Caffeine Supplementation on Arsenic Induced Histopathological Alterations In The Gills of Freshwater Bivalve, Lamellidens corrianus (Lea)

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ABSTRACT

Aim: Histopathological study of caffeine supplementation on arsenic induced alterations in the gills of freshwater bivalve, Lamellidens corrianus (LEA).

Methods: Study was conducted under five groups of freshwater bivalves Lamellidens corrianus (LEA). Group A was control; B group was exposed to acute dose (LC50/2) of sodium arsenate (0.672 ppm As+++). Group C was exposed to acute dose (LC50/2) of sodium arsenate (0.672 ppm As+++ with caffeine (1, 3, 7-Trimethylxanthine) (5 mg/l.). After 4 days bivalves from group B were divided into D and E. D group bivalves pre exposed to acute dose (LC50/2) of sodium arsenate were allowed to cure in normal water. E group bivalves pre exposed to acute dose (LC50/2) of sodium arsenate were exposed to caffeine (5 mg/l.) for recovery.

Results: Histopathological study on gills of bivalves was screened from all groups. The present investigation indicates that, the histological structure of gills showed less damage in the presence of caffeine and the recovery from the arsenic induced alterations in the structure of gills was faster in presence of caffeine.

Conclusions: The caffeine has the capacity to protect the damage of tissues against the arsenic induced toxic impact.

KEYWORDS: Caffeine; Arsenic; Lamellidens corrianus; Gills; Histopathology

RUNNING TITLE: Caffeine supplementation on arsenic
INTRODUCTION
The end of 19th century and significant awareness has been developed in recent days. The problems of pollution are different in water bodies. Usually in ponds and lakes, the water contains high quantities of pollutants.
Generally, water is contaminated by toxic heavy metals and pesticides. Heavy metals are elements having atomic weight between 63.546 and 200.590 and a specific gravity greater than 4.0. Heavy metals are among the most dangerous contaminants. All heavy metal exits in the surface waters in colloidal, particulate, and dissolved phases, although dissolved concentrations are generally low. The colloidal and particulate metal may be found in 1) hydroxides, oxides, silicates, or sulfides or 2) adsorbed to clay, silica, or organic matter. The soluble forms are generally ions or unionized organometallic chelates or complexes. The solubility of heavy metals in surface water is predominately controlled by the pH of water, the type and concentration of ligands on which the metal could be absorbed, the oxidation state of the mineral components and the redox environment of the system (Connell and Miller, 1984; Kennish, 1992).
Metals are into three categories [1] Non critical, for e.g. sodium, potassium, calcium, magnesium and iron, [2] Toxic but very rare, or very insoluble e.g. rare metals like thorium and [3] very toxic, soluble and relatively accessible e.g. selenium, arsenic, zinc, mercury, lead, copper, cobalt, nickel etc. classified (Wood, 1974). The metals in the 3rd group are highly toxic.
Symptoms of acute poisoning are pharyngitis, gastroenteritis, vomiting, nephritis, hepatitis and circulatory collapse while chronic poisoning may cause liver damage, neural damage, and teratogenesis (USEPA, 1987). Arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, molybdenum, nickel, lead, vanadium and show deleterious effects on the water quality, soil quality, enters plants and animals through the food chain, and finally reaches in man in toxic concentration.
A new general approach to the mechanism of action of heavy metals has been pioneered by Rothstein (1959). It is based on the assumption that the cell membrane is the first point of attack by heavy metals. The studies show that heavy metals bind to the cell membrane and the first detectable change in cellular function are due to changes in the membrane either in inhibition of active transport process or in an increase in passive permeability. This membrane concept and the relevant studies up to 1960 have been the subject of a general review (Passow et al., 1961).
Arsenic ingestion can cause severe toxicity through ingestion of contaminated food and water causing, vomiting, diarrhea, and cardiac abnormalities. After acute exposure the deposition in tissues is, in the order, liver, kidney, intestine, spleen, and lung (Dubois and F.M.K, 1959). Arsenic appears in hair about 2 weeks after the first exposure, where it is bound to the sulfide linkages in keratin, chronic exposure leads to accumulation in hair, bone, and skin (Joseph, 1971). Arsenic may be found in high concentrations in the hair; year after cessation of exposure and after most of the metal has been removed from the soft tissues (Gaddum, 1959). Acute poisoning occurs because arsenic, especially in the form of As2O3 which is readily available, practically tasteless, has the appearance of sugar, and is quickly absorbed from the gastrointestinal tract. Oral intake is followed by a symptomatic period of about 30 minutes (Hyden et al., 1997). The throat and stomach pain, vomiting may ensure, which can develop the life risk. Depressed urine flow is
characteristic of acute arsenic intoxication. Death usually results in 1 to 3 days after arsenic poisoning due to nephritis. Diarrhoea and vomiting occur, but are less pronounced than in case of acute poisoning of arsenic (Dubois and F.M.K, 1959).

Hyden et al., 1997 reported that many well water have arsenic concentrations in the range of 100-800 ppb. Continued exposure to arsine gas generally results in symptoms similar to the picture of arsenic poisoning. However, a destruction of red cells takes place, resulting in a steady level of anemia. Skin keratoses result from prolonged exposure to arsenic and may become malignant. It seems unlikely that arsenic causes cancer in other tissues. Heavy metals are known to interfere with functional groups of micro molecules, the presence of heavy metal above threshold level results in irrevocable alterations in the microenvironment of the cell. Therefore, a continuous series of investigations have been performed using aquatic animals (Shastry and Sunita, 1984; Singh and Sahai, 1984).

Heavy metals enter the system of aquatic organisms via three main pathways. 1) Free metal ions and metal ions adsorbed on the particles that are absorbed through respiratory surface (e.g. gills) readily diffused into the blood stream. 2) Free metal ions that are absorbed by the body surface are passively diffused into the blood stream. 3) Metals that are adsorbed on to food and particulates may be ingested; as well as their free ions are ingested with water (Connell and Miller, 1984).

Arsenic is the most potent and versatile pollutant. Their physiological hazards lie in the fact that is not organ specific like some of the organic pesticides e.g. the organophosphates, which are neuro inhibitors and organochlorine compounds like DDT; which primarily accumulates in the gonads. Heavy metals are hepatotoxic agents, which induce neoplastic lesions in various tissues and cause general histological damages. Histopathological changes in the hepatopancreas due to industrial pollutant and chlorinated hydrocarbons are on record (Eller, 1971; Mukharjee and Bhattacharya, 1975; Dubale and Shah, 1979). Saxena, 1981 studied that Neoplasia in the kidney of freshwater teleost fish, *Channa punctatus* due to cadmium exposure. The history of gill and intestine of fish *Mystus vittatus* exposed to chromium stress under the scanning electron microscope (Shrivastava and Maurya, 1991). Acute and sub lethal effect of cadmium on histology of kidney of *Tilapia mossambica* reported (Usharani, 1986).

The histopathological studies show that heavy metal caused tissue damage in aquatic organisms. It has been previously noted in a variety of animals by many workers, in crabs exposed to mercury (Vernberg and Vernberg, 1972). Nimmo, et al., 1971 in shrimps exposed to cadmium, Shrivastava et al., 1982 a & b in fish exposed to chromium. In two species of prawns exposed to copper, (Ghate and Mulherkar, 1977) and in the fish *Punctius sophore* exposed to mercury is note worthy (Khangarot and Somani, 1980).

All scientists have proved that the heavy metal increases the risk of life in various ways leading to the death of organisms. Heavy metals are very difficult to remove from body; the damage of tissues caused by heavy metals may be recovered. Various antioxidants are use for recovery or reduce the damage of tissue due to heavy metals. Vitamin C and E are common antioxidants in the diet.

The caffeine molecule is a bitter alkaloid, which contributes to both acidity as well as the bitter properties of coffee. Chemically caffeine is 1, 3, 7-Trimethylxanthine and is structurally related to uric acid. It gets metabolized in the body by the
biochemical processes of demethylation and oxidation. The main urinary metabolites are 1-methylxanthine, 1-methyluric acid, and an acetylated uracil derivative.

The most profound action of methylxanthine, the major metabolite of caffeine in the human body is to bind to the adenosine receptors of cells, as the structure of this chemical compound is very similar to ‘adenosine’, which is a chemical naturally produced by the nerve cells.

Caffeine has similar effect on several organ systems. They differ mainly in their relative potencies. Caffeine has an ability to produce central stimulation, thus is usually classified as central nervous system stimulant. However, it has other important effects, such as relaxation of smooth muscles (particularly in the bronchioles and certain arterioles), stimulation of both cardiac and skeletal muscle and diuresis.

Caffeine protects the damage of tissues chemicals and genetic materials of organisms from the heavy metals generated free oxygen radicals. MSH calcium ion release channel protein due to caffeine stimulation of malignant hyperthermia susceptible sarcoplasmic reticulum was reported by Shomer (1994).

The present research work, on the study of caffeine supplementation on Arsenic induced histopathological alterations study on the gills of an experimental model animal, the freshwater bivalve, *Lamellidens corrianus* has been carried out to study the efficacy of caffeine.

**MATERIALS AND METHODS**

Selected experimental model, the freshwater bivalve, *Lamellidens corrianus* were acclimatized in the laboratory condition at room temperature for five days. The healthy and active acclimatized bivalves of approximately same size were divided into three groups A, B and C.

(1) A group bivalves were maintained as control, (2) B group bivalves were exposed to acute dose (LC502) of sodium arsenate (0.672 ppm As+++).

(3) C group bivalves were exposed to acute dose (0.672 ppm As+++ ) of sodium arsenate with 5 mg caffeine.

After 4 days bivalves from group B were divided into two groups D and E. (4) D group bivalves pre-exposed to acute dose of sodium arsenate were allowed to cure in normal dechlorinated water.

(5) E group bivalves pre-exposed to acute dose of sodium arsenate were exposed to 5 mg caffeine of dechlorinated water.

The experimental bivalves of A, B and C group were dissected after 24 hrs and 96 hrs and from D and E groups of recovery after 2 days and 4 days and gills were fixed in Bouin’s fluid, for 24hrs washed and dehydrated in alcohol grades, cleared in toluene and embedded in Paraffin wax (58-60°C). Prior to fixation gonads were screened by smear, techniques and only testis were fixed. Prepared blocks of tissues were cut at the thickness of 6μ and stained with Mallory’s Tripple Stain. Stained slides with serial sections were examined under light microscope for histopathological impact. Gills of bivalves from all groups i.e. control, exposed and recoveries were screened and photomicrographs are presented in Figure No.1 and 2.

**OBSERVATIONS AND RESULTS**

The effect of arsenic on gills of *Lamellidens corrianus* after exposure to 0.672 ppm (As+++), arsenic with, without caffeine, and during recovery has been shown in the figures.

The histological structure of gill from different groups indicates the effect of acute exposure to arsenic, As+++ with caffeine and recovery. Bivalves are filter –feeders. As they pump the water, the gills filter out particles and remaining suspended material from the water. Gill is the main respiratory organ in the aquatic animals,
which is directly exposed to the chemicals and contaminants or heavy metals present in water.

Histological Structure of Gill:

Histological structure of gill shows mainly gill lamellae with the ciliated epithelium, lamellar junctions, chitious rods and water tubes as shown in Figure No.1.

Figure No.1: Photomicrograph (Magnification X 100) of Gill of *L. corianus* on exposure to, Normal (Control), Sodium Arsenate for 24 hrs (A), Sodium Arsenate + Caffeine for 24 hrs (B), Sodium Arsenate for 96 hrs (C), Sodium Arsenate + Caffeine for 96 hrs (D).

Legends: C - Connective Tissue, CE - Ciliated Epithelium, DCE - Damaged Ciliated Epithelium, WT - Water Tube.
Histological changes after exposure to acute concentration of arsenic (0.672 ppm) with and without caffeine for 96 hrs and during recovery are shown in Figure No.2.

**Figure No. 2:** Photomicrograph (Magnification X 100) of Gill of *L. corrianus* during recovery after pre-exposure to Sodium Arsenate on exposure to, Normal Water for 2 days (A), Caffeine for 2 days (B), Normal Water for 4 days (C), Caffeine for 4 days (D).

**Legends:** C-Connective Tissue, CE- Ciliated Epithelium, DCE- Damaged Ciliated Epithelium, DLCE- Delaminated Ciliated Epithelium, WT- Water Tube

After sodium arsenate exposure, the lamellae of gills showed various changes, such as rupture of the ciliated epithelium, increase in the size of lamellae, increase in space between the inter lamellar junctions and increase in space between the water tubes and inter lamellar junctions. Normal structure of gills is totally damaged or disturbed showing the fusion and atrophy of secondary gill lamellae, displacement and necrosis of outer layer of gill lamellar epithelium due to the sodium arsenate.

After 24 hrs and 96 hrs of exposure to sodium arsenate, changes in the cytoarchitecture of gills of *Lamellidens corrianus* were severe as compared to the gills of those bivalves exposed to sodium arsenate with caffeine. During the period of recovery, the gills in normal water showed space between inter lamellar junctions and water tubes, and damaged ciliated epithelium, while the recovery was faster in gills of caffeine exposed bivalves with respect to these changes after 2 days and 4 days.
During recovery, the nature of damage of lamellar ciliated epithelium was less than sodium arsenate exposed gill lamellae. Caffeine exposed bivalves showed that the caffeine increased the rate of recovery and reduced the damage as compared to the normal recovery in water indicating its role in detoxification of arsenic. The nature of damage in gills observed in exposed to sodium arsenate was more. Simultaneous use of caffeine showed protection by caffeine on heavy metal induced alterations. Faster recovery was observed in all tissues on exposure to caffeine.

The present investigation indicates that, caffeine has a protective and curative role in the arsenic induced alterations. The histological structure of gills showed less damage in the presence of caffeine and the recovery from the arsenic induced alterations in the structure of gills was faster in presence of caffeine.

**DISCUSSION**

The histopathological study shows that, alterations caused by arsenic in various gill is specific and time dependent. Histological approach is the most valuable tool for assessing the action of toxicant at tissue level and for its manifestation of structural and functional changes. Histopathological abnormalities caused due to toxicity of heavy metals in animals have been reported earlier (Shrivastava et al., 1982 a & b; Khalid et al., 1986). The effect of various compounds of the heavy metals is mainly studied in invertebrates, amphibians and mammals (Laborda et al., 1986; Joshi and Patil, 1995).

Gills function as the major route for the uptake of heavy metals as they are the most permeable regions of the body (Victor, 1993a; Victor, 1994). Effect of copper and mercury on the fresh water bivalve *Corbicula striatella* and observed that the gills are highly affected due to continuous exposure to toxicants (Mahajan and Zambare, 2001). The heavy metals cause severe damage to gill surface and reduce oxygen uptake capacity of respiratory organs (Nonnotte et al., 1993). Prasad et al., 2000 observed the damage of the gill tissue marked by curling of secondary lamellae, rupture of gill rakers, displacement and necrosis of outer layer of lamellar epithelium due to the exposure to toxicants.

Heavy metals interferes the respiratory mechanism by disrupting the structure of gills in crustaceans and fishes (Jones, 1975; Victor, 1985; Victor, 1993b; Vogen et al., 2001). The overall factor influencing the accumulation of metals is absorption of ions by membrane, interference in gills and the uptake or diffusion by active or passive mechanisms (Carpene and George, 1981). The effect of chromium stress on gill and intestine of *Mystus vittatus* for prolonged period of 75 days observed (Shrivastava and Maurya, 1991). The histological changes observed in the organs were the normal irregular concentric ridges on primary gill lamellae and smooth surface of secondary gill lamellae, changes to symmetrical concentric ridges resulting into crevices and furrows on the surface.

After short-term exposure to copper sulphate, gill arch remained unaffected, while in the case of lead nitrate slight damage was seen in the cartilaginous and muscular part of the gill arch. After long term exposure of copper and lead, severe damage was seen in the gill arch. Most of the chondroblast cells were found in shrunken condition. The damage was more severe in the case of lead nitrate in comparison to copper sulphate. Gupta and Rajbanshi, (1979) studied that the degeneration of blood cells, blood capillaries and cartilaginous cells in the gills of *Heteropneustes fossilis*.

After long term exposure to copper sulphate, primary gill lamellae showed hyperplasia at
certain places while on exposure to lead nitrate, heavy necrosis was noticed by various reporters. Matei et al., (1993) have also reported degeneration, hypertrophy and hyperplasia in the secondary gill lamellae. Sub lethal concentration of toxicants induced fusion and atrophy of secondary gill lamellae (Vijayalakshmi and Tilak, 1996).

Gill injuries such as separation of gill epithelium, fusion of secondary lamellae, degeneration, of epithelial cells, nuclei and dissolution of the basement membrane have also been reported in the freshwater fish, Aplocheius and Puntisus sophore after the treatment of heavy metals like Cu and Hg (Khangarot and Somani, 1980). Kshemkalyani et al., (1990) reported that histopathological changes in the gill and liver of fish Hepidocephalus guttæ,(Ham), after 96 hrs of exposure showed swelling of secondary gill lamellae, loss of epithelial cells in the gills and swelling of nuclei and necrosis of liver cells. Reduction in the size of primary and secondary gill lamellae and necrosis of tissue in the copper, lead and zinc treated fishes were observed in contrast to control fish.

Hosaka et al., (2001) has observed the inhibition of hepatocarcinogenesis by caffeine in Ag1 rats treated with 2-acetylaminofluorene and has proposed that caffeine inhibited hepatocarcinogenesis induced by 2-acetylaminofluore. Mahajan, (2005) investigated that caffeine have the capacity to reduce the heavy metals, arsenic trioxide in snail, Bellamya bengalensis. Chung Fung – Lung, (1999) suggested that caffeine have the capacity to reduce the tissue damage and protect the hepatopancreas. caffeine when given in drinking water at a concentration identical to that found in 2% tea was able to inhibit lung tumors induced by 4 (methylNitrosamino)-1-(3-pyridyl)-1butanol (NNK). Caffeine stimulation of malignant hypothermia susceptible sacroplasmic reticulum Ca$^{2+}$ release, and suggested that caffeine sensitivity of Malignant Hypothermia Susceptible (MHS) skeletal muscle fiber bundles is due to an altered caffeine sensitivity of MHS calcium ion release channel protein.

Puming et al., (2001) studied suppression of lipopolysaccharide induced liver injury by various types of tea and coffee in D-galactosamine sensitized rats and suggested that caffeine containing beverages generally suppress, lipopolysaccharide induced liver injury according to their caffeine content. Inhibition of ATM and ATR kinase activities by the radio sensitizing agent, the caffeine and suggested that the radio sensitizing effects of caffeine are related to inhibition of the protein kinase activities of ATM and ATR and that both proteins are relevant targets for the development of novel anticancer agents (Sarkaria, et al., 1999). Caffeine increases endurance and attenuates force sensations during the first 10-20 second of concentration. The rapidity of this effect suggests that caffeine exerts its effects naturally (Plaskett and Cafarelli, 2001). Caffeine has been found to increase glutathione synthetase and reduced glutathione in liver and lungs of mouse (Shelar, 2002). Oxygen at second and sixth position of caffeine probably forms the chelate with the metal and hence caffeine-metal-chelate complex can reduce the activity of metal and complex can be excreted out as it has low molecular weight.

The present investigation indicates that, caffeine has a protective and curative role in the arsenic induced alterations. The histological structure of gills showed less damage in the presence of caffeine and the recovery from the arsenic induced alterations in the structure of gills was faster in presence of caffeine. The caffeine has the capacity to protect the damage of tissues against the arsenic induced toxic impact.
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