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***Research Article***

# CAFFEINE SUPPLEMENTATION ON HEAVY METAL SALTS INDUCED BIOCHEMICAL ALTERATIONS IN THE GILLS AND FOOT OF FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS*

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## ABSTRACT
The present study was carried out to study the preventive role of caffeine (1, 3, 7-Trimethylxanthine) on arsenic induced alterations in the protein contents of an experimental freshwater bivalve, *Lamellidens marginalis*. The effect on bivalve was studied under five groups. A group bivalves were kept as control, B of bivalves were exposed to chronic dose ($LC_{50/10}$) of arsenic trioxide (0.236ppm) C group of bivalves were exposed to chronic dose ($LC_{50/10}$) of arsenic trioxide with caffeine (5 mg/L.) up to 9 days. Protein contents in selected tissues from each group were estimated after 3, 6 and 9days. After 9 days bivalves from group B were divided into two groups D and E. D group of bivalves pre exposed to chronic dose ($LC_{50/10}$) of arsenic trioxide were allowed to cure in normal water. E group of bivalves pre exposed to chronic dose ($LC_{50/10}$) of arsenic trioxide were exposed to caffeine (5 mg/L) for recovery up to18 days. From D and E groups of bivalves, Protein contents in selected tissues were estimated after 12, 15 and 18 days. The protein content was decreased due to arsenic and increased to caffeine in presence of arsenic. During recovery protein content increased and the increased was higher with caffeine. The rapid recovery by caffeine shows that preventive role towards arsenic.

***Keywords:*** *Caffeine, Protein Content, Arsenic, Lamellidensmarginalis*

## INTRODUCTION
Pollution of an environment is mostly due to man's intervention and his rapid progress in colonization, urbanization, industrialization, agriculture, mining, transportation and chemical technology; there by the marine and freshwater habitats have become the repositories of pollutants released from all those anthropogenic activities. Mining and smelting operations and discharge of most of the industrial wastes into the aquatic environment lead to the accumulation of inorganic pollutants like mercury, cadmium, copper, lead, chromium, iron and zinc in dissolved and suspended forms (Chukwu and Ugbeva, 2003). Humans are exposed to arsenic primarily from air, food and water. Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals.

However, elevated arsenic level in drinking water is the major cause of arsenic toxicity in the world. Reports of arsenic contamination in water are available from more than 30 countries in the world (Chakraborti and Datta, 1979) studied and concluded, In India, though cases of arsenic toxicity including liver fibrosis due to drinking of arsenic contaminated water were reported from Chandigarh in early 1978, occurrence of large number of cases of arsenic induced skin lesions were reported from Kolkata, West Bengal in 1984 (Garai *et al.*, 1984). Most of the reports of chronic arsenic exposure in man focus attention on skin manifestations because of their diagnostic specificity. However, data derived from population based studies, clinical case series and reports relating to intake of inorganic arsenic in drinking water, medications or occupational and environmental exposure, show that chronic arsenic exposure adversely affects multi organ systems of human body. The symptoms of chronic arsenic toxicity (arsenicosis) are insidious in onset and are dependent on the magnitude of the dose and duration of its exposure. There is a wide variation of occurrence of symptoms in an arsenic exposed population.

Protein acts as enzyme, antibody, hormone and basic structural component of the animal. Protein is key substance to show the effect of heavy metal. Proteins respond to stress condition for better survival by altering their levels. These heavy metals enter into the body of animals including man through the non vegetarian and vegetarian diet, drinking water and air and accumulate in the tissues, usually react with proteins and interfer the physiological activities and thus increase the risk of life in various ways. They are difficult to remove from the body. The trace metals are known to be non biodegradable and highly toxic to most organisms (Kaoud and Dahshan, 2010). Mc Laughlin (2000) of CSIRO, Australia has found that coffee has capacity to bind with heavy metals. Heavy metal content of water was much reduced after addition of caffeine. Dissolved heavy metal ions are positively charged and coffee contains uncharged and negatively charged molecules, the metals ions might be taken out of solution by binding to negatively charged molecules in the coffee granules. Liguori (1997) reported that, caffeine from coffee or other beverages is absorbed by the small intestine within 45 minutes of ingestion and then distributed throughout all tissues of the body. Metabolites of caffeine also contribute to caffeine's effects. Paraxanthine is responsible for an increase in the lipolysis process, which releases glycerol and fatty acids into the blood to be used as a source of fuel by the muscles. Theobromine is a vasodilator that increases the amount of oxygen and nutrient flow to the brain and muscles. Theophylline acts as a smooth muscle relaxant that chiefly affects bronchioles and acts as a chronotrope and inotrope that increases heart rate and force of contraction (Dews, 1984). Maughan (2003) suggested that, caffeine equivalent to 2–3 cups of coffee are administered to people who have not consumed caffeine during prior days, they produce a stimulation in urinary output.

The molecules of coffee being small, it's chelate with heavy metal can be easily excreted out by the biological system. This property of caffeine indicates that caffeine can have the capacity to remove the heavy metals from the living organisms. However no attempt has been made to study the role of caffeine supplementation on heavy metal detoxification.

## MATERIALS AND METHODS

The bivalves, *Lamellidens marginalis* were acclimatized to laboratory condition for 2-3 days and healthy active bivalves of approximately medium size and weight were chosen. These bivalves were divided into three groups, such as group A, B and C. The bivalves of group A were maintained as control. The bivalves from group B were exposed to chronic concentration (LC 50 value of 96 hr/10) of heavy metal salt, Arsenic trioxide ( 0.236 ppm) upto18 days, while bivalves from group C were exposed to chronic concentration of along arsenic trioxide with 5 mg/lit caffeine upto 18 days. After exposure for 18 days to heavy metals, the bivalves from group B were divided into two subgroups, such as D and E groups. The bivalves of group D were allowed for self cure naturally in normal water while the bivalves of group E were exposed to 5 mg/lit caffeine up to 9 days. The experimental bivalves from A to C groups were dissected after 3,6 days and 9 days and from each recovery group (E and D) were collected after 12,15 and 18 days. The gills and foot, from all experimental and recovery group were dried at 80 0C in an oven until constant weight was obtained. The dried powders of these tissues of control, experimental and recovery group animals were used for estimation of their protein contents. Total protein was estimated by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin as standard from each powder. The average results of three repeats are presented in the table No. 1 and are expressed as percentage of dry weight. Percent variations were calculated and are expressed in respective tables.

## RESULTS AND DISCUSSION

### *Observation and Results*

Protein contents in different tissues of *Lamellidens marginalis*after exposure to Arsenic trioxide (0.236 ppm) along with caffeine and during recovery have been summarised in tables.

Table A shows that the protein contents in Gills and foot of *Lamellidens marginalis*in presence of arsenic trioxide (0.236 ppm) decreased with the increase in exposure period. The protein contents were more in heavy metal with caffeine exposed bivalves as compared to those exposed to only heavy metal salts forthe

corresponding period of exposure. Table B shows that the bivalves preexposed to heavy metal salts showed fast recovery in the alteration of protein in presence of caffeine than those allowed to cure naturally. Therefore after studies the effect of caffeine on heavy metal it is proved that ,The caffeine possess binding site to connect heavy metal salts and due to this effect of heavy metal is less in animals those exposed in caffeine as compared to those exposed only in heavy metal salts.

**Table A: Protein content in selected tissues of *Lamellidens marginalis*, after chronic exposure to heavy metal salt, arsenic trioxide without and with Caffeine**

| Treatment | Sr No. | Body Tissue | The protein content (%) ± S.D. 3 Days | 6Days | 9Days |
|---|---|---|---|---|---|
| (A) Control | i | G | 26.5 ± 0.0091 | 26.2 ± 0.0091 | 25.5± 0.0069 |
| | ii | F | 19.5 ± 0.0074 | 18.9 ± 0.0074 | 17.6 ± 0.0114 |
| (B) 0.236 ppm $As_2O_3$ | i | G | 21.3± 0.008$^{***}$ - 0.052$^{\bullet}$ | 19.6 ± 0.008$^{***}$ - 0.066$^{\bullet}$ | 18.0 ± 0.0089$^{***}$ - 0.075$^{\bullet}$ |
| | ii | F | 14.5 ± 0.008$^{***}$ - 0.050$^{\bullet}$ | 13.7 ± 0.0072$^{***}$ - 0.052$^{\bullet}$ | 13.5 ± 0.0226$^{***}$ - 0.041$^{\bullet}$ |
| (C) 0.236 ppm $As_2O_3$+ 5mg/lit Caffeine | i | G | 22.3 ± 0.008$^{***}$ - 0.42$^{\bullet}$, +0.01$^{\Delta}$ | 21.2 ± 0.0071$^{***}$ -0.50$^{\bullet}$, +0.016$^{\Delta}$ | 19.7 ± 0.0065$^{***}$ -0.58$^{\bullet}$, +0.017$^{\Delta}$ |
| | ii | F | 16.5 ± 0.008$^{***}$ - 0.03,$^{\bullet}$+0.02$^{\Delta}$ | 15.6 ± 0.0101$^{***}$ -0.033$^{\bullet}$, +0.019$^{\Delta}$ | 14.8 ± 0.0074$^{***}$ -0.028$^{\bullet}$, +0.013$^{\Delta}$ |

**Table B: After 9 days exposure to 0.236 ppm $As_2O_3$**

| Treatment | Sr No. | Body Tissue | The protein content (%) ± S.D. 12 Days | 15 Days | 18 Days |
|---|---|---|---|---|---|
| (D) Normal Water | i | G | 25.5 ± 0.0074$^{***}$ - 0.01$^{\bullet}$, + 0.00$^{\square}$ | 25.6 ± 0.0033$^{***}$ - 0.006$^{\bullet}$, +03.001$^{\square}$ | 26.4 ± 0.0065$^{NS}$ +0.009$^{\bullet}$, - 0.011$^{\square}$ |
| | ii | F | 17.8 ± 0.0068$^{***}$ - 0.002$^{\bullet}$, + 0.043$^{\square}$ | 18.2 ± 0.0048$^{***}$ - 0.002$^{\bullet}$, + 0.047$^{\square}$ | 19.1 ± 0.004$^{NS}$ + 0.015$^{\bullet}$, + 0.056$^{\square}$ |
| (E) Normal Water + 5mg/ lit. Caffeine | i | G | 25.7 ± 0.0088$^{***}$ - 0.002$^{\bullet}$, 0.077$^{\square}$ | 26.5 ± 0.0072$^{***}$ - 0.01$^{\bullet}$, + 0.075$^{\square}$ | 26.5 ± 0.0048$^{NS}$ + 0.01$^{\bullet}$, + 0.085$^{\square}$ |
| | ii | F | 18.0 ± 0.0072$^{***}$ - 0.00$^{\bullet}$, + 0.045$^{\square}$ | 18.2 ± 0.0065$^{NS}$ - 0.002$^{\bullet}$, + 0.047$^{\square}$ | 18.6 ± 0.0034$^{NS}$ + 0.006$^{\bullet}$, + 0.051$^{\square}$ |

*G.- Gills, F– Foot/N.S. -Non Significant,● -Compared with respective A, * -P < 0.005, ** -P <0.01 , *** - P < 0.001, □ -Compared with respective 9 days of B*

***Discussion***

The change in biochemical composition of an organ due to heavy metal stress indicates the change in activity of an organism . It reflects light on the utilisation of their biochamical energy to counteract the toxic stress. Heavy metal salts affect the metabiolism of the fresh water bivalves, *Lamellidensmarginalis* Alterations in metabolic pocesses, following exposure to heavy metal stress have been always used as an indicator of stress. Protein as one of the main sources of energy and it plays an important role in the maintenance of blood glucose (Jrueger *et al.,* 1968). It is the most fundamental and abundant biochemical constituent present in the animal body and the estimation of protein is considered to be important (Ravichandran *et al.,* 1994). Mule and Lomte (1995) have reported that the protein content of an animal is an important organic constituent, which plays a major role in cellular metabolism.

In present study, in the *Lamellidens marginalis* the protein contents in the selected tissues was decreased in chronic concentration of arsenic trioxide as compared to the control and LC50/10 concentration with 5

mg/lit caffeine. According to Abel (1974) the decrease of protein may be due to alterations of membrane permeability. The depletion in the protein content was reported from the muscles of fish, *Clariasbatrachus* after treatment with pesticide by the Yagana Bano *et al.,* (1981). Nagabhushanm and Kulkarni (1979) studied variation in protein metablism in *Barytelephusacunicularis*. Joseph *et al.,* (1987) observed the effect of copper on biochemical composition of *Cyprinuscarpio* and found that total protein content of the brain, liver and muscles was declined. Mukherjee and Sinha (1993) studied the effect of heavy metal toxicity on haematological and biochemical aspect in the fresh water major carps, *Labeorohita*. Katticaran *et al.,* (1995) studied the copper induced alterations in total carbohydrate and protein level in the bivalve, *Sunettascripta*.

In present stress, ionic arsenic trioxide might have caused severe disturbances of the metabolism in the animal. Chronic exposure of copper sulphate alone showed a remarkable decrease in protein content in*Lamellidensmarginalis* than the chronic doses of heavy metal salts with caffeine. Starvic (1994) after the study of role of chemo preventer in human diet suggested that most of the chemo preventive strategies have been based on the modification of metabolism at one or many steps such that these agents can block the metabolic activition or enhance the detoxification. Caffeine is well-known nervous system stimulant but besides it, it is now observed that it has antioxidant activity. This activity of caffeine can protect the damage of tissues chemicals & genetic materials from heavy metal generated free oxygen radicals. Oral administration of tea has been found to moderately enhance the activities of lipid Peroxidase. catalase, glutathione-s-transferase which in turn protect against cancer by blocking the reaction of electrophilic carcinogens with cellular micromolecules (Madal and Maity, 1999). Caffeine being water soluble and common cheaper beverage, it will be cheapest preventive and curative medicine. Takayamas (1982) long term study on the effect of caffeine in wister rats, has proved that caffeine belongs to a group of compound known as methylxanthine and it is non carcinogenic in animal model. It has also been reported to antagonize the carcinogenic effects of chemicals in vitro. Under in vitro condition caffeine has been reported to enhance or inhibit tumorigenesis induced by various carcinogenic agents, mercury, arsenic and lead is the known carcinogenics. Wattenberg (1992) reported that any compound that can block the metabolic activation step, scavenge the reactive inter mediate or enhance detoxification would be potential chemopreventiveagent. Chung (1999) reported that caffeine when given in drinking water at a concentration identical to that found in 2% tea was able to inhibit lung tumours induced by 4-(Methylnitrosoamino)-1-(3-pyridyl)-1 furane (NNK). Hosakas *et al.,* (2001) has observed that caffeine inhibite hepatocarcinogenesis induced by 2- acetylaminoflurene. Gandhi and Khanduja (1992) studied the action of caffeine in altering the carcinogen activating and detoxofying enzymes in mice and reported an induction of xenobiotic detoxifying enzyme as an additional mechanism by which plant product may act as anticarcinogens, since this induction of detoxifying enzyms is capable of competing with steps in xenobiotic activation. Caffeine has been found to increase glutathione synthetase and reduced glutathione in liver and lungs of mouse (Gandhi and Khanduja, 1992).

Lu *et al*., (2001) studied the stimulatory effect of oral administration of green tea or Caffeine on ultraviolet light induced alterations and suggested that green tea & caffeine inhibits UV- induced carcinogenesis. Matsumura *et al.,* (2000) reported that, the ATP – requring priming stage is lacking in the process of caffeine induced exocytosis in bovine adrenal chromaffincells. Hove-madsen (1999) suggested caffeine exposure incresed pulse duration to 85m/s and slowed the inactivation of the Ca2+ current (Ica). Leoty *et al*., (2001) found that caffeine stimulates the reserve mode of NA(Sup+)/Ca(SUP2+) exchanger in ferreteventrecular muscles. and indicated that the increase in resting tension following exposure to caffeine was mediated by Na+/Ca2+ exchanger, Which represents an additional element of coplexity in caffiene action on cardiac muscles. Massey *et al.,* (1993) indicated the increased urinary excretion of calcium, magnesium, sodium and chloride after oral doses of caffeine which indicates the chelated caffeine with heavy metal is excretable. In Sept. (2001), Women's Health Weekly also reported that, the caffeine in the drinks was primarily responsible for excess calcium excretion. Caffeine is used as an ergogenic aid because multiple well-controlled experiments have found that moderate doses of caffeine (3-6 mg/kg) can improve performance in athletes (Graham, 2001; Flinn *et al.,* 1990). The ergogenic effect

of caffeine ingestion before exercise has been reported above all in high intensity aerobic conditioning programs (Bruce *et al.*, 2000; Jackman *et al.*, 1996; Wiles *et al.*, 1992).

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