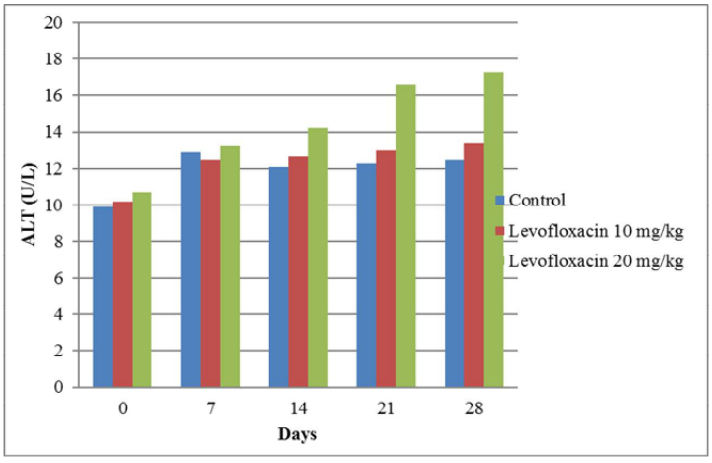

Fig .2.Effect of levofloxacin on Alanine aminotransferase (U/L) in dual purpose chicken

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RESEARCH ARTICLE

Varietal Susceptibility of Rice Weevil, *Sitophilus oryzae* on Different Wheat Varieties

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ABSTRACT

The study on susceptibility of different wheat varieties against rice weevil, *Sitophilus oryzae* were carried out at Department of Entomology, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, Gujarat during 2010. The total twenty two varieties *viz.,* HI-1567, GW-366, GW-173, LOK-62, HI-8691, GW-273, GW-411, GW-1139, HI-1568, AKOW-4021, VAS-321, AKAW-4493, GW-1255, HW-5207, GW-322, HI-8498, MACS-3744, HI-8704, GW-496, GW-503, LOK-1 and MACS-6274 were tested to study and found the overall results of various parameters of growth and development of rice weevil, it could be concluded that variety HI-1568 and GW-366 were found resistant to rice weevil, while the some cultivars (HI-1567, GW-173, LOK-62, HI-8691, GW-273, GW-411, GW-1139, AKOW-4021, VAS-321, AKAW-4493, GW-1255, HW-5207, GW-322, HI-8498, HI-8704, GW-496, GW-503, LOK-1 and MACS-6274) proved more susceptible to rice weevil. Among them MACS-3744 variety was found highly susceptible to *S. oryzae* under the laboratory stored condition.

Keywords : Wheat, Varietal susceptibility, *Sitophilus oryzae* L., Biological parameters.

INTRODUCTION

In India, post harvest losses caused by unscientific storage, rodents, insects, microorganisms, moisture etc. account for about 10.0 per cent of total food grains. A world survey conducted by FAO indicated about 5.0 per cent loss of cereals has been caused annually in storage. Stored product pests have the capacity to infest both raw and processed

agricultural products. However, large numbers of insect species infesting wheat grains in storage. The weevils *viz., Sitophilus oryzae* (Linnaeus), *Sitophilus granaries* Linnaeus and *Sitophilus zeamais* (Motsch.) are classified in the most important primary pests of stored wheat, whose adults damage grains, and larvae inhabit and feed inside the grain (Rees, 2004; Beckett, *et al.,* 2007). The rice weevil, *S. oryzae* (Coleoptera: curculionidae), is the most widespread and destructive major insect pest of stored cereals throughout the world. *S. oryzae* causes substantial losses to stored grain accounting 18.30 per cent (Adams and Schulten, 1978). This species has a relatively short developmental period and high populations can easily be built up (Aitken, 1975). It feed internally by boring into stored grains. Adults of *S. oryzae* feed mainly on the endosperm, thus reducing the carbohydrate content, but larvae feed preferentially on the germ of the grain and remove a large percentage of the proteins and vitamins (Belloa, *et al.,* 2000). Moreover, the kernel damage caused by *S. oryzae* larvae enables other species, the external feeders, which are not capable of infesting sound grain, so increase the damage rapidly.

In the present era of organic farming, massive overuse and frequent misuses of synthetic organic insecticides against stored grain pest has led to problems of 3R's viz; Resistance, Resurgence and Residues as well as toxicity hazards to man and domestic animals resulting in environmental degradation. Sarin and Sharma (1983) have revealed that all the stored grain pests exhibit the phenomenon of preference or non-preference for the grains of different varieties. A number of characteristics are known to render the cultivars less suitable or unsuitable for feeding, oviposition and development of insect pests. These mechanisms may induce due to biochemical attributes which can modify behavioral responses of insect (antixenosis) or affect its development (antibiosis) (Throne, *et al.,* 2000). There has been little emphasis in breeding for grain resistance to insect pests of stored grain products. In the countries where storage facilities are inadequate, stored grain resistance might be used either alone or along with other protective methods. By keeping this in mind the present investigation were conducted to study the varietal susceptible of wheat grain on the basis of biological parameters of *S. oryzae* and biochemical variation among different varieties of wheat. The data generated from present study will make a platform in indentifying resistant and susceptible reaction against *S. oryzae*, which can also be useful in further breeding programme.

MATERIALS AND METHODS

To screen out different wheat varieties for their relative resistance/susceptibility against rice weevil, *S. oryzae,* an experiment was conducted under laboratory condition in the Department of Entomology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during the year 2009-2010. The total twenty two varieties viz., HI-1567, GW-366, GW-173, LOK-62, HI-8691, GW-273, GW-411, GW-1139, HI-1568, AKOW-4021, VAS-321, AKAW-4493, GW-1255, HW-5207, GW-322, HI-8498, MACS-3744, HI-8704, GW-496, GW-503, LOK-1 and MACS-6274 were tested. Experiment was conducted in Completely Randomized Design (CRD) with 3 repetitions. The details of different methodologies used were furnished as hereunder.

Adult emergence, grain damage and weight loss

To study the relative susceptibility of wheat cultivars on the basis of adult emergence, grain damage and weight loss against *S. oryzae,* 50 g grains from each variety were taken in plastic jar per treatment per repetition and five pairs of newly emerged adults of *S. oryzae* was released in each jar. These jars were kept in Biological Oxygen Demand (B.O.D.) incubator at constant ely temperature of 27±2°C and 70±5 per cent relative humidity and following parameters were taken to study the relative susceptibility. The grains were examined 60 days of released the pest and newly adult emergence was recorded for different varieties of wheat.

The healthy and damaged grains were also counted separately from each container and per cent grain damage was worked out from following formula.

$$\text{Grain damage (\%)} = \frac{\text{Number of damaged grains}}{\text{Total number of grains used}} \times 100$$

Moreover, to study the per cent weight loss adult weevils were removed from sample and the damaged and healthy grains were weighed with electric balance. The per cent loss in weight of pods was calculated by adopting the following formula:

$$\text{Per cent weight loss} = \frac{I - F}{I} \times 100$$

Where,

I = Initial weight of seeds

F = Final weight of seeds

RESULT AND DISCUSSIONS

Adult emergence: The result (Table 1) revealed that the maximum numbers of adults were found in variety MACS-3744 (49.2) and it was at par with AKAW- 4493 (40.2) and MACS- 6274 (40.1). While HI-1568 (18.3) variety had minimum adults and it was at par with the variety GW-366 (21.2), GW- 408 (23.33) and LOK-1 (24.2). Thus, the twelve wheat varieties *viz.,* HI-1568 (18.3), GW-366 (21.2), GW- 408 (23.33), LOK-1 (24.2), GW-273 (24.6), LOK-62 (26.5), HW-5207 (27.2), GW-173 (28.3), HI-8498 (29.1), GW-1255 (30.3), HI-1544 (31.0) and GW-1139 (31.1) had less number of adults than the average of 31.29 adult.

Per cent grain damage: It was clearly marked from the data (Table 1) that none of the variety was free from grain damage and maximum percentage of grain damage was found in variety MACS-3744 (82.68) and it was at par with AKAW-4493 (76.93) and MACS-6274 (72.54). While the minimum percentage of grain damage were found in variety HI-1568 (16.93) and it was at par with the variety GW-366 (21.0), GW-408 (23.06) and LOK-1 (25.15). Thus, the varieties *viz.,* HI-1568 (16.93), GW-366 (21.0), GW-408 (23.06), LOK-1 (25.15), GW-273 (26.2), LOK-62 (26.6), HW-5207 (27.4), HI-1567 (29.8), GW-173 (31.5), HI-1544 (33.06), GW-411 (33.72), HI-8498 (36.3), GW-503 (36.55), GW-1255 (38.58) and GW-496 (38.58) were least preferred by *S. oryzae.*

Per cent weight loss: The data presented in Table 1 revealed that per cent weight loss varied with the variety. The maximum percentage of weight loss (11.67) was found in variety MACS-3744 (11.69%). While the minimum percentage of weight loss (2.24) was found in variety HI-1568 (2.24%). The sixteen wheat varieties *viz.,* HI-1568 (2.24%), GW-366 (2.67%), GW-408 (3.07%), LOK-1 (3.32), LOK-62 (3.32), HW-5207 (3.38), GW-273 (3.44), HI-1567 (3.51), GW-173 (3.64), HI-1544 (3.90), GW-411 (4.04), HI-8498 (4.25), GW-1255 (4.46), GW-496 (4.53) and GW-503 (4.75) had lowest weight loss than the average (4.78 %) and such varieties were less preferred by *S. oryzae.*

The present finding of varietal susceptibility on the basis of biological parameters are supported by prior report of Chahal and Singh, 1974 wherein they noted that varieties *viz.,* WL 218, WL 221 and WL 224 showed relatively resistant reaction, while Lerma roja, WL 237 and K 69-508 were found to be susceptible on the basis of total numbers of adult emergence and total life span. Khokhar and Gupta, 1974 observed that none of the wheat variety was immune to *S. oryzae* and reported that the varieties HD 1944, C 281 and *Kalyansona* were found tolerant and Lerma rosa was most susceptible. However, Tiwari, *et al*., 1989 observed that the maximum weight loss (48.37%) was against

S.oryzae in K.W. variety and minimum (14.52%) in Shekhar variety of wheat at 150 days of storage. Thereafter, Suleman, *et al.,* 2000 reported that cultivar C 591 showed partial resistant reaction, with *Khyber* 87 as susceptible to highly susceptible, Pak 81 as susceptible to partially resistant and Rawal 87, Inqilab 91, Sariab 92, Bakhtawar and Faisalabad 85 were not significantly differed when subjected to three different tests (free choice, confinement and antixenosis. Rao and Sharma, reported that MACS 3744 was susceptible to the *S. oryzae.*

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Table 1: Performance of certain wheat varieties against *S. oryzae* in storage condition

Sr. No.	Variety	Adult emergence*	% grain damage**	% weight loss **
1	GW-366	4.65 (21.2)	27.3 (21.0)	9.11 (2.67)
2	HI-1567	5.36 (28.2)	33.1 (29.8)	10.8 (3.51)
3	LOK-62	5.19 (26.5)	31.1 (26.6)	10.5 (3.32)
4	GW-173	5.36 (28.3)	34.2 (31.5)	11.0 (3.64)
5	HI-8691	6.31 (39.3)	47.1 (53.66)	14.1 (5.93)
6	GW-273	5.0 (24.6)	30.8 (26.2)	10.7 (3.44)
7	GW-411	5.49 (29.7)	35.5 (33.72)	11.6 (4.04)
8	GW-1139	5.62 (31.1)	40.5 (42.17)	12.8 (4.90)
9	HI-1568	4.32 (18.3)	24.3 (16.93)	8.62 (2.24)
10	AKOW-4021	5.81 (33.2)	42.3 (45.29)	13.2 (5.91)
11	VAS-321	6.05 (36.0)	46.5 (52.6)	13.9 (5.77)
12	AKAW-4493	6.32 (40.2)	61.3 (76.93)	16.1 (7.69)
13	GW-1255	5.55 (30.3)	38.4 (38.58)	12.2 (4.46)
14	HW-5207	5.26 (27.2)	37.6 (27.4)	10.6 (3.38)
15	GW-322	5.68 (31.7)	40.1 (41.4)	12.7 (4.83)
16	HI-8498	5.44 (29.1)	37.1 (36.3)	11.9 (4.25)
17	MACS-3744	7.06 (49.2)	65.38 (82.68)	20.0 (11.69)
18	HI-8704	5.93 (34.6)	41.0 (43.04)	13.0 (5.06)
19	GW-496	5.84 (33.6)	38.4 (38.58)	12.3 (4.53)
20	GW-503	5.72 (32.2)	37.2 (36.55)	12.6 (4.75)
21	LOK-1	4.96 (24.2)	30.1 (25.15)	10.5 (3.32)
22	MACS-6274	6.38 (40.1)	58.4 (72.54)	15.2 (6.87)
23	GW-408	4.87 (23.3)	28.7 (23.06)	10.1 (3.07)
24	HI-1544	5.61 (31.0)	35.1 (33.06)	11.4 (3.90)
25	Local variety	6.30 (39.1)	53.2 (64.11)	14.6 (6.35)
	Mean	31.29	40.75	4.78
	S. Em. ±	0.89	1.09	0.35
	C. D. at 5%	2.52	3.09	0.98
	C. V. %	4.92	4.77	4.85

* = Square root transformed values
** = Arcsin √ percentage transformation
Figures in the parenthesis are retransformed value.

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RESEARCH ARTICLE

Copper-Charged Water may Give Relief in Cramping during Gestation: a Preliminary Study

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ABSTRACT

Yoga has proven to be the best form of exercise as it works at all the levels of human existences physical, physiological, psychological, intellectual and spiritual.Hence yoga provides complete health to the humans, as per the definition of WHO (World Health Organization). It is the best form of exercise during pregnancy as it is the only non impact form of exercise, which is soft in nature. Yogasanas nourish the tissues of the mother and the baby in gentle way.While giving Yoga sessions to pregnant women researcher came across a major problem of muscles cramps among them especially in the third trimester. After studying the problem, copper charged water was given to them for one month. Outcome of the research data was very optimistic and realistic. Researcher has mentioned and discussed those points in this manuscript.

Keywords: Pregnancy, gestation, cramps, yogasanas, copper charged water.

INTRODUCTION

Pregnancy triggers a wide range of changes in a woman's body leading to various musculoskeletal dysfunctions. Most commonly reported musculoskeletal discomforts by pregnant women are low back pain and symphysis pubis pain and cramps.The culture and the environmental factors may influence the discomforts experienced by a pregnant woman.In this paper we are going to discuss the reason for cramping during gestation especially in the third trimester. The researcher took 50 primigravidae in her experimental group of the same age group, economic status

and life style. They joined regular yoga sessions between 12 to 16 weeks of their conceiving. The sessions given to them were one hourly class, five days a week. Pregnancy brings day-to-day problems for the expectant. These problems keep on changing with the change in the trimester. Researcher observed that the expecting mothers faced a common problem of leg cramps especially in their third trimester.

Previous studies

Not much work has been done in past regarding cramping during pregnancy. Researchers have shown that the problem of the contractions of gastrocnemius muscle and the foot muscle increases considerably in the third trimester [1]. The table 1 clearly shows a considerable increase in the painful tetanic contractions of the various muscles in the third trimester.According to the study made by Kehner, during the latter part of pregnancy 75% women demonstrate a positive chvostek sign indicating a heightened neuro-muscular irritability [2].Researcher too observed the gravity of muscle cramps especially during the third trimester. Not many papers have been devoted on the cramping problem during pregnancy.

Etiology and treatment as per literature review

1. *Margaret Robinson*, 1947 – there was an accepted concept that cramps occurred due to the deficiency of calcium and hence it played a part in etiology. Margaret Robinson rejected this and proposed that the depletion of sodium chloride was the cause of cramps and replenishing of sodium chloride would give relief.
2. There was no specific reason given as etiology of cramps but quinine was advocated for the relief, but only in non-pregnant women. Quinine is prohibited during pregnancy hence this was no answer to the expecting mother's problem[3].
3. *Mendenhall, Drake & Northrup* – these researchers again established the view that calcium deficiency was relatively the cause of leg cramps in pregnancy and the treatment was replenishing it [4].
4. Another studies have shown various causes of cramping during late pregnancy:
    a) The round ligament pain – the infamous round ligament pain strikes pregnant women in their second and third trimester. As the uterus expands the ligaments stretch to support it. This leads to cramping.
    b) Pre-term labour – cramping, mild or severe diarrhea and back pain can be indicators of pre-term labour
    c) Braxton-Hicks contractions – they are irregular intermittent contractions that occur in third trimester
    d) Labour – during labour cramping and back pain are common symptoms [5].

The above-mentioned causes (a-d) are very different and they occur in the abdominal region. In this study we are focusing on the cramps in the other body parts especially legs and feet.In this paper we are going to discuss the role of the trace element or the micro nutrient copper in cramping during pregnancy especially in the third trimester. Researcher has tried to prove the etiology and has also copper charged water as the solution to the problem.

Role of copper in human body [6-9]

Copper plays a very important role in the human life [6]. Though a trace element or micro nutrient it is essential for maintenance, growth and development of bones, brain, heart, connective tissues and many other organs. Copper synthesizes and releases life-sustaining proteins and enzymes which in turn produce cellular energy, regulate nerve transmission, blood clotting and oxygen transport.Copper is also involved in the formation of red blood cells, absorption and utilization of iron, metabolism of cholesterol and glucose. Copper also stimulates the immune system to fight infections, to repair injured tissues and to promote healing. It also helps to neutralize free-radicals which can cause severe damage to cells [7,8]. A list of some key copper-containing enzymes and their functions is summarized in table 2[9].

Fetus health and copper

Copper is essential for the normal growth and development of the human fetus.The human fetus accumulates copper rapidly in its liver during the third trimester during pregnancy. At birth, a healthy infant has four times the concentration of copper than a full grown adult. As the human milk is low in copper, the neonates' liver stores of copper fall down rapidly after birth, as it supplies copper to the fast growing body [10].These supplies are necessary to carry out metabolic functions as cellular respiration, melanin pigment and connective tissue synthesis, iron metabolism, free-radical defense, gene expression and the normal functioning of heart and immune system.Severe deficiency of copper in pregnant women increases the risk of health problem and cramping is one of them [11]. While carrying out the ante-natal classes, researcher found a common complaint of cramps which would be giving too much discomfort to the expecting mother. Being a naturopath researcher advised them to consume copper charged water for one week [12].

MATERIALS AND METHODS

The materials and equipment used in the present study are very simple and easily available in every household, which include water, a copper utensil, and a small wooden table or stool.The subjects were asked to fill the copper container with one liter water and place it on a wooden table overnight.The wooden table or board was important as it worked as insulation and prevented the charge being earthed.The subjects were asked to consume one to two glasses early morning empty stomach.The rest they had to consume during the daytime.They were asked to drink this water for a fortnight daily.

RESULTS AND DISCUSSION

To the biggest surprise of the researcher, most of them did not get cramps just after two days of treatment. Rests of all were relieved of the cramps considerably within few days.

Interfering effect of silver

Once a subject of experimental group when advised for copper water said that she was taking it regularly but there was no relief in the frequency or gravity of cramps.This was surprising for the researcher as per her previous experience, where all the subjects were relieved of the problem after consuming copper charged water. After talking at length finally she remembered that her in-law used to put a silver coin in the copper container. And, she was consuming that water. Researcher immediately advised her not to put silver coin in the water. Subject when consumed that water without silver coin was relieved of the cramping problem.To confirm this phenomenon researcher advised the future subjects to add a silver coin in the copper jar of water. The result showed that there was no relief in the problem at all. The subjects were then advised to avoid silver coin and consume the copper charged water. The results were fantastic. Cramping was relieved and the frequency became negligible.

The above experiment shows that copper is a very important micro-nutrient for the human body and is very essential for the fetal and infant development. As the human milk is low in copper nature does the preparation and the fetus starts storing copper in its liver nearly four times the concentration of adult human body. This depletes the copper very fast from the expecting mother hence creating a deficiency in her body.This leads to cramping. When this deficiency was replenished through copper-charged water, the cramping reduced remarkably.Silver is considered cool by nature and copper is considered warm according to Ayurveda. Hence, according to researcher, the interference of silver neutralized the effect of copper and the result becomes nil.

CONCLUSION

Researcher draws the conclusion that copper-charged water should be advised to the expecting mothers in order to get rid of the cramping problem especially in the third trimester.

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Table-1.Prevalence of musculo-skeletal dysfunctions across trimester in percentage [1]

Types of cramps	First trimester	Second trimester	Third trimester
Calf cramps	26.7%	47.8%	64.6%
Foot muscle cramps	3.3%	9.3%	15.1%
Trapezius spasms	0.00%	1.5%	1.7%

Table-2.Copper-containing enzymes and their functions [9]

Enzymes	Function
Amine oxidases	Group of enzymes oxidizing primary amines (e.g., tyramine, histidine and polylamines)
Ceruloplasmin (ferroxidase I)	Multi-copper oxidase in plasma, essential for iron transport
Cytochrome c oxidase	Terminal oxidase enzyme in mitochondrial respiratory chain, involved in electron transport
Dopamine β-hydroxylase	Involved in catecholamine metabolism, catalyzes conversion of dopamine to norepinephrine
Hephaestin	Multi-copper ferroxidase, involved in iron transport across intestinal mucosa into portal circulation
Lysyl oxidase	Cross-linking of collagen and elastin
Peptidylglycine alpha-amidating mono-oxygenase (PAM)	Multifunction enzyme involved in maturation and modification of key neuropeptides (e.g., neurotransmitters, neuroendocrine peptides)
Superoxide dismutase (Cu, Zn)	Intracellular and extracellular enzyme involved in defense against reactive oxygen species (e.g., destruction of superoxide radicals)
Tyrosinase	Enzyme catalyzing melanin and other pigment production

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RESEARCH ARTICLE

Seasonal Variations of Physico-chemical Parameters of Groundwater of Manora Taluka, Dist-Washim, M.S. in Relation to its Drinking Standards

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ABSTRACT

Groundwater is required for continuity of life and sustainability of ecosystem. Hence, this study aimed at assessing the groundwater quality in Manora Taluka, Dist-Washim,M.S.India.Groundwater samples were collected from 4 different sampling spots of Manora Taluka and assessed for various physico-chemical parameters including pH, Total Dissolved Solids, Free CO_2, Dissolved Oxygen, Total Alkalinity, Chlorides, Total Hardness, Magnesium content, Sulphates, Phosphates and Nitrates. The observed values of physico-chemical parameters were compared with the WHO water quality standards. Studies of these physico-chemical characteristics indicate that in some of the studied samples, water was contaminated and not suitable for drinking purpose. The drinking water of the groundwater sources needs some degree of treatment before consumption and preventive steps need to be taken to stop its contamination.

Keywords : Groundwater quality, Seasonal variations, Physico-chemical analysis, drinking standards.

INTRODUCTION

Groundwater is an important natural source of water supply all over the world. Safe potable water is absolutely a good source of fresh water available on the earth. Groundwater is ultimate and most suitable fresh water resource for human consumption in both urban as well as rural areas. Water sources available for drinking and other domestic purposes must possess high degree of purity, free from chemical contamination and micro-organisms. There are several states in India where more than 90% populations are dependent on groundwater for drinking and other

purpose (Ramchandraiah, 2004). In India, there are over 20 million private wells in addition to the government tube wells (Datta, 2005). The wells are generally considered as the worst type of ground water sources in the term of physico-chemical contamination due to lack of concrete plinth and surrounding drainage system (WHO, 1997). Most of the industries discharge their effluent without proper treatment into nearby open pits or pass them through unlined channels, resulting in the contamination of groundwater (Jinwal and Dixit, 2008). The wastewater is highly viscous with high suspended solids and total dissolved solids. Therefore, pollution of water resources needs a serious and immediate attention through periodical checkup of water quality. The present work makes an attempt to carry out qualitative analysis of some physico-chemical parameters of groundwater in study area.

MATERIALS AND METHODS

Washim is one of the 11 districts of Vidarbha Region of Maharashtra, India. The entire district occupies an area of 5196 sq. km. It is situated in the North latitudes $19^0$59' & $21^0$16' and East longitude $76^0$07' & $77^0$14'. The district is divided into 6 Talukas. It has a total population of 10,19,000 as per 2001 census. The district has 789 villages, 4 Nagar Parishads, 6 Panchayat Samitis and 493 Gram Panchayats.Manora Tehsil of Washim District, M. S. India is situated above 1318 feet of mean sea level. For groundwater assessment, 4 sampling spots of Manora Tahsil i. e; Spot 1 as Manora (Injori), Spot 2 as Manora (Dapura), Spot 3 as Manora (Bhoyni), Spot 4 as Manora (Kupta) were selected. From the groundwater sources, water samples were collected seasonally i. e, in Monsoon 2012, winter 2012 and summer 2013 for the physico-chemical analysis to check its potability. Collection of water samples were done during early morning hours. The physico-chemical parameters were analysed with the standard methods given by APHA (1998) as illustrated in Table 1.

RESULTS AND DISCUSSION

The average results of the physico-chemical parameters for groundwater samples are presented in Table 2, Fig. 1. and Fig. 2.

pH: In the present investigation, the maximum value of pH was found as 8.00 at spot no. 4 of Manora Tehsil i. e, Kupta near bus stop in Summer 2013. This value of pH is near the permissible limit (6.5-8.5) as suggested by WHO (2004). It may be due to the percolation of sewage disposal containing carbonate and bicarbonate substances from very big drain through cracks of hand pump in respective groundwater. Our findings are in conformity with the results of Rao *et. al*., (2012) while carrying out physico-chemical analysis of water samples of Nujendla area in Guntur District, Andhra Pradesh, India.

TDS: Maximum value of TDS in the present investigation was found at spot no. 4 as 937.23 mg/l of Manora Tehsil i. e, Kupta near bus stop which is very close to permissible limit i. e, 1000 mg/l as suggested by WHO (2004). Our results are parallel with Ezeribe et al., (2012) who reported that high value of TDS at some location could be due to differences in organic matter of poorly constructed septic tank residing nearby it that remains as residue in the groundwater of that locality.

Total Alkalinity: Throughout the investigation, the highest value of total alkalinity was found at spot no. 3 of Manora Tehsil in Summer 2013 as 552.35 mg/L at Bhoyni which is located near Krishi seva Kendra. This value of total alkalinity is much above the permissible limit i. e, 200 mg/L as suggested by WHO (2004). Similar results were found by Prajapati and Raol (2010) who also found highest total alkalinity values during summer months which were due to absence of the hydroxide and carbonate ions in the water samples and disposal of surrounding garbage. They further observed that bicarbonate ions and free CO_2 were present in higher concentration during summer months and these two important factors could be reason for contributing to higher total alkalinity.

Chloride:The highest value of chloride throughout the study period was found at spot no. 2 as 230.21 mg/L in Manora Tehsil in Winter 2012 at Dapura, located near bus stop. In the present study, the chloride value is near the permissible limit which is 250 mg/L as suggested by WHO (2004). Similar trend of chloride was observed by Jha and Verma (2000) who found that high concentration of chloride occurrence may be due to the invasion of domestic wastes, garbage effluents and disposals by human activities.

Free CO_2 : The highest free CO_2 value throughout the investigation was recorded as 42.17 mg/L at spot no. 2 of Manora Tehsil at Dapura near bus stop in Summer 2013 which is near bus stop and it is above the permissible limit i. e, 22 mg/L as suggested by WHO (2004). However, Prajapati and Raol (2010) found higher free CO_2 values during summer months. They reported that free CO_2 is accumulated in the water due to microbial activity and respiration of organisms. It means that microbial activity and respiration might be higher during the summer.

Total Hardness: In the present investigation, the highest value of total hardness was found at spot no. 2 of Manora Tehsil i. e, Dapura near bus stop in Summer 2013 i. e, 582.21 mg/L which is above the permissible limit i. e, 500 mg/L as suggested by WHO (2004). The source of groundwater studied in the present investigation is handpump located in Murtizapur, Panchashilnagar and polluted by garbage effluents. Similar trend of total hardness was observed by Olufemi et al., (2010) who found that the high values of total hardness may be due to the introduction of polyvalent cations into the groundwater system.

Sulphate: The highest value of sulphate throughout the investigation was recorded as 2.431 mg/l at spot no. 2 of Manora Tehsil in Winter 2012 which is within permissible limit i. e, 500 mg/l as suggested by WHO (2004). Our results of sulphates are in conformity with Majolagbe et al., (2011) who also found low value of sulphate, less than permissible limit. Such low value shows that the underground under review are free from possible sulphate toxicity which includes gastrointestinal irritation. The low level of sulphate could be as a result of microbial action capable of reducing SO_4^{2-} to S^- leading to depletion of sulphate in study areas.

Total Phosphorous: The highest value of total phosphorous was found as 9.134 mg/l at spot no. 4 at Kupta of Manora Tehsil in Summer 2013 which is within permissible limit i. e, 800 mg/l as suggested by WHO (2004). Similar results were found by Prasath *et al*., (2013) who also observed the higher phosphate values during monsoon months which were due to rain, surface water runoff, agriculture runoff, garbage effluents, washer man activity, which could have also contributed to the inorganic phosphate content.

Nitrate: The highest value of nitrate throughout the investigation was recorded as 3.515 mg/l at spot no. 2 of Manora Tehsil i. e, Dapura near bus stop in Winter 2012 which is within the permissible limit i. e, 50 mg/l as suggested by WHO (2004). Our results are in conformity with Connelly and Taussiq (1994) who found high values of nitrate observed at some stations due to the infiltration of pollutants from various sources like industries and also percolation of household sewage and presence of improper septic tanks in that sites.

CONCLUSION

The groundwater quality was studied in Manora Tehsil of Washim District M. S. India. In general, water samples from Spot 1 and 4 were exceeding the maximum limit in various parameters as compared to WHO drinking water standards. It is suspected that this may be due to indiscriminate disposal of domestic wastes. The groundwater of these spots needs some degree of treatment before drinking and it needs to be protected from contamination so as to prevent adverse health effects on human beings.

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Table 1: Analysis of Water Quality Parameters

Sr. No.	Water Quality Parameters	Method of Determination	Method By
1	Hydrogen ion concentration (pH)	pH metry	APHA, 1998
2	Total Dissolved Solids (TDS)	Evaporation method	APHA, 1998
3	Total Alkalinity	Titrimetry	APHA, 1998
4	Chlorides	Titrimetry	APHA, 1998
5	Dissolved Oxygen	Titrimetry	APHA, 1998
6	Free Carbon dioxide	Titrimetry	APHA, 1998
7	Total Hardness	EDTA-Titrimetry	APHA, 1998
8	Calcium (ca++)	EDTA-Titrimetry	APHA, 1998
9	Magnesium	EDTA-Titrimetry	APHA, 1998
10	Sulphate	Spectrophotometry	APHA, 1998
11	Phosphate	Spectrophotometry	APHA, 1998
12	Nitrate	Spectrophotometry	APHA, 1998

Table 2: Seasonal analysis of various physico-chemical parameters of groundwater of karanja tahsil of selected sampling spots during 2012-13.

Sr. No.	Physico-chemical Parameters (mg/l)	WHO Limit	Monsoon (2012)				Winter (2012)				Summer (2013)			
			Spots				Spots				Spots			
			1	2	3	4	1	2	3	4	1	2	3	4
1	pH	6.5-8.5	7.27	6.40	7.42	6.50	6.37	7.51	6.67	6.27	6.63	7.71	7.05	8.00
2	TDS	1000	747.06	937.11	831.13	721.04	583.09	621.14	733.35	685.37	655.10	433.17	789.19	937.23
3	Free CO2	22	14.12	15.17	27.09	31.06	23.09	19.14	21.21	29.28	38.11	42.17	32.19	38.31
4	Dissolved Oxygen	5-7	11.39	13.47	16.25	19.53	15.17	18.25	22.15	9.29	9.27	12.53	8.55	10.65
5	Total Alkalinity	200	373.20	430.17	457.24	437.05	437.29	541.22	467.47	415.44	546.17	532.25	552.35	508.35
6	Chlorides	250	81.09	140.06	75.03	70.14	97.26	230.21	100.15	141.29	137.23	193.29	153.37	167.21
7	Total Hardness	500	311.13	421.19	343.10	300.08	383.12	415.24	391.30	313.10	472.15	582.21	488.31	438.37
8	Calcium Hardness as CaCO3	100	273.10	261.03	157.15	183.17	181.09	310.03	185.01	210.05	258.35	405.40	222.73	300.50
9	Calcium Hardness as Ca++	100	93.15	87.10	51.17	73.13	53.10	110.03	57.13	83.07	103.50	162.44	89.30	120.54
10	Magnesium Content	150	9.24	38.92	45.18	28.41	49.09	25.57	50.13	25.04	51.95	42.96	64.53	33.50
11	Sulphates	500	1.130	1.387	1.213	0.633	1.351	2.431	1.561	1.311	1.430	1.370	1.930	1.230
12	Total Phosphorous	800	0.215	0.347	0.757	0.685	5.831	3.635	7.713	4.373	7.314	7.650	6.453	9.134
13	Inorganic Phosphorous		0.003	0.001	0.002	0.001	3.219	1.263	5.353	2.515	0.653	0.981	0.123	0.817
14	Organic Phosphorous		0.212	0.346	0.755	0.684	2.612	2.372	2.36	1.858	6.661	6.669	6.33	8.317
15	Nitrates	50	1.883	1.137	2.471	1.475	2.467	3.515	1.531	2.715	1.770	1.510	1.350	1.830

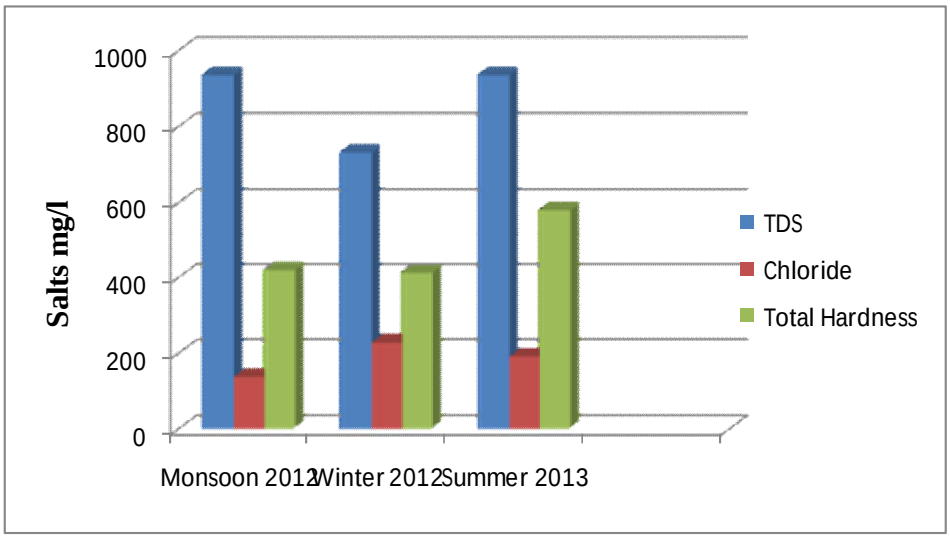

Fig 1: Seasonal variations of TDS, Chloride and Total Hardness in Manora Taluka.

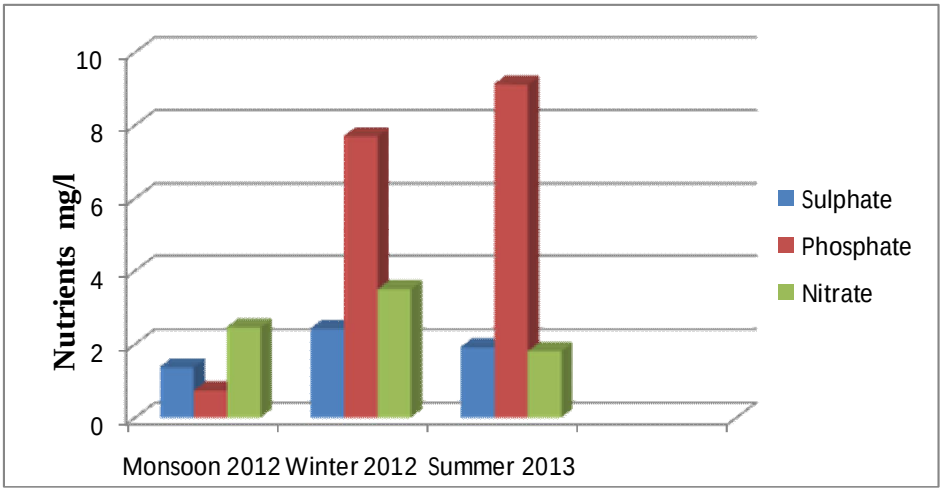

Fig 2: Seasonal variations of Sulphate, Phosphate and Nitrate in Manora Taluka.