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# MERCURY CHLORIDE INDUCED RENAL LESIONS IN THE MULLET *LIZA PARSIA* (HAMILTON-BUCHANAN)

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Abstract

*Liza parsia* were exposed to 0.05 and 0.1 ppm $HgCl_2$ for 15 days. The treatments resulted in an initial swelling (hypertrophy) of the tubular lining epithelial cells. Thereafter, in proximal segments, the tubular epithelium became nectrotic at several places. These necrosed cells contained eosinophilic hyaline droplets in their cytoplasm. Glomerular capillaries became more permeable and proteinaceous fluid appeared in many Bowman's capsule. These degenerative changes were more pronounced in the fish maintained at higher concentration of Hg. The probable interactions of heavy metal pollutant with fish immune system has also been discussed.

## Introduction

Urbanization and industrialization cause serious deterioration of aquatic environment and pose threat to the aquatic life (Kimura, 1988; Chua *et al.*, 1989). Accumulation of industrial effluents in water bodies has become a major concern (FAO, 1986). Mercury (Hg) forms one of the important components in these effluents and can cause serious damage to the life systems (Moore and Ramamoorthy, 1983). Hg has been reported to cause severe poisoning to fish (Carpenter, 1927; Irukayama, 1967; WHO, 1976; McKim *et al.*, 1976; Olson *et al.*, 1978; Armstrong, 1979; Sastry and Sharma, 1980; Sastry and Rao, 1981; Weis and Weis, 1982; Ram and Sathyanesan, 1983, 1986). Several workers observed pathological lesions in various organs of the teleosts due to excessive amount of Hg in water (Gill and Pant, 1981; Srivastava, 1982; Bhattacharya *et al.*, 1985). Kidney accumulates the highest concentration of Hg and thus suffers extensive damage (Runnells *et al.*, 1965; Backström, 1969; Trump *et al.*, 1975; Jones and Hunt, 1983). Studies have demonstrated the distribution of Hg along the Indian Coasts (Tejam and Halder, 1975; Singbal *et al.*, 1978; Sanzgiry *et al.*, 1979, 1988; Zingde and Desai, 1981; Krishnakumar and Pillai, 1990). Further, Kureishy *et al.* (1979, 1983) reported the mercury accumulation in marine fishes collected from the Indian Ocean and Andaman waters. Since we lack information regarding the effect of Hg on kidney of an estuarine teleost, an attempt has, therefore, been made to fill this gap.

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## Material and Methods

Immature specimens of *Liza parsia* (average length 6.5 cm) were collected from the Chinese dip net at Fort Cochin (09°57′ N;

76°14′ E) and were brought to the laboratory. They were kept in aquaria of 120 litres capacity containing well-aerated sea water (salinity 30 ppt, average water temperature 28°C) for a week prior to use. Then, they were randomly divided into three equal groups consisting of 40 specimens and were subjected to the following treatments :

Group A : Fish in the sea water served as controls.

Group B : Fish were kept in 0.05 ppm $HgCl_2$ in sea water.

Group C : Fish were maintained in 0.1 ppm $HgCl_2$ in sea water.

Since fish actively extract Hg from the water, the medium was renewed every alternate day. Fish were not fed during the entire course of investigation.

Five specimens from each group were sacrificed on day 2, 4, 7, 10 and 15 after onset of the experiment. Dead fish were discarded from the study. The kidney were extirpated and fixed immediately in the freshly prepared Bouin's solution. After 24 hours, the tissues were washed in running tap water and routinely processed in the graded series of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections were cut at 6µ and stained in hematoxylin-eosin.

## Results

Kidney of *Liza parsia,* maintained in sea water (controls), consisted of secretory, excretory, haemopoietic and endocrine (interrenal) components which were interspersed in stroma of the reticuloendothelial tissue. The reticular tissue and numerous blood sinuses, arising out of renal portal vein, constituted the reticuloendothelial system. Sinuses were lined by the endothelial cells. The kidney of mullets did not show any zonal structures like medulla and cortex. Anterior portion of the kidney exclusively contained haemopoietic and endocrine tissues whereas posterior segments comprised excretory as well as haemopoietic tissues.

The nephron consisted of a tuft of capillaries forming glomerulus which was surrounded by Bowman's capsule, lined by squamous epithelial cells. Bowman's capsule was followed by a short neck which had ciliated cuboidal epithelium. There were two proximal segments separated by a ciliated segment in between. The first segment was formed by cuboidal to low columnar epithelium with prominent brush border and basal nuclei. Whereas the second segment was lined by acidophilic columnar epithelium having brush borders. Nuclei were centrally placed and cytoplasm of these cells were giving striated appearance in basal regions. The second proximal segment was connected to the collecting duct by a short distal tubule formed by low columnar to cuboidal epithelium. The interstitial space was occupied by actively dividing haemopoietic tissue (Pl. I A, B). Islands of polygonal cells interspersed between haemopoietic tissue constituted the cortical adrenal tissue whereas the chromaffin cells seen among the interstitial tissue formed the adrenal medulla. Numerous melanomacrophages centers consisting of the pigmented cells were also seen in the interstitial tissue.

Kidney of 0.05 ppm $HgCl_2$ - treated fish (Group B) revealed swelling (hypertrophy) of the epithelial cells of the proximal segments after 7 days of treatment. Also, eosinophilic casts appeared in the tubular lumen and there were shrinkage in the glomeruli which resulted in the dilation of Bowman's space (Pl. I C). In kidney collected after 15 days of exposure severe changes were observed. Tubular epithelium of the proximal segments became

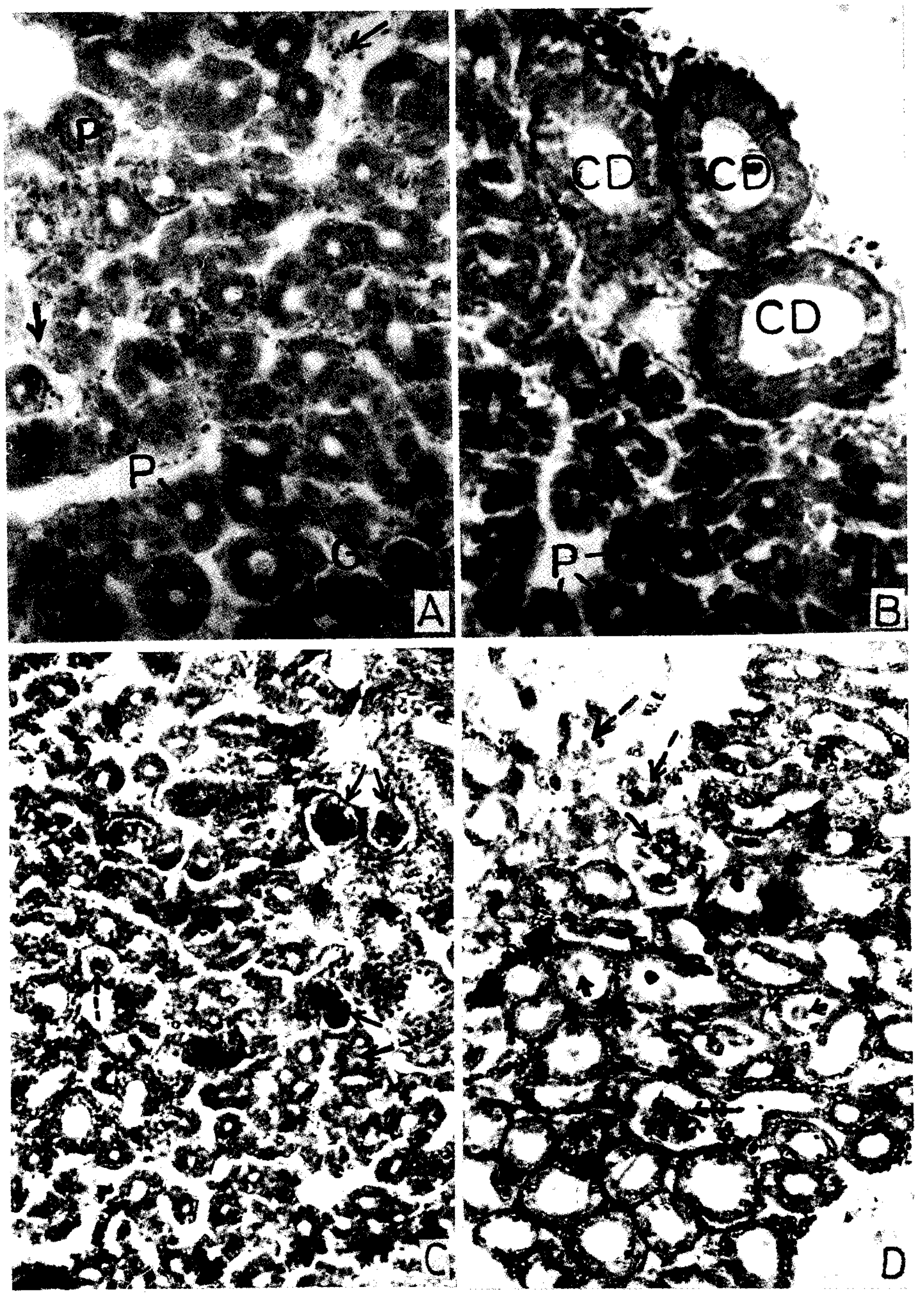


PLATE I A. Posterior kidney of control *Liza parsia* showing the proximal convoluted tubules (P), glomerulus (G) and haemopoietic tissue (arrow), X 200; B. Kidney of control *Liza parsia* exhibiting collecting ducts (CD) and proximal convoluted tubules (P), X 200; C. On day 7 of 0.05 ppm $HgCl_2$ treatment - Note the eosinophilic cast in the lumina of the proximal tubules (broken arrow) and shrinkage in the glomeruli (arrow), X 200 and D. Exposed to 0.05 ppm $HgCl_2$ for 15 days, showing the presence of eosinophilic casts in the proximal tubules (arrow head), sclerosis of the glomerular capillaries (arrow) and focal tubulonecrosis (broken arrow), X 200, Hematoxylin-eosin.

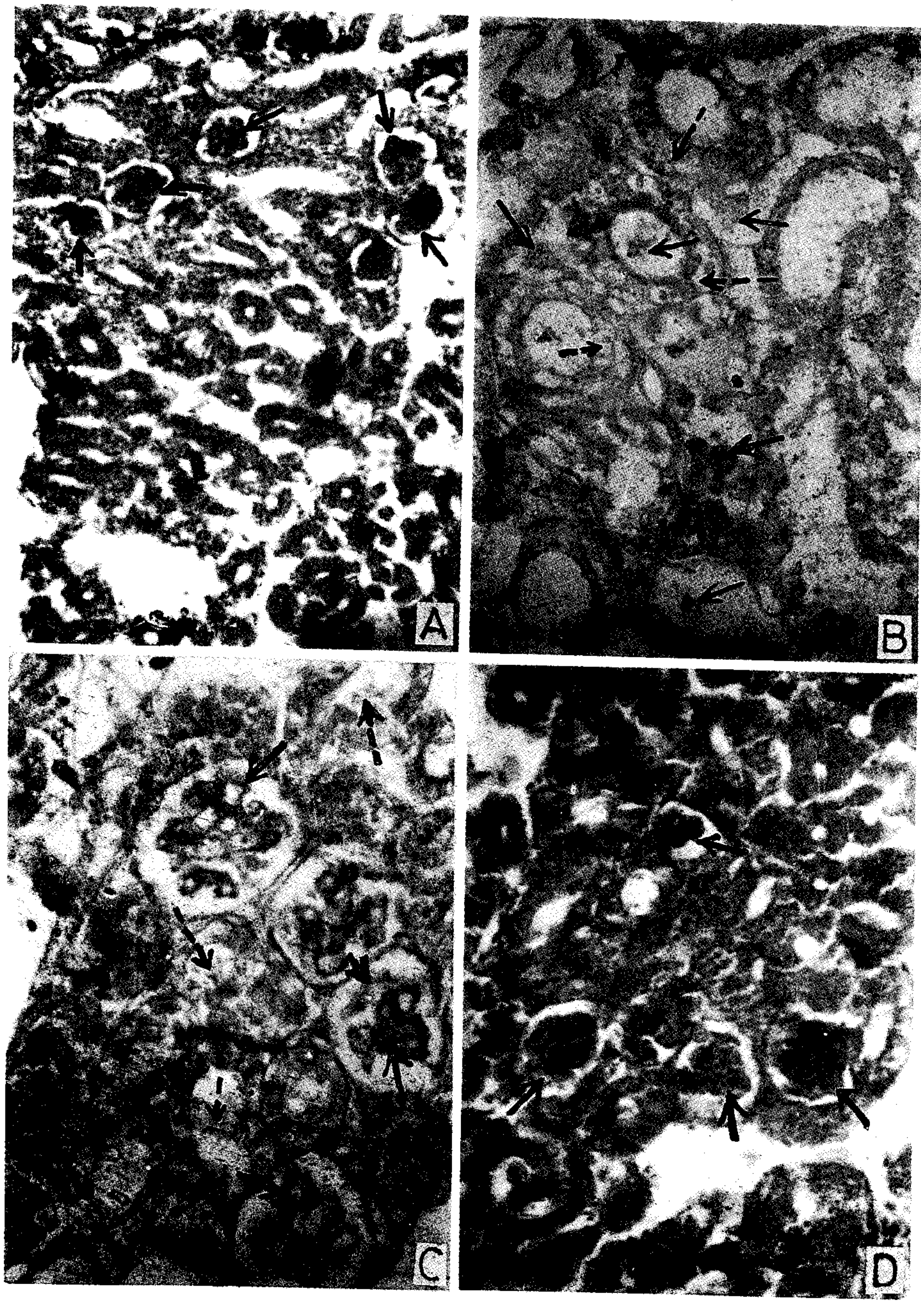


PLATE II A. Kidney of ***Liza parsia*** on day 7 of 0.1 ppm $HgCl_2$ treatment depicting shrinkage in the glomeruli (arrow) and dilation of the Bowman's space. Also, mark the focal tubulonecrosis, X 200; B. Exposed to 0.1 ppm $HgCl_2$ for 7 days showing the eosinophilic casts in the lumina of proximal tubules (arrow) and vacuolation of the epithelial cells (broken arrow). At places complete necrosis of the epithelium is also seen, X 400; C. After 15 days of 0.1 ppm $HgCl_2$ treatment exhibiting eosinophilic cast in the Bowman's capsule (arrow head) and in the lumina of proximal tubules (broken arrow). Mark the sclerosed glomerular capillaries (arrow), X 400; and D. Exposed to 0.1 ppm of $HgCl_2$ for 15 days. Mark the presence of uroliths in the renal tubules, X 200. Hematoxylin-eosin.

necrotic in many areas. These necrosed cells contained eosinophilic hyaline droplets in their cytoplasm. The swelling of cells were seen in most of the epithelial lining. Tubules also contained hyaline casts. In glomeruli, the capillaries became more permeable and proteinaceous fluid appeared in many Bowman's capsules. Partial sclerosis of the blood capillaries were also noticed (Pl. I D).

In mullets exposed to the higher concentration (0.1 ppm) of $HgCl_2$ (Group C), degenerative changes like swelling (hypertrophy) of the tubular epithelial cells started appearing within 96 hours (4 days). In 7 days treated fish, extensive changes were noticed in tubules and glomeruli. Tubular changes included swelling and vacuolation of the epithelial cells, desquamation or exfoliation of cells and accumulation of hyaline casts in the tubules. Eosinophilic hyaline droplets were also present in cytoplasm of the epithelial cells. Further, tubulonecroses were seen at many places. Glomerular changes consisted of hyperpermeability of capillaries, thickening of the capillary walls, increased cellularity, mesenchymal proliferation and progression of these lesions to the complete sclerosis of capillaries (Pl. II A, B).

Histological changes in the kidney of 15 days exposed fish remained almost same, however, many tubules contained amorphous material casts. Also, glomerular changes became more extensive and many of the glomeruli were sclerosed and appeared shrunken. Further, dilation of Bowman's space was also recorded (P. II C, D).

## DISCUSSION

Kidney of *Liza parsia* exhibits almost similar histological structures as described for a number of euryhaline teleosts (Trump *et al.*, 1975; Roberts, 1978; Oguri, 1982; Hwang and Wu, 1987).

In $HgCl_2$-treated *Liza parsia* (Groups 2 and 3), extensive changes were observed in the nephrons which increased in severity in proportion to the concentration of Hg and duration of the exposure. Initially, only tubular changes like degeneration of epithelial cells, vacuolation and desquamation or exfoliation of cells as well as eosinophilic caste in the tubular lumina were noticed. These changes are very close to the lesions reported in mammals and fish for heavy metal toxicity and anoxic conditions (Runnells *et al.*, 1965; Gardner and Yevich, 1970; Wobeser, 1975; Koyama and Ituzwa, 1977; Kumar and Pant, 1981 Jones and Hunt, 1983; Cruz and Tamse, 1986; Forlin *et al.*, 1986; Kirubagaran and Joy, 1988). However, appearance of hyaline droplets in the tubular epithelium was a remarkable feature in our study. In mammals, such structures are associated with the increased glomerular permeability to plasma proteins (Jones and Hunt, 1983). Though may glomeruli exhibited hyperpermeability in the kidney of Hg $Cl_2$-treated *Liza parsia*, the mechanism of production of these lesions might be different in fish. It is known that in fish kidney plays a major role in excretion of metallic ions and tubular epithelium is associated with this process (Trump *et al.*, 1975; Suzuki, 1977; Roberts, 1978). It is assumed that the increased load of metallic (Hg) ions damaged the membrane proteins by binding with sulphydril (-SH) groups (Trump *et al.*, 1975).

In fish exposed to higher concentrations of Hg (Group 3) for 15 days, mineral casts were observed in the kidney tubules, (Pl. II D). Such conditions of nephrocalcinosis or urolithiosis in fish have been described earlier by Trump *et al.* (1975) and Roberts (1978). Occurrence of such lesions has been ascribed to the excessive tubulonecrosis, imbalance in diet and excessive carbon dioxide in waters (Roberts, 1978). Though excessive tubular necrosis was found in our study, the exact mechanism involved in the production of such lesions is not known.

In Hg $Cl_2$-treated *Liza parsia* (Groups 2, 3), the glomeruli showed changes like increased permeability, thickening of capillaries, proliferation and sclerotic changes. These lesions appears similar to the common glomerulonephritis of other vertebrates which are attributed to the immunologically-mediated inflammatory reactions (Cassey *et al.,* 1979; Slauson and Lewis, 1979; Jones and Hunt, 1983). According to Roberts (1978), the glomerular lesions are frequent in fish though the causative factors are not known. Autoimmune reactions and deposition of immune complexes were suspected to cause glomerulonephritis (Cassey *et al.,* 1979; Slauson and Lewis, 1979; Jones and Hunt, 1983). Chemicals are reported to alter tissue antigen. Thus, new antigenic sites are produced which might stimulate host's own immune systems to react (Jones and Hunt, 1983). The glomerular lesions observed in *Liza parsia* were also noticed by other workers in experiments where zinc, copper, cadmium, potassium and insecticides were used (Gardner and Yevich, 1970; Kumar and Pant, 1981; Cruz and Tamse, 1986; Forlin *et al.,* 1986; Dinesan, 1988). It is worth to take up studies to understand the exact mechanism(s) involved in the production of glomerular lesions in fish.

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