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Contribution of Biological Research for Sustainable Development

Chief Editor
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Assist. Prof. (Marathi)
MGV'S Arts & Commerce college,
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Caffeine On Arsenic Induced Alterations On The Acid Phosphatase Activity In *Lamellidens Corrianus* (Lea)

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ABSTRACT

During this investigation, group A bivalves, *Lamellidens corrianus* were maintained 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 each of five groups, their acid phosphatase activity in gills, testis and digestive glands of bivalves was estimated. The acid phosphatase activity in various tissues was found to be significantly increased after acute treatment by arsenic. The acid phosphatase activity in these tissues was least affected when exposed to arsenic with caffeine. During recovery, the bivalves showed drastic decrease in the acid phosphatase activity in presence of caffeine than those allowed to cure naturally. The results indicate the protective effect of caffeine on arsenic induced alterations.

KEYWORDS

Caffeine; arsenic; acid phosphatase; *Lamellidens corrianus*

INTRODUCTION

The studies of enzymes create special interest because it lies just on the borderline where biological and physical sciences met. On the other hand, enzymes are of supreme importance in biology. Life depends on complex network of chemical reactions brought about by specific enzymes, and any modification of the enzyme pattern may have far-reaching consequences for the living organisms. The mechanism of enzymes is itself one of the most fascinating fields of scientific investigation.

Heavy metals have high biological activity and have a tendency to accumulate in organisms, making adverse effects possibly at very low levels of exposure. Many enzymes are metalloenzymes, which need a specific metal as co-factor for its activity. Some non-target highly reactive metals can bind with these enzymes and thus reduce the activity of that enzyme. Secondly, some heavy metals bind at –SH groups of enzymes thus the tertiary structure of enzyme is affected and the enzyme looses its enzyme activity.

Acid phosphatase, a nonspecific monoesterase, pre-eminently regarded as the marker enzyme has been found in Golgi cisternae and lysosomes. The lysosomal enzymes undergo metabolic transformation in vivo resulting in change of substrate specificity [1]. Acid phosphatase enzyme helps in the metabolism and transphosphorylation [2]. The enhancement of acid phosphatase is quite conceivable in animals under morbidity. In crustaceans, the distribution of phosphatase and their activity in the haemolymph, hepatopancrease (Digestive Gland) [3], Cuticle [4, 5] and gastrolith walls [6], have been observed by histochemical procedures.

The impact of heavy metals and other biocides on tissue phosphatases activity of organisms are well documented but studies in relation to endotoxin toxicity or infection in animals are rather meager. The alterations in the acid phosphatase activity in various organs of snails, the intermediate hosts for trematodes have been reported by number of workers, [7-9].

Acid phosphatase is responsible for transphosphorylation and has an important role in the general energetic of an organism. Lysosomal enzymes and trematode infection in a snail, *Thiara tuberalata* (Muller) reported by [10] while Acid phosphatase activities in hepatopancreas of trematode on toxicant treatment to *Melania tuberculata* [11]. There is considerable amount of literature developed for the study of effect of heavy metals concerning the enzyme systems of various animals [12-14].

Molluscs have exhibited ability to adopt heavy metals or toxicants up to certain level in different types of habitats. The studies on the enzymes of lamellibrancs were first exhibited by in *Oyster* in India [15]. All enzymes are proteins in nature and they control sub cellular functions. In the metabolism of protein,

involvement of many enzymes, co-enzymes, intermediate proteins and amino acids are studied in many animals [16].

Heavy metal ions have affinity towards the different groups of the enzymes like suphydryl groups where they tightly bound, while the amino, hydroxyl, carbaryl, imidazole and phosphate groups where they showed loose binding. Thus, metal ions act as potent enzyme inhibitors [17-21] observed the inhibition of different enzymes after the exposure to heavy metals.

More recent research indicates that as compared to arsenite, trivalent methylated arsenic metabolites exert a number of unique biological effects, which are more cytotoxic, and genotoxic, and are more potent inhibitors of the activities of some enzymes [22,23] because each arsenic species (e.g. As (III), As (V), AsB, MMA^V, MMA^{III}) exhibits different toxicities. It may be important to take into account the fraction of total arsenic present in the inorganic and organic forms while estimating the potential risk posed to human health through the consumption of arsenic contaminated fish and shellfish.

Arsenic toxicity can cause a wide range of problems including severe injury to the body organs and the brain. To reduce the heavy metal load from the body, some chelating agents are used. Dimercaprol (BAC) is used for lead, arsenic and mercury toxicity and is given intramuscularly, Calcium disodium verasenate (CaNa2- EDTA) can be used in conjunction with BAL in lead toxicity, it never be used alone in treating lead toxicity because it chelates only extra cellular lead but lead is usually intracellular.

Caffeine being water soluble and common cheaper beverage, it will be cheapest preventive and curative medicine. Hepatocarcinogensis inhibition by caffeine in Agl rats treated with 2-acetybrminoflurened and has showed that caffeine inhibited hepatocarcinogensis induced by 2-acetylaminoflure [24]. 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-(Methylnitrosomino)-1-(3-pyridyl)-1-butonone (NNK) [25]. These agents can block the metabolic activation steps, scavenge the reactive intermediates or enhance the detoxification [26]. Any compound that can block the metabolic activation step, scavenge the reactive intermediate or enhance detoxification would be a potential chemo preventive agent [27].

Action of caffeine in altering the carcinogen activating and detoxifying enzymes in mice and reported the induction of xenobiotic detoxifying enzymes as an additional mechanism by which plant products may act as anticarcinogens, since, the induction of detoxifying enzymes is capable of competing with step in xenobiotic activation. Caffeine have been found to increase glutathione synthetase activity and reduced glutathione in liver and lungs of mouse [28].

Hence, an attempt has been made to study the effect of arsenic on an experimental model, the freshwater bivalve, *Lamellidens corrianus* with respect to changes in the levels of acid phosphatase enzyme activity and the preventive impact of caffeine on the alterations in the acid phosphatase activity.

MATERIALS AND METHODS

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 ($LC_{50/2}$) of sodium arsenate (0.672 ppm As^{+++}).
(3) C group bivalves were exposed to acute dose (0.9 ppm equivalent to 0.672 ppm As^{+++}) of sodium arsenate with 5 mg $caffeine^{-1}$.

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 $caffeine^{-1}$ of dechlorinated water.

The bivalves from A, B and C group were dissected after 24 hrs and 96 hrs and from D and E groups after 2 days and 4 days during recovery and gill, testis and digestive glands were separated. After removing these tissues from bivalves, 1% homogenate of each tissue was prepared in ice-cold citrate buffer of pH 4.9. The homogenate was centrifuged and supernatant was used to estimate the acid phosphatase activity.

Acid phosphatase activity was measured by the Gutman and Gutman method [29]. The activity was carried out in reaction mixture comprising of 1 ml (0.1 M) disodium phenyl phosphate, 2 ml citric acid buffer pH 4.9 and 0.5 ml tissue homogenate. The mixture was incubated at 37 ^{0}C for 1 hour. The reaction was inhibited by the addition of 1 ml of Folin and Ciocalteu's Phenol reagent and mixture was centrifuged at

2000 rpm for 10 minutes. To the supernatant 2 ml of 15% sodium carbonate was added. The blue colour complex developed was read at 660 nm. The blank readings were taken without incubation.
The calibration of standard curve was developed by using phenol as a standard. The activity was expressed as KA units/100gm/hr at 37°C at pH 4.9. Standard deviation and student't' test of significance are calculated and expressed in the Table.

RESULTS AND DISCUSSION

Effects of acute concentration of arsenic on acid phosphatase activity of gills, testis and digestive glands of *Lamellidens corrianus* without and with caffeine and after 2 and 4 days of recovery with and without caffeine are given in Table.

Table - Changes in the acid phosphatase activity in selected tissues of *Lamellidens corrianus* after acute exposure to As^{+++} without and with caffeine and during recovery. (Values represent KA units/100 gm of tissue/hr at 37 ^{0}C and pH 4.9)

Treatment		Tissue	24 hrs	96 hrs	Recovery 2 days	Recovery 4 days
(A) Control		Gills	2.433 ± 0.0742	2.521 ± 0.0693	-	-
		Testis	3.324 ± 0.0697	3.389 ± 0.0597	-	-
		Digestive Glands	3.821 ± 0.0571	3.844 ± 0.0762	-	-
(B) 0.672 ppm As^{+++}		Gills	2.617± 0.0632❖ (-7.562)	3.764±0.0451❖❖❖ (-49.305)	-	-
		Testis	3.491± 0.0525❖❖ (-5.024)	4.821± 0.0433❖❖❖ (-42.254)	-	-
		Digestive Glands	4.364±0.0453❖❖❖ (-14.210)	5.684± 0.0455❖❖❖ (-47.866)	-	-
(C) 0.672 ppm As^{+++}+ 5mg-l Caffeine		Gills	2.077± 0.0401❖❖ (-14.682)	3.053± 0.0311❖❖❖ (-21.102)	-	-
		Testis	3.156± 0.0375❖❖ (-17.403)	4.753± 0.0424❖❖❖ (-19.592)	-	-
		Digestive Glands	3.033±0.0309❖❖❖ (-20.622)	5.423± 0.0435❖❖❖ (-41.077)	-	-
After 96 hrs Expo-sure to 0.672 ppm As^{+++}	**(D) Normal Water**	Gills	-	-	3.521± 0.0633■■ [+6.455]	3.389± 0.0866■■ [+9.962]
		Testis	-	-	4.429± 0.0871■■ [+8.831]	3.763± 0.0451■■■ [+21.945]
		Digestive Glands	-	-	5.245±0.0655■■■ [+7.723]	4.869± 0.0875■■■ [+14.338]
	(E) Normal Water + 5mg-l Caffeine	Gills	-	-	2.827±0.0399■■■ [+24.893]	2.673± 0.0355■■■ [+28.985]
		Testis	-	-	4.263±0.0401■■■ [+11.574]	3.389± 0.0481■■■ [+29.703]
		Digestive Glands	-	-	5.157±0.0421■■■ [+9.271]	4.64 ± 0.0397■■■ [+18.121]

Legends- i) Values in the ( ) brackets indicate percent change over respective control, ii) Values in the [ ] brackets indicate percent change over 96 hrs respective (B), iii) ❖ - Compared with respective (A), iv) ■ - Compared with respective 96hrs of (B),v) ❖/ ■ - $P < 0.005$, vi) ❖❖/■■ - $P < 0.01$, vii) ❖❖❖/■■■ -$P < 0.001$

The data from the table reveals that after acute exposure to sodium arsenate (0.672 ppm As^{+++}), there was increase in the acid phosphatase activity in gills, testes and digestive glands of experimental bivalves, *Lamellidens corrianus* as compared to the control bivalves. The acid phosphatase activity was less affected in sodium arsenate with caffeine (5 mg/l) exposed bivalves than those exposed to only sodium arsenate. The bivalves showed faster recovery of tissues acid phosphatase activity in presence of caffeine than in normal water. Digestive glands being the main detoxification center in the bivalves, the impact of the arsenic was maximum on its acid phosphatase activity, while the testes were the least affected.

The acid phosphatase activity was marginally affected in arsenic with caffeine-exposed bivalves than those of only arsenic ones. The bivalves showed faster recovery of tissues acid phosphatase activity in presence of caffeine than the normal water.The arsenic enters in to the organisms either through the skin, gills or through the food and accumulates in the organisms. Acid phosphatase is responsible for transphosphorylation and has an important role in the general energetic of an organism. Decrease or increase in the enzyme activity represents the stress on any organism that results in metabolic burden [30].

In prawn *M. rosenbergii* inoculated with *Pseudomonas aeruginosa,* both acid phosphatases significantly increased in the haemolymph and body muscle and decreased in the hepatopancreas after 96 hrs of inoculums

treatment. Acid phosphatase, pre-eminently regarded as the marker enzyme, has been found in Golgi cisternae and lysosomes [31].
Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme, which was kept in a latent state inside the membrane of lysososmes, due to disruption of the membrane [32]. Sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatase [33]. Degradation and necrosis induced by toxicants in hepatopancreas cause release of acid phosphatase [34].

Acid phosphatase, a lysosomal enzyme, serves as a biochemical marker for specific androgen dependent steps in spermatogenesis and is very important for tissue reorganization and tissue repair. Acid phosphatase activity increased in sertoli cells with severe cell damage and in germ cells with heavy exfoliation [35]. Similar increase of acid phosphatase has been observed in organisms such as *Bubalis bubalis* exposed to Malathion [36]. The increase of acid phosphatase activity in the haemolymph and body muscle might be attributed to increased substrate pressure consequent to endotoxin stress. The increased acid phosphatase activity to alteration of pH in the tissue microenvironment consequent to toxicity stress mediated acid products of metabolism [37].
Generally, the increased activity of acid phosphatase has been attributed to the activation of the enzyme, which was stored in a latent state inside the membrane of lysosomes, due to disruption of the membrane [38]. The inflammatory changes by the inoculums of *Vibrio parahemdytcas* MTCC 451 on muscle might have caused disruption of lysosomes and enhanced the activity of acid phosphatase [39].

Caffeine exposure increased pulse duration and showed the inactivation of the Ca^{2+} current [40]. On administration of sodium selenite in combination with mercury, partially or totally alleviated the toxic effects of mercury on ACP and ALP in rat and concluded that selenium could be able to antagonize the toxic effects of mercury [41].

In present study, the main cause of increased acid phosphatase activity can be the increase in the necrotic regions due to the stress of the arsenic. Our study indicates much distortion and damage of the tissues on exposure to the arsenic. The caffeine binding with arsenic might have reduced the toxic effect of the arsenic. Secondly, the free reactive oxygen generated due to the arsenic could have reacted with the caffeine as it is an antioxidant and hence has reduced the severity of the tissue damage. The improvement in the enzyme level in the presence of the caffeine indicates reduction in the tissue necrosis.

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