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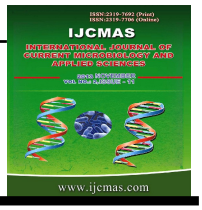

Original Research Article

Role of Caffeine (1, 3, 7-Trimethylxanthine) on Arsenic Induced Alterations of DNA Level in the Freshwater Bivalve, *Lamellidens corrianus* (Lea)

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Caffeine; arsenic; DNA; *Lamellidens corrianus.*

A B S T R A C T

The present study was carried out the probable role of caffeine (1, 3, 7-Trimethylxanthine) on arsenic induced alterations on the experimental freshwater bivalve, *Lamellidens corrianus.* The effect on bivalve was studied under five groups. A group bivalve was kept as control; B group bivalves were exposed to acute dose ($LC_{50/2}$) of sodium arsenate (0.672 ppm As^{+++}). C group bivalves were exposed to acute dose ($LC_{50/2}$) of sodium arsenate with caffeine (5 mg/L.). After 4 days bivalves from group B were divided into two groups D and E. D group bivalves pre exposed to acute dose ($LC_{50/2}$) of sodium arsenate were allowed to cure in normal water. E group bivalves pre exposed to acute dose ($LC_{50/2}$) of sodium arsenate were exposed to caffeine (5 mg./l) for recovery. From all five groups, some bivalves were removed from each group and their DNA content in selected tissues of bivalves was estimated. Results indicate that the DNA level was decreased due to arsenic and increased to caffeine in presence of arsenic. During recovery DNA content increased and the increased was higher with caffeine. Investigation concluded that the caffeine has the protective and curative role in DNA level caused due to the exposure to arsenic.

Introduction

Arsenic poisoning is difficult to diagnose. The great challenge is for removing arsenic from water and body of animals, and from global environment. Arsenic contamination has been found in many areas in West Bengal (India), Bangladesh and several other countries. The chemistry of arsenic in aquatic system is quite complicated, however, in ground water the arsenate ($H_3 AsO_4$, $H_2 AsO_4^{-1}$, $H AsO_4^{-2}$) and arsenite ($H_3 AsO_3$, $H_2 AsO_3^{-1}$, $HAsO_3^{-2}$) species are more predominant. Arsenic can be remediated by oxidation, coagulation, sedimentation, filtration, adsorption, ion change and reverse osmosis from biological materials studied

by Johnson and Heijnen, (2001). Coagulation involves the removal of colloidal (0.001 - 100μ) stable particles and co-precipitation occurs when arsenic forms an insoluble complex with coagulant. Lastly, it causes death.

Nucleic acid contents are considered as an index of capacity of an organism for protein synthesis. Different hormones and stress conditions may exert control over synthesis, activity and break down of nucleic acids. The nucleic acid contents can cause alterations in genetical information and genome functioning so it is important to investigate the levels of DNA and RNA periodically in different tissues of the organisms undergoing stress conditions.

The binding of metal ions and their complex to DNA or, more generally to nucleotides, there are several different coordination sites available. The metal centers can bind to the negatively charged oxygen atoms of (poly) phosphate group or the nitrogen and oxygen atoms of purine and those containing large JL systems as ligands should also be able to inter catalane between two base pairs, possibly even in a sequence specific fashion. Lastly, coordinated ligands with, for example, amine or hydroxyl functions may form hydrogen bonds with protein acceptor components of the polynucleotides.

Interactions of metal ions or metal complexes with nucleic acid play an important role:

1. In sustaining the conformation such as DNA or RNA through electrostatic effects,
2. In the nucleic acid metabolism particularly in phosphoryl transfer,
3. In the regulation, replication and transcription of genetic information,
4. For efforts directed at specific DNA cleavage with synthetic probes and
5. For metal induced mutagenesis.

Such mutations can be due to genometric distortions of the DNA through unphysiological cross linking or to the stabilization or a wrong nucleobase tautomer complex nucleic acid inter actions and their physiological consequences can thus be quite varied, even in the extensively studied platinum compounds they are far from being fully understood.

The platinum complexes act as anticancer agents by interacting with DNA and Trans platinum bind most strongly to RNA than to DNA and least strongly to proteins. When the activity was assessed as the ability to suppress the synthesis of DNA, RNA and protein, only the synthesis of DNA was suppressed.

The selectivity of platinum complexes in attacking tumor cells rather than normal cells, even through there is little or no preferential uptake of platinum in tumour cells, has led to the suggestion that cancer cells are deficient in some DNA repair mechanisms. DNA is constantly being damaged but various repair proteins can recognize the damaged segment and cause the repair. Platinum specifically binds DNA at the minor groove.

Arsenic is known to cause DNA damage and related events, such as DNA protein cross-links, micronuclei etc. (Schaumloffel and Gebel, 1998), DNA strand breaks, or alterations in DNA repair enzymes. Supper oxide scavengers such as Cu, Zn - SOD suppress arsenic induced DNA damage (Hartwing, 1998; Lynn *et al*., 1998).

Tong Lu *et al*. (2001) studied that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers as compared with those of controls, damage was also observed due to involved arsenic in the DNA of respective cells. Zhao *et al*., (1997) studied the association of arsenic induced malignant transformation with DNA hypomethylation and aberrant gene expression. Detmar and Andrea (1992) studied that cobalt is an essential trace element for mammalian nutrition, but also is classified as carcinogenic with the fidelity of DNA synthesis. Regarding anti and co-mutagenic mechanisms, the evidence for interference of Co (II) with DNA repair processes is known.

Heavy metals enter into the body of organism through the respiratory organs like gill, lung, etc. and through food and drinking water. It is hazardous to aquatic ecosystem and disturbs the food chain. This disturbed phenomenon has been expressed in the biochemical contents of tissues of animals.

Chelation therapy for metal ion toxicity has been reported by Sharma (1995). Caffeine molecule is having a site that usually binds divalent cations Ca^{++} and affects activity of Ca^{++} dependent enzymes. The binding constants of Ca^{2+} and Mg^{2+} with caffeine to be 29.8 and 22.4 M^{-1} respectively reported by Nafisi *et al*., (2002). Kolayly *et al*., (2004) found that the eight metal ions, CA^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+} and Cr^{2+} investigated, formed complexes with caffeine in varying capacities but these were very weak in strength when compared to EDTA. EDTA shows 10^{10} fold higher metal binding activity compared to caffeine.

Caffeine chelate with heavy metals can be easily excreted out by the biological system. Molecular weight of caffeine is 194.2 Daltons thus caffeine metal chelate will be having less than 500 Dalton molecular weight and hence it can easily pass through membranes. Therefore, caffeine can have the capacity to remove the heavy metals from the body of living organisms and prevent the damage of tissues and can indirectly save the life of living organisms. Secondly, it is known that caffeine increases the rate of urine formation.

Materials and Methods

Selected experimental model animals, the freshwater bivalve, *Lamellidens corrianus* were collected from the Paithan dam at Paithan Tq. Paithan. Dist. Aurangabad (M.S.).After collection, bivalves were acclimatized in the laboratory condition at room temperature for 2 - 3 days. The healthy and active acclimatized bivalves of approximately same size were selected for experiment.

Before starting the experiment, these bivalves were divided into three groups such as A, B and C.

(1) A group bivalves were kept as control,
(2) B group bivalves were exposed to acute dose ($LC_{50/2}$) of sodium arsenate (0.672 ppm As^{+++}).

(3) C group bivalves were exposed to acute dose ($LC_{50/2}$) of sodium arsenate with caffeine (5 mg/L.).

After 4 days bivalves from group B were divided into two groups D and E (4) D group bivalves pre exposed to acute dose ($LC_{50/2}$) of sodium arsenate were allowed to cure in normal dechlorinated water.

(5) E group bivalves pre exposed to acute dose ($LC_{50/2}$) sodium arsenate were exposed to caffeine (5 mg. /l).

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. Gills, testis and digestive glands from all five groups of bivalves were dried at 80^{o}C in an oven until constant weight was obtained. The dried powders of these different tissues of control and experimental animals were used for estimations of their DNA contents. DNA contents were estimated by Diphenylamine method (Schender, 1967).The results are presented in the Table as percent changes of three repeats and are expressed as percentage of dry weight. Standard deviation and student't' test of significance are calculated and expressed in respective Tables.

Result and Discussion

In the control bivalves, DNA content in the gills after 24 hrs was 1.218 and after 96 hrs was 1.220. In the bivalves treated with acute concentration of sodium arsenate (0.672 ppm As^{+++}), the DNA content was 1.031 and 0.854 respectively for 24 and 96 hrs of exposure periods while in the bivalves exposed to sodium arsenate with caffeine (5 mg/l), the DNA content was 1.135 and 0.972 respectively for 24 and 96 hrs of exposure periods.
During recovery from sodium arsenate intoxication, the DNA content was 0.865 and 0.931 in normal water after 2 and 4 days while in normal water with caffeine (5 mg/l), the values for corresponding periods were 0.952 and 1.127.

In the control bivalves, DNA content in the testis after 24 hrs was 2.41 and after 96 hrs were 2.3. In the bivalves treated with acute concentration of sodium arsenate (0.672 ppm As^{+++}), the DNA content was 1.624 and 1.236 respectively for 24 and 96 hrs of exposure periods. While in the bivalves exposed to sodium arsenate with caffeine (5 mg/l), the DNA content was 2.030 and 1.473 respectively for 24 and 96 hrs of exposure periods. During recovery from sodium arsenate intoxication, the DNA content was 1.315 and 1.437 in normal water after 2 and 4 days while in normal water with caffeine (5 mg/l), the values for corresponding periods were 1.624 and 1.977.

In the control bivalves, DNA content in the digestive glands after 24 hrs was 2.218 and after 96 hrs were 2.113. In the bivalves treated with acute concentration of sodium arsenate (0.672 ppm As^{+++}), the DNA content was 2.002 and 1.653 respectively for 24 and 96 hrs of exposure periods. While in the bivalves exposed to sodium arsenate with caffeine (5 mg/l), the DNA content was 2.083 and 1.961 respectively for 24 and 96 hrs of exposure periods. During recovery from sodium arsenate intoxication, the DNA content was 1.721 and 1.906 in normal water after 2 and 4 days while in normal water with caffeine (5 mg/l), the values for corresponding periods were 1.831 and 1.993.

Lastly, it was observed that after acute exposure to sodium arsenate, there was decrease in the level of DNA content in various tissues of experimental bivalves as compared to those of control bivalves. The DNA contents were higher in sodium arsenate with caffeine-exposed bivalves as compared to those exposed to sodium arsenate. The bivalves showed the faster rate of recovery of tissue DNA level in presence of caffeine than those allowed curing naturally.

Table.1 DNA content in selected tissues of *Lamellidens corrianus* after acute exposure to As^{+++} without and with caffeine and during recovery. (Values represent percentage in dry weight)

Treatment		Tissue	24 hrs	96 hrs	Recovery	
					2days	4days
Control (A)		Gills	1.218 ± 0.0389	1.220 ± 0.0471		
		Testis	2.410 ± 0.0582	2.300 ± 0.0354		
		Digestive Glands	2.218 ± 0.0589	2.113 ± 0.0892		
0.672 ppm As^{+++} (B)		Gills	1.031 ± 0.0710NS (-15.353)	0.854 ± 0.0913❖❖ (-30)		
		Testis	1.624 ±0.0600❖❖❖ (-32.614)	1.236 ±0.0693❖❖❖ (-46.260)		
		Digestive Glands	2.002 ± 0.0468❖❖ (-9.738)	1.653 ± 0.0663❖❖ (-21.769)		
0.672 ppm As^{+++}+ 5mg/lit. Caffeine (C)		Gills	1.135 ± 0.0345❖❖ (-6.814)	0.972 ± 0.0564❖❖ (-20.327)		
		Testis	2.030 ± 0.0613❖❖ (-15.767)	1.473 ±0.0375❖❖❖ (-35.956)		
		Digestive Glands	2.083 ± 0.0349❖ (-6.086)	1.961 ± 0.0368NS (-7.193)		
After 96hrs Exposure to 0.672 ppm As^{+++}	**Normal Water (D)**	Gills			0.865 ± 0.0473NS [+1.346]	0.931 ± 0.0552NS [+9.016]
		Testis			1.315 ± 0.0935NS [+6.391]	1.437 ± 0.0254■■ [+16.242]
		Digestive Glands			1.721 ± 0.0753NS [+4.113]	1.906 ± 0.0118■■ [+15.305]
	Normal Water + 5mg/lit. Caffeine (E)	Gills			0.952 ± 0.0364NS [+11.475]	1.127 ± 0.0571■ [+31.967]
		Testis			1.624 ± 0.0834■■ [+31.391]	1.977 ± 0.0332■■■ [+59.951]
		Digestive Glands			1.831 ± 0.0376■■ [+10.768]	1.993 ± 0.0482■■ [+20.568]

Values in the ( ) brackets indicate percent change over control
Values in the [ ] brackets indicate percent change over 96hrs of respective (B)
❖ - Compared with respective (A); ■ - Compared with respective 96hrs of (B); NS - Non Significant
❖/ ■ - P < 0.005, ❖❖/■■ - P < 0.01, ❖❖❖/■■■ - P < 0.001

Heavy metals are very harmful pollutants as they remain in the nature for a very long period. Uptake of heavy metals through food chain in human being may cause various physiological disorders like hypertension, sporadic fever, renal damage, liver cirrhosis etc.

DNA (Deoxyribose Nucleic Acid) contents can be the index of capacity of an organism for protein synthesis in the different stress conditions affected by heavy metals or any toxic metals or pesticides. Structural changes in the DNA can be monitored using biochemical methods and usually low quantitative changes are observed on heavy metals exposure. DNA strand scission can also be sensitively monitored, and even more importantly, the specific nucleotide position cleaved can be pin pointed by biochemical methods. This methodology has been applied successfully in monitoring both the efficiency of DNA strand scission by metal complexes and the specific sites cleaved, and where the complexes are specifically bound on the helical strand.

Tong Lu *et al*., (2001) observed that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers compared to controls. The differentially expressed genes induced those involved in cell cycle regulation, apoptosis, DNA damage response, and intermediate filaments. The observed gene alterations appear to be reflective of hepatic degenerative lesions seen in the arsenic exposed patients. This array analysis revealed important patterns of aberrant gene expression occurring with arsenic exposure in human liver. Aberrant expressions of several genes were consistent with the results of array analysis of chronic arsenic exposed mouse livers and chronic arsenic - transformed rat liver cells.

Rao *et al*., (1998) studied the effect of Fluoride toxicity on the nucleic acid contents of freshwater crab, *Barytelphusa cunicularis*. They observed that the level of DNA in muscles and hepatopancreas were found to be elevated initially and then a gradual decrease was noted in gills, testes and ovaries. The decreased levels of DNA were observed by Chaudhari *et al*., (1993) in *Thiara lineata* under various different toxic stresses.

Detoxification can be used as a beneficial curative measure and as a tool to increase overall health and vitality. Detoxification treatment has become one of the cornerstones of alternative medicine. Detoxification therapies are having increasing importance and popularity. Chelation therapy is the application of chelation techniques for the therapeutic or preventive effects of removing unwanted metal ions from the body combination therapy. Dimercaprol (Bal) is used for metal intoxication as a cheater to remove arsenic, mercury, cadmium and lead poisoning. EDTA is useful antidote in lead poisoning and lead encephalopathy. EDTA is administered intravenously or intramuscularly (Chelationtherapy, Journal of Envt. Engg. Vol. 127, No.3).

Mapengo, (1990) studied that the fusion temperatures of caffeine – 1, 3 - (CD3) 2, caffeine – 3, 7- (CD3) 2, Caffeine 1, 3, 7- (CD3) 3 were 0.4 - 1.7^{o}C) higher than for caffeine, indicating a higher degree of hydrogen bonding in the crystalline forms of these compounds. Plaskett and Cafarelli (2001) studied that caffeine has known exogenic effects, some of which have been observed to increase the T lymphocytes. The radio sensitizing effects

of caffeine is associated with the disruption of DNA damage responsive cell cycle checkpoints. The caffeine might inhibit one or more components in an ATM dependent Checkpoint medium pathway in DNA damaged cells. Caffeine inhibits the catalytic activity of ATM and the related kinase and DNA damage as was studied by Sarkaria *et al.,* (1999).

Harish *et al.,* (2000) observed that the effect of caffeine as α reflective DNA synthesis inhibitor or given as pre-inter and post treatments on the ethyl methano sulphonate (EMS) induced adaptive responses in vivo mouse bone marrows cells was studied in order to understand the influence of caffeine on the adaptive response. Matsumura *et al.,* (2000) studied that lack of Ca_2^+ and ATP dependent priming stage in caffeine induced exocytosis in bovine adrenal chromaffin cells in comparison with Ca^{++}. They suggested that the ATP requiring priming stage is lacking in the process of caffeine-induced exocytosis in bovine adrenal chromaffin cells.

Effect of caffeine and zinc on DNA and protein synthesis of neonatal rat cardiac mussel cell in culture was studied by Kanemaru *et al.,* (1992) and they found that the effect of caffeine (0.2^{-2} mM) inhibited both DNA and protein synthesis of the cells. Addition of EDTA in the growth medium inhibited both DNA and protein synthesis without caffeine and in the presence of lower concentration of caffeine (0.2 mM) in the growth medium, 10 micron of zinc concentration reversed DNA synthesis, which was inhibited by the chelating agent (EDTA).

Limited research has been reported on detoxification of heavy metals, DNA damage due to arsenic as well as caffeine as a chelator towards arsenic. Thus, the present investigation carried out and results indicate changes in DNA level of different tissues of *L. corrianus* on acute exposure to arsenic with and without caffeine and during recovery. It was observed that after acute exposure of arsenic, there was decrease in the level of DNA content in various tissues of experimental bivalves as compared to those of control bivalves. The DNA contents were higher in arsenic with caffeine-exposed bivalves as compared to those exposed to only arsenic. The bivalves showed the faster rate of recovery of DNA level in presence of caffeine than those allowed curing naturally. This investigation concluded that the caffeine has the protective and curative role in DNA levels caused due to the exposure to arsenic.

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