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Cadmium-Induced Changes in the Histology of Kidneys in Common Carp, *Cyprinus carpio* (Cyprinidae)

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Heavy metals pose a wide variety of challenges to aquatic organisms, especially to fish. These pollutants are uncommon (Clarke 1993), but are a major problem because of their toxicity, persistence and tendency for bio-accumulation in food chains (Wani 1986).

Cadmium is important because of its use in various industrial processes, and as a by product of zinc mining, fossil fuel, base metal smelting, combustion and atmospheric transport. Since this biologically non-essential element is highly toxic to aquatic organisms and is a known renal toxicant (Mueller 1993), there is a need to understand its effects on the kidneys of an edible fish *Cyprinus carpio communis.* This fish offers some unique experimental advantages for the study of cadmium renal toxicity due to its tolerance to temperature variations, toughness and biennial breeding (Singhal 1995). The natural level of cadmium - a stable non-volatile element, varies from 0.1 to 10 µg/1 in freshwater. However, anthropogenic processes discharge can increase this level from 50 µg/1 (Singhal and Abusaria 1990) to 10,000,000 µg/l (Ringwood 1992).

MATERIALS AND METHODS

Cyprinus carpio communis was regularly procured from the National Fish Seed Farm (NFSF) Jyotisar. Adult healthy specimens of both sexes, about 12-15 cm long were selected as experimental animals. These animals were maintained in the laboratory in dechlorinated tap water at 22-25°C for two weeks. Fifty acclimated *C. carpio* in triplicate were released in 100 l plastic aquaria containing cadmium chloride ($CdCl_2$ 2.5 H_2O) solutions of different concentrations i.e. 58 µg/l, 43.5 µg/l, 29.0 µg/l and 14.5 µg/l (pH 8-9).

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The exposure system consisted of 15 circular plastic containers of 100 1 capacity. To each container about 90 l of test solutions were added. Each container was well aerated for a week before the animals were introduced, Acclimated *C. carpio* were released in aerated containers at 22-25°C (pH 8-9) in a semi-static bioassays. All tests were conducted in triplicate using 50 animals (fed *ad libitum* fish feed manufactured by Lipton India Ltd., Vijayawada).

One test animal from each container was taken out at an interval of seven days. All these animals were quickly cut open in a humane manner approved in accordance with the Guide to the Care and Use of Experimental Animals by the Animal Care Committee of Kurukshetra University, to remove the kidneys which were fixed in refrigerated 10% buffered formalin for 24-72 h, dehydrated in a graded ethanol series, and then embedded in paraffin following standard histological procedures. Sections were cut transversally at 6-8 µm, stained in hematoxylin and eosin (H and E) for studying the histopathological changes in cadmium stressed kidneys of *C. carpio.*

RESULTS AND DISCUSSION

The first sign of cadmium damage in the kidneys of *C. carpio* was observed 7 days after exposure to 29 µg/l W/V conc. of cadmium chloride. At this conc. the stainability of the nucleus and the cytoplasm of the lymphoidal cells in the walls of nephrons was reduced and in comparison to control kidneys the necrotic centres and the tubular walls appeared abnormally stretched (Fig. 1). After 14 days exposure to this concentration the uriniferous tubules were often found constricted by the swollen walls and the inner lining of these tubules left the wall. Subsequently, some of the tubules were broken and the gaps appeared therein (Fig. 2). Higher exposure for 4 weeks at 50% W/V cadmium chloride resulted in the degeneration of the tubules (Fig. 3) and in the release of tubular epithelial cells.

With increasing cadmium concentrations and exposure time, more pathological changes were observed, including, damage to parenchyma, toxic necrobiosis and supperative inflammation. These exposures i.e.

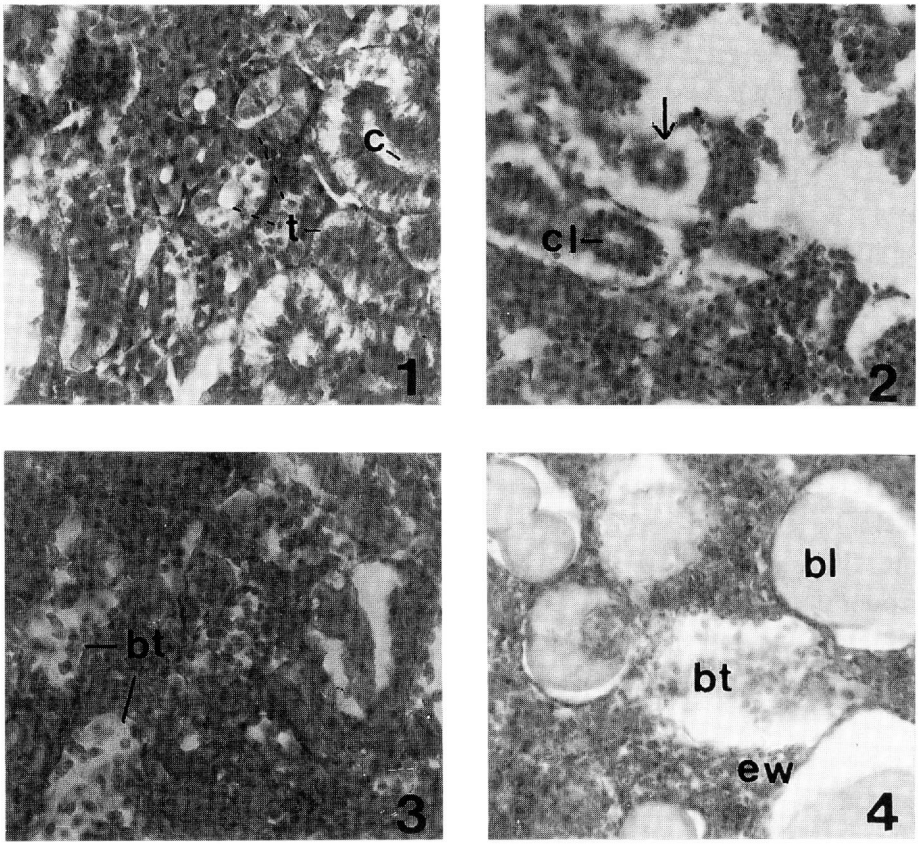

Figure. 1. Cross section of kidney of control *Cyprinus Carpio* showing the presence of intact tubules (t) with cilia (c) (H & E x 330).

Figure.2. Cross section of kidney of *C. carpio* exposed to 29 µg/l (50% W/V) cadmium chloride for 2 weeks; showing the lumen of tubules constricted by swollen walls and low staining capacity of the cytoplasm and nucleus in the necrotic centres (arrow), cl-constricted lumen (H& E x 330).

Figure.3. Cross section of kidney of *C. carpio* exposed to 29 µg/l (50% W/V) cadmium chloride for 4 weeks. Broken tubules (bt) are visible (H & E x 330).

Figure.4. Cross section of kidney of *C. carpio* exposed to 29 µg/1 (50% W/V) cadmium chloride for 4 weeks. This picture shows the separation of glomerular basal lamina (bl) from epithelial wall (ew) and broken tubules (bt) (H & E x 825).

50% - 75% W/V cadmium chloride conc. for 3-4 weeks showed lesions in the various regions of the kidneys wherein the lumen of the Bowman's capsule was reduced due to its thickening. Further, diffusively thickened glomerular basement membrane was separated from the basal lamina with a space between this and the parietal cells (Fig. 4). Compared to the controls, cadmium stressed *C. carpio* kidneys showed reduction in the number of lymphocytes thus enlarging the intertubular space. Other changes, such as, the reduction in the cilia in the ciliated neck region, disappearance of brush border in the initial proximal and second proximal segments, reduction in the number of collecting ducts, extreme swelling and simultaneous disintegration of the renal tubules, and displacement of the nucleus in the renal tubules were also observed after 4 weeks exposure at 75% W/V cadmium chloride conc. In animals exposed to 58 µg/1 (100% W/V) cadmium chloride for 2 weeks the coagulative necrosis was recognizable as an acidophilic area in the kidneys where the cell nuclei were destroyed but the outlines of the cells were still visible (Fig. 5). In these animals the stages of necrosis were best assessed in terms of the nucleus. They were (1) pyknosis, (Fig. 6), (2) karyohexis or rupture of the nuclear membrane and fragmentation of the nuclear chromatin (Fig. 7), and (3) karyolysis (Fig. 7).

Kidneys of carp with maximum cadmium concentration exposure i.e. 58 µg/l (1OO% W/V) for 4 weeks showed extreme necrotic and ulcerative inflammation. In these conditions the localized tissues disintegrated and left behind more or less extensive defects (Fig. 7).

In order to assess renal damage already caused by cadmium toxicity some of the exposed animals were kept in the normal water for the period for which they were exposed to cadmium. Upon histological examination of the kidneys of these animals, it was observed that the renal damage showed no reversal. Instead, the renal damage continued resulting in greater degenerative changes (Fig. 8).

The cadmium induced histopathological changes observed in the kidneys of *C. carpio* were similar to pathological changes observed in other fishes due to heavy metal toxicity (Singhal *et al.* 1996). Physiological consequences can be inferred from some of the morphological lesions reported (Singhal and Davies 1987). The lesions in nephrons and

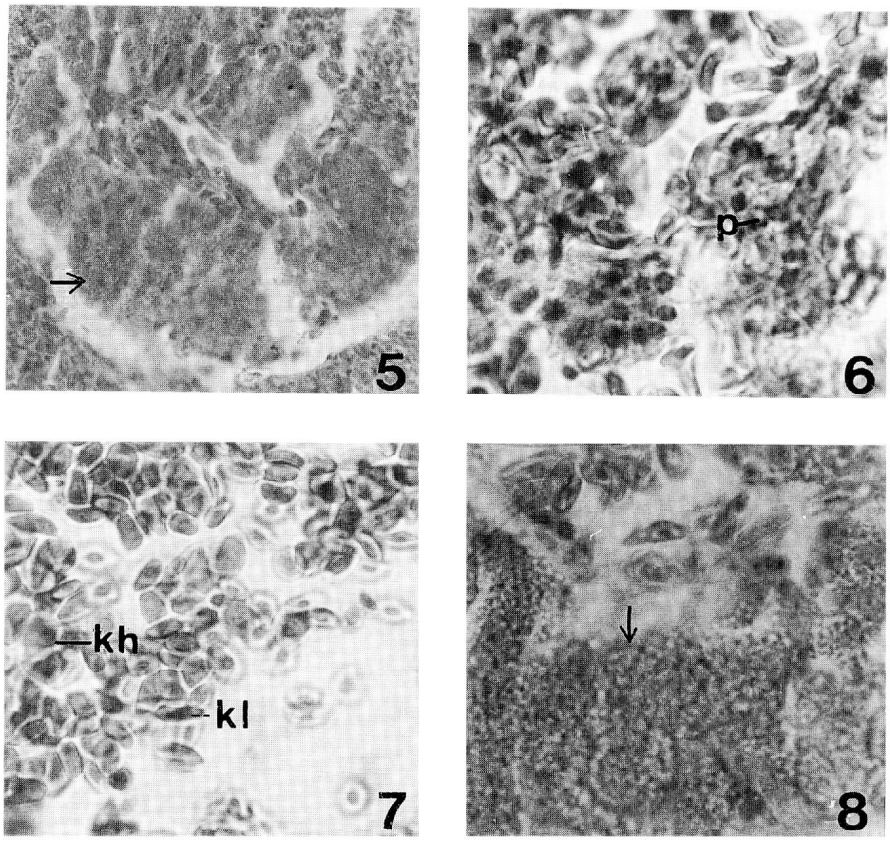

Figure.5. Cross section of kidney of *Cyprinus carpio* exposed to 58 µg/l (100% W/V) cadmium chloride for 1-2 weeks showing complete disappearance of tubules and loss of nuclei of cells (arrow) (H& E x 330).

Figure.6. Cross section of kidney of *C. carpio* exposed to 58 µg/1 (100% W/V) cadmium chloride for one week showing Pyknosis (p) (H & E x 825).

Figure.7. Cross section of kidney of *C. carpio* exposed to 58 µg/1 (100% W/V) cadmium chloride for 4 weeks showing karyohexis (kh) and karyolysis (kl), (H & E x 825).

Figure.8. Cross section of kidney of *C. carpio* exposed to 43.5 µg/l (75% W/V) cadmium chloride for 3 weeks and revived for the same period in normal water. This picture shows the damaged tubules (arrow) (H& E x 330).

haemopoietic tissues suggested that both osmotic and ionic regulation were impaired upon exposure to different concentrations of cadmium (Jarup *et al.* 1993).

Roberts (1978) and Schaperclaus *et al.* (1991) reported the reduced stainability in the hepatic cells in heavy metal stressed teleosts, as is reported here in kidney tissues. Brightly staining hyaline droplets deposition within the cells of proximal tubules can often appear to displace the nucleus causing nuclear necrosis and represent protein which has been reabsorbed from the glomerular filtrate. Since the renal tubular epithelium has its major function in the excretion of divalent ions, pollution with heavy metals such as cadmium is highly likely to affect these cells (Schaperclaus *et al.* 1991). The results of this study confirm the earlier findings (Jarup *et al.* 1993) that cadmium induced tubular necrosis and renal dysfunction were irreversible. The impairment in glomerular function seems to be irreversible, even after exposure to cadmium is stopped. In conclusion, our results indicate that *C. carpio* is sensitive to cadmium. It may either be due to the high cadmium molecules that are actively taken up by the regulatory mechanism which is active immediately following exposure thus increasing the toxic action of cadmium in *C. carpio.*

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