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HISTOMORPHOLOGICAL ALTERATIONS INDUCED BY HEAVY METAL, CHROMIUM IN THE DIGESTIVE GLAND OF AN ESTUARINE CLAM, *Mactra violacea* (BIVALVIA: MOLLUSCA)

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ABSTRACT

The median lethal concentration (LC_{50}/96h) of Chromium Chloride for the estuarine clam, *Mactra violacea* was recorded to be 2.4 mg/l. Acute chromium exposure (0.24 mg/l = 1/10th of LC_{50}/96h) to *M. violacea* caused histological alterations in the chief metabolic organ namely digestive gland. Histological studies showed that the digestive gland tissues suffered more on 72h and 96h exposures than 24h and 48h exposures. Distinct histomorphological alterations namely *epithelial degeneration, necrosis, vacuolation and granular cytoplasm* were noticed in acute treated digestive diverticula of *M. violacea*. Results of histopathology in the present study were dependent on the period of exposure.

INTRODUCTION

Over recent years, estuaries get contaminated by a variety of toxicants such as heavy metals via natural processes or natural deposits and anthropogenic activities (Moriarty, 1983; Francis, 1994 and Sharp and Hagan, 2013). Metal contamination of estuarine environment has been and is monitored through analysis of water, sediment and estuarine species samples in order to elucidate the contamination status, distribution and possible pollution sources to assess the risks on estuarine organisms and human (Coombs, 1972; Roseman, *et al.,* 1994; Blasco, *et al.,* 1999; Suresh, 2001 and Abdullah, *et al.,* 2007). Among important estuarine organisms, bivalves are sessile and filter feeder species, able to accumulate organic or toxic elements in their tissues (Simkiss, *et al.,* 1982; Langston, 1986; Livingstone, *et al.,* 1988; Andral, *et al.,* 2004; Zhou, *et al.,* 2008; Taleb, *et al.,* 2008 and Levent Bat, *et al.,* 2012). Bivalves such as mussels and clams accumulate chemical contaminants particularly in the digestive gland. Digestive gland cells are not only involved in digestive and absorptive processes but are also involved in the bioaccumulation and bioconcentration (Patel and Anthony, 1991; Elfwing, *et al.,* 2002; Fang, *et al.,* 2003; Rainbow, 2006; Azarbad, *et al.,* 2010 and Ponnusamy, *et al.,* 2014). Though several studies have shown the impact of petroleum hydrocarbons and organotins on the vital functioning of digestive gland of bivalves (Lee, *et al.,* 1974; Carles, 1984; George, *et al.,* 1986; Bright and Ellis, 1988; Langston and Burt, 1991 Pope 1998; and Shah, *et al.,* 2003) very few studies are available on metal toxicity and its impact on the histology of vital metabolic organ, digestive gland of bivalves (Nambisan, *et al.,* 1977; Tripp, *et al*., 1984; Babu Kutty and Chacko, 1992; Bush, *et al.,* 1992 and Sreekala Pillai, 1993). Heavy metal, Chromium occurs naturally in the Earth's crust and it is ubiquitous in water, soil and biological materials. It is released in aquatic bodies and get accumulated in animal tissues through abiotic and biotic processes. In the present study, the impact of sublethal toxicity of chromium chloride on the morpho–functional features of an estuarine edible clam, *Mactra violacea* is reported.

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MATERIALS AND METHODS

Mactra violacea were collected from the mud flats of Agniar estuary (Habib Mohamad and Abdul Rehman, 1987) and were kept in glass circular pneumatic troughs (40L x 30B x 30Hcm) containing 10L of freshly collected estuarine water in each

trough for 7 days for acclimation to the laboratory conditions (Temp: 26 ± 1ºC; pH: 7.8 ± 0.2). The medium water was renewed daily to avoid faecal contamination.

Preparation to Chromium Chloride concentration

Chromium concentrations for bioassay studies were prepared in. From 10% stock solution, varied concentrations in mg/l ranging from 0.5 to 5.0 were prepared in double distilled water by v/v method.

LC_{50} determination

Healthy and active specimens of *M. violacea* of uniform shell length (70–75 mm) were chosen from the stock culture to determine LC_{50} value. From the mortality values obtained at different concentrations of $CrCl_2$ (0.5 to 5.0 mg/l), a concentration rendering 50 percent of the population dead, i.e., LC_{50}/96h was computed adopting a method described by Hamilton *et al.* (1977). The results were confirmed by repeating the experiments three times.

Bioassay studies

Acclimated *M. violacea* of uniform body size (70 – 75 mm shell length) were chosen for the bioassay studies. Two groups were formed. One was considered as the experimental and exposed to acute toxicity of $CrCl_2$. The acute treatment consisted of exposure of 0.24 mg/l of $CrCl_2$ upto 96 hours. The other group was treated without $CrCl_2$ and was considered as control. Ten test clams in each set of both control and experimental groups were maintained simultaneously. The clams both control and acute treated were scarified at intervals of 24 hours for histomorphological studies.

Histomorphology of digestive gland

The digestive gland of both control and acute treated clams were dissected out, fixed and stored in aqueous Bouin's fluid for 24 hours. Tissues were subsequently dehydrated and blocks were prepared in paraffin wax (50º to 60ºC) (Steedman, 1960). Tissue sections of 5µ to 6µ were cut and stained with Haemotoxylin–Eosin stains (Humason, 1972).

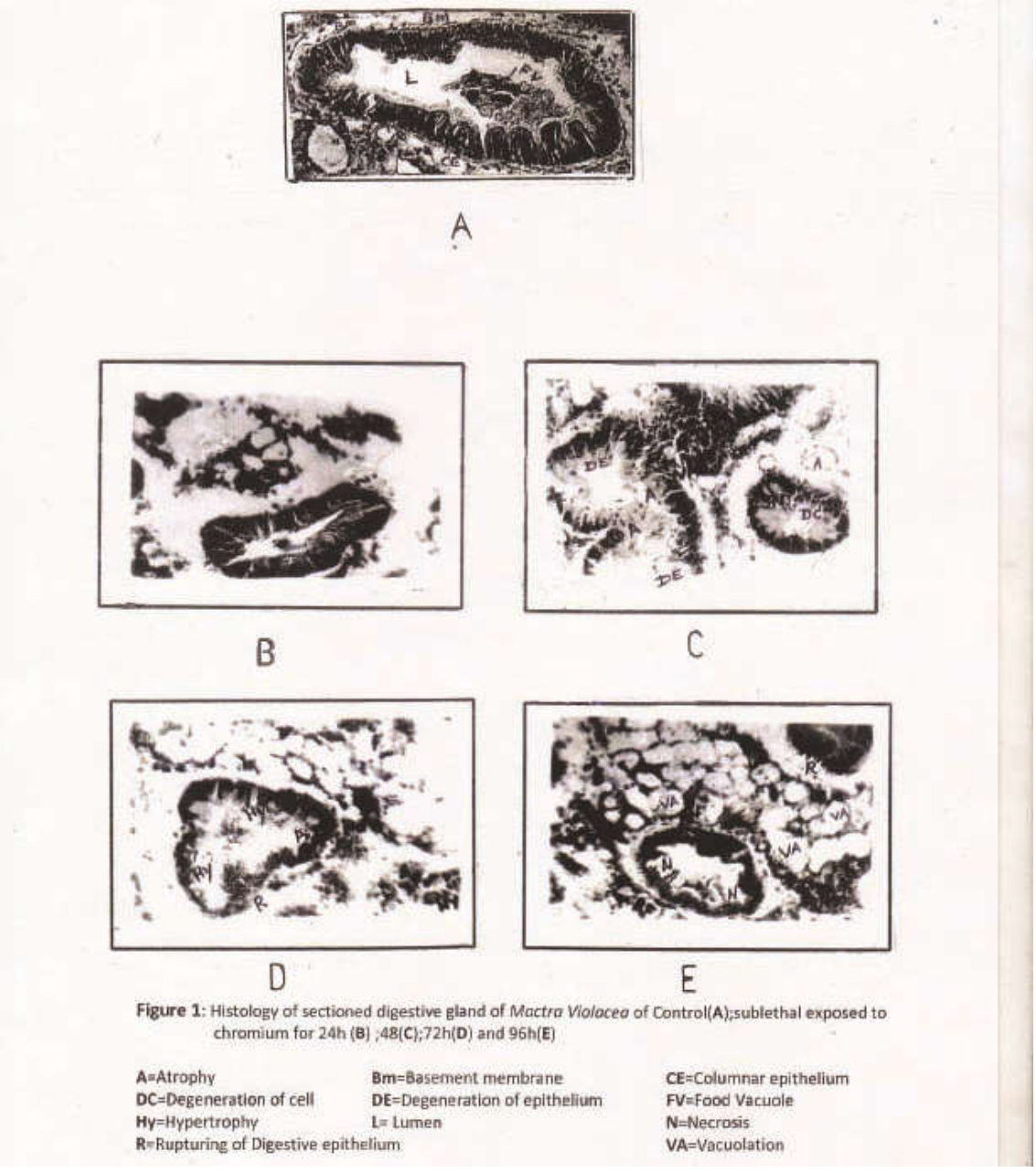

Figure 1: Histology of sectioned digestive gland of *Mactra Violacea* of Control(**A**);sublethal exposed to chromium for 24h (**B**) ;48(**C**);72h(**D**) and 96h(**E**)

A=Atrophy
Bm=Basement membrane
CE=Columnar epithelium
DC=Degeneration of cell
DE=Degeneration of epithelium
FV=Food Vacuole
Hy=Hypertrophy
L= Lumen
N=Necrosis
R=Rupturing of Digestive epithelium
VA=Vacuolation

Inspection of sectioned digestive gland of *M. violacea* revealed that the digestive tubules underwent certain histological alterations.

RESULTS

Histology of digestive gland under acute exposure of $CrCl_2$

Digestive gland also called hepatopancreas consists of numerous digestive or hepatic labules. Figure 1 – A. shows histology of Digestive gland in control *M. violacea*, each tubule is lined by columnar epithelial cells and secretory cells resting on basement membrance. In the case of digestive tubule there is a narrow luman. Interlobular space is filled by thin layer of connective tissue. Precipitation of cytoplasm and nucleus, degeneration of digestive cell epithelium and precipitation of microvilli cells were totally missing but hypertrophy in the nucleus, atrophy in the digestive cells and rupturing of digestive cells membrance were noticed rarely at 24h, 48h and 72h exposure (Figure 1: B–D). Vacuolation (digestive cells), hypertrophy (nucleus); atrophy (digestive cells), rupturing (cell membrane) were noticed at 96hr exposure while traces of precipitation both in cytoplasm mucus and microvilli cells and degeneration of digestive cells epithelium were found (Figure 1.E).

DISCUSSION

Estuarine pollution has changed dramatically in recent decades. The use of bivalves as biomonitors among estuarine organisms is very important as tools to assess the toxicity of pollutants such as heavy metals entering the estuarine environment and being take up into the body tissues of bivalves species. General histological make up the digestive gland of untreated *M. violacea* is illustrated in (Figure 1:A). The digestive gland exhibited morpho functional characters namely such as digestive tubules consisting of digestive cells which formed the entire digestive gland. These findings are in agreement with those of previous studies in estuarine and marine bivalve speciets (Owen, 1956, 1970; Parmar, 1978; Robinson and Langton, 1980; Platt, 1981; Sreekala Pillai, 1993 and Shah, *et al.,* 2003; Andhale *et al*., 2011) The histological alterations observed in the digestive gland of *M. violacea* to actue $CrCl_2$ exposure (Figure. 1; B, C, D and E) are indentical with those of observations already reported by Shah, *et al*. (2001). More frequent vacuolation in the digestive cells, followed by *hypertrophy, atrophy* during acute exposure of $CrCl_2$ indicated the damages which entered the respiratory tissues (gills), then to digestive tract (digestive gland) and finally to other parts of body along with food and thus accumulated within the body tissues. These accumulated $CrCl_2$ resulted in the damaging of cellular make up metabolic tissues such as digestive gland.

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