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Protective Role of Caffeine (1, 3, 7-Trimethylexanthine on Lead Induced Alterations in Protein Content of Different Tissues of Fresh Water Bivalve, *Lemellidens corrianus* (Lea)

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ABSTRACT: The present communication deals with effectiveness of caffeine (1,3,7-Trimethylexanthine) in lead induced toxicity in an experimental model, the freshwater bivalve, *Lamellidens corrianus*. The effect on bivalve was studied under five groups. Group A bivalves were maintained as control, B group bivalves were exposed to chronic dose ($LC_{50/10}$) of lead nitrate (6.81 ppm) for 20 days. Group C bivalves were exposed to respective chronic concentration of lead nitrate along with caffeine (5mg/l). Protein contents in selected tissues from each group were estimated after 10 and 20 days. Bivalves from group B were divided for recovery into two groups D and E after 20 day exposure to lead. D group bivalves were allowed to cure in normal water, E group bivalves were exposed to caffeine (5mg/l) up to the 9 days. From each of recovery groups, some bivalves were removed and protein contents in selected tissues of bivalves were estimated after 3, 6 and 9 days. The protein level was significantly decreased on exposure to lead while the decrease in presence of caffeine was less when exposed simultaneously than when exposed individually. During recovery protein contents recovered and the rate of recovery was faster in caffeine exposed bivalves as compared to those recovered in normal water. The probable role of the caffeine (1,3,7-Trimethylexanthine) is discussed in the paper.

Key words: caffeine, protein, *Lamellidens corrianus*

INTRODUCTION

Heavy metals are one of the most common forms of anthropogenic pollutants in the aquatic environment. Industrial wastes are generally loaded with these toxic substances. Most toxic metals are lead, mercury, cadmium, zinc, arsenic and copper which enters the environment and deteriorates water quality. It has been observed that heavy metals can cause biochemical effect, such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer (Underwood, 1971; Zemansky, 1974; Lucky & Venugopal, 1977). Heavy metals mainly react with proteins and adversely alter the physiological activities hence cause risk of life in different way. Lead is well known severe environmental pollutant, considering as furtive villain (Raghuram, 2000). Protein acts as enzyme, antibody, hormone and basic structural component of the animal. Protein is key substance to show the effect of heavy metal. Proteins respond to stress condition for better survival by altering their levels.

The trace metals are known to be non bio-degradable and highly toxic to most organisms (Kaoud and Dahshan, 2010).

Detoxification has also become a prominent treatment as people have become more aware of environmental pollution. Dimercaprol (BAL), Calcium EDTA, penicillamine etc. are used in metal intoxication as chelators to remove As, Hg, Pb, and Cd poisoning. There are number of chelators used for the remediation of metal toxicity. Chelators are particular substances that bind to heavy metal and speed their elimination (Hammand, 1971; Graziano *et.al*.,, 1985**).** Antioxidant plays a protective role in the treatment of lead poisoning (Gurer *et.al*, 2001**).**

Caffeine is found to have antioxidant activity. Caffeine being water soluble and common cheaper beverage, caffeine will be cheapest preventive and curative medicine. The protective action of caffeine from damage of tissues biomolecules and genetic material due to heavy metal generated free oxygen radicals might be because of its antioxidant property (Mahajan,2005)

Though there are known chelators to remove the toxic heavy metals, they are not usually practiced unless heavy doses are taken. These chelators are also having the toxic effects. It is therefore necessary to know the remedy which is in the diet of regular food as the heavy metals also enters in the body in small doses regularly. There immediate removal will be helpful in the present scenario to protect the body. The present work was carried out to study the protective as well as curative role of caffeine individually on chronic exposure of lead nitrate and during recovery on the experimental model, *Lamellidens corrianus*.

MATERIALS AND METHODS

The freshwater bivalves, *Lamellidens corrianus* were collected from the Nathsagar 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. These bivalves were divided in to five groups and were treated as follows.

First group was maintained as Control. In second group the bivalves were exposed separately to chronic doses (LC50values of 96 hr/10) of Lead nitrate (6.81 ppm) up to the 20 days. Third group bivalves were exposed separately to chronic concentration of Lead along with of caffeine (5mg/l) up to the20 days.

After 20 days exposure to lead nitrate, bivalves from group B were divided in to two subgroups as D and E for recovery studies. Bivalves pre-exposed to chronic doses of lead nitrate were allowed for self cure in normal water while the bivalves of group E were exposed to5mg/1caffeine up to 9 days.

The experimental bivalves from A to C groups were dissected after 10 days and 20 days and from each recovery group (E and D) were collected after 3,6 and 9 days. The digestive glands and testis, from all experimental and recovery group were dried at 80 0C in an oven until constant weight was obtained. The dried powders of these tissues of control, experimental and recovery group animals were used for estimation of their protein contents. Total protein was estimated by Lowry's method (Lowry *et. al.*, 1951) using bovine serum albumin as standard from each powder. The average results of three repeats are presented in the table No. 1 and are expressed as percentage of dry weight. Percent variations, standard deviation, and "t" test of significance were calculated and are expressed in respective tables.

RESULTS AND DISCUSSION

Table 1: Protein content in Testis of *Lamellidens corrianus* (Lea) after chronic exposure to Lead nitrate without and with Caffeine and during recovery. (Values are in percent of dry weight) Values in ( ) indicate percent change over control.

Treatment		**10 days**	**20 days**	**Recovery**		
				3 Days	**6 days**	**9 days**
Control		33.65 ±2.2203	34.48 ±2.2203			
Lead		27.09±2.2181NS (-16.64)	22.16 ±2.8653* (-35.73)			
Lead +Caff		29.16 ±2.2181NS (-13.34)	27.0 ±1.8 110NS (-15.37)			
After 20 days exposure to 6.81 ppm Lead nitrate	Normal Water			22.63 ±1.8110 NS [+2.21]	21.55 ±2.2203 NS [+28.83]	32.40 ±2.2196* [+46.20]
	Normal Water + 5 mg/L. Caffeine			24.63 ±2.4819 NS [+11.14]	27.5 2 ±2.2203 NS [+24.18]	31.29 ±2.2196* [+41.20]

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

Table 2: Protein content in Hepatopancreas of *Lamellidens corrianus* (Lea) after chronic exposure to Lead nitrate without and with caffeine during recovery.(Values are in percent of dry weight).

Treatment		**10 days**	**20 days**	**Recovery**		
				3 Days	**6 days**	**9 days**
Control		9.55 ±2.86	32.01 ±2.8635			
Lead		24.63 ±2.5612 NS (-16.64)	19.70 ±2.2181* (-38.45)			
Lead + Caff		27.0 ±2.218 1NS (-8.32)	29.55 ±2.8600NS (-7.68)			
After 20 days exposure to 6.81 ppm Lead nitrate	Normal Water			22.16±1.8110 NS [+12.48]	26.55±2.2181NS [+34.77]	31.48±1.5684** [+54.72]
	Normal Water + 5 mg/L. Caffeine			25.40 ±1.8179NS [+18.78]	36.33 ±2.2181* [+33.65]	26.26±2.2203** [43.45]

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

Table 1 and 2 clearly indicates that, after chronic exposure of lead nitrate (6 .81ppm), there was high depletion in protein level in gonads and digestive glands of *Lamellidens corrianus* as compared to respective control. The protein content in tissues of bivalves exposed to lead with caffeine showed least decrease in protein content. During recovery after 20 days lead exposure, bivalves showed faster recovery in caffeine, which is fast than those bivalves which are allowed to cure naturally in normal water.

Pollutants comprising heavy metals may alter cellular functions, ultimately affecting physiological and biochemical mechanism of animals (Radhakrishnan *et.al*.1991). during acute and chronic heavy metal stress depletion of protein level was observed in gonads and digestive gland.

Andhale and Zambare (2011), studied the nickel induced biochemical alterations in freshwater bivalve, *Lammellidens marginalis* and reported that the protein contents were decreased in treated animals than the control. In the present study ascorbic acid recovered the total protein content and it play important role as detoxication of nickel which recovered the protein content.

The depletion in protein level during heavy metal exposure might be due to increase in protein catabolism to overcome against the stress of lead. The heavy metals bind with the DNA and cause the DNA damage, also the RNA polymerase are inactivated by heavy metals. These facts can reduce the rate of transcription and hence, the rate of the protein synthesis (Mahajan, 2006).

Heavy metal, mercury affects DNA, contracts the chromatin and disturbs the protein synthesizing machinery of the cell resulting in to the decreased enzyme synthesis in hepatopancrease (Zambare and Mahajan 2001).

Rao *et al.* (1994) recorded that the content of sperm protein in cauda epididymis reduced significantly on exposure to mercury for 60 days. Rao et al. (1987) found decrease in protein levels in the hepatopancreas of *Indonaia caerules* on exposure to fluorides. Mohanty *et.al*., (2005) analyzed and compared protein profile from gill, foot, and mantle

of two freshwater bivalves, *L. corrianus* and *L. marginalis* and found protein markers which helps to study the molluscan taxonomy. Decrease in protein content in mantle, foot, gill, gonad and hepatopancrease of bivalve, after exposure to $HgCl_2$ and $CuSo_4$ treated animal might be due to alteration of membrane permeability (Abel, 1975).

Caffeine has capacity to bind with heavy metals. Heavy metal content of water was reduced after addition of coffee. Caffeine binds divalent cations of calcium in Ferrete ventricular muscle (Leoty *et..al*., 2001).

Dissolved heavy metal ions are positively charged and caffeine contains uncharged and negatively charged molecules. Metal ions might bind to negatively charged groups. This reduces the charged active heavy metal ions which indicates that caffeine have capacity to remove the heavy metal from the living organism. Gandhi and Khanduja, (1992) studied action of caffeine in altering the carcinogenic activating and detoxifying enzymes in mice. Caffeine has been found to increase glutathione synthetase activity in liver and lungs of mouse

The present investigation concluded that caffeine have a capability to reduce stress effect of lead nitrate. Caffeine has more efficient protective action against lead toxicity. Also it was noticed that they show accelerated curative rate than individual cure of animal stressed by lead intoxication.

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