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Histopathological changes in the hepatopancreas of *Lamellidens marginalis* exposed to cadmium in winter

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Abstract
In the present study, the destruction of the basement membrane of the hepatic tubules and cell surface of the digestive cells after cadmium exposure probably caused disfunction of the surface receptors resulting in the disturbance of the lysosomal system functioning.

Keywords: Cadmium, bivalve molluscs, toxicity.

INTRODUCTION

The bivalve molluscs appear to have particularly high capability for concentrating metals in body along with other foreign materials found in their environment when they filter food particles during feeding. Because of the ability of many metals to form complexes with organic substances there is tendency for them to be fixed in the different tissues and not to be excreted so they have a large biological half life, therefore one of the major problem that metal posses with respect to their effect on aquatic organisms [1].

Pollution an undesirable changes in the physical, chemical and biological characteristic of air, water and soil, which is harmful to living organisms. Whatever may be the mode of contamination polluted environment is not suitable for existing life forms.

Cadmium accumulated in various parts of the body (liver, pancreas, kidney etc.) is known to cause painful bone disease called Itai-Itai. India is one of the country which shows marked increase in cadmium level in industrialized area specially Ludhiana and Faridabad of Punjab and Haryana states respectively. The survey of literature on cadmium toxicity clearly shows that this heavy metal is implicates as sensitive agents of several pathological disorders both in laboratory animals and human beings. Biologically cadmium is neither essential nor beneficial.

The bivalve's mollusks are known to be used as indicators of contamination [2, 3, 4 and 5]. It is becoming important to have a broad understanding of metal levels in a large number of bivalve mollusks from different habitats to establish a base one for comparison with possible future contamination in marine estuarine and freshwater ecosystems [6 and 7].

Background levels of cadmium in food, water and ambient air may not be as health concern for the general population except for smoking individuals or individuals in industry producing or using cadmium. Chronic exposure, however, can be a major concern because cadmium has a tendency to accumulate, primarily in the kidneys and liver and affect primarily the kidneys, cardiovascular system, and skeletal system [8 and 9]. Cadmium also has been associated with an increased frequency of prostate carcinoma [9, 10,11 and 12].

MATERIALS AND METHODS

The bivalve molluscs, *Lamellidens marginalis* inhibiting is an enclosed water body in Kutluq Lake at Daultabad, District Aurangabad, is chosen for the study. After collection of the animals from habitat they were immediately transported to the laboratory. After bringing to the laboratory, the fouling biomass and mud on shell valves were removed without disturbing the siphonal regions. The equal sized animals (90–100mm shell length) were grouped and kept in sufficient quantity of water (animal / liter) in aquaria with aeration for 24 hrs. to adjust the animals to laboratory conditions (with renewal of water at interval of 12 to 13 hrs). No food was given during this time and during experiments. After 24 hrs animal of equal size (90–100mm shell length) were grouped in 10 and exposed to different test concentrations of cadmium for static bioassay tests.

The stock solution of cadmium was prepared by dissolving appropriate quantity of cadmium chloride ($CdCl_2$. 2 $\frac{1}{2}$ H_2O AR Grade CDH Bombay) in double distilled water. The pH of the water is brought between 6.9 to 7.1 by adding 1N HCl (due to insolubility of cadmium in reservoir water having 7.6 to 8.1). Appropriate test concentrations were then prepared and animals were exposed. The experiments were conducted in natural day-night rhythm. The experiments were repeated three times for confirming observed Lc_0 and Lc_{50} values. The 96 hrs. acute test was recorded.

After 96 hrs. acute toxicity test using Cd for histological studies, hepatopancreas from control, Lc_0 and Lc_{50} groups were fixed in aqueous Bouin's Hollande fluid for 48 hrs. The dissected tissues were dehydrated with alcohol and toluene and embedded in paraffin wax (58 to 60^0). Hepatopancreas sections were cut down at 6 - 7µ.

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For histopathological study of hepatopancreas Mallory's triple stain was used. All the photomicrography was made under light-microscope Labo VT – 20 Scan model.

RESULTS AND DISCUSSION

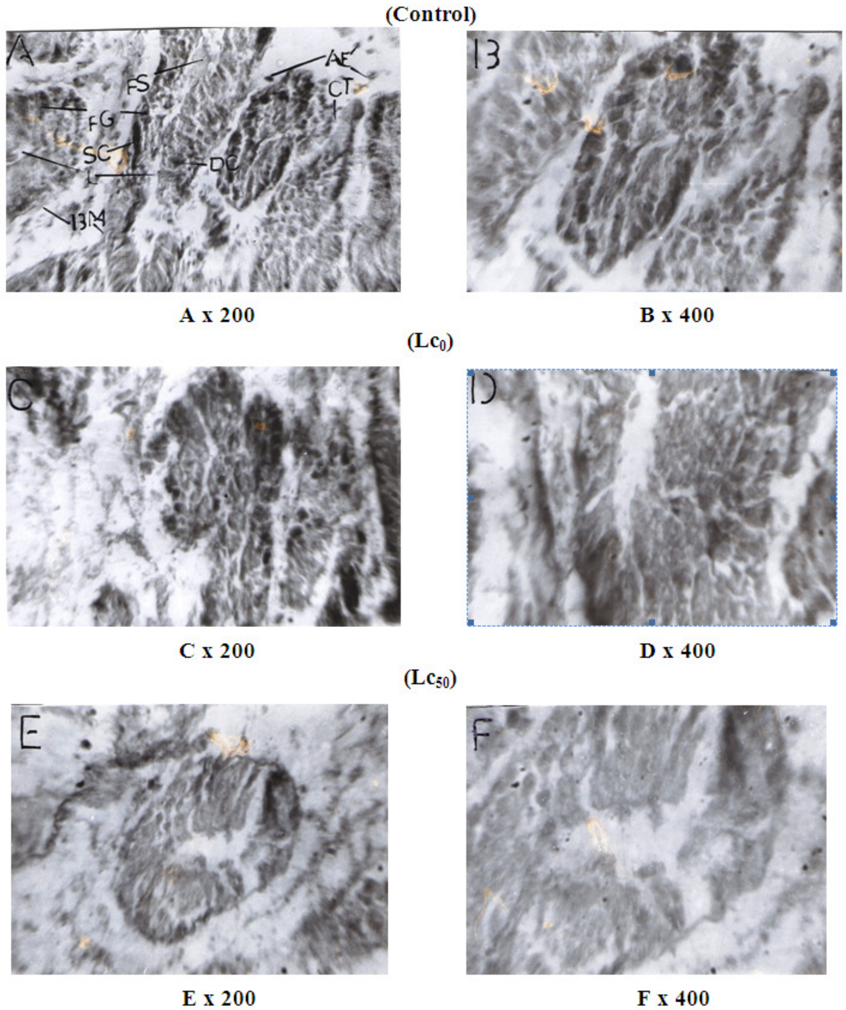
The changes are hepatic tubules due to cadmium toxicity along with control are shown in Figs. A – F. In (control) group, *Lamellidens marginalis* the hepatopancreas consists of ducts and digestive tubules group in the form of bundles indistinctly separated and connected inter-lobular connective tissue of collagenous fibres. Each tubule is bounded by thin muscle fibres which form the basement membrane. Each digestive tubule consists of digestive cells or columnar type, vacuolated and acidophilic, and secretary or pyramidal type and basophilic type cells.

Lc_0

In Lc_0 group the tubules held original shape as in control but the basement membrane was completely dissoluted the fragmentation spherules appeared mucoidal.

Lc_{50}

In Lc_{50} groups, the effect of cadmium was more pronounced vacuolization was more evident than the Lc_0 group. The tubules were completely distorted, lost normal shape and appeared lengthened. Karyolitic and necrotic conditions were visible. The amoebocyted almost disappeared from the connective tissue and totally infiltered tubules.



BM = Basement membrane, L = Lumen, CT = Connective tissue, AC = Acidophilic cells , DC = Digestive cells, BC = Basophilic cells

Fig. Histological changes in the hepatopancreas of *Lamelliden marginalis* exposed to cadmium in winter

Histologically, the hepatopancreas possess basal oval nuclei with prominent nucleoli. The acidophilic cells are responsible for intracellular digestive of food referred to as digestive cells [13]. The cells in the digestive tubule accept the food particles and are responsible for absorption and intracellular digestion. It has been well established that particulate material from the lumen of the tubule is taken up by digestive cell by pinocytosis and the pinocytotic vacuoles so formed appear to fuse giving rise to heterophagosomes [13]. The fate of the residual bodies has been shown to vary from species to species but in *Mytilus edulis* they migrate to the apical region of the cell and are rejected into the tubule lumen. Similar structure is also found in present study, the residual bodies or fragmentation spherules were comparatively more in the lumen in monsoon than in winter and summer. Large number of amoebocytes and heamocytes are also found the interlobular connective tissue. The lumeny of the tubule increases or decreases on the basis of the amount or food particles accepted for digestion. During this process fragmentation spherules are budded off from the apex of the digestive cells into the lumen. Haematocyte acculumation in between the hapatopancreas tubules in supposed to act as a plug to repair the damage [14]. The amoebocytes are known to take part in the process of digestion and mercury is reported to inhibit number of enzymes in vitro including lysosomal enzymes [15]. In the present study it was observed that cadmium affected the amoebocytes, the affect was disintegrated to cells in Lc_{50} group.

The result revealed that the severity of affect was more pronounced in Lc_{50} groups than in Lc_0 groups. The clumping of food particles, swelling of the tubules in Lc_0 group and distorted tubules in Lc_{50} group, and infiltration of the amoebocytes in the lumen of the tubules in Lc_0 group and total disappearance of these cells in Lc_{50} group. It is pertinent to note that acute toxic effect of cadmium brought significant increase in the physiological demand through biochemical reserves [16]. These mussels are known to respire at a higher rate and commence reproduction in monsoon soon after first few rains [16]. In the bivalves the cells of the tubules and the ducts are actively involved in absorption and intracellular digestion of food material [17].

Vacuolization in the tubule cells as a result of stress has been reported in bivalve mollusks [18 and 19]. This could be a step towards the detoxification process that the liver is responsible for. Disintegration of tubules and necrosis of hepatic cells as a result of stress is also a common feature in many aquatic animals [20]. Loss of cytoplasm organization and integrity seriously affect the functioning of hepatopancreas and may prove fatal to animals [21]. Akarte (1985) [22] observed that the pesticides like Folithion and Lebaycid were effective in all the season to Indonaia caeruleus and *Lamellidens marginalis* to cause cellular destruction of hepatic tubules. The changes were loss of conceptive tissue, shrinked or desquamated basement membrane, necrosis or loss of orientation of digestive or secretory cells, vacuolization or swelling of cells and pinosis or karyolysis. However, the degree of such deteriorative effects varied with season.

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