Orginal article

Caffeine (1,3,7-Trimethylxanthine) as a modulator of Arsenic bioaccumulation in the experimental *Pelecypod* model, *Lamellidens corrianus*

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ABSTRACT

Aims: The present investigation was carried out to study modulator effect of caffeine (1,3,7-Trimethylxanthine) on Arsenic-induced alterations on freshwater bivalve, *Lamellidens corrianus.*

Materials and Methods: Freshwater *Pelecypod Mollusc, L. corrianus* were exposed to acute dose of arsenic (0.672 ppm As^{3+}) for 4 days. Arsenic exposed *Bivalves* were allowed to cure naturally and with caffeine. Testis and digestive glands from different groups were removed and dried in the oven. The dry powders were digested in nitric acid and perchloric acid in 4:1 ratio at hot temperature till dryness. The digest was dissolved in double glass distilled water and the arsenic contents were estimated by the Atomic Absorption Spectrophotometer.

Results: After 4 days of exposure, the amount of bioaccumulated arsenic in the testis and digestive glands was 1.044 and 1.119 µg/g. During the recovery after 4 days, the bioaccumulated arsenic was reduced to 0.969 and 1.067 µg/g of normal water while in caffeine exposed *Bivalves* was reduced to 0.917 and 0.975 µg/g in testis and digestive glands respectively.

Conclusion: Rapid reduction in the arsenic contents in caffeine exposed *Bivalves* indicates the role of caffeine in arsenic excretion. Therefore, the caffeine (1,3,7-Trimethylxanthine) as a modulator of Arsenic bioaccumulation in the experimental *Pelecypod* model, *L. corrianus*

Key words: Arsenic, Bioaccumulation, Caffeine, *Lamellidens corrianus*

INTRODUCTION

The *Bivalves* being bottom dwellers are exposed to higher doses of the toxic metals as most metal precipitates and increases in the sediments.[1,2] The high metal contents in the surrounding make them the best bioaccumulators.[3,4] These mussels are useful indicators of the abundance and spatial distribution of metals in aquatic ecosystem.[5-7]

Investigation results those acute disorders in aquatic organisms due to toxic heavy metals.[8] Heavy metals are very

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harmful pollutants as they remain in the nature for a very long period. The bioaccumulation of heavy metal toxicants in aquatic animals depends on availability and persistence of the contaminants in water and the physico-chemical properties of toxicants. The metal content of mollusk is controlled by the surface to volume ratio of the animal.[9,10]

Arsenic is a chemical that bioaccumulates in tissues of aquatic organisms but does not biomagnify in the aquatic food chain.[11-16] The consensus in the literature is that 85- 90 percent of arsenic found in edible portions of marine fish and shellfish is organic arsenic Arsenobetaine (ASB), Arsenocholine, Dimethylarsinic Acid, Monomethylarsonic Acid (MMA) and that appropriately 10 percent is inorganic arsenic.[17-20] Less is known about the forms of arsenic in freshwater fish, but there is evidence that organic arsenic may be prevalent, Field based study or considerably less.[14,21-25]

More recent research indicates that as compared to arsenite, trivalent methylated arsenic metabolites exerts a number of unique biological effects, are more cytotoxic and genotoxic, and are more potent inhibitors of the activities of some enzymes.[26,27] Because each arsenic species (As^{III}, As^{V}, ASB, MMA^{V}, and MMA^{III}) exhibits different toxicities, it may be important to take into account the fraction of total arsenic present in the inorganic arsenic and organic arsenic forms while estimating the potential risk posed to human health through the consumption of arsenic contaminated fish and shellfish.[13]

The removal of toxic metal through chelating allows the body functions at an optimal level. Dissolved heavy metal ions are positively charged, caffeine contains uncharged or negatively charged molecules, and the metal ions can be taken out of solution by binding to negatively charged molecules in the coffee which indicates that caffeine can have the capacity to bind the heavy metals from living organisms. According to investigator, oxygen from 2nd and 6th position of caffeine forms chelate with heavy metal.[28]

Therefore, it is proposed to study the efficacy of caffeine in the detoxification of arsenic with respect to their tendency of bioaccumulation in an experimental animal model, the freshwater bivalve, *Lamellidens corrianus*.

MATERIALS AND METHODS

L. corrianus collected from the Nathsagar Dam, Paithan were acclimatized in the laboratory condition at room temperature for 5 days. The healthy and active acclimatized *Bivalves* of approximately the same size were divided into two groups A and B.

1. A group *Bivalves* were maintained as a control,
2. B group *Bivalves* were exposed to acute doses (LC_{50}) of sodium arsenate (0.672 ppm As^{3+}). After 4 days *Bivalves* from group B were divided into two groups C and D.
3. C group *Bivalves* pre-exposed to acute dose of sodium arsenate were allowed to cure in normal dechlorinated water.
4. D group *Bivalves* pre-exposed to acute doses of sodium arsenate were exposed to 5 mg caffeine^{-1} in dechlorinated water.

The experimental *Bivalves* of A and B group were dissected after 24 h and 96 h and from C and D groups of recovery after 2 days and 4 days. Testis and digestive glands of bivalves from all groups were dried at 80°C in an oven till constant weight was obtained. The powders obtained were stored in airtight specimen bottles by waxing the cork outside.

The 500 mg tissue powder was digested in 10 ml of acid mixture (HNO_3: Perchloric acid) in (4:1) ratio on a hot plate till dryness. The digested mixture was cooled and diluted to 50 ml by double distilled glass water in a volumetric flask and was filtered by (Whatman grade 541) filter paper. From each sample, arsenic was estimated by Atomic Absorption Spectrophotometer (Chemito)

The concentration of arsenic accumulated in the tissue of each exposure period in each tissue was recorded and the results are given in the Table 1 and Figure 1a and b.

Table 1: Arsenic content (µg/g dry weight) in testis and digestive glands of *Lamellidens corrianus* after acute exposure to As^{3+} and during recovery

Treatment		Tissue	Exposure period		Recovery Period	
			24 h	96 h	2days	4days
Control (A)		Testis	0.016±0.006	0.016±0.002	—	—
		Digestive Glands	0.011±0.004	0.011±0.003	—	—
0.672 ppm As^{3+} (B)		Testis	1.026±0.051	1.044±0.037	—	
		Digestive Glands	1.089±0.023	1.119±0.016	—	—
After 96 h Exposure to 0.672 ppm As^{3+}	Normal Water (C)	Testis	—	—	1.010±0.021 [−3.256]	0.969±0.012 [−7.183]
		Digestive Glands	—	—	1.105±0.014 [−1.251]	1.067±0.024 [−4.647]
	Normal Water + 5mg/l Caffeine (D)	Testis	—	—	0.942±0.036 [−9.770] (− 6.732)	0.917±0.052 [−12.167] (−5.366)
		Digestive Glands	—	—	1.073±0.028 [−4.110] (−2.895)	0.975±0.011 [−12.868] (−10.032)

Values in the [ ] brackets indicate percent change over respective B of 96 h Values in the ( ) brackets indicate percent change over respective C

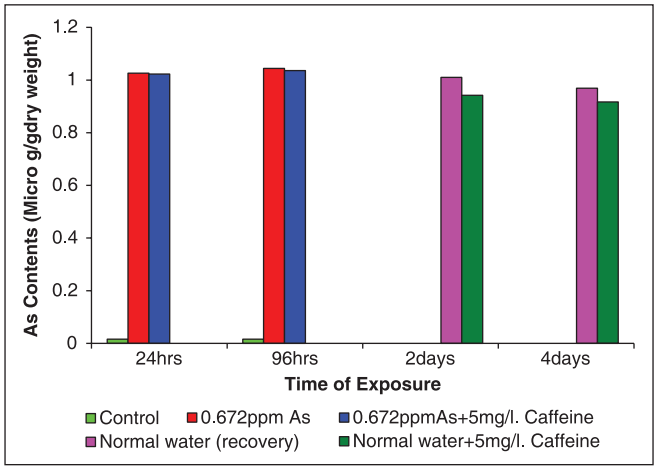

Figure 1a: Arsenic contents in testis of *L. corrrianus* after acute treatment and recovery

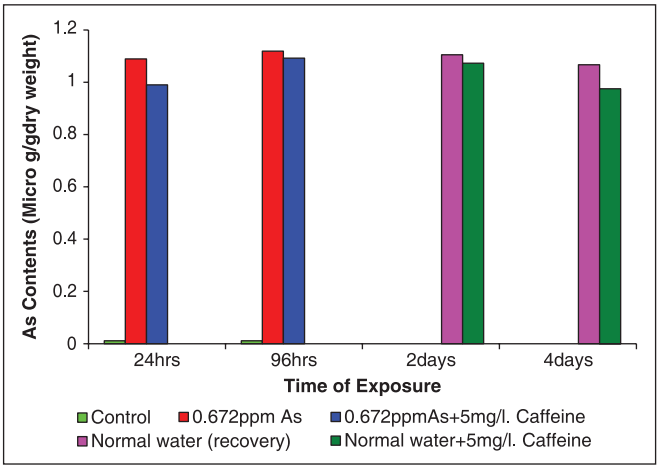

Figure 1b: Arsenic contents in digestive glands of *L. corrianus* after acute treatment and recovery

RESULTS

Bioaccumulation of arsenic in testis and digestive glands of *L. corrianus* exposed to arsenic (0.672 ppm As^{3+}) and during recovery has been summarized in the Table 1 and Figure 1a and b.

Bioaccumulation of arsenic

Testis

Bivalves from control group showed 0.016 μg/g arsenic in testis while the amount of accumulated as in presence of sodium arsenate (0.672 ppm As^{3+}) for 24 h was 1.026 μg/g and after 96 h was 1.044 μg/g. The *Bivalves* pre-exposed to as showed fast arsenic depletion with caffeine than those allowed to cure naturally. The amount of accumulated as observed after 2 days was 0.942 μg/g and after 4 days was 0.917 μg/g while in those *Bivalves* allowed to cure naturally, the amount of accumulated arsenic after 2 days was 1.010 μg/g and after 4 days was 0.969 μg/g.

Digestive glands

The control group of animals showed 0.011 μg/g arsenic in digestive gland. The amount of bioaccumulated as in presence of sodium arsenate (0.672 ppm As^{3+}) for 24 h was 1.089 μg/g and after 96 h was 1.119 μg/g. The *Bivalves* pre-exposed to sodium arsenate showed fast removal of As with Caffeine than those allowed to cure naturally. The amount of accumulated As observed after 2 days was 1.073 μg/g and after 4 days was 0.975 μg/g in caffeine exposed *Bivalves* and in those allowed to cure naturally, the amount of accumulated $As^{3+}$for 2 day was 1.105 μg/g and 4 days was 1.067 μg/g.

DISCUSSION

Heavy metal uptake and concentration in the food chain, especially those terminating in the human beings have renewed interest largely due to several instances of human intoxication.[29,30] The accumulation of metal in different species is the function of their respective membrane permeability and enzyme system.[31] Toxicity and bioaccumulation of arsenic and chromium in epigen and hypogen in freshwater macro-invertebrates tolerates, and higher values of Zn, Ni, Hg, Cr and Pb were observed in comparison to water in fish.[32,33]

Higher amount of As, Hg, Mn, Ni, Cr, Cd, Pb, Fe, Cu and Zn in the *Bivalves*, *Ruditapes decussatus* and *Ruditapes philippinarum* from the Atlantic coast of Southern Spain observed.[34] *Bivalves*, *Pinctadea radiata* and *Brachidontes pharaonis* were bioaccumulate fairly large amount of Cd, Fe, Zn, Cu.[35]

The ratio between bioaccumulation and exposure concentration with periods of exposure has been shown by various investigators.[36-40] The accumulation of several metals is due to the low capacity of these mollusks for discriminating among metals, which are similar in some characteristics such as ionic radius.[41,42] Mussels also possess a variety of effective detoxification mechanisms to reduce the toxicity of the metal uptake.[43-45]

Caffeine binds divalent cations of calcium, which indicates that caffeine can bind other reactive divalent cations of heavy metals. Calcium association with caffeine is an indicator of such binding.[46]

Suppressive effect of caffeine on hepatitis and apoptosis induced by tumor necrosis factor L studied.[47] The interactions between caffeine and metal ions can be through its oxygen and nitrogen atoms, Because of the blockage on N1, N3 and N7 atoms by methyl groups. Caffeine probably binds to metal ions through its oxygen atom of 2nd and 6th position. Caffeine would bind to metal ions through its second and sixth O atoms in the gaseous phase.[48]

Caffeine inhibited hepatocarcinogenesis induced by 2-acetylaminoflure showed.[49] Oral administration of

green tea and caffeine inhibits Ultra Violet-induced carcinogenesis.[50]

The binding capacities of caffeine with different micronutrients is weaker than that of the EDTA (Ethylene Diamine Tetra Acetic acid) and since all nitrogen groups are blocked by methylation, metals probably form complexes with second and sixth oxygen of caffeine.[28] Though the binding strength of caffeine with metal ions is weaker than that of EDTA, it is sufficient to drag the metal ions bound to the proteins because the metals are usually bound to –SH groups of proteins and the bond energy of the oxygen metal complex is stronger than sulphydril-metal bond. EDTA is very toxic as compared to the caffeine, and it removes essential micronutrients from the body.

The kidney manufacture and discharge up to 100% more urine on caffeine intake, investigation state the increased urinary excretion of calcium, magnesium, sodium and chloride after oral doses of caffeine.[51] The caffeine metal-chelate complex being of small molecular weight can easily pass through the membranes of the cells of the epithelia of the tubules of the kidney and can be effectively excreted out.

The bioaccumulation of arsenic in *L. corrianus* was studied. The arsenic accumulation increased with the increase in exposure period of acute concentration of arsenic (0.672 ppm As^{3+}). The amount of arsenic accumulation is less when exposed with 5 mg/l caffeine in presence of respective concentration of arsenic as compared to those exposed to only arsenic. The *L. corrianus* pre-exposed to arsenic (0.672 ppm As^{3+}) showed rapid removal of the arsenic in presence of caffeine than those maintained in normal water. Therefore, the caffeine (1,3,7-Trimethylxanthine) as a modulator of Arsenic bioaccumulation in the experimental *Pelecypod* model, *L. corrianus*.

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