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SYNERGISTIC PROTECTIVE ROLE OF CAFFEINE AND L-ASCORBIC ACID ON LEAD INDUCED ACID PHOSPHATASE PROFILE ALTERATIONS IN VARIOUS TISSUES OF *LEMELLIDENS CORRIANUS* (LEA)

Nawale S. P.

Department of Zoology, Walchand College of Arts and Science, Solapur. (M.S.) India.

E-mail- nawalesachin7@gmail.com

Abstract: **The present communication deals with individual and synergistic effectiveness of caffeine and L-ascorbic acid on acid phosphatase activity profile in lead induced toxicity in an experimental model, freshwater bivalve, Lamellidens corrianus. The effect on bivalve was studied under nine groups. From each treated and recovery groups, some bivalves were removed and enzyme activity in selected tissues of bivalves were estimated. The acid phosphatase activity was significantly increased on exposure to lead while the increase in presence of caffeine + ascorbic acid was less when exposed simultaneously than when exposed individually. During recovery acid phosphatase activity recovered and the rate of recovery was faster in caffeine + ascorbic acid exposed bivalves as compared to those recovered individually and in normal water. The probable role of the caffeine (1,3,7-Trimethylexanthine) and L-ascorbic acid is discussed in the paper.**

Key words: *Synergistic, Caffeine, L-Ascorbic acid, Lead, Acid phosphatase , Lamellidens corrianus*

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I INTRODUCTION

Pollution is not merely the addition of a substance to the aquatic environment, but its addition at rate faster than the environmental degradation can accommodate it. There are natural levels of chemicals such as arsenic and mercury in the environment but only if their levels exceed critical values, they can be considered as pollutants. Biochemical reactions proceeding in a cell are influenced by catalytic action of catalytic agents, termed as enzymes, which are unique biocatalysts. Enzymes accelerate the rate of chemical reaction without altering themselves and remain unaffected after overall changes. Shift in the biochemical and physiological features and histological atlas of the organ system is essential parameter which comes to our rescue in analyzing the state of health of the organisms in a stressed environment.

Bivalves are aquatic molluscs, which represents benthic fauna of fresh and marine water ecosystems. They have an inherent ability to act as sedentary filter feeder, absorb and accumulate metal ion in their tissues, providing information on the exert of contamination in aquatic environment Most of the enzymes play a vital role by various metabolic pathways. Altered pattern of enzymes activity, induced by heavy metals, is the surest indication of functional disorder. Hence, enzyme bioassay becomes useful technique in looking for sub lethal effects of toxic heavy metal pollution. The impact of heavy metals on aquatic as well as terrestrial ecosystem has been widely studied and well documented (Hatchinson and Whithy, 1974; Bonsova et.al., 1987). Metals are known to decrease the energy level by interfering with the metabolic pathway (Torreblanca et. al., 1992). Lead is most electropositive and fairly reactive heavy metal without any biological function (Silbergeld, 1992). Lead has been reported as an important contributor to health problem on its exposure (Bellows and Rudolph,1993). Goyer (1986) documented severe lead poisoning which affects haemopoietic, neuromuscular and urinary dysfunction. Constantly increasing lead level in the ecosystem due to

industrial waste, dyes, petrochemical industry and agriculture enhances the threat to aquatic organisms.

Vitamin C is an antioxidant vitamin. By this function, it helps prevent oxidation of water-soluble molecules that could otherwise create free radicals, which may generate cellular injury and disease. Chemically caffeine is 1, 3, 7-trimethylexanthine which have structural similarity with uric acid. Caffeine undergoes demethylation and oxidation in the body during metabolic biochemical processes. Caffeine acts on several organ systems as it has an ability to stimulate central nervous system Acid phosphatase is regarded as marker enzyme found in Golgi cisternae and lysosomes. Lysosomal enzymes undergo metabolic transformation in vivo resulting in change of substrate specificity (Ide and Fischman, 1969).

In present study, freshwater bivalve Lamellidens corrianus is used as test model to detect the role of caffeine and ascorbic acid individually and synergistically for the detoxification of lead. Acid phosphatase profile is studied as the indicators from different tissues. Reduction of toxicant reduces the stress and hence reduces level of stress effect. Protective and curative role of caffeine and ascorbic acid individually as well as synergistically was observed after heavy metal treatment and during recovery in experimental model L. corrianus.

Materials and Methods:

The freshwater bivalves, Lamellidens corrianus were collected from the Nathsagar dam at Paithan, Aurangabad (M.S.). 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. The effect on bivalve was studied under nine groups. Group A bivalves were maintained as control, B group bivalves were exposed to chronic dose (LC50/10) of Lead nitrate (6 ppm) for 20 days. Group C bivalves were exposed to respective chronic concentration of Lead nitrate along with caffeine (1mg/l), Group D bivalves were exposed to respective chronic concentration of Lead nitrate along with L-ascorbic acid (25 mg/L.). Group E bivalves were exposed to respective chronic concentration of Lead nitrate along with caffeine + ascorbic acid. Bivalves from group B were divided for recovery into four groups F, G, H and I after 20 day exposure to arsenic. F group bivalves were allowed to cure in normal water, G group bivalves were exposed to caffeine (1mg/l), H group bivalves were exposed to ascorbic acid (25 mg/L) for recovery while I group bivalves were exposed to caffeine (1mg/l) with ascorbic acid (25mg/l).

During treatment gills, gonads and digestive glands from each group bivalves were removed after 10 and 20 days. Similarly during recovery after 5 days and 10 days tissues were taken from recovery groups. Acid phosphatase activity was measured by the method of Gutman and Gutman (1940). The activity was carried out in reaction mixture comprising of 1ml (0.1M) Disodium phenyl phosphatase, 2ml of citric acid buffer pH 4.9 and 0.5ml tissue homogenate. The mixture was incubated at 37 0C to 1hr. the reaction was inhibited by the addition of 1ml of Folin Ciocaltaeus phenol reagent and mixture was centrifuged at 2000 rpm for 10 minutes. To the supernatant 2ml of 15% sodium carbonate was added. The blue colour complex developed was read at 660 nm. The blank readings were taken without incubation.

The caliberation of standard curve was developed by using phenol as a standard. The activity was expressed as KA units/100gm/hr at 37 0C at pH 4.9. Standard deviation and student “t” test of significance were calculated and expressed in respective tables.

Results and Discussion:

Heavy metals comprise important dangerous toxicants which encounters in various occupational and environmental circumstances. Many studies have been carried out on effect of heavy metal on marine and freshwater invertebrates and fishes. Toxic Heavy metals have the capability that severely alters the important biological structure and system in to irreversible conformation leading to deformity or death (Lucky et.al., 1977).

Acid phosphatase Enzyme actively involve in metabolic action and transphosphorylation. Acid phosphatase being a lysosomal enzyme hydrolyses phosphate esters in acidic medium (De duve, 1963). Lead can interfere with the cells ability to remove reactive oxygen species (ROS) and thus interfere with normal metabolism and vital cellular functions like electron transport chain (crucial to both mitochondrial and chloroplast function). Additionally, lead can decrease the number of mitochondrial cristae which in turn decreases oxidative phosphorylation and ATP synthesis. Synergistic effects of lead, cadmium and manganese are poorly understood (Hensley, 2004) Although zinc is a normal co-factor required in trace amounts to plant and animal systems, the effects of zinc in combination with lead are still under investigation.

L-ascorbic acid reduces the clastogenic effect generated by certain chemical agents in the vivo and in vitro assays (Khan et.al. 1996). Ascorbic acid is thought of excellent reducing agent which is able to serve as donor antioxidant in the free radical mediated oxidation processes and is able to reduce metal such as Cu and Fe.Caffeine protects the damage of tissues chemical and genetic material of organism from heavy metal generated free oxygen radicals.The decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson et. al., 1992).

Reduction in the enzyme activity in fishes was observed in response to heavy metals. Chandravathy and Reddy (1994, 1995) studied the effect of lead on Anabas scandens and found that there was decrease in the activity of lactate dehydrogenase and succinatedehydrogenase.

Zambare and Mahajan (2001) observed toxic effect of heavy metals on corbicula stritella and reported suppressed enzyme activity namely protease and lipase. Mahajan SS (2007) concluded that both induction and inhibition of phosphatase takes place depending on the concentration of metal. Ahmad et.al., (1997) investigated and reported decreased level in acid and alkaline phosphatase activity in muscle, hepatopancrease and haemolymph due to toxic effect of copper on S. serrata.

Table No. 1.1: Profiles of Acid phosphatase activity in gills of *L. corrianus* after chronic exposure to Lead nitrate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery (Values are in KA units/100gm tissue/hr at 37 ^{0}C)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		0.968 ±0.0484	1.097 ±0.0346		
Lead nitrate		1.921 ±0.0452*** (+98.45)	2.421 ±0.0787*** (+120.69)		
Lead nitrate + Caff		1.258 ±0.0562** (+29.95)	1.65 ±0.0546*** (+50.41)		
Lead nitrate + AA		1.174 ±0.0490** (+21.28)	1.454 ±0.0235*** (+32.54)		
Lead nitrate + Caff + AA		1.06 ±0.0423* (+9.50)	1.274 ±0.0355** (+16.13)		
After 20 days exposure to Lead nitrate	Normal Water			1.977 ±0.0342▪▪▪ [-18.33]	1.613 ±0.0511▪▪▪ [-33.37]
	Normal Water + Caff			1.629 ±0.0367▪▪▪ [-32.71]	1.423 ±0.0422▪▪▪ [-41.22]
	Normal Water + AA			1.577 ±0.0416▪▪▪ [-34.87]	1.162 ±0.0564▪▪▪ [-52.00]
	Normal Water + Caff + AA			1.338 ±0.0488▪▪▪ [-44.73]	1.129 ±0.0435▪▪▪ [-53.36]

Table No. 1.2: Profiles of Acid phosphatase activity in Gonad of *L. corrianus* after chronic exposure to Lead nitrate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery (Values are in KA units/100gm tissue/hr at 37 ^{0}C)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		0.677 ±0.0406	0.684 ±0.0598		
Lead nitrate		0.968 ±0.0571** (+42.98)	1.568 ±0.0507*** (+129.2)		
Lead nitrate + Caff		0.823 ±0.0299** (+21.56)	1.229 ±0.0476*** (+79.67)		
Lead nitrate + AA		0.793 ±0.0309** (+17.13)	1.162 ±0.0510*** (+69.88)		
Lead nitrate + Caff + AA		0.713 ±0.0341NS (+5.31)	0.629 ±0.0543NS (+8.04)		
After 20 days exposure to Lead nitrate	Normal Water			1.313 ±0.0489▪▪ [-16.26]	1.123 ±0.0566▪▪▪ [-28.38]
	Normal Water + Caff			1.128 ±0.0724▪▪▪ [-28.06]	1.068 ±0.0407▪▪▪ [-31.88]
	Normal Water + AA			1.089 ±0.0531▪▪▪ [-30.54]	1.025 ±0.0501▪▪▪ [-34.63]
	Normal Water + Caff + AA			0.957 ±0.0587▪▪▪ [-38.96]	0.877 ±0.0455▪▪▪ [-44.06]

Table No. 1.3: Profiles of Acid phosphatase activity in Digestive glands of *L. corrianus* after chronic exposure to Lead nitrate without and with caffeine, ascorbic acid, with caffeine + ascorbic acid and during recovery (Values are in KA units/100gm tissue/hr at 37 ^{0}C)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		0.957 ±0.0550	0.968 ±0.0498		
Lead nitrate		1.907 ±0.0298*** (+99.26)	2.677 ±0.0541*** (+179.7)		
Lead nitrate + Caff		1.774 ±0.0546*** (+85.37)	1.946 ±0.0410*** (+103.3)		
Lead nitrate + AA		1.621 ±0.0623*** (+69.38)	1.868 ±0.0309*** (+92.97)		
Lead nitrate + Caff + AA		1.307 ±0.0519*** (+36.57)	1.674 ±0.0435*** (+72.93)		
After 20 days exposure to Lead nitrate	Normal Water			2.497 ±0.0551▪ [-6.72]	2.243 ±0.0433▪▪▪ [-16.21]
	Normal Water + Caff			2.307 ±0.0399▪▪▪ [-13.82]	2.07 ±0.0654▪▪▪ [-22.67]
	Normal Water + AA			1.923 ±0.0612▪▪▪ [-28.16]	1.68 ±0.0540▪▪▪ [-37.24]
	Normal Water + Caff + AA			1.65 ±0.0463▪▪▪ [-38.36]	1.484 ±0.0422▪▪▪ [-44.56]

Lead nitrate = 6 ppm, Caff =1mg/l Caffeine, AA = 25mg/l Ascorbic acid

Values in ( ) indicate percent change over control

Values in [ ] indicates percent change over respective metal treated of 20 days

^{NS} - Non significant, *-compared with control, ▪- compared with respective metal treated of 20 days

*/▪- P< 0.005, **/▪▪-P< 0.001, ***/▪▪▪- P<0.01

Increased acid phosphatase activity suggested increased glycolysis during metal toxicity and enhanced breakdown of phosphatase to release energy in view of impaired ATPase system during metal stress (Reddy et.al., 1994). Phosphatase play an important role in carbohydrate metabolism (Goodman and Rothstein, 1957).Lead has several biochemical effects that interfere with many enzymes involved in the heme synthesis such as the erythrocytic delta-aminolevulinic acid dehydratase which, in this respect, is considered one of the best indicators of recent lead intoxication (Zielhuis, 1975; Hernberg, 1980; Tsuchiya, 1986). Wayker and Lomte, (2002) reported in protease activity in hepatopancrease of bivalve after pollutant treatment.

Chelation is a unique useful detoxification therapy for removal of toxicants from body of organism. Present observation clearly indicates that caffeine can act as a chelator in toxic stress condition. Caffeine intake increases pulse duration and showed inactivation of Ca2+ current (Hove-Madsen, 1999). Ascorbic acid prominently involve as chelator for reducing the heavy metal load in stressed metabolic condition. Ascorbic acid is a well known hydrophilic molecule; it is cheapest prophylactic and curative naturally available drug. On enzymatic level, role of ascorbic acid traced to hydroxylation, oxygenation and oxidation of corticosteroids (Chatterjee, 1967).

Hence, Present study was carried out to analyze effect of heavy metals lead and arsenic on enzyme ALP and ACP activity of of freshwater bivalve Lamellidens corrianus and to notice the chelating and antioxidant role of caffeine and ascorbic acid individually and in combination during recovery of bivalve.

CONCLUSION:

The present investigation concluded that caffeine and ascorbic acid individually have a capability to reduce stress effect of Lead nitrate. Synergistically caffeine with ascorbic acid has more efficient protective action against Lead toxicity. Also it was noticed that in combination they show accelerated curative rate than individual cure of animal stressed by Lead intoxication.

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