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Hepatocyte Damage in Indian Major Carp, *Labeo rohita* with Respect to Accumulation and Elimination of Mercury

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Abstract : Fresh water fish *Labeo rohita* (Hamilton) was exposed to identical concentrations of $HgCl_2$ (inorganic mercury) and CH_3HgCl (methyl mercury chloride) separately and the liver was studied for mercury bioconcentration and histopathological changes. Mercury bioconcentration and histological damage were found to be relatively more in fishes exposed to methyl mercury at all intervals of time. When the fishes were brought to control conditions after 60 days of exposure, mercury level in liver declined from 19.2µg/g to 16.3µg/g in the case of inorganic mercury treated fish. But interestingly liver mercury residue level increased from 37.4µg/g to 39.8µg/g in the case of methyl mercury treated group. A parallel histopathological study revealed that the reduction in mercury level in the former brought about a corresponding improvement in the diseased condition of the liver, however the histological picture continued to show further degenerative changes in the methyl mercury treated group even though the fishes were brought to control conditions.

Key words : *Labeo rohita*, liver, inorganic mercury, methyl mercury, histology, accumulation

Introduction :

Public health concern over mercury exposure to human beings due to consumption of contaminated fish has been a topic of political and medical debate. Pathological effects due to inorganic mercury in certain fishes have been reported. (Kendall, 1975; Sastry and Gupta, 1978; Khangrot, 1980; Naidu *et al*., 1983). In the aquatic environment inorganic mercury is converted to methyl mercury and is the predominant form of mercury reported in fishes caught from contaminated waters (Ann Houck *et al.* 2004). Since the Minamata disease, methyl mercury has been considered as one of the most dramatic and best documented examples of bioaccumulation of metals in the environment, particularly in aquatic food chain. Organic mercury is more toxic (Clarkson, 1994) and is rapidly accumulated in aquatic organisms than inorganic forms (Taylor, 1979; Paulose, 1988; Mason *et al*, 1995). Methyl mercury has been implicated as a neurotoxicant, a mutagen and a teratogen. The present author has reported the comparative effects of inorganic and methyl mercury in the gills (Paulose, 1987b and 1989) and intestine (Paulose, 2003) of certain commercially important fishes. The present paper reports the comparative histological damage induced by inorganic and methyl mercury in the liver of Indian major carp *Labeo rohita*, which constitute an important fishery in India. An attempt has also been made to correlate the histopathological changes with respect to mercury accumulation and elimination from the liver.

Materials and Methods:

Labeo rohita obtained from a local source were acclimated to the laboratory condition for 20 days. They were fed with pellets prepared from oil cake and wheat bran in the ratio 1:1. The 96-hour LC_{50} values were determined by static bioassays according to Sprague (1973). The values

were 0.180 and 0.068 ppm Hg as $HgCl_2$ and CH_3HgCl, respectively. 0.01 ppm was selected for long term studies. Fish with an average weight of 25.6 ± 0.85 g were divided into 3 groups of 50 each. One of these groups was maintained as control whereas the other two groups were exposed separately to 0.01 ppm Hg in water as $HgCl_2$ and CH_3HgCl. The experiments were carried out in plastic pools with 1250 litre of water. The exposure medium was changed daily to replenish the mercury concentration. After 60 days of exposure, the two experimental groups were brought to control condition to study the recovery and clearance rate of accumulated mercury in the liver. Fishes were fed with pellets during the experimental period. The physico-chemical characteristics of the water were analyzed according to APHA (1985), and the values (mg/l) were: total alkalinity, 141 ± 8, total hardness 220 ± 12, chloride 78 ± 4, dissolved oxygen, 7.2 ±0.6 and the pH 7.4 ±0.2 .The water temperature varied between 19°C to 23°C.

For histopathological studies, six fishes from each group were sacrificed at intervals of 7, 15, 30, 45, 60 and 75 days. The liver was fixed in Bouin's fluid, washed, dehydrated in ethanol series, cleared in xylene and embedded in paraffin. Sections were cut at 5-µm thickness and stained with haematoxylin and eosin.

Total mercury in the liver was determined at the same time intervals as described above in the histopathological protocols. Mercury was determined by cold vapour atomic absorption spectrophotometry according to Uthe *et al*. (1970). The details of tissue processing for this purpose have been reported elsewhere (Paulose, 1987a).

Results :

Bioconcentration of mercury at different intervals is reported in Table 1. In both the experimental groups, liver mercury increased continuously till the end of the exposure. However, the rate of accumulation at all intervals of time was greater in the methyl mercury treated group. The accumulation pattern was more or less similar in both, inorganic and methyl mercury treated groups. When the fishes exposed to inorganic mercury were brought to control condition the mercury residue in liver decreased from 19.2µg Hg /g at day 60 to 16.3µg Hg /g at day 75. On the contrary, the level continued to rise in the methyl mercury exposed group even after discontinuation of treatment. The values were 37.4 µg Hg/g and 39.8 µg Hg/g at 60 and 75 days, respectively. Fishes in control groups were also analyzed for background level of mercury and the values were found always less than 0.02 µg Hg/g tissue.

Table 1 : Mercury concentration in the liver (µg Hg/ g) tissue of *Labeo rohita* at varying intervals of exposure (0.01ppm) to $HgCl_2$ and CH_3HgCl. (Values are mean ± SE of 6 fish in each group)

	Days					
	7	15	30	45	60	75
$HgCl_2$	2.21±0.23	4.84±0.44	13.64±0.68	17.13±0.92	19.23±1.34	16.30±1.88*
CH_3HgCl	4.63±0.49	7.57±0.72	23.55±1.19	32.11±2.06	37.44±2.22	39.88±2.41*

* Value after 15 days of depuration.

Liver damage was also found more in methyl mercury treated group. Healthy hepatocytes of *Labeo rohita* are polyhedral cells having uniformly spherical nuclei placed centrally or eccentrically. The hepatocyte nucleus has large and prominent nucleolus (Figure 1). In the inorganic mercury treated fish though the gross structure of the liver appeared normal throughout the experimental period yet cellular and nuclear hypertrophy were observed in the hepatic cells. Mild vacuolation of the cytoplasm was evident in the initial stage of exposure. As the exposure continued further the cytoplasm became granular and showed wide spread vacuolation. At day 60, the vacuolation increased further and extensive necrosis of the hepatic parenchyma gave a spongy appearance to the liver (Figure 2). However, regeneration of protoplasmic material was observed when the mercury treatment was stopped. Restoration of control condition for 15 days resulted in remarkable recovery so much so that the hepatocytes appeared regenerating their structural organization by reconstituting the cell membranes. Mild vacuolation of the cytoplasm was, however, still persisted (Figure 3).

All the symptoms observed in inorganic mercury treated group were also found in fishes treated with methyl mercury, but the extent of damage in the latter was severe and rapid. Vacuolation of cytoplasm was so acute in the methyl mercury treated group that some cells were completely filled with a large vacuoles presenting a balloon shaped appearance (Figure 4). In many cases cell membranes ruptured and naked nuclei were found scattered in the hepatic parenchyma (Figure 5). Instead of recovery, the histological picture deteriorated further during depuration studies. The typical arrangement of the hepatocytes was completely lost and many of the cells were found devoid of protoplasmic contents (Figure 6).

Discussion :

Inorganic mercury salts are unable to cross tissue-blood barriers and is eliminated at a faster rate than methyl mercury (Ulfvarson, 1966). Animals start accumulating mercury when the rate of uptake exceeds the rate of elimination. In the present study, when inorganic mercury treatment was stopped, the elimination of accumulated mercury resulted in the decline of its residue level in liver and this brought about a corresponding histological recovery.

The picture was, however, different in the methyl mercury treated group. Methyl mercury accumulates quickly and depurates very slowly. The accumulated methyl mercury is excreted slowly due to its high lipid solubility (Rucker and Amend, 1969) and tends to be cumulative in tissues like brain and liver (Clausing *et al*., 1984). The reason for increased level of mercury in liver even after depuration may be due to its redistribution from other tissues. McKim *et al.*, (1967) found a decline in mercury level in the blood of *Salmo gairdneri* exposed to methyl mercury, was brought to control condition, but it registered a further increase in kidneys. Giblin and Massaro (1978) have reported withdrawal of mercury from the blood and its redistribution to other tissues after a single intra-gastric dose of CH_3HgCl in *Salmo gairdneri*. Mature and immature red blood cells in this fish were found accumulating CH_3HgCl more rapidly than $HgCl_2$. It was observed that these cells bind over 90% of CH_3HgCl with haemoglobin and readily transfer to other tissues. In his previous studies the first author has found a decline in mercury level in gills and intestine when methyl mercury

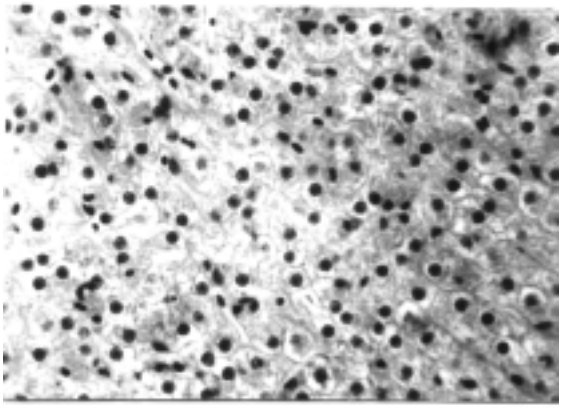

Fig. 1 : Control. x 400

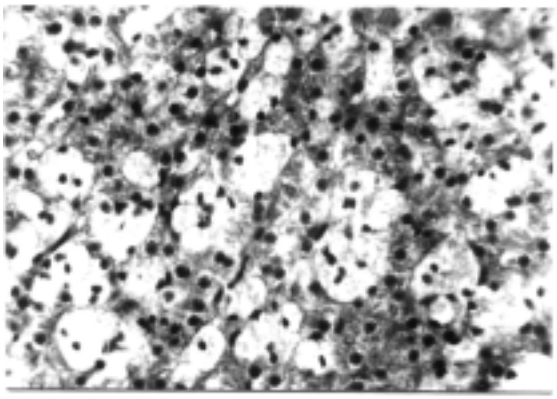

Fig. 2 : Fish exposed to $HgCl_2$ for 60 days. Note widespread necrosis in the liver cells giving a spongy appearance. x 400

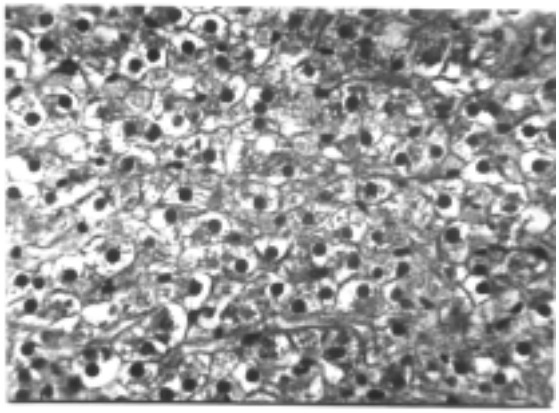

Fig. 3 : Fish maintained in control condition for 15 days after exposure to $HgCl_2$ for 60 days. Note regenerative changes such as reconstitution of cell membrane. x 400

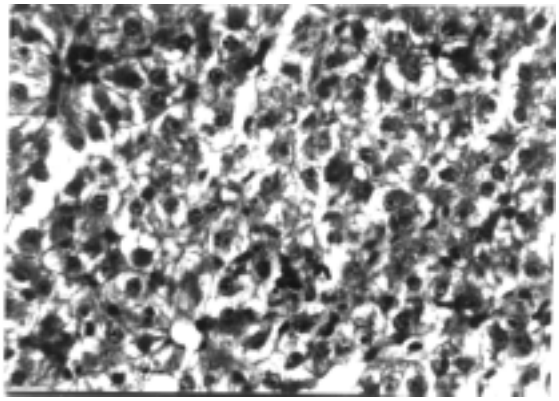

Fig. 4 : Fish exposed to CH_3HgCl for 30 days. Note necrotic and naked nuclei and degeneration of cytoplasm. x 1000

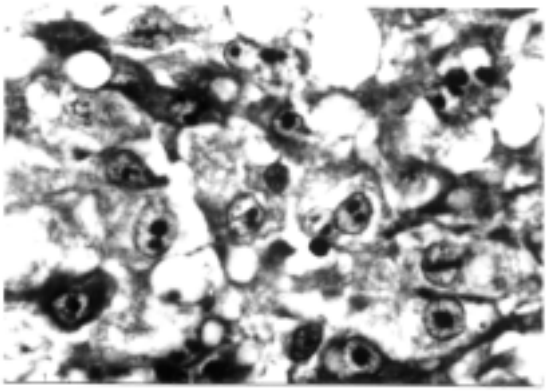

Fig. 5 : Fish exposed to CH_3HgCl for 60 days. Note the advanced stage of degeneration. The structural organization of the heatocytes is lost due to complete dissolution of cell membrane. x 1000

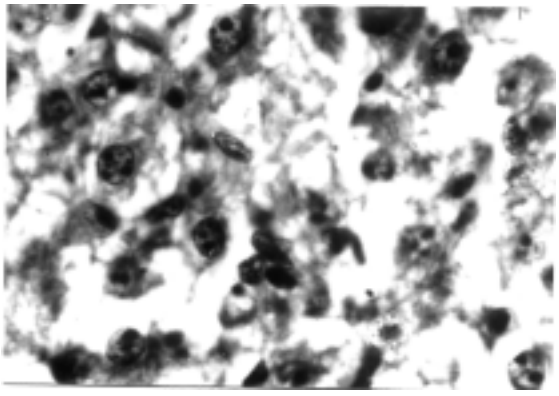

Fig. 6 : Fish maintained for 15 days in mercury free water after exposing to 60 days to CH_3HgCl. Note further damage of to hepatocytes instead of recovery. x 1000

treated fishes were brought to normal waters (Paulose, 1989 and 2003). Mercury might have been transported to liver from these sites during depuration. Methyl mercury becomes firmly bound to sulfhydryl-containing macromolecules resulting a long half-life for elimination. Although mercury sulfhydryl bond is stable, it is labile in the presence of other free sulfhydryl groups; therefore, methyl mercury will be redistributed to other competing sulfhydryl legends. This is the basis of chelation of heavy metals with sulfhydryl compounds. The binding and dissociation of these mercury–thiole complexes are believed to control the movement of mercury and its toxic effects in the body (Lyn Patrick, 2002)

The continuation of histological damage in methyl mercury treated fish even during depuration may be due to high level of residue present in liver. The percentage of cells undergoing apoptosis was reported which is dependent on the mercury content of the medium, regardless of the form of mercury (Mare and Joseph, 2000). Mercury can inflict cellular damage wherever it accumulates in sufficient concentrations (Miura *et al*., 1999, Zalups, 2000). This may be the reason for the selective toxicity of mercury in relation to distribution.

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