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

CAFFEINE IS THE REMEDY FOR PROTECT EFFECT OF ARSENIC ON HISTOLOGICAL STRUCTURE OF HEPATOPANCREAS IN FRESH WATER BIVALVE, *LEMELLIDENS MARGINALLIS*

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ABSTRACT
To study remedial role of caffeine (1,3,7-Trimethlaxanthine) against arsenic trioxide induced histopathological alteration on hepatopancreas in an experimental model the fresh water bivalve,*Lamellidens marginallis*. The effect of As_2O_3 and caffeine were studied under three groups. Group A bivalves was maintained as control, Group B was exposed to chronic $LC_{50}/_{10}$ doses of $CuSO_4$ (2.12 ppm) for 18 days, while group C bivalves were exposed to respective chronic concentrations of heavy metals with 5 ml/lit caffeine. The changes are observed at 6, 12 and 18 days. In hepatopancreatic structure showed more disintegration of basement membrane due to damaged epithelial cells, disruption of hepatic tubules, and increase in internal luminar area in bivalves exposed to copper sulphate as compared to those exposed in copper sulphate along with caffeine. The probable remedial role of caffeine is discussed in the paper.

***Keywords**: Lamellidens marginallis, copper sulphate, Hepatopancreas, Histopathology, Caffeine*

INTRODUCTION

Bivalves can take up contaminants from sediments, suspended particulate materials, water column and also food sources (Laffon *et al.,* 2006 and Livingstone, 1993). They have been widely used for many years as bioindicator organisms in monitoring of chemical pollutants and biomonitoring in aquatic ecosystems. This is particularly due to their sedentary nature or immobility, filter-feeding activity, low metabolism, contact with sediments, suitable size for biochemical analysis, wide distribution in marine, estuarine and freshwater environments, practicality in collection, ability to bioaccumulate pollutants and high tolerance to chemical exposure due to a remarkably active immune system (Emmanouil, Kypriotakis, Kungolos, & Machera, 2008; Gupta & Singh, 2011).

Copper sulphate is frequently used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae, and to control certain fish diseases (Tucker, 1990). Consequently, treating plankton with copper compounds might lead to copper bioaccumulation reaching a toxic stage in fish. The toxic effect of copper is correlated to its aptitude for catalyzing oxidative reactions, leading to the production of reactive oxygen species (Lopes *et al.,* 2001). Contamination of fresh water with a wide range of pollutants has become a matter of concern over last few decades (Vutukuru, 2005). Heavy metals have devastating effects on ecological balance of the recent environment and a diversity of aquatic organisms (Farombi *et al.,* 2007). 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 main constituent a coffee is caffeine. 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. Dissolved heavy metal ions are positively charged and caffeine contains uncharged and negatively charged groups. The metals ions might be taken out of solution by binding to negatively charged groups of caffeine in the coffee granules.The molecules of caffeine being small (Mole wt.193.), its 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).The aim of our study was to determine the histological effect in the hepatopancreatic cells of freshwater bivalves, *Lamellidens consobrinus* after heavy metal stress.

MATERIALS AND METHODS

Chronic study

Lamellidens consobrinus are the fresh water bivalves occurring abundantly in Hartala pond at Hartala, Dist-Jalgaon of Maharashtra.For acute studies LC50 (lethal concentration with 50% mortality) was calculated for 96 hours (APHA, 2005) by probit regression analysis (Finney, 1971). Water was replaced every day and fresh toxicant was added after water renewal for 96/10 hours. Bivalves were monitored at regular intervals and dead bivalves were removed from aquaria. A total of 30 mussels were collected and divided into three groups of 10 each. The first group A was consisted by control bivalves while the second B group was exposed to $LC_{50/10}$ concentration of 96 hrs. CuSO4 (2.12 ppm) and groupd C bivalves exposed to $LC_{50/10}$ concentration of 96 hrs. CuSO4 (2.12 ppm) along with 5 mg/lit. Caffeine upto 18 days.

Histological studies

The experimental bivalves from A,B and C group were dissected after 6,12,and 18 days. The hepatopancreas from all experimental group were removed and fixed in Bouin's fluid for 24 hours;washed and dehydrated in alcohol grades, cleared in toluene and embedded in paraffin wax (58-60^0c). Serial sections of 5 u thickness were cut and stained with Mallory's triple stain. The stained sections were examined under light microscope for histopathological impact of heavy metal salts. Hepatopancreas of bivalves from all groups of control and exposed were screened and data is presented and compared.

Tissue processing

The removed wet tissue of digestive gland from all groups were homogenate in blender with M/150 phosphate buffer at 1-4^0c and centrifuge.stir sediment with cold phosphate buffer and allows standing in the cold with shaking occasional then repeating the extraction once or twice and using the supernatant for assay of catalase.

RESULTS

The accumulation of heavy metals in the body of the organism leads to the formation histological lesions in the organs. chronic exposure of *Lamellidens consobrinus* to copper sulphate causes severe histological

lesions in the hepatopancreas as compared to those exposed to $LC_{50/10}$ concentartion of $CuSo_4$ with 5mg/lit. Caffeine and control group of bivalves.

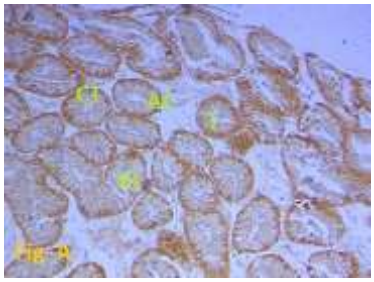
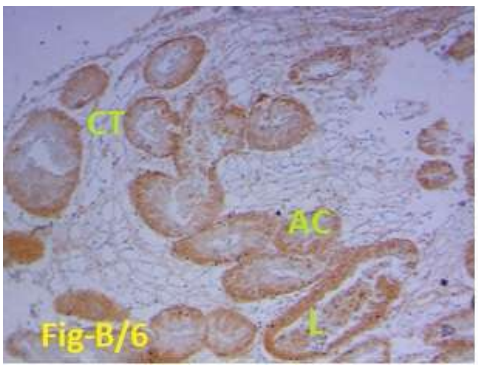
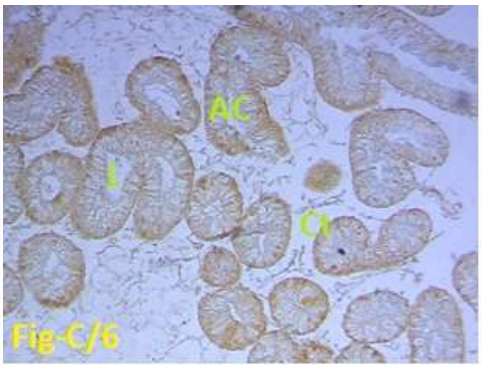
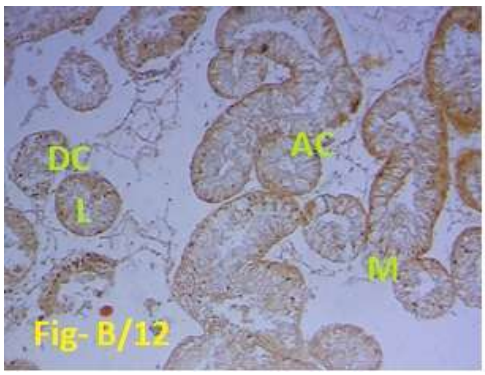
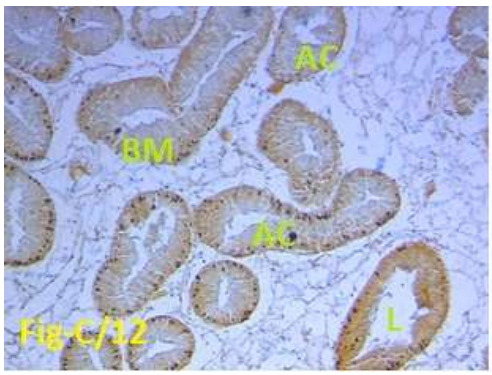


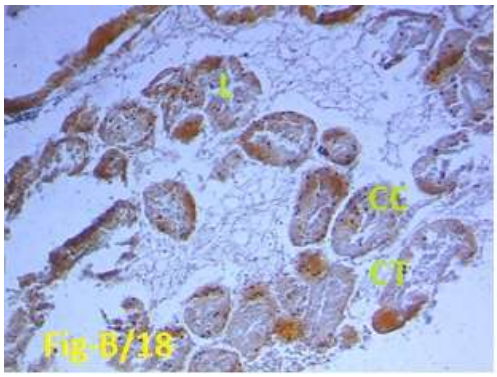
Figure 1A: Normal histological structure of hepatopancreas











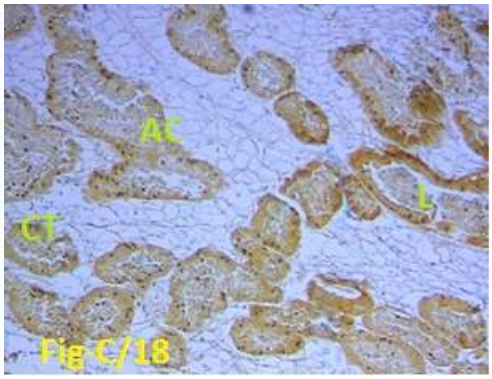


Fig-B/6,B/12 and B/18
Exposed to LC50/10 conc.of $CuSO_4$

Fig-C/6,C/12 and C/18
LC50/10 conc.of $CuSO_4$+5mg/lit.caffeine

Histological study shows different types of cells in hepatic lobules , the typical liver cells or digestive cells (columnar epithelial cells) and secretory cells,absorptive cells,blood lacunae. The cells rest on basement membrane. The lumen present inside the lobules. The lobules of the gland are bound together by the thin connective tissue layer as shown in fig. A . The deformity in the cytoarchitecture of hepatopancreas due to heavy metal stress definitely reflect in it's metabolic activity.The histological changes in the hepatopancreas of *Lamellidens consobrinus* exposed to $LC_{50/10}$ concentration, 2.12 ppm for 6 days,12days and 18 days of $CuSO_4$, show in fig.B/6,B/12 and B/18. The deformity in the histologic structure of digestive gland is more in 18 days exposure group of bivalves as compared to those bivalves exposed to $LC_{50/10}$ concentration of $CuSO_4$ with 5mg/lit. caffeine and control group.

DISCUSSION

The alterations observed in histo-architecture in the various tissues are tissue specific and time dependent.When *Channa punctatus* was exposed to mercuric chloride for a prolonged span of 30 days profound histological changes in the liver were observed which induced necrosis, vacuolation and degeneration of hepatocytes (Sastry and Gupta, 1978).The histological techniques are the promising area of research in aquatic toxicology as it gives the real picture of the effects imposed and the involvement of the xenobiotics in either disturbing or destroying the vital organs of living organisms. Many workers have reported the degenerative changes in selected tissues of the animals in response to pollution by various toxicants (Shaikh, *et al.,* 2010; Andhale, *et al.,* 2011).Victor *et al.* (1990) observed histopathological changes in the hepatopancreas of *P. hydrodromous* in response to cythion resulting in reduction in the height of tubular epithelium, enlargement of lumen, vacuolation and atrophy. The histopathological changes indicated that the animals were not able to digest and store food properly. The exposure of the bivalves to $LC_{50/10}$ concentrations of heavy metals ($CuSO_4$) caused varying degrees of histological alterations in the organs examined hepatopancreas. In the hepatopancreas, there was a prevalence of hepatocellular foci of cellular alterations (FCA) in bivalves exposed to $LC_{50/10}$ concentrations of copper sulphate. Peripheral thickening and inflammation of hepatic tubules of the hepatocytes were also observed in exposed bivalves .Less damge in histological structure in bivalves those exposed to $LC_{50/10}$ concentration of copper sulphate with 5 mg/lit.caffeine .Over all study indicates that, caffeine has a protective and curative role in the heavy metal induced alterations. The damages in the histological structure of hepatopancreas are less in presence of caffeine and the recovery from the heavy metal induced damage in the structure of hepatopancreas was faster in presence of caffeine.

Caffeine has antioxidant activity. This activity of caffeine can protect tissues from heavy metal induced free radical damage of the tissues. Shomer (1994) studied caffeine stimulation of malignant hyperthemia-susceptible sarcoplasmic reticulum Ca2+ release, and suggested that caffeine sensitivity of malignant hyperthermia-susceptible (MHS) skeletal muscle fiber bundles is due to an altered caffeine sensitivity of the MHS calcium ion release channel protein. Chung Fung- Lung (1999) reported that caffeine when given in drinking water at a concentration indentical to that found in 2 % tea was able to inhibit lung tumours induced by 4-(Methylnitrosoamino)-1- (3-pyridyl) - 1 butanol (NNK).Hosta *et. al;* (2001) has studied hepatocarcinogensis inhibition by caffeine in AGI rats treated with 2-acetylaminoflurence and has showed that caffeine inhibited hepatocarcinogensis induced by 2-acetylaminoflure. Break of DNA strands in hepatopancreas of mussels exposed to resin acids was reported by Gravato *et al.,* (2005).

The results of the present study indicated the positive role in detoxification of heavy metals by caffeine. It's antioxident property, capacity to chelate the heavy metal and induced increase in urine formation mey be the cause of hepatoprotection.

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