Bull Environ Contam Toxicol (2012) 89:479–483
DOI 10.1007/s00128-012-0743-3

Histopathological Effects and Bioaccumulation of Mercury in the Kidney of an Indian Major Carp, *Labeo rohita* (Hamilton)

Debasree Ghosh · Dipak Kumar Mandal

Received: 23 October 2011 / Accepted: 10 July 2012 / Published online: 19 July 2012

Abstract The effect of mercuric chloride on the histomorphology and bioaccumulation in the kidney of an Indian major carp, *Labeo rohita* (Hamilton) were examined after exposing the fish (15–20 cm) to three sublethal concentrations (0.033, 0.066 and 0.132 mg/L) of $HgCl_2$ for 30 days. Mercury deposition in kidney tissues had increased significantly with dose and exposure duration dependant manner. Several histological changes were noted in the kidney of all treated groups in compare to control group.

Keywords *Labeo rohita* · Mercuric chloride · Kidney · Bioaccumulation · Histopathology

Mercury has been recognized as one of the most hazardous aquatic pollutants due to its toxicity, bioaccumulative and non-biodegradable properties. Mercury and its compounds have no known biological function, and if present in any living organisms, may cause cytochemical and histopathological effects. Three general types of mercury are found as elemental, inorganic and organic. Elemental mercury is not well absorbed by the gastrointestinal tract (GI), but its vapour is well absorbed by the lungs. Organic mercury compounds, particularly methyl mercury, can evaporate and undergo pulmonary absorption, and are also well absorbed by ingestion. Very little amount of this compound is absorbed through skin.

The inorganic mercury is used extensively in various fields like paint and colour industries, in pharmaceutical industries for drugs manufacture, disinfectants and antiseptic dressing, in formulation of a large number of fungicides. As a result a huge quantity of mercury is being disposed into the aquatic system in every year. Mercuric mercury, as water soluble salts, is highly potent poison. In many carcinogenicity and toxicity studies, the effects of mercuric chloride have been assessed. After absorbed, it is dispersed in all tissues and some amount of it can easily cross the blood brain barrier and the placenta (Clarkson and Magos 2006).

Labeo rohita (Hamilton), a freshwater Indian major carps (IMC), is a rich source of protein for human beings. It is a column feeder and feeds zooplankton (juveniles) and phytoplankton (adults). It is economically important due to its high commercial demand on markets.

Kidney maintains a stable internal body environment in relation to electrolytes and water balance (Ortiz et al. 2003) and excretes nitrogenous product. It is the primary target site where mercuric chloride accumulates after chronic exposure (WHO 1991). Since, fish are an important source of the human food, may indirectly influence the harmful effects of mercury to the human beings.

Many authors have observed histopathological effects of different heavy metals on fish kidneys (Banerjee and Bhattacharya 1994; Gill et al. 1989). Cadmium shows affinities for renal tissue as a site of accumulation. A huge concentration of this metal is found in kidney exploring dietary or through water (McCoy et al. 1995). Although copper has no specific affinity for the kidneys, it produces significant structural damage to both glomeruli and renal tubules (Bakshi 1991).

D. Ghosh · D. K. Mandal (✉)
Fish Biology Laboratory, Department of Zoology,
School of Life Sciences, Visva-Bharati University,
Santiniketan 731 235, West Bengal, India
e-mail: dkmandal.vb@gmail.com

D. Ghosh
e-mail: debasree.zoology@gmail.com

Many reports have indicated the adverse effect of mercury on different fish tissues (Ram and Sathyanesan 1986; Kirubagaran and Joy 1988). The organic mercury compounds as well as mercury vapour affects central nervous system (Vahter et al. 2000) whereas liver, GI tract and kidney is the main target organ of mercuric chloride (Ghosh and Sil 2008). Proteins bind mercuric ions to sulfhydryl groups resulting in structural as well as functional protein impairment.

The present investigation has been carried out to observe the nephrotoxicity of fish *Labeo rohita* by histopathological analysis and accumulation of mercury after continuous exposure of mercuric chloride up to 30 days.

Materials and Methods

A group of healthy, disease-free fingerlings of *Labeo rohita* (length 15–20 cm and weight 30–40 gm) were procured from local fish ponds, Santiniketan (23.68°N 87.68°E), West Bengal, India. They were acclimatized for 2 weeks under laboratory condition with proper aeration. The fish were fed with commercially available feeds. Different parameters of water body can alter the levels of mercury. Owing this, the physico-chemical parameters of laboratory water condition were analysed by the methods following APHA (2010). The water parameters of all aquarium were maintained as natural habitats of this fish species viz – temperature 20–22°C; pH 7.2–7.5; dissolved oxygen 4.16 mg/L, total alkalinity 162.4 mg/L, and total hardness 166.4 mg/L. No mortality was observed during this period.

Prior to the experiment, we determined 72 h LC_{50} value of $HgCl_2$ for *Labeo rohita* following the Probit analysis method (Finney 1971). Then, for this experiment, after acclimatization of fish, a set of 4 aquariums were taken with same water source and the fish were randomly divided into four groups containing equal number (10 fish/group) in which the first one was kept as control (without contamination) and other 3 groups were exposed to three different sublethal concentration of mercuric chloride [1/5th (0.132 mg/L), 1/10th (0.066 mg/L) and 1/20th (0.033 mg/L) dose of 72 h – LC_{50}] for 30 days with continuous aeration. The above experiment was done in triplicate. The fish were fed ad libitum. Water was exchanged and the doses were repeated in every alternate day. After completion of experiment, fish were anaesthetized with MS 222 and intact kidney tissues were collected carefully from both control and treated fish.

For histological study, the kidney tissues were fixed in aqueous Bouin's fixative for 20 h. Fixed tissues were washed in distilled water repeatedly, dehydrated through graded series of ethanol followed by acetone and cleared in benzene. Then the tissues were embedded in paraffin wax (Merck, India) of 56–58°C under a thermostat vacuum paraffin-embedding bath for a period of 1 h. Serial thin (4 μm) sections of the paraffin block of the tissue were obtained using a rotary microtome (Wesnox, India). The serial sectioned tissues were stretched on Mayer's albuminised glass slides. Ten sections were taken in each slide in two rows for both control and treated groups. The sections were deparaffinized with xylene, hydrated through down-graded ethanol up to distilled water. Then the slides were stained with Mayer's Haematoxylin and counter stained with eosin (1 %). After staining, slides were dehydrated in graded ethanol, cleared in xylene and mounted with DPX. For each $HgCl_2$ treated group, randomly collected six fish were dissected and five slides were made for each. Hence, a total of 30 slides were observed per treatment per organ. Stained slides were examined, compared to control group and photographed under Olympus BX52 Compound Research Microscope. Histopathological alterations in the kidney tissues were assessed semi-quantitatively by using a score ranging from – to +++ depending on the degree of alteration: (–) none, (+) mild alteration, (++) moderate alteration and (+++) severe alteration.

To determine the accumulation of mercury in the fish kidney through atomic absorption spectrophotometric study, the kidney tissues of three fish were taken and pooled together to obtain ten samples from both control and treated groups. They were weighed, each sample was digested in aqua regia (nitric acid: hydrochloric acid = 1:3 v/v) and diluted with double distilled water. The rate of accumulation of mercury was analysed by AAnalyst 200 Perkin-Elmer atomic absorption spectrophotometer by cold hydride process followed by the standard curve provided by Perkin-Elmer and the results were given as μg/g wet weight.

Data were presented as mean ± SD. Statistical analysis for accumulation of mercury in each treated group was carried out by one-way analysis of variance (ANOVA) to determine the significance level and results were compared to f-table values at the p values <0.05 probability level. Fisher's t test was performed to test the significance between control and treated groups ($n = 10$). All the analysis was done using MATLAB 2007.

Results and Discussion

Kidney is one of the major organs for detoxification and elimination of metallic pollutants. In our present study, the histological observation in *Labeo rohita* revealed that, the kidney was formed of numerous renal corpuscles with well-developed glomeruli, tubules and collecting ducts, embedded in the interstitium which contained hemopoetic

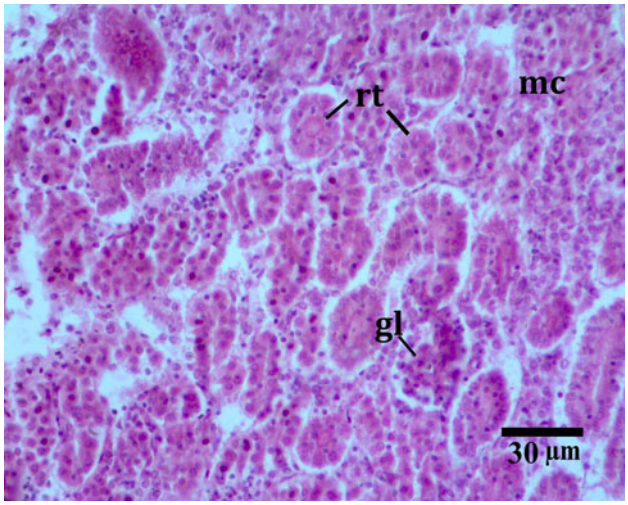

Fig. 1 Kidney section of *Labeo rohita* showing normal structure with well-formed glomerulus (gl), renal tubules (rt) and mesenchymal cells (mc). H & E

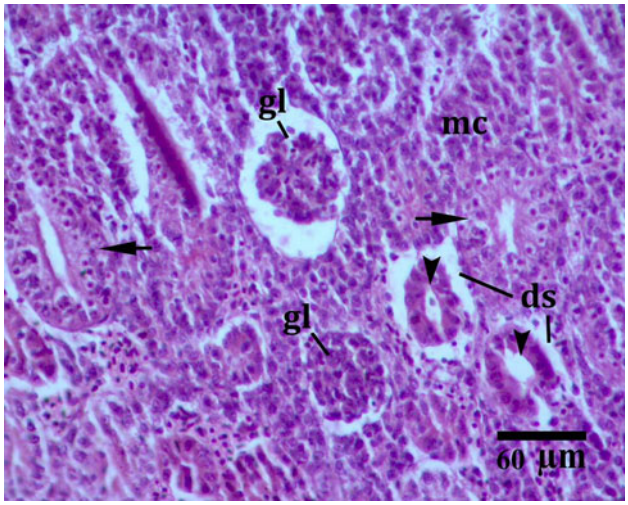

Fig. 2 Section of kidney of *Labeo rohita* after exposure at 0.033 mg/L $HgCl_2$ showing enlargement of renal tubules (*arrow*), dilation of tubule lumen (*arrowhead*), desquamation of epithelial lining (ds), shrinkage of glomeruli (gl) and hypertrophied mesenchymal cells (mc). H & E

tissues. The glomerulus was formed of capillaries surrounded by Bowman's capsule. The microscopic structure of glomerulus showed that the capillaries were covered by endothelial cells. On the other hand, the tubules (extended from Bowman's capsule) were covered by tall columnar epithelial cells with oval or round nuclei, showing apical microvilli or brush border towards the lumen (Fig. 1).

The kidney of inorganic mercury treated fish exhibited marked differences from control group when the fishes were treated with low dose (0.033 mg/L) of mercuric chloride. The renal tubules became expanded; their epithelial lining was separated from the tubular cells. Desquamation of epithelial lining was distinct. The lumen of the tubules became dilated. Shrinkage of the glomerulus increased space within the Bowman's capsule. Necrosis and pyknotic nuclei have also been observed in mesechymal tissue along with hypertrophied cells in renal tubules (Fig. 2). When the fish were exposed to moderate dose (0.066 mg/L) of $HgCl_2$ up to 30 days the renal tubules became more dilated followed by loss of cellular integrity. Hypertrophied epithelial cells of renal tubules were also seen. Some tubules became vacuolated due to loss of cells. Shrinkage of glomerulus and necrosis in mesenchymal cells were the prominent features (Fig. 3). Fish exposed at high dose (0.132 mg/L) of mercuric chloride for same duration, exhibited disintegration in the histological structure of renal tissue. The tubules lost their cellular integrity due to necrosis and destruction of epithelial and mesenchymal cells (Fig. 4). Heavy metals contaminated fish showed pathological alterations, inhibition in metabolic processes, haematological variations and marked changes in their fertility and survival (Kaoud and El-dahshan 2010). In this study, noticeable histological alteration in kidney of all $HgCl_2$ exposed fish group was evidenced (Table 1). The

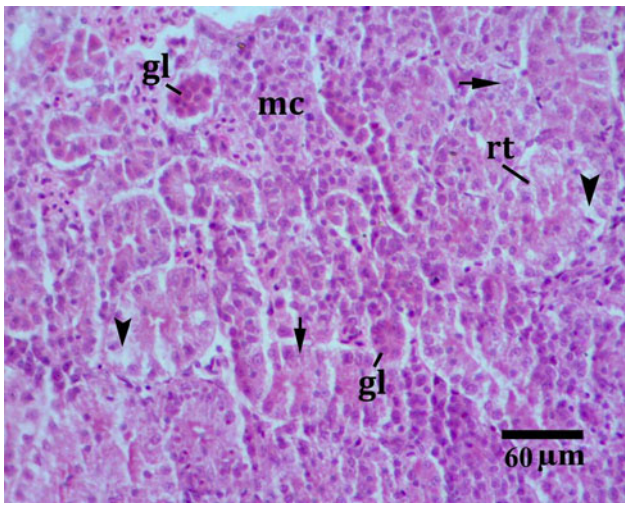

Fig. 3 Section of kidney of *Labeo rohita* after exposure at 0.066 mg/L $HgCl_2$ for 30 days showing enlargement of renal tubules (rt), necrosis (*arrow*) and vacuole formation in renal tubule (*arrowhead*), shrinkage of glomeruli (gl) hypertrophy in the mesechymal cells (mc). H & E

fish kidney receives much of the largest proportion of postbranchial blood, and therefore, any deformity in renal structure is a prominent indicator of environmental pollution (Ortiz et al. 2003). Long term exposure in inorganic mercury affected degeneration and disorganization of hemopeotic tissue, thickening of uriniferous tubules, indicated disturbance in normal functioning of kidney. It can also disturb several cellular processes such as enzyme function inhibition; blocked the cellular receptors in ion channels.

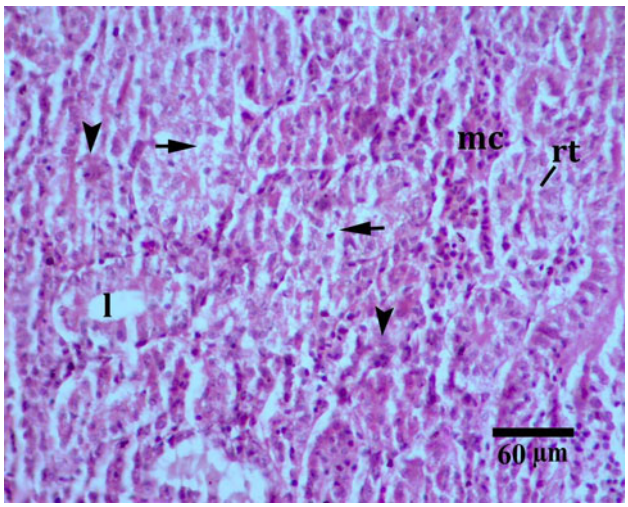

Fig. 4 Section of kidney of *Labeo rohita* after exposure at 0.132 mg/L $HgCl_2$ for 30 days showing enlarged renal tubules (rt) with dilated lumen (l), vacuole formation (*arrow*) and necrosis of cells (*arrowhead*). Mesenchymal tissue (mc) degenerated. H & E

Table 1 Semi quantitative scoring of the histopathological changes in the kidney of *Labeo rohita* after 30 days exposure of mercuric chloride

Histopathological changes	Mercuric chloride concentration (mg/L)		
	1/20th dose (0.033 mg/L)	1/10th dose (0.066 mg/L)	1/5th dose (0.132 mg/L)
Enlargements of renal tubules	+	+++	+++
Vacuole formation in renal tubules	–	++	+++
Hypertrophy in mesenchymal cell	++	++	+++
Shrinkage of glomeruli	+	++	+++
Necrosis of cells	+	++	+++
Degeneration of mesenchymal tissue	–	++	+++

Score: –, no alteration; +, mild alteration; ++, moderate alteration; +++, severe alteration

In *Labeo rohita*, histopathological damage in the kidney coincided with mercury accumulation in this tissue. Mercury has affinity to sulfhydryl groups on the cell membrane and thus inhibits active transport and cell functions (Passow et al. 1961). Dallinger et al. (1987) reported a high accumulation of several heavy metals in liver and kidney and suggested that these organs were target for final deposition of various heavy metals. In this study, significant level of increase of mercury accumulation in the kidney was observed in dose dependent manner after 30 days exposure to different sublethal concentrations of

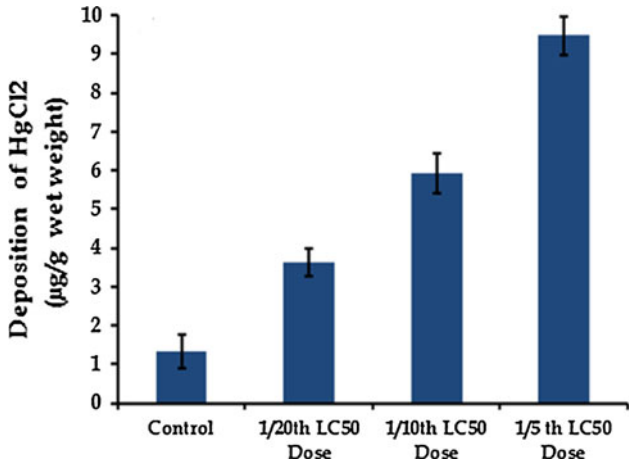

Fig. 5 Graphical representation of accumulation of mercury (Mean ± SD) in kidney tissue (n = 10) of *Labeo rohita* following exposure at different sublethal concentrations of mercuric chloride for 30 days

mercuric chloride (Fig. 5). Fisher's *t* test revealed that, all the mercuric chloride treated groups were significantly different to each other and also from control group ($p < 0.05$). In fish, the kidney accumulates any form of mercury at a rate of high concentration, but, the accumulation factors and rate of excretion are different (Bäckström 1969). Jernelöv and Lann (1971) stated that, the microorganisms in the fish intestine synthesized methyl mercury from inorganic mercury though it was not clear whether the fish itself is able to do this or not. Here, in our study we tested with inorganic mercury. Therefore, it could be expected that, the mercury had accumulated as its inorganic form in fish kidney. Hence, to confirm the form of mercury deposition, it needs further investigation. Thophon et al. (2003) and Gupta and Srivastava (2006) reported the effects of mercuric chloride in fish kidney varied from slight disruption of the brush borders of the proximal tubular cells to swelling and vacuolization. Increased cell death was observed by necrosis and apoptosis when they exposed the fish to cadmium and zinc respectively. We had seen the similar observations in the mercuric chloride exposed fish including the cell death by necrosis. The dilation of the lumen of the kidney tubules, degeneration and necrosis of mesenchymal cells were observed at low dose treated fish. Similar observations were reported by Gill et al. (1988) and Prashanth (2011) when fish were exposed to various pollutants. It was observed that if the concentration of heavy metals was very high in the tissue, it might cause severe structural damage. Any degenerative changes in kidney might lead to excretory disorders. It could also been suggested that damaged nephrons may cause disbalance in osmotic and ionic regulation.

This study clearly revealed that $HgCl_2$ is a nephrotoxic agent and its toxicity caused severe damage and altered the

histomorphology of kidney of the *Labeo rohita* and thus might adversely affect the kidney function.

Acknowledgments The authors are thankful to the University Grant Commission, New Delhi, India for financial support and to the Head, Department of Zoology, Visva-Bharati University, Santiniketan, West Bengal for providing the laboratory facilities. We are also thankful to Mr. Amitabha Ghosh, Metallurgy Department, Jadavpur University for his assistance during atomic absorption spectrophotometry study.

References

APHA (2010) Standard methods for the examination of water and wastewater. In: Clesceri LS, Greenberg AE, Eaton AD, 20th edn.

Bäckström J (1969) Distribution studies of mercuric pesticides in quail and some freshwater fishes. Acta Pharmacol Toxicol 27:5–103

Bakshi N (1991) Toxicity effect of copper on the survival, behaviour and histopathological changes in the liver, intestine and kidney in a freshwater teleost, *Heteropneustes fossilis* (Bloch). Uttar Pradesh J Zool 11:117–120

Banerjee S, Bhattacharya S (1994) Histopathology of kidney of *Channa punctatus* exposed to chronic non-lethal levels of elsan, mercury and ammonia. Ecotoxicol Environ Safe 29:265–275

Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36:609–662

Dallinger R, Prosi F, Segner H, Back H (1987) Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia 73:91–98

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge, pp 25–26

Ghosh A, Sil PC (2008) A protein from *Cajanus indicus* Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. Biol Pharm Bull 31:1651–1658

Gill TS, Pant JC, Pant J (1988) Gill, liver, and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (*Puntius conchonius* ham.). Bull Environ Contam Toxicol 41:71–78

Gill TS, Pant JC, Tewari H (1989) Cadmium nephropathy in a freshwater fish, *Puntius conchonius* hamilton. Ecotoxicol Environ Safe 18:165–172

Gupta P, Srivastava N (2006) Effects of sublethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus* (Bloch). J Environ Bio 27:211–215

Jernelöv A, Lann H (1971) Mercury accumulation in food chain. Oikos 22:403–406

Kaoud HA, El-dahshan AR (2010) Bioaccumulation and histopathological alterations of the heavy metals in *Oreochromis niloticus* fish. Nat Sci 8:147–156

Kirubagaran R, Joy KP (1988) Toxic effects of three mercurial compounds on survival, and histology of the kidney of the catfish *Clarias batrachus* (L.). Ecotoxicol Environ Safe 15: 171–179

McCoy CP, O'Hara TM, Bennett LW, Boyle CR, Lynn BC (1995) Liver and kidney concentrations of zinc, copper and cadmium in channel catfish (*Ictalurus punctatus*): variations due to size, season and health status. Vet Human Toxicol 37:11–15

Ortiz JB, Canales MLGD, Sarasquete C (2003) Histopathological changes induced by lindane (γ-HCH) in various organs of fishes. Sci Mar 67:53–61

Passow HA, Rothstein H, Clarkson TW (1961) The general pharmacology of the heavy metals. Pharmacol Rev 13:185–225

Prashanth MS (2011) Histopathological changes observed in the kidney of freshwater fish *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin. Rec Res in Sci Tech 3:59–65

Ram RN, Sathyanesan AG (1986) Effect of mercurial fungicide on the gonadal development of the teleostean fish *Channa punctatus*(Bloch). Ecotoxicol Environ Safe 11:352–360

Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S (2003) Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ Poll 121:307–320

Vahter M, Åkesson A, Lind B, Björs U, Schütz A, Berglund M (2000) Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ Res Section A 84:186–194

WHO (1991) Inorganic mercury. Environmental health criteria 118. International programme on Chemical Safety, World health Organization, Geneva