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Histopathological alterations induced in the liver of an estuarine mullet, *Liza parsia* by mercuric chloride and DDT

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ABSTRACT

Liza parsia were exposed to $HgCl_2$ (acute, 1.0 ppm; sublethal, 0.5 ppm) and DDT (acute, 0.1 ppm) for 15 days. In control mullets, the liver was comprised of polygonal hepatocytes with centrally placed nucleus. Exposures to $HgCl_2$ and DDT induced dilation of blood sinusoids, vacuolization and granular degeneration of hepatocytes. The complete necrosis of hepatocytes at places (focal necrosis) and fibrosis were noticed on day 15 in the experimental fishes. Blood sinusoids also depicted complete disorganization by close of the experiment.

Introduction

Mercury (Hg), the most toxic and non-essential heavy metal, has a wide distribution in earth's crust and aquatic environments (Ruvio, 1972). With its extensive uses in various industries for preparation of medicines and a large number of insecticides, fungicides and bactericides, its concentration is increasing in the aquatic ecosystem (FAO, 1986). Recent studies have shown the distribution and/or accumulation of Hg in the water, sediments, zooplankton and fish of Indian seas (Singbal *et al.*, 1978; Kureishy *et al.*, 1979, 1983; Sanzgiry *et al.*, 1979, 1988; Krishnakumar and Pillai, 1990). Though the levels of Hg in fish is far below the recommendations made by WHO, there are certain 'hot spots' of mercurial pollution off Bombay, Karwar and between Mangalore and Calicut of the west coast (Tejam and Halder, 1975; Singbal *et al.*, 1978; Zingde and Desai, 1981; Sanzgiry *et al.*, 1988) and Adyar (Madras) as well as Rishikulya (Orissa) estuaries of the east coast of the country (Nammalwar, 1985; Zingde, 1989). Further, there exist reports on the presence of DDT in water and sediments from Bay of Bengal (Sarkar and Sen Gupta, 1980; Sen Gupta and Qasim, 1985). Since liver is an important target organ affected by pollutants in freshwater fish (Bhattacharya *et al.*, 1975, 1985; Dubale and Shah, 1979a, b; Naidu *et al.*, 1983; Sultan and Khan, 1983; Gill *et al.*, 1990), an attempt was made to record the histopathological changes in the hepatic tissue of the estuarine mullet, *Liza parsia*, exposed to mercuric chloride ($HgCl_2$) and DDT.

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Materials and methods

Immature specimens of *Liza parsia* (Hamilton - Buchanan) (average length 6.5 cm, weight 5.8±0.8 g) were collected from the Chinese dip net at Fort Cochin (09° 57' 06" N, 76° 14' 2" E) and were transported to the laboratory. They were kept for acclimatization in aquaria of 120 litre capacity containing well-aerated sea water (salinity 28 ppt, average water temperature 26°C) for a period of one week prior to use. Thereafter, they were randomly divided into 4 equal groups of 40 specimens each and subjected to the following treatments:

Group A : Fishes were maintained in sea water and served as control.

Group B : Mullets were exposed to acute concentration (1.0 ppm, two-third of LC50 value for 96 hrs) of $HgCl_2$ in sea water.

Group C : Fishes were exposed to the sublethal concentration of $HgCl_2$ in sea water (0.5 ppm, one-third of LC50 value for 96 hrs).

Group D : Mullet were treated with acute concentration (0.1 ppm, one - half of LC50 value for 96 hrs) of DDT. DDT was initially dissolved in acetone and the required concentration was maintained by adding sea water.

The media were renewed every alternate day. The fish did not accept formulated feed under the experimental condition and dead fishes were removed from the study.

Five specimens each from all the groups (A, B, C, D) were killed on days 2, 4, 7, 10 and 15 of initiation of the experiment. Their liver were surgically removed and fixed immediately in freshly prepared aqueous Bouin's solution. After 24 hrs, the tissue were washed thoroughly in running tap water, dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Serial sections were cut at 6μ and stained in hematoxylin - eosin (H & E). The cells were measured along its long and short axes with the help of ocular micrometer and the mean values were recorded.

Results

Liver of control *Liza parsia* comprised of continuous mass of hepatocytes arranged in irregular cords. The hepatic cells were large and polygonal in shape with almost centrally placed nucleus. The cytoplasm of hepatocytes stained homogenously with eosin (Fig. 1). Hepatic cells measured 6-8 and 4.5-6 μ in long and short axes, respectively. A large number of blood sinusoids were also seen around the hepatocytes.

Not much of changes were noticed in the liver of fish on day 2 of $HgCl_2$ or DDT treatments. Thereafter, all the mullets exposed to the toxicants exhibited hepatopathy. On day 4, a slight dilation of blood sinusoid was observed in 0.5 ppm $HgCl_2$ treated fish, however, this change was more marked at 1.0 ppm concentration (Fig. 2). In DDT - treated fish, bile canaliculi appeared more prominent due to accumulation of secretion and in a few hepatocytes signs of vacuolization appeared resulting in the displacement of nuclei towards the periphery (Fig. 3). By day 7 of $HgCl_2$ treatment, vacuolization was more pronounced and pycnotic changes were observed in the nuclei. Also, blood clots were frequently seen in the sinusoids

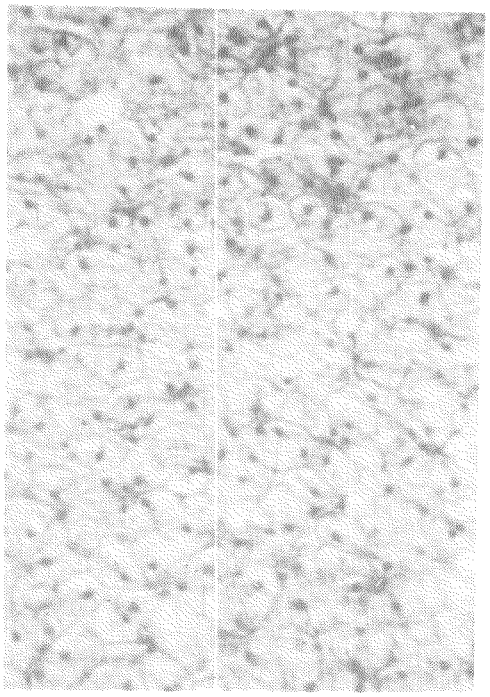

Fig. 1. Liver of control *Liza parsia* showing the continuous mass of hepatocytes arranged in cords. The hepatic cells are large and polygonal in shape with centrally placed nucleus. Mark the homogenously stained cytoplasm of hepatocytes. H & E. x 500.

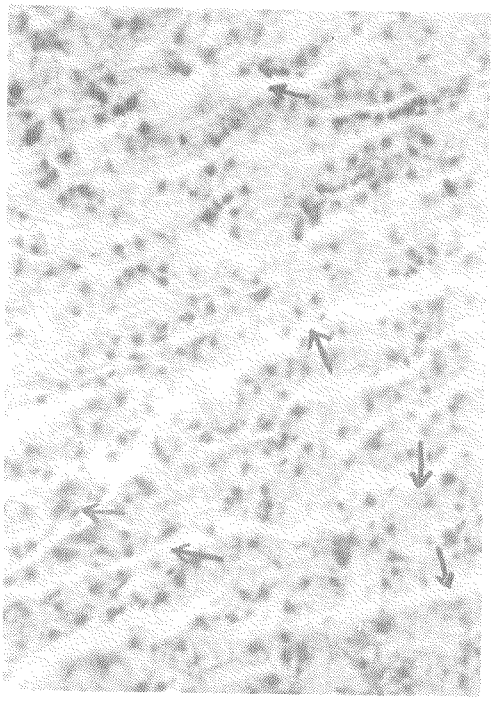

Fig. 2. Liver of *Liza parsia* on day 4 of 1.0 ppm $HgCl_2$ treatment exhibiting the dilated sinusoids (arrow). H & E. x 500.

(Fig. 4). Liver of DDT - treated fish also depicted a similar response on day 7 of exposure.

Hepatic lesions among $HgCl_2$ and DDT - treated mullets on days 10 and 15 were characterised by complete destruction of cytoplasmic and nuclear materials, vacuolization of the hepatocytes and proliferation of fibroblasts (Fig. 5). However, such changes were more drastic in DDT-treated fish where clumps of degenerating cells were observed very frequently (focal necrosis) and blood sinusoids exhibited complete disorganization (Fig. 6).

Discussion

Histological structures of liver of the estuarine mullet, *Liza parsia,* are almost similar to those described for a number of freshwater teleosts namely *Salmo clarkii* (Eller, 1971), *Channa punctatus* (Anees, 1978; Dubale and Shah, 1979 a, b; Bhattacharya *et al.,* 1985), *Carassius auratus* (Sultan and Khan, 1983), *Oreochromis mossambicus* (Naidu *et al.*, 1983), *Salmo salar* and *S. gairdeneri* (Roberts, 1989). However, we could not find any pancreatic tissue in the liver of *Liza parsia* as reported in *Clarias batrachus* (Bhattacharya *et al.*, 1975) and *Channa punctatus* (Bhattacharya *et al.*, 1985).

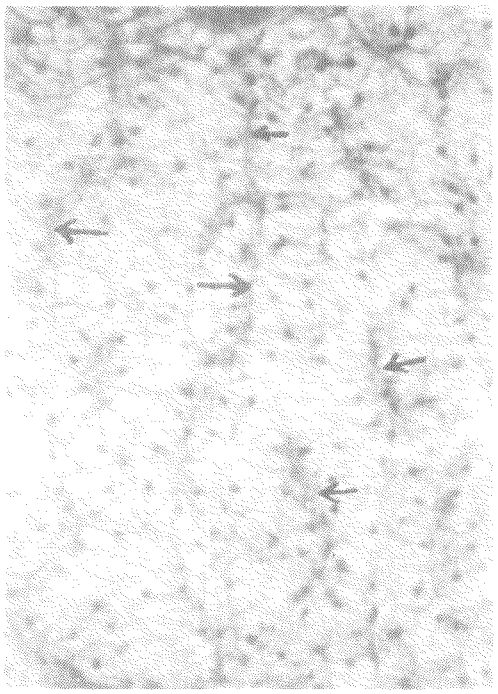

Fig. 3. Liver of *Liza parsia* on day 4 of DDT (0.1 ppm) treatment depicting the accumulation of secretion in the bile canaliculi (arrow). Some hepatocytes exhibit the displacement of nucleus towards the periphery. H & E. x 500.

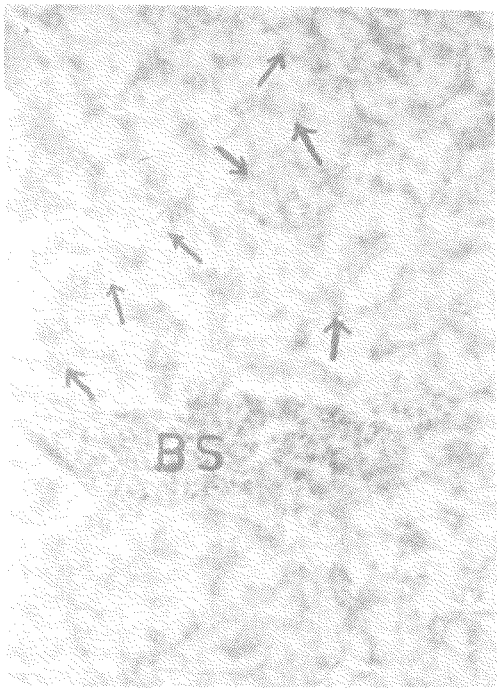

Fig. 4. Liver of *Liza parsia* showing cytoplasmic vacuolization and pycnotic nuclei (arrow) on day 7 of $HgCl_2$ (1.0 ppm) treatment. Also, mark the enlarged blood sinusoids (BS). H & E. x 500.

Helmy *et al.* (1979) and Krishnakumari *et al.* (1983) have demonstrated the toxicity of Hg in the estuarine fishes *Liza macrolepis* and *Therapon jarbua,* respectively. Our studies revealed that both $HgCl_2$ and DDT induced pathological changes in the liver of *Liza parsia*. Jackim *et al.* (1970) have shown that the salts of Pb, Hg and Cu caused detrimental effects on the hepatic enzymes in *Fundulus heteroclitus*. Dubale and Shah (1979 a) reported degenerative changes like vacuolization of cytoplasm and shrinkage in nuclei of *Channa punctatus* exposed to varying concentrations (0.001, 0.02, 0.05 ppm) of cadmium nitrate. Destruction of cytoplasmic materials and vacuolization of hepatocytes of *Carassius auratus* in response to acute (1000 µg/l) and chronic (100 µm/l) exposures of $CuSO_4$ were observed by Sultan and Khan (1983). Liver of *Sarotherodon (Oreochromis) mossambicus* exhibited engorged blood sinusoid, vacuolization, granular degeneration of hepatocytes, oedema, focal necrosis and proliferation of fibroblasts to lethal (1.5 ppm) and sublethal (0.1 ppm) $HgCl_2$ treatments (Naidu *et al.*, 1983). Recently, Bhattacharya *et al.*

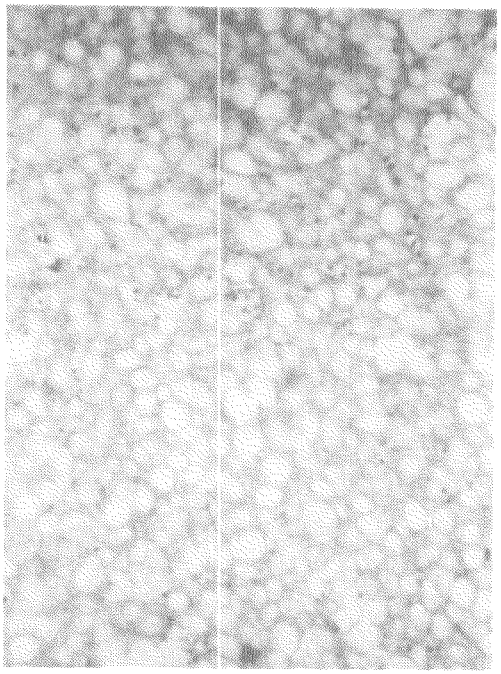

Fig. 5. Liver of *Liza parsia* on day 15 of $HgCl_2$ (0.5 ppm) intoxication depicting necrotic changes in hepatocytes. Cytoplasm is filled with vacuoles and nuclei show pycnosis. H & E. x 500.

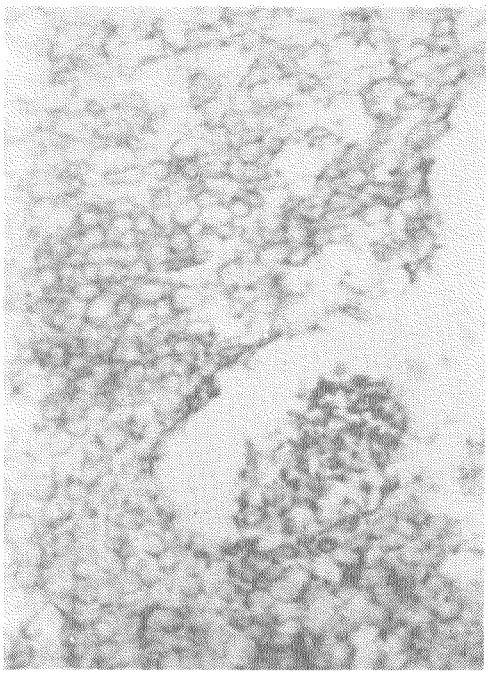

Fig. 6. Liver of *Liza parsia* exhibiting complete necrosis of the hepatocytes and disorganization of sinusoids by day 15 of DDT (0.1 ppm) treatment. H & E. x 500.

(1985) also noticed clumping of cytoplasmic materials, displacement of nuclei towards periphery and coagulation of blood in sinusoids of *Channa punctatus* treated with 0.60 ppm $HgCl_2$, however, complete vacuolization of most of the hepatocytes and extrusion of nuclear materials were recorded among murrels at 0.96 ppm concentration. The observed progressive degenerative changes in the liver of *Liza parsia* in response to $HgCl_2$ intoxication supports the findings in *Sarotherodon (Oreochromis) mossambicus* (Naidu *et al.*, 1983) and *Channa punctatus* (Bhattacharya *et al.*, 1985).

Histopathological changes in the liver of freshwater fishes caused by pesticides/insecticides intoxication have been recorded by Gill *et al.,* (1990). Eller (1971) observed swollen hepatocytes and progressive degeneration of liver of the cutthroat trout, *Salmo clarkii,* exposed to high levels of endrin. In *Channa punctatus,* Anees (1978) reported cytoplasmic granulation following acute exposure to sublethal concentration of dimethoate. Degenerative changes were also noticed in *Channa punctatus* in response to dieldrin, lindane, endrin and dimecron intoxications (Mathur, 1976; Sastry and

Sharma, 1978; Sastry and Malik, 1979). Dubale and Shah (1979 b) studied exhaustively the effects of malathion on the hepatic lesions in *Channa punctatus* and noticed an initial precipitation of cytoplasmic materials, vacuolization and shrinkage and disintegration of the nuclei of hepatocytes. The treatments elicited necrotic changes in the hepatocytes at longer durations. Mathur (1962) reported hepatic lesions in *Channa punctatus, Heteropneustes fossilis, Trichogaster fasciatus* and *Barbus stigma* exposed to the varying concentrations (3-60 ppm) of DDT. Our results on the histopathological changes in *Liza parsia* to 0.1 ppm DDT intoxication correspond well with the observations made in 1.0 mg/litre malathion-treated *Channa punctatus* (Dubale and Shah, 1979 b) because in both these cases severe necrotic changes were seen after day 7 of the experiment. The present study clearly demonstrates that the liver is an important target organ for Hg and DDT pollutants in the estuarine fish, *Liza parsia*.

Acknowledgements

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