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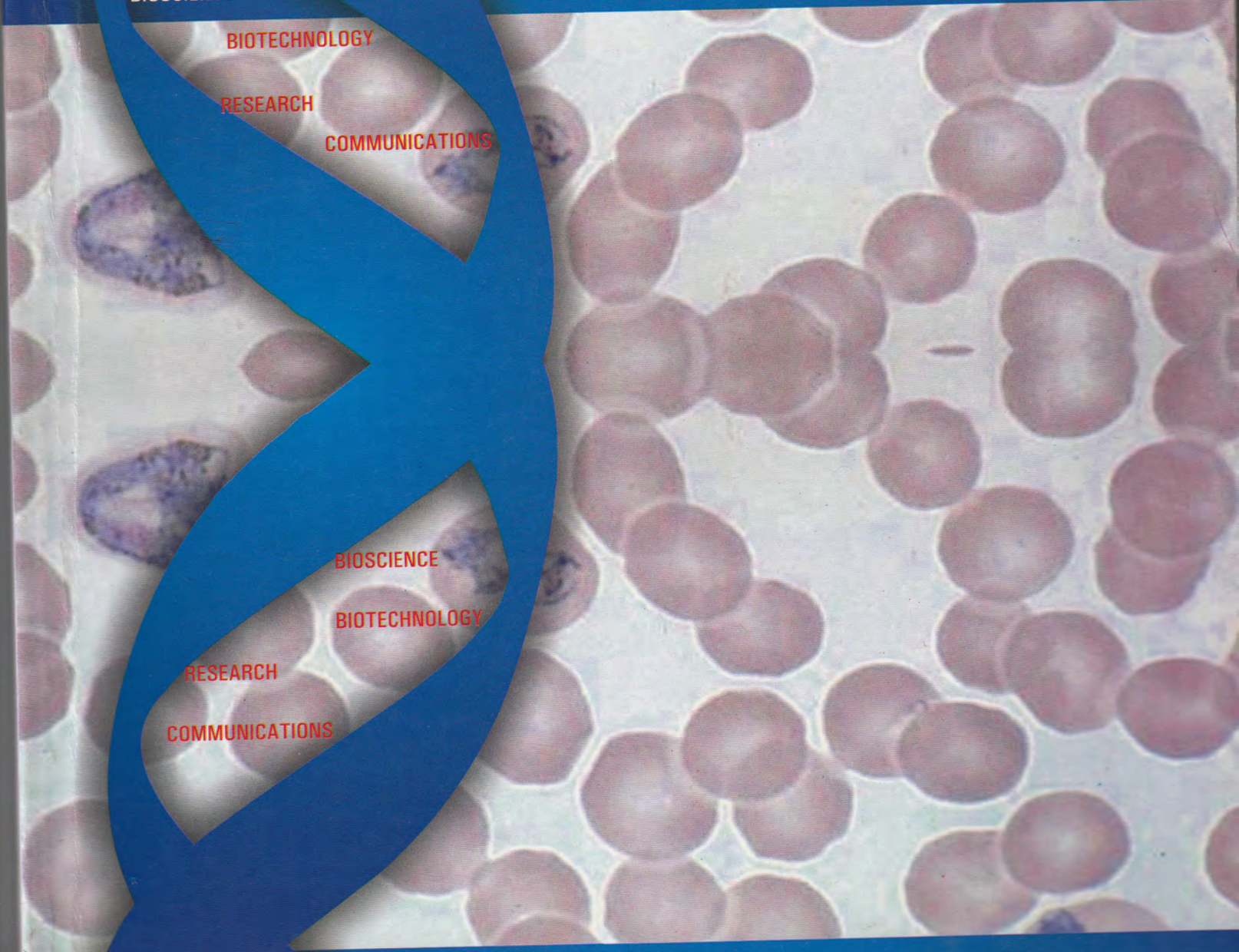
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EDITORS COMMUNIQUE

Heartiest greetings to you all!

It is indeed a great privilege and honor to don the mantle of Editor of Bioscience Biotechnology Research Communications, an international biannual journal, which will try to make an impact on advancement of science and technology, particularly in basic and applied areas of life sciences .With India's strong research bull on a move, though with a lot of goading, good journals have a vital role to play for taking science ahead. Young researchers particularly students of colleges, universities and R&D institutions have a long way to go to really compensate for the country's paradox of huge scientific manpower sans quality.

Over the last 25 years great accomplishments have floored us, both economically and scientifically, areas of applied sciences have leapt and unleashed their power which has to be maximally tapped and used judiciously. But the warnings and premonitions of our scientific advisers who have toiled hard as research students must be making them uneasy as the current trend of shortcuts to fame and name is jeopardizing the progress of science and mankind.

The present decline of interest in basic sciences for the shining lures is worrying, if the new generation is not encouraged and molded in the old cast of sincerity, honesty and commitment which are the hallmark of basic research, the future of science will be bleak. Is our science education at the foundation levels worthy of facing the challenges of future? Are our universities colleges and research institutions on the right track? Are we encouraging our teachers and the taught? Are our scientific policies of fund managing accountable and productive? Are we satisfied ethically and morally? The questions are unending but we hope there will be the right answers at the right time and Indian science will be a forerunner, as it has been.

A huge nation with an awakened democracy will not remain a mute spectator and the youth will take it to dazzling heights. The young BBRC team will try to do its job well of course with the help and cooperation of the scientific fraternity and create its own niche.

The first issue though in haste must be subjected to healthy criticism to make it better. A couple of special pages for scientific correspondence and a section for young investigators will certainly become a lively part of this new journal to encourage them. In this issue there are two communications of graduate and post-graduate students.

Thanks once again in anticipation of your magnanimous academic cooperation.

Amicably yours

Dr. Sharique Ali

(Chief Editor, Bioscience Biotechnology Research Communications)

Recent Laboratory Techniques for Diagnosis of Bacterial Meningitis.

Manoj K. Patel[1], Suman[2] and Ashok Kumar[1,*]

[1]Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India
[2] Amity Institute of Advance Research and Studies (Material and Devices), Amity University Sector-125, Noida- 201303, India. e-mail: ashokigib@rediffmail.com

Abstract

Meningitis is an infection of the fluids in a spinal cord and of the fluids that surrounds the brain causing damage of meninges. It can be caused by either viral or bacterial infection. Viral meningitis is less severe and resolves without specific treatment while bacterial meningitis can be severe and may results brain damage and finally death. Therefore, early diagnosis and treatment are very important in meningitis. In this paper different serogroups, virulent genes causing bacterial meningitis, recent laboratory diagnostic techniques and biochemical methods which are mostly used in different labs have been described. PCR and recent development of DNA biosensor for meningitis can play important role for rapid diagnosis and save lives of several people infected with *Neisseria meningitidis.*

Keywords: Bacterial meningitis, DNA biosensor, *Neisseria meningitidis,* Meningitis diagnosis

Introduction

Neisseria meningitidis is one of the major causes of bacterial meningitis in human beings, it is a serious infection of the fluids in the spinal cord and brain, which affects the membrane (meninges) surrounding the brain. Meningitis is also caused by a virus which is usually mild and clear within one or two weeks. Viral meningitis is also called aseptic meningitis. Bacterial meningitis is more serious and causes severe brain damage and even death (Pahari *et al.,* 2004). Meningitis caused by *Neiseria meningitidis* is also called meningococcal meningitis. Bacterial meningitis is mostly caused by three types of bacteria *i.e. Haemophilus influenza* type b (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae*. Due to immunization in children Hib disease occurrence is reduced whereas other two bacteria are the leading causes of bacterial meningitis which is found worldwide. The bacterial infections are spread by direct close contact with the discharge from the nose or throat of an infected person. The bacteria live harmlessly in person's mouth and throat. In rare instances, they can break through the body immune defense and travel to the fluids surroundings the spinal cord and brain .There they begin to multiply quickly causing their membrane that covers the spinal cord and brain (called meninges) becomes swollen (inflamed) leading to the classic symptoms of bacterial meningitis such as high fever, headache and stiffneck.These symptoms develop in 1 or 2 days. Other symptoms may be nausea, vomiting, light sensitivity, confusion and sleepness. Pathologically, diagnosis and confirmation is based on the isolation of *Neisseria meningitidis* from cultures (Tseng *et al.*, 2004). The majority of the patients with bacterial meningitis may survive, but neurological sequelae occur in as many as one-third of all survivors especially newborns and children (Gray and Fedorko, 1992). Bacterial meningitis constitutes a significant global public health problem, in both developed and developing countries. Meningococcal infection is present as an endemic and an epidemic disease. This disease is manifested not only as meningitis but also as meningococcemia. The global persistence of *Neisseria meningitidis* is due to the significant number of carriers and the dynamics of transmission and disease. Multiple factors have been identified that predispose to the transmissibility of *Neisseria meningitidis*. Upper viral respiratory tract infections, drought seasons and over crowding. Meningococcal disease was described by Vieusseux in 1805 during an outbreak and 33 deaths in the vicinity of Geneva, Switzerland (Vieusseux *et al.,* 1805). The Italian pathologists Marchiafava and Celli first described intracellular oval micrococci in a sample of cerebro spinal fluids (CSF). However, Anton Weichselbaum in 1887 first identified bacterium causing meningococcal disease in the CSF of six of eight patients of bacterial meningitis and the bacterium was named *Neisseria*-intracellular is (Marchiafava *et al.*, 1884). *Neisseria meningitidis* causes a disease spectrum ranging from occult sepsis with rapid recovery to fulminant disease. Before the 1920s, meningococcal disease was fatal in 70 % cases (Flexner, 1913). Serum

therapy with serum from immunized horses, introduced at the beginning of this century by Jochmann in Germany and Flexner in the United States, could reduce mortality from nearly 100% to 30%. The discovery of sulfonamides and other antimicrobial agents led to a further decline in the case fatality rates. Despite treatment with appropriate antimicrobial agents and optimal medical care, the overall case fatality rates have remained relatively stable over the past 20 years, at 9 to 12%, with a rate of up to 40 % among patients with meningococcal sepsis (Rosenstein, 2001). 11 - 19% of Survivors of meningococcal disease have sequelae, such as hearing loss, neurological disability (Manchanda *et al.*, 2006).

Meningococcal infections occur worldwide as endemic disease (Schwartz *et al.*, 1989, Achtman, 1995, Caugant, 1998) capsular polysaccharides provide the basis for grouping the organism. Twelve serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W135). The most important serogroups associated with disease in humans are A, B, C, Y, and W135. Serogroups A, B and C account for most cases of meningococcal disease throughout the world, with serogroups A and C predominating throughout Asia and Africa and serogroups B and C responsible for the majority of cases in Europe and the America (Schwartz *et al.*, 1989, Achtman, 1995, Caugant, 1998, WHO, Rosenstein, 1999). The numbers of cases of serogroup Y has reported in United States (Connolly and Noah, 1999) and only 4% cases of serogroup W-135 was reported in United States. In India serogroup A is associated with all the repeated outbreaks of meningitis during both epidemic and endemic periods, whereas serogroup B and C were also detected in a few sporadic cases (Ayyagari *et al.*, 1987, Paul *et al.*, 1987, Ichhpujani *et al.*, 1990, Verheul *et al.*, 1993, Suri *et al.*, 1994). *Neisseria meningitidis*, a gram negative bacterium is surrounded by outer membrane composed of lipids, protein and lipopolysaccharides (LPS). All known group A strains have the same protein serotype antigens in the outer membrane. Another serotyping system exists based on the antigenic diversity of meningococcal lipooligosaccharide (LOS). Meningococcal meningitis occurs both sporadically (mainly groups B and C meningococci) and in epidemics (mainly group A meningococci), with the highest incidence during late winter and early spring. Whenever group A, strains become prevalent in the population, the incidence of meningitis increases markedly (Todar, 2005).Meningococcal disease is endemic in Delhi and sporadic cases of meningococcal meningitis have been occurring in the past. An outbreak of pyogenic meningitis occurred in western part of India (Surat, Gujrat) during 1985-87 (Bhavsar *et al.*, 1989).The outbreak of meningitis is mostly occur in the spring and winter season. In Delhi till June 2008, 138 meningitis cases were reported out of which 14 were died, which is less than the year 2007 (25 death out of 129), year 2006 (34 death out of 486) and year 2005 (60 death out of 441) (Fig.1.).

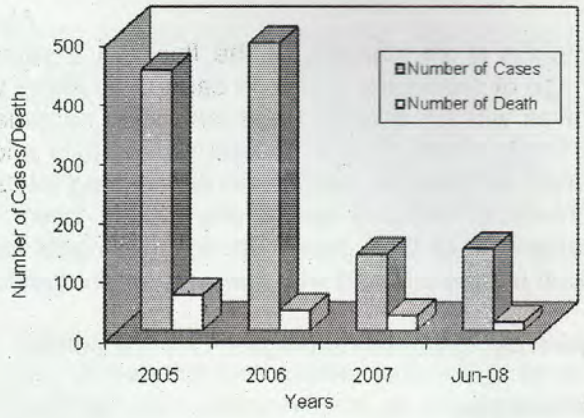


Fig.1. Meningitis cases reported in Delhi

Pathogenic *Neisseria meningitidis* possesses several surface-expressed adhesions. The type IV pilus mediates initial interactions with host cells. The opacity proteins Opa and Opc are phase-variable outer membrane proteins believed to play a role in later stages of adhesion and invasion, and enable adhesion in the absence of pili. The lipopolysaccharide (LPS) molecules display variation in side chains; the unsialylated forms of LPS might contribute to adhesion by binding to asialoglycoprotein receptors. The polysaccharide capsule is believed to inhibit adhesion to cells, possibly due to negatively charged molecules such as sialic acid that repel host cells or by masking the exposure of other surface adhesins (Anne *et al.*, 2004). Early diagnosis and treatment are very important. Bacterial meningitis can be treated with a number of effective antibiotics. It is important to start treatment as early as possible. Penicillin is the drug of choice to treat meningococcemia and meningococcal meningitis. Although penicillin does not penetrate the normal blood brain barrier, it readily penetrates the blood brain barrier when the meninges are acutely inflamed.Chloramphenicol or cefotaxime or ceftriaxone is used in persons allergic to penicillin. Meningococcal disease is contracted through association with infected individuals, as evidenced by the 500 to 800 fold greater attack rate among household contacts than among the general population, because such household members are at high risk, they require chemoprophylaxis.

Sulphonamides were the chemoprophylactic agent of choice until the emergence of sulphonamide-resistant meningococci. At present, approximately 25 percent of clinical sample isolates of *Neisseria meningitidis* in the United States are resistant to sulphonamides. Now a day, rifampicin is the chemoprophylactic agent of choice (Todar, 2005).The early prevention of sporadic meningococcal disease is antimicrobial chemoprophylaxis of close contacts of infected persons. Mass chemoprophylactic programs are not recommended to control large outbreaks of disease. Disease can be prevented by the eradication of the carrier status in subjects likely to harbor virulent meningococci, e.g., housemates of an index patient. Thus, effective control of emergence of secondary cases is attributed to effective chemoprophylaxis in household members, contacts at day care centers and anyone else directly exposed to an infected patient's oral secretions, e.g., through kissing or mouth-to-mouth resuscitation. Chemoprophylaxis should be administered to close contacts as soon as possible (ideally within 24 hours after identification of the index patient). Given more than 14 days after the onset of disease, chemoprophylaxis has no benefit. Rifampin, ciprofloxacin, ofloxacin, minocycline, cefixime, sulfadiazine and ceftriaxone are all 90-95% effective in reducing nasopharyngeal carriage of *Neisseria meningitidis* and are acceptable alternatives for chemoprophylaxis (Table 1). Meningococcal Routine vaccination is not currently recommended because the risk of infection is low (Manchanda *et al.*, 2006). The group B capsular polysaccharide is a homopolymer of sialic acid and is not immunogenic in humans. A group B meningococcal vaccine consisting of outer membrane antigens has developed. Vaccine potential of meningitis virulent genes are listed in the Table 2. At present meningococcal polysaccharide vaccines against serogroups A, C, Y and W135 have been commercially available (Verheul *et al.,* 1993). The vaccines are highly effective in preventing disease in adults and older children. They are also effective in controlling epidemics in India. However, these vaccines have several short comings that preclude their incorporation into routine immunization programs or the expanded programme on immunization. They have limited efficacy in young children, the group most at risk of disease. They do not provide long lasting protection. Polysaccharide vaccines may not protect against all forms of carriage. They may not protect against nasopharyngeal carriage. Repeated doses of the capsular polysaccharide have been associated with hypo responsiveness. For these reasons, polysaccharide vaccines have been used for control of epidemics, or for use in travelers to areas of the world where disease is present. In India the repeated vaccination of the broad target age group is very difficult. A conjugate vaccine that provides longer term protection at all ages.

Laboratory techniques for diagnosis of meningitis

Bacterial meningitis is one of the serious infectious diseases of children and epidemic cause devastating impact on entire populations. Diagnosis of bacterial meningitis presented in this review includes culturing CSF, methods of detecting bacterial antigens and bacterial components in CSF (latex agglutination), use of the polymerase chain reaction and testing CSF for bacterial antigens (Gray and Fedorko, 1992). The laboratory investigation of suspected cases is critical for the early detection of epidemics and evaluating the impact of immunization services. Routine bacterial meningitis surveillance focuses on meningitis and other "invasive" infections such as sepsis, which can be diagnosed with microbiological tests on cerebrospinal fluid (CSF) and blood. In laboratory diagnosis of bacterial meningitisdepends on the examination of CSF (Table3). The identification of type of bacteria responsible for meningitis is essential for correct selection of antibiotic treatment. Bacterial meningitis is usually confirmed by three methods: (1) Culture method: isolation of a bacterial pathogen from a normally sterile clinical specimen such as CSF. (2) Antigen detection methods: identification of a bacterial antigen CSF by latex agglutination or counter immuno electrophoresis. (3)Gram stains results. The most conventional tests carried out in laboratory are as follows:

Bacterial culture and oxidase test

The diagnosis is usually made by growing bacteria from sample of CSF. The CSF is obtained by inserting a needle into the lower back and removes some fluid from the spinal cord.CSF samples from patients were inoculated onto Muller Hinton (MH), Chocolate Agar (CA) plates containing 10% sterile defibrinated sheep blood. The agar plate placed into candle jar (5% CO_2) and incubated at 37 ^{0}C for 48 h. After 48 h brown colonies were collected from CA and tested for oxidase production by spreading the colonies on a piece of filter paper soaked with the reagent tetramethyl-p-phenylene diamine.The development of purple color was recorded as a positive reaction (Table 4). Colonies collected from CA was also Gram stained. The presence of Gram negative diplococcus along with a positive oxidase test confirmed the presence of *Neisseria meningitidis* in CSF (Filippis *et al.*, 2005).

Catalase test

Catalase is an enzyme that decomposes hydrogen peroxide (H_2O_2) into oxygen and water. Catalase is a haemoprotein, similar in structure to haemoglobin, except that the four iron atoms in the molecule are in the oxidized (Fe^{2+}) rather than the reduced (Fe^{3+}) state. Excluding the streptococci, most aerobic and facultaitive anaerobic bacteria possess catalase activity. Most anaerobic that decomposes H_2O_2 with peroxidase enzyme work in a similar to the catalase except only one ferric ion is contained per molecule. *Neisseria meningitidis* is catalase positive (Table 4).

Rapid acid detection test

Patterns of acid production from the carbohydrates (glucose, maltose, lactose, sucrose and fructose) are used to identify *Neisseria* and related species. In contrast to most bacteria which produce acid by a fermentative pathway, *Neisseria* species produce acid by an oxidative pathway. This is an important distinction because more acid is produced by fermentation than by oxidation. In addition to producing less acid from carbohydrates, many *Neisseria* species produce ammonia from peptone which may neutralize the acid produced from carbohydrates. "Acid detection' is probably the most accurate term for describing the results obtained in tests designed to detect acid production from carbohydrates. The terms 'carbohydrate utilization' and 'fermentation' should not be used to describe acid production by *Neisseria* species.

Several rapid tests have been developed to detect acid production from carbohydrates by *Neisseria* species. These tests depend on enzymes present in heavy suspensions of the microorganism to produce detectable acid from carbohydrates. Phenol red is used as the pH indicator in most rapid acid detection tests for *Neisseria* sp. Organisms which produce acid from carbohydrates will produce sufficient acid to exceed the buffering capacity of the substrate medium and cause a color change from red (alkaline) to yellow (acid).

Nitrate reduction test

In human *Neisseria* and related species such as *Neisseria mucosa*, *Moraxella catarrhalis*, and *Kingella denitrificans* reduce nitrate. The nitrate reduction test is a critical test for differentiating between *Neisseria gonorrhoeae* and *Kingella denitrificans*, particularly when strains of *Kingella denitrificans* appear to be gram-negative diplococci in stained smears. *Neisseria meningitis* shows nitrate reduction test negative (Table 4).

Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. Among the *Neisseriaceae* of Human origin, strains of *Neisseria mucosa*, *Moraxella catarrhalis*, and *Kingella denitrificans* reduce nitrate. Strains of *Moraxella catarrhalis* and *Kingella denitrificans* have been misidentified as *Neisseria gonorrhoeae*. The nitrate reduction test permits differentiation between these species which are nitrate positive and *Neisseria gonorrhoeae and Neisseria meningitidis* (nitrate-negative). The reduction of nitrate may be coupled to anaerobic respiration in some species. The nitrate reduction test is based on the detection of nitrite and its ability to form a red compound when it reacts with sulfanilic acid to form a complex (nitrite-sulfanilic acid) which then reacts with alpha-naphthylamine to give a red precipitate (prontosil). A red color will be produced in the medium only when nitrite is present in the medium. Lack of a red color in the medium after the addition of sulfanilic acid and α-naphthyl amine means nitrite is not present in the medium.

Enzyme-substrate test

The production of three enzymes-a glycosidase (β-galactosidase) and two amino peptidases (γ-glutamyl aminopeptidase and hydroxyl prolylaminopeptidase) have been used to differentiate between *Neisseria* and related species isolated on selective media. Although this test is easy to perform, nongonococcal isolates including *Neisseria* species and *Kingella denitrificans*, which may grow on selective media, may be misidentified as *Neisseria gonorrhoeae* if additional tests are not performed. Enzyme substrate tests should not be used as the only test for identifying *Neisseria gonorrhoeae* (Table 4). *Gonochek II* is an enzyme test kit that is designed to differentiate between *Neisseria lactamica*, *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Moraxella catarrhalis*. The enzymes produced by these species are detected in a single test by the production of colored end products from colorless substrates. β-galactosidase, produced by *Neisseria lactamica* strains, hydrolyzes 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (BCIG) to produce a blue-colored end product. *Neisseria meningitidis* produces γ-glutamyl amino peptidase which hydrolyzes γ-glutamyl-p-nitroanilide (GPNA) releasing a yellow p-nitro aniline end product. Hydroxy prolyl amino peptidase produced by *Neisseria gonorrhoeae* strains, hydrolyzes a β-naphthyl amino acid derivative (prolyl-4-methoxy naphthyl amide (PMNA) and releases a free β-naphthyl amine derivative which then complexes with a diazonium salt to produce a red-pink color. *Moraxella catarrhalis* has none of these enzymes; therefore, strains of *Moraxella catarrhalis* produce no distinct color change in this test.

Table 1. Antibiotics recommended in different age groups of meningitis patients.(Johri *et al.*., 2005).

Drug	Age	Dosage
Ciprofloxacin (Oral)	Adult	20 mg/kg, single dose
Rifampicin (Oral)	<1 month	5 mg/kg, twice a day for 2 days
	≥1 month	10mg/kg, twice a day for 2 days
	Adults	600 mg, twice a day for 2 days
Ceftriaxone	<15 years	125 mg, single dose, intramuscular
	≥15 years	250 mg, single dose, intramuscular
Oflaxacin (Oral)	Adults	400 mg, single dose
Azithromycine (Oral)	Adults	500 mg , given in a single dose

Table 2. Potential vaccines based on different virulent genes for meningitis.
(Verheul *et al.*, 1993, Segal and pollard, 2005,Roos *et al.*, 1997)

Virulent genes	Characteristics	Vaccine potential
PorA	Cation porin	Induces protective form
Por B	Anion porin	Induces immunity
Rmp M	Reduction modifiable protein	No protective response
Opa/Opc	Opacity proteins, involved in adhesion/invasion	Induces protective Ab
LbpA/Lbp B	Lactoferrin binding , Iron regulated	Induces protective Ab
Nsp A	Immunogenic surface protein A	Induces protective Ab

Table 3. Characteristics of cerebrospinal fluids(CSF) in different types of meningitis

Diseases	Micro-organisms	Physiological appearance	Cell concentration (Cells/$mm^{3)}$	Microbiological study	Glucose
Normal	-	Clear and colorless	0-5 lymphocytes	Sterile	2.9-3.9 mmol/L
Bacterial meningitis	*Neisseria meningitis, Haemophilus influenza, Streptococcus pneumoniae*	Turbid	500-20000, mainly polymorphs few lymphocytes	Bacteria in gram stain deposit, Growth on culture, PCR	Reduced or absent
Viral meningitis	Enteroviruses, Mumps virus	Clear /slight turbid	10-500, mainly lymphocytes	Viruses rarely isolated from CSF, Diagnosis by stool culture (enteroviruses) , or serology (mumps),PCR	Normal
Tuberculous meningitis	*Mycobacterium tuberculosis*	Clear/slight turbid	10-500, mainly lymphocytes,Poly-morphs in early stages	AAFB in ZN-stained deposit - often scanty, Growth on LJ culture, PCR	Usually reduced
Cerebral Abscess	*Streptococcus milleri,Bacteroides sp., Staphylococcus aureus, Proteus sp.*	Clear /slight turbid	0-500, mainly polymorphs some lymphocytes	Organism mostly absent in CSF	Normal

(AAFB: Acid and alcohol-fast bacilli, ZN: Ziehl - Neelsen, L J: Lowenstein-Jensen.)

Table 4. Biochemical tests for the identification of *Neisseria meningitidis*

Tests	Results
Cell morphology	Gram negative, diplococcus
Colony morphology	Smooth, circular, whitish
Pigmentation	Pink, brown
Oxidase	(+) ve
Enzyme-substrate test	(+) ve
Nitrate reduction Test	(-) ve
Polysaccharide	
DNase production	(-) ve
Superoxol Test	Variable
Catalase Test	(+) ve
Colistin antibiotic	Resistant
Glucose	(+) ve
Maltose	(+) ve
Sucrose	(-) ve
Fructose	(-) ve
Lactose	(-) ve

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Role of Tumor Markers in Cancer.

Govind Pandey[1*] and Madhuri S.[2]

[1]In-charge of Rinder Pest (Animal Husbandry Dept., Govt. of M.P.), Jabalpur Division, Jabalpur
[2]Senior Research Fellow of C.S.I.R., Department of Zoology & Biotechnology,
Model Science College Jabalpur- 482002 (M.P.)
***Corresponding author:** 157/1, North Milloniganj, Jabalpur- 482002 (M.P.)
E-mail: drgovindpandey@rediffmail.com; drmadhurig8@yahoo.co.in

Abstract:

Cancer (malignant tumor) is the second leading cause of human deaths in the world and claims over 6 millions annually..The diagnosis of cancer is also very critical at its early stages. Hence, in the recent years, various tumor markers have been used in the diagnosis, management and treatment of cancer. Tumor markers are biochemical indicators of the presence of tumors, selectively produced by the neoplastic (cancerous) tissues. They are released into blood or in other body fluids. Monoclonal antibody technique is the most commonly used method for identification of specific marker in tissue, urine or blood sample. Reversed transcriptase and polymerase chain reaction and immunohistochemistry are also commonly used methods in various tumor assays. Current tumor markers include glycoproteins (AFA and CEA), hormones (ACTH and HCG), enzymes (PSA and PAP), mucins and other molecular species. Proper utilization of serum marker facilitates an evidence-based approach to medicine in cancer therapy. Tumor marker tests are generally not diagnostic, but they help in the diagnosis by providing information that contributes to confirmatory diagnosis of cancer. The assay of various tumor markers can be utilized for population screening, tumor detection, diagnosis, staging, prognosis or follow up of cancer. A tumor marker assay used for the diagnosis of cancer should be easy to perform, have low cost and be acceptable to patient. Such a diagnostic test is designed to identify asymptomatic individuals with a high likelihood of having the cancer.

Keywords: Cancer/ tumor, tumor markers, role of tumor markers in diagnosis of cancer.

Introduction

"Tumor (Tumour) or neoplasm" is a mass of tissues formed as a result of abnormal, excessive, autonomous and purposeless growth of cells. Tumor is of two types: benign and malignant. Benign tumor is harmless; while the malignant tumor, known as "Cancer" is very harmful, leading to death of human beings as well as animals. Cancer, may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes. It is caused by complex, poorly understood genetic and environmental factors (Loose and Stancel, 2006; Pandey, 2007; Pandey and Madhuri, 2006). Cancer represents the largest cause of mortality in the world and claims over 6 millions. It kills annually about 3500 per million populations (Kathiresan *et al.,* 2006; Pandey, 2007; Madhuri *et al.,* 2007). In another report, it has been cited that the incidence of cancer is increasing worldwide, and it is the second leading cause of human deaths in the world (Pandey, 2007).

It is difficult to cure cancer as the cells proliferate to their maximum till it starts showing clinical symptoms. Therefore, the diagnosis of cancer is also very critical at its early stages. In recent years, various tumor markers have been used in the diagnosis, management and treatment of cancer, and they have played an important role. The European Group for Tumor Markers (1999) has described the term "Tumor marker" as a spectrum of molecules of widely divergent characteristics, but sharing an association with malignancy that facilitates their application in clinical detection (diagnosis and screening) and management (monitoring and prognosis) of cancer patients. A very broad definition of a tumor marker given by Diamandis (2002) is "a tool that enables the clinician to answer clinically relevant questions regarding a cancer disease". However, most would frequently accept the definition of a tumor marker as "a molecule, a process, or a substance that is altered quantitatively or qualitatively in precancerous or cancerous conditions, the alteration being detectable by an assay" (Hayes *et*

al., 1996). Finally, the tumor markers are biochemical indicators of the presence of tumor. They include cell surface antigen, cytoplasmic proteins, enzymes and hormones. Robbins and Cotran (2006) and Sidransky (2002) explained that a tumor marker clinically refers to molecules which can be detected in plasma or other body fluids.

Regardless of which definition is preferred, the tumor marker itself can be DNA, mRNA, protein or processes (apoptosis, angiogenesis, proliferation, etc.) measured quantitatively or qualitatively by an appropriate assay. In addition, the types of specimen in which the tumor marker is detected can be different; tissue, blood (plasma/ serum), saliva, urine, etc. are all used. The tumor marker assays can be of very different formats ranging from complex animal models to immunohistochemical test kits. The most commonly used format is probably the immunoassay, which is a well characterized methodology. However, this field is progressing rapidly and advanced assays, e.g. microarrays and mass spectrometry are newly introduced technologies in tumor marker research (Schrohl *et al.,* 2003). Tumor markers have now been expanded to quantitatively or qualitatively in tissues and in other body fluids including urine and cerebrospinal fluid (CSF), and even the assay of genes and oncogenes (Schwartz, 1998). Classically, tumor markers are synthesized by malignant cells or certain benign conditions and released into the blood stream; however, markers may be produced by the host tissues in response to direct invasion or metabolic changes induced by the tumor (Michael and Torosian, 1998).

During the last four decades since the introduction of diagnostic immunopathology, tumor markers have been widely accepted for the diagnosis and management of cancer. They could be used for population screening and for detection, diagnosis, staging, prognosis or follow up of malignant diseases (Lindblom and Lijegren, 2000). The end aim of tumor markers is to develop a test to detect cancer at its early stages, while treatment is most effective and complete cure is more reasonably attainable. An ideal tumor marker as described by Chin Loy *et al.,* (2003) should be: detectable only when malignancy is present; specific for the type and site of malignancy; corrected with the amount of malignant tissue present; responded rapidly to a change in tumor size; and easy cheap to measure from a laboratory point of view. Unfortunately, at present no ideal tumor marker fulfils all of the above criteria to satisfy various clinical uses with adequate sensitivity and specificity (Michael and Torosian, 1998). Nevertheless, a tumor marker assay used for the diagnosis of cancer should be easy to perform, have low cost and be acceptable to patient. Such a diagnostic test is designed to identify asymptomatic individuals with a high likelihood of having the cancer. To determine the effectiveness of therapy during the course of treatment following surgery, it is essential to determine a patient's response to therapy.

Classification and Nature of Tumor Markers

Class I (Tumor specific proteins): A specific tumor marker is expressed only in tumor cells. Various experimental model systems have revealed that many chemically induced tumors express private or unique antigens not shared by other histopathologically identical tumors induced by the same chemical even in the same animal (Vegad, 2000). The fusion protein has been elucidated to be associated with malignant process in which oncogene is translocated and fuse to an active promoter of another gene (Lindblom and Lijegren, 2000). The result is a constantly active production of the fusion protein, leading to the development of a malignant clone.

Class II (Non specific proteins or markers related to malignant cells): Most tumor antigens are unique (specific) to the individual tumor. In fact, they are expressed by normal adult tissue. Oncofoetal antigen (OFA) or embryonic antigens, e.g. Alfa fetoprotein (AFP) and carcino embryonic antigen (CEA) are non specific proteins and less stringent but still very useful. These are expressed in cells during embryological development and in cancer cells. The CEA is expressed in all gastrointestinal, pancreas and breast cancers (Hunerbein, 1998; Mohan, 2006), whereas according to Lamerz (1997) the AFP is used to diagnose hepatocellular carcinoma but also expressed in nonseminomatous germ cell tumors of testis and ovarian cancers.

Class III (Differential specific proteins): Some antigens are expressed by both cancer and normal adult tissue. Differential specific proteins are expressed by normally differentiated cells but are expressed at higher rates (over expressed) in the corresponding tumor cells, that is why a relative increase in serum concentration can be used as tumor marker; this is the case with prostate specific antigen (PSA) concentration in prostate cancer (Kuban, 1998). Cell specific proteins have been reported (Shivers, 1998) to be useful for diagnostic purpose, e.g. tyrosinase protein is expressed in melanocyte in malignant melanoma. Vegad (2000) pointed that the differential specific protein antigens

also serve as useful differentiation marker in the diagnosis of lymphoid and prostatic cancers in humans as well as animals.

Various tumor markers with different biochemical natures, usually released into blood specimen (Robbins and Cotran, 2006; Mohan 2006) are summarized in Table1.

Major Tumor Markers and Related Cancers:

New molecular markers: Some new tests in an active area of research have been developed to detect cancer markers in blood and body fluids. Some of the markers being evaluated include: the detection of mutated APC, p53, and RAS in the faces of patients with colorectal carcinoma; the presence of mutated p53 and hypermethylated genes in spectrum of patients with lung cancer and in the saliva of patients with head and neck cancers; and the detection of mutated p53 in the urine of patients with bladder cancer (Robbins and Cotran, 2006; Sidransky, 2002).

Alfa fetoprotein (AFP): This is a glycoprotein, synthesized normally by foetal liver cells. Their levels are elevated in hepatocellular carcinoma and non seminomatous germ cell tumors of testis. Certain non-neoplastic conditions also have increased levels of AFP, e.g. in hepatitis, cirrhosis, toxic liver injury and pregnancy (Mohan 2006).

Carcino embryonic antigen (CEA): It is also a glycoprotein, normally synthesized in embryonic tissues of gut, pancreas and liver. Their levels are high in the cancers of pancreas, gastrointestinal tract and breast. As in AFP, CEA levels are also elevated in certain non-neoplastic conditions, e.g. in ulcerative colitis, Crohn's disease, hepatitis and chronic bronchitis (Mohan 2006).

Some of the major tumor markers with their biochemical properties and uses in various cancers (Baral, 2005; Bose and Mukherjee, 1994; Mohan, 2006; Robbins and Cotran, 2006; Seifert and Resman-Targoff, 2006; Sturgcon, 2002) have been described in Table 2.

General Methods for Identification of Tumor Markers:

1. Immunohistochemistry: This method is used to identify tumor marker proteins, and can be used directly on the tumors for diagnostic purposes, as is done in melanoma, or to identify the protein in serum, as is done in thyroid cancer. Efforts have also been made to identify tumor marker proteins that are detectable in peripheral blood, bone marrow or lymph nodes to improve the staging of disease and to obtain prognostic information (Pelkey *et al.,* 1998). The use of immunohistochemical staining method has been demonstrated by Marein *et al.* (1995) for analysis and expression of some tumor markers routinely processed samples for canine liver carcinoma suggesting that some tumor markers, e.g. keratins are correlated with histological type of tumor.

2. Monoclonal antibodies (MAbs) technique: It is also commonly used to identify tumor marker proteins. The tumor marker detection system may be based on radioimmunoassay (RIA) or enzyme linked immunoassay (EIA). The RIA refers to technique used to detect and quantify the presence of tumor antigen in patient's blood. The assay is based on a 'sandwich' technique using a radio-labeled antibody as 'detector' and 'capture' antibody bound to solid phase substrate or bead. The RIA can be produced in manual assay format or automated format. The enzyme label or tag is used for the detection system and linked to an antibody, which detects the amount of antigen presents in patient's blood. Tumor associated antigen (TAA) is an example of tumor marker in sera of canine mammary carcinoma patient. SB_2 is a murine MAb generated against a canine mammary carcinoma cell line by immunizing laboratory mice with a cell line derived from canine mammary carcinoma which circulates in patients with mammary carcinoma (Wang et al., 1995).

3. Reversed transcriptase and polymerase chain reaction (RT-PCR): The RT-PCR was first used to show the bcr/ abl translocation in patients with chronic myeloid leukemia (Blennerhassett *et al.,* 1988) but has now been used experimentally for detecting micrometastases in a wide variety of malignant diseases (Raj *et al.,* 1998). This technique has been available for simple and rapid amplifying of DNA- the polymerase chain reaction. This technique can also be applied to RNA, after RNA is first altered into DNA by using an enzymatic reaction with reversed transcriptase. This method- "RT-PCR" makes it possible to study very small amounts of gene expression and has been shown to be a much more sensitive method for detecting micrometastasis (Raj *et al.,* 1998). Amplification by polymerase chain reaction allows detection of transcripts from a single tumor cell among 10 to 100 million normal cells.

Utility of Tumor Markers

The utility of many tumor markers in cancer has been reported by various authors. According to Slamon *et al.,* (1989), the overexpression of oncogene is being related to poor prognosis in

breast cancer. Ladenson (1996) described that the tissue specific expression of thyroglobulin (TG) and parathyroid hormone (PTH) is commonly used for diagnosis and follow up of patients with thyroid cancer. Translocations creating fusion proteins are commonly involved in haematological diseases. The Philadelphia chromosome in chronic lymphocytic leukemia is the best known example (Forestier *et al.,* 1997). The detection of circulating prostate cancer cells in the blood stream by using a RT-PCR assay for PSA has been reported (Olsson *et al.,* 1997) as a predictor of surgical failure, and further development and use of this technique could give clinically relevant information. The PSA and prostate specific membrane antigen, diagnosed with immunohistochemistry, have long since been used as serum tumor markers of relapse in prostate cancer (Montie and Meyers, 1997). Both these tumor markers have been clinically accepted for screening and diagnostic purposes (McCarthy, 1998).

The cancer specific marker, CEA has been used for a long time in follow up to detect early relapse in colorectal cancer. However, the benefit of the use of this marker in the clinic is unclear (Ratto *et al.,* 1998). It was recently shown that for detecting CEA by using RT-PCR on lymph nodes at surgery for colorectal cancer is a prognostic factor in stage II of the disease, and it is hoped that this method can be used to identify a subgroup of patients who will benefit from adjuvant treatment (Liefers *et al.,* 1998). According to American Society of Clinical Oncology (1998), the only tumor marker routinely used clinically is the estrogen receptor (ER) protein, the status of which is useful when deciding on adjuvant hormone treatment. Pegram *et al.,* (1998) reported that the antibodies directed against this protein are used in clinical trials. Shivers *et al.,* (1998) reported that the tyrosinase is used in the diagnosis of melanoma. Many types of sarcoma have been reported (Arden *et al.,* 1996; Kawai *et al.,* 1998) to be associated with chromosomal translocations and fusion proteins. The specific embryological markers, HCG and AFP are used for diagnosis and follow up of patients with testicular cancer (Dean and Moul, 1998). In ovarian cancer, the CA-125 marker is used for follow up, and an increase in serum concentrations may predict recurrent disease or serous adenocarcinoma. The CA-19-9 (or CA72-4) marker has been used experimentally in a similar way to follow the patients with ovarian mucinous adenocarcinoma. The data are still not sufficient for recommendation of routine use of any of the breast or ovarian cancer markers (American Society of Clinical Oncology, 1998).

The tumor marker, alkaline phosphatase (AP) differentiates the source of tumor among liver, bone ovarian and germ cell origin; non diagnostic by itself, it helps to confirm malignancy in a small number of patients (Michael and Torosian, 1998). Tumor marker can also be used to aid in differentiating malignant from benign disease, in the diagnosis of metastatic cancer of unknown origin and with conventional imaging tests in difficult diagnostic cases. It can be used to distinguish tumor type and origin as well as to distinguish primary from metastatic tumors. Tumor markers can be used in addition to other methods to forecast a patient's response to therapy, thereby enabling the physician to appropriately adjust or determine the level of treatment needed to manage disease. The tumor markers can also be used as 'predictive indicator' to predict a patient's response to a given chemotherapy or outcome. The ER status is a tumor marker which is used as a predictive indicator (Pegram *et al.,* 1998). ER level are determined in women diagnosed with breast cancer for predicting their response to hormone therapy and assisting in the choice of appropriate therapy. American Society of Clinical Oncology (1998) has recommended that the ER assay can be performed in all patients diagnosed with breast cancer. Furthermore, the European Group for Tumor Markers (1999) suggested that the tumor marker is helpful in differential diagnosis (e.g. in germ cell cancer where they may be different cell types) and especially in metastatic deposits but the primary site is unknown, e.g. NSE used in differential diagnosis of lung carcinoma, neuroblastoma, apudoma and prostatic cancer, and CA-15-3 in breast cancer. Screening is different from diagnosis in that it attempts to identify a disease or condition at an early stage prior to the appearance of clinical symptom. Eche *et al.,* (2001) reported that the Pap test can be used for screening of cervical cancer, and the fecal occult blood test for colorectal cancer. It has been further stated that the tumor markers can be used to aid in the management of cancer patients, such as for screening and diagnosis of cancer, to monitor the effectiveness of therapy, for detection of early recurrence, for differential diagnosis and as prognostic and predictive indicators (Chin Loy *et al.,* (2003). At the completion of primary chemotherapy, a tumor marker assay can be used to determine the persistence of malignancy. An elevated marker level may indicate the presence of a tumor, although a low level does not necessarily mean that no tumor is present. A tumor marker can also be used serially to determine recurrences.

Conclusion

In conclusion, the tumor markers are important tools which play an important role to aid clinicians and oncologists with regard to early diagnosis of cancer, estimation of patient prognosis, prediction of therapy response and disease monitoring. Nonetheless, in parallel to the comprehensive requirements for federal approval of therapeutic drugs, tumor markers should undergo extensive studies of validation and quality assessment at several levels prior to introduction into the clinical setting. The tumor markers must prove usefulness in improving the cancer patient outcome or quality of life, or in lowering costs of care. Although for some markers, considerable progress has been made in the standardization of methods and assay protocols, efforts should be continued as only the stringent application of quality control systems enables a consistent assessment of the utility of tumor markers in cancer.

Table 1: Tumor markers and their nature

S. No.	Tumor markers	Nature
1.	Prostate specific antigen (PSA; a serine protease), prostate acid phosphatase (PAP), creatinekinase; alkaline phosphatase (AP), lactate dehydrogenase (LDH), 5' nucleotidase, amylase and neuron specific enolase (NSE)	Enzyme
2.	Ectopic hormones, e.g. human chorionic gonadotropin (HCG), growth hormone (GH), GnRH and renin, calcitonin, vanillyl mandelic acid (VMA), catecholamines, and adrenocortico-tropic hormone (ACTH)	Hormone
3.	Onco foetal antigen (OFA), e.g. alfa fetoprotein (AFP) and carcino embryonic antigen (CEA), tissue polypeptide antigen (TPA) and tissue polypeptide specific antigen (TPSA)	Glycoprotein
4.	Ovarian cancer antigen (CA-125), breast cancer antigen (CA- 15-3), and colorectal and pancreatic cancer antigen (CA-19-9)	Mucin and other glycoproteins

Table 2: Some major tumor markers and related cancers

Tumor marker	Biochemical properties	Cancer in which used
AFP	Glycoprotein, 4% carbohydrate and albumin	Liver, testicular and canine multicentric lymphomas
AP	Non specific zinc-metallo enzymes	Liver cancer and osteogenic sarcoma
CA-125	Mucin identified by MAbs	Ovarian cancer
CA-15-3	Mucin identified by MAbs	Breast cancer
CA-19-9	Glycolipid carrying Lewis blood group determinant	Pancreatic, colon and gastro-intestinal carcinomas
CEA	Family of glycoprotein and 45-60% carbohydrate	Pancreatic, gastrointestinal, lung and breast carcinomas
Calcitonin	Hormone	Medullary-thyroid C cells
ER	Nuclear transcription factor	Breast cancer
Heat shock protein (HSP) 60 and HSP 70	Protein	Canine transmissible venereal tumor
HCG	Glycoprotein hormone consisting of two non-covalently bound subunits	Placental (trophoblastic) and testicular tumors
Lactate dehydrogenase (LDH)	Enzyme	Various cancers
LDH- 1	Isoenzyme of LDH	Germ cell tumors (tetratomas, testicular seminomas, ovarian dysgeminomas)
NSE	Dimer of enolase enzyme	Small cell lung carcinoma, apudoma, neuroblastoma and prostate cancer
Placental AP (PLAP)	Heat stable isoenzyme of alkaline phosphatase (AP)	Ovarian and germ cell tumors (seminomas)
Prostate acid phosphatase (PAP)	Enzyme	Prostate cancer
PSA	Glycoprotein serine protease	Prostate cancer
Progesterone receptor	Nuclear transcription factor	Breast cancer
Sqamous cell carcinoma antigen	Glycoprotein subfraction of tumor antigen T4	Sqamous cell carcinomas
TG	Hormone	Thyroid cancer
Tissue polypeptide antigen	Fragments of cytokeratin 8, 18 and 19	Bladder and lung carcinomas
Tissue polypeptide specific antigen	Fragments of cytokeratin18	Metastatic breast carcinoma

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Studies on Identification and Population Dynamics of Anopheline Mosquitoes from Jeddah Province of Saudi Arabia.

Khalid Al-Ghamdi , Masroor Alikhan *; Jazem Mahyoub and Z.I. Afifi
Department of Biological Sciences, King Abdul Aziz University, Jeddah. KSA
*Mosquito Taxonomist , Al-Amana, Jeddah,KSA.

Abstract:
Anopheline mosquito species have been studied in Jeddah Province of Saudi Arabia. 11 species have been found to be prevalent in this region. The identification of the species has been based on the morphological characters of the adult mosquitoes. The population dynamics of the species with reference to optimum temperature and humidity has been discussed along with the ratio of male and female catches .
Keywords: Identification, population, dynamic , anopheline mosquitoes, Saudi Arabia.

Introduction:

Several reports are available on the Anopheline mosquitoes of Saudi Arabia, but there is no authentic record available on the anopheles species of Jeddah province, which occupies a unique place in Saudi Arabia being a gate way of Mecca. It is located on the middle of the Red Sea coast and is the major urban center of western part of the country. It is located at the junction of latitude 21^0 29 north and longitude 39^0 07 east having a population of 4.5 million . The climate of this region is warm like other cities of the country. Summer temperatures are considered very hot and break the $+40^0$ C mark and in winter $+25^0$ C. Rain is very scanty and only a small amount of it falls usually in winter. Air pollution remains high, particularly on the hot summer days. Being a coastal city Jeddah suffers from high humidity on most days of the year, especially during summer. During winter humidity generally remains low. There is no proper sewage disposal network, growth of slums is rapid, and is widely exposed to many different cultures through large number of pilgrims visiting Mecca via Jeddah through out the year. All the above conditions play a very important role on the density of Anopheline mosquitoes.

Detailed reports of Saudi Arabian mosquito species are available in the literature, (Knight & Mattingly (1956); Beccari (1971); Zaher (1973); Buttiker (1981); Glick (1992); Abdullah & Mardan (1995). All these workers have recorded many anopheles species of different regions of Saudi Arabia, but none of them have studied the fauna of Jeddah province in particular. Buttikar (1981) has mentioned that all inspections were negative for anopheline mosquitoes from Jeddah. Abdullah & Mardan (1995) reported only 4 species from South West region of Saudi Arabia, but have not included Jeddah in their report. Knight & Mattingly (1956) have reported some 13 species from Saudi Arabia. They have identified *Anopheles coustani* Str. *A. coustani* var. *tenebrosus, A. cinereus, A. culicifacies, A. culicifacies* ssp. *Adenensis, A. d'thali, A. fluviatilis. A. gambiae, A. multicolor, A. pharoensis, A.pretoriensis, A. pulcherrimus, A. rhodesiensis* ssp.*rupicolus, A. sergenti, A. stphensi, A. turkhudi*, but have mentioned that only 4 species viz , *Anopheles d'thali, A.gambiae , A. multicolor and A. sergenti are found in Jeddah province.*

Recently there have been reports of Malaria from this region of the country, hence there was an urgent need to study the Anopheline species of Jeddah province, As per the bulletin of the Ministry of health of Saudi Arabia, during 2006 there were 39 cases of *Plasmodium falciparum* infections and 31 cases of *Plasmodium vivax infections* ,and during 2007 some 75 cases of *P.falciparum* and 32 cases of *P.vivax* were recorded by the Ministry of Health in Jeddah province. The risk of mosquito-born diseases depends on the population size of the mosquitoes and incidence rate of disease. Collecting the information on adult mosquito populations and their identification is thus important for the proper control and to predict the disease outbreak. Looking into the importance of malaria the present work was undertaken to identify the potential of Anopheline malaria vectors. The present study has identified some 11 species of Anopheles mosquito from Jeddah province.

Material and Methods:

To study the different species of Anopheline mosquito of Jeddah province adult, a thorough sampling was carried out by installing the light traps in various habitats of the province. Powered aspirators were also used to collect the mosquitoes from their resting areas. Trapping of adult mosquitoes gives information on the relative population size and the species composition. The Black hole light traps were used during the whole

study to maintain the uniformity of the collection. 504 different spots were carefully chosen from the 14 districts of Jeddah province with 85 samples per day. We covered different human dwellings areas such as congested housing, markets, restaurant, mosques, tourist spots, parks, breeding points, water storage tanks & sewage water lakes etc. The surveillance work was carried out for whole one year. It was started in January 2007 up to December 2007.Live adult mosquitoes were brought to the laboratory and were immobilized by keeping them in the deep freezer for 15 minutes. Later we assorted them out firstly by genera and species wise and than sex wise (male & female). With the help of different standard identification keys (Glick,1992; Knight & Mattingly,1956; Fauna of British India,19933 and Smart,1956) for Anopheline mosquitoes, the species were identified under binocular microscope and their scientific out lined drawings were drawn to reconfirm the species. Identification is mainly based on adult morphological characters. While compiling the data the relevant information on the environmental features of Jeddah were also taken into consideration to find out the optimum temperature and humidity in relation to the densities of the Anopheles species.

Results and Discussion:

A total of 1091 adult Anopheles mosquitoes were obtained from the collection (Table 1), out of which 768 were females (70.39%) and 323 were males (29.6%) which were trapped. Maximum number of Anopheles mosquitoes were collected from the district Asfaan (21.92 N; 39.357 E) followed by Bariman (21.58N; 39.24 E) & Thoal (22.28 N; 39.09E) which is about 45.48%, 21.82% & 21.73% respectively of the total collection. The minimum number of adults were collected form district Obhor (21.62N; 39.23E) & Al Sharfia (21.55N; 39.16 E) which is about 0.09% and 0.18 % respectively (Fig. 1&2). Most of the species recoded during this study are already reported from different parts of Saudi Arabia, but unfortunately few of them are mentioned in the literature from Jeddah Province. Knight & Mattingly, (1956) had carried out an extensive work on the mosquitoes of Arabia and in their report they had mentioned 13 species from Saudi Arabia but reported only 4 species (*Anopheles d'thali, A. gambiae, A multicolor and A. sergenti*) from Jeddah. Recently, Azzaam, (2006) in his report recorded the larvae of 5 species from Jeddah (*Anophelesturkhudi, A. subpict A. stephensi, A. rhodensiensis & A. d'thali*). Abdullah & Merdan (1995) had earlier identified only four species (*Anopheles arbiensis, A. sergenti, A. multicolor & A. tenebrosus*) from South Western region of Saudi Arabia and they had excluded Jeddah province from their studies.

In the present study we have identified 11 species of Anopheles from Jeddah province out of which some of them are already reported form other parts of the country. The recorded species are as follows : *Anopheles culicifacies* Giles; *Anopheles thurkhudi* Liston; *anopheles pharoensis* Theobald; *Anopheles gambae* ; *Anopheles multicolor* Camboulin; *Anopheles d'thali* Patton; *Anopheles sergenti* Theobald; *Anopheles subpictus* Grassi; *Anopheles stephensi* Liston; *Anopheles rhodesiensis* Theobaldi; *Anopheles fluviatilis* James. We have based our identification of the anopheline species on the keys given by Glick,1992 & Smart,1956.

Table.1Four weekly population density of anopheles mosquitoes in Jeddah

No. of weeks	No. of Anopeles	Months
4	19	Jan.
4	23	Feb
5	35	Mar.
4	122	Apr.
4	141	May.
5	327	Jun.
4	42	Jul.
4	40	Aug.
4	97	Sep.
4	82	Oct.
4	70	Nov.
5	93	Dec.
51	1091	

Table.2 monthly population density in relation to Temperature & humidity

Anopheles	Hum.	Tem.	Months
19	71.285714	27.45238	Jan
23	80.75	28.07143	Feb
35	81.607143	30.6746	Mar
122	80.035714	33.39286	Apr
141	71.714286	37.24206	May
327	83.857143	37.30952	Jun
42	79.75	40	Jul
40	83.5	39	Aug
97	84	37.5	Sep
82	80.75	37.75	Oct
70	80.25	36.25	Nov
93	78.3	30.8	Dec

The activity of mosquitoes of Saudi Arabia has been studied by many workers. Abdullah & Merdan (1995); Azzaam (2006), & Al Kheerji (2005), but very little work has been done on the anopheline mosquitoes specially with reference to temperature and humidity as these two are the main factors which are affecting their activity in this region of the country. The activity was recorded through out the year. In the present study we observed relatively low activity during winter specially in the month of January & February when the average temperature was recorded below 28 ^{0}C and humidity was below75% and during summer months of July and August when the average temperature was above +40 ^{0}C (Fig.3; Table 2). The highest peak active period was recorded during the month of May & June when the temperature was about 37^0 C and the humidity was 84% (Fig.4;Table1). The effect of maximum and minimum temperatures & humidity is one of the most important factors determining the catches and activity of the mosquitoes. The most suitable temperature for their activity is in between 33^{0}C to 37^0 C and suitable humidity between 75% to 84 %.(Table 2) The ratio of male and female anopheles trapped was also determined. The females accounted for 70.39% while males were only 29.61% of the total collection.(Fig 5)

Correct identification of anopheline mosquitoes is essential to the effective control of the malaria vectors and malarial parasites. We have prepared a simple guide to identify the species of Jeddah province to facilitate the correct identification of the adults of anopheles species found in this area. Our identification is based on morphological characters which can be easily recognized.

1.*Anopheles dthali* Patton

Identifying characters:

-Female palpi with thin and uniform thickness. The last segment is very short, uniformly dark except small pale spots.

-Wings with 4 pale spots which are confined to costa, subcosta and radius (R_1), wing field is with dark veins with out pale spots.

-Head scales erect, narrow & light colored.

-Legs uniformly dark but pale, hind tarsomere 4&5 dark.

- Thorax shiny & without scales.

2 .*Anopheles culicifacies* Giles

-Female palpi with a very short terminal segment. The apical band narrow ,pale white.

- Wing with pale spot at the base of costa with an interruption and opposite to it a dark spot on Radius (R_1)

-Vein 3 (R_{4+5}) all dark, but occasionally a pale area in the middle.

-Thorax usually with out scales.Middle area with dark chaetae.

-Legs uniformly dark with no pale areas, tarsi entirely unbanded.

***3. Anopheles gambiae* Giles**

-Female palpi with long pale apical segment with depressed scales.

-Wings with average amount of dark and pale scales. Costa with 2 interruptions at the base. R_1 with the pale spots ; vein 3 (R_{4+5}) with two dark spots at the beginning and end while in the middle pale. Anal vein with 3 dark spots.

-Apical segment of tarsus dark; femora & tibiae pale spotted.

-Abdomen without scales except on cerci.

-Thorax with scales in the middle.

***4. Anopheles thurkhudi* Liston**

-Female palpi long and thin, apical segment dark except for its basal portion, tip with black chaetae.

-Base of the Costa mainly dark with pale interruption near the base. Middle dark spot with a pale area on R_1.

- Vein R_{4+5} is usually pale with light scales.

- Femora dark through out.

- Abdomen light with light hairs & with out scales.

- Thorax uniformly colored.

***5.Anopheles phorensis* Theobald**

-Female palpi with four pale bands.

-Costa is pale underside, vein R_1 light at base and middle spot is with a small light area.

- Vein R_{4+5} with light scales except 3 dark spots one in the middle and two at both ends.

-Hind femur and tibia are not dark, hind tarsus with light spots and pale apically.

- Abdominal segments all with projecting postero-lateral dark scale tufts.

***6.Anopheles sergenti* Theobald**

-Female palpi of uniform thickness, but apical segment short and pale.

-Costa is dark at base, R_1 pale at base near humeral dark spots.

- Vein R_{4+5} usually dark or with only a small pale spot. Fringe with well marked spots at all veins except anal vein area.

- Thorax darker at sides than middle area.

- Front femora not swollen at base, Uniformly dark with pale marking at tips of femora and tibiae of all legs. Tarsi with out bands.

- Abdomen dark with out scales.

***7.Anopheles multicolor* Camboulin**

-Female palpi long, thin & smooth, apical band dark except basally, tip with brush like dark chaetae.

- Tarsi not banded, front femora not swollen at base.

-abdomen with out scales.

- Costa pale at base and humeral area. Middle dark costal spot on vein R_1 shorter at inner end . some dark spots on wing field.
-Vein R_{4+5} mainly pale.
-Abdomen devoid of scales.

***8.Anopheles fluviatilis* James**

-Female palpi with sub apical narrow pale band, intervening dark areas much broader.
- Inner Costa with out any pale interruption, R_1 pale at base middle spot shorter than Costa.
-R_{3+4} usually extensively pale.
- Pale fringe spots on all veins except anal vein.
- Thorax pale in the middle than the sides which are dark .
- Joints of hind and for tarsi without distinct white bands.

***9.Anopheles subpictus* Grassi**

-Female palpi shaggy, apical segment large with pre apical band about the same length as the apical band.
- Base of Costa with three small dark spots; middle dark spot on R_1 is shorter than spot on costa.
-Front femora swollen at the base, femora and tibia not speckled, joints between fore tarsomeres with broad basal apical bands.
Abdomen thickly covered with golden hairs and light colored.

***10.Anopheles stephensi* Liston**

-Female palpi slightly thick, cylindrical, smooth, apical segment and its lower segment pale, some light spots on segment three.
- R_1 usually with prehumeral dark costal spots. Dark spots on the base of R_2 and R_{4+5}. Anal vein has 3 dark spots.
- Femora and tibiae speckled. First tarsal segment with some pale spots, other segments dark with basal pale bands.

***11.Anopheles rohdesiensis* Theobald**

-Female palpi dark and narrow.
-Head scales erect, broad and pale on the vertex and dark brown laterally and posteriorly.
- wing spots like *A. d'tathli* (with which it is usually been confused). Pale spots confined to costa and R_1
-Apical segment of hind taursus dark.
- Femora and tibiae not pale spotted.
- Thorax not shiny like *A.d'thali*, with some scales.
- Abdomen without scales.

Conclusion:

Identifying mosquito sampling and monitoring is extremely helpful and can be used to study the mosquito infestation level to initiate proper control and management . The present data were collected from the potential malaria vectors in Jeddah province. Although we took utmost care in identifying the different anopheline species, but we feel that some species have got away and still need to be identified, hence there is an urgent need to carry out some more work in establishing their proper identification, with regard to their population dynamics as well.

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Fig.1 Monthly fluctuations & Temperture

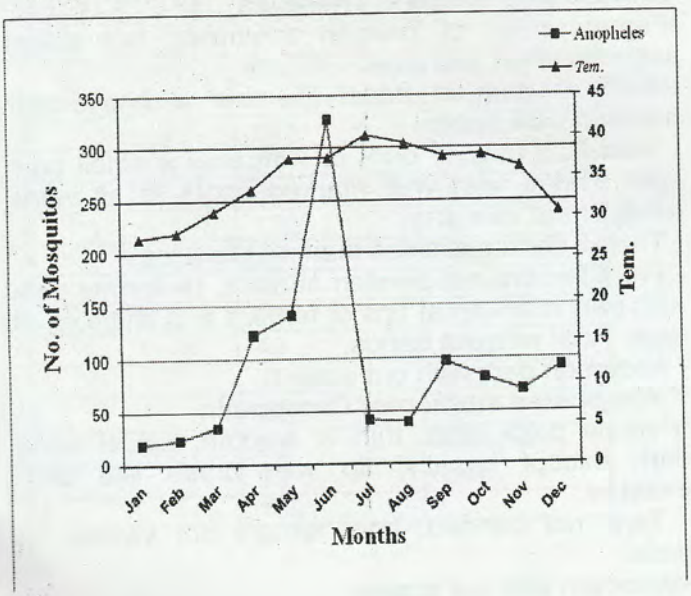


Fig 2 Monthly fluctuations and Humidity

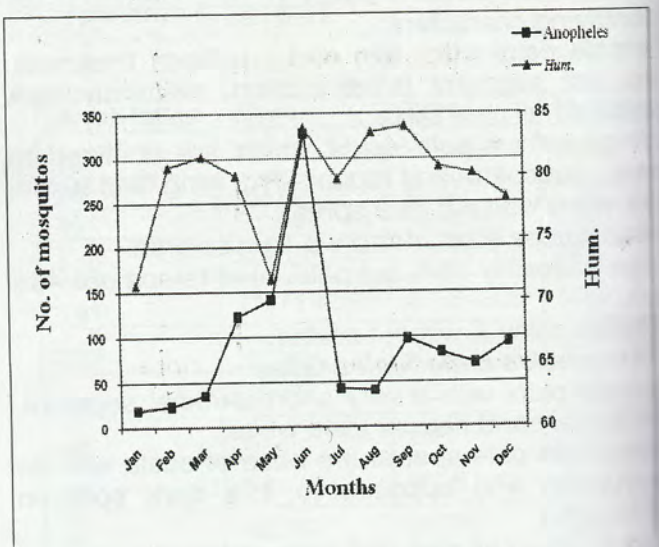


Fig.3 Annual population density of Anopheles mosquito In Jeddah Province.

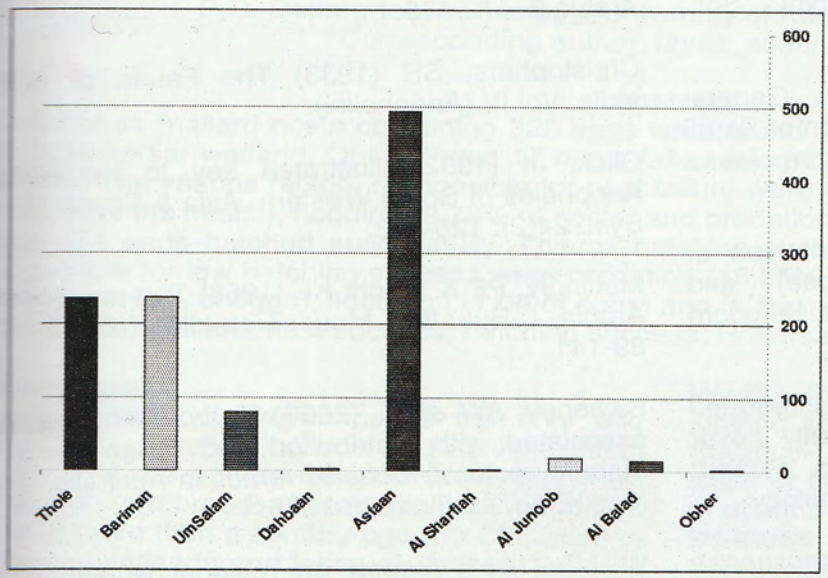


Fig.4 Percent variation of the population density of male and female adults during a year

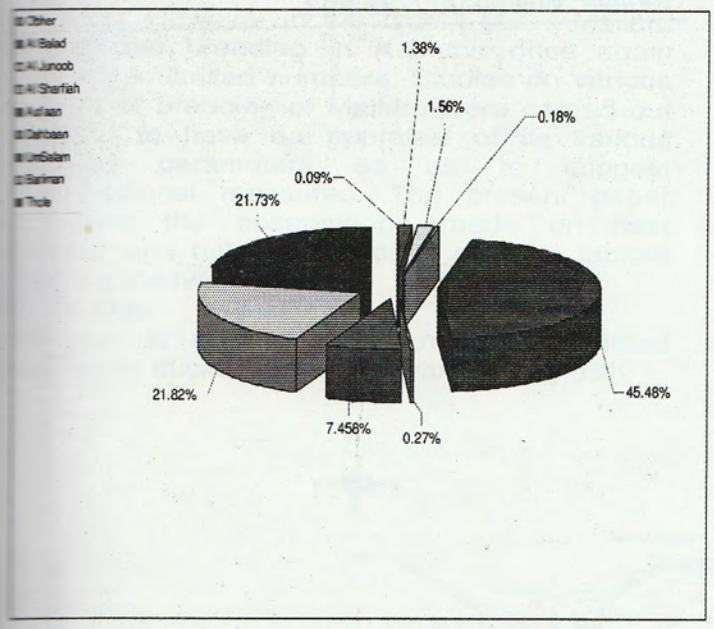
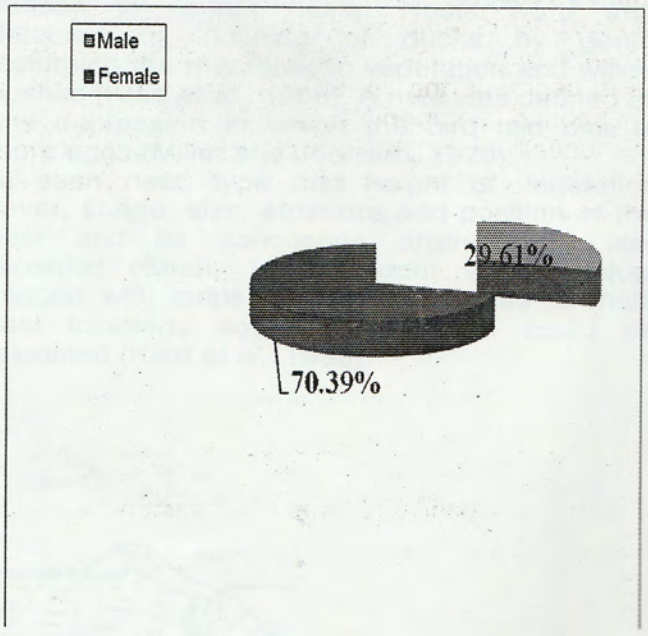


Fig.5 Percent variation in the population density of adult population in Jeddah province



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Factors affecting nest success and hatching success of mallard (*Anas platyrhynchos*) in Hokersar wetland of Kashmir.

G. Mustafa Shah, Ulfat Jan and Fayaz Ahmad Ahanger*
P.G. Department of Zoology, University of Kashmir, Srinagar 190006.
*Corresponding author: fayaz_sultan@yahoo.co.in

Abstract

A total of 41 mallard nests containing 326 eggs were found during the two year study period (2004-05) in Hokersar wetland. Out of these 17 nests failed completely to hatch. Overall nest success was 58.54%. The various factors responsible for nest failure were the abandonment of nest by the female (17.07% of the nests.), flooding (9.76% of nests) and predation (14.63% of nests). Out of the total 326 eggs 174 eggs hatched successfully. Overall hatching success was 53.37%. The various factors responsible for low hatching success were predation (15.65% of eggs), abandonment of nests by the female (14.11% of eggs), flooding (11.04 of eggs) and faulty incubation and infertility (5.83% of eggs).

Key words: Mallard, nest success, hatching success, Hokersar

Introduction

Mallard (*Anas platyrhynchos*) is the only wild duck that presently breeds in Kashmir. Infact Kashmir is the only territory within Indian limits where Mallard breeds (Bates and Lowther 1952). Almost more than a century ago this bird used to breed in wetlands and lakes of Kashmir in large numbers (Bates and Lowther (1952). With destruction of wetland habitat in general and nesting sites of the bird in particular, illegal egg collection and unfriendly attitude of man, the bird altogether abandoned its breeding in Kashmir and restricted itself to the valley only for wintering. However in the recent years Mallard has started breeding in Kashmir once again although in limited numbers. Studies on various aspects of breeding of Mallard were carried out in order to have an appraisal of its various breeding parameters so as to suggest conservational measures. The present paper discusses the observations made on nest success and hatching success and the factors affecting the two.

Study area

Hokersar is an important and well-protected reserve for ducks and geese managed by J&K Wildlife Protection Department. The wetland is located about 10kms. to the west of Srinagar on Srinagar- Baramullah highway. The wetland is more or less semicircular in outline with an area of about 5 sq. km

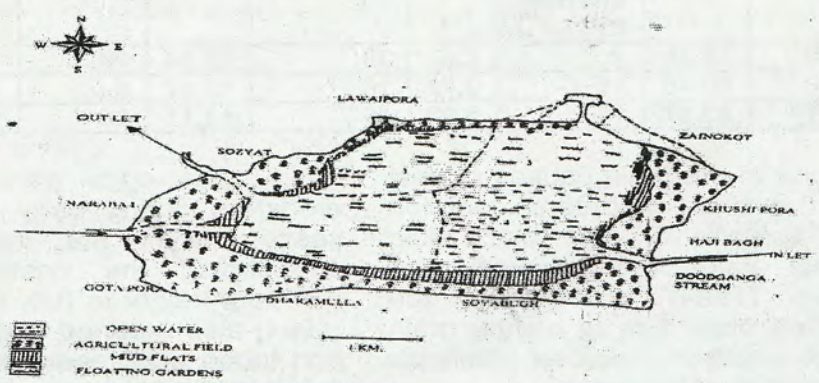
Methods

The present studies was carried for a period of two years (2004 and 2005)

(i). Nest searching

Tall and dense macrophytic vegetation, willow (*Salix* sp.) bushes and hollows of old willows were thoroughly searched during breeding season to detect nests. Nests were also detected by flushing of ducks by gently disturbing the macrophytic vegetation and willow bushes (Klett *et al.*, 1988). A nest was defined as any depression in which the bird laid one or more eggs (Miller and Johnson, 1978).

At each nest, type and height of vegetation cover, shape, size, structure and position of the nest and its concealing arrangement was recorded (Shah, 1984).Slender willow stakes flagged with strips of cloth were used to mark nest locations so that the nests could be relocated (Klett *et al.*, 1988)



Map of Hokersar wetland

For the determination of nest success and hatching success, the nests were followed till the hatching or the period till nest was live.

(ii). Nest success and hatching success

Nest and hatching success was calculated as per Teal (1965), Shah (1984) and Fazili (2002).

Nest success was calculated as

$$\frac{NS}{NS + NU} \times 100$$

NS = Number of successful nests.
NU = Number of unsuccessful nests

Hatching success was calculated as

$$\frac{\text{Number of eggs hatched}}{\text{Total number of eggs observed}} \times 100$$

Results and Discussion

Nest success

In all 41 nests were studied during the two year study period, 19 nests in 2004 and 22 nests in 2005. 17 nests failed completely to hatch. Nesting success for the year 2004 and 2005 was 47.37% and 68.18% respectively where as the overall nest success was 58.54%.

Table 1: Fate of nests

Year	Total Nests found	Number of Success-ful nests	%	Number of Aband-oned nests	%	Number of Flooded nests	%	Number of Pred-ated nests	%
2004	19	9	47.37	4	21.05	3	15.79	3	15.79
2005	22	15	68.18	3	13.64	1	4..54	3	13.64
Total	41	24	58.54	7	17.07	4	9.76	6	14.63

Hatching success:

326 eggs from 41 nests were used for hatching success calculations. 154 eggs from 19 nests were studied during 2004 for hatching success calculations while as the number of eggs studied during 2005 was 172 from 22 nests. Hatching success was 45.45% for the year 2004 and 60.46% for the year 2005. The overall hatching success for the two years was 53.37%

Table 2: Fate of eggs.

Year	Total Eggs	Eggs Hat-ched	%	Eggs lost to faulty incub-ation & infertile-ity	%	Eggs lost to abandon-ment	%	Eggs lost to flood-ing	%	Eggs lost to preda-tion	%
2004	154	70	45.45	4	2.6	28	18.18	28	18.18	24	15.58
2005	172	104	60.46	15	8.72	18	10.47	8	4.6	27	15.7
Total	**326**	**174**	**53.37**	**19**	**5.83**	**46**	**14.11**	**36**	**11.04**	**51**	**15.65**

Predation was one of the main causes of nest failure. Among the avian predators, common crow (*Corvus splendens*) and black kite (*Milvus migrans*) were the principal egg predators. Shah (1984) also found the common crow and black kite to be the main egg predators of common moorhen (*Gallinula chloropus)* in wetlands of Kashmir

The eggs were most vulnerable to predators during early part of the breeding season when the macrophytic vegetation surrounding the nests had not attained sufficient height to fully conceal the nests. Hill (1984) also reported that proportion of mallard and tufted duck nests destroyed by predators generally decreased as the minimum vegetation height around the nest increased.

Predation on nests has been found to be the main factor for decreased nest success in mallards in North America (Greenwood et al., 1995; Beauchamp et al., 1996)

Abandonment (desertion of the nests or eggs) by the female was other major factor responsible for egg loss. Abandonment of eggs may have been caused by several reasons like the disturbance of nest, disturbance of the duck at the nest, poaching or predation of the duck etc. Greenwood et al (1987, 1995) found that 14% of mallard nests were lost to abandonment.

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Biochemical Investigations of Osteo-Hepatic Syndrome in Fluorotoxicity

Shashi A.*, Kaur K and Bhardwaj M
Department of Zoology, Punjabi University, Patiala – 147 002, India
Corresponding Author: Dr. Shashi Aggarwal, Reader, Department of Zoology, Punjabi University, Patiala-147002, Punjab, India. E-mail: shashiuniindia@yahoo.co.in

Abstract - The present study has been conducted to investigate biochemical alterations in some diagnostic liver function indexes in osteofluorosis caused by excessive fluoride ingestion in drinking water. The concentration of fluoride in drinking water varied from 3.7 to 22.5 mg /L. The age range of the patients was from 20 years to 80 years with a maximum number of cases in the age group of 50-60 (22.87%). In the studied fluorotic population, 63.4% were males and 36.6% were female, thereby showing male preponderance in fluorosis in the present investigation. Clinical symptoms of fluorotic patients included lower back pain (75.2%), hand and feet tingling (60.1%), neck pain (35.2%), muscle weakness (45.1%), loss of appetite (36%), abdominal pain (24.8%), constipation (19.6%), bone deformities (13.7%), dental fluorosis (94.8%), polydypsia (29.4%), polyuria (24.8%), bloated feeling (29.4%), and mouth sores (8.5%). Serum fluoride levels were significantly ($p<0.001$) elevated in fluorotic patients of both sexes as compared to control. There was a positive correlation between the serum fluoride concentration and prevalence of skeletal fluorosis. Grade II (Moderate) was the most prevalent grade affecting 51% of patients studied. Next was grade I (Mild) which has distribution of 28.1% and least prevalent was grade III (Severe) affecting 20.9% of the studied population. There was significant ($p<0.001$) positive correlation between drinking water fluoride and serum fluoride. Biochemical analysis of liver function markers such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, acid phosphatase, and lactate dehydrogenase showed highly significant ($p<0.001$) elevation in the fluoride exposed patients compared to controls. The total and direct bilirubin pigment in the serum were also significantly ($p<0.001$) raised compared to the control subjects. Data suggest that fluoride exposure affect hepatic functions in osteofluorosis.
Key words: AST, ALT, ALP, ACP, LDH, Bilirubin, Liver function, Osteofluorosis

Introduction

Endemic fluorosis is related to the high concentration of fluoride in the drinking water. An endemic fluorosis belt has been reported in Punjab and it covers about 12 districts. Fluoride causes damage to many animal and human organs (Li and Cao, 1994, Franklin *et al*, 1999), predominately the skeletal systems and teeth as well as the structures and functions of the non skeletal systems, such as brain, liver, kidney, reproductive organs and spinal cord (Guan *et al.*, 1998; Shashi, 2003; Wang *et al*, 2004).The amount of alanine aminotransferase, aspartate aminotransferase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase, and bilirubin pigment are considered as markers of liver function. Studies of blood biochemical changes in response to fluorosis showed increase in the activities of alanine aminotransferase, aspartate aminotransferase, and serum lactate dehydrogenase (Ferguson and Stephen, 1981; Kessabi *et al.*, 1983). Calcium is important for bones and teeth. In cells calcium combine with fluoride, which results in formation of calcium fluoride. The element is deposited in various forms in cells and tissues. Some enzymes like alpha – amylase, peptidase are activated by calcium ions and are inhibited by added fluoride. (Machoy, 1987).Some animal and epidemiological studies showed that exposure to excessive fluoride could induce damage to the liver, kidney and muscles (Wang *et al.*, 1993; Michael *et al.*, 1996). Furthermore some animal experiments revealed that a significant dose effect relationship was detected between water fluoride level and damage to soft organ functions (Liu *et al.*, 1994; Guan *et al.*, 1998; Shashi and Thapar, 2000; Shashi, 2003). Surprisingly, few studies have examined the effects of fluoride on the function of human liver, kidney and muscle and the possible dose-effect relationship between drinking water fluoride levels and damage to human liver and kidney functions. The aim of the present study was to analyze some sensitive and biochemical indexes that reflect hepatic functions in persons exposed to high level of water fluoride in residential areas with different levels of drinking water fluoride in three villages of Sangrur district of Punjab state, India.

Material and Methods

The study was conducted in three villages namely Chhajli, Arkwas and Lehar Khurd of Sangrur district, Punjab. The levels of drinking water were different i.e. 3.7, 4.5 and 22.5 mg /L respectively. The Raipur village of Patiala district with low fluoride levels of drinking water (0.57 mg /L) was included in the study as a control village. The study population consisted of 221 subjects including 68 controls randomly selected.

Clinical Examination

Clinical examination for dental mottling and signs of skeletal deformities such as genu varum, genu valgum, neck rigidity, stiffness of joints, tingling sensation, kyphosis, bone tenderness, and muscle weakness was carried out on all the subjects in the affected villages.

Biochemical Analysis

The fluoride levels in the drinking water and serum were estimated by fluoride ion selective electrode (Harwood, 1969). A sample of 5ml blood was collected from each patient and control. The serum was separated immediately and was used for further analysis. The activities of aspartate aminotransferase, alanine aminotransferase, Alkaline phosphatase, acid phosphatase, Lactate dehydrogenase and total bilirubin and direct bilirubin levels were estimated in the serum samples of fluorotic patients and controls using the test kits supplied by Bayer Diagnostic Pvt. Ltd., Medsource Ozone Biochemical Pvt. Ltd. and Reckon Diagnostics Pvt. Ltd. During analysis, working reagents were freshly prepared by following methods mentioned in their respective kits.

Statistical Analysis

All data were represented as mean ± standard deviation (SD). The mean values were compared between control and fluoride affected groups by using t – test, analysis of variance (ANOVA) with subsequent Z mode test, Chi square test, Pearson correlation coefficient using analysis software (SPSS, G STAT, CPCS). A difference at $p<0.05$ was considered statistically significant.

Results

The fluorotic patients and control were divided into six age groups *viz.*, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80 years. The average age of control and patients of fluorosis are presented in Fig.1. The male and female ratio of fluorotic patients was 1.7: 1. Z – mode analysis showed a value of 4.71, which indicates significant ($p<0.001$) difference between number of male and female patient (Fig. 2).

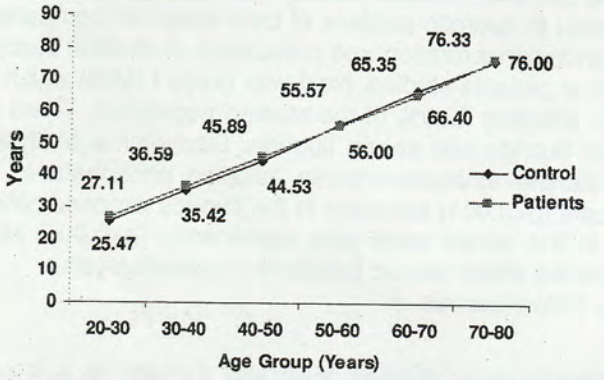


Fig. 1. Average Age of control and patients of fluorosis.

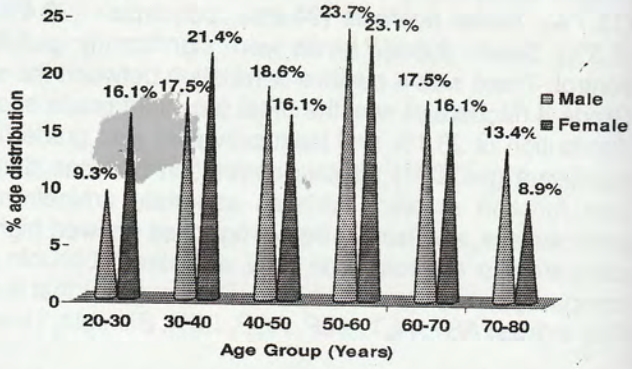


Fig. 2. Percentage distribution of patients of fluorosis on the basis of age and sex.

Clinical manifestations

The patterns of clinical symptoms based on physical examination of fluorotic patients are presented in Fig. 3. Highest cases were of dental fluorosis (94.8%), lower back pain (75.2%), hand and feet tingling (60.1%), followed by muscle weakness (45.1%), gastrointestinal disturbances (19.6 – 35.9%) and lowest were of mouth sores (8.5%). The grading of fluorotic patients was done by the method of Teotia *et al.*, (1985). Majority of cases were affected by Grade II type skeletal fluorosis (51%), followed by Grade I (28.1%) while only 20.9% were suffering from severe Grade III skeletal fluorosis. (Fig. 4).

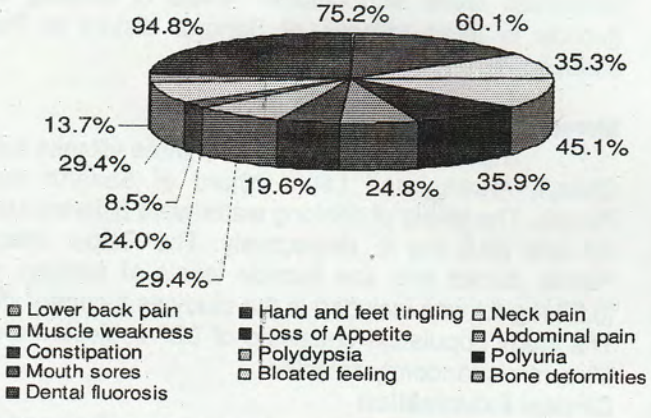


Fig. 3. Symptomatic distribution of fluorotic patients

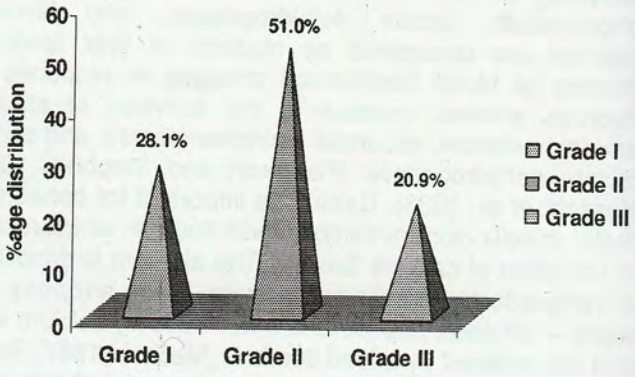


Fig. 4. Grading of patients of skeletal fluorosis

The fluoride content in drinking water

The level of fluoride in drinking water varied from [illegible] to 22.5 mg/L. A significant positive relationship was also found between the level of fluoride in drinking water and serum of the fluorotic patients. As the fluoride level in drinking water increases, so did the serum fluoride level (Pearson correlation coefficient = 0.670, p<0.001)(Fig. 5).

Serum fluoride level showed a significant (p<0.05) increase with advancing age. The average age difference of 3.75 years for males and females with fluorosis in affected villages was significant (p<0.05) (Fig. 6). There was a significant (p<0.001) increase in serum fluoride in patients of fluorosis of both sexes as compared to control. The level of serum fluoride revealed a significant (p<0.05) elevation in male as compared to female (Table 1, Fig. 7).

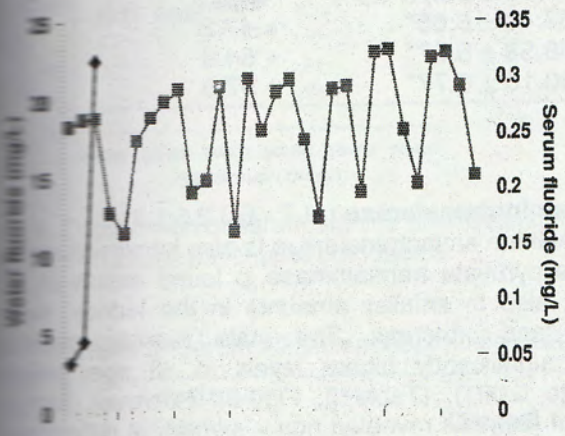


Fig. 5. Correlation between fluoride in drinking water and serum.

(Pearson Correlation = 0.670, p<0.001)

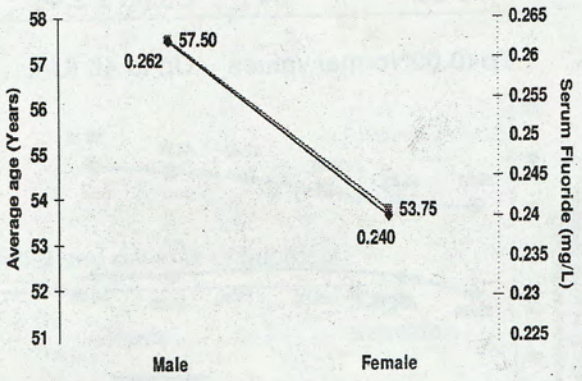


Fig. 6. Average age and fluoride in blood serum of males and females with skeletal fluorosis

Table 1. Fluoride in blood serum of male and female patients of fluorosis and control

Gender	No.of subjects	Fluoride in serum (mg/L)	
		Mean ± S.D	Range
Male	18	0.262 ± 0.05*	0.160 – 0.323
Female	12	0.240 ± 0.04*	0.159 – 0.291
Control	05	0.026 ± 0.003	0.06 – 0.10

*P<0.001

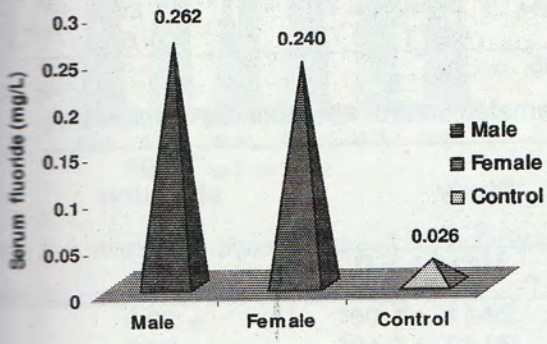


Fig. 7. Fluoride in blood serum of male and female patients of fluorosis and control

Enzymes

Aspartate aminotransferase (AST - EC 2.6.1.1)

Liver and skeletal muscles are rich in AST. Table 2 shows that a highly significant (p<0.001) increase was observed in the activity AST in patients of fluorosis as compared to control. The percentage elevation ranged from 37.8 to 57.9% in patients of fluorosis (Table 2, Fig. 8).

Table 2. The changes in the activity of aspartate aminotransferase (IU/L) in control and patients of fluorosis

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	15	33.06 ± 2.28	18	50.83 ± 6.09*	+ 53.8
30-40	19	36.94 ± 2.17	29	50.82 ± 5.18*	+ 37.8
40-50	19	38.80 ± 5.20	27	54.58 ± 5.87*	+ 40.5
50-60	06	39.00 ± 2.00	35	57.40 ± 6.65*	+ 47.2
60-70	05	37.8o ± 6.09	26	58.58 ± 5.67*	+ 54.0
70-80	04	38.00 ± 2.44	18	60.10 ± 6.77*	+ 57.9

*p<0.00Normal values = Up to 46 IU/L

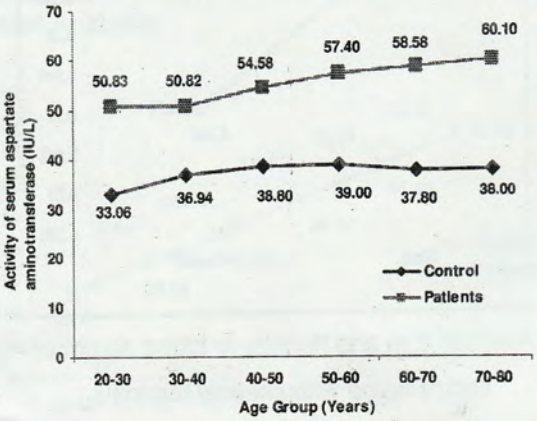


Fig. 8. Aspartate aminotransferase activity in male fluorotic patients and contr

Alanine aminotransferase (ALT - EC 2.6.1.2)

Alanine aminotransferase is also known as serum glutamate pyruvate transaminase is found mainly in the liver, but also in smaller amounts in the kidney, heart muscles, and pancreas. The male fluorotic patients revealed significantly higher levels in all age groups ($p<0.05$ to 0.001). (Table 3, Fig. 9). However, female patients of fluorosis revealed non – significant differences in the activity of alanine aminotransferase in females of 50 – 70 year age groups. However, a highly significant ($p<0.05$ to 0.001) increase was observed in other age groups. The maximum percentage activity of alanine aminotransferase was seen in 20 to 50 year old patients (21.4 to 36.6%) (Table 4, Fig. 9).

Table 3. Alanine aminotransferase (IU/L) activity in male control and patients of fluorosis.

Age group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	08	32.75 ± 2.49	08	48.87 ± 1.95*	+ 49.2
30-40	08	35.37 ± 4.92	10	49.60 ± 3.65*	+ 40.2
40-50	08	37.50 ± 2.56	10	49.50 ± 2.95*	+ 32.0
50-60	04	41.75 ± 0.95	10	51.60 ± 3.43*	+ 23.6
60-70	03	43.66 ± 4.04	10	52.50 ± 2.75*	+ 20.2
70-80	02	46.50 ±2.12	07	49.71 ± 3.76**	+ 6.9

*p<0.001; **p<0.05

Table 4. Activity of alanine aminotransferase (IU/L) in females control and patients of fluorosis

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	07	22.57 ± 1.27	09	29.11 ± 1.90*	+ 29.0
30-40	10	23.50 ± 2.01	10	32.10 ± 3.10*	+ 36.6
40-50	10	24.72 ± 1.42	06	30.00 ± 2.82*	+ 21.4
50-60	02	27.50 ± 2.12	10	30.50 ± 2.83	+ 10.9
60-70	02	27.50 ± 2.12	07	31.85 ± 2.34	+ 15.8
70-80	02	26.00 ± 1.40	04	30.25 ± 1.25**	+ 16.4

*p<0.001 ,**p<0.05 as compared to control, Normal value < 30 IU/L

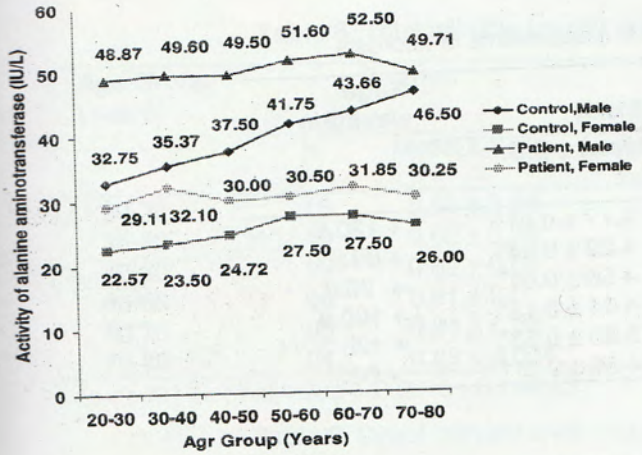


Fig. 9. Comparison of serum alanine aminotransferase activity in fluorotic patients of both sexes.

Alkaline phosphatase (ALP - EC 3.1.3.1)

Data showed a statistically significant increase ($p<0.001$) in the activity of alkaline phosphatase in patients of fluorosis. The percentage elevation in the activity of serum alkaline phosphatase observed was from 65.5% to 92.4% in patients of fluorosis (Table 5, Fig. 10)

Table 5. Serum alkaline phosphatase (IU/L) activity in control and patients of fluorosis

Age group (Years)	Control		Study		%age elevation
	N	Mean ± S.D	N	Mean ± S.D.	
20-30	*15*	*92.26 ± 3.22*	*18*	*177.55 ± 5.53**	*+ 92.4*
30-40	19	98.36 ± 6.95	29	184.86 ± 2.96*	+ 87.9
40-50	19	108.26 ± 5.33	27	190.95 ± 5.68*	+ 76.5
50-60	06	118.66 ± 5.39	35	196.33 ± 6.21*	+ 65.5
60-70	05	114.21 ± 3.49	26	196.81 ± 2.38*	+ 72.3
70-80	04	107.75 ± 3.40	18	199.83 ± 5.30*	+ 85.5

*p<0.001 Normal value = 60 – 170 IU/L

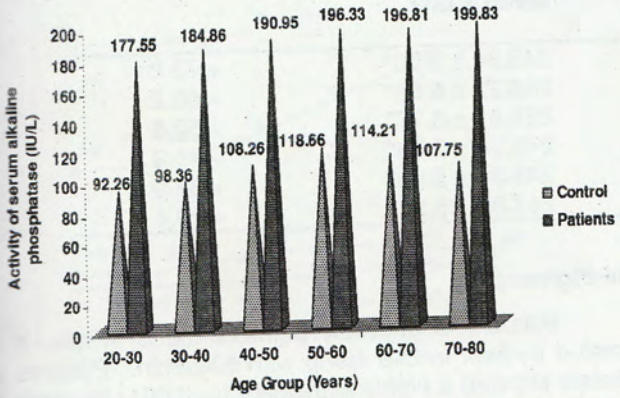


Fig. 10. Serum alkaline phosphatase (IU/L) activity in control and patients of fluorosis

Acid phosphatase (ACP - EC 3.1.3.2)

Acid phosphatase is an enzyme used to free attached phosphate groups from the molecules during digestion. Patients of fluorosis showed a significant ($p<0.001$) increase in ACP activity in all the age groups as compared to control. Patients exhibited the maximum increase in 60-70 (126.3%) and 20-30 (120.6%) year age groups(Table6,Fig.11).

Table 6. The activity of serum acid phosphatase (KA) in control and patients of fluorosis

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	08	1.89 ± 0.02	10	4.17 ± 0.01*	+ 120.6
30-40	08	2.26 ± 0.03	10	4.29 ± 0.04*	+ 89.8
40-50	10	2.33 ± 0.02	11	4.50 ± 0.04*	+ 96.0
50-60	06	2.46 ± 0.03	10	4.94 ± 0.13*	+ 100.8
60-70	05	2.43 ± 0.01	09	5.40 ± 0.33*	+ 126.3
70-80	04	2.31 ± 0.01	08	4.36 ± 0.21*	+ 88.7

*p<0.001 Normal value = 1.0 – 4.0 KA units

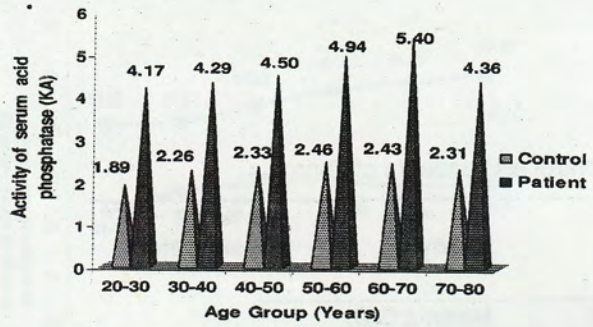


Fig. 11. Serum acid phosphatase (KA) activity in control and patients of fluorosis

Lactate dehydrogenase (LDH - EC 1.1.1.27)

LDH is an enzyme associated with carbohydrate metabolism. It is present in liver, muscles, heart and kidney. A highly significant (p<0.001) increase in LDH activity was recorded in patients of fluorosis. The higher percentage increase was 73.8% in 20-30 years age group and subsequently showed downward trend in remaining age groups (Table 7, Fig. 12

Table 7. Serum lactate dehydrogenase activity (IU/L) in control and patients of fluorosis.

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	15	141.14 ±5.87	18	245.34 ± 5.30*	+ 73.8
30-40	19	154.94 ± 5.98	29	248.22 ± 6.66*	+ 60.2
40-50	19	164.87±3.80	27	251.61 ± 6.77*	+ 52.6
50-60	06	176,21± 9.07	34	249.04 ± 7.08*	+ 41.3
60-70	05	185.52 ± 7.59	26	248.36 ± 5.85*	+ 33.9
70-80	04	205.87 ± 1.84	18	247.81 ± 6.99*	+ 20.4

*p<0.001 Normal value = 114 – 240 IU/L

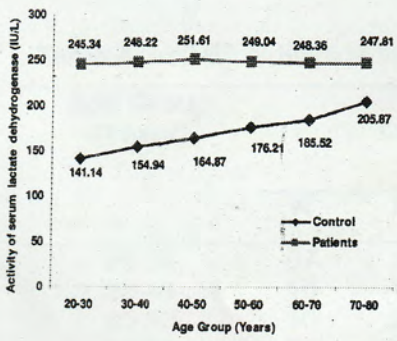


Fig. 12. Serum lactate dehydrogenase activity (IU/L) in control and patients of fluorosis

Bile Pigment

Bilirubin is a yellow pigment found in bile. It is secreted by liver in bile along with biliverdin. Patients of fluorosis showed a highly significant (p<0.001) increase in total bilirubin as compared to control. Maximum elevation was showed by patients of 30-40 years age group (58.1%) (Table 8).

A highly significant (p<0.001) increase in direct bilirubin level of patients of fluorosis as compared to control. The percent elevation was maximum in 40 – 50 years age group (47.4%) (Table 9, Fig. 13)

Table 8. Total bilirubin (mg/dl) in the control and patients of fluorosis.

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	15	0.59 ± 0.05	18	0.84 ± 0.07*	+ 42.4
30-40	19	0.62 ± 0.08	29	0.98 ± 0.10*	+ 58.1
40-50	19	0.69 ± 0.04	27	1.06 ± 0.12*	+ 53.6
50-60	06	0.81 ± 0.01	34	1.13 ± 0.13*	+ 39.5
60-70	05	0.94 ± 0.02	26	1.09 ± 0.09*	+ 15.9
70-80	04	0.89 ± 0.004	18	1.11 ± 0.08*	+24.7

*p<0.001 Normal value = 0.00 – 1.00 mg/dl

Table 9. Direct bilirubin level (mg/dl) in control and patients of fluorosis.

Age Group (Years)	Control		Study		%age elevation
	N	Mean ± S.D.	N	Mean ± S.D.	
20-30	15	0.18 ± 0.02	18	0.23 ± 0.04*	+27.8
30-40	19	0.19 ± 0.02	29	0.24 ± 0.04*	+ 26. 3
40-50	19	0.19 ± 0.02	27	0.28 ± 0.04*	+ 47.4
50-60	06	0.21 ± 0.02	34	0.31 ± 0.03*	+ 46.7
60-70	05	0.25 ± 0.04	26	0.31 ± 0.04*	+ 20.0
70-80	04	0.22 ± 0.02	18	0.27 ± 0.04*	+22.7

*p<0.001 Normal value = 0.00 – 0.25 mg/dl

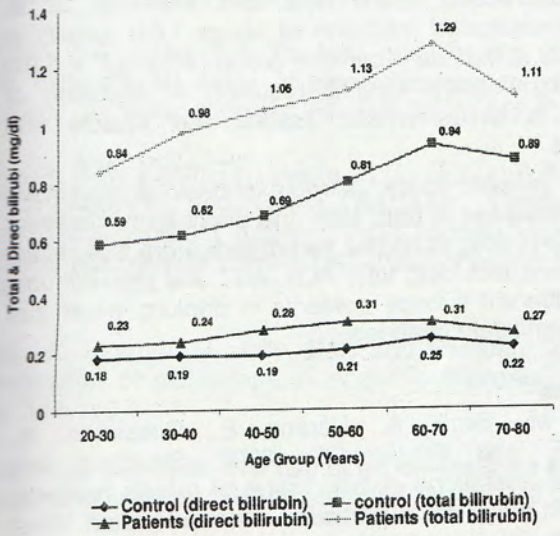


Fig. 13. Comparison of serum total and direct bilirubin (mg/dl) between control and patients of fluorosis.

Discussion

Serum fluoride concentration is recognized as a good indicator of fluoride exposure and provides an important basis for endemic fluorosis. (Parkin, 1974; Kono *et al.*, 1992; Wan *et al.*, 2000: Gupta *et al.*, 2001). In the present study, a significant (p<0.001) increase in the level of serum fluoride was noted. Serum fluoride levels showed a significant elevation with advancing (Li and Ke 1990; Fan *et al.*, 1992). Song *et al.*, (1995) also found that the severity of skeletal fluorosis is directly related to elevated serum fluoride. Barot, (1998) reported increase in serum fluoride (2.81 ± 0.179) in skeletal fluorosis. Li *et al.* (1996) demonstrated positive significant dose response relationship between the serum fluoride concentration and the prevalence of skeletal fluorosis. The correlation coefficient between the serum fluoride level and prevalence of skeletal fluorosis was 0.88. In the study of Xiang *et al.*(2005) the correlation coefficient was 0.95 by the model of quadratic fit. In the present study, the Pearson correlation coefficient was 0.670, which showed a highly significant (p<0.001) positive dose response relationship between serum fluoride concentration and drinking water fluoride level. Xiong *et al.*, (2007) also reported higher fluoride levels in the serum of children with dental fluorosis from high-fluoridated drinking water areas.

Serum AST and ALT, well-known marker of liver function were significantly elevated in the fluorotic patients in all age groups in the present study. The maximum percent elevation in AST (50%) and ALT (49.2%) was

recorded in male fluorotic patients which indicate disturbance in hepatic functions (Shashi, 2003).

Elevated serum AST and ALT has also been reported earlier by many workers. Plaa and Hewit (1982) showed marked increase in the activities of AST and ALT in fluoride exposed humans. Chinoy *et al.*(1992) found not only higher SGOT and SGPT levels demonstrating impaired liver function, but also reported cases with hepatic cellular and damage. Similar findings of significant levels of increased SGOT and SGPT was noted in a study of Michael *et al.* (1996) among the fluoride-exposed individuals of endemic region of North Gujarat.

Fluoride has been reported to cause a depression in DNA and RNA synthesis in cultured cells (Strochkova *et al.*, 1984). Fluorine is known to inhibit protein synthesis mainly due to impairment of peptide chain initiation and by interfering with peptide chain on ribosomes. Fluoride inhibits nucleic acid synthesis and attachment of m-RNA to ribosome. The decrease in RNA content of rabbit brain was observed during acute and chronic fluoride-intoxication seems to be due to fluoride-induced inhibition of protein syntheses (Shashi *et al.*, 1994). Fluoride has been reported to cause reduction in DNA and RNA synthesis as well as DNA/RNA and DNA/protein ratio, indicating probable disturbances in the process of transcription, translation, as well as mitotic cycles and chromosomal alterations (Memon and Chinoy, 2000). A per found ameliorative effect of black tea extract on NaF induced reduction in DNA, RNA, and protein contents in liver and kidney of mice and SGPT and SGOT activities in blood has been reported (Trivedi *et al.*, 2008). Acute hepatitis and light degeneration in the liver develop following fluoride intake (Kessabi and Hamliri, 1986). As a result of this serum transaminase activity increases (Fidanci and Sel, 2001).

In the present study, the activities of acid and alkaline phosphatase showed a statistically significant ($p<0.001$) increase in phosphatases enzyme activity. The maximum elevation of 126.3% and 92.4% was observed in acid phosphate and alkaline phosphate in the serum of fluorotic patients respectively. Similar results have been reported in earlier studies on fluorotic individuals (Shivashankara *et al.*, 2000; Khandare *et al.*, 2005) and on experimental analysis (Singh *et al.*, 1998; Liangx, 1999).

The serum levels of ALKP activity are generally an index of bone activity. Elevated plasma alkaline phosphatase and parathyroid hormone levels support the diagnosis of associated metabolic bone disease and secondary hyperparathyroidism in fluorosis (Teotia and Teotia, 1988). High levels of ALKP activity may suggest the presence of bone disturbances resulting in bone destruction. High levels of ALKP activity were reported to be associated with fluorosis, which increases osteoblastic activity (Jeji *et al.*, 1985; Khandare *et al.* 2005; Shivashankara *et al.* 2000). Fluorine exerts an inhibitory effect on many metabolic enzymes of various tissues. The ncreased level of ALKP and SGPT in fluoride exposed individuals affects renal and hepatic functions in endemic areas of India (Ravichandran *et al.*, 2006).

The fluoride in vitro causes alkaline phosphatase to increase the bone cell and plasma (Miller and Shupe, 1962;Farley *et al.*, 1983). Miller *et al.* (1977) reported that increased ALP activity is directly proportional to the fluoride concentration of the bone. Serum fluoride ALP activity was found to be higher ($p<0.001$) in sheep of Yatagan area than that in Ortaca area of Turkey (Fidancy and Sel, 2001).

LDH is a cytosolic enzyme of carbohydrates metabolism present in liver, muscles, kidney and heart and is also used in the diagnosis of endemic fluorosis (Rzeuski *et al.*, 1998). In the present study significant ($p<0.001$) increase in activity of LDH was noted in fluorotic patients. The elevated fluoride load increased as the drinking water fluoride concentration increased, which may cause damage to liver function (Xiong *et al.*, 2007).

It is reported that serum LDH activity is increased in rabbits (Akdogan *et al.*, 2002) and rats (Guan *et al.*, 1989) and sheep (Cenesiz *et al.*, 2005). Similarly, the addition of fluoride to culture media of myocardial cells increased LDH levels due to increased membrane permeability (Wang *et al.*, 1998).In fluoridated mice, a significant decrease ($p<0.001$) was observed in LDH activity in brain as well as gastrocnemius muscles, suggests reduction in glycolytic activities which may lead to an active pentose phosphate pathway for obtaining energy (Vani and Reddy, 2000). Zabrawska *et al.* (2002) observed that the LDH activity in sperm samples with three lower concentrations of fluoride (20, 100 and 200 Mmol/L) decreased nearly nine fold. Mamczer *et al.* (2007) demonstrated inhibition of serum LDH activity in serum of rat in fluoride + caffeine group (4.9 mg F^- + 3 mg caffeine/ kg / body weight) resulting in inhibition of glycolysis in extra hepatic tissues like muscle and erythrocyte.

In present study, bilirubin showed a significant ($p<0.001$) increase in both total and direct form. However Liang *et al.* (1999) analyzed serum indicators that reflect liver functions including total ALB, AST and total bilirubin from six different fluoride contents in drinking water and found no significant changes.

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Effect of *Achyranthes aspera* Leaf Extracts on the Wound Healing Activity of Albino Rats

V.Srividhya[a], A.Mazumder[b], L.K.Ghosh[c], **K.Nagarajan**[d*].

a)Post Graduate Research scholar, Karpagam College of Arts and science, Coimbatore, India. b)Professor & Head, School of Pharmacy, Technology & Management, NMIMS University, Mumbai, India.c)Professor, Dept. Of Pharmaceutical Technology, Jadavpur University, Kolkata, India. d)Assistant Professor & Head, Division of Bio-Medicinal chemistry R&D Laboratory, Department of Pharmacy, IIMT College of Medical Sciences, Meerut-250001, India.

Abstract:

The wound healing activity of ethanol extract of *Achyranthes aspera* (Amaranthaceae) incorporated in to ointments with yellow soft paraffin has been investigated on Wistar albino rats. Wound healing activity was studied using excision wound model in rats following topical application. The ethanolic leaf extract of the plant produced a significant response with the above wound model studied. The wound contracted in 16 days in case of *Achyranthes aspera* leaf extract ointment (10% w/w) and 18 days in case of leaf extract ointment (5% w/w), as compared with the standard drug Nitrofurazone ointment (0.2% w/w), having 18 days for complete healing of the wound. The ethanol extract have shown predominant wound healing activity which is mainly due to the presence of alkaloidal components.

Key words: *Achyranthes aspera*; Wound healing activity; Leaf ethanol extract; Excision model; wound contraction.

Introduction:

Wound may be defined as loss of breaking of cellular and anatomic or functional continuity of living tissues (Yeo *et al.,* 2000). The process of wound healing occurs in four phases namely, coagulation which prevent blood loss, inflammation and debridment of wound, repair, including cellular proliferation and tissue remodeling and collagen deposition. Wounds are visible results of individual cell death or damage and classified by site, size, depth and causation-surgery, accident or circulatory failure. Many growth factors usually polypeptide, for example, platelet derived growth factor (PDGF) and growth factors derived from epidermal cells (EGF) and macrophages (MDGF) are likely to be involved in healing and regeneration. Wound healing by first intention occurs in clean, incised wound with good apposition of the edges – particularly planned surgical incisions. Wound healing by second intention occurs in open wounds, particularly when there has been significant loss of tissue necrosis or infection. The indigenous system of medicine namely ayurvedic, siddha and unani have described several drugs from indigenous plant sources for the wound healing activity (Nair *et al.,* 1982). *Achyranthes aspera* is an erect herb found throughout India. It has astringent, emetic, antitumour and antiinflammatory activity (Kirtikar & Basu, 1994). The cancer chemopreventive activity of *Achyranthes aspera* leaves on Epstein – barr virus activation in methanolic extract in two stage mouse skin carcinogenesis suggests that *Achyranthes aspera* leaf extracts and the non alkaloidal fractions have valuable antitumour promoters in carcinogenesis (Asima *et al.,* 2002). *Achyranthes aspera* possess anti-inflammatory and anti-arthritic activity and support the rationale behind the traditional use of these plants in inflammatory conditions in rats and mice (Gokhale *et al.,* 2002). It has been found to elevate thyroid hormone levels and decrease hepatic lipid peroxidation in male rats, it appears that the *Achyranthes aspera* leaf extract is both prothyroidic and anti-peroxidative in nature (Pankaj and Anandkar, 2000). The effect of *Achyranthes aspera* leaf extracts on the immunity and survival of *Labeo rohita* infected with *Aeromona hydrophila* suggest that it stimulates immunity and increase resistance to infection in the fish (Vasudeva *et al.,* 2006). *Achyranthes aspera* was also found to stimulate the immunity and enhanced the antigen clearance in *Catla catla* (Rina and Rao, 2005). The *in vitro* determination of the contraceptive spermicidal activity of a composite extract of *Achyranthes aspera* and *Stephania hernandifolia* on human semen have also been shown and the data suggested that plant extracts possess potential contraceptive spermicidal activity *in vitro.* Based on the traditional classification, the present study aims at evaluating the wound healing potency of leaf extracts of *Achyranthes aspera.*

Material and Methods:

Leaves of *Achyranthes aspera* were collected in Coimbatore during the month of October 2006. The collected plants were botanically identified by an acknowledged Botanist and the voucher specimens are deposited at Tamil Nadu Agricultural University (TNAU), Coimbatore.

500 gms of leaf powder were extracted with ethanol, acetone and chloroform successively under 60-80ºC by continuous hot percolation process using Soxhlet apparatus. After completion of extraction, the corresponding extracts from ethanol were purified by bent tube and the solvent was removed by distillation under reduced pressure. Green waxy residue was obtained. The residue was stored in dessicator. The ethanol free semisolid mass thus obtained was used for the experiment Phytochemical screening shows the presence of alkaloids as predominant constituents.

Two types of ointment formulation were prepared from the extract 5% w/w, 10%w/w, where 5 gms and 10 gms of the extract were incorporated in 100gms of the simple ointment base B.P respectively. Nitrofurazone ointment (0.2% w/w, Smithkline Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

Wound healing activity:

Wistar Albino rats (150-180 gms) were selected for these studies. The animals were maintained at animal house of Periyar College of Pharmaceutical Sciences for girls, Trichy, which is approved by CPCSEA (F234). Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water adlibitum.

Excision wound model:

4 groups with 6 animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500mm square full thickness of skin from the depilated area (Saha *et al.*, 1997), the wound were left undressed to open environment. Then the drugs, that is the reference standard (0.2% w/w) Nitrofurazone (NFZ) ointment, Simple ointment B.P, Extract ointment of *Achyranthes aspera* (5% w/w) and *Achyranthes aspera* extract ointment (10% w/w) were applied once daily till the wound was completely healed. This model was used to monitor contraction and wound closure time (Morton *et al.*, 1972).

Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically (Prasad *et al.*, 1995) by tracing the wound margin on graph paper every alternate day.

Results and Discussion:

The extracts were screened for the phytochemical constituents like alkaloids, glycosides and steroids. The presence of alkaloids was observed in ethanolic extract. Absence of glycosides and steroids was observed in all the extracts of ethanol, acetone and chloroform. The results are expressed in Table 1.

The wound healing effect of *Achyranthes aspera* extract and Nitrofurazone on excision wound model was evidenced by percentage increase of wound contraction as the days increased. After 6 days of treatment with extracts, 5% w/w of *Achyranthes aspera* extract ointment showed better wound healing effect with an wound area of 228 sq.mm (56.4% increase of wound contraction), which was more efficient than the standard Nitrofurazone ointment (0.2% w/w), having the wound area of 268 sq.mm (49% increase of wound contraction), whereas 10% w/w extract ointment showed wound area of 298 sq.mm (42.4% increase of wound contraction), hence a substantial increase was observed among the extract combination. All the results are shown in table2.

Table 1. Phytochemical screening of various extracts from *Achyranthes aspera*

Active constituents	Ethanol	Acetone	Chloroform
Alkaloids (Dragendroffs reagent)	+	-	-
Alkaloids (Hagers reagent)	+	-	-
Steroids (Libermann Burchard reagent)	-	-	-
Glycosides	-	-	-

(+)-*Presence of Phytoconstituent* (-) -*Absence of Phytoconstituent*

Table 2. Effect of *Achyranthes aspera* extract and Nitrofurazone on excision wound model.

Post Wounding Days	Wound Area (mm²)			
	Simple Ointment (Control)	Nitrofurazone Ointment (0.2% W/W)	Extract Ointment (5% W/W each)	Extract combination (10% W/W each)
0	529 +/- 33.6(0)	526 +/- 36.8 (0)	523 +/- 24.0(0)	518 +/- 39.8(0)
2	509 +/- 18.6(3.7)	458 +/- 36.8(12.9)	468 +/- 19.8(10.5)	438 +/- 14.8(15.4)
4	453 +/- 13.8(14.3)	321 +/- 12.6*(38.9)	371+/- 17.9*(29)	341 +/- 18.6*(34.1)
6	428 +/- 30.1(19)	268 +/- 14.7*(49.0)	228 +/- 13.3*(56.4)	298 +/- 19.4 **(42.4)
8	379 +/- 14.8(28.3)	191 +/- 11.4**(63.6)	151 +/- 14.5**(71.1)	103 +/- 9.8**(80.1)
10	345 +/- 23.6(34.7)	108 +/- 8.6**(79.4)	89 +/- 10.1**(82.9)	71 +/- 5.9**(86.2)
12	269 +/- 14.3(49.1)	73 +/- 6.3**(86.1)	53 +/- 6.4**(89.8)	38 +/- 2.1**(92.6)
14	215 +/- 11.3(59.3)	31 +/- 1.6**(94.1)	32 +/- 4.8**(93.8)	13 +/- 1.3**(97.4)
16	189 +/- 14.3(64.2)	10 +/- 1.9**(98.0)	18 +/- 1.2**(96.5)	0.0**(100)
18	171 +/- 15.1(67.6)	0.0**(100)	0.0**(100)	----------

Meanwhile, as the days increased, the results varied considerably with increased dose of extract ointment. Thus 10% w/w extract ointment was very effective after 12 days in healing the wound with a wound area of 38 sq.mm (92.6% increase of wound contraction) in comparison with the 5% w/w extract ointment (53 sq.mm; 89.8%).

Both the standard drug and 5% w/w extract ointment completely healed the wound on 18th day, (0.0 sq.mm, 100%), while the 10% w/w extract ointment completely made a full fledged recovery against wound by healing it on 16th day onwards (0.0 sq.mm; 100%).

Among 5% w/w and 10% w/w ointment formulation, 10% w/w extract of the *Achyranthes aspera* ointment showed better wound healing activity in comparison with standard Nitrofurazone ointment. Clinically one may come across poor healing or non healing which may be due to infection of NSAIDS (Lee, 1968; Brandit *et al.*, 1984) and steroids (Ehrlich *et al.*, 1968; Ehrlich *et al.*, 1973). An increase in wound breaking strength of treated wounds may be due to increase in collagen concentration and stabilization of fibres (Udupa *et al.*, 1995). In recent years, oxidative stress has been implicated in a variety of degenerative processes and diseases. These include acute and chronic inflammatory conditions such as wound healing (Maiere *et al.*, 2002). Oxygen free radicals play an important role in the failure of ischemic wound healing and antioxidants improve the healing in Ischemic skin wounds (Senel *et al.*, 1997). The present potent wound healing action of *Achyranthes aspera* extract combination is mainly due to the presence of alkaloids as major phyto constituents, as described earlier in the literature with regard to the plant extracts having wound healing activities(N air *et al.*, 1982).

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Fluoride Induced Depletion of Mucosubstances in Rat Brain Cortical Slices

Shashi A*, Neetika S. And Bhardwaj M.
Department of Zoology, Punjabi University, Patiala 147 002, India
E-mail: shashiuniindia@yahoo.co.in

Abstract - The effect of fluoride on concentration of mucosubstance in cortical regions of brain of rats intoxicated with 30, 45, and 75 mg NaF/kg bw/day respectively for 20 and 35 days was investigated. The control rats were injected double distilled water 1cc/kg bw/day for the same period. The mucosubstance content in the brain of fluoride treated rats was studied by PAS staining method and it was noted that there was an overall decline in the cerebral mucosubstances as compared to control. Its content was decreased in the neural membrane and neuroplasm of glial cells in cerebral cortex of brain of rats exposed to 30 mg NaF/kg bw/day, while in rats treated with 45 mg NaF/kg bw/day, a decrease in PAS positive mucosubstances in neural membrane and neuroplasm but enhancement in nucleus was noticed. In rats of 75 mg NaF group, the mucosubstances were decreased in all cell regions of glial cells of cerebral hemisphere.
Corresponding Author: Dr. Shashi Aggarwal, Reader, Department of Zoology, Punjabi University, Patiala-147002, Punjab, India.
Key words *: Brain, Cerebellum, Cerebrum, Glial cells, Mucosubstances, Neural membrane, Neuroplasm.*

Introduction

Elevated level of long-term ingestion of fluoride cause fluorosis in bones and teeth (Li *et al.* 1995). Recent investigation point to an inverse relationship between early fluoride ingestion and intelligence, indicating a lower intelligence quotient in children living in villages with elevated fluoride intake from food and drinking water (Geereats *et al.* 1986). Fluoride is known to cross the blood brain barrier and accumulate in the central nervous system. Accumulation of fluoride induce wide variety of changes in the concentration of different chemical constituents viz. lipids (Stanley, 1972), proteins (Shashi, 1992) and nucleic acids (Lu *et al.* 2000). Over uptake of fluoride for a long term could cause potential increase in the level of oxidative stress in brain tissue (Shao *et al.*, 2000). The increased oxidative stress could be one of the mediating factors in the pathogenesis of fluoride toxicity in the brain (Shivarajshankara *et al.*, 2002). Brain and muscles are affected by fluoride with inhibition of some enzymes associated with free radical metabolism, energy production and transfer, membrane transport and synaptic transmission, but with enhanced activity of xanthine oxidase enzyme (Vani and Reddy, 2000).

The present study is designed to elucidate alterations in the level of mucosubstances in different regions of brain of rat in experiment fluorosis.

Materials and Methods

Design of study

In the present study Sprague Dawley albino rats of both sexes acclimatized for one week to laboratory conditions, and fed on pellet diet obtained from Hindustan Lever Limited, Mumbai, India. Water was supplied *ad libitum*. The animals were injected with NaF dissolved in deionized double distilled water /kg bw/day as depicted in Table I:

Experimental protocol

Group	Dose mg NaF/kg bw/day	Treatment (Days)	Day of autopsy (Days)	Number of animal used
Control (I)	1 cc double distilled water	20 & 35	Sacrificed along with treated	6
II	30 mg NaF	20	21	9
III	45 mg NaF	20	21	9
IV	75 mg NaF	20	21	9
V	30 mg NaF	35	36	9
VI	45 mg NaF	35	36	9
VII	75 mg NaF	35	36	9

After the completion of treatment period, the overnight fasted rats were sacrificed, brain tissues were taken out in chilled saline. Small pieces of brain were fixed in Bouin's fluid (24 hours). The tissues were dehydrated in 95% alcohol for 45 minutes, in tertiary-butyl alcohol for 6 hrs, cleared in amyl acetate for overnight and embedded in paraffin wax (temperature 60-62ºC). Wax blocks were then prepared and 7µm thin serial sections were cut with rotary microtome. The cytochemical localization of mucosubstances was studied by Periodic acid Schiff's method of staining (Pearse, 1968).

Result and Discussion

Group I- Control

The cerebral cortex in control rat reveals uniform distribution of PAS positive mucosubstances in different glial cells (Fig. 1).

During first phase of experiment, the rats were treated with 30, 45, and 75 mg NaF/kg bw/day for 20 days.

Group II- 30 mg NaF/kg bw/day for 20 days

The cerebral cortex showed neurodegenerative changes with reduced intensity of mucosubstances. The level of PAS positive mucosubstances were elevated in nucleus in comparison to neuroplasm and neural membrane of nurogalial cells of cerebral cortex of fluoride treated rats (Fig. 2).

Group III- 45 mg NaF/kg bw/day for 20 days

Deposition of mucosubstances in patchy form was observed in cerebral cortex of fluoride treated rats. The mucosubstances content in the neural membrane and nucleus showed elevation whereas it was decreased in the neuroplasm of the glial cells (Fig. 3).

Group IV- 75 mg NaF/kg bw/day for 20 days

The cerebral mucosubstances declined in the glial cells, neural membrane and neuroplasm. The nucleus showed increase in the intensity of PAS positive mucosubstances. The accumulation of mucosubstances in patchy form was prominent feature (Fig. 4).

During second phase of experiment the rats were treated with 30, 45, and 75 mg NaF/kg bw/day for 35 days.

Group V- 30 mg NaF/kg bw/day for 35 days

A few glial cells revealed increase in deposition of PAS positive mucosubstances. Neural membrane and nucleus revealed elevation in mucosubstances content while decline in its content was observed in neuroplasm (Fig. 5).

Group VI- 45 mg NaF/kg bw/day for 35 days

The intensity of mucosubstances elevated in some neuroglial cells of the cerebral cortex. The neural membrane, nucleus and neuroplasm revealed increase in intensity of PAS positive mucosubstances (Fig. 6).

Group VII- 75 mg NaF/kg bw/day for 35 days

The accumulation of carbohydrates in the form of small darkly stained patchy granules in cerebral cortex were clearly visible. The vacuolated degenerative neurons showed less intensity of mucosubstances (Fig. 7).

During present investigation an overall decrease in level of mucosubstances in different brain regions was observed. Very less literature is available on the direct effect of fluoride on carbohydrate synthesis in brain, however effect on enzymes involved in carbohydrate metabolism was studied by Vani and Reddy (2000), who reported accumulation of fluoride and altered activities of some enzymes involved in free radical metabolism and membrane function in brain and gastrocnemius muscle of female mice treated with 20 mg NaF/kg bw/day for 14 days. The enzymes viz; superoxide dismutase, glutathione transferase and catalase decreased significantly ($P<0.01$) in contrast to xanthine oxidase activity which moderately increased. Succinate dehydrogenase, lactate dehydrogenase, alanine amino transferase, aspartate amino transferase, creatine phorphokinase, activities, and membrane bound enzymes viz Na^+, K^+, Mg^{++} and Ca^{++} ATPase, and acetylcholinestrase were decreased significantly ($P<0.01$) in both brain and gastrocnemius muscle. This study suggested that fluoride affect enzymes like lactate dehydrogenase, which involved in carbohydrate metabolism.

Earlier workers reported the effect of fluoride on the cytochemical localization of other chemical components of brain like Gaun *et al.,* (1998) analyzed brain membrane lipids in rats being fed either 30 or 100 ppm fluoride for 3, 5, and 7 months. The protein content of brain with fluorosis decreases. After 7 months of fluoride treatment, the total brain phospholipids content decreased by 10% and 20% in the 30 and 100 ppm groups respectively. The main phospholipids influenced by fluorosis were phosphotidylethanolamine, phosphotidylcholine and phosphotidylserine. Tsunoda *et al.,* (2005) recorded the levels of regional brain neurotransmitter and their metabolites in different brain regions i.e. cerebellum, cerebrum, medulla, hypothalamus, mid brain and striatum. Decrease in the level of homovanillic acid was observed in the hypothalamus. Borlongan *et al.,* (2005) reported that methane sulphonyl fluoride treatment produced about 90% inhibition of acetylcholinesterase in the brain. They noted that MSF, possibly by reserving the functional cholinergic system, attenuated stroke-induced deficits in a simple learning and memory task.

The present study completely insures that fluoride caused deleterious effect on rat brain mucosubstances. The mucosubstances decline during initial phases, but were elevated as the dose of fluoride administration was increased.

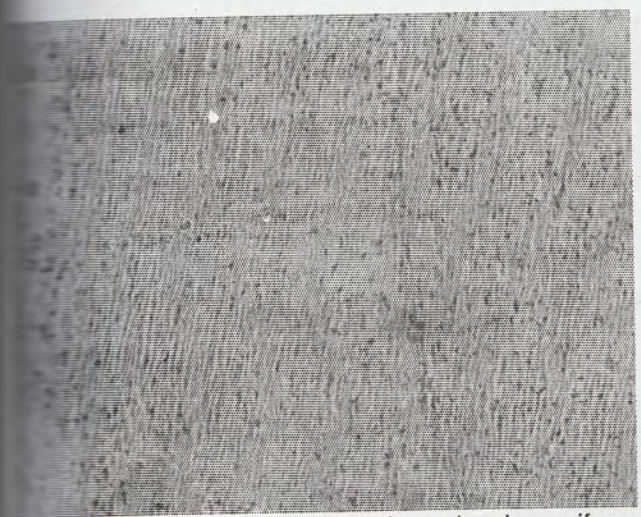


Fig.1 T.S. of cerebrum of control rat showing uniform distribution of mucosubstances in all neuronal regions. PAS (X 100).

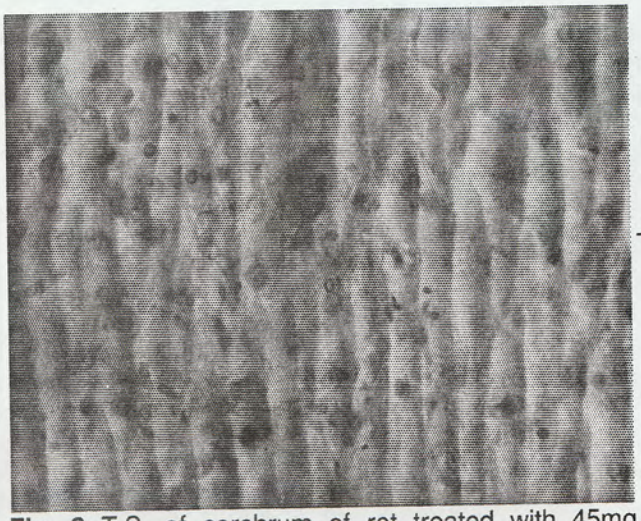


Fig. 3 T.S. of cerebrum of rat treated with 45mg NaF/kg bw/day for 20days showing decrease in mucosubstance content and its accumulation in particular region. PAS (X 400).

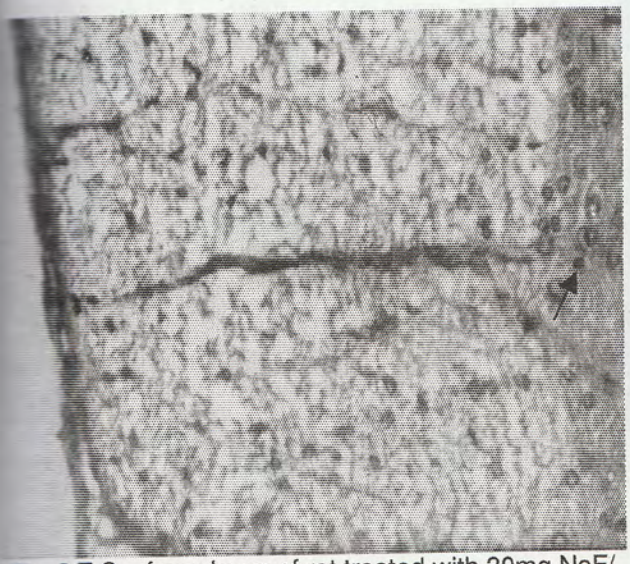


Fig. 2 T.S. of cerebrum of rat treated with 30mg NaF/kg bw/day for 20 days showing accumulation of mucosubstances in a single layer. PAS (X 400).

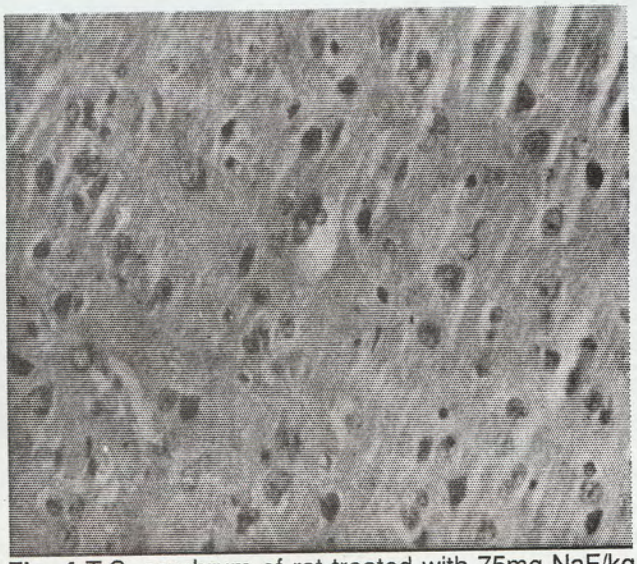


Fig. 4 T.S. cerebrum of rat treated with 75mg NaF/kg bw/day for 20 days showing decline in the mucosubstance content and its accumulation in small dense area. PAS (X 400



Fig. 5 T.S. of cerebrum of rat treated with 30mg NaF/kg bw/day for 35 days showing accumulation of mucosubstances in small patchy forms. PAS (X 400).

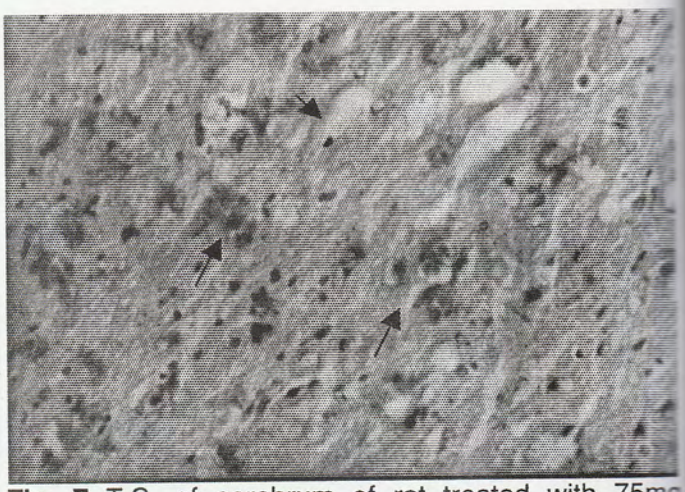


Fig. 7 T.S. of cerebrum of rat treated with 75mg NaF/kg bw/day for 35 days showing accumulation of PAS positive substances in the small granular form, formation of fibrous structure also observed. PAS (X 400).



Fig. 6 T.S. of cerebrum of rat treated with 45mg NaF/kg bw/day for 35 days showing accumulation of mucosubstances in the granular form in the neuroplasm of glial cells. PAS (X 400).

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Incidence of Brucellosis in rural areas of Qasseem region of Saudi Arabia: Case Reports.

Farah Sultan.
Al Butain Primary Health Center, Saudi Arabia
Present Address: 112 N, Pennock Avenue, Upper Darby, PA 19082, USA

Abstract:

Case reports of Brucellosis from rural areas of Qasseem region of Saudi Arabia along with symptoms of the disease and line of treatment are reported in present communication. These typical and rare cases of Brucellosis were only found in Qasseem region of Saudi Arabia. In the first case study blood transfusion and in second case study infected livestock was the cause of Brucellosis respectively.

Key words: Brucella. Livestock. Hooknoses. Pasturization . Blood transfusion . Undulant fever

Introduction:

Brucella is a coccobacillus, gram negative, non-sporing and non-motile aerobic bacterium whose hosts are mostly animals T-3 Brucellosis is a major zoonotic disease, where the bacterium possesses an unconventional non endotoxic lipopolysaccharide that confers resistance to antimicrobial attacks and modulates the host immune response . *Brucella* species are facultative intracellular pathogens that have the ability to survive and multiply in professional and non professional phagocytes and cause undulant fever in humans(Matyas and Fujikara, 1984 & Al Eissa *et al.*, 1990) It has four species: *abortus*, *canis*, *melitensis* and *suis* , *Brucella melitensis* is found primarily in goats, sheep and camels; *Brucella abortus* is found in cows; *Brucella suis* in pigs; and *Brucella canis* in dogs. The *Brucella* species differ in degree of virulence and invasiveness. *Brucella melitensis* is the most invasive and produces the most severe disease. *Brucella abortus* is the least invasive and causes the mildest illness. In Saudi Arabia, human infection with *Brucella melitensis* is commonly encountered (80%-100%), and infection with *Brucella abortus* is less frequent, but infection with other species has not been reported. The natural reservoir of control of brucellosis in agricultural animals is a prerequisite for the prevention of the disease in humans.

Brucellosis constitutes a major health and economic problem in many parts of the world, including countries of the Mediterranean Basin, the Middle East and the Arabian Gulf. It is endemic in Saudi Arabia (national prevalence, 15%) Kasab *et al.*, 1988. Endemicity in Saudia Arabia results from the persistence of domestic animal reservoirs for *Brucella* species and the human consumption of unpasteurized products . It is primarily a contagious disease of domestic animals—goats, sheep, cows, camels and dogs. Human-to-human transmission is uncommon but it has been rarely reported.3-5 after blood transfusion, bone marrow transplantation and possibly during sexual intercourse . Humans are commonly infected through ingestion of raw milk, cheese or meat, or through direct contact with infected animals, products of conception or animal discharges (e.g., among shepherds, farmers and veterinarians), and through inhalation of infectious aerosols (e.g., by workers in abattoirs and microbiology laboratories).

Although sporadic human cases of brucellosis were reported between 1956 and 1982 in Saudi Arabia, Arrighi 1986, it was not until the early 1980s that the disease emerged as a major public health problem of almost epidemic proportions. Kambal *et al.,* 1983, Radwan *et al.,* 1983;Norton 1984 Madkour *et.al.,* 1985; Al-Freihi , *et al.,*1986;.

In the large tertiary care hospitals, the increased rates of annual admissions for brucellosis were documented in the 1980s (Norton. 1984;, Talukdar *et al.,* 1985;, Al-Freihi *et al.,* 1986;, Hadad& , Smith 1986 ;Kiel &, Khan,1987;, Al-Eissa *et al.,* 1990; This surge in incidence of brucellosis in animals and humans during this period has been strongly linked to the uncontrolled importation of potentially infected animals with brucellosis, widespread animal husbandry, and the prevailing habit of ingesting raw milk or its products among the population of nomadic background. During the times of booming

economic prosperity of this country in the late 1970s, the government encouraged and subsidized the establishment of many dairy farms/industries and intensive sheep/cattle breeding projects with minimal veterinary resources. This led to the uncontrolled importation of animals that were poorly screened for infection, and subsequent introduction of brucellosis into the Kingdom. Hafez 1986;. It is of interest to note that seroepidemiologic surveys for brucellosis antibodies carried out on imported and local animals sacrificed during the Hajj season of 1977 revealed higher incidences of infection among imported animals; The prevalence of human brucellosis correlates closely with the extent of animal infection in a given country. .(Radwan *et al.,* 1983)

There has been a steady increase in the incidence of *Brucella* infection in the livestocks in Saudi Arabia over the past two decades. In 1977, the incidence of brucellosis in goats in Makkah, Saudi Arabia was found to be 0.8%, in sheep 0.5%, in camels 2.8% and in cows 3.6% (Radwan *et al.,*1983). In 1987, the incidence of brucellosis had gone up to 18.2% in goats, 12.3% in sheep, 22.6% in camels and 15.5% in cows in the Asir region(Bilal *et al.,*1991).Morbidity in humans in the Saudi population continues to be reported with increasing frequency from various regions of the country, particularly from the rural areas, and human infection is in the range of 1.6%-2.6%. Al-Nasser A *et al.,* 1991; Al-Sekait , 1992;Al-Balla Suliman 1995.The disease presents in both sexes and in all ages. A natural survey of 23,613 subjects was conducted in 1997, in order to assess the seroprevalence of brucellosis in Saudi Arabia. The result of the study revealed that the seroprevalence of brucellosis was 15%. The seroprevalence increased by age, and was higher in rural areas, and among people in high-risk occupation. With humans, the main form of acquiring brucellosis is through ingestion of raw milk and milk products obtained mainly from infected goats or camels, a traditional custom fostered by the nomadic heritage and dietary habits of the people. Human brucellosis is notoriously a multisystem disease with varied manifestations, and the onset may be either acute or insidious. The latter mode of presentation causes more difficulties in diagnosis. The diverse and sometimes deceptive manifestations of localized, sub-acute or chronic infection may lead to missing or delaying the diagnosis if the attending clinician has a low index of suspicion (Al-Eissa *et al.,*1991). Morbidity depends largely upon the speed of diagnosis and the initiation of specific antimicrobial therapy. Fever with drenching sweats, lassitude, arthralgia, body aches, anorexia and weight loss are common symptoms in acute brucellosis. Fever occurs in all patients at some time during their illness, but it can wax and wane over a period of weeks to months when untreated, hence the term undulant fever. Hepatosplenomegaly and lymphadenopathy are the usual physical findings.

Hepatitis probably occurs in most infected patients during the course of their illness, and liver function tests commonly demonstrate hepatic involvement. (Al-Eissa *et al.,*1990; Al-Aska , 1989).Most patients experience nausea, vomiting, anorexia and weight loss. Rare cases of ileitis, colitis and spontaneous peritonitis have been reported. Al-Eissa *et al.,*1991. Halim *et al.,*1993. Hematologic complications of mild anemia and leucopenia have been frequently associated with acute brucellosis, (Al-Eissa *et al.,*1993) but pancytopenia and thrombocytopenia are less frequently seen.(Al-Eissa *et al.,*1993). Cardiovascular localization of *Brucella* infection can result in endocarditis, myocarditis or pericarditis. (Al-Kasab *et al.,* 1988). The aortic valve has been commonly involved, followed by the mitral valve alone or both valves concurrently. Meningitis, encephalitis, meningoencephalitis, cerebellar ataxia, myelitis, radiculitis, peripheral neuritis, Guillain-Barré syndrome, and cranial nerve palsy have occurred in patients with brucellosis and involvement of the nervous system(Bashir *et al.,* 1985;Al-Orainey *et al.,* 1987;Al-Eissa ,1995; Al-Eissa & Al-Herbish, 1996). Psychiatric disturbances seem to be frequent, but they are not clinically recognized (Al-Eissa Y *et al.,*1991). Epididymo-orchitis is the most common genitourinary complication (Patel 1988). A variety of pulmonary, ophthalmic and cutaneous manifestations of brucellosis have been reported (Al-Eissa *et al.,*1991; Al-Eissa , 1993; Patel *et al.,*1988; Abdelrazak , 1991; Al-Orainey *et al.,* 1988)

The definitive diagnosis of brucellosis is made when the organisms are isolated from blood, bone marrow, or other body fluids or tissues. A presumptive diagnosis can be made by demonstrating significant *Brucella* titers.

Although most patients with brucellosis have agglutination titers of 1:160 or more, the significance of the titer level must be interpreted in light of epidemiologic evidence of a possible source of infection and clinical manifestations compatible with the disease. *Brucella* serology does not distinguish between the various species because the conventional serum agglutination test (SAT) equally detects antibodies to *Brucella melitensis*, *Brucella abortus* and *Brucella suis*, due to crossreactivity. Among the newer serologic tests, enzyme-linked immunosorbent assay (ELISA) appears to be the most sensitive and it may replace the SAT in the future. Polymerase chain reaction (PCR) methods using specific primers have been developed, but further evaluation and standardization of these methods are required. The microplate agglutination test (MAT) was developed as screening test and successfully utilized in population surveys,(Rienthaler *et al.,* 1998; Ndyabahinduka and Chu . 1984. MAT is probably the ideal method for population surveys because it has the advantage of being more rapid, economical, and highly sensitive. The efficacy of antimicrobial therapy for any infection is largely judged by the rate of cure and the incidence of sequelae. Human brucellosis continues to pose a therapeutic problem despite the susceptibility of the organisms to several antibiotics; this has been attributed to the intracellular localization of the brucellae within the reticuloendothelial cells of the host, a site that is relatively inaccessible to antibiotics.

The unpredictable relapses after treatment are almost invariably associated with inappropriate choice, dosage, and length of antimicrobial therapy, or failure of patients to take prescribed drugs. Antibiotic-resistant *Brucella* strains are rarely a cause of therapy failure. Hence, the institution of a proper combination of antibiotics for a longer duration seems warranted to improve outcome and prevent relapses. The standard therapy for acute brucellosis has been a combination of orally administered tetracycline or doxycycline for six weeks, plus streptomycin intramuscularly for the first two weeks. Treatment with orally administered rifampin, together with tetracycline or doxycycline for six weeks, is an effective alternative regimen. Treatment with cotrimoxazole is an acceptable alternative to tetracycline for use in children less than nine years of age. Relapses occur in fewer than 10% of cases when the patient receives proper therapy early in the course of illness and rapidly responds to a repeat course of antimicrobial therapy. Use three different antibiotics for as long as 3 months if the central nervous system is infected. For localized, serious, or chronic infection, a prolonged course of 2 to 4 combined antimicrobial drugs may be required to improve outcome and prevent relapses. Clinical trials with other antibiotics, including cephalosporins, new macrolides, and fluoroquinolones, have either given inferior results or have been too limited for proper evaluation. A safe and effective vaccine for use in humans is not yet available, but recent work on *purE* mutants of *B. melitensis* has given promising results in animals and may lead to clinical trials.

The magnitude of human *Brucella* infection in Saudi Arabia can serve as a barometer of the prevalence of the disease in domestic animals. Eradication of brucellosis in animals is the key to prevention in humans. Attempts to eliminate brucellosis have been successful in many developed countries. These countries maintain their brucellosis-free herds by serologic testing, quarantine, and other precautionary measures at their frontiers to prevent importation of infected animals. The situation in Saudi Arabia is unique and more complicated because of continuous importation of millions of slaughter animals annually, poor animal quarantine procedures and lack of legislation to control marketing and movement of animals. These major constraints are barriers to the achievement of control and eradication of the disease in this country.

Although attempts have been made for surveillance, testing and massive immunization of animals in certain areas with high infection rates, an organized national brucellosis control program to eradicate the disease has not been undertaken. Human brucellosis acquired from milk is preventable, and legislation should be enacted to strictly require pasteurization of milk and dairy products. Nevertheless, public health education assumes an important role in preventing the transmission of brucellosis from animals to humans. *Brucella melitensis* remains the principle cause of human brucellosis in Saudi Arabia, causing 88-93% of the cases. Recent national statistics indicate that the disease incidence in humans is close to 40 cases per 100,000. The eradication of human brucellosis in Saudi Arabia will ultimately depend on the eradication of animal

brucellosis. There is an urgent need for a national program for controlling brucellosis in the Kingdom. The components of this program will include recruitment and training of qualified veterinarians, development of an adequate number of animal quarantine centers and implementing legislation to control marketing and movement of animals.There is controversy about the relationship between brucellosis and the outcome of pregnancy (Hackmon *et al.,* 1998; Harefuah 1998). There is some evidence that brucellosis causes a higher rate of complications such as abortion, preterm labour and IUFD more frequently than do other bacterial infections (Hackmon *et al.,* 1998). Harefuah. 1998). It is postulated that maternal bacteremia, toxemia, acute febrile reaction and DIC are causes of spontaneous abortion and IUFD in brucellosis.

Objective:

My objective for reporting these Cases of Acute human brucellosis is that in my career of 13 years practice as a physician in different countries like India, Saudi Arabia and United States of America, I came across these Typical & Rare cases of Brucellosis only in Qasseem region of Saudi Arabia. In my first case study Blood Transfusion and in second study infected Livestock was the cause of Brucellosis.

CASE STUDIES ON BRUCELLOSIS

The purpose of this study was to study rare disease like Brucella in the general population in different endemic areas of Saudi Arabia.

Materials and Methods

Saudi Arabia covers an area of about two million square kilometers, and has an estimated population of about 16 million, with almost 70% living in urban areas. About 7% of the population led a nomadic lifestyle in tent settlements (National Population Atlas, Kingdom of Saudi Arabia, 1998). Case study was done in Al Qassim region of Saudi Arab, Al Butain and Al Fawarah Primary Health Centers. Investigations included interviews, clinical examination and blood sampling for antibody determination, using the standard tube agglutination test (STAT).

Case1.

HISTORY: A 32 years old pregnant female reported to me in her first trimester with the complaints of generalized weakness, pain abdomen, severe backache and night sweats. She gave the history of blood transfusion for Anemia in her first trimester of pregnancy. Case was reported in Al Butain in the year 1999.

Vitals P 99/min, B.P 90/70, RR 28/min (tachypneic), febrile 38 degree centigrade,

HEENT Headache, blurred vision

NECK has no lymphadenopathy

CHEST was clear, no rales, rhonchi or wheezing.

HEART has irregular rate & rhythm, normal S1/S2, no murmurs, rubs or gallops.

PER ABDOMEN soft, non tender, BS+, hepatomegaly +, splenomegaly +

JOINTS Spinal tenderness + , no joint tenderness

EXTREMITIES: edema+, clubbing -, cyanosis -Blood work showed moderate anemia, and pancytopenia. Serological Test such as the standard tube agglutination test (STAT) showed the titer of 1:320 (B.abortus) and positive blood cultures done on 10/1999. Lymphocytosis or evidence of hemolysis was not found.

The female patient was referred to Tertiary Hospital for follow-up and was treated with a combination of Rifampicin and Trimethoprim-sulfamethoxazole for 6 weeks, and blood transfused for anemia. Result was that she aborted in early third trimester.

Case 2.

A 30 year old Saudi female, presented to me with the complaints of severe sore throat, intermittent fever, joint pains mainly involving the weight bearing joints of lower extremities, sweats & 3 pounds weight loss since 4-5 weeks. She gave the dietary history of ingestion of undercooked meat, unboiled milk and a close contact with sheep & goats as she was a shepherd by occupation. She reported to me on 1/2001 in the Primary Health Center of a small desert of Al- Fawarah, a place famous for rearing livestocks like goats, sheep & camels or in other words Brucellosis Endemic area.

Vitals P 102/min (tachycardiac), B.P 120/80, RR 24/min (tachypneic), febrile 39 degree centigrade,

HEENT WNL,

NECK has no lymphadenopathy

CHEST was clear, no rales, rhonchi or wheezing.

HEART has regular rate & rhythm, normal S1/S2, no murmurs, rubs or gallops.

PER ABDOMEN soft, non tender, BS+, hepatomegaly +, splenomegaly -

JOINTS knee movement's restricted, spinal tenderness -

EXTREMITIES: No clubbing, cyanosis, or edema.

Blood examination showed mild anemia, leucopenia & lymphocytosis. Serological Test such as the standard tube agglutination test (STAT) showed the titer of 1:160 (B.melitensis) and positive blood cultures done on 1/2001. Thrombocytopenia, Pancytopenia, or evidence of hemolysis was not found.

The patient was treated with a combination of Rifampicin and Doxycyclin for 6 weeks. Fatima responded to the treatment well and fully recovered from Brucellosis. Result was that due to drug interaction between Rifampicin & Oral contraceptive pills (OCPS) she was using, Rifampicin impairs the effectiveness of OCPs and she conceived soon after and gave birth to a healthy baby boy.

Progress: The patient was found to progress good & was symptom free with no signs of relapse, gave follow-up visits at 3 months & 6 months interval.

Conclusion : The effectiveness of vaccination programs can be evaluated by investigating incidence rate in humans, especially people at high risk, before and after vaccination. It is recommended that surveillance of brucellosis should be strengthened. Cooperation and joint supervision between the Ministries of Health, Agriculture, and Municipality and Rural Affairs, and cooperation with neighboring Countries, should also be encouraged. Furthermore, we should adopt health education programs that aim at stopping the spread of infection among animals and then to humans. Regulations concerning the adoption of hygiene measures among high-risk population, especially. These two case studies confirm that ignorance is one of the important reversible factors behind this rare disease in Saudi Arabia specially in small deserts. So people should be oriented & educated to ingest pasteurized milk, fully cooked meat products and avoid intimate contact with infected animals like cattle, sheep, goats, camels and so on. Follow basic precautions to prevent disease transmission when handling animals. Wear waterproof gloves and thoroughly wash hands after handling, especially with pregnant animals at the time of birth.

Along with timely reporting to physician if these symptoms arise and maintaining the compliance to antimicrobial treatment will reduce the morbidity and major complications like infection of the CNS or the lining of the heart (endocarditis). Brucellosis remains a major public health problem in many developing countries.5-20 Saudi Arabia is one of those countries where control measures are either lacking or difficult to implement. These countries do not have good standardized and effective public health & animal disease control programs. A Brucellosis Eradication Program has been established to eradicate the disease from cattle's in United States. Post exposure prophylaxis recommendations are Doxycyclin 100 mg orally twice a day plus Rifampicin 600mg orally once a day for 21 days.

In the United States, frequency is related to the number of infected animals. Infected animals are rare in the United States, and pasteurization of milk has eliminated that potential reservoir, thus infection generally occurs via occupational exposure (cattlemen, veterinarians, slaughterhouse workers). The incidence is approximately 200 per year or 0.04 per 100,000. Patients in the United States are primarily found in Texas, California, Virginia, and Florida.

Frequency of brucellosis varies across nations but obviously is higher in more agrarian societies and in places where handling of animal products and dairy products is less stringent. The highest incidence is observed in the Middle East, Mediterranean region, China, India, Peru, and Mexico. Currently, central and southwest Asia is seeing the greatest increase in cases.

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Inhibitory effect of *Glycyrrhiza glabra* extract on deoxyribose degradation.

Kavita Shakya, Renu Mishra & Smitha Sam*
Dept. of Microbiology, Shri Satya Sai College, Bhopal
* Research Centre, Jawaharlal Nehru Cancer Hospital, Bhopal

Abstract

Reactive oxygen species (ROS) have been known to damage many biological macromolecules, of which DNA is the most significant target. Oxidative DNA damage is considered to be a good biomarker relevant to carcinogenesis and aging. In the present study, the antioxidant activity of methanolic extract of *Glycyrrhiza glabra* was investigated by evaluating its inhibitory effect on deoxyribose degradation using Fentons method. The activity was found to increase in a concentration dependent manner and the results were comparable to that of the standard antioxidant, alpha tocopherol, whose percentage inhibition of thiobarbituric acid reactive substances (TBARS) was only slightly higher than that of *Glycyrrhiza glabra.* This analysis can also be used to assess the capacity of *Glycyrrhiza glabra* to scavenge of OH· rivaled by 2-deoxy-D-ribose and to inhibit the metal-ion dependent generation of OH·.

Keywords: Antioxidant activity, Reactive Oxygen Species, Thiobarbituric Acid Reactive Substances, Fentons

Introduction

Recent years have witnessed a renewed interest in plants as pharmaceuticals. This interest has been focused not only on the discovery of new biologically active molecules by the pharmaceutical industry, but also on the adoption of crude extracts of plants for self-medication by the general public (Devasagayam, and Sainis, 2002). Within this context, considerable interest has arisen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients with antioxidant properties, such as vitamin E, vitamin C, ß-carotene and plant phenolics. There is high demand for natural antioxidants in the food, cosmetic and therapeutic industry, due to their low cost, high stability, high compatibility with dietary intake and no or very less harmful effects inside the human body, unlike some synthetic antioxidants BHA (Butylated Hydroxyl Anisole) and BHT (Butylated Hydroxy Toluene) which are carcinogenic in nature (Aherne *et al.*, 2007).

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals, hydroxyl radicals (OH.) and non free-radical species such as H_2O_2 and singlet oxygen ($.O_2$), are various forms of activated oxygen. The importance of free radicals and ROS has attracted increasing attention over the past decade. These molecules are exacerbating factors in cellular injury and aging process. ROS have aroused significant interest among scientists. Their broad range of effects on biological and medicinal systems has drawn the attention of many experimental works (Gouindarajan *et al.*, 2003; Gayathri *et al.*, 2005; Aqil *et al.*, 2006; Kumar *et al.*, 2006). Iron is an essential element for all living organisms, but it can be toxic when not carefully treated by proteins and also under diseased conditions. DNA is known to bind various metal ions because of its polyanionic nature and is therefore especially prone to iron-dependent site-specific oxidative damage. Iron-mediated oxidative damage to DNA has been implicated in several diseased conditions. The mechanism by which iron can cause this deleterious effect is that ferrous ion (Fe^{2+}) can react with hydrogen peroxide (H_2O_2) to produce the hydroxyl radical (HO*) via the Fenton reaction. By this reaction, a standardized solution of Fe–EDTA complex or ferrous iron catalyst reacts with hydrogen peroxide, leading to the formation of hydroxyl radicals. The peroxide is broken down into a hydroxide ion (-OH) and a hydroxyl free radical (HO*). The hydroxyl free radical is the primary oxidizing species and can be used to oxidize and break apart organic molecules resulting in the release of thiobarbituric acid reactive substances (TBARS) (Halliwell and Gutteridge, 1989; Desmarchelier *et al.*, 2005).

The Fenton reaction generates hydroxyl radicals (OH*), which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Addition of low concentrations of transition metal ions such as

iron to DNA causes degradation of the sugar into malondialdehyde (MDA) and other related compounds which form a chromogen with thiobarbituric acid reactive substances (TBARS) Antioxidants suppress the production of TBARS that can be measured by spectrophotometer and the inhibition of colour development is defined as antioxidant activity (AOA). (Ishikawa *et al,* 2004; Gulcin *et al*, 2007). *Glycyrrhiza glabra* (Leguminosae), a hardy herb and grows in subtropical climates in rich soil to a height of four or five feet, is commonly used for the treatment of many ailments such as sore throat, colds and cough for about a hundred years. Later, Glycyrrhizin (GL), a compound isolated from its extract was examined by several researchers for its many activities including anti-inflammatory effects. Its antioxidant properties are evident through its inhibition of lipid peroxidation in rat liver, and its protective action on mitochondrial functions under oxidative stress, where the isoflavones (hispaglabridins A and B, glabridin A and 4-O-methylglabridin), the chalcones (isoliquiritigenin and isoprenylchalcone) and the isoflavone formononetin played an essential role (Jingnan *et al.*, 2007).

The purpose of this study was to determine the effect of methanolic extract of *Glycyrrhiza glabra* on the iron catalyzed HO* formation as compared to the activity of Vitamin E (alpha tocopherol) to inhibit the iron dependent HO* formation, by using the HO* generating system Fe^{3+} / H_2O_2 system.

Materials and Methods:

Collection and Extraction of Plant materials

The roots of *Glycyrrhiza glabra* were collected from Botanical gardens and forests areas near Bhopal. The collected plant materials were cleaned to remove any foreign material thereon. This cleaning was achieved by manually separating the dirt and other foreign matter adhered to the plant and repeatedly washing the plant with tap water at room temperature. These were kept in shade at room temperature ($25^0C \pm 2^0C$) for drying. The cleaned materials were grounded to powder in order to obtain a particulated mass. The dried and powdered plant materials were extracted with petroleum ether. This removes fats, oils, terpenes, waxes etc. After removing the petroleum-ether layer, the remaining material was extracted with suitable solvent system using separating funnel for maceration and percolation. The entire set up was kept at room temperature with intermittent shaking. The material was then extracted with 50% methanol at room temperature. After filtration it was dried at 40^0C to make free from the solvent. Finally, it was concentrated in hot air oven at 40^0C to obtain the desired drug powder. Weighed quantity of the extract was suspended in DDW (Double Distilled Water) to prepare appropriate dilutions.

Determination of inhibitory effect on deoxyribose degradation

Inhibitory effect of *Glycyrrhiza glabra* extract on deoxyribose degradation was determined by measuring the production of 2-thiobarbituric acid-reactive substances (TBARS) (Halliwell *et al,* 1987; Lee *et al*, 2003). For this scavenging assay, a 0.1 ml aliquot of different dulutions (10ug/ml, 20ug/ml, 30ug/ml, 40ug/ml, and 50 ug/ml) of different concentrations (100%, 75%, 50%, and 25%) of *Glycyrrhiza glabra* extract was mixed with 1 ml of reaction buffer (100 IM $FeCl_3$, 104 IM EDTA, 1.5 Mm H_2O_2, 2.5 mM deoxyribose, and 100 IM L-ascorbic acid, pH 7.4) and incubated for 1 h at 37 $\pm^0C$. A 1 ml aliquot of 0.5% 2-thiobarbituric acid (TBA) in 0.025 M NaOH and 1 ml of 2.8% trichloroacetic acid (TCA) were added to the mixture and it was heated for 30 min at 80 $\pm^0C$. The mixture was cooled on ice and the absorbance was measured at 532 nm. Percentage inhibition of TBARS (Thiobarbituric Acid Reactive Substance) was calculated using following formula:

$$\% \text{ Inhibition of TBARS} = \frac{\text{Absorbance(testsample)} - \text{Absorbance(control)}}{\text{Absorbance (control)}} \times 100$$

Graph was plotted for parentage inhibition vs concentration.

Statistical analyses

Experimental results were mean ± S.D of three parallel measurements. Statistical analysis was performed according to the student's t-test. Analysis of variance was performed by ANOVA procedure.

Results

The effect of *Glycyrrhiza glabra* extract on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the Fe^{2+} - dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH·), which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Figure 1 shows the effect of the concentration of deoxyribose on oxidative degradation induced by the Fe^{3+} / H_2O_2 system with control. The study was done in triplicats and the results were expressed as

percentage inhibition of thiobarbituric acid reactive substances (TBARS). It was observed that the absorbance of methanolic extract of *Glycyrrhiza glabra* increases in a concentration dependent manner. For 100% concentration of the extract the absorbance (Mean ± SD) increases from 0.7053 ± 0.00 (10ul) to 3.93 ± 0.06 (50ul); for 75%, from 0.519 ± 0.005 (10ul) to 1.523 ± 0.002 (50ul); for 50%, from 0.5463 ± 0.001 (10ul) to 0.983 ± .00 (50ul); and for 25%, from 0.311 ± 0.00 (10ul) to 1.146 ± 0.001. These values were in comparable to that of the positive control, alpha tocopherol, whose absorbance increases from 0.72633 ± 0.00153 (10ul) to 3.99367 ± 0.01484 (50ul), from 0.52067 ± 0.00306 (10ul) to 1.545 ± 0.005 (50ul), from 0.553 ± 0.00265 (10ul) to 1.10767 ± 0.00551 (50ul), and from 0.35367 ± 0.00321 (10ul) to 1.215 ± 0.00 (50ul), for 100%, 75%, 50% and 25% concentration respectively. The results were statistically significant at $P<0.001$, as compared to control. The antioxidant mechanism by which extract inhibits deoxyribose degradation could be by hydroxyl radical trapping or by ferrous chelating action. Results indicate that the extract of *Glycyrrhiza glabra* has the ability to protect against oxidative damage to DNA, but was not as effective as that of Vitamin E or alpha tocopherol (At).

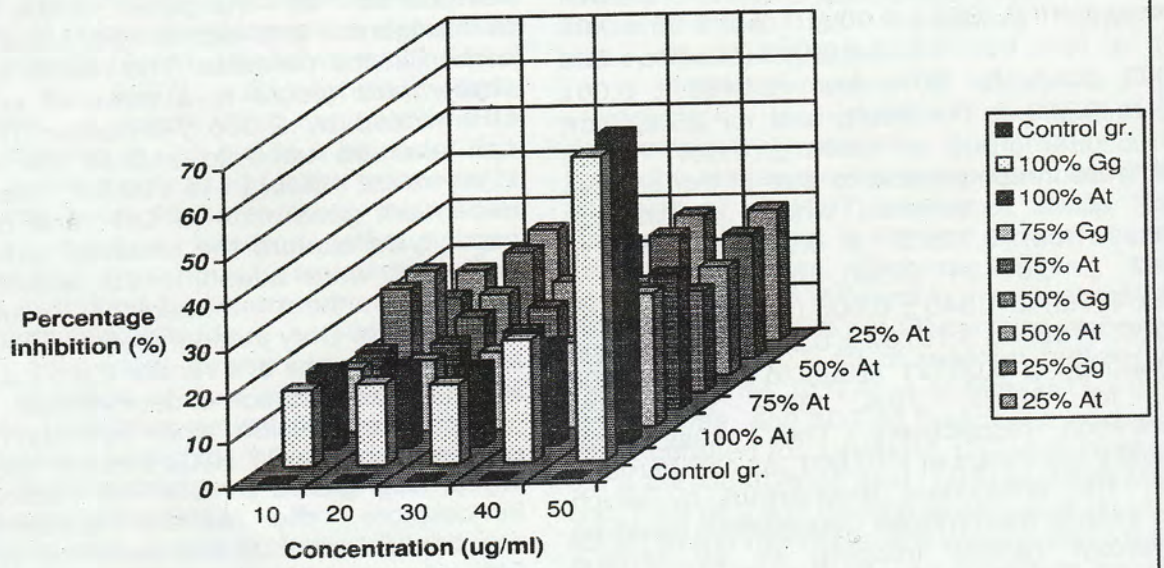
Discussion

Free radical scavenging property of the methanolic extract of *Glycyrrhiza glabra* has been evaluated in previous studies using different methods like inhibition of lipid peroxidation in rat liver, and its protective action on mitochondrial functions under oxidative stress (Jingnan *et al*, 2007). The antioxidant property was further verified by deoxyribose assay. In the assay, free radicals were generated by Fentons reaction, which react with deoxyribose and produce MDA. MDA on being incubated with thiobarbituric acid (TBA) produces a pink chromogen. Free radical scavenging compounds scavenge the free hydroxyl radical generated by the Fenton reaction and limits its availability to react with the deoxyribose, thus reducing the formation of pink chromogen. The change in the color of the reaction mixture was quantified to evaluate the scavenging property of the tested compound. Deoxyribose assay therefore further authenticated the free radical scavenging property of the extract (Ashvin, and Mishra, 2007; Duan *et al*, 2007). One primary advantage of the Fenton's Reaction is that it does not produce further organic compounds or inorganic solids such as permanganate and dichromate, since there is no carbon in the peroxide. The results showed that *Glycyrrihiza glabra* is a powerful scavenger of OH· rivaled by 2-deoxy-D-ribose. This analysis can also be used to assess the capacity of *Glycyrrihiza glabra* to inhibit the metal-ion dependent generation of OH·, and not only the capacity to capture the released radicals. When Fe^{3+} ions were added to the reaction mixture, some of them formed a complex with deoxyribose. Only these molecules that were able to chelate Fe ions and render them inactive, could inhibit the degradation of deoxyribose.

In conclusion, when hydroxyl radicals are generated by Fe^{3+} / H_2O_2 system, the extract of *Glycyrrhiza glabra* inhibits the Fenton reaction. Furthermore, the extract accelerates Fe^{2+} autoxidation and thus decreases the availability of Fe^{2+} for participation in hydroxyl radical generating Fenton reactions. These results suggest that *Glycyrrhiza glabra* works as an effective antioxidative herb against iron-catalyzed hydroxyl radical formation. Moreover, *Glycyrrihiza glabra* and Vitamin E might be useful for the prevention of iron-mediated oxidative stress related diseases. *Glycyrrihiza glabra* exhibited excellent antioxidant activity as suggested in many studies. In the present study, it was found that they also acted as strong electron-donating agents in the Fe^{3+} to Fe^{2+} assay.

Since *Glycyrrihiza glabra* is used in both traditional and formal medicine to treat a number of conditions, possibly its effectiveness is partly due to its capacity to neutralize free radicals hydroxyl radicals. These properties make *Glycyrrihiza glabra* applicable to both food and pharmaceutical industries. Hence, natural antioxidants strengthen the endogenous antioxidant defences from ROS damage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention.

Fig.1. Inhibitory effect of Glycyrrhiza glabra (Gg) and Alpha tocopherol (At) on deoxyribose degradation



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Certain biochemical studies on the leaves of medicinal plant, *Eclipta alba.*

Hemant Pandey* Ayesha S Ali , Mohd. Sajid and Sharique A. Ali
Post Graduate Department of Biotechnology, Saifia College of Science and Education, Bhopal – 462001 India.
*Post-graduate student of M.Sc.(Final) Biotechnology.

Abstract:
The medicinal plant *Eclipta alba* is very significant in traditional medicines as it is used as anthelmintic, expectorant, antipyretic, antiasthmatic and has even antivenom and memory enhancing properties is also used as deobstruent in hepatic and spleen enlargement . A phytochemical screening of leaves of *Eclipta alba* was assessed to evaluate its medicinal properties. The qualitative and quantitative phytochemical analyses were performed which revealed that the leaves of the plant contain saponins, alkaloids, phenols and flavonoids Among all the phytochemical constituents saponins, alkaloids and phenols were found in significant quantity which ranged between 7.8±0.30 to 1.39±0.12. A fine chemical analysis on methanolic leaf extracts through LC/MS which showed that they contained isoflavonoids; wedelolactone and demethylwedelolactone. These isoflavonoids can be used for hepatoprotection and also as antibacterial and anti hemorrhagic agents.

Introduction:

Medicinal plants are of great importance to the health of individuals and communities, consequently there has been resurgence in the consumption of medicinal plants, which greatly invokes the need to study them medicinal in more scientific manner to meet their ever increasing demands. The medicinal value of any plant lies in some or many chemical substances that produce a definite specific action. The most important among these bioactive constituents of the plants are – saponins, alkaloids, phenolic compounds, flavonoids or tannins (Mojab *et al.*, 2003, Edeoga *et al.*, 2005). The knowledge of the phytochemical constituents is desirable, not only for the discovery of therapeutic compounds, but also because such information may be of greater value in disclosing new sources of many economic materials. Among the various medicinal plants, *Eclipta alba* is a noteworthy herb which has significant medicinal value in hepatic and splenic disorders and various chronic skin maladies. It is a small, multi branched annual herb, commonly called as "bhringraja", which belongs to *Asteraceae*, is native to the tropical and sub-tropical regions of the world. It contains mainly coumestan derivatives – wedelolactone and demethylwedelolactone, saponins, alkaloids, phenolic compounds, tannins and other substances that are important in supporting this herb as a medicinal wonder (Edeoga *et al.*, 2005; Khanna *et al.*, 2007). In view of the increasing demand of medicinal plants and there active ingredients, the present study has been taken to screen the crude leaves of *Eclipta alba* with regard to their important phytochemical constituents. Also, the leaf extracts were subjected to phytochemical screening using hyphenated techniques of liquid chromatography and mass spectrometery (LC/MS).

Materials and methods: Collection and Preparation of Plant Materials:-The fine powder of the leaves of *Eclipta alba* was obtained from Sanjivani Ayurveda, Bhopal (Government of M.P) and phytochemical screening was performed on crude alcoholic and aqueous leaf extracts each of 10% concentration. The alcoholic leaf extract was prepared with 80% methanol and distilled water using Soxhlet assembly according to the method of Sablon (1996).

Phytochemical screening:-

Qualitative and quantitative chemical analyses were carried out using the above materials by following the standard procedures to identify the constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993).To test the presence of saponins, alkaloids and phenolic compounds, relative tests were performed according to standard procedures described by Kokate (1999), Evans (1997) and Mace (1963) respectively. The presence of flavonoids, proteins and carbohydrates were also detected

according to procedures reported by Mace (1963), Plummer (2002) and Thimmaiah (1999).For determination of the consistence of tannins, terpenoids and phlobatannins respective tests were performed on the basis of methods given by Mace (1963).

Quantitative determination of the chemical constituents of leaves of *Eclipta alba*:-

Determination of Saponin:

25 ml of 20 % aqueous ethanol with 5 gm powder of leaves were heated at 55°C for 4 hrs. The mixture was re-extracted with the residue with 50 ml of 20% aqueous ethanol. The extract was reduced upto half at 90°C and the concentrate was transferred into separating funnel and 7.5 ml of diethyl ether. Aqueous layer was recovered while ether layer was discarded. The purification process was repeated (Obadoni and Ochuko, 2001).

Determination of Alkaloid:

One gram leaf powder was mixed with 40 ml of 10% acetic acid in ethanol, covered for 4 hours. Settled precipitate obtained was washed with dilute ammonium hydroxide, filtered, dried and weighed (Harborne, 1973).

Determination of Total phenols:

Centrifuge test solution of ethanolic extract (10% solution from 80% ethanol) was centrifuged at 10,000 rpm twice. A dilution series from 0.2 to 2.0 mg/ml was made from standard solution of resorcinol, maintaining of 3 ml in each tube by distilled water. To this was added 0.5 ml of Folin reagent and 2 ml of 20% Na_2CO_3 followed by taking of absorbance using spectrophotometer at 650 nm (Thimmaiah, 1999).

Determination of Flavonoids:

1 gram leaf powder was extracted repeatedly with 20 ml of 80% aqueous methanol. The solution was filtered by Whatmann filter paper No. 42 (125 mm). The filtered solution was evaporated and weighed (Boham *et al.*, 1974).

Determination of Proteins:

It was determined hereby following Lowry's method. As a standard, egg albumin solution (0.2 mg/ml) was prepared (Plummer, 2002).

Determination of Carbohydrates: Carbohydrate was determined by following Anthron's test. 1 ml of aqueous extract was diluted by 10 times with water and standard was prepared from glucose (20 mg) in 200ml (Thimmaiah, 1999).

Fine Chemical analysis:

A chemical screening was done using hyphenated techniques of Liquid Chromatography and Mass Spectrometry (LC/MS) at Sophisticated Analytical Instrument Facility (SAIF) IIT, Mumbai from methanolic crude leaves extracts.

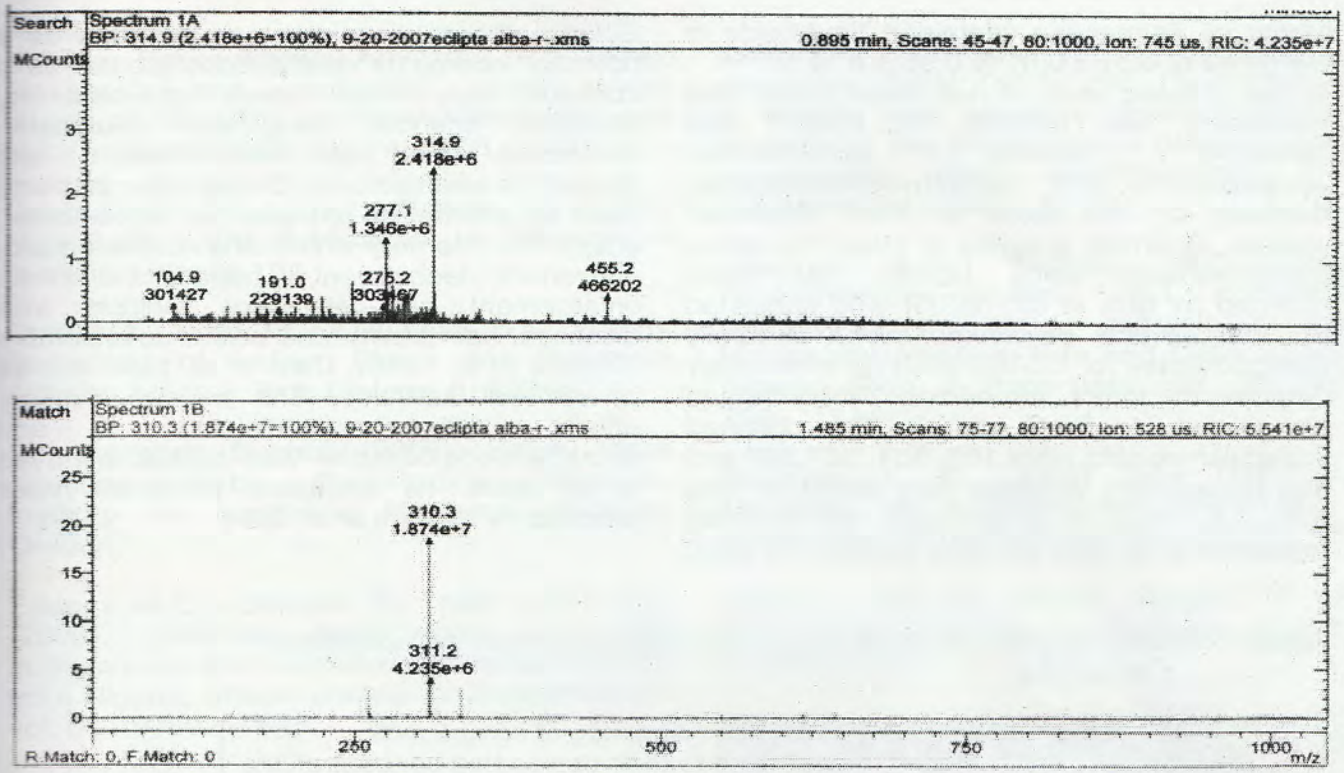
Results and Discussion:

The present study on the leaves of plant materials of *Eclipta alba* revealed the presence of several medicinally active constituents summarized in Table 1. The investigated plant *Eclipta alba* was found to be contain saponins, alkaloids and phenols in significant amounts, ranging from 7.8 ± 0.30% to 1.39 ± 0.12% while flavonoids, proteins, carbohydrates, tannins, terpenoids and phlobatannins were found ,in low concentrations ranging from 0.57 ± 0.20% to 0.15 ± 0.03%.

The quantity of certain phytochemicals, which were found to be present in extracts of leaves had been successfully estimated (in percentage). The quantitatively estimated phytochemicals in the samples were saponins, alkaloids, phenols, flavonoids, proteins and carbohydrates and their concentrations are summarized in Table 2.

The fine chemical analysis of methanolic extracts of leaves of *Eclipta alba* revealed the peaks with the molecular weight of 314.9, peak area of 2.418 e+6 and retention time of 3.687 for wedelolactone (a major constituent) assured to be present in leaves of *Eclipta alba*. Similarly, for demethylwedelolactone (nor – wedelolactone) obtained molecular weight was 310.3 (appx. with Na^+ impurities) the peak area was of 1.874 e+7 and the retention time of 2.784 as shown in figure 1 (a) and (b).

Figure 1 : (a) and (b) showing molecular weight and peak area of wedelolactone and demethylwedelolactone respectively.



The present qualitative and quantitative estimation of the average concentration (in percent) crude yields of biochemical constituents of leaves of the plant *Eclipta alba* showed that they are rich in saponins (7.8 ± 0.30%), alkaloids (1.55 ± 0.20%) and phenols (1.39 ± 0.12%) while the leaves of same plant contained lower yields of flavonoids (0.57 ± 0.20%), proteins (0.44 ± 0.19%) and carbohydrates (0.15 ± 0.03%). The results of present study are in full collaboration with the findings of Khanna *et al* (2007) who have also reported that the leaf extracts of *Eclipta alba* contains saponins in significant quantities. Edeoga *et al* (2005), who studied two plants of *Asteraceae* named *Emilia coccinea* and *Tridxa procumbens*, it has been reported that phlobatannins were not present in both plants while terpenoids were not present in *Tridxa procumbens*. On the other hand in the present study the leaf extracts of *Eclipta alba* have been found to contain phlobatannins and terpenoids which is being reported for the first time in *Asteraceae*. Also, in the present study alkaloids and phlobatannins have been detected in *Eclipta alba* which the earlier workers have not reported in the same plant (Khanna *et al*, 2007). On detailed review of literature, it becomes evident that, Edeoga *et al.*, (2005) have reported that leaves of *Emilia coccinea* and *Tridxa procumbens* contained saponins in the range of 1.70 ± 0.01 to 2.30 ± 0.20%, whereas in the present study significantly greater amounts of saponins have been detected quantitatively, which is about 7.8±0.30%. One of the reports which comes very close to the present data with regard to the content of saponins in this species i.e. *Eclipta alba* is of Khanna *et al,* (2007) who have reported saponins of 4.5% in the leaf extracts of same plant.

Many workers have reported that the alkaloid content in the various plants of *Asteraceae* are found in the range of 0.007 to 0.92% (Edeoga *et al*, 2005; Dharmananda, 2006). While in the present study in the leaf extracts of *Eclipta alba*, the alkaloids have been in much greater quantity than reported by earlier workers i.e. 1.55%. The quantitative estimation of phenols was found to be 1.39 ± 0.12%. In contrast to the present findings, the amount of phenols reported earlier in the plants of *Asteraceae* family is quite low being in the range of 0.06 ± 0.33% to 0.81 ± 0.10% (Edeoga *et al.,* 2005). In the present study, the flavonoids were present in 0.57 ± 0.20% which is in full collaboration with the findings of Edeoga *et al* (2005) who reported that leaves of some

plants of *Asteraceae* contained flavonoids in the range of 0.61 ± 0.01 to 0.96 ± 0.10%.

In the present study it has been found that methanolic leaf extracts of *Eclipta alba* contained specific isoflavonoids; wedelolactone and demethylwedelolactone, detected on the basis of their molecular weights. A similar analysis of major bio-active phytochemicals using LC/MS has been reported by Sun *et al.*, (2005) who subjected the leaf extracts of *Ginkgo biloba* of family *Ginkgobiaceae* for LC/MS analysis where they detected the major terpenoids- bilobalide and ginkgolides (A, B, C and J). The obtained molecular weights were 163, 351, 367,367 and 386 respectively whereas their retention time was 7.6, 11.2, 11.5, 9.3 and 9.0 minutes respectively. In view of these results, the plant *Eclipta alba* studied here can be seen as a potential source of useful constituents, as it contains very high levels of saponins, alkaloids, phenols along with coumestan derivatives such as wedelolactone and demethylwedelolactone. *Eclipta alba* is being used as alternative anthelmintic, expectorant, antipyretic, memory enhancing, antiasthmatic, antivenom, deobstruent in hepatic and spleen enlargement and also for multiple skin diseases, hair growth and colour development (Chopra *et al*, 1966). Dhar *et al*, 1968 studied its antiviral activities. The trypsin inhibitory effect of wedelolactone and demethylwedelolactone was studied by Syed *et al*, 2003. Its analgesic properties were reported by Sawant *et al*, 2004.

Table 1: Qualitative analysis of the phytochemicals of aqueous leaf extracts of *Eclipta alba*.

S. No.	Phytochemicals	Presence / Absence of compounds, according to their decreasing concentrations
1.	Saponins	+ + + + +
2.	Alkaloids	+ + + +
3.	Phenols	+ + + +
4.	Flavonoids	+ + +
5.	Proteins	+ + +
6.	Carbohydrates	+
7.	Tannins	+
8.	Terpenoids	+
9.	Phlobatannins	+

+:- Presence of the compound
-:-Absence of the compound

Table 2: Qualitative analysis of phytochemicals present in the leaf extracts of plant; *Eclipta alba*.

S. No.	Name of component	Minimum	Maximum	Average	Standard deviation
1.	Saponins	7.5	8.1	7.8	± 0.30
2.	Alkaloids	1.35	1.75	1.55	± 0.20
3.	Phenols	1.27	1.51	1.39	± 0.12
4.	Flavonoids	0.37	0.77	0.57	± 0.20
5.	Proteins	0.25	0.63	0.44	± 0.19
6.	Carbohydrates	0.12	0.18	0.15	± 0.03

- All values are mentioned in (%) Percent

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Pharmacological Effects of Lead Nitrate, Adrenaline and Potassium on Isolated Fish Melanophores.

Sharique A. Ali,S. Malik, Keisham V. Meitei ,Tahira Sultan, Mohd. Sajid and Mohd. Ovais*
Department of Biotechnology Saifia College of Science and Education, Bhopal-462001 and
*Department of Bioscience , Barkatullah University, Bhopal 4620026 India . Email:
drshariqali@yahoo.com

ABSTRACT :

In the present study, agonistic effects of lead nitrate , adrenaline and potassium chloride have been studied on the isolated scale melanophores of a teleost fish, *Cirrhinus mrigala*, using the mean melanophore size index assay . It has been found that adrenaline ,lead nitrate and potassium chloride all aggregated the isolated scale melanophores in the decreasing order. This is for the first time that lead in molar concentrations has been found to induce dose dependent melanin aggregation within the fish melanophores along with the known adrenergic agonists like adrenaline and potassium chloride .

Key Words: Isolated fish scale melanophores aggregation adrenergic agonists

Introduction

The intracellular activities of the pigment granules within the melanophores of fishes, is responsible for the pigmentary responses of the animal as a whole. There are several reports available in the literature regarding the effects of pharmacological, hormonal and miscellaneous substances on the pigment cells of fishes. However, there are few reports regarding the effects of heavy metals such as lead, mercury, chromium and cadmium on physiological and pharmacological aspects of coloration of fishes. Interestingly, a couple of reports available in the literature regarding the effects of lead on fish pigmentation (Davis *et al.*, 1976; Pace *et al.*, 1977; Hodson *et al.*, 1978) only indicate that lead in high concentrations has toxicological effects on the melanophores, however, pharmacological information regarding the effects of very low doses of lead is almost nil. In view of these lacunae the present study has been undertaken where the effects of lead nitrate *per se* and in combination with some adrenergic stimulating agents have been studied using the dermal scale melanophores of *Cirrhinus mrigala*, in order to find out if lead nitrate has any pharmacologically significant actions on the fish melanophores which are disguised type of smooth muscle cells. It has also been attempted to investigate whether lead nitrate in extremely low concentrations can stimulate the cellular receptors present on the fish melanophores as other adrenergic agents like adrenaline and potassium do.

Material and Methods

Young *Cirrhinus mrigala* (Ham) after procuring them, were kept in the laboratory for 48-72 hours for acclimatization. Injured or inactive fish were discarded and only healthy fish were used in present study. The aquaria were maintained as per recommended methods. Water was changed twice in a week and was regularly aerated. Fish were given a local fish feed once a week. The scales were removed from the dorsal region below the head and lateral sides of the live fishes and immediately immersed in 0.7% sodium chloride solution and Fish Ringer solution according to the method of Spaeth (1913). Care was taken to hold the scales by their non-pigmented sides. To observe the effect of drug, 0.5 ml of 0.7% sodium chloride or Fish Ringer solution was added to the Petri dishes using hypodermic syringes and equal amount of drug was kept in to maintain the volume of the solution. The drugs were allowed to be mixed with the scales through the solution for 5-10 minutes, the control as well as treated scales were first placed on a glass slide with dermal side down with a little incubation medium and covered with a glass cover slip. The slide was mounted, placed on the microscope and observed accordingly. The reading was taken with the help of ocular micrometer fitted in the microscope calibrated earlier. The reading was taken on a 0 10 100 scale, then mean was obtained according to the method of Bhattacharya *et. al* (1976), modified by Ali (1983). In order to achieve the accuracy,

individual melanophore was measured in low power microscope through the method described above. Mean Melanophore Size Index (MMSI) was prepared and statistical analyses with the levels of significance , wherever necessary were calculated using the standard methods of statistical analysis (Lewis, 1971).

Results and discussion

In a series of experiment, effects of lead nitrate, adrenaline and potassium chloride in varying concentrations have been studied in fish Ringer with doubled calcium chloride. The effect of lead nitrate was observed on the dermal melanophores in a concentration ranging from 10^{-11} to 10^{-6} M. The melanophores responded by distinct dose dependent aggregation as shown in figure 1a. Effects of potassium chloride on melanophores have also been studied. It was found to aggregate the melanophores, the MMSI decreased from control value of 6.35$\pm$ 0.058 to 4.17 $\pm$ 0.252 as seen by the highest concentration of 10^{-4} M of potassium chloride (Figure 1) Effects of adrenaline were also studied and it was found that there was a marked increase in degree of aggregation of all the dermal melanophores as compared to lead nitrate and potassium chloride. The melanophores showed a similar response, that they aggregated severely when they were treated with different concentrations of adrenaline (10^{-12} to 10^{-6} M) in fish Ringer with doubled calcium chloride (Figure 2). On comparing the results of all the three used agents i.e., lead nitrate, adrenaline and potassium chloride , it was found that adrenalin aggregated the melanophores of *C. mrigala* maximally. The order of melanophore aggregation of all the three adrenergic agents was as follows: adrenaline> lead nitrate> potassium chloride.

In concentrations of the earlier series of experiments, effects of lead nitrate in different concentrations along with adrenaline and potassium chloride have also been studied in fish Ringer without calcium chloride but in presence of EDTA (Ethylene diaminetetra acetic acid (disodium salt). It was found that the dermal melanophores responded by distinct aggregation as shown in fig. 3a. The MMSI decreased from a control value of 4.68 $\pm$ 0.127 to 4.46 $\pm$ 0.112 and, further followed by the decrease in the MMSI (3.18 $\pm$ 0.1125) in response to the maximum concentration of 10^{-6} M (fig.3). In the next set of experiments , adrenaline was used in varying concentrations. It was observed that adrenaline aggregated the melanophores severely and the MMSI decreased from a control value of 4.68 $\pm$ 0.127 to 4.36 $\pm$ 0.165 by the first dose of 10^{-12} M of adrenaline, followed by more aggregation in all the melanophores by increased doses of Adrenaline. The last concentration of 10^{-5} M decreased the MMSI 2.40 $\pm$ 0.163 (fig.3).

In the next set of experiments, potassium chloride was used and it aggregated all the melanophores, the MMSI decreased from control value of 4.68+0.127 by the initial dose of 10^{-10} M., further doses increased the degree of melanophore aggregation. In a dose range of 10^{-6} to 10^{-4} M the MMSI values were 3.93 $\pm$ 0.09 and 3.11$\pm$ 0.071 respectively (fig.3). On comparing the results of all the three agents in figure 4. It was found that adrenaline aggregated the melanophores maximally followed by lead nitrate and potassium chloride (fig.2). In another set of experiments effects of lead nitrate, adrenaline and potassium chloride in varying concentrations have been studied in fish Ringer without calcium chloride. It was found that lead nitrate aggregated the dermal melanophores in concentrations ranging from 10^{-12} to 10^{-6} M. the MMSI decreased from a control value of 4.54 $\pm$ 0.32 to 4.37 $\pm$ 0.31 by the first initial dose of 10-12 M of lead nitrate. This was followed by further aggregation of melanophores (3.37 $\pm$ 0.12) by increasing the doses of lead nitrate. As in the case of adrenaline and potassium chloride, the response was of aggregation in all the dermal melanophores, however the degree of aggregation differed (figure 4). On comparing the effects of lead nitrate, adrenaline and potassium chloride, it was found that adrenaline aggregated the melanophores more distinctly followed by lead nitrate and potassium chloride (Fig. 4).

In another set of experiments effects of lead nitrate and adrenaline have been studied on the isolated scale melanophore in keeping the concentration of lead nitrate fixed, whereas, the concentration of adrenaline was increased dose dependently. In fig. 4 there was aggregation of melanophores, whereas the MMSI decreased from a control value of 5.01 $\pm$ 0.329 to 3.22 $\pm$ 0.26 by the first dose of adrenaline (10^{-11}) with lowest dose of lead nitrate that was 0.5 x 10^{-7} M. With further increase in the concentration of adrenaline (10^{-5}) the MMSI had become 1.75 $\pm$ 0.185. In figure 4, the effect of adrenaline in varying

concentration with lead nitrate (1.86×10^{-7} M) is shown; the melanophores are more aggregated as compared to above. The MSI had become 3.32 ± 0.09 from the control value of 5.01 ± 0.329 in the first dose, that was 10^{-11} M. With further increase in the dose of adrenaline 10^{-5} the MSI had become 1.35 ± 0.13.

Fig. 4 shows the melanin aggregatory effect of adrenaline in varying concentrations. The MSI had become 4.23 ± 0.33 in response to the dose of 10^{-11} M, further decreased as dose of adrenaline were increased. Fig. 4d shows severe melanophore aggregating effects of adrenalin with highest dose of lead nitrate (2.965×10^{-7}). In the first dose of adrenaline (10^{-11} M) with lead nitrate (2.965×10^{-7}) the MSI was 3.56 ± 0.37 and in the second dose (10^{-10} M) the MSI was 3.19 ± 0.09. when the doses of adrenaline were further increased the melanophores gradually decreased in size, finally became very small dot like structures (10^{-5} M) where the MSI recorded was 0.92 ± 0.022. On comparing the results of effects of adrenaline *per se* and with lead nitrate (0.05×10^{-7} M, 1.86×10^{-7} M and 2.965×10^{-7} M) it was found that adrenaline with highest concentration of lead nitrate (2.965×10^{-7} M) showed severe aggregation than other combination. The order of aggregation of the melanophores was as follows: adrenaline> lead nitrate > potassium chloride.

The results of the present study regarding the effects of lead nitrate on the isolated scale melanophore of *Cirrhinus mrigala*, a teleost fish, reveal several aspects of physiology and pharmacology of melanophores with reference to lead nitrate and its mechanism of action. The responses of melanphores to specific agonist and antagonist alongwith lead nitrate used were found to be more or less similar whether they were of epidermal or dermal nature. On comparison of the responses of epidermal melanophores or dermal melanophores, it was found that the responses of melanophores to exogenously applied agonists as well as lead nitrate were quite weak as compared to the latter. This is in fairly good consistence with the findings of earlier workers like Bagnara and Hadley (1973), Ali (1983, 1986), Ovais and Ali (1984), Ali *et. al* (1985a, 1985b, 1986, 1993, 1995, 1996, 1998,) and Parveen (1994) who have reported that dermal melanophores are more sensitive and respond strongly to pharmacological agents like adrenalin, noradrenalin, histamine, 5-hydroxytryptamine etc. on a host of vertebrate species including fishes.

In the present study it has been observed that lead nitrate, adrenaline and potassium chloride all caused significant melanophore aggregation dose dependently (fig 2). The results of the present study as shown in the figures clearly demonstrate that lead nitrate *per se*, adrenaline *per se* and potassium chloride *per se* all caused significant dose dependent melanophore aggregating effects which are statistically significant.

The site of action of all the three melanophore aggregating agents that is lead nitrate, adrenaline and potassium chloride whether in fish Ringer with doubled calcium chloride or without calcium chloride in fish Ringer and without calcium chloride in Ethylene diaminetetraacetic acid (EDTA) clearly suggest that the melanin aggregating effects are quite powerful and are due to the stimulation and involvement of dominantly present adrenergic receptors which have also known to be present in several teleostean fish species as reported by workers like Ali and Ovais (1983), Ali (1983, 1985a,b), Jain and Patil (1992), Ovais (1994). These workers have provided detailed information that adrenergic agonists like adrenaline, potassium chloride and lithium chloride cause distinct melanophore aggregation in teleost fishes via., alpha-adrenergic receptor activation.

Ali and Ovais(1983) have found that exogenously applied alkaline earth ions K+, Ba++ and Li++ ions produced pigment aggregation in scale melanophores of *C. punctatus*. It appears that these ions do not act directly on the melanophores , but exert their effects on the nerve endings, liberating an adrenergic neurotransmitter which cause the centripetal movement of the pigment granules. Ca++ and Mg++ ions induce pigment dispersion indirectly affecting the ionic influx.

Comparing the data of the present study, regarding the effects of lead nitrate, adrenaline and potassium chloride it becomes clear that adrenaline was the most powerful melanin aggregating agonist followed by lead nitrate and potassium chloride. Out of these above three melanophore aggregating compounds adrenaline and potassium chloride have been reported since long to be powerful melanophore aggregating agents by several

past workers like Fuji(1961), Scheline (1963),Reed and Finnin (1972), Fernando and Grove (1974) and Fuji and Miyashita (1975) in several teleost fishes. As mentioned earlier, workers like Ali (1983, 1985a), Morishita (1987), Jain and Patil (1992), Patil and Jain(1993), Katayama *et al.*, (1990), Ovais (1994) and Parveen (1994) have also found that adrenaline and potassium chloride caused melanophore aggregation in different species of teleost fishes via alpha adrenergic receptor stimulation.

These melanophore aggregating effects have been found to be initiated by stimulation of alpha-adrenergic receptors which gets considerable support from the studies of earlier workers on several fishes like *Chasmicthys* (Fuji, 1961), *Labrus ossifagus* (Scheline, 1963), *Scopthalmus aquasus* (Scott, 1965), *Phoxinus* (Healey and Ross, 1966), *Fundulus* (Abbot, 1968) and in many others (Iga, 1968;Read and Finin, 1972; Fernando and Grove, 1974; Fuji and Miyashita, 1975; Katyama, 1980 and Fuji and Oshima,1986).

However, the data of the present study regarding the melanophore aggregating effects of lead nitrate on the melanophores of *C. mrigala* are interesting as well as are being reported for the first time in literature particularly its role as a melanophore aggregating agent in teleost fish *C. mrigala*.It appears that lead nitrate has a role in liberating adrenaline or adrenaline like substance at the neuromelanophore junction , which induces melanin aggregation in this species of teleost fish.

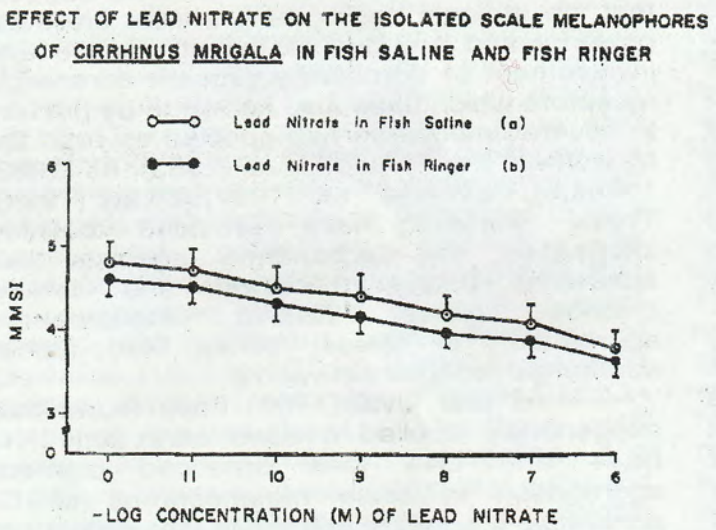


Fig.1

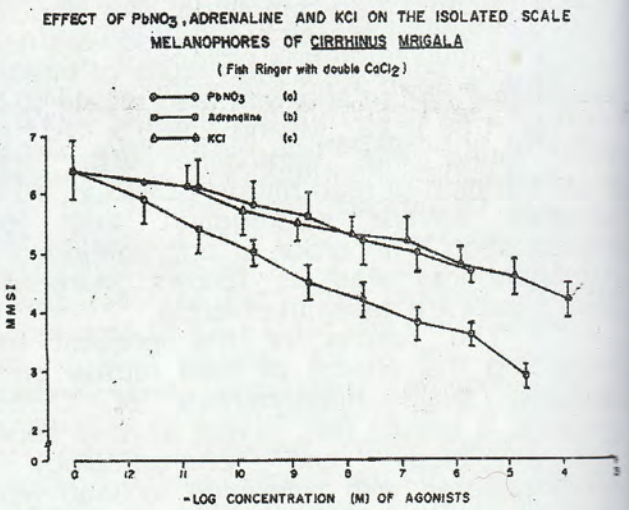


Fig.2

Fig.1.(a)Showing the melanin aggregating effects of lead nitrate per se in Fish Saline(10^{-11} to 10^{-6} M).
(b) Showing the melanin aggregating effects of lead nitrate per se in Fish Ringer (10^{-11} to 10^{-6} M).
Vertical bars represent the SE $\pm$ of the Mean
Fig.2. Showing the different melanophore aggregatory effects of lead nitrate (a) (10-12 to 10-6 M), adrenaline.(10^{-12} to 10^{-5} M) (b) and Potassium chloride(10^{-11} to 10^{-4} M) (c) on the MMSI of isolated scale melanophores of *C. mrigala* in Fish Ringer with doubled Calcium chloride.

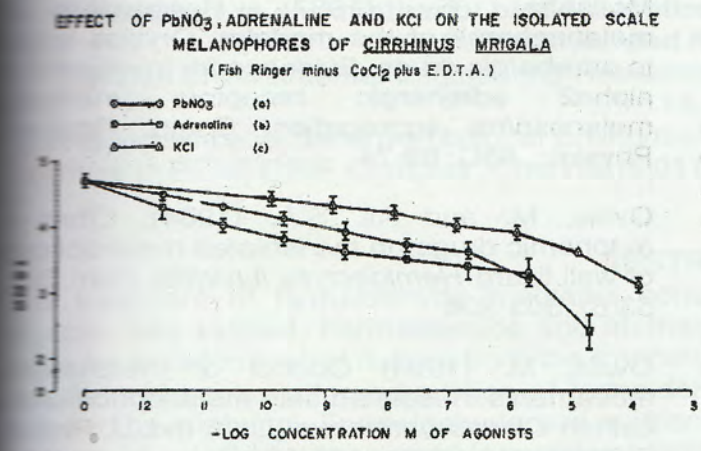


Fig.3

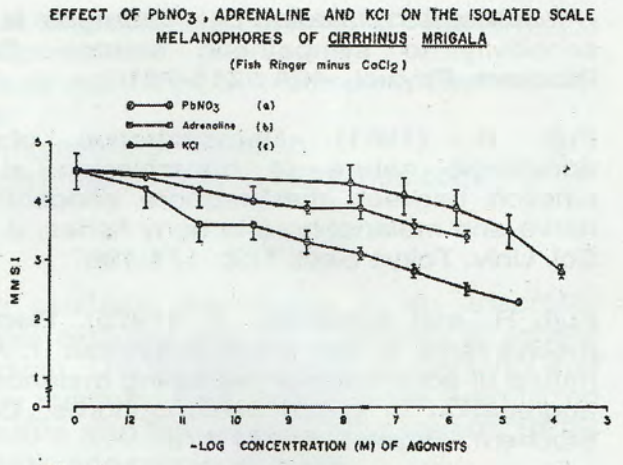


Fig.4

Fig.3. Showing the different melanophore aggregatory effects of lead nitrate (a) (10-12 to 10-6 M), adrenaline.(10^{-12} to 10^{-5} M) (b) and Potassium chloride(10^{-10} to 10^{-4} M) (c) on the MMSI of isolated scale melanophores of C. *mrigala* in Fish Ringer with EDTA and without Calcium chloride. Fig.4 Showing the melanophore aggregatory effects of lead nitrate (a) (10-12 to 10-6 M), adrenaline.(10^{-10} to 10^{-5} M) (b) and Potassium chloride(10^{-8} to 10^{-4} M) (c) on the MMSI of isolated scale melanophores of *C. mrigala* in Fish Ringer without Calcium chloride.

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Formaldehyde and methanol biodegradation from the mixture in anaerobic digester.

S.Sandhya* and K.Swaminathan

National Environmental Engineering Research Institute (NEERI) CSIR Complex, Chennai 600113, India

*Correspondence: S.Sandhya National Environmental Engineering Research Institute (NEERI) CSIR Complex, Chennai 600113, IndiaEmail : sswami_in@yahoo.com

ABSTRACT

The treatment of formaldehyde methanol containing synthetic wastewater in an anaerobic digester was studied. Formaldehyde and methanol were oxidized with the formation of volatile acids by mixed microbial culture from the synthetic medium. The biodegradation of methanol and volatile acid begins after exhaustion of formaldehyde in medium. First volatile acids are utilized followed by methanol. Formaldehyde and methanol mixture also follows the same pattern. HPLC analysis of volatile acids showed formation of formic, acetic and propionic acids.

Key words: Anaerobic digester, formaldehyde/ methanol biodegradation

Introduction

Formaldehyde and methanol are common chemical solvents widely used in the chemical, textile, paper and wood processing industries. Besides this they are important industrial solvents in the manufacturing of resins, plastics, adhesive and many other products. At higher concentrations, both the pollutants are known to produce adverse effect on humans such as vomiting, gastrointestinal problems *Prado et al.,* 2004. Formaldehyde is also used as an active ingredient in preservative and disinfectants. Its disposal directly to sewage treatment plant after use may imbalance the performance, due to its antimicrobial effects. The carcinogenicity of formaldehyde to animal and human is well known Swenberg *et al.,* 1980. Nevertheless, formaldehyde at lower concentration is known to be biodegradable in both aerobic Yamazaki *et al.,* 2001; Hidalgo *et al.,* 2002 and anaerobic systems Qu,M. *et al.,* 1997; Omil,F *et al.,* 1999. Hidago *et al.,* reported the removal of formaldehyde by suspended cells of heterotrophic bacterium *Rhodococcus erythropolis* UPV1 in defined mineral medium supplemented with phenol, formaldehyde. Gerike and Gode 1990 reported formaldehyde degradation at 2-5 mg l^{-1} in activated sludge. Bonastre et al., 1986 observed the partial degradation of formaldehyde at 2100 mg l^{-1} in activated sludge. Garnido *et al.,*2001 worked with a multiple up flow filter under anoxic conditions using formaldehyde as carbon source. They could eliminate 0.5 kg formaldehyde $m^{-3}d^{-1}$efficiently at 1 to 0.3 d. hydraulic retention time. Anaerobic degradation is an alternative to aerobic degradation for formaldehyde removal from wastewater. Formaldehyde concentrations of 20-250 mg l^{-1} resulted in 50% reduction in methanogenesis activity Todini *et al.,* 1992; Sharma *et al.,* 1994. The main purpose of the study was to biodegrade formaldehyde, methanol, individually and in mixture by anaerobic /facultative mixed microorganisms in anaerobic digester. The degradation pattern in presence of co-substrates and identification of volatile acids formed during biodegradation using HPLC for predicting the probable metabolic pathway of degradation.

Materials and Methods

Organism and culture isolation. The formaldehyde degrading bacteria were isolated by successive enrichments of sewage containing 0.05% formaldehyde. The enriched microorganisms were always maintained in mineral salts medium containing formaldehyde. The mineral salt medium used contains mgl^{-1}; ammonium chloride 280; potassium di-hydrogen phosphate 250; magnesium sulfate 110; calcium chloride 20; yeast extract 20; trace element solution.

Reagents and Chemicals Methanol and Formaldehyde used in study are commercially available solvents and other chemicals used were all reagent grade.

Analytical determination Formaldehyde was determined by Nash procedure using UV-Visible spectrophotometer Nash *et al.,* 1953. Methanol was measured using a Perkin Elmer gas chromatograph equipped with Nukol column (30x0.25id) and flame ionization detector. Nitrogen (1.5 cm^3 min^{-1}) was utilized as carrier gas. The injector and detector temperature were 250°C and 270°C respectively. Volatile acids were determined using Hawlett Packard 1100 Liquid Chromatogram equipped with a C18 ODS column (25 cmsx4m id) and a diode-array UV detector. The mobile phase was acetonitrile - phosphoric acid (80:20) at a flow rate of 1. Detection was performed at 210 nm.

Biodegradation studies The biodegradation studies were conducted in anaerobic digester of one liter capacity with 500 ml working volume with outlet to remove samples periodically. Microorganisms were enriched from municipal sewage in presence of formaldehyde and methanol.

Results and Discussion

The growth of microorganism(s) on formaldehyde (420 mg l^{-1}) as sole carbon source has been studied in the laboratory. The degradation of formaldehyde led to the simultaneous appearance of methanol and volatile acid as shown in Figure 1. As soon as volatile acids are formed, there is decrease in pH from 7 to 4.2 followed by increase to 6.5, indicating utilization of formed volatile acid. Methanol is also formed as an intermediate after three days and remains unchanged till nineteen days and followed by removal after 21 days. Formaldehyde was first totally converted to methanol. It has also reported that formation of methanol from formaldehyde during biodegradation of formaldehyde under anaerobic condition occurs. Eiora *et al.*,2006 have also reported the formation of methanol and formic acid during formaldehyde degradation. This is also in agreement with results obtained by Kato *et al.,* 1983 studying formaldehyde biodegradation. They found out an enzyme, which catalyzed dismutation of formaldehyde to form methanol and formic acid. Adroer *et al.*,1990 have also reported the formaldehyde biodegradation is initiated by a dismutation reaction, and the biodegradation led to the simultaneous appearance of methanol and formic acid. The degradation of methanol and volatile acid begins after exhaustion of formaldehyde in medium. Table 1 shows the results of formaldehyde degradation achieved during 21 days in anaerobic digester operation under identical conditions at 70-988 mg l^{-1} of initial formaldehyde concentration. The influent COD was 78 – 618 mg l^{-1} with COD removal efficiency of 81-94%. The ratio between the COD and formaldehyde concentration in the influent was 0.62-1.11, but this ratio was found to vary for effluent samples, reaching the new value of 0.59 – 1.4. The maximum influent concentration of 988 mg l^{-1} was treated in anaerobic digester. Olivera *et al.,* 2004 had studied formaldehyde degradation in an anaerobic packed bed reactor and reported that 1158 mg HCHO $l^{-1}d^{-1}$ was applied as formaldehyde loading rate resulting in 99.7% formaldehyde and 92% COD removal efficiencies, where as Gurado *et al.,* 2001 worked with a modified up flow filter under anoxic conditions using formaldehyde as carbon source and obtained 65% removal.

Batch growth of microorganisms on 536 mg l^{-1} of methanol as sole source of carbon has shown the formation of volatile acid (452 mg l^{-1}), formaldehyde (240 mg l^{-1}) within two days of incubation (Figure 2). This was followed by decrease in formaldehyde to nondeductible limit with the concentration of volatile acid remaining constant after 7th days. Methanol concentration decreased gradually till 10th day and reduced to almost nil after 14th day. Table 2 shows the results of methanol degradation achieved during 21 days in anaerobic digester operation under identical conditions at 96-972 mg l^{-1} of initial methanol concentration. The influent COD was 170 – 1721 mg l^{-1} with COD removal efficiency of was 67 - 91%. The ratio between the COD and methanol concentration in the influent was 1.77 at all the concentrations and remains constant for effluent samples also. The maximum influent methanol concentration of 972 mg l^{-1} was treated in anaerobic digester. Eiora *et al.,* 2006 have also observed slow methanol removal and formation of formaldehyde and formic acid as product of degradation. Methanol as single carbon source was removed in few days, but in presence of formaldehyde its removal took longer time. This could be due to the inhibition of microorganisms caused by formaldehyde. However, this inhibition could also be caused by the presence of formic acid, since inhibition remained after formaldehyde had been completely removed.

The removal of formaldehyde or methanol by adsorption in the digester can not be ruled out. But it seems to be negligible. Qu ano Bhattacharya 1997 and reported only 10-11% of a biotic removal in anaerobic reactor.

When formaldehyde (250 mg l^{-1}) and methanol (100 mg l^{-1}) were added together, formaldehyde was preferentially utilized and exhausted within 10 days. The degradation of formaldehyde and methanol in mixture is shown in Figure 3. But there was formation of volatile acid with decrease in pH and it was utilized first followed by methanol. Biological conversion of formaldehyde to methanol is evidence by increase in methanol concentration from 100 mg $l^{-1}$to 134 mg l^{-1} till 3 days, but started decreasing after words. An inhibitory effect of methanol on volatile acids degradation was observed. There exist a possibility that volatile acid formation might be inhibitory to methanol dehydrogenase enzyme and therefore only after its removal it is been expressed. The enzymatic assay has not been investigated, but the results obtained are in agreement with basic characteristics of growth on multiple substrates.

When formaldehyde (500 mg l^{-1}) and methanol (500 mg l^{-1}) were added to gather, formaldehyde was preferentially utilized and exhausted within 14 days. The degradation of formaldehyde and methanol in mixture is shown in Figure 4. At higher concentration of formaldehyde methanol mixture the degradation pattern was different. Volatile acids formation started after 3 days of incubation and decreased after 14 days. The quantity of the formation of volatile acids was less as compared to single compound degradation. The decrease in pH was observed and formaldehyde was utilized first within 17 days followed by methanol. Only 79% of initial methanol was degraded within 21 days. COD removal was gradual and maximum during 21 days of incubation. Methanol concentration has increased to 520 mg l^{-1} within 7 days again indicating biological conversion of formaldehyde to methanol.

The degradation of formaldehyde via methanol is thermodynamically favored as it generates more energy. At low concentrations of volatile acids and methanol, direct conversion to methane is favored. But at higher concentration, methanol degradation is slow and effluent shows presence of methanol and organic COD. Florencio *et al.*,1994 showed that the affinity of methalotrophic methanogens for methanol is 60 times higher than acetogens. Therefore, a low methanol concentration, methalotrophic methanogens are survived. When methanol concentration in effluent is high, the production of volatile acids was strongly stimulated and acetogens survival rate is higher. Methanogens cannot compete over acetogens.

The HPLC analysis of different volatile acids at the end of formaldehyde and methanol degradation is given in Figure 5 A for volatile acids formed after biodegradation of formaldehyde. At 500 mg l^{-1} of initial formaldehyde concentration, when biologically treated effluent was analyzed, it showed presence of formic acid (1.0 mg l^{-1}), acetic acid (2.2 mg l^{-1}), propionic acid (3.6 mgl^{-1}). The HPLC analysis is shown in Figure 5B for volatile acids formed after biodegradation of methanol. A traces of formic acid (0.49 mg l^{-1}), acetic acid (1.8 mg l^{-1}) and propionic acid (4.2 mg l^{-1}) were detected as by product of methanol degradation.

The presence of volatile acids indicated that the formaldehyde conversion pathway in the anaerobic digester was similar to the pathway presented by Gonzeatee Gil *et al.*,1999. According to them the formaldehyde was transformed to formic acid and methanol. The HPLC analysis suggests that oxidation of formaldehyde to formic and acetic acids and methanol. Oliveira et al.,2004 have observed immediate consumption of formic acid formed. The proposed mechanism suggests the oxidation of formaldehyde into formic acid and its rapid reduction to methanol. Eiroa *et al.*,2006 also detected formic acid and methanol as formaldehyde degradation product and have suggested that formaldehyde consumption under anaerobic condition is either by direct methane conversion or by methanol generation. Oliveirs *et al.*, 2004 have also detected low quantities of acetic acid in effluent samples of anaerobic packed bed reactor treating formaldehyde. They have further suggested that consumption of formed formic acid leads to generation of acetic acid and have suggested that biological or chemical synthesis was occurring in the reactor.

The possible biodegradation mechanism seems to be similar to "Cannizzaro Reaction' Oliveira et al., 2004. Biodegradation of methanol shows generation of formaldehyde and formic acid as intermediate product of degradation. Acetic acid was not detected in

the degradation product indicating formic acid to methanol conversion is reversible reaction. Lu and Hegemann 1998 reported that there exist a possibility that formaldehyde resistant bacteria may be converting formaldehyde to methanol and formic acid using formaldehyde dismutase

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Table 1 Influent and effluent formaldehyde and COD concentration observed in anaerobic sludge digester

Formaldehyde, mgl^{-1}			COD, mg l^{-1}		
Influent	Effluent	Removal, %	Influent	Effluent	Removal, %
70	10	85.71	78	14	82.0
228	40	82.45	165	31	81.0
492	30	93.90	340	19	94.0
719	60	91.65	458	36	92.0
988	135	86.33	618	80	87.0

The average values are based on a minimum of five observations

Table 2 Influent and effluent methanol and COD concentration observed in anaerobic sludge digester

Methanol, mgl^{-1}			COD, mg l^{-1}		
Influent	Effluent	Removal, %	Influent	Effluent	Removal, %
96	15	84.37	170	17	90.0
232	32	86.20	410	36	91.0
462	127	72.51	818	144	82.4
689	325	52.83	1220	367	70.0
972	502	48.35	1721	567	67.0

The average values are based on a minimum of five observations

Figure 1 Biodegradation of formaldehyde and product formation

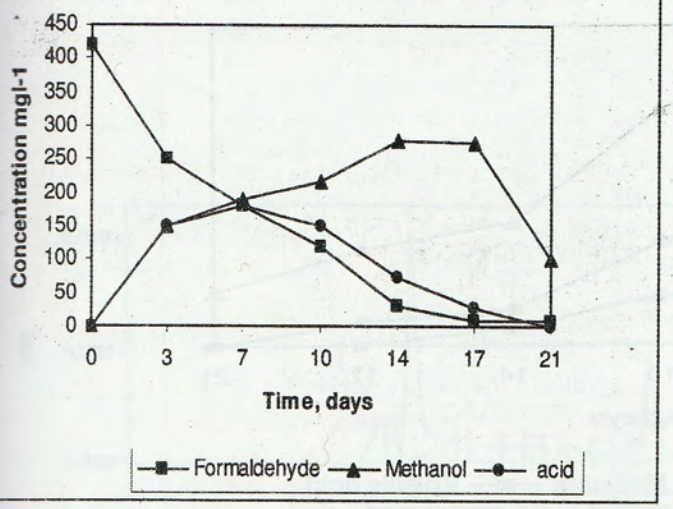


Figure 2 Biodegradation of methano and product formation

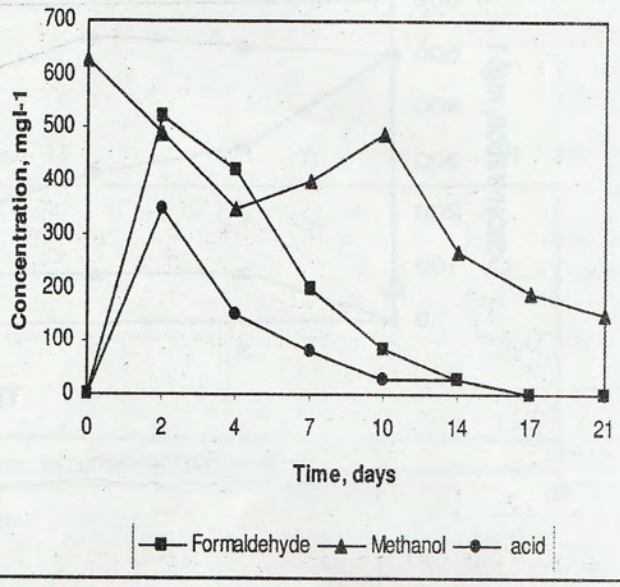


Figure 3 Biodegradation of formaldehyde and methanol in mixture (Formaldehyde 250 mgl-1 and Methanol 100 mgl-1)

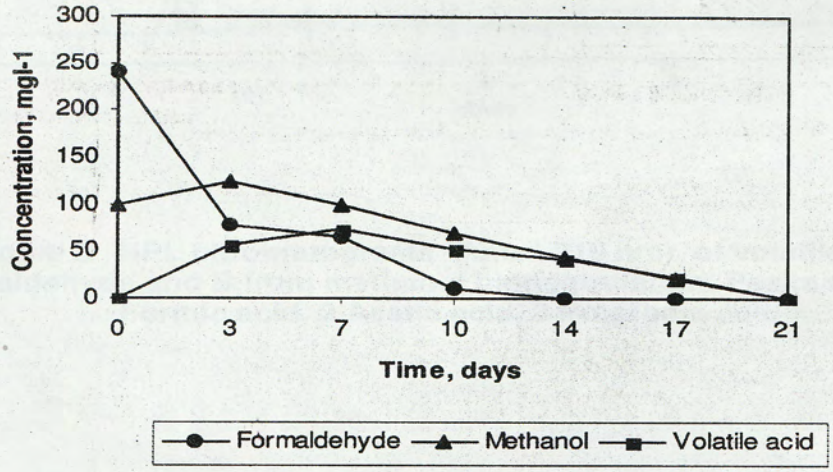
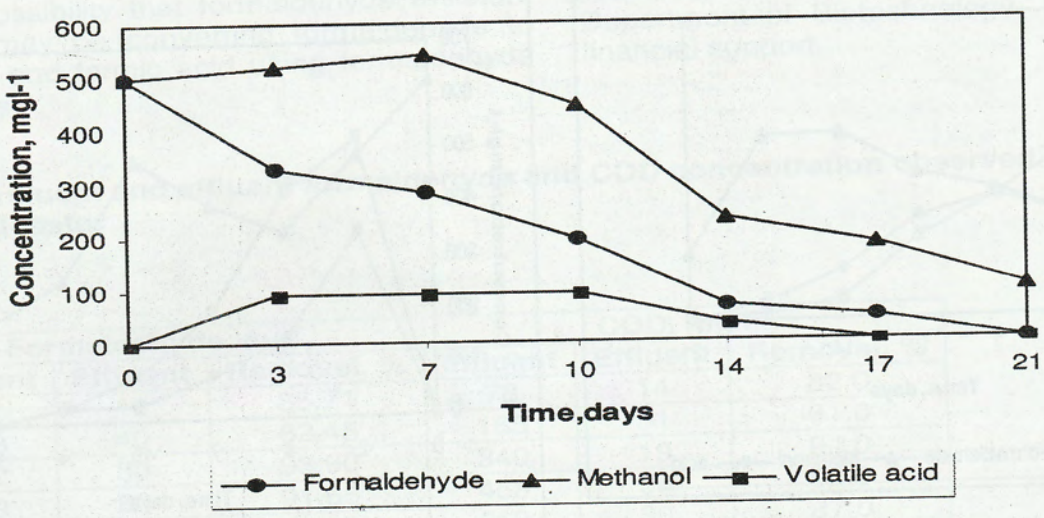


Figure 4 Biodegradation of formaldehyde and methanol in mixture (Formaldehyde 500 mgl-1 and Methanol 500 mgl-1



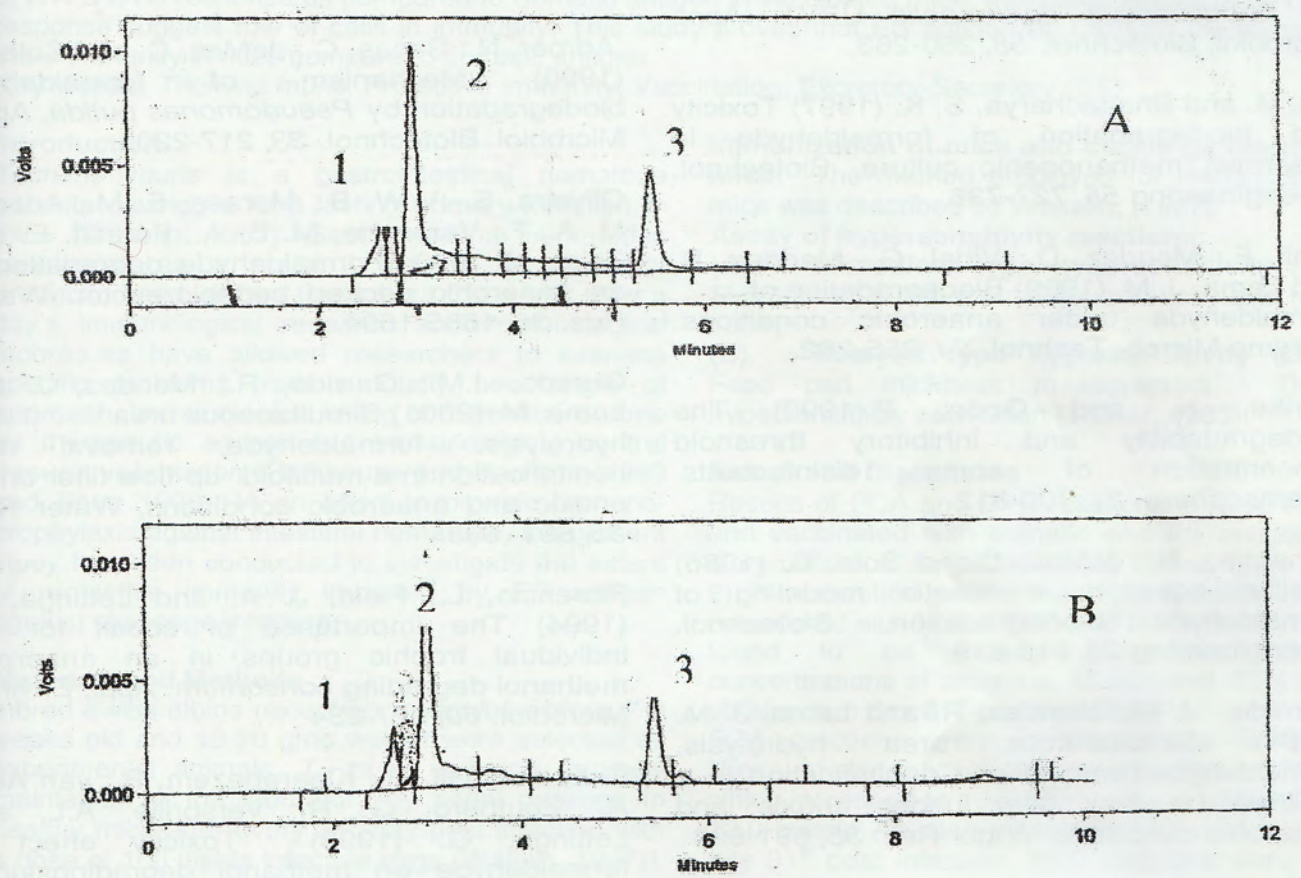


Figure 5 **HPL chromatograms (λmax 210 nm) of volatile acids A from formaldehyde and B from methanol biodegradation. Peaks correspond to 1, Formic acid, 2 Acetic acid, 3 Propionic acid**

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Immediate and Delayed Hypersensitivity Response in Mice Infected and Vaccinated with *Trichuris muris* Antigen.

S. Gaherwal, R.R. Kanhere* and M.M. Prakash.
Department of Zoology, Govt. Holkar Science College, Indore (M.P.)
*Department of Zoology, Govt. P.G. College, Barwani (M.P.)

Abstract

The ability of immune response to *Trichuris muris* infection has been studied by increase in ITH & DTH response. In the present investigation Exeretory-Secretory antigen of *Trichuris muris* showed highest values of ITH & DTH response as compared to Somatic antigen in vaccinated mice. Increased values of ITH & DTH response suggest role of cells in immunity. This study proves that ES antigen of *Trichuris muris* imparting more immunity in mice compare to somatic antigen.

Key words: *Trichuris muris*, Protective immunity, Vaccination, Excretory-Secretory (ES)

Introduction

Trichuris muris is a gastrointestinal nematode parasite causing a long lasting primary infection in mice (Wakelin, 1967). Gastrointestinal nematodes considered as a major constraint on ruminant production and welfare throughout the world. Now a day's immunological research and immunological techniques have allowed researchers to examine specific problems from a totally new angle of approach. Immunological killing of helminths is done by therapeutic agents and immunological control through vaccination (Parkhouse and Harrison, 1989 and Sher, 1998). In an effort to provide immuno-prophylaxis against intestinal nematode, the present study has been conducted to investigate the extent of protective immunity imparted by ES antigen against challenge infection.

Materials and Methods

Inbred Swiss albino mice *Mus musculus* albinos 7-9 weeks old and 18-20 gms weight were selected as experimental animals. *T. muris* is being routinely maintained in the laboratory by serial passage in healthy mice after every 31st day post infection with a dose of 100 viable infective eggs (Walelin, 1967)). The infected animals provide the various stages of the parasite for experimental purpose.

Preparation antigens

ES Antigen: The 500 adult worms kept in 10 ml of culture medium and placed in an incubator at 39^{0}C. After 48 hours, the culture medium (now containing the ES products) from each tube was centrifuged at 500 rpm for 10 minutes and carefully decanted. This culture medium was lyophilized and kept a 4^{0}C for further experimental use.

Somatic Antigen: Somatic antigens were prepared by homogenization and lyophilization. The adult stages worms were washed thoroughly and homogenized separately in the protein free culture medium. The homogenate were lyophilized and kept at 4^{0}C.

Immunization of mice and challenge infection of mice: The method employed for immunization of mice was described by Wakelin, (1967).

Assay of hypersensitivity reactions:

(A) Immediate type hypersensitivity (ITH) : Skin testing passive cutaneous anaphylaxis (Overy, 1964).

(B) Delayed type hypersensitivity (DTH) : Food pad thickness measurement : Delayed hypersensitivity estimation (Talwar, 1983).

Result and Discussion

Results of PCA and DTH reactions in mice infected and vaccinated with somatic and ES antigens are summarized in the tables (1 and 2) and figure (1 and 2). Skin reaction greater than 5 mm in diameter was considered significant. PCA and DTH reactions were found to be directly proportional to the concentrations of antigens. All ITH and DTH Values obtained on day 31st post infection.

PCA reactions were in the groups $SoAg_1$-Ag_5. Minimum skin reaction 9.2 mm was observed in Ag_1 which increased to 10.5 mm in Ag_2, 11.5 mm in Ag_3, 12.0 mm in Ag_4 and maximum 12.8 mm in Ag_5 on day 31st post infection. PCA reactions were in the groups $ESAg_1$-Ag_5. Minimum skin reaction 13.2 mm was observed in Ag_1 which increased to 14.6 mm in Ag_2, 15.5 mm in Ag_3, 16.2 mm in Ag_4 and maximum 17.7 mm in Ag_5.. Minimum PCA reaction 9.2 mm was observed at Somatic Ag_1 and maximum 17.7 mm in ES Ag_5.

DTH reactions were in the groups $SoAg_1$-Ag_5. Minimum skin reaction 4.8 mm was observed in Ag_1 which increased to 6.0 mm in Ag_2, 6.5 mm in Ag_3, 7.3 mm in Ag_4 and maximum 8.1 mm in Ag_5.. DTH reactions were in the groups $ESAg_1$-Ag_5. Minimum skin reaction 6.2 mm was observed in Ag_1 which increased to 9.4 mm in Ag_2, 10.8 mm in Ag_3, 12.2 mm in Ag_4 and maximum 12.8 mm in Ag_5. Minimum DTH reaction 4.8 mm was observed at Somatic Ag_1 and maximum 12.4 mm in Ag_5.

All the values obtained in various experimental groups were statistically significant when compared to those of the controls. Thus results obtained showed that ES antigen is highly antigenic than that of somatic antigen. ES antigen is not allowing the worms to develop in the intestine (Else *et at.*, 1992; Brancroft *et al.*, 2001; Else, 2003). Immediate type hypersensitivity is an allergic reaction induced by specific antigen provoked by re-exposure to the same antigen mediated by specific IgE antibodies, and produced by the cellular release of histamine and other vasoactive mediators, resulting in an immediate local or system i.c. reaction, IgE antibodies that constitutively express high affinity surface receptors for the Fc component of IgE (Roitt *et al.*, 1993). Lilywhite *et al.*, (1991) and Blackwell and Else (2001) reported the antibody responsible for protective immunity to *T.muris* may be involved in IgG, or IgE immunoglobulin.

In cell mediated hypersensitivity CD_8 cytotoxic T-cell and CD_4 helper T-cells recognize either intracellular or extracelluarly synthesized antigen. Macrophages function as antigen-presenting cells and release interleukin-1, which promotes the proliferation of helper T-cell, which release interferon-gamma and interleukin-2, which together regulate delayed hypersensitivity reactions centered on macrophage activation and T-cell mediate immunity. Activated macrophages express increased phagocytic activity when confronted with intracellular pathogens, which undergo granulomatous transformation in to epitheloid and multinucleated giant cell (Roitt *et al.*, 1993).

Table: 1 - PCA response in *T. muris* infected mice. Vaccinated with different concentrations of somatic and ES antigen (Ag.).

Group No.	Groups Name	PCA response in mm.	
		Somatic Ag.	ES Ag.
1	$INVC_2$	7.4 ±0.624	8.2 0.624
2	Ag_1	9.2 ±0.837	13.2 1.475
3	Ag_2	10.5 ±0.423	14.6 1.847
4	Ag_3	11.5 ±1.874	15.5 2.945
5	Ag_4	12.0 ±1.243	16.2 0.475
6	Ag_5	12.4 ±2.761	17.7 0.267

Fig. 1 - PCA response in *T. muris* infected mice. Vaccinated with different concentrations of somatic and ES antigen (Ag.).

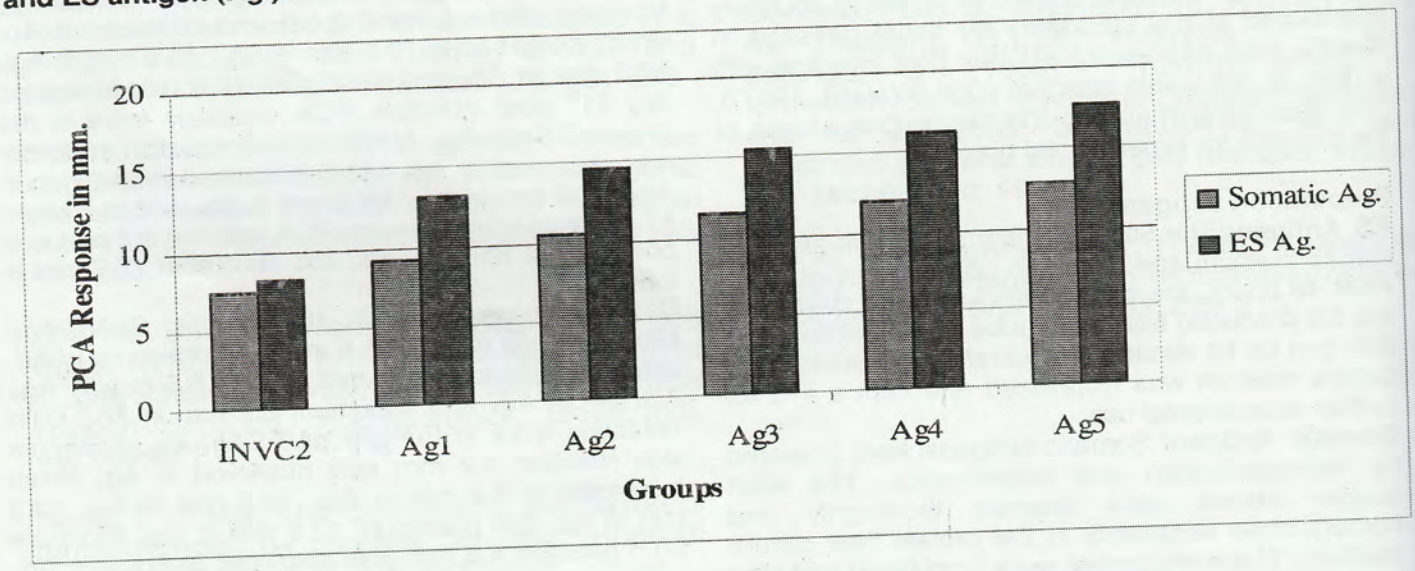
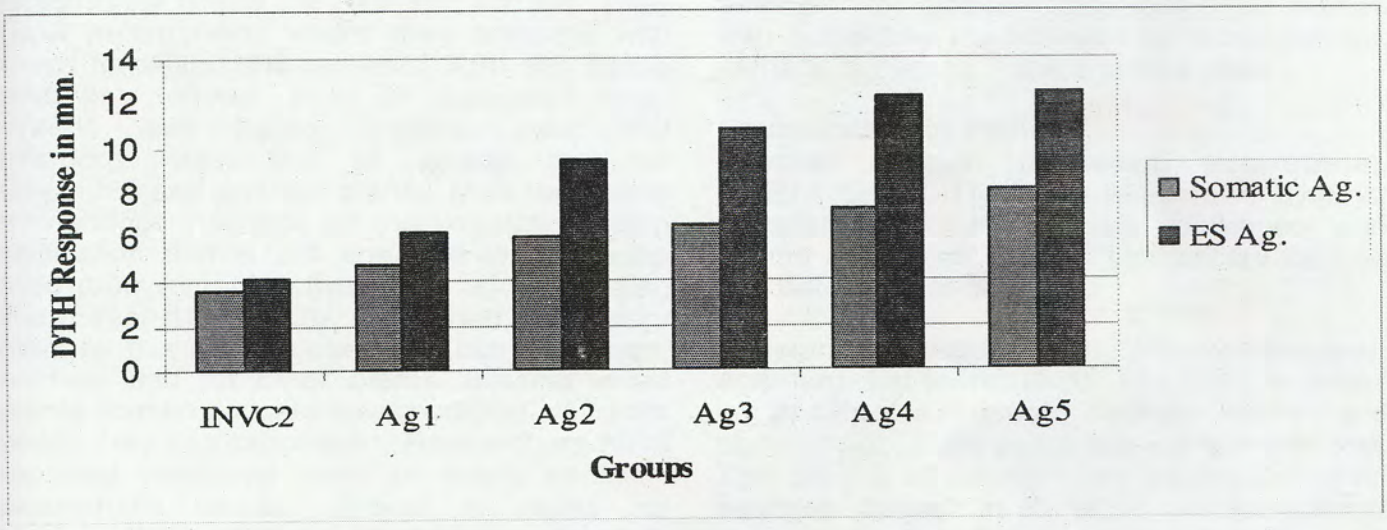


Table: 2 - DTH response in *T. muris* infected mice. Vaccinated with different concentrations of somatic and ES antigen (Ag.).

Group No.	Groups Name	DTH response in mm.	
		Somatic Ag.	ES Ag.
1	$INVC_2$	3.6 ±0.121	4.2 0.121
2	Ag_1	4.8 ±1.456	6.2 0.352
3	Ag_2	6.0 ±2.652	9.4 1.982
4	Ag_3	6.5 ±0.746	10.8 1.547
5	Ag_4	7.3 ±1.543	12.2 0.441
6	Ag_5	8.1 ±1.823	12.4 0.441

Fig. 2 - DTH response in *T. muris* infected mice. Vaccinated with different concentrations of somatic and ES antigen.



The results of the present study also corroborate with those of the observations of the above-mentioned authors. In comparison to the percentage protection provided by ES antigen is greater which may be because of the fact that these are excreted or secreted by the parasite *in vivo* too and thus easily recognized by the immunological system.

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Some Commonly Occurring Weeds of Bhopal (Madhya Pradesh) With Medicinal Importance

Hitendra K. Ram*, Abhay K. Patil and Deepesh Awasthi
Minor Forest Produce Processing and Research Centre (MFP PARC),
Barkheda Pathani, BHEL, Bhopal-462021 (M.P.)
*- Corresponding Author

Abstract:

Medicinally important weed plant species of Bhopal have been investigated in the present study. About 30 angiospermic taxa belonging to 20 families have been collected. These weed species have been studied as potential medicinal plants. In the present study an exhaustive idea has been given about the medicinal uses of these species and also future prospects regarding the cultivation of these weeds. The collected specimens were identified with the help of flora of Madhya Pradesh (BSI). Medicinal properties of the species have been indicated by consulting standard literatures. Present study concludes that due to human interference and other biotic pressure the weed population is highly affected.

Introduction:

Weeds as all of us know are the plants which grow where they are not wanted. They grow in the fields where they compete with crops for water, soil nutrients, light and space and thus affects yield of cultivated crop. Weeds also harbor insects, pest and microorganisms. But all weeds are not unwanted and useless plants. Man has made tremendous influence on the vegetation since civilization and is still engaged in sustaining food procurement activities. In ancient Indian literatures, it is clearly mentioned that every plant on this earth is useful for human beings, animals and for other plants. Several weed plants contain considerable medicinal value for which they can be utilized. Researchers have reported medicinal uses of many common problematic weeds. Bhopal is capital of Madhya Pradesh and it is situated at 23.20 N latitude and 77.30 E longitudes. Temperature in Bhopal varies usually from minimum 08.2 ^{0}C to maximum 42.7 ^{0}C. Bhopal city have rich botanical wealth in the form of urban forestry. During the investigation of medicinally important weed plant species of Bhopal; about 30 angiospermic taxa belonging to 20 families have been collected. These weed species have been studied as potential medicinal plants. In the present study an exhaustive idea has been given about the medicinal uses of these species and also future prospects regarding the cultivation of these weeds. The collected specimens were identified with the help of flora of Madhya Pradesh (BSI). Medicinal properties of the species have been indicated by consulting standard literatures such as Chopra *et al.,* (1956), Kirtikar and Basu (1935). In the enumeration of plants arranged in alphabetically botanical names with authorities are followed by family names, vernacular names, habit and their uses

Enumeration of Plants

Abutilon indicum (L.)Sweet. (Malvaceae). Kanghi, Shrub, The plant is useful is dysuria, diabetes fever, thirst, piles, diarrhoea and worms (Chunekar, 1982). Leaves are cooked and eaten in bleeding piles.

Achyranthes aspera Linn. (Amaranthaceae). Apamarg, Lat jeera, Herb, The plant is useful in pneumonia, cough, kidney stone and hemorrhoids. Seed paste applied to insect bite. This plant is effectively used against bronchial astyhma (Suresh et al. 1985) and is reported to have contraceptive property (Wadhwa *et al.,* 1986).

Argemone maxicana Linn.(Papaveraceae). Satyanashi, Annual Herb, it cures rhemautism, constipation, dysentery, colic and other intestinal affections. Seed oil applied to ulcers, herpetic eruptions and other skin diseases.

Barleria prionitis Linn., (Acanthaceae). Katsareya, Sub Shrub, A paste of the root applied to boils and glandular swellings. Leaves chewed to relieve toothache.

Blumea lacera (Burm.f.) DC., (Asteraceae). Jangle Muli, Herb, It is astringent, acrid, anti-inflammatory, ophthalmic, digestive, anthelmintic, liver tonic, expectorant,

antipyretic, diuretic and stimulant (Warner *et al*,1996). It is given in bleeding piles, root with black pepper is given in cholera.

Caesulia axiliaris Roxb., (Asteraceae). Bangra, Herb, Essential oil obtain from extract of this plant is perform antimicrobial activity on human pathogenic bacteria and fungi.

Calotropisgigantean(L.)R.Br., (Asclepiadaceae).Safed Aak, The plant has purgative property. It can be used in small pox, dropsy, leg and chest pain, skin diseases, poison, ulcers, enlargement of spleen, muscular pain, tongue paralysis and piles.

Cassia tora Linn., (Caesalpiniaceae). Puwar, charouta, Herb, It is used for skin diseases and cures eczema, dermatosis, itching, cough and other respiratory problems. Seeds are used for the cure of arthritis, poisonous affection and hemicrania.

Chenopodium album Linn., (Chenopodiaceae). Bathua, Herb, The plant is used in intestinal ulcers, piles, throat and eye troubles. Decoction as tonic, diuretic and aphrodisiac; given in hepatic disorders and spleen enlargement.

Clerodendrum indicum (L.)Kuntze., (Verbenaceae). Bharangi, Shrub, it is used in fever, atrophy, cholera, cough, bronchitis.

Commelina benghalansis Linn., (Commelinaceae). Kanchara, Herb, The plant is bitter, emollient, laxative and beneficial in leprosy.

Corchorus aestuans Linn., (Tiliaceae). Titapat, Herb, Seed are stomachic, cardiotonic and used in pneumonia.

Cynodon dactylon (L.)Pers., (Poaceae). Doob, Herb, Whole plant is used as the drug and it is a good remedy in epitaxis, haematuria and scabies.

Cyperus iria Linn., (Cyperaceae). Galmotha, Herb, The plant is used as stringent, stimulant, tonic and stomschic.

Echinops echinatus Roxb., (Asteraceae). It is diuretic, nerve-tonic used in horse cough, hysteria, dyspepsia and ophthalmia.

Eclipta prostrata(L.) L.Mant., (Asteraceae). Bhrigraj, Herb, It is used in hepatic and spleen enlargement and in skin troubles.

Elephantopus scaber Linn., (Asteraceae). Gobhi, Herb, It cures cold cough and other bronchial troubles, diabetes, ulcers and fever.

Euphorbia hitra Linn., (Euphorbiaceae). Dudhi, herb, Leaves and seeds are given in bowel complaints and worms of children. The plant extract is reported to have antiviral, spasmagenic, and anticancer, anti-bacterial properties.

Evolvulus alsinoides (L.)L. (Convolvulaceae). Sankhahuli, Herb, It is an excellent bone growth promoter.

Gomiphrena celosioides Mart. (Amaranthaceae). Herb, The plant extract is diuretic.

Hygrophila auriculata (Schum.)Heine, (Acanthaceae). Talmakhana Sub Shrub, the plant used in rheumatism, inflammation, jaundice, hepatin obstruction, pain etc. (shanmugasundaram & Venkataraman,2005). The plant is also known to possess antitumor (Ahmed *et al.*, 2001).

Lantana camara Linn., (Verbenaceae) *Ghaneri*, Shrub, The plant is Diaphoretic, carminative, stimulant and cures sprains, respiratory diseases. Leaf decoction is used in treating constipation.

Leucas aspera (Willd.)link., (Lamiaceae). Drodapuspi, Herb, It is used as antipyretic. Juice of the leaves applied externally in psoriasis, chronic skin eruptions and painful swellings. It is as such used for the treatment of chronic malaria and asthma (Ghosh,1988).

Oxalis corniculata Linn., (Oxalidaceae).Amrul sak, herb, It is beneficial in indigestion, dyspepsia, dysentery, piles and fever. Leaves are used externally to remove corns, warts and other excrescence on the skin.

Phyllanthus reticulates Poir., (Euphorbiaceae). Panjoli, Shrub, Leaves are diuretic.

Sida acuta Burm., (Malvaceae). Khareti, Herb, The plant is tonic, anti-pyretic, diuretic and

emollient. Root is used for nervous and urinary disorders.

Solanum nigrum Linn., (Solanaceae). Makoi, Herb, Fresh extract of plant has been recommended in dropsy, inflammatory swellings and enlargement of liver and spleen. The ripe fruits are effective in treating the disease of heart and eye, piles, leucuderma, asthma and jaundice due to presence of solasodine (Afaq. *et al.*, 1985).

Sphaeranthus indicus Linn., (Asteraceae). Gorakhmundi, The plant is used for jaundice, piles, hepatic disorders, tonic for eyes. Seeds are used in chest troubles.

Tephrosa purpurea (L.) Pers., (Fabaceae). Sarphokha, The plant is laxative, diureyic and deobstruent ; used in bronchitis. Decoction of root given in dyspepsia, diarrhoea, rheumatism, asthma and urinary disorders.

Trianthema portulacasturm Linn., (Aizoaceae). Lal sabuni, Herb, Leaves diuretic, used in dropsy, poisoning, also used in rheumatism and as vermifuge. The root applied to the eye cures corneal ulcers, itching, dimness of sight, night blindness (Nandkarni, 1976).

Conclusion

Several studies reveal that human population pressure is constantly threatening the biodiversity day by day. Present study concludes that due to human interference and other biotic pressure the weed population is highly affected. These weed plant species having lot of medicinal value. Villagers can collect these useful weeds and earn additional income by selling the different weeds to drug manufacturers or local buyers. It should be protected for different aspect of remedies and be promoted for cultivation also.

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Isolation of pathogenic *Corynebacterium renale* from the uterus of a bitch.

Priya, P.M [1*], Mini, M [2] and Ajithkumar, S[3]
Department of Veterinary Microbiology
College of Veterinary & Animal Sciences, Mannuthy
Thrissur, Kerala, 680 651

1* : Corresponding author: Assistant Professor, Department of Veterinary Microbiology , College of Veterinary and Animal Sciences , Mannuthy, Thrissur, Kerala . 680651.
2 : Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Pookot, Wayanad, Kerala . 673576.
3 : Associate Professor , Department of Clinical Medicine , College of Veterinary and Animal Sciences , Mannuthy, Thrissur, Kerala . 680651.

Abstract:

Corynebacterium renale was reported from a case of pyometra in a bitch as a primary etiological agent. There are very few reports regarding the isolation of *Corynebacterium renale* from uterus of bitches in India and has not been reported in Kerala. The present paper deals with the report of isolation of *Corynebacterium renale* from the uterus of a bitch.

Key words: *Corynebacterium renale*, pyometra, bitch, Kerala.

Corynebacterium renale, Corynebacterium pilosum and *Corynebacterium cystitidis* are opportunistic pathogens that inhabit the urinary tract of cattle and other domestic animals. Cystitis and pyelonephritis due to *Corynebacteriu .renale* has been reported rarely in bitches (Carter and Cole, 1990). *Corynebacterium renale* is the most important of these three, based on the incidence of infection. The vulva may be an important portal of entry for *Corynebacterium renale* into the urinary tract. This *Corynebacterium renale* and *Corynebacterium pilosum* adhere more readily to vulvar epithelial cells than to uroepithelial cells (Hayashi *et al.,* 1985).

The major risk factors are the shortness of the female urethra and the effects of pregnancy and parturition, pyometra occurs more frequently in mature animals.

There are very few reports regarding the isolation of *Corynebacterium renale* from bitches in India and has not been reported in Kerala. The present paper deals with the report of isolation of *Corynebacterium renale* from a bitch.

A three-year-old bitch (Doberman) was brought to Veterinary College Hospital, Mannuthy with a history of purulent vaginal discharge. The animal was whelped one month back and had given birth to eight pups. The uterine discharge was collected aseptically for detailed investigation. The sample was streaked on brain heart infusion agar and incubated at 37^{0}C both under aerobic and anaerobic conditions. No growth could be detected in anaerobically incubated plates for the first 48 hours. Few colonies were noticed in aerobically incubated plates within 24 hours, and were later identified as *E. Coli* based on morphological, cultural and biochemical characters. On further incubation, minute opaque colonies were developed after two days in plates incubated under aerobic and anaerobic conditions. Anaerobic incubation was found to present more growth. These colonies were identified as *Corynebacterium renale* on the basis of morphological, culture and biochemical characters as per Cowan (1991).

Based on the number of recovered colonies from the samples for *E. coli* and *C. renale*, it may be assumed that *Corynebacterium renale* could be the primary etiological agent for pyometra.

The animal was successfully treated with Moxel, which confirmed the involvement of *Corynebacterium renale* in causing pyometra in bitch.

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Efforts of MP Biotechnology Council in development of Biotechnology in the State of Madhya Pradesh.

S. K Panwar
IFS Chief Executive Officer , MP Biotechnology Council
.26, Kisan Bhaawan, III[rd] Floor, Arera Hills BHOPAL – 462011, India

The Government of Madhya Pradesh has displayed a strong commitment for the socio-economic development of the citizens of the state. Conservation of the state's rich biodiversity, its sustainable use for the expanding livelihood security and ensuring equitable sharing of its benefits have been part of this larger commitment. For this envelopment of Biotechnology and the spread of benefits through widespread use of its application have emerged as one of the leading intellectual enterprises of the scientific community the world over. While the Biotechnology field transcends state and national boundaries and thereby necessarily involves the Government of India, the State Government has made proactive efforts to harness the potentials of biotechnology by promulgating the Madhya Pradesh Biotechnology Policy. The Biotech Policy entails Inter alia promotion of biotech education, research and industry in the state.

Institutional Mechanism of the Biotechnlology Council

A State Biotechnology Council has been established under the chairmanship of the Chief Minister. The council is overseeing the implementation of the Biotechnology Policy of the State. It has as its members from eminent biotechnologists, educationists and industrialists. The Department of Biodiversity and Biotechnology is the nodal department to coordinate activities in the biotechnology sector.

Strategic Initiatives:

To translate the salient features of the biotechnology policy into action a set of strategic initiatives are on the anvil on which action shall be commenced as follows:

Setting up of a world class **'Institute of Life Science and Technology'** The key objectives of the institute will be create a state- of-art facility for frontline and relevant research in Life Science and to generate technologies, products and processes based innovation in biology and also to generate skilled human resources and provide entrepreneurship training and contribute towards socio-economic development of the State.

It will be based on an ideal synthesis of top quality education and cutting edge research and aim at global excellence with the local relevance. In other words, the Institute, while sustaining the highest level of scholarly endeavors, will contribute to the development of the people of India, and state of MP in particular. The Institute will have carefully crafted flexible, modular and interdisciplinary programs.

Establishing a Biotechnology Park with emphasis on agri-biotechnology and pharma-biotechnology units. The biotechnology park would comprises the following facilities: Technology incubation centre (TIC) consisting of laboratories and instrumentation facility, Micro propagation facility, Common facilities including animal testing facilities, Business support facilities (BSF), Pilot plan for biofertilisers, Pilot plan for Medicinal plant extraction, Business enterprise zone (BEZ).

Initially, the park management will set up the Technology incubation centre, Common facilities and the Business support facilities. The pilot plants for scale up facilities will be set up based on the actual requirements of tenants from time to time. The Business enterprise zone will house regular commercial units for the whom plots of land will be given on lease or sale, to set up the units at the clients cost. In addition a pilot plant conforming to BSL3 standards can be set up.

Promotion of Biotechnology Industry:

An appropriate policy framework for biotechnology industry has been created. Under this framework while all incentives and concessions available to large, medium and small scale industry has been made available to biotechnology units, package of additional fiscal and non fiscal incentives has been launched in the Industrial Promotion Policy of the state.

Promotion of Research in Biotechnology

Biotechnology is research led and capital intensive. The council is forging alliances between capital intensive researches and the scientific research of the state by providing financial assistance to foster BT research. The MP Biotech council is engaged to address Biotechnology related regulation, to create an environment conducive for the application of biotechnology tools to further its commitment for socio-economic development.