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HISTOPATHOLOGY OF THE INTESTINE OF *CLARIAS BATRACHUS* L. UNDER HEAVY METAL STRESS

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ABSTRACT

Histopathological effect of three heavy metals- cadmium, chromium and mercury on intestine of *Clarias batrachus* L. was studied under laboratory condition. The common histopathological changes that took place under heavy metal exposure were disintegration of mucosal epithelium, hypertrophied epithelial cells, increased number of goblet cells, sloughed off epithelial cells and mucous in the lumen, degeneration of the connective tissue of sub-mucosa and hyperemic blood vessels. Separation of mucosal epithelium, especially in the villi and increased goblet cells were prominent in Cd-exposed fish. Clumping of the epithelial cells at the base near the basement membrane was prominent in the case of the Cr- and Hg-exposure. Connective tissue degeneration in the lamina propria and submucosa of the intestine and dilated blood vessels with congestion of blood were prominent in the Cr and Hg exposed fish.

Key words : Histopathology, mucosal epithelium, hypertrophy, goblet cell.

INTRODUCTION

In nature, heavy metals are present in very small concentration, but beyond certain low limit, they become highly toxic to living organisms. Indiscriminate disposal of wastes produced due to various anthropogenic activities are held responsible for the rise of heavy metal concentration in nature. Water receives heavy metals and an array of other pollutants through land drainage, industrial effluents, organic wastes, sewage sludge, etc. (Ching and Hongxiao 1985, Kay *et al.* 1986). Once dispersed in the biosphere, heavy metals can not be recovered or degraded. They become free in nature, accumulate in agricultural fields, and in organisms causing reduction of biodiversity, poor production, and many other anomalies (Badsha and Goldspink 1982, Singh 1988).

Among the metal toxicants cadmium, chromium and mercury, the heavy metals with which the present work deals, have been detected in many waterbodies of Bangladesh in relatively high concentrations (Anonymous 1998,

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Quraishi and Akhter 2005). The present work is an attempt to investigate the toxic effect of three heavy metals- cadmium (Cd), chromium (Cr) and mercury (Hg) on the intestine of *Clarias batrachus* L.

MATERIALS AND METHODS

Fresh and live specimens of *Clarias batrachus* L. (average length 16 ± 3 cm and weight 70 ± 5 g) was bought from Chittagong city market and acclimatized for 7 days in 25 L aquarium in nonchlorinated tap water. Fish species were given oligochaetes, prawn and small pieces of *Harpodon nehereus* as food once a day, and water was changed at 24h interval. Following it, 15 fish specimens in each 25L aquarium were exposed to each sub lethal conc. of heavy metals (Cd- 9 ppm, Cr- 12 ppm and Hg - 0.3 ppm) for 28 days along with a control without heavy metal. Test fishes in the aquarium were fed once a day with a change of solution after each feeding.

Method of Humason (1961) was followed for histopathological study. At the end of the exposure period intestine from the metal exposed as well as control fishes were isolated, cleaned with physiological saline solution, cut into pieces and fixed in freshly prepared Bouin's fixative. After 24 hours tissues were transferred to 70% alcohol for preservation. For histological slide preparation tissues were dehydrated in graded alcohol, cleared in benzene and embedded in paraffin. Thin sections ($3-5\mu$) were cut by a rotary microtome, double stained by eosin-haematoxylin, mounted with DPX for examination under microscope. Photomicrographs were taken by Ricoh 35 mm SLR camera.

RESULTS AND DISCUSSION

In control specimen, the intestine wall of *C. batrachus* was composed of four layers viz, mucosa, submucosa, muscularis and serosa (Plate 1). The mucosa was thrown into prominent finger like projections forming villi. The mucosa was composed of columnar epithelium consisting of absorptive and mucous secreting cells or goblet cells. The submucosa, made up of loose connective tissue, was vascular and extended into the villi as lamina propria which formed the core of the villi. The muscularis was formed of the inner circular and the outer longitudinal layer of smooth muscle fibres. Serosa formed the outermost thin layer of flattened epithelial cells.

The common histopathological changes that took place under heavy metal exposure were, damages of the mucosal epithelium, detachment of epithelium

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from the basal layer, especially at the tips of the villi, vacuolation in the epithelium, hypertrophy and degeneration of the mucosal epithelial cells (Plates 2-8).

Degeneration of epithelium and subepithelial tissues of the intestine was noted by Konar (1975) in the carp *Labeo rohita* and catfish *Heteropneustes fossilis* due to DDVP and phosphamidon pesticides, while Kabir and Begum (1978) noted lesion of villi, and necrosis of epithelial cells of the intestine of fish exposed to diazinon. Sastry and Gupta (1979) observed degeneration of intestinal villi in *H. fossilis* exposed to cadmium. Separation of the hypertrophied mucosal epithelium from the basement, especially in the villi was prominent in case of Cd-exposed fish in the present experiment. Moitra and Sadhu (1982) while working with *Channa striatus* exposed to phosphamidon reported that the toxic agents induced damage to the mucous membrane and the basement layer of the intestine. All these findings fully agree with the findings of the present work. Test fish of the present work revealed derangement of the epithelial cells of the mucous membrane, rupture of the epithelial cells and formation of cluster by the distorted cells or nuclei at some places indicative of necrosis. The epithelial cells were hypertrophied with pyknotic nuclei and clear cytoplasm. Some degree of necrosis was also observed by Kabir and Begum (1978) in the intestine of fish exposed to diazinon. Clumping of the epithelial cells at the base near the basement membrane seemed to be a primary stage of rupturing or erosion of the epithelium from the basal layer. Such change was prominent in the case of the Cr- and Hg-treated *C. batrachus* (Plates 4, 7). Virk *et al.* (1987) observed disappearance of the goblet cells in the pesticide exposed fish, *Mystus tengara*, which is contrary to the finding of the present work where increased number of goblet cells was noted in the mucous membrane of the Cd-, Cr- and Hg- treated fishes which, however, corroborates with the findings of others (Annes 1976, Sastry and Gupta 1979, Sastry and Malik 1979, Jauhr and Kulshrestha 1983, Pandey *et al.* 1994). Crespo *et al.* (1986) in an investigation on intoxication due to dietary cadmium and lead, observed increased goblet cells and enlarged apical pits and suggested that heavy metal exposure triggers mucous cell activity and production of mucous as a protective measure from the irritating agents.

In the present study, connective tissue degenerations were noticed in the lamina propria and submucosa of the intestine. Such degenerative changes were prominent particularly in the Cr and Hg exposed fish (Plates 5 – 8). Besides, dilated blood vessels with congestion of blood were also noticed in those fishes.

The above findings corroborate with the results of earlier investigators (Konar 1975, Bengeri and Patil 1985, Virk *et al.* 1987, Begum *et al.* 2001). Crespo *et al.* (1986) observed oedematous epithelial and connective tissue, and dilated blood vessels in trout intestine due to cadmium exposure. They regarded those symptoms as response to the irritation of internal mucosa by the metal.

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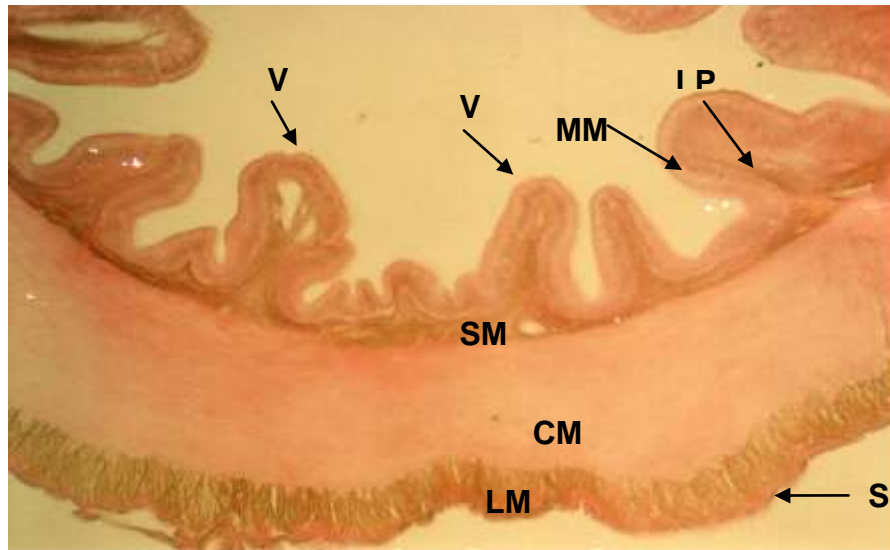


PLATE 1: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF *CLARIAS BATRACHUS* OF CONTROL SPECIMEN SHOWING VILLI (V), MUCOSAL MEMBRANE (MM), LAMINA PROPRIA (LP), SUBMUCOSA (SM), MUSCULARIS COMPRISING CIRCULAR MUSCLE LAYER (CM) AND LONGITUDINAL MUSCLE LAYER (LM), AND SEROSA (S). H&E 10×10.



PLATE 2: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF CD-EXPOSED *CLARIAS BATRACHUS* SHOWING DETACHMENT OF THE MUCOSAL EPITHELIUM (ME) FROM THE BASAL LAYER; VACUOLATION AND CONGESTION OF BLOOD IN LAMINA PROPRIA (LP) AND SUBMUCOSA (SM); AND A TUMOUR LIKE GROWTH (ARROW) IN SUBMUCOSA. H&E 10×10.

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PLATE 3: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF CD-EXPOSED *CLARIAS BATRACHUS* SHOWING SEPARATION OF MUCOSAL EPITHELIUM (ME) CONTAINING HYPERTROPHIED EPITHELIAL CELLS AND GOBLET CELLS AND CONGESTION OF BLOOD IN LAMINA PROPRIA (LP). H&E 10×40.

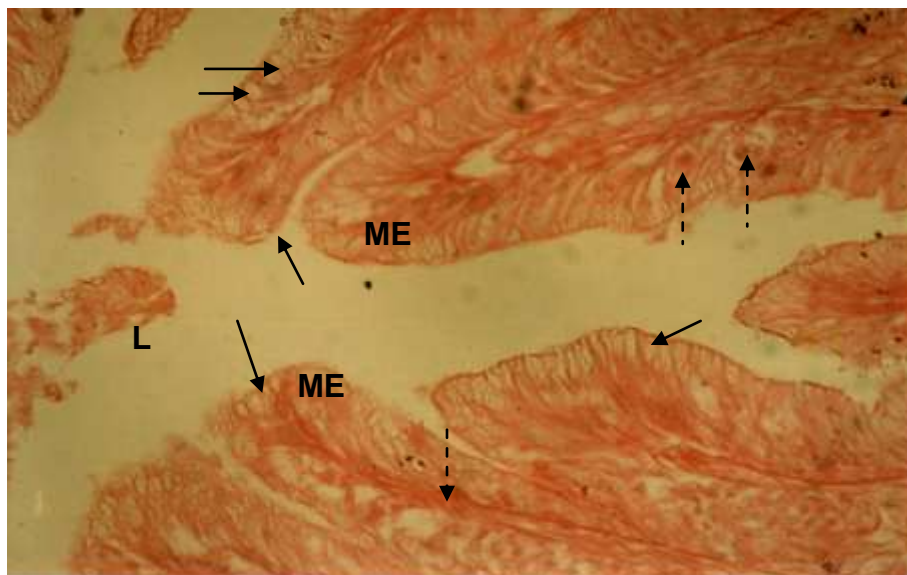


PLATE 4: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING HYPERTROPHIED EPITHELIAL CELLS WITH CLEAR CYTOPLASM OF MUCOSAL EPITHELIUM (ME), DISARRANGEMENT OF THE EPITHELIAL CELLS, INCREASED NUMBER OF GOBLET CELLS (ARROW), SLOUGHED OFF EPITHELIAL CELLS WITH MUCOUS IN THE LUMEN (L) OF INTESTINE AND NECROSIS IN MUCOSAL LAYER (BROKEN ARROW). H&E 10×10.

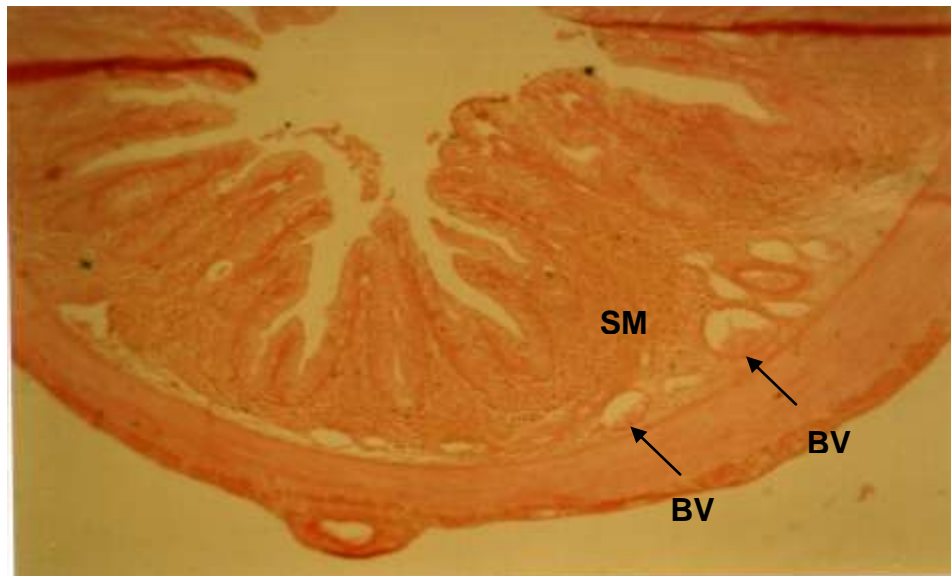


PLATE 5: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING DEGENERATION OF CONNECTIVE TISSUE OF SUBMUCOSA (SM) AND DILATED BLOOD VESSELS (BV). H&E 10×10.

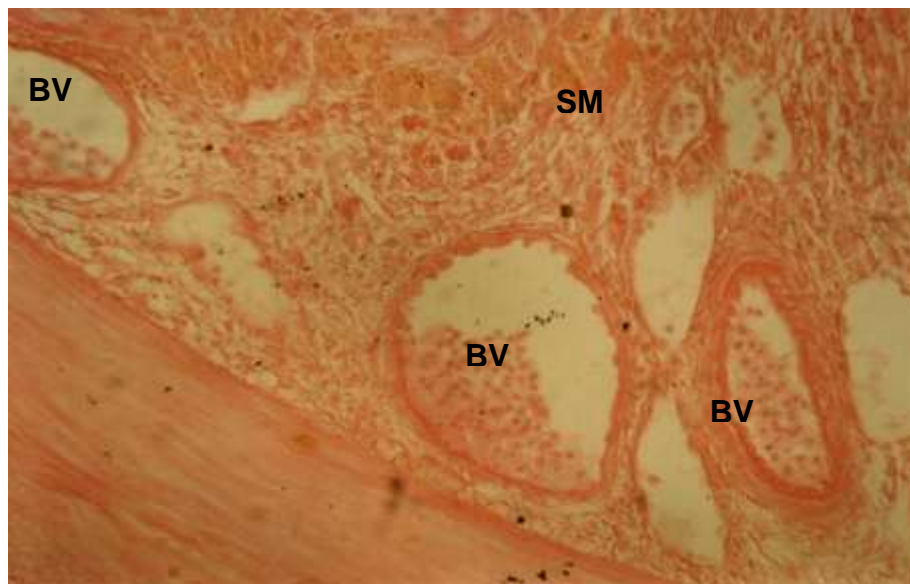


PLATE 6: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF CR-EXPOSED *CLARIAS BATRACHUS* SHOWING DEGENERATION OF CONNECTIVE TISSUE OF SUBMUCOSA (SM) FORMING CLUSTERS AND DILATED BLOOD VESSELS (BV) WITH EXCESS BLOOD CELLS. H&E 10×40.

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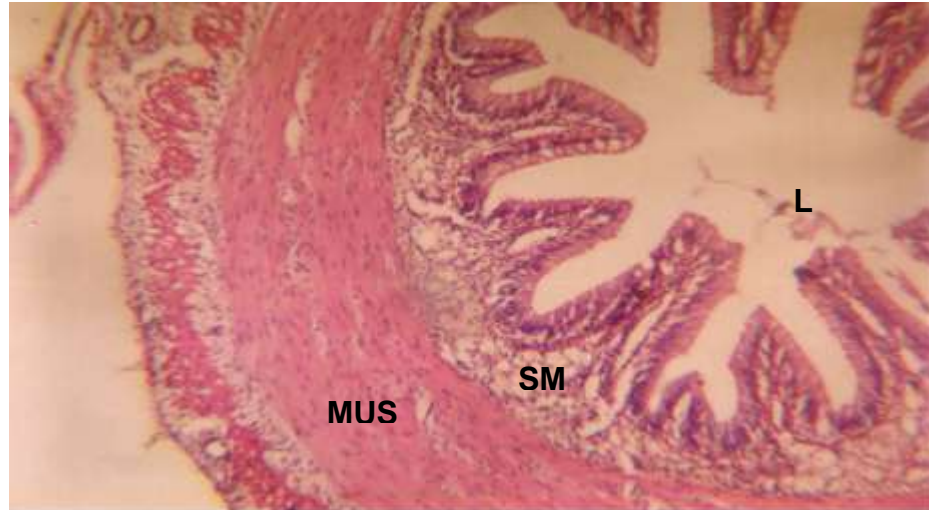


PLATE 7: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF HG-EXPOSED *CLARIAS BATRACHUS* SHOWING MUCOUS IN THE LUMEN (L) OF INTESTINE, DISTORTED CONNECTIVE TISSUE OF SUBMUCOSA (SM), RUPTURE IN MUSCLE LAYER (MUS) AND SCATTERED BLOOD CELLS ALL OVER THE TISSUE. H&E 10×10.



PLATE 8: PHOTOMICROGRAPH OF THE SECTION OF INTESTINE OF HG-EXPOSED *CLARIAS BATRACHUS* SHOWING EXTENSIVE DAMAGE OF THE MUCOSAL EPITHELIAL CELLS WHICH WERE HYPERTROPHIED WITH PYKNOTIC NUCLEI; RUPTURED AND NECROSED MUCOSAL EPITHELIUM (ME), MANY GOBLET CELLS (ARROW), AND DISTORTED LAMINA PROPRIA (LP) AND SUBMUCOSA (SM). H&E 10×40.

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