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Effect of Cadmium on the Histology of Hepatopancreas and Foot of the Freshwater Mussel *Lamellidens marginalis* (Lam.)

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ABSTRACT

Histopathological studies would help in evaluating the extent of damage caused to the tissues under stress. Hence, in the present study an attempt has been made to observe the structural changes in the hepatopancreas and foot of the mussel *Lamellidens marginalis* exposed to acute (11.0mg/L) and subacute (1.10mg/L) concentrations of cadmium. The changes are observed at day 1 and day 3 in acute concentration and day 10 and 30 in subacute concentration, one at short- term and the other at long-term exposure. In hepatopancreas enlargement of intertubular area, cell necrosis and the formation of sinuses and in foot loss of connective tissue, vacuolization, disarrayed fibres and nuclear pyknosis were seen on exposure to acute concentration of cadmium at day 1. Similar changes with mild degree of recovery were observed at day 3. In subacute concentration some degenerative changes like mild tubular atrophy and cell necrosis in hepatopancreas and fibrillation, intermuscular oedema and pyknotic nuclei in foot of mussel were observed at day 10, however, at day 30 the tissues appeared more or less similar to those of controls with insignificant changes.

INTRODUCTION

The physico-chemical properties of heavy metals in aquatic systems are the principal factors for their accumulation in animals (Davyd Kova et al. 2005). Heavy metals are posing great threat to the health of Indian aquatic ecosystems due to their toxicity and accumulation behaviour. Many effluents, discharged into nearby ponds and drains without any treatment, contain highly toxic dyes, bleaching agents and heavy metals (Mathur et al. 2005). The common feature of these metals is that they can be easily accumulated by the aquatic biota and become relatively toxic even at fairly low concentrations. Some of these metals are essential in limited quantities to normal growth of animals and human body. Even essential metals are toxic if concentration exceeds the normal requirement. Metals like mercury, copper, cadmium, lead, chromium, nickel and zinc are very toxic, of which except copper and zinc the others are non-essential. Cadmium is the second member of Group II B triad (Zn, Cd, Hg) in the periodic classification of elements. It has the atomic weight 112.4, atomic number 48, density 8.6, melting point 320.9°C and boiling point 765°C. It is a hexagonal crystalline, silver-white malleable metal with stable oxidation state +2. It has a medium class B character compared to zinc and mercury. This character imparts moderate covalency in bonds and high affinity for sulphydryl groups leading to increased lipid solubility, bioaccumulation and toxicity. The chloride, sulphate and nitrates of cadmium are soluble compounds whereas carbonate and hydroxides are not. Cadmium is one of the most toxic and widespread heavy metals, and is a recognized carcinogen in mammals (Pruski & Dixon 2002). There has been rapid and continuous increase in the worldwide production and use of cadmium since 1925. It is used in a number of industrial processes. Because of its ability to protect iron rusting, it is used for coating items of iron by electroplating. Cadmium

coated parts of automobiles are most resistant to rust than zinc coated (galvanized) objects. Cadmium sulphide is used as colour pigment in plastics and in various types of paints. Cadmium stearate is used as a stabilizer in plastics. Cadmium reaches the water bodies primarily from the industrial sources such as zinc melting and electroplating, combustion of fuels, plastics, phosphate fertilizers, pesticides, domestic wastes, oil refineries, etc.

The study of environmental effects of cadmium is now very timely. Cadmium has become the focus of intense research globally because of its toxicity to humans and terrestrial and aquatic organisms. Exposure to cadmium causes anaemia, and hepatic, renal and cardiovascular diseases. Cadmium accumulated in endocrine glands exerting its effect on gametes quality and reproductive functions, and its role as apoptotic factor was evidenced in different cell types and tissues (Migliarini et al. 2005). The reports available though indicate an imbalance in metabolic homoeostasis of animals exposed to cadmium, studies on histology are scanty. As the prime recipients of cadmium contaminated effluents are freshwater bodies and the shellfish inhabiting them are first prone than the finfishes to the effects of it. The authors feel necessary to make a histopathological study on the effects of cadmium in a freshwater shellfish, the bivalve *Lamellidens marginalis.*

MATERIALS AND METHODS

The freshwater mussels weighing 25 ± 2 g were collected from the local ponds and lakes and maintained in laboratory in 5' × 3' × 3' cement tanks. Water from the local wells was used for their maintenance. It has temperature 28 ± 1°C, pH 7 ± 0.1, total hardness 100 ± 5 mg/L, chlorinity 0.08 ± 0.003% and dissolved oxygen 5.8 ± 0.4 mg/L (Sivaramakrishna & Radhakrishnaiah, 2000). The mussels were fed *ad libitum* with freshwater plankton. The animals were adapted to laboratory conditions for ten days prior to the experimentation.

A stock solution of cadmium was prepared by dissolving 2.74g of cadmium nitrate in one litre of distilled water, which consists of 1 g of cadmium. Appropriate amount of stock solution was taken to obtain the desired concentrations of cadmium. 96h LC_{50} was determined by exposing the mussel to different concentrations of cadmium (Finney 1971). Based on the percent and probit mortality curves as well as through Dragstedt and Behren's method, the 96h LC_{50} obtained for the mussel was 11.0 mg/L. This concentration was considered as acute, and one tenth of it, i.e., 1.100 mg/L was considered as subacute concentration. Further, as the period of exposure is an important factor in assessing the effects of a metal on an organism, 1 and 3 days in acute and 10 and 30 days in subacute were selected to observe short-term and long-term effects. Controls were maintained alongside for comparison. After the period of exposure the mussels were sacrificed and hepatopancreas and foot were dissected and transferred into 10% formalin solution for histological sectioning. The block making, section cutting and staining were done by adopting the procedure as described by Humason (1972).

RESULTS

The histological sections of hepatopancreas and foot of mussels exposed to acute concentration of cadmium at day 1 and 3 and subacute concentration at day 10 and 30, besides those of control animals, were photographed and are presented in Figs. 1 and 2. The healthy hepatopancreas is a large dark brown or green gland of irregular shape that occupies the dorsal part of the mantle cavity surrounding the stomach. The section of the control animal showed a compound branched blind ending tubules connected with primary and secondary ducts. The tubules appeared either round or oral, and were lined with a single layer of epithelial cells on a prominent basement membrane. In

some tubules the outline of the lumen was undulated owing to a variation in the height of the lining cells. The primary ducts of the hepatopancreas possessed wide lumens, each comprising of simple columnar epithelium. The nuclei of them were oval in shape. Basally, some vacuoles were observed in the apical pole of these cells. The tubules and ducts were surrounded by loose connective tissue. The intertubular space was less. The digestive and calcium cum excretory cells were darkly stained under the basement membrane (Fig. 1a).

Figs. 1b and 1c represent the sections of the hepatopancreas of the mussel at day 1 and 3 of exposures respectively to acute concentration of cadmium. At day 1 of exposure greater degree of damage to the intertubular connective tissue was prominently noticed. The tubules appeared as small masses of cells which is a characteristic feature atrophy. Viable cells were not found, necrotic changes appeared leading to the fragmentation of the basement membrane and maximum loss of structural and functional organization of the hepatopancreas (Fig. 1b). At day 3 exposure mild degeneration in the intertubular connective tissue was observed with a rupture in the basement membrane. The number of tubules reduced with shrinking of tubular diameter; but their lumen was found enlarged. Similarly, the intertubular spaces were also less enlarged (Fig. 1c). In subacute concentration, the histological damage was less when compared with acute concentration of cadmium both in hepatopancreas and foot of mussels. Figs. 1d and 1e represent the sections of the hepatopancreas at 10 and 30 days of exposures respectively. A mild degree of degenerative changes like the loss of basement membrane of hepatic tubules, atrophy and cell necrosis, with partial dissolution of the intertubular connective tissue were noticed at day 10 (Fig. 1d). At day 30 exposure the structure of the hepatopancreas was not much disturbed, and appeared almost similar to the structure of control. But the intertubular connective tissue was found not compact and there was a certain degree of vacuolization in the intertubular spaces. However, the tubules appeared round/oval in shape surrounded by the basement membrane. Appearance of digestive and calcium cum excretory cells beneath this membrane was also noticed (Fig. 1e).

The ventral muscular foot of the control mussel is a distinct tissue from rest of the body. It is the locomotory organ chiefly employed for burrowing. The wedge shaped foot was adapted for progression in mud or sand where the animal lives. The section of foot taken from the control animal clearly revealed the organized arrangement of dorsoventral muscle bundles that are compactly packed. The transverse muscles were also thickly packed in the connective tissue and were seen running over the dorso-ventral muscle fibres. Uniform round to spindle shaped nuclei were found distributed. The intermuscular spaces consisted of pedal sinuses or haemocoelic cavities. The muscle bundles were thick and dense showing no mark of splitting of fibres.

Figs. 2b and 2c represent the sections of foot of the mussels at 1 and 3 days respectively on exposure to acute concentration of cadmium. At day 1 exposure drastic degenerative changes like splitting of muscle bundles and severe degree of muscular atrophy were observed. The connective tissue has lost its integrity leading to the loosening of the muscle bundles. The nuclei became distorted in shape and exfoliation was noticed at certain places. The muscle bundles appeared pallid and toneless, and showed intramuscular oedema (Fig. 2b). The foot of mussels at day 3 exposure also clearly exhibited some degenerative changes. They included a disarrayed condition of the muscle bundles and dissolution of sinuses. Most of the nuclei were spindle shaped and showed a pyknotic tendency. The muscle fibres showed oedematic condition and tendency of splitting down to fibrillar structures (Fig. 2c). Figs. 2d and 2e represent the sections of the foot of mussels at 10 and 30 days respectively in subacute concentration of cadmium. A moderate degree of destructive changes like

fibrillation, atrophy of muscle bundles, intermuscular oedema and pyknotic nuclei with less viable cells were seen at day 10. The transverse muscle fibres were also not conspicuous. Thus, there was some disorganization in the structural integrity of foot musculature (Fig. 2d). However, at day 30 the section revealed some restructuring of the foot architecture with little difference compared to the foot of the control animals. A mild degree of thinning of fibres with pyknotic nuclei and hypertrophic condition was noticed (Fig. 2e).

On the whole, severe degenerative changes were observed in hepatopancreas and foot of the mussel at day 1 of exposure to acute cadmium concentration; but at day 3 a slight recovery was noticed but with significant damage. In subacute concentration of cadmium mild degree of degenerative changes were observed in both the tissues of mussel at day 10, whereas at day 30 the tissues appeared more or less normal with insignificant changes.

DISCUSSION

In the present study the progressive degenerative changes observed in the histology of the organs of mussels exposed to the acute concentration of cadmium are direct indications of the severity of metal stress on animals. The histological changes observed in hepatopancreas, like the degeneration in the intercellular connective tissue, reduction in glandular cells, and dissolution of tubular cells at day 1 of exposure to acute concentration are expression of cadmium toxicity. These degenerative changes result in the impairment of physico-metabolic processes of mussels. Break of DNA strands in hepatopancreas of mussels exposed to resin acids was reported by Gravato et al. (2005). Such irrecoverable damage was also reported in the hepatopancreas of a few shellfishes exposed to lethal concentration of heavy metals (Usha Rani & Ramamurthi 1989, Bano & Mahdi Hasan 1990, Hemelraad et al. 1990 a,b, Marigomez et al. 1990 b, Nott et al. 1993, Etxeberria et al. 1994, Marigomez et al. 1996, Ptashynski et al. 2002). Less damage noticed at day 3 exposure indicates partial recovery, may be due to the initiation of metal detoxification mechanisms. Chabicovsky et al. (2004) reported a specific function of epithelial cells of hepatopancreas in metal detoxification or in clearing of cellular debris from cell death in snail *Helix pomation* on exposure to cadmium toxicity.

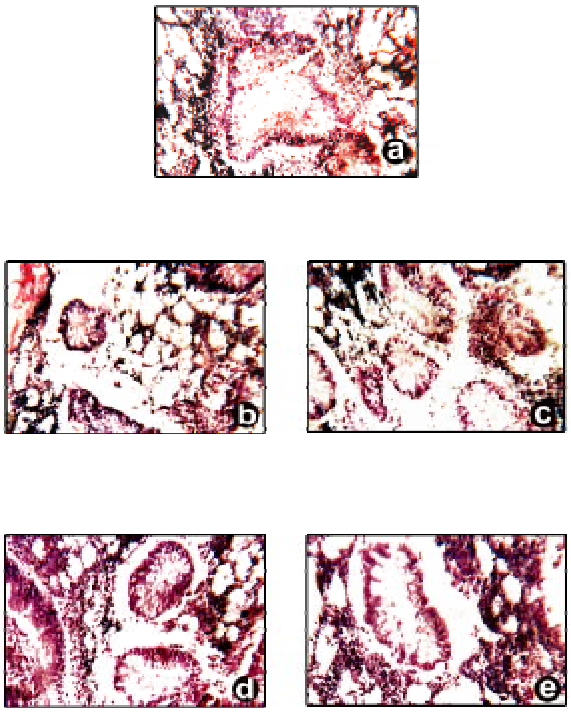

Fig. 1 a,b,c, d and e: Transverse sections of the hepatopancreas of mussel *L. marginalis* of the control (a) and at days 1 (b) and 3 (c) on exposure to the acute and days 10 (d) and 30 (e) to subacute concentrations of cadmium × 150 (H & E).

The foot too exhibited some degenerative changes in acute concentration of cadmium. There appeared a dissolution

of intermuscular connective tissue, thinning down of muscle fibres and nuclear pyknosis followed by heavy vacuolization, muscular atrophy, loss of integrity of muscle fibres and destruction of muscular architecture. This could lead to the failure of a number of biochemical activities as well as osmo and iono-regulatory functions of the foot. The copious amounts of mucus secretion observed near the shell valves of mussels exposed to acute concentration of cadmium could be due to the oedematic condition of the foot muscle. Similar observations were also reported by Chetty et al. (1988) in the freshwater mussel *Lamellidens marginalis* exposed to acute concentration of methyl parathion. Partial recovery observed at day 3 could indicate the animals effort to overcome the stress.

In subacute concentration of cadmium, the extent of tissue damage in the organs of mussels is considerably less compared to the damage observed in acute concentration. Mild destruction in tubular epithelial cells, decrease in number of globules and loss of integrity of secretory cells in the hepatopancreas, and the enlargement of pedal sinuses, the pyknotic appearance of nuclei, thinning down of muscle fibres and a mild degree of oedema in the foot of mussels at day 10 exposure indicate the initial impact of cadmium on the structural integrity of mussels. To overcome the stress the animal might have elevated the protein synthesis ability and started repair of the structural damage. So no significant changes were noticed in hepatopancreas and foot of mussels at day 30. Probably, on prolonged exposure the animal could able to reorganize its organ structures to normal level. Further, the synthesis of metal binding proteins might have prevented the metal to intervene with structural dissolution. This adaptive ability at the structural level shows that mussels have a high detoxification ability without any significant structural damage on prolonged exposure to subacute cadmium stress.

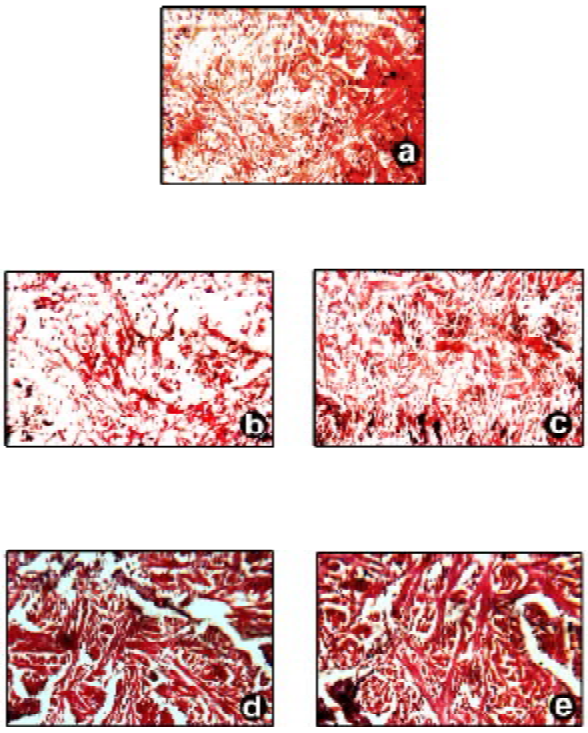

Fig. 2 a,b,c, d and e: Transverse sections of the foot of mussel *L. marginalis* of the control (a) and at days 1 (b) and 3 (c) on exposure to the acute and days 10 (d) and 30 (e) to subacute concentrations of cadmium × 150 (H & E).

On the whole, the acute concentration of cadmium caused severe damage to the organs of mussel *L. marginalis.* Though a partial recovery in the damage was noticed at day 3 exposure, but it is not significant. Whereas, the subacute concentration though caused initially a mild damage to the organs of mussel but on prolonged exposure the animals could develop enough resistance and replenish the loss, may be by activating the protein synthesis machinery. Thus, the bivalves could serve as biodetectors of cadmium pollution and also can evaluate the water concentrations of cadmium depending on the damage caused to their soft tissues.

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