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RESEARCH ARTICLE

TOXICITY OF CADMIUM ON THE BIOCHEMICAL COMPOSITION AND HISTOLOGY OF ESTUARINE CLAM MERETRIX CASTA

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ABSTRACT

The toxic effect of the heavy metal cadmium on the biochemical constituents and histology of digestive gland clam *Meretrix casta* was studied. The clam were exposed for 10, 20 and 30 days in 10% sub lethal concentration of 96 hr LC_{50} of cadmium (1.25 mg/l). There is significant decrease in the carbohydrate, protein and lipid profiles in the digestive gland and gill after exposed to sublethal concentration of cadmium. In clam exposed to sub lethal concentration of cadmium various histopathological alterations are observed after 10 days of exposure. Shrunken of digestive and secretory cells and enlarged lumen were noticed . At 20 days of exposure, rupturing of cell membrane and degeneration of digestive tubules were also observed. After 30 days of treatment, the hepatopancreas was highly damaged vacuolization, necrosis and atrophy of digestive cells were also observed.

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INTRODUCTION

Pollution is an undesirable change in the physical, chemical or biological characteristics of air, water and land that may or will harmfully affect human life, industrial progress, living conditions and cultural assets. The pollution is the negative feedback of the environment, which affects living organisms. Human beings have been responsible for water pollution, as they have dumped directly or indirectly, large amounts of industrial wastes, heavy metals effluents, chlorinated hydrocarbons oil, titanium dioxide, etc., into the aquatic environment. Estuaries and rivers have not been spared with the result of the deleterious effects have paved the way for health hazards to human beings (Gesamp, 1984). In recent times attention has been focused on rivers and estuaries as these are considered as major sources of pollutants of coastal areas and oceans. Molluscs are generally viewed as reliable indicators of contamination because of their sedentary and filter feeding habits. Monitoring of heavy metals in fresh water system, using fresh water bivalves was carried out by many authors (Merlini 1996; Mathis and Cummings, 1973; Forester, 1980; Heit *et al.,* 1980; Adams *et al*, 1981; Salanki *et al.,* 1982; Schmitt and Finger, 1982; Hameed and Mohanraj, 1989; and Amanulla Hameed, 1994). The histological methods have been used to assess the effect of pollutants on aquatic organisms, since such studies bear a direct testimony to the deleterious effects of toxicants (Hinton *et al*., 1973). Further, histological examinations of tissues may also be used for the assessment of water quality in the environment (Bhatnagar *et al*., 1987). The hepatopancreas is the main organ of reserve and detoxification of xenobiotics in mollusks and is highly sensitive to physiological and environmental changes (Simkiss and Mason, 1983). The hepatopancreas in mussel which is analogous to liver in higher vertebrates always form the prime target of heavy metal toxicity (Sreekala Pillai and Menon, 1998). Through much data are available on the acute toxicity of many species, information on sub lethal effects of metal on the rate of enzyme activity and histopathology of hepatopancreas are scanty. The present work has been carried out with a view to the effect of heavy metal cadmium on biochemical constituents and histopathology of hepatopancreas of the estuarine clam, Meretrix casta.

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MATERIALS AND METHODS

The acute bioassay was analysed following the method of Litchfield and Wilcoxen (1949). The 96 h LC50 value for cadmium in Meretrix casta was found to be 1..25 mg/l. The clams were maintained in four groups. Group1 served as controls, Groups 2, 3 and 4 were exposed to 10% sub lethal concentration of cadmium for 10, 20 and 30 days respectively. At the end of the treatment period, the control and treated clam were dissected and the gill and digestive gland tissues were

collected to analysis the nutritive value. The carbohydrate, protein and lipid estimations were carried out by following the methods of Bradford, 1976, Reddy *et al*., 1989 and Folch *et al*., 1957 respectively.

RESULTS

Carbohydrate content in the hepatopancreas

The total carbohydrate content of the hepatopancreas in control and experimental clams are shown in the table 1. Exposure of *Meretrix casta* to the heavy metal reduced the level of carbohydrate in the hepatopancreas at all days of exposure. The percentage decreased over the control were -29.09 %, -45.92 % and – 69.50 % respectively at 10,20and 30 days of exposure.

Protein content in the hepatopancreas

When the clam was exposed to a sublethal concentration for a period of 30 days, the protein content decreased. The percentage over control after 10, 20 and 30 days of exposure were – 9.80%, - 27.22% and – 44.39% at sublethal concentration, which is found to be dependent upon duration of exposure (Table 1).

Lipid content in the hepatopancreas

The lipid content decreased in the hepatopancreas at all the exposure periods, when the clam exposed to sublethal concentration (Table 1). The decreased of the lipid were -34.57 %, -45.59 % and – 36.60% after 7, 14 and 21 days of exposure.

Table 1. Effect of sublethal concentration of Cadmium on biochemical constituents in the hepatopancreas of estuarine clam *Meretrix casta* (mg/100mg)

Biochemical constituents	Medium	Period of exposure (days)		
		10	20	30
carbohydrate	Control	8.66 ± 0.732	8.71 ± 0.671	8.56 ±0.267
	10% SLC	6.14 ± 0.366	4.71 ± 0.315	2.61 ±0.672
	Variation (%)	-29.09	-45.92	-69.50
Protein	Control	8.16 ± 0.319	8.41 ± 0.216	8.47 ±0.369
	10% SLC	7.36 ± 0.327	6.12 ± 0.309	4.71 ±0.261
	Variation (%)	-9.80	-27.22	-44.39
Lipid	Control	3.76 ± 0.316	3.97 ± 0.331	3.86 ±0.236
	10% SLC	2.46 ± 0.615	2.16 ± 0.371	1.68 ±0.269
	Variation (%)	-34.57	-45.59	-36.60

Each value is the mean ± SD of five individual observations.
(-) denotes % decrease over control

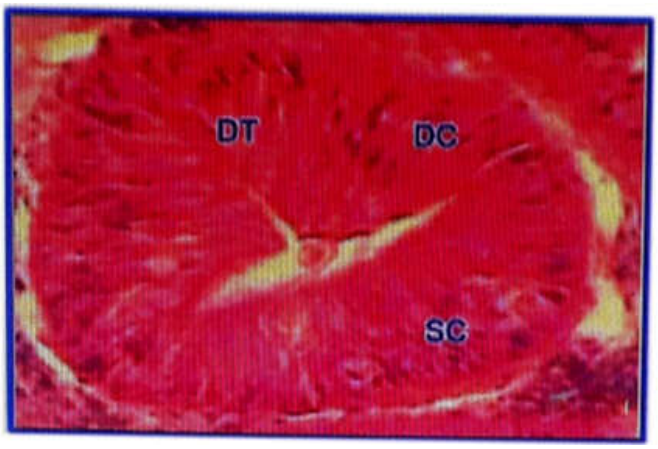

Fig. 1. Control clam hepatopancreas

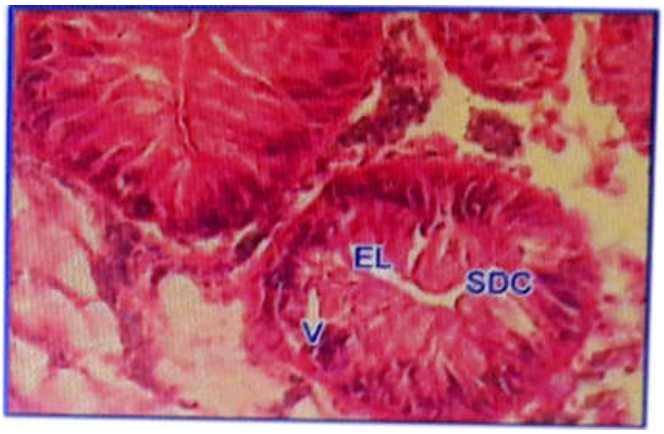

Fig. 2. 10% SLC of cadmium treated clam hepatopancreas for 10 days

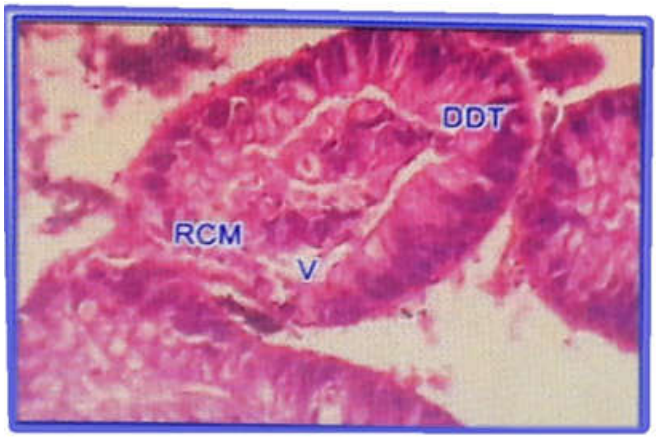

Fig. 3. 10% SLC of cadmium treated clam Hepatopancreas for 20 days

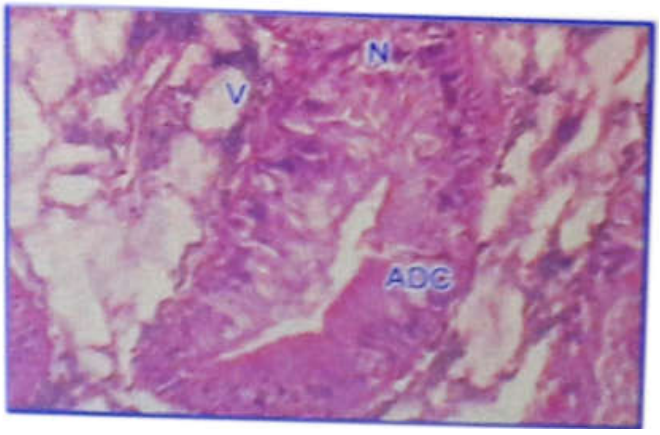

Fig. 4. 10% SLC of cadmium treated clam hepatopancreas for 30 days

Histology of hepatopancreas

The digestive gland is made up of a number of fine digestive tubules (DT) which communicate with the lumen of stomach through partially ciliated main ducts and non ciliated secondary ducts. The ducts could be circular or oval in shape (Fig.1). Two types of cells namely digestive cell (DC) and secretory cells (SC) could be differentiated in the digestive tubules of hepatopancreas in control mussels (Fig.5). The digestive cells are responsible for absorption and intracellular digestion of most of the food ingested. The secretory cells produce digestive enzymes.

Effect of cadmium on histology

In clam exposed to sub lethal concentration of Cadmium various histopathological alterations are observed after 10 days of exposure. Shrunken of digestive and secretory cells and enlarged lumen were noticed (Fig.2). At 20 days of exposure, rupturing of cell membrane and degeneration of digestive tubules were also observed (Fig.3). After 30 days of treatment, the hepatopancreas was highly damaged vacuolization, necrosis and atrophy of digestive cells were also observed (Fig.4).

DISCUSSION

The carbohydrate of aquatic organisms comprised mainly glycogen and total free sugars and the fluctuations in the carbohydrate content may be due to accumulation and utilization of glycogen and total free sugars at different stages like gametogenesis and spawning. In aquatic organisms, generally the carbohydrate reserves may be rapidly utilized under unfavorable conditions and the great variation found in the tissues indicates that the level of mobilizable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in the nutritional state of the animal. In the present study the percentage of carbohydrate content decreased in the hepatopancreas of mussels exposed to sub lethal concentration of heavy metal (Fig.2). Patil and Mane (1992) have observed that the mercury exposed *L. marginalis* showed a decrease in the carbohydrate content in the various tissues. The decrease in total carbohydrate level signifies its utility possibly to meet the higher energy demands of the mussel reeling under heavy metal toxicity. The synthesis and utilization of carbohydrate are therefore, altered in the organism subjected to heavy metal stress.

Carbohydrates which supply the major portion of the metabolities for the energy requirements in a normal individual is oxidized for the energy requisites. Carbohydrates may be converted to glycogen or shunted in the metabolic pathway to supply the carbon chain for amino acids or converted into fat (Priscilla, 1985). At sub lethal concentration when the liver carbohydrate content decreased blood sugar level increased which suggests the breakdown of liver glycogen (glycogenolysis) . The mobilization of glucose from the liver to the blood and its availability for utilization by the needy tissues for ensuring normal metabolic processes in the body appears inevitable when the mussel is exposed to toxic medium. Mane and Kulkararni(1999) observed a fall in hepatopancreas carbohydrate level in *L. marginalis* when exposed to different sub lethal concentration of cadmium. Mali (2003) have reported the depletion of glycogen content in hepatopancreas of freshwater crab, *Barytelphusa guerini* when exposed to copper sulphate. Vijayavel *et al*. (2006) studied the effect of naphthalalene on carbohydrate metabolism of crab, *Scylla tranquebarica*. These obsevations are in conformity with the reports on the fall in hepatopancreas glycogen level in *Meretrix casta*, when exposed to TBTO. (Shah *et al.,*1998). Studies in general have suggested that exposure to heavy metal treatment interferences with the carbohydrate metabolism. A greater decrease of carbohydrate content indicates greater utilization of carbohydrate to cope with enhanced metabolism under stressful situation. Despite a continuous and rapid release of glucose by glycogenolysis in the hepatopancreas, to meet the energy requirement for the increased muscular activity, a fall in the overall in mussels subjected to heavy metal treatment. Kambale and Nanaware (2008) observed similar type of depletion in glycogen level in a freshwater snail *Bellamya bengalensis* due to Cadmium toxicity.

Proteins are mainly involved in the architecture of the cell. Protein is the most important constituent in living tissues, which is of considerable metabolic and structural value. Therefore, any change in this constituent indicates the stress inflicted on the metabolic functions required for maintaining a healthy physiological state. In this work the protein content of *Meretrix casta* at sub lethal concentration decreased in all exposure periods. (Fig.3). The depletion in digestive gland protein of *Meretrix casta* indicates rapid utilization of energy stores to meet the energy demands warranted by the environment. The observed depletion in tissue protein on treatment with sub lethal doses of heavy metal is suggestive of proteolytic activity, possibly to meet the excess energy demands under toxic conditions. Ramana Rao and Ramamurthy (1980) reported depletion in the protein content in the freshwater snail *Pila globosa* exposed to sumithion. Khan *et al*. (2008) recorded decrease in protein content of marine edible gastropod *Natica picta* exposed to sublethal concentrations of Cadmium chloride. A significant decrease was reported in the protein content of the hepatopancreas in the prawn *Penaeus indicus* after exposure to sublethal concentration of lead (Sathyavathi and Prabhakara Rao,2002). Senthil kumar *et al.* (2007) observed decrease in protein content in all the experimental tissues of field crab *Spiralothelphusa hydrodroma* due to the effect of heavy metal copper.

Lipid is an important constituent of animal tissue, which plays a prime role in energy metabolism. Lipids are also important in cellular and sub-cellular membranes. A gradual decrease in lipid content in digestive gland tissues of *Meretrix casta*after chronic treatments of heavy metal of various periods of exposure are shown in Table 1. Earlier researchers like Anusha *et al*. (1996) also suggested that the decrease in lipid content in *C. carpio* may be either due to the uptake of lipid by the tissue for utilization at cellular levels or due to increased lipolysis or mitochondrial injury, which affect the fatty acid oxidation mechanism as suggested by Ware (1980). Sivaprasanna Rao and Raman Rao (1979) studied the considerable decrease in total lipid in tissues might be due to drastic decrease in glycogen content in the same tissue which is an intermediate source of energy during toxic stress conditions . After glycogen, lipid content may be used for energy production to overcome toxic stress. Some workers support these results in which lipid content decreased in animals after exposure to pollutants. Hameed and Muthukumaravel (2006) reported significant decrease in lipid of *L. rohita when* exposed to heavy metal cadmium.

Villalan *et al*. (1990) observed the effect of cadmium on *Macrobrachium idella* and reported that lipid content was decreased. Similar decrease in lipid content level has also been observed by Saravana Bhavan and Geraldine (1997) in *Macrobrachium malcolmsonii* when exposed to endosulphan and suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to the pesticide toxicity. Lomte and Muley (1993) reported the decrease in lipid level in the freshwater snails *Thaira tuberculata* and *Parresia courrugata* exposed to copper toxicity. The hepatopancreas is the centre for metabolism and detoxification in molluscs (Thompson *et al.,* 1974). On chronic exposure to Cadmium, the hepatopancreas exhibited several pathological changes including shrunken of digestive and secretory cells, vacuolation, rupturing of cell membrane, atrophy of digestive cells and necrosis. These changes were linearly proportional to exposure period. The present study closely agrees with a similar report by Muley and Mane (1986) in the hepatopancreas of lamellibranch molluscs, *L. corrianus* and *L. marginalis* exposed to pesticide endosulfan. The histopathological alterations observed, were ruptured basement membrane and muscular layer and shrunken of digestive and secretory cells. The vacuolation, atrophy and rupturing of cell membrane in *L. marginalis* by Amanulla Hameed *et al.* (2005) due to copper toxicity have been reported. Tubular damage and heavy vacuolization in tubules in *Perna viridis* (Sreekala Pillai and Menon,1998) and reduction in the size of digestive cells of *Mactra violacea* (Shah *et al* 2003) have been reported. Auffret (1988) observed that *Mytilus edulis* exposed to high concentrations of diesel oil and copper mixture showed severe degenerative changes in the epithelium of the digestive gland. Epithelial cell shrinkage, erosion of cells and large vacuoles in the digestive tubules noticed in the present instance have been suggested as the effects of copper and cadmium by Mathew and Menon (2005) in the mussel *Perna indica.* The present study clearly demonstrates that the hepatopancreas is an important target organ for heavy metal pollutants in the estuarine clam *Meretrix casta*.

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