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Research Article

PROTECTIVE ROLE OF CAFFEINE (1, 3, 7-TRIMETHYLEXANTHINE) ON LEAD INDUCED ALTERATIONS IN PROTEIN CONTENT OF HEPATOPANCREAS AND GONADS OF FRESH WATER SNAIL, *BELLAMYA DISSIMILIS*

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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 snail, *Bellamya dissimilis*. The effect on snail was studied under five groups. Group A snails were maintained as control, B group snails were exposed to chronic dose ($LC_{50/10}$) of lead nitrate (6.77 ppm) for 18 days. Group C snails 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 9 and 18 days. Snails from group B were divided for recovery into two groups D and E after 18 day exposure to lead. D group snails were allowed to cure in normal water, E group snails were exposed to caffeine (5mg/l) up to the 9 days. From each of recovery groups, some snails were removed and protein contents in hepatopancreas and gonad of snails 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 snails 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 Content, Lead, Bellamya Dissimilis*

INTRODUCTION
Heavy metals are recognized as a strong biotoxicants, because of their persistent nature and cumulative action to the aquaticflora and fauna (Sharma and Agrawal, 2005). The discharge of heavy metals by industries pose a serious water problem due to the toxic properties of these metals and their adverse effects on aquatic life. According to the survey conducted by Central Inland Fisheries Research Institute (CIFRI, 1981), these heavy metals are well kowan pollutants which are often encountered in many rivers of India, and there is every possibility of deterioration of water quality and hence including man and various organisms are presenting a potential threat for survival. Heavy metals are economic poisons used to control a wide range of animal and plant pests. The fresh water environment is becoming increasingly polluted throughout the biosphere with various heavy metals and as heavy metals are non-biodegradable, their concentration in the environment increases. The effect of heavy metals on animal life in fresh water is an important aspect of pollution and the information available on the physiological effects of exposure to different pollutants is meagre (Kleerkoper, 1974). These environmental pollutents bring about damage to different organs or disturb the physiological and biochemical processes within the organism. 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.
Wood (1974) classified metals into non-critical, very toxic and toxic metals. He classified arsenic, lead and mercury as very toxic heavy metals. These heavy metals enter into the body of animals including man

through the non vegetarian and vegetarian diet, drinking water and air and accumulate in the tissues, usually react with proteins and interfer the physiological activities and thus increase the risk of life in various ways. They are difficult to remove from the body. The trace metals are known to be non biodegradable and highly toxic to most organisms (Kaoud and Dahshan, 2010).

Micke Mc Laughlin (2000) of CSIRO, Australia has found that coffee has capacity to bind with heavy metals. Heavy metal content of water was much reduced after addition of caffeine. Dissolved heavy metal ions are positively charged and coffee contains uncharged and negatively charged molecules, the metals ions might be taken out of solution by binding to negatively charged molecules in the coffee granules. Liguori (1997) reported that,caffeine from coffee or other beverages is absorbed by the small intestine within 45 minutes of ingestion and then distributed throughout all tissues of the body.Metabolites of caffeine also contribute to caffeine's effects. Paraxanthine is responsible for an increase in the lipolysis process, which releases glycerol and fatty acids into the blood to be used as a source of fuel by the muscles. Theobromine is a vasodilator that increases the amount of oxygen and nutrient flow to the brain and muscles. Theophylline acts as a smooth muscle relaxant that chiefly affects bronchioles and acts as a chronotrope and inotrope that increases heart rate and force of contraction (Dews, 1984). Maughan (2003) sugested that, caffeine equivalent to 2–3 cups of coffee are administered to people who have not consumed caffeine during prior days, they produce a stimulation in urinary output.

The molecules of coffee being small, it's chelate with heavy metal can be easily excreted out by the biological system. This property of caffeine indicates that caffeine can have the capacity to remove the heavy metals from the living organisms. However no attempt has been made to study the role of caffeine in heavy metal detoxificatioin.

MATERIALS AND METHODS

The snails, *Bellamya dissimilis* were acclimatized to laboratory condition for 2-3 days and healthy active snails of approximately medium size and weight were chosen. These snails were divided into three groups, such as group A, B and C. The snails of group A were maintained as control. The snails from group B were exposed to chronic concentration (LC 50 value of 96 hr/10) of heavy metal salt, Lead nitrate (6.77 ppm) upto 21 days, while snails from group C were exposed to chronic concentraction of along lead nitrate with 5 mg/lit caffeine upto 18 days. After exposure for 18 days to heavy metals, the snails from group B were divided into two subgroups, such as D and E groups. The snails of group D were allowed for self cure naturally in normal water while the snails of group E were exposed to 5 mg/lit caffeine up to 9 days. The experimental snails from A to C groups were dissected after 9 days and 18 days and from each recovery group (E and D) were collected after 3,6 and 9 days. The hepatopancreas and gonads, 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 1 and are expressed as percentage of dry weight. Percent variations were calculated and are expressed in respective tables.

RESULTS

Protein contents in different tissues of *Bellamya dissimilis* after exposure to lead nitrate (6.77 ppm) along with caffeine and during recovery have been summarised in tables.

Table 1 shows that the protein contents in whole body, hepatopancreas and gonad *Bellamya dissimilis* in presence of lead nitrate (6.77 ppm)decreased with the increase in exposure period. The protein contents were more in heavy metal with caffeine exposed snails as compared to those exposed to only heavy metal salts for the corresponding period of exposure.Table 2 shows, that the snails preexposed to heavy metal salts showed fast recovery in the alteration of protein in presence of caffeine than those allowed to cure naturally.Therefore after studies the effect of caffeine on heavy metal it is proved that the caffeine

posseses binding site to connect heavy metal salts and due to this effect of heavy metal is less in animals those exposed in caffeine as compared to those exposed only in heavy metal salts.

Table 1: Protein content in selected tissues of *Bellamya dissimilis*, after chronic exposure to heavy metal salt, lead nitrate without and with Caffeine

Treatment	Sr No.	Body Tissue	The protein content (%) ± S.D. 9Days	18Days
(A) Control	i	H	38.75 ± 0.0043	36.25± 0.0043
	ii	G	34.0 ± 0.0042	32.5± 0.0069
(B) 6.77ppm $PbNo_3$	i	H	35.75 ± 0.0001*** - 8.391$^{\bullet}$	34.5 ± 0.004*** - 5.072$^{\bullet}$
	ii	G	32.25 ± 0.0033*** - 5.426$^{\bullet}$	31.0 ± 0.0031*** - 4.838$^{\bullet}$
(C) 6.77ppm $PbNo_3$+ 5mg/lit Caffeine	i	H	37.5 ± 0.0069*** -3.200$^{\bullet}$, +4.800$^{\Delta}$	35.0 ± 0.0069*** -3.571$^{\bullet}$, +1.428$^{\Delta}$
	ii	G	33.0 ± 0.0028*** -2.272$^{\bullet}$, +2.272$^{\Delta}$	32.25 ± 0.0047*** -0.775$^{\bullet}$, +3.875$^{\Delta}$

Table 2: After 18 days exposure to 6.77 ppm $PbNO_3$

Treatment	Sr No.	Body Tissue	The protein content (%) ± S.D. 3 Days	6 Days	9 Days
(D) Normal Water	i	H	33.75 ± .0066*** - 14.814$^{\bullet}$, + 3.703□	35.5 ± 0.0033*** - 2.112$^{\bullet}$, + 8.450□	36.25 ± 0.0043^{NS} +2.600$^{\bullet}$
	ii	G	31.50 ± 0.003*** - 7.936$^{\bullet}$, + 6.349□	32.25 ± 0.0055*** - 0.775$^{\bullet}$, + 8.521□	32.50 ± 0.0028^{NS} + 2.307$^{\bullet}$
(E) Normal Water + 5mg/ lit. Caffeine	i	H	34.5 ± 0.0074*** - 12.318$^{\bullet}$, + 5797□	36.75 ± 0.0017^{NS} + 1.360$^{\bullet}$, + 11.564□	37.5 ± 0.004^{NS} + 5.333$^{\bullet}$, + 13.333□
	ii	G	32.25 ± 0.0055*** - 5.426$^{\bullet}$, + 8.527□	33.0 ± 0.0028^{NS} + 1.515$^{\bullet}$, + 10.606□	33.75 ± 0.0026^{NS} + $^{5.925}$, +12.592 □

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 days of B*
*** - *P < 0.001*

DISCUSSION

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 biochamical energy to counteract the toxic stress. Heavy metal salts affect the metabiolism of the fresh water snails, *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.Protein content in the tissue of animal is an important essential organic constituent which plays a vital role in the cellular metabolism. All enzymes are proteins in nature and they control subcellular functions and accelarate the rate of metablic action in the body of organism. Ramanarao and Ramamurthi (1978) studied the protein content in the tissue of *Pila globosa* after exposure to pesticide.

In present study, in the *Bellamya dissimilis* the protein contents in the selected tissues was decreased in chronic concentration of copper sulphate as compared to the control and LC50/10 concentration with 5 mg/lit. According to Abel (1974) the decrease of protein may be due to alterations of membrane permeability. The depletion in the protein content was reported from the muscles of fish, *Clarias batrachus* after treatment with pesticide by the Yagana Bano *et al.,* (1981). Nagabhushanm and Kulkarni (1979) studied variation in protein metablism in *Barytelephusa cunicularis*. Joseph *et al.*, (1987) observed the effect of copper on biochemical composition of *Cyprinus carpio* and found that total protein content of the brain, liver and muscles was declined. Mukherjee and Sinha (1993) studied the effect of heavy metal toxicity on haematological and biochemical aspect in the fresh water major carps, *Labeo rohita*. Katticaran *et al.,* (1995) studied the copper induced alterations in total carbohydrate and protein level in the bivalve, *Sunetta scripta*.

In present stress, ionic copper sulphate might have caused severe disturbances of the metabolism in the animal. Chronic exposure of copper sulphate alone showed a remarkable decrease in protein content in *Bellamya dissimilis* than the chronic doses of heavy metal salts with caffeine. Starvic (1994) after the study of role of chemopreventer in human diet suggested that most of the chemopreventive strategies have been based on the modification of metabolism at one or many steps such that these agents can block the metabolic activition or enhance the detoxification . Caffeine is wellknown nervous system stimulant but besides it, it is now observed that it has antioxident activity. This activity of caffeine can protect the damage of tissues chemicals and genetic materials from heavy metal generated free oxygen radicals.Oral administration of tea has been found to moderately inhance the activities of lipid Peroxidase catalase, glutathione-s-transferase which in turn protect against cancer by blocking the reaction of electrophilic carcinogens with cellular micromolecules (Madal and Maity, 1999). Caffeine being water soluble and common cheaper beverage , it will be cheapest preventive and curative medicine. Takayamas (1982) long term study on the effect of caffeine in wister rats, has proved that caffeine belongs to a group of compound known as methylxanthine and it is non carcinogenic in animal model. It has also been reported to antagonize the carcinogenic effects of chemicals in vitro. Under in vitro condition caffeine has been reported to enhance or inhibit tumorigenesis induced by various carcinogenic agents, mercury, arsenic and lead are the known carcinogenics. Wattenberg (1992) reported that any compound that can block the metabolic activation step, scavenge the reactive inter mediate or enhance detoxification would be potential chemopreventive agent. Chung Fung-Lung (1999) reported that caffeine when given in drinking water at a concentration identical to that found in 2% tea was able to inhibit lung tumours induced by 4-(Methylnitrosoamino)-1-(3-pyridyl)-1 furane (NNK). Hosakas *et al.,*(2001) has observed that caffeine inhibite hepatocarcinogenesis induced by 2- acetylaminoflurene.

Gandhi and Khanduja (1992) studied the action of caffeine in altering the carcinogen activiting and detoxofying enzymes in mice and reported an induction of xenobiotic detoxifying enzyme as an additional mechanism by which plant product may act as anticarcinogens, since this induction of detoxifying enzyms is capable of competing with steps in xenobiotic activation. Caffeine have been found to increase glutathione synthetase and reduced glutathione in liver and lungs of mouse (Gandhi and Khanduja, 1992). Lu *et al.,* (2001) studied the stimulatory effect of oral administration of green tea or Caffeine on ultraviolet light induced alterations and suggested that green tea & caffeine inhibits UV-induced carcinogenesis. Matsumura *et al.,* (2000) reported that, the ATP – requring priming stage is lacking in the process of caffeine induced exocytosis in bovine adrenal chromaffin cells. Hove-madsen (1999) suggested caffeine exposure incresed pulse duration to 85m/s and slowed the inactivation of the Ca^{2+} current (Ica). Leoty *et al.,* (2001) found that caffeine stimulates the reserve mode of NA(Sup+)/Ca(SUP2+) exchanger in ferrete ventrecular muscles and indicated that the increase in resting tension following exposure to caffeine was mediated by Na+/Ca2+ exchanger,Which represents an additional element of coplexity in caffiene action on cardiac muscles. 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 with heavy metal is excretable. In Sept. (2001), Women's Health

Weekly also reported that, the caffeine in the drinks was primarily responsible for excess calcium excretion. Caffeine is used as an ergogenic aid because multiple well-controlled experiments have found that moderate doses of caffeine (3-6 mg/kg) can improve performance in athletes (Graham, 2001 and Flinn *et al.*, 1990). The ergogenic effect of caffeine ingestion before exercise has been reported above all in high intensity aerobic conditioning programs (Bruce *et al.*, 2000; Jackman *et al.*, 1996 and Wiles *et al.*, 1992). The mechanism for the caffeine-improved performance is not clear but several possibilities have been proposed such as the ntagonism of adenosine receptors (Davis *et al.*, 2003), the attenuation of effort perception or reduction of muscle pain (Doherty *et al.*, 2004 and O'Connor *et al.*, 2004) and the increase in catecholamine release (Greer *et al.*, 2000; Graham *et al.*, 2000; Jackman *et al.*, 1996 and Van Soeren *et al.*, 1993). Caffeine has been reported as a protective substance on cellular damage (Kamat *et al.*, 2000 and Krisko *et al.*, 2005) with beneficial antioxidant effects (Nikolic *et al.*, 2003); probably due to the main metabolites of caffeine, 1- methylxanthine and 1-methyluric acid, that are highly effective antioxidants and are able to prevent LDL oxidation in vitro (Lee, 2000).

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