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COMBINED CURATIVE ROLE OF CAFFEINE (1,3,7-TRIMETHYLEXANTHINE) AND L-ASCORBIC ACID ON LEAD INDUCED ALTERATION IN DNA CONTENT IN VARIOUS TISSUES OF AN FRESHWATER BIVALVE, *LAMELLIDENS CORRIANUS* (LEA)

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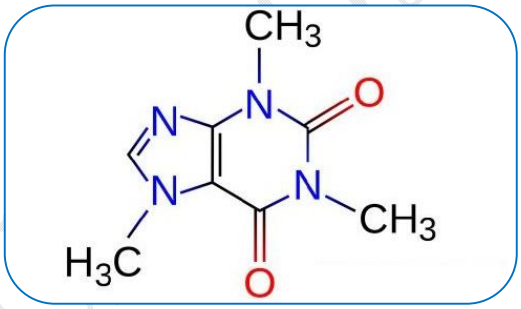
ABSTRACT :



The present communication deals with individual and synergistic curative role of caffeine and L-ascorbic acid 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 DNA contents in selected tissues of bivalves were estimated. The DNA level was significantly decreased on exposure to lead while the decrease in presence of caffeine + ascorbic acid was less when exposed simultaneously than when exposed individually. During recovery DNA contents 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.

KEYWORDS : *curative, caffeine, L-ascorbic acid, lead, DNA, Lamellidens corrianus*

INTRODUCTION

Exposure of aquatic animals to non essential metals like lead causes severe health hazards. These metals have great retention capability in almost all tissues of animal and their adverse effect can be observed on their normal physiology. Heavy metals are also reported to have a role in oxidative damage of cellular membrane. It may be due to generation of free radicals (Amoruso *et. al.,* 1982, Ochi *et.al.,* 1987, Myung *et. al.,* 1989). Free radicals are certain molecule or molecular segment with unpaired electron in their outer orbit. Due to this unpaired electron, free radicals show certain characteristic properties, like paramagnetism. As free radicals are highly reactive, free radical generated by various pathways in aerobic cellular activities such as generation of intracellular radical in mitochondria, conversion of xanthine dehydrogenase to xanthine oxidase, arachidonic cascade activated neutrophils auto oxidation of catecholamins (Maeda *et. al.* 1987).

Heavy metals are most hazardous pollutants because of their non degradable nature and property to affect all kinds of ecological systems. The salt of metals, which find their way in to commercial, industrial applications process certain biocidal properties. Lead may exert toxic effects on several organ systems, but those in kidney are the most insidious. Intracellular lead is associated with specific high affinity proteins and can also bind to metallothionein.

Lead affects almost every organ or system in the body. It is absorbed into the body and distributed to the blood, soft tissue and bone. The central nervous system is most vulnerable to lead toxicity, particularly in developing children. Heavy metal toxicity reflects a suppressing effect on various biochemical constituents such as proteins, nucleic acids, lipids etc in the molluscs. Krishnamoorthy and

Subramanian (1995) reported decrease in protein contents in muscles, gills and hepatopancrease of *Macrobrachium lamarrei* on exposure to copper.

L-ascorbic acid have some characteristic points concerning the chemistry which are important in connection with its determination. Vitamin C is being shown through continued research to stimulate the immune system; through this function, along with its antioxidant function, it may help in the prevention and treatment of infections and other diseases. Caffeine molecule has structure closely related with uric acid. Biochemical process such as demethylation and oxidation helps to caffeine for getting metabolized in the body. As the metabolic intermediates of the caffeine metabolism retains the chelation capacities, it reduces the interactions of the heavy metals with other groups of the proteins .

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. DNA 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 (6ppm) 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 Lead. 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. DNA contents were estimated by Diphenylamine method (Schenider, 1967)

RESULTS AND DISCUSSION:

Heavy metals are also reported to have a role in oxidative damage of cellular membrane. It may be due to generation of free radicals (Amoruso *et. al.,* 1982, Ochi *et.al.,* 1987, Myung *et. al.,* 1989). Misra *et.al.*(1990) reported the damage of lipids, cell membranes and DNA in tissues, due to increased level of an active oxygen species. It may also damage the genetic material and hence contribute in carcinogenic effect. Rehman (1984) showed 10 fold increases of MDA (malonaldehyde) levels in brain homogenates of adult rats exposed to lead acetate. Various metal ions have been confirmed to accelerate free radical reaction in vitro (Quinlan, 1988). Postmortem studies have proposed oxidative injury by oxidative damage to proteins, lipids, and DNA, although the initiating causes of these events have not been identified (Agar and Durham, 2003). There was also gradual decrease in the brain protein level showing significant alterations but the brain ascorbic acid level showed no significant alterations (Desai *et. al.,* 2002).

Table No. 1.1: DNA content 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 mg/100mg of dry weight)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		1.218 ±0.048	1.220 ±0.034		
Lead nitrate		1.025 ±0.0563** (-15.84)	0.91 ±0.0178*** (-25.40)		
Lead nitrate + Caff		1.103 ±0.0345* (-9.44)	0.98 ±0.0325*** (-19.67)		
Lead nitrate + AA		1.111 ±0.0354* (-8.78)	1.08 ±0.0345** (-11.47)		
Lead nitrate + Caff + AA		1.135 ±0.0652^{NS} (-6.81)	1.119 ±0.0246** (-8.27)		
After 20 days exposure to Lead nitrate	Normal Water			0.92 ±0.0245^{NS} [+1.09]	0.94 ±0.0532^{NS} [+3.29]
	Normal Water + Caff			0.94 ±0.0354^{NS} [+3.29]	0.97 ±0.0254▪ [+6.59]
	Normal Water + AA			0.95 ±0.0563^{NS} [+4.39]	0.99 ±0.0153▪▪ [+8.79]
	Normal Water + Caff + AA			1.08 ±0.0538▪▪ [+18.68]	1.13 ±0.0294▪▪▪ [+24.17]

Table No. 1.2: DNA content in Gonads of *L. corrianus* after chronic exposure to Lead nitrate without and with caffeine, ascorbic acid and with caffeine + ascorbic acid and during recovery (Values are in mg/100mg of dry weight)

Treatment	10 days	20 days	Recovery	
			5 days	10 days
Control	2.410 ±0.0782	2.400 ±0.057		
Lead nitrate	2.135 ±0.192^{NS} (-11.41)	1.83 ±0.108*** (-23.75)		
Lead nitrate + Caff	2.2 ±0.0876* (-8.71)	2.02 ±0.0837** (-15.83)		
Lead nitrate + AA	2.25 ±0.0673*** (-6.63)	2.14 ±0.0872** (-10.83)		
Lead nitrate + Caff + AA	2.49 ±0.0268^{NS} (-3.31)	2.31 ±0.0927^{NS} (-3.75)		

After 20 days exposure to Lead nitrate	Normal Water			1.86 ±0.0245^{NS} [+1.63]	1.97 ±0.194^{NS} [+7.65]
	Normal Water + Caff			1.96 ±0.0279^{NS} [+7.10]	2.1 ±0.0789▪ [+14.75]
	Normal Water + AA			1.98 ±0.0693^{NS} [+8.19]	2.16 ±0.0925▪▪ [+18.03]
	Normal Water + Caff +AA			2.09 ±0.0674▪ [+14.20]	2.32 ±0.0835▪▪ [+26.77]

Table No. 1.3: DNA content in Digestive glands of *L. corrianus* after chronic exposure to Lead nitrate without and with caffeine, ascorbic acid and with caffeine + ascorbic acid and during recovery (Values are in mg/100mg of dry weight)

Treatment		10 days	20 days	Recovery	
				5 days	10 days
Control		2.218 ±0.0825	2.213 ±0.102		
Lead nitrate		1.83 ±0.0698** (-17.49)	1.63 ±0.0918** (-26.34)		
Lead nitrate + Caff		1.95 ±0.0590^{NS} (-12.08)	1.81 ±0.190* (-18.21)		
Lead nitrate + AA		2.01 ±0.0892* (-9.37)	1.86 ±0.0781** (-15.95)		
Lead nitrate + Caff + AA		2.13 ±0.0690^{NS} (-3.96)	2.05 ±0.0980^{NS} (-7.36)		
After 20 days exposure to Lead nitrate	Normal Water			1.72±0.0604^{NS} [+5.52]	1.81±0.0809▪ [+11.0]
	Normal Water + Caff			1.79±0.0945^{NS} [+9.81]	1.86±0.0649▪ [+14.11]
	Normal Water+ AA			1.81±0.0892^{NS} [+11.04]	1.93±0.0910▪▪ [+18.40]
	Normal Water + Caff + AA			2.05±0.0484▪▪ [+25.76]	2.13±0.0945▪▪ [+30.67]

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

Anti-oxidant plays a role in the treatment of lead poisoning (Gurer *et. al.,* 2001). Ascorbic acid supplementation was found to recover the lead induced kidney function in rat (Sumathi and Jeyanthi, 2005). Puzas *et.al.,* (2004) reported that the constant and high environmental Pb exposure from 1940"s to 1960's, women's currently going through menopause were at additional risk of osteoporosis. Pb becomes sequenced in the skeleton, incorporated in to hydroxyl apatite crystals during calcification and remains there until the bone is reabsorbed or remodeled (Wittmers *et.al.,* 1998).

DNA content is drastically affected due to the stress condition caused by toxic exposure of heavy metals or pesticides. Lead exposure caused the DNA damage, and strand breakage in foot tissue of *Anodonta grandis* (Black *et.al.*1996) It occurs only due to certain physiological burden or achieved exposure duration. The toxic effect of pesticide stress on DNA and protein of shrimp larvae, *Litopenaeus stylirostris* of the California gulf was studied by Galindo *et.al.,* (2002). Tong lu *et.al.* (2001) investigated that approximately 60 genes (10%) shows differential expression in human liver exposed to arsenic as compared to control. Variety of gene expression might play an integral role in arsenic hepatotoxicity and causes the carcinogenic effect. The metal may be carcinogenic because of their ability to generate oxygen species and other reactive intermediates which directly damage the DNA structure (Bryan *et.al.* 1971).

Metals have the ability for DNA damage through inhibition of DNA repair enzymes (Hartwig *et.al.* 2002). Free radical of metal ultimately targets the DNA and causes the carcinogenicity and genotoxic effect. Mayer *et. al.,* (1998) observed that Ni^{++} is weakly genotoxic and causes mutagenic alteration in DNA.

Caffeine is well known nervous system stimulant. 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. Harish *et.al.,*(2002) detect the effect of caffeine as α reflective DNA synthesis inhibitor or as pre-inter and post treatment on ethyl methano sulphonate (EMS) induced adaptive response in vivo mouse bone marrow cell was studied to understand influence of caffeine.

L-Ascorbic acid play curative role against heavy metal induced biochemical alteration and cures structural damages caused by metals in the animal body. Somasundaram *et.al.,*(1978) reported that concentration of ascorbic acid depended upon the physiological state of organism. Kutzly, (1973) called ascorbic as anti-stress factor. It was likely that increased mobilization and utilization of ascorbic acid is closely linked in to regenerative process occurring in the reproducing organ following Cd stress.

Mahajan (2007) reported the decrease in collagen, ascorbic acid, protein, content of the tissue of freshwater bivalve *Lamellidens marginalis*, on exposure to heavy metal cadmium and arsenic and lead but it is less in metal with Ascorbic acid and during recovery with Ascorbic acid improves biochemical level of all tissues of bivalve faster than normal recovery.

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 nitrate 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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