REVIEW OF RESEARCH
ISSN: 2249-894X
IMPACT FACTOR : 5.7631(UIF)
VOLUME - 11 | ISSUE - 7 | APRIL - 2022

CURATIVE ROLE OF CAFFEINE AND L-ASCORBIC ACID ON LEAD INDUCED ALKALINE PHOSPHATASE ENZYME ACTIVITY IN VARIOUS TISSUES OF *LEMELLIDENS CORRIANUS* (LEA)

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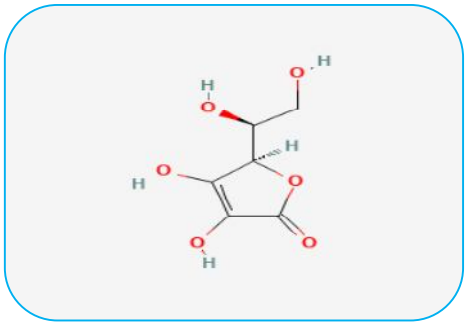
ABSTRACT:



The present communication deals with individual and synergistic effectiveness of caffeine and L-ascorbic acid on alkaline 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 alkaline 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 alkaline 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: *curative, caffeine, L-ascorbic acid, lead, alkaline phosphatase , Lamellidens corrianus.*

INTRODUCTION

Living cell is a cascade of number of biological reactions. These 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. Toxicants in the environment may exert stress; it may also create a state of exhaustion, if the stress is too severe or long lasting. 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 (Raungwises and Raungwises,1998). This unique property has qualified them to recognize as water pollution indicator (Perez *et.al.,* 2001).

Heavy metals disrupt a vast array of metabolic processes. Heavy metals alter pro-oxidant/antioxidant balance and bind to free sulfhydryl groups, resulting in inhibition of glutathione metabolism, numerous enzymes and hormone functions. Nutritionally, heavy metals are directly

antagonistic to essential trace elements and compete with nutrient elements for binding sites on transport and storage proteins, metalloenzymes and receptors.

Lead is well known for their direct, destructive effects on neuronal function and direct adverse effects on cellular processes. Lead poisoning is another serious source of environmental pollution which causes water pollution. Pb poisoning is characterized by CNS damage, anemia and deposition of Pb in bones and teeth. The major sources of this pollutant are paint manufacturing industries/factories, lead smelting works; petrol engines discharged inorganic Pb salts, metallic Pb and organic Pb respectively. Pb $(C_2H_4)_4$ is used as an anti knock in petrol engines and is a pollutant

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

All enzymes are protein in nature and they control sub cellular function and accelerate the rate of metabolic action in the body of organism. Alkaline phosphatase is known to be involved in mineralization, possibly through hydrolysis of organic phosphatase to raise the product (Ca^{+}) and (iP) to a level where precipitation occurs. Moog (1945) first studied the functional important role of alkaline phosphatase. Alkaline phosphatases from different sources exhibit three types of activity; hydrolytic, phosphotransferase and pyrophosphatase.

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. Alkaline 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 ($LC_{50/10}$) 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. Alkaline phosphatase activity was measured by the method of King (1951). The reaction mixture consisted of 1ml (0.01M) Disodium phenyl phosphate, 2ml carbonate-bicarbonate buffer of pH 10 and 0.5 ml tissue homogenate. It was incubated at 37 ^{0}C for 1 hr. The reaction was terminated by the addition of 1ml of Folin ciocaltaeu phenol reagent and centrifuged at 2000 rpm for 10 min. To the supernatant, 2ml of 15% sodium carbonate was added. The blue colour complex developed was read at 660 nm. The blank readings were obtained without incubation. The initial reading of the reaction before incubation was subtracted from the final reading after the enzyme activity of the incubation.

The caliberation of standard curve was developed by using phenol as a standard. The activity of alkaline phosphatase enzyme was expressed as KA units/100gm/hr at 37 at pH 10 (K.A. unit = King-Armstrong unit).

RESULTS AND DISCUSSION:

According to Burton *et.al.,* (1972) and Alam and Lomte (1984) heavy metals like mercury, arsenic, lead, and cadmium are mostly non essential elements. Fenvalerate induced alterations in the activities of acid and alkaline phosphatases were quantified in the catfish *Heteropneustes fossilis.* The fish, exposed to different graded concentrations of fenvalerate for 30 d, elucidated an elevation in the activity of acid phosphatase, and inhibition in the activity of alkaline phosphatase in muscle, liver and kidney (Johal *et.al.,* 2003). The alkaline phosphatase activity is quite conceivable in animals and in crustaceans under morbidity. The distribution of alkaline phosphatase enzymes and their activity in haemolymph (Hernberg, 1980), digestive gland (Vallee BL and Ulmer DD, 1972), cuticle (Travis, 1957, 1966) and gastrolith wall (Travis, 1963) have been observed by histochemical techniques.

L-ascorbic acid reduces the clastogenic effect generated by certain chemical agents in the vivo and in vitro assays (Khan *et.al.* 1996). According to Buttner and Jurkiewicz (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.

Table No. 1.1: Profiles of Alkaline 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		2.125±0.0456	2.13±0.0331		
Lead nitrate		2.912±0.0518***(+37.03)	3.675±0.0455***(+72.53)		
Lead nitrate + Caff		2.625±0.501***(+23.52)	3.35±0.0433***(+57.27)		
Lead nitrate + AA		2.496±0.0434***(+17.45)	3.017±0.0572***(+41.64)		
Lead nitrate + Caff +AA		2.325±0.0591**(+9.41)	2.812±0.0347***(+32.01)		
After 20 days exposure to Lead nitrate	Normal Water			3.425±0.0899▪▪[-6.80]	3.175±0.0633▪▪▪[-13.60]
	Normal Water + Caff			3.221±0.0788▪▪▪[-12.35]	2.962±0.0592▪▪▪[-19.40]
	Normal Water + AA			3.13±0.0434▪▪▪[-14.82]	2.862±0.0487▪▪▪[-22.12]
	Normal Water + Caff + AA			2.925±0.0435▪▪▪[-20.40]	2.775±0.0467▪▪▪[-24.48]

Table No. 1.2: Profiles of Alkaline 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		1.775 ±0.1034	1.87 ±0.0918		
Lead nitrate		3.35 ±0.0819***(+88.73)	4.162 ±0.106***(+122.5)		
Lead nitrate + Caff		3.195 ±0.110***(+80.0)	3.55 ±0.0879***(+89.83)		
Lead nitrate + AA		3.362 ±0.176***(+89.40)	3.625 ±0.0927**(+93.85)		
Lead nitrate + Caff +AA		2.812 ±0.0981***(+58.42)	3.25 ±0.168***(+73.79)		
After 20 days exposure to Lead nitrate	Normal Water			3.98 ±0.0818NS[-4.37]	3.45 ±0.120▪▪▪[-17.10]
	Normal Water + Caff			3.55 ±0.0824▪▪▪[-14.70]	3.29 ±0.137▪▪▪[-20.95]
	Normal Water + AA			3.45 ±0.0912▪▪▪[-17.10]	3.14 ±0.0971▪▪▪[-24.55]
	Normal Water + Caff + AA			3.041 ±0.118▪▪▪[-26.93]	2.775 ±0.131▪▪▪[-33.32]

Table No. 1.3: Profiles of Alkaline 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		2.425 ±0.0893	2.53 ±0.0919		
Lead nitrate		3.512 ±0.0899***(+44.82)	4.725±0.112***(+86.75)		
Lead nitrate + Caff		3.158 ±0.154***(+30.22)	4.245 ±0.109***(+67.78)		
Lead nitrate + AA		3.035 ±0.104***(+41.48)	3.85 ±0.145***(+52.17)		
Lead nitrate + Caff +AA		2.895 ±0.136**(+19.38)	3.55 ±0.0659***(+40.31)		
After 20 days exposure to Lead nitrate	Normal Water			4.305 ±0.112▪▪[-8.88]	4.125 ±0.133▪▪[-12.69]
	Normal Water + Caff			3.927 ±0.102▪▪▪[-16.88]	3.55 ±0.144▪▪▪[-24.86]
	Normal Water + AA			3.875 ±0.172▪▪[-17.98]	3.225 ±0.0997▪▪▪[-31.74]
	Normal Water + Caff + AA			3.362 ±0.234▪▪▪[-28.84]	3.125 ±0.187▪▪▪[-33.86]

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**

Caffeine protects the damage of tissues chemical and genetic material of organism from heavy metal generated free oxygen radicals. MSH calcium ion release channel protein due to caffeine stimulation of hyperthermia susceptible sarcoplasmic reticulum was reported by Shomer (1994). This antioxidant property of caffeine can prevent the tissue damage, also the alteration in metabolic and biochemical reactions in the body and damage of genetic material of organism from the heavy metal generated free radicals.

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 Ca^{2+} 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). 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)

Zambare and Mahajan (2001) observed toxic effect of heavy metals on *corbicula stritella* and reported suppressed enzyme activity namely protease and lipase. 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.

Norseth, (1967) reported decrease in acid phosphatase activity was due to accumulation of mercury in the lysosomes and blockage in the release of enzymes. Generally, the increased activity of acid phosphatase was attributed to the activation of the enzymes, which was kept in a latent state inside the membrane of lysosome, due to disruption of the membrane (De duve *et.al.*, 1955).There might be multiple causes of death due to heavy metal poisoning depending on mainly time and concentration combination. However no clear cut explanation regarding mode of action of various heavy metal causing the mortality of aquatic animal. Heavy metals are reported disrupt enzyme system (Gould and Krolus, 1974, Jakim *et.al.*, 1970; Gopal and Balapermeswara Rao, 1985) and also noticed that responsible for damage to the tissues and organ (Gardner and Yevich, 1970). Bedse and Karyakarte

(1995) observed that increase in acid phosphatase and alkaline phosphatase activity in snail *Melenia tuberculata* infected with *cercaria beglanensis*, also reported that degree of enzyme activity correlated with degree of infection

Poisoning of an enzyme system depends on its capacity to react with legends. The living cells are centers of majority of enzyme canalization reaction. Alteration enzyme occurs in cell functioning due to toxic substances. Enzymes leak through membrane in to circulating fluid, some of cytoplasm enzymes leaked out of tissue from damaged cells resulting decreased enzyme activities in tissues and corresponding increase in the fluid, which ultimately results in quantitative altered enzyme activity. Thus, by diagnostic means, enzyme bioassay support to asses change or injury to organism due to pollutant exposure. In clinical serum enzyme analysis used to diagnose both the site and exert of organ injury (Schmidt and Schmidt 1976). Wayker and Lomte, (2002) reported in protease activity in hepatopancrease of bivalve after pollutant treatment.

Hence, Present study was carried out to analyze effect of heavy metals lead and arsenic on enzyme alkaline phosphatase 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.

The alkaline phosphatase activities in all tissues were found to be significantly increased after chronic treatment of heavy metal salt as compared to the control. After 20 days exposure to heavy metal salt, the bivalve's showed faster loss in alkaline activity during recovery in gills, gonad and digestive gland with synergistic effect of caffeine and ascorbic acid than the bivalves recovered with caffeine or ascorbic acid individually; The result profiles indicate the protective and curative role of caffeine and ascorbic acid, individually and in synergistic way on heavy metal induced alterations. The increase in the levels of alkaline activities suggests the damage of the tissue and rapid loss of the alkaline activity in caffeine and ascorbic acid during the recovery indicates rapid recovery of the damaged tissue.

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