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***Research Article** (Open Access)*

CAFFEINE, PREVENT THE LOSS OF GLYCOGEN CONTENT IN VARIOUS TISSUES OF SNAILS, *BELLAMYA BENGALENSIS* (LAMARCK) AFTER ARSENIC INTOXICATION

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ABSTRACT
The present study was conducted to evaluate the effectiveness of caffeine (1,3,7- Trimethylxanthine) in arsenic induced toxicity in an experimental model, the fresh water gastropod snail's, *Bellamya bengalensis*. The effect on snail's was studied under five groups. Group A was maintained as control, group B of snail's were exposed to chronic $LC_{50/10}$ dose of As_2O_3 (1.069 ppm) for 21 days, while group C of snail's were exposed to respective chronic concentraction of arsenic trioxide along with 5 mg/lit of caffeine. Glycogen content in selected tissues of *Bellamya bengalensis.*from all groups were estimated after 7, 14 and 21 days. Snail's from B groups were divided into two groups after 21 days exposure to arsenic trioxide in to D & E groups. Snail's of D groups were allowed to cure naturally while those of E were exposed to caffeine (5 mg/lit.). Glycogen contents in selected tissues from these D & E group snail's were stuided after 7, 14 and 21 days. Significant decrease in glycogen content was observed in As_2O_3 exposed snail's as compared to control. The groups exposed to As_2O_3 along with caffeine showed more glycogen content in the tissues than those exposed to heavy metal. Pre-exposed snail's to As_2O_3 showed fast recovery and higher glycogen content in caffeine treated snail's than those which were allowed to cure naturally. The probable preventive role of caffeine is discussed in the paper.

***Keywords**: Caffeine, glycogen content, Arsenic trioxide and Bellamya bengalensis*

INTRODUCTION
The present day through the use of pesticides and fertilizers has increased to a great extent to increase agriculture production, but these are causing serious problem of water pollution as they reach the ponds, lakes, rivers and other water bodies along with run-off water. Concentration of toxic agents in the ecosystem has increased tremedously,specifically in terrestrial and aquatic environment. Balkas *et.al* (1982).reported cotamination of terrestrial and aquatic ecosytem by heavy metal and became serious enviornmental cocern.Heavy metals are highly active contaminants of biotic material, which enters the aquatic environment through a number of routes (Bryan and Langston,1992).

Fresh water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals. Therefore, increase of arsenic level in drinking water is the major cause of arsenic toxicity in the world. Reports of arsenic contamination in water are available from more than 30 countries in the world (Chakraborti et.al,1992).Arsenic contamination has been found in the States of Bihar, Uttar Pradesh, Jharkhand,Assam,Chhattisgarh and Andhra Pradesh (IARC,WHO,2004 and Nickson et.al,2007). Metals are into three categories [1] Non critical, for e.g. sodium, potassium, calcium, magnesium and iron, [2] Toxic but very rare, or very insoluble e.g. rare metals like thorium and [3] very toxic, soluble and relatively accessible e.g. selenium, arsenic, zinc, mercury, lead, copper, cobalt, nickel etc. classified (Wood, 1974). The metals in the 3rd group are highly toxic. Toxic heavy metal such as arsenic enter into the body of living organism including man through non-vegetarian and vegetarian diet and drinking water and accumulate in the tissues. A main problem in toxic effect of heavy metals is that they are very difficult to remove from the body of animal, because they are usually bound to some legends. The heavy metals bind to the cell membrane. Therefore, they are very difficult to remove from

cell membrane.Preventative effects of caffeine in rodent HCC models have been demonstrated (Balansky *et al.,* 1994 and kim and Lee, 1992). However, the exact molecular mechanisms by which caffeine exerts beneficial effects on hepatocarcinogenesis are poorly defined.

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; Guaguang and Liu, 2003 and Sevgi *et al.,* 2004).Nuenberg (1984) and Kiffney (1993) reported that,the molluscs were the best indicator of heavy metal pollution.higher and unlimmited concentration of heavy metal affected biological processes including feeding ,growth,reproduction and other metabolic activities leading to maturity of animal (Coughtrey, 1976).

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 detoxificatioin.

MATERIALS AND METHODS

The snails, *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} value of 96 $hr/_{10}$) of heavy metal salt, As_2O_3 (1.069 ppm) upto 21 days, while snail's from group C were exposed to chronic concentraction of along As_2O_3 with 5 mg/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 snail's were fed on fresh water algae. The hepatopancreas and gonads of snail's from all groups were collected after every seven days and were dried at 80^0 C in an oven till constant weight was obtained. Glycogen contents in the dried tissues were estimated by the method of Dezwann and Zandee (1972) using anthrone reagent and glucose as standard. The values of glucose obtained were converted to glycogen values by multiplying with the factor 0.927.

RESULT

Glycogen contents in different tissues of *Bellamya bengalensis* after exposure to As_2O_3 (1.069 ppm) along with caffeine and during recovery have been summarised in tables.

Table-A, shows , that the glycogen contents in tissues of hepatopancreas and gonads of *Bellamya bengalensis* in presence of As_2O_3 (1.069 ppm) decreased with the increase in exposure period as compared to those exposed to heavy metal salt; arsenic trioxide along with 5 mg. Caffeine / lit.

Table-B, shows, the snail's preexposed to heavy metal salts showed fast recovery in the alteration of glycogen in presence of caffeine than those allowed to cure naturally.

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 biochemical energy to counteract the toxic stress. Heavy metal salts affect the metabolism of the fresh water snails, *Bellamya bengalensis*. 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 .

Table A: Glycogen Content In Selected Tissues Of Bellamya Bengalensis After Chronic Exposure To Heavy Metal Salts, AS_2O_3 without And With Caffeine.

Treatment	Sr No.	Body Tissue	The Glycogen content (%) ± S.D.		
			7 Days	14 Days	21 Days
(A) Control	I	H.	1.0887 ± 0.0007	1.0665± 0.0005	1.0665 ± 0.0010
	II	G.	0.6666 ± 0.0008	0.6443± 0.007	0.6221 ± 0.008
(B) 1.069 ppm As_2O_3	I	H.	1.0443 ± 0.0005*** - 4.251•	1.0221 ± 0.007*** - 4.343•	0.9999 ± 0.0006*** - 6.660•
	II	G.	0.6221 ± 0.0008*** - 7.153•	0.5999 ± 0.0005*** -7.401•	0.5777 ± 0.0007*** - 7.685•
(C) 1.069 ppm As_2O_3 + 5mg/lit Caffeine	I	H.	1.0665 ± 0.0005*** -2.081•, +2.081Δ	1.0443 ± 0.0005*** -2.125•, +2.125Δ	1.0443 ± 0.0008*** -2.125•, +4.251Δ
	II	G.	0.6443 ± 0.0000*** -3.461•, +3.445Δ	0.6221 ± 0.0010*** -3.568•, +3.568Δ	0.5999 ± 0.0008*** -3.700•, +3.700Δ

Table B: After 21 Days Exposure To 1.069 As_2O_3

Treatment	Sr No.	Body Tissue	The ascorbic acid content (%) ± S.D.		
			28 Days	35 Days	45 Days
(D) Normal Water	I	H.	1.0221 ± 0.0007*** - 6.515•, + 2.171□	1.0443 ± 0.0005*** - 2.125•, + 4.251□	1.0665 ± 0.0005NS - 0.000•, + 6.244□
	Ii	G.	0.5999 ± 0.0008*** - 11.118•, + 3.700□	0.6221 ± 0.0005*** - 3.568•, + 7.137□	0.6221 ± 0.0010NS - 0.000•, + 7.137□
(E) Normal Water + 5mg/ lit. Caffeine	I	H.	1.0443 ± 0.0005*** -4.251•, + 4.251□	1.0443 ± 0.0010*** - 2.125•, + 4.251□	1.0887 ± 0.0007NS - 2.039•, + 8.156□
	Ii	G,	0.6221 ± 0.0010*** - 7.153•, + 7.137□	0.6443 ± 0.0003NS - 0.000•, + 10.336□	0.6443± 0.0007*** - 3.445•, + 10.336□

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

The storage of carbohydrate in invertebrates and vertebrate body is important as they have reserves of calories (energy) for the body tissues. Glycogen (animal starch) accumulates in liver and is an important

reserve fuel found in the all higher invertebrate and vertebrate. The glycogen stored in the digestive gland, renal organ was used as energy source. Decrease in stored glycogen indicates a change in carbohydrate metabolism, which might be due to enhancedglycolysis to meet the high energy demand during toxic stress and overcome this stress, stored glycogen, was used. The biochemical components in *Bellamya bengalensis* after exposure to Malathion and showed that glycogen content in digestive gland decrease significantly (Lomte and Alam, 1982). The effect of sublethal concentration of sumithion on some biochemical constituents of the snail, *Pila globosa* and found a decrease in glycogen content. Other researchers reported the depletion of tissue glycogen and also decrease in the glucose 6- phosphate activity (Goel and Agrawal, 1982). Other researchers observed depletion in glycogen content in digestive gland of snail, *Thiaratuberculate* when exposed to mercuric chloride (Muley and Lomte,1992).

In present study, in the *Bellamya bengalensis* the glycogen contents in the selected tissues was decreased in chronic concentration of As_2O_3 as compared to the control and $LC_{50/10}$ concentration with caffeine 5 mg/lit.Due to caffeine has antioxident activity. This activity of caffeine can protect the damage of tissue, chemicals and gentic material from heavy metal generated free oxygen radicals. In Mc.Mag Lin 2000 Australia has found that caffeine has the capacity to bind with heavy metals. Heavy metals ions are positively charged, and caffeine contains unchrged and negatively charged molecules, the metal ions may form a chelate to negatively charged group of caffeine molicule. This reduces the charged aactive heavy metal ions and hence a activirty of heavy metal. The caffeine is diuretic and has low molecular wieght .There property of caffeine indicates that caffeine can have the capacity to remove the heavy metals from the living organism. caffeine is found in the routine beverages. Such as tea, coffee and some cold drinks. The caffeine contents of a cup of tea varies from 50 to 80 mg. Caffeine being water soluble and common cheaper beverage, it will be cheapest reventive and curative medicine. In 1982, Takayamas, long term study on the effect of caffeine in Wistar rats, *Gann* has proved that caffeine belongs to a group of compound known methylxanthine and it is not carcinogenic in animal model. It has also been reported to antagonize the carcinogencic effects of chemicals in vitro. Under in vitro condition, caffeine has been reported to enhance or inhibit tumorgensis induced by various crcinogenic agents. 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 are related to inhibition of the protein kinase activities of ATM and ATR and that both proteins are relevant targets for the development of novel anticancer agents (Sarkaria, *et al.*, 1999). Caffeine increases endurance and attenuates force sensations during the first 10-20 second of concentration. The rapidity of this effect suggests that caffeine exerts its effects naturally (Plaskett and Cafarelli, 2001). Caffeine has been found to increase glutathione synthetase and reduced glutathione in liver and lungs of mouse (Shelar, 2002). 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. 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.

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