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Research Article

CAFFEINE, PREVENT THE BIOACCUMULATION OF COPPER IN WHOLE BODY TISSUES OF FRESH WATER GASTROPOD SNAIL, *BELLAMYA BENGALENSIS* AFTER CHRONIC INTOXICATION

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ABSTRACT
The effect of Caffeine (1, 3, 7-trimethylxanthine) on bioaccumulation of heavy metal sal (copper) in whole body mass during chronic copper intoxication of the freshwater gastropods snail's, *Bellamya bengalensis* has been studied. The bioaccumulation of heavy metal salts in snail's was studied under three groups. Group A was maintained as control, group B snail's were exposed to chronic $LC_{50/10}$ dose of Cupric oxide (1.382 ppm) and group of snail's C exposed to $LC_{50/10}$ dose of Cupric oxide (1.382 ppm) with 5 mg/lit. caffeine up to 21 days. After 21 day the group B snail's divided into two group such as group D and group E. The snails of group D alloweded to cure naturally in normal water while snail's from group E exposed to normal water along with 5 mg/lit. Caffeine for self cure. Bioaccumulation level in whole body mass of *Bellamya bengalensis*' from all groups were collected after every seven days and were dried at 80^{oc} in an oven till costant weight was obtained. The sample were analysed on the instrument atomic absorption sepctrophotometer (Chemito). It was found that the group B snails showed higher concencentration of Cu bioaccumulation as compared to group C and group A. The bioaccumulation in whole body in snails those alloweded for self cure with and without caffeine showed recovery in bioaccumulation. The more recovery occure in snails those allowed with caffeine. In present studies the probable preventive role of caffeine is discussed in the paper.

Keywords: *Copper, Caffeine, Bioaccumulation, B.Bengalensis*

INTRODUCTION
Metals enter the fresh water bodies from a variety of sources, including: rocks and soils directly exposed to waters, dead and decomposing vegetation and animal matter, wet and dry fallout of atmospheric particulate matter and human activities, including the discharge of various treated and untreated wastes to the water body (Abo El Ella *et al.,* 2005). Metals are non-biodegradable and consider as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al.,* 2003). Aquatic organisms have ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharge of waste water (Goodwin *et al.,* 2003). The heavy metals have prolonged persistence i.e. non-biodegradability in the nature. Excess deposition of heavy metals and its accumulation in organism causes toxic effect over the body (Kaur, 2012 and Kwon, 2001).

Copper (Cu), an essential metal, is known to show evidence of undesired physiological effects both to humans and aquatic animals at levels beyond allowable limits. A four-day average copper concentration for freshwater aquatic life criteria by Environmental Protection Agency are 6.5, 12, and 21 µg/L for 50, 150, and 200 mg/L water hardness, respectively. Human consumption not exceeds 1 µg/L at any time (Watersheds' heavy metals 2007). Human cosumed mollusc as a food in some of the country and mollusk have ability to heavy metal accumulate in their tissues, which in turn make them accessible for human consumption through the food chain. It is therefore imperative to monitor them for public safety and other ecological concerns. Copper found to be essential for metabolism; consequently, if concentration enhanced may cause tissue damage (Brian, 2003).

Heavy metals are very difficult to remove from body; the damage of tissues caused by heavy metals may be recovered. Various antioxidants are use for recovery or reduce the damage of tissue due to heavy

metals. Vitamin C and E are common antioxidants in the diet. The caffeine molecule is a bitter alkaloid, which contributes to both acidity as well as the bitter properties of coffee. 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. The metals ions might be takenout of solution by binding to negatively charged groups of caffeine in the coffee granules. The molecules of caffeine being small (Mole wt.193.), 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 organism, and prevent the damage of tissues. Coffee is the most widely consumed natural beverage by the people around the world. It contains caffeine as major bioactive constituent along with caffeic acid, chlorogenic acid, kahweol palmiate, and cafestol palmiate as trace amounts. Caffeine acts as neurostimulator and exerts protective effect against genotoxic/carcinogenic activity of environmental chemicals in *in vitro* and *in vivo* assay system (Ferguson, 1994; Abrahm, 1984; Aeschbacher and Jaccaud, 1990; Stavric, 1992). These elaborate findings indicate caffeine is a chemopreventive drug against mutagens and carcinogens. The presence of higher amount of heavy metals in any part of the body will induce changes in biochemical metabolisms, serum biochemical changes, histopathological changes and other induced stresses. Therefore, the studies on the accumulation of heavy metals in various organs of the gastropod snails were very much important. Bioaccumulation alterations in snails under the influence of heavy metals can be used as a reliable indicator of aquatic pollution. 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 preventive role of caffeine in heavy metal bioaccumulation.

MATERIALS AND METHODS

The snail, *Bellamya bengalensis* were acclimatized to laboratory condition for 2-3 days and 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 to chronic concentration $LC_{50/10}$ value of 96 hr. of heavy metal salt, Cupric oxide (1.382 ppm) and group C were exposed to $LC_{50/10}$ conc. of CuO with 5mg/lit. caffeine upto 21 days. After exposure for 21 days to heavy metals, the snail's from group B were divided into two subgroups, such as D and E groups. The snail's of group D were allowed for self cure naturally in normal dechlorinated water while the snail's of group E were exposed to 5 mg/lit caffeine up to 21 days. During experimentation snails were fed on fresh water algae. The whole body mass of snails from all groups were collected after every seven days and were dried at 80^c 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 ($HCL:HNO_3$ 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 (Chemito). The concentration of Cu accumulation in the whole body tissue of each exposure period was recorded and the results are given in the table.

RESULTS AND DISCUSSION

Copper contents in *Bellamya bengalensis* after exposure to concentrations of Cupric oxide (1.382 ppm) up to 21days. After 7,14 and 21 days of chronic exposure to heavy metal, it was observed that there was an increased in concentration of accumulated heavy metals in the body of *B. bengalensis* with respect to time as compared to those of control snails.

The accumulation data from table and Figure indicates that the concentration of bioaccumulation Cu in presence of CuO (1.382 ppm) increased with increse in exposure period as compared to control. The Cu content is expressed in µgm/kg. dry weight. The control group of animals showed minute quantity of Cu as compared to the experimental groups. The control group of animal showed 656.0 µgm/kg, Cu in whole body tissue while the bioaccumulated Cu in presence of CuO (1.382 ppm)) after 7 days exposure was 1360.0µgm/kg. The concentration in the tissues was raised after 14 days to 2345.0µgm/kg, while after 21 days increases to 2890.0µgm/kg. The Cu content in snails those exposed in CuO LC50/10 concentration along with 5 mg/lit. caffeine is 969.0 µgm/kg , 1390.0 µgm/kg and 1950.0µgm/kg after 7, 14 and 21 days exposure respectively. There was more change in the bioaccumulation of Cu in control and heavy metal exposure group of animals. After exposure for 21 days to heavy metals, the snail's alloweded to cure naturally as well as with caffeine from group D and E showed less accumulation of Cu. The Cu content in less in group E snails as compared to group D due to 5 mg/lit. caffeine. The self cure group of snail in normal water showed 2475.0 µgm/kg,1995.0 µgm/kg, 1520.0 µgm/kg after 28, 35 and 42 days respectively. The snails groups of exposed in normal water with 5mg/lit. caffeine showed 1930.0 µgm/kg, 1472.0 µgm/kg and 1242.0 µgm/kg of Cu after 28 ,35 and 42 days respectively.

Table A): Copper Content (µGm/Kg Dry Weight) in Whole Body of *Bellamya Bengalensis* After Chronic Treatment of Cupric Oxide

Treatment	Tissue	Cu content (µgm/kg Dry Weight)		
		7 Days	14 Days	21 Days
(A) Control	W.B.	656.00	660.00	628.00
(B) 1.382 ppm CuO	W.B.	1360.00 + 7.04•	2345.00 + 16.85•	2890.00 + 22.65•
(C)1.382 ppmCuO + 5 mg/lit.Caffeine	W.B.	969.00 + 3.13•	1390.00 + 7.03•	1950.00 +13.22•

Table B): After 21 Days Exposure to 1.382ppm CuO Snails Alloweded for Cure Naturally and with Caffeine

Treatment	Tissue	Cu content (µgm/kg Dry Weight)		
		28 Days	35 Days	45 Days
(D) Normal Water	W.B	2475.00 +18.19•	1995.00 +13.35•	1520.00 + 8.92•
(E) Normal Water + 5mg/ lit. Caffeine	W.B	1930.00 +12.74•	1472.00 +8.12•	1242.00 + 6.14•

W.B.- Whole Body / • -Compared with respective A

Discussion

In the present study, the freshwater gastropod snail's *Bellamya bengalensis* were exposed to $LC_{50/10}$ concentrations of Cuo and LC 50/10 concentration of Cuo with 5mg/lit. Caffeine for twenty one days in the laboratory to examine the bioaccumulation of the heavy metals on their survival and their fate through their soft parts. From the obtained results it is clear that the analysis of the investigated gastropods snail's (whole body tissue's) indicated that those organisms exposed only in heavy metal salts, was more accumulate Cu in high concentrations in their whole bodies as compared to those exposed in heavy metal

salts with caffeine. After 21 days exposed snails alloweded for self cure in normal water and normal water with caffeine. The result showed that, the detoxification of heavy metal in animals those exposed with caffeine.

Bivalve are one of the most important groups of animal for metal bioaccumulation as was also evident from some of the earlier studies. *B*ryan (1980) reported that, heavy metals are among the pollutants causing fish mortality in both lotic and lentic water bodies, Cadmium could increase GSH concentrations by reducing glutathione peroxidase activity.

According to the Gundacker (1999), a zebra mussel accumulates high amounts of potentially toxic metals and was widely used as a bio-monitoring organism. Avelar *et al.,* (2000) reported that Oyster and mussels can accumulate Cd in their tissues at levels up to 100,000 times higher than the levels observed in the water in which they live.

Passow *et al.*, (1961) reported that lead can induce synthesis of specific proteins which selectively bind them. Inhibition of enzyme activities by heavy metals is either due to the direct binding with enzyme protein or due to damage of cell organelles or by toxic effect produced. The specific amoebocytes and or digestive vesicles within the cell may engulf metals outside the cell membrance (i.e. in the human digestive tract), then move back into the tissue carying their particulate burden (Owne *et al.,* 1966). The potential toxicity of Cu carbonate to snails dissociated through biological and chemical reactions. Carbonate would be available for shell development, Cu found to be accumulated in soft tissue.In juvenile apple snail *Pomacea paludasa* Cu found bioaccumulated in soft tissue (about 60% in the viscera and 40% in the foot) and its shell contained was <4% of total accumulated copper (Hoang and Rand, 2009 and Hoang *et al*., 2008).

Conclusion

The snails are the aquatic resources and mainly consumed by many people in the various contries. Hence, it is ascertain to know the suitability and safety for the people regarding their edible snails. This study was undertaken to view toxicological importance of these edible snails to know its variations in heavy metals contamination. Hence, care should be taken for avoid to reach the metal concentrations above the permissible limits. The heavy metals contamination not only affects the snails but also other aquatic life gets affected. Hence, a proper detoxification of heavy metals in effluent and municipal waste is necessary before discharge into the water body. In present studies conclude that, Caffeine has ability to detoxification and prevent the accumulation of heavy metal salts.

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