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CAFFEINE (1,3,7-TRIMETHYLAXANTHINE) AS A MODULATOR OF LEAD BIOACUMULATION IN THE EXPERIMENTAL MUSSELS MODEL, *LAMELLIDENS CORRIANUS*

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ABSTRACT

The present study was carried out to study modulator effect of caffeine (1,3,7-Trimethylxanthine) on Lead induced alterations on freshwater mussels, *Lamellidens corrianus.*The Freshwater mussels, *L. corrianus* were exposed to chronic $LC_{50/10}$ dose of Lead nitrate (6.321 ppm) for 12 days.After 12days Lead exposed mussels were allowed to cure naturally and with caffeine. The digestive glands and gills from different groups were removed and dried in the oven. The dry powders were digested in nitric acid and perchloric acid in 4:1 ratio at hot temperature till dryness. The digest was dissolved in double glass distilled water and the lead and cadmium contents were estimated by the Atomic Absorption Spectrophotometer.After 12 days of exposure, the amount of bioaccumulated lead in the digestive glands and gills was 750 to 1.230 and 489 to 894 µgm/Kg respectively. 568 to 932 and 415 to 675 µgm/Kg in Pb in with caffeine. During the recovery after 12 days, the bioaccumulated lead was reduced to 1.057 to 835 and 728 to 518 µgm/Kg of normal water while in caffeine exposed mussels was reduced to 950 to 680 and 635 to 494 µgm/Kg in digestive glands and gills respectively. Decrease in the lead contents in caffeine exposed mussels indicates the role of caffeine in lead excretion. Therefore, the caffeine (1, 3, 7-Trimethylxanthine) as a modulator of lead bioaccumulation in the experimental mussels model, *L. Corrianus*

KEYWORDS: Bioaccumulation, lead, Caffeine and Lamellidens corrianus.

INTRODUCTION

The presence of toxic metals poses environmental problems due to their non-degradable and persistent nature (Sarabject and Dinesh, 2007). The pollution of aquatic environment by heavy metals affects aquatic biota posse's considerable environmental risks and concerns (Amisah *et al.*, 2009). Many aquatic organisms have the ability to accumulate and biomagnify metals (Davies et al., 2006), which leads to concentrations several orders of magnitude higher than those of the surrounding water (Casas et al., 2008). Usually, the level of pollutant accumulated in such organism's tissues is used for assessing the level of pollution in its habitat (Abdallah and Moustafa, 2002). These mussels are useful indicators of the abundance and spatial distribution of metals in aquatic ecosystem. (Doherty et al, 1993; Oertel, 1998 and Sures et al,1999).

Lead and Cadmium are non essential elements that accumulatein tissues of living organisms, causing toxicity problems to local biota and to man (Forstner and Wittman, 1983).

Lead (pb) is a heavy metal which occurs naturally or result from industrial contamination, or be leached from lead pipes in some water systems. Lead is widely used in paint industry, pigments, dyes, electrical components, plastic chemicals and in various other industries (Hodson *et. al;* 1984). Ibrahim and Mat (1995) shown that the total metal content is directly proportional to the body weight. The body burdens of metals in most bivalves have been used to identify and map areas with exceedingly high levels of trace metals and organic pollutants; hence they can be used as biomonitors for aquatic environment. In order to use the bivalve as bioindicators in pollutionmonitoring programmes, there is a need to develop a bioaccumulation database using various bivalve species.

Many studies have attracted considerable attention due to the antioxidant, anticancer properties and health benefits of tea. However, the caffeine may be caused a higher risk of developing bone problems, including osteoporosis, problems in metal absorption, excretion. It is also caused reabsorption processes in intestines, kidney, iron deficiency animia specially for people consuming high

amounts of caffeine (Borse et al, 2002; Chen and Whitford, 1999 and Pan et al, 2003). A study done in Glasgow, employing whole coffee fruit extracts and subsequent characterization of its caffeine components showed the presence of caffeoylquinic acids, feruloylquinic acids, dicaffeoylquinic acids, caffeoylferuloylquinic acids etc. along with a methyl ester of 5-caffeoylquinic acid. These extracts also demonstrated some antioxidant activity (Mullen *et. al;* 2011).

The removal of toxic metal through chelating allows the body functions at an optimal level. Dissolved heavy metal ions are positively charged, caffeine contains uncharged or negatively charged molecules, and the metal ions can be taken out of solution by binding to negatively charged molecules in the coffee which indicates that caffeine can have the capacity to bind the heavy metals from living organisms. According to investigator, oxygen from 2^{nd} and 6^{th} position of caffeine forms chelate with heavy metal (Kolayly et.al, 2004). Therefore, it is proposed to study the efficency of caffeine in the detoxification of lead and cadmium with respect to their tendency of bioaccumulation in an experimental animal model, the freshwater mussels, *Lamellidens corrianus.*

MATERIALS AND METHODS

The mussels, *Lamellidens corrianus* were acclimatized to laboratory condition for 2-3 days and healthy active mussels of approximately medium size and weight were chosen. These mussels were divided into three groups A, B and C. The group A were maintained as control. The mussels from group B were exposed to chronic concentration (LC 50 value of 96 hr/10) of heavy metal, Lead nitrate (6. 321 ppm) while mussels from group C were exposed to chronic concentraction of Lead nitrate with 5mg/lit. caffeine up to 12 days. During experimentation mussels were fed on fresh water algae. The digestive glands and gills of mussels from all groups were collected after every four days and were dried at 80^{0C} in an oven till constant weight was obtained. The 500 mg sample was taken for digestion. The tissue was digested in 10 ml of acid mixture (HCL: HNO3 in (3:1) ratio) on hot plate till dryness. The digested mixtures were kept in water bath for 6-7 hours until the samples were cooled. Cool digested samples were filtered (Whatman grade 541). The total volume was diluted to 50 ml by double glass distilled water in volumetric flask. The sample were analysed on the instrument Atomic Absorption Sepctrophotometer (AAS, Chemito). The concentration of Pb accumulation in the tissue of each exposure period was recorded and the results are given in the tables.

OBSERVATIONS AND RESULTS

Bioaccumulation of lead in digestive glands and gills of *Lamellidens corrianus* exposed to lead (6.321 ppm) and lead with caffeine has been summarized in the Table -A.

The biaccumulation data from table A, indicates that the amount of Pb in tissues of digestive glands and gills on exposure to $PbNO_3$ (6.321ppm) increased with increase in exposure period as compared to control. The lead contents are expressed in µgm/Kg dry weight. The control group of animals showed minute quantity of lead as compared to the experimental groups. The control group of animal showed lead in digestive glands and gills is 310 to 300 µgm/Kg and 285to 285µgm/Kg respectively. Lead bioaccumulate in group B of mussels is 750 to 1.230µgm/Kg and 489 to 894 µgm/Kg respectively while in group of mussels C bioaccumulate Pb is 568 to 932 µgm/Kg and 415 to 675 µgm/Kg respectively.

TABEL – A: Lead content (µgm/Kg dry weight) in digestive gland and gill tissues of *Lamellidens corrianus* after chronic treatment of $PbNo_3$.

Treatment	Sr.No.	Body Tissue	Pb content (µgm/kg dry weight)		
			4 Days	8 Days	12 Days
(A) Control	I	D.G	310.00	310.00	300.00
	II	G	285.00	280	280
(B) 6.321 ppm $PbNo_3$	I	D.G	750.00,(141.93 %)	968.00,(212.25%)	1.230,(310.00%)
	II	G.	489.00,(71.57%)	654.00,(133.57%)	894.00,(219.28%)
(C) 6.321 ppm $PbNo_3$ + 5G/lit.caffeine	I	D.G	568.00,(83.22%)	764.00,(146.45%)	932.00,(210.66%)
	II	G	415.00,(45.61%)	510.00,(82.14%)	675.00,(141.07%)

D.G.- Digestive gland,G.- Gill /Value in bracket indicates % Variation compared with control.

The mussels pre-exposed to as showed fast lead depletion with caffeine than those allowed to cure naturally. The data of recovery mussels showed from table B. The amount of Pb accumulated as observed after 4,8 and 12 days in digestive gland and gills was1.057 to 835µgm/Kg and 728 to 518 µgm/Kg respectivelyin mussels allowed to normal water for natural cure while in those mussles exposed to cffeine for cure, the amount of accumulated Pb after 4,8 and 12 days in digestive glands and gills was 950 to 680 µgm/Kg and 635 to 494 µgm/Kg.

DISCUSSION

The accumulation of metal in different species is the function of their respective membrane permeability and

enzyme system(Mitra et.al, 2000). The accumulation of metal in different species is the function of their respective membrane permeability and enzyme system. (Jeffree RA et al, 1993; Metcalfe Smith JL, 1994). Heavy or toxic metals are metals with a density at least five times that of water. They are stable elements (meaning they cannot be metabolished by the body) and bio-accumulative are (passed up the food chain to humans). These include : mercury, lead, nickel, arsenic, cadmium, aluminium, platinum and copper.Heavy metals besides micronutrients have no fuction in the body and can be highly toxic. Studies confirm that heavy metals can directly influence behaviour of living organism including man. Two obvious methods exist for expressing the heavy metal component of living organisms. Absolute may be assessed by considering the organisms, metal contens i.e. body burden and the metal component may be expressed as a fuction of the weight of individual organism.

The caffeine metal-chelate complex being of small molecular weight can easily pass through the membranes of the cells of the epithelia of the tubules of the kidney and can be effectively excreted out.(Hove-Madsen, 1999). Wang et al. (2002) reported that the interactions between metal geochemistry and animal physiology determine the differences in the bioavailability among metals. Beeby and Richmond (1987) also suggested that Pb assimilation and excretion is under control of a physiological mechanism that is able to adapt to high concentrations of this metal in its food. This ability of the snails to adapt to lead contamination and accumulate high concentration of the metal in the edible part therefore implies an urgent need to institute a continuous monitoring of the snails and soils where they are reared or collected to avert a looming public health problem that might arise under a no-control situation. The pond snail (*Lymnaea stagnalis* L.), which is one of the most common snails of freshwater habitats in central Europe, have a good indicator potential, since more information about the features of heavy metal accumulation, toxic pollution tolerance and impact of metals on the physiology of the genus *Lymnaea* are known (Bogatova 2009). The bioaccumulation of lead in *L. corrianus* was studied. The lead accumulation increased with the increase in exposure period of chronic concentration of lead nitrate (6.321ppm). The amount of lead accumulation is less when exposed with 5 mg/l caffeine in presence of respective concentration of lead as compared to those exposed to only lead. The *L. corrianus* pre-exposed to lead (6.321ppm) showed rapid removal of the lead in presence of caffeine than those maintained in normal water. Therefore, the caffeine (1,3,7-Trimethylxanthine) as a modulator of lead bioaccumulation in the experimental mussels, *L. corrianus.*

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