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Research Article

ANTIOXIDANT PROPERTIES OF CAFFEINE (1,3,7-TRIMETHYLXANTHINE) ON LEAD INDUCED ALTERATION IN LIPID CONTENT OF DIFFERENT TISSUES OF FRESH WATER GASTROPOD SNAIL, BELLAMYA (VIVIPARUS) BENGALENSIS

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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 gastropod snail's *Bellamya bengalensis*. The effect on snails was studied under five groups. Group A snails's were maintained as control, B group snails's were exposed to chronic dose (LC50/10) of lead nitrate (6.753 ppm) for 21 days. Group C snails's were exposed to respective chronic concentration of lead nitrate along with caffeine (5mg/l). Lipid contents in selected tissues from each group were estimated after 7, 14 and 21 days. Snails from group B were divided for recovery into two groups D and E after 21 day exposure to lead. D group snails's were allowed to cure in normal water, E group snails's were exposed to caffeine (5mg/l) up to the 42 days. From each of recovery groups, some snails's were removed and lipid contents in selected tissues of snails's were estimated after 7,14 and 21 days. The lipid 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 lipid contents recovered and the rate of recovery was faster in caffeine exposed snails as compared to those recovered in normal water.The probable role of the caffeine is discussed in the paper.

Keywords: *Lipid Content, Lead Nitrate, Caffeine, Bellamya Bengalensis*

INTRODUCTION
Water pollution is the biggest menace of urbanization, industrialization and modern agricultural practices. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as that of the environment (Indra and Sivaji, 2006). The biochemical changes in the organs of animal exposed to heavy metals have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract toxic stress. The biochemical changes occurring in the body gives the important indication of stress (Peter, 1973).
Lead is a persistent metal, however, and is still present in the environment in water, brass plumbing fixtures, soil, dust, and imported products manufactured with lead. Lead is a highly toxic substance. There are many ways in which humans are exposed to lead: through deteriorating paint, household dust, bare soil, air, drinking water, food, ceramics, home remedies, hair dyes and other cosmetics. Lead is of microscopic size, invisible to the naked eye. When a pregnant woman has an elevated blood lead level, that lead can easily be transferred to the fetus, as lead crosses the placenta. The pollution of the aquatic environment by heavy metals is a subject of great concern. Heavy metals pose a serious threat to the aquatic environment because of their toxicity, persistence, tendency to accumulate in organism & undergo food chain amplification (Weis and Weis, 1977a, b).They cause severe damage to the aquatic fauna, including molluscs, fishes etc, thereby telling up on their health & population. They affect the activity of biologically active molecule such as glycogen, protein & lipid (Ghosh and Chatterjee, 1985). The heavy metals can cause biochemical alterations such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer (Underwood, 1971; Lucky and Venugopal, 1977). The biochemical changes occurring in the body gives first indication of stress. It has been reported that acute or chronic treatment of pesticide cause biochemical alterations in the organs involved in detoxification mechanisms

(Shobana *et al.*, 2000; Prabhakara and Prasadrao, 2002). Lipids, being energy reserves and raw materials for membrane system of the cells, play a very vital role in pysiological activities of the orgnisms. Membrance structure and function point towards the complex role of lipid in environmental adaptation.
Cappuzzo and Lancaster (1981) studied the lipid utilization in post larval lobstar *Homarus americanus* when exposed to pollutant. Deshmukh and Lomte (1998) studied the effect of copper sulphate on lipid metabolism of the freshwater bivalve, *Parreysia corrugata.* Very little information is available on the heavy metal induced variations in glycogen protein, lipid and ascorbic acid content in freshwater gastropd snails. Many workers studied the lipid alterations in various animals after exposure to toxicants (Zambare, 1991; Deshmukh, 1995; Waykar and Lomte, 2004; Shaikh, 2011).
The alkaloid caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and prooxidant properties. Caffeine and its metabolites may also contribute to the overall antioxidant and chemo preventive properties of affeinebearing beverages, such as tea (Azam *et al.*, 2003). Caffeine is found to have antioxidant activity. This activity of caffeine can protect the damage of tissues, biochemicals and gentic materials of organisms from the heavy metal generated free oxygen radicals. In Feb, 2002 Miike Mclaughlin of CSIRO, Australia has found that coffee has capacity to bind with heavy metals. Heavy metal content of water was much reduced after adddition of coffee. The main constituent a coffee is caffeine. Dissolved heavy metal ions are positively charged and caffeine contains uncharged and negatively charged groups.
Caffeine has been reported as a protective substance on cellular damage (Kamat *et al.*, 2000; Krisko *et al.*, 2005) with beneficial antioxidant effects (Nikolic *et al.*, 2003). Therefore, the present investigation carried out for the study of antioxidant role of caffeine towards lead. The present study investigates the antioxidant properties of caffeine against the lead nitrate in lipid contents of the various tissues (whole body, hepatopancreas, and gonads) of the fresh water gastropods snail's, *Bellamya bengalensis after* chronic exposure.

MATERIALS AND METHODS

Attempts will be made in this study to select Fresh water gastropod snails, *Bellamya bengalensis* were collected from of Suki dam which is about at the distance of 31 K.M. away from Savda City of Maharashtra State. First they were made acclimatized to laboratory condition for 2-3 days . The water in the aquarium was changed regularly after every 24 hours. After the acclimatization, The healthy active snail's of approximately medium size and weight were chosen. These snail's were divided into three groups, such as group A,B and C . The snail's of group A were maintained as control. The snail's from group B were exposed t o chronic concentration (LC 50 value of 96 hr/10) of heavy metal salt, lead nitrate (6.753 ppm) and the snail's from group C were exposed to chronic concentration (LC 50 value of 96 hr/10) of heavy metal salt, lead nitrate (6.753 ppm) with 5mg/lit.caffeine upto 21 days. During experimentation snail's were fed on fresh water algae. The hepatopancreas and gonad of snail's from B and C group's were collected after every seven days and tissues of hepatopancres and gonad were dried in oven at 75 0C to 80 0C till constant weight was obtained and blended into dry powder. The total lipids from the tissues were esstimated by vanilline reagent method as given by Barnes and Blackstock (1973) using Cholesterol as standard.All values are the averages of three repeats and are expressed as percentage of dry weight. Standard deviation and students 't' test of significance are calculated and expressed in respective tables.

RESULTS AND DISCUSSION

The biochemical estimation of lipid contents was estimated from the different tissues that is whole body, hepatopancreas and gonad of experimental model, the freshwater snail's *Bellamya bengalensis* from control and experimental groups are presented in respective tables. Thus from the above investigation the results obtained indicates that there was severe alteration in the lipid metabolism in the fresh water snail's *Bellamya bengalensis* after exposure to lead nitrate. In the present study, significant decrease in the lipid content was observed in the whole body, hepatopancreas and gonad of experimental snail's as compared

to those of the control snail's. There was increase in the lipid content in heavy metal salts with caffeine-exposed snail's as compared to those exposed to only lead nitrate. The snail's show fast recovery in tissue lipid level in presence of caffeine than those allowed to cure naturally. When the snail's exposed for 21 days to lead was allowed to recover, lipid recovery was at a very slow rate in naturally curing snail's in normal water as compared to those allowed to cure with caffeine. Lipid contents recovered faster in all tissues in caffeine as compared to normal water. Rate of recovery was better in caffeine than in normal water recovery.

The change in biochemical composition of an organ due to heavy metal stress indicates the change in activity of an organism . It reflects light on the utilisation of their biochemical energy to counteract the toxic stress. Heavy metal salts affect the metabolism of the fresh water snail's, *Bellamya dissimilis*. Alterations in metabolic pocesses, following exposure to heavy metal stress have been always used as an indicator of stress. But there is a vast difference in the pattern & metal induced physiological alterations from metal to metal & animal to animal . Caffeine (1,3,7-trimethyl xanthine), an ingredient of coffee, has been investigated for its potential antioxidant activity against oxidative damage to rat liver microsomes. Such damage was induced by three reactive oxygen species of cardinal importance in causing membrane damage in vivo namely hydroxyl radical (.OH), peroxyl radical (ROO.) and singlet oxygen (1O2). The results obtained showed that caffeine was an effective inhibitor of lipid peroxidation by Devasagayam *et al.*, (1996).

A)Lipid content in selected tissues of *Bellamya bengalensis* after chronic exposure to heavy metal salt,$PbNO_3$.

Treatment	Sr No.	Body Tissue	The lipid content (%) ± S.D. 7 Days	14 Days	21 Days
(A) Control	i	W.B.	0.510 ± 0.0007	0.490 ± 0.0007	0.480 ± 0.0005
	ii	H.	1.420 ± 0.0008	1.400± 0.0006	1.400 ± 0.0047
	iii	G.	0.990 ± 0.0011	0.980 ± 0.0010	0.960 ± 0.0007
(B)6.753 ppm $PbNO_3$	i	W.B.	0.430 ± 0.0008***	0.340 ± 0.0011***	0.290± 0.0016***
			-18.604$^{\bullet}$	-44.117$^{\bullet}$	-65.512$^{\bullet}$
	ii	H.	1.270 ± 0.0013***	1.080 ± 0.0098***	0.990 ± 0.0007***
			-11.811 $^{\bullet}$	- 29.629$^{\bullet}$	- 41.414$^{\bullet}$
	iii	G.	0.740 ± 0.0004***	0.670 ± 0.0010***	0.590 ± 0.001***
			- 21.818$^{\bullet}$	- 46.268$^{\bullet}$	- 61.711$^{\bullet}$
(C) 6.753 ppm $PbNO_3$+ 5mg/lit Caffeine	i	W.B.	0.460 ± 0.0007***	0.410 ± 0.0018***	0.370 ± 0.0005***
			-10.869$^{\bullet}$, +6.521$^{\Delta}$	-19.512$^{\bullet}$, +16.097$^{\Delta}$	-29.729$^{\bullet}$, +21.621$^{\Delta}$
	ii	H.	1.310 ± 0.0258***	1.190 ± 0.0007***	1.160 ± 0.0008***
			-8.396$^{\bullet}$, +3.053$^{\Delta}$	-17.647$^{\bullet}$, +9.243$^{\Delta}$	-20.689$^{\bullet}$, +14.655$^{\Delta}$
	iii	G.	0.810 ± 0.0026***	0.760 ± 0.0007***	0.660 ± 0.0001***
			-22.222$^{\bullet}$, +8.641$^{\Delta}$	-28.947$^{\bullet}$, +11.842$^{\Delta}$	-45.545$^{\bullet}$, +10.606$^{\Delta}$

Caffeine is the most commonly consumed psychoactive substance in the world. Caffeine occurs naturally in more than 60 plants including coffee beans, tea leaves, kola nuts used to flavor soft drink colas, and cacao pods used to make chocolate products. Dorea *et al.*, (2005) showed that ,epidemiological and experimental studies have shown positive effects of regular coffee drinking on various aspects of health, such as psychoactive responses (alertness, mood change), neurological condition (infant hyperactivity,

Parkinson's disease) and gonad and liver function. Caffeine's popularity worldwide can be attributed to its ability to promote wakefulness, enhance mood and cognition, and produce stimulatoryeffects (Haskell *et al.,* 2005; Lieberman *et al.,* 2002).

B) After 21 days exposure to6.753 ppm $PbNO_3$

Treatment	Sr No.	Body Tissue	The lipid content (%) ± S.D. 28 Days	35 Days	45 Days
(D) Normal Water	i	W.B.	0.330 ± 0.0008***	0.380 ± 0.0005***	0.410 ± 0.0018***
			- 54.545•, + 12.121□	- 28.947•, + 23.684□	- 17.073•, + 29.268□
	ii	H.	1.040 ± 0.0098***	1.120 ± 0.0010***	1.180 ± 0.0008***
			- 36.538•, + 4.807□	- 2.500•, + 11.607□	-18.644•, + 16.101□
	iii	G.	0.630 ± 0.0010***	0.690 ± 0.0011***	0.770 ± 0.0005***
			- 42.857•, + 6.349□	- 42.028•, + 14.492□	- 24.675•, + 23.376□
(E) Normal Water + 5mg/ lit. Caffeine	i	W.B.	0.360 ± 0.0007***	0.420 ± 0.0019***	0.430 ± 0.0020NS
			- 41.666•, + 19.444□	- 16.666•, + 30.952□	- 11.627•, + 32.558□
	ii	H.	1.090 ± 0.0011***	1.210 ± 0.0007***	1.230 ± 0.005NS
			- 30.275•, + 0.921□	- 15.715•, + 18.181□	- 13.821•, + 19.512□
	iii	G.	0.680 ± 0.0010***	0.730 ± 0.0010***	0.830 ± 0.0037***
			- 45.588•, + 13.235□	- 34.246•, + 19.178□	- 15.662•, + 28.915□

W.B.- Whole Body *N.S. - Non Significant* • - *Compared with respective A*
H. - Hepatopancreas * - *P < 0.005* Δ - *Compared with respective B*
G - Gonads ** - *P < 0.01* □ - *Compared with respective 21*
*** - *P < 0.001*

The protective action of caffeine against a variety of chemical carcinogens was established by several studies, carried out by Abraham (1989; 1991). 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.

Lipids are also one of the most important energy reservoirs and these are stored and transported in the form of di and tri glycerol's and esters. Massey *et al.,* (1993) indicated the increased urinary excretion of calcium, magnesium, sodium and chloride after oral doses of caffeine which indicates the chelated caffeine treatment with heavy metal is excretable. In September (2001), Women's Health Weekly also reported that, the caffeine in the drinks was primarily responsible for excess calcium excretion. Deshmukh and Lomte (1998) reported significant decrease in the lipid contents in all tissues of *Parreysia corrugata* after exposure to copper sulphate.

Bhavani and Dawood (2003) reported decrease in levels of lipids in the body tissue of *Perna viridis,* due to metal toxicity. Waykar and Lomte (2004) reported significand reduction in the lipid content from soft tissues of freshwater bivalve, *Parreysia cylindrical* after exposure to pesticide. Patil *et al.,* (2011) reported a decrease in lipid content in the foot of a freshwater snail, *Indoplanorbis exustus* exposed to heavy metals.

The lipid alterations in various animals after exposure to toxicants were studied by (Caley and Jenson, 1973; Coopage *et al;* 1975; Bhagyalakshmi, 1981; Chaudhary, 1988; Zambare, 1991). In the present study it is proved that,Caffeine posses antioxidant ability because it recovered the total lipid content and it play important role as detoxication of lead which recovered the lipid content.

Conclusion

It is concluded from this study that, overall decrease in lipid levels in different soft tissues of fresh water gastropod snail's, *Bellamya bengalensis* was due to chronic treatment of lead nitrate. It has been also observed that severity of depletion in lipid content was less in snail's those exposed heavy metal salts along with 5mg caffeine/lit.The studied was indicates that,caffeine plays a important role in removal of heavy metal salts outside the body.

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