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Cadmium induced histopathological alterations in the kidney of freshwater catfish *Heteropneustes fossilis* (Bloch.)

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Abstract

Effect of sublethal concentration of cadmium chloride (12 ppm; 10% of 96h LC_{50}) on kidney of the freshwater catfish *Heteropneustes fossilis* was studied for a period of 45 days under laboratory conditions. The alteration in histology was found in the proximal tubule as deformation of brush border, gradual atrophy of basal cytoplasm and condensation of nuclear material leads to pycnosis of nuclei. Degenerated cells were frequently seen extruding into the lumen of tubules and were filled with fragments of cellular components. Focal degeneration of tubular cells as well as more extensive necrosis of the whole nephron. Infiltration of leucocytes and macrophages surrounded the tubules.

Keywords: Cadmium toxicity, histopathology, *Heteropneustes fossilis*, kidney.

1. Introduction

Water pollution by agricultural, municipal and industrial sources has become a major concern for the welfare of humanity (Leland and Kuwabara, 1985; Scot and Sloman, 2004). Water soluble toxicants from industrial and municipal wastes, leached soils and the atmosphere have rapidly transferred to natural bodies of water. While some of the pollutants decompose or volatilize, others form insoluble salts, which precipitate and get incorporated into the sediment (Bowen, 1979). Uptake of such toxicants by aquatic organisms like fish may be followed by metabolism of the toxicants into more toxic derivatives (Webb, 1975; Duffus, 1980). Among heavy metals cadmium, copper, mercury and zinc are reported to be ubiquitously present in the waste water which are generally released into aquatic ecosystems and toxic for aquatic organisms (Olsson, 1998; Kumar *et al.,* 2007; Kumar *et al.,* 2009) by changing genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Among the aquatic habitants, fish are the most susceptible to these elemental contaminants and more vulnerable to metal contamination than any other aquatic habitant (Alinnor, 2005). Cadmium is a non-essential, non-biodegradable element with no known biological function and is reported to be a major contaminant of aquatic ecosystems causing adverse effects on aquatic organisms (Hollis *et al.,* 1999). It is released from diverse sources such as electroplating, paper, PVC plastic, pigments and ceramic industries, battery, mining and smoldering units and many other modern industries (Gupta *et al.,* 2003). The toxic effect of cadmium is primarily the inactivation of enzymes and proteins by binding to sulfydryl groups. It also impairs Ca^{2+} uptake and causing ionoregulatory damage. Hence the present investigation aims to study the sublethal effect of cadmium on kidney of *Heteropneustes fossilis.*

2. Materials and methods

Irrespective of sex, healthy specimens of *H. fossilis* having 18 to 20 cm length and 36 to 38 g weight belonging to a single population were collected locally at Chidambaram, Tamilnadu, India and were acclimated to the laboratory condition for 30 days (d). Water was renewed after every 24 hours (h) with routine cleaning of the aquaria. Fish were fed with minced goat liver everyday. Prior to the commencement of the experiment, 96 h median lethal concentration (96 h LC_{50}) of cadmium chloride (99% pure, E – Merck, India) was estimated following trimmed spearman karber method (Hamilton *et al.,* 1997) and 24 h renewal bioassay system and was found to be 120 ppm after 5% trimming. For the present study 12 ppm (10% of 96h LC_{50}) was selected as sublethal concentration as cadmium present in natural water is very low. A group of 10 fish were exposed to 12 ppm cadmium chloride solution prepared in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mg L^{-1} and water temperature 28 ± 2˚C (APHA, 2005). Parallel group of 10 fish were kept in separate aquaria containing 50 Liters tap water without the addition of cadmium chloride as control. Feeding was allowed in the experimental as well as control groups' everyday for a period of 3h before the renewal of the media throughout the tenure of the experiment. After the expiry of 45 days of exposure, 5 fish each from the respective experimental as well as control groups were sacrificed and the kidney of control and treated fish were excised and fixed in boun's fluid for 24 hours at room temperature. Tissues were repeatedly washed with 70% alcohol till all the traces of bouin's fluid were removed. Dehydration process was carried out by washing the tissue with alcohol (90% and 100%), this is followed by cleaning process by alcohol-benzene in different ratios(3:1, 1:1 and 1:3) followed by pure benzene and benzene-paraffin wax (1:1) then embedded in paraffin wax. 5µm sections were stained with haematoxylin and eosin. Permanent slides were prepared with Canada balsm and observed under light microscope.

3. Results

Control: The control kidney consists of a renal corpuscle, (Bowman's capsule and glomerulus) and the renal tubules (proximal, intermediate and distal tubule) and collecting duct. Proximal tubules have prominent brush borders (Microvilli) bathed in the vascular bed in the interstitial tissues. Distal tubules and collecting ducts, both devoid of brush borders, and are sparsely distributed (Figure1). The intermediate segments between proximal and distal tubules are rarely seen. The renal corpuscles are located in close vicinity of renal tubules and blood vessels in the interstitial tissue. Pigments and leucocytes are very common in the interstitial tissue (Figure 2).

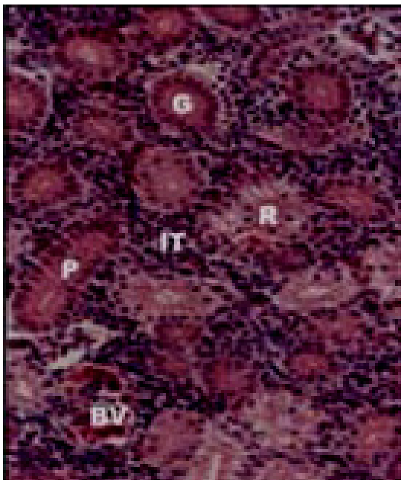

Figure 1: Section of kidney of control fish showing normal structure.
(P- Proximal tubule, G -Glomerulus, BV-Bloodvessels, DT- Distal tubule and IT-Interstitial tissue, R- Renal Tubule.) (H/ E X 400).

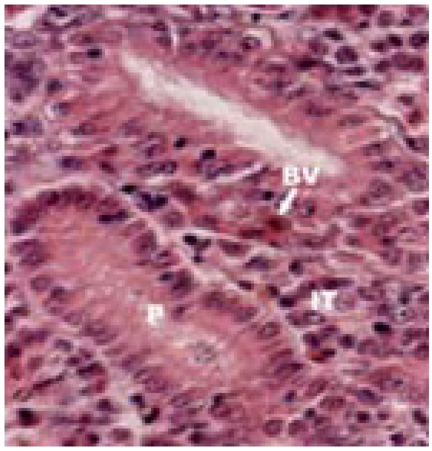

Figure 2: Enlarged view of Proximal tubules (P) with Blood vessels (BV) and Interstitial tissue (IT) of control fish (H/ E X 1000).

Experimental: After treatment with sublethal concentration of cadmium for 45 days, the kidney showed reduction in renal cell number in the proximal and distal tubules, which resulted in narrowing of lumen. The tubular cells showed hypertrophy and some of the renal tubules have lost their normal shape. Vacuolization due to degeneration of cytoplasm is quite obvious (Figure 3). The nuclei of epithelial cells have become quite dominant and are found infiltrating into the surrounding tissue. The perforation of kidney tubules is commonly observed. The kidney showed hyperplasia, vacuolization, degeneration and necrosis leading to complete necrosis. Cuboidal epithelial cells lining the tubules showed complete vacuolization with degenerating cytoplasm and more nuclear division and disorderly scattered nature (Figure 4). The hemopoietic tissue was fully studded with lymphatic cells at the highest rate of nuclear division. The lumen of the tubules was found to be dilated (Figure 4).

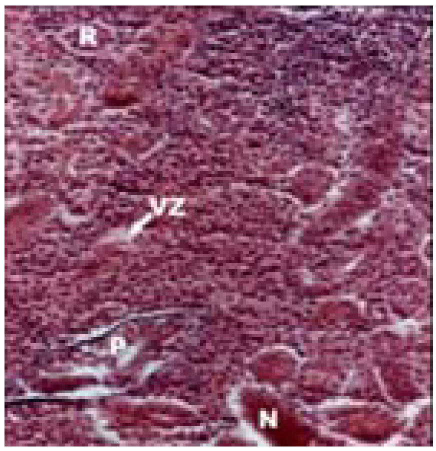

Figure 3: Section of kidney of fish exposed to cadmium showing enlargement of tubular lumen after 45 days.

(Vacuolization (VZ), Necrotic material (N) and damage of Proximal tubule (P), Renal Tubule (R). (H/ E X 400).

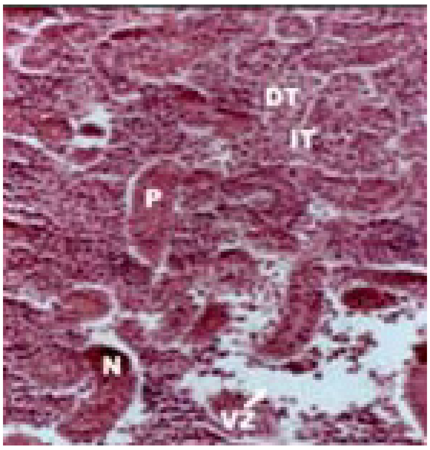

Figure 4: Section of kidney of fish exposed to cadmium showing extensive vacuolization after 45 days. (Vacuolization (VZ), Necrotic martial (N) and damage of Proximal tubule (P). (H/ E X 400).

4. Discussion

Sublethal cadmium exposure induced marked abnormalities in the kidney initiated with disruption of tubular organization. Thereafter degeneration of tubular epithelial cells and lympocytic infiltration was evident. Most of these pathological changes persisted with vacuolation, clotting of blood in some sinusoids and glomerular degeneration. The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.,* 2003). Exposure to metals frequently causes alterations in the tubules and glomerulus as described by Thophon *et al.* (2003) for the perch *Lates calcarifer* exposed to cadmium. Handy and Penrice (1993) found swollen Bowman´s capsule cells and melanomacrophages in the kidney of trout *Salmo trutta* and tilapia *Oreochromis mossambicus* exposed to mercuric chloride. Similar alterations were found in fishes exposed to organic contaminants (Veiga *et al.,* 2002) and mixed environmental contaminants (Schwaiger *et al.,* 1997; Pacheco and Santos, 2002). These reports suggest that the histopathological changes in the kidney could not be considered specific to the stressors. In the present study, kidney of the fish often showed cloudy swelling in tubule cells. This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm, which takes on the appearance of a net. This initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Takashima and Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995).

The presence of tubule degeneration coupled with necrosis in the kidney in the present study, the presence of tubule degeneration coupled with necvesis in the kidney indicates that indicates that the kidney suffered damage after exposure to the water contaminated with cadmium. Moreover, Cadmium accumulates preferentially in the kidney, new proteins such as metallothionein are synthesized in the liver and kidney (Ooi and Law, 1989). The membranous organelles, such as mitochondria,endoplasmic reticulum and nuclear envelope, are most easily affected by pollutants in which disorganization, rearrangement and malfunction may occur. Thus, the proximal tubules which posses numerous mitochondria rather than the distal tubules are easily damaged by cadmium (Dumitrescu *et al.,* 2010). The appearance of atrophic or pycnotic nuclei in fish kidney increases with the increase in duration of exposure. The phenomenon of nuclear changes in fish is probably similar to that found in other animals (Copius-Peereboom and Stegeman, 1981). It has been suggested that a nuclear and nucleolar changes are induced preceding atrophy and necrosis of cells in other animals (Dumitrescu *et al.,* 2010). At the beginning, the change may probably form part of a diffuse mechanism, leading to activation of synthetic or other activities in the cell as synthesis of metallothionein (Dallinger *et al.,* 1997; Klassen and Liu, 1997; Hollis *et al.,* 2001). Leucocytes are common in the interstitial space of control fish, but they are rarely aggregated so densely and abundantly as in treated renal tissue (Hickman and Trump, 1969). The increase of leucocytes shows inflammatory response. Leucocytes may either remove or engulf injured and non functional cells.

The dilation of the lumen of the kidney tubules, degeneration, tissue rupture in the collecting tubules and necrosis as observed in the present investigation have also been reported in various fish exposed to pollutants (Kumar and Pant, 1984; Sukumar and Karpagaganapathy, 1986; Gill *et al.,* 1988 and Vardhani and Gowri, 2002). According to Dubale and Shah (1981) the process of destruction is a function of dosage and period of exposure and they opined that the renal tubules of kidney are the first to be affected by toxicant stress. Vacuolisation of epithelial cells of renal tubules and pronounced enlargement of the tubules were also observed. The proximal tubule in mammals and fishes is involved in reabsorption and lysosomal degradation of macromolecules (Hickman and Trump, 1969). After reabsorption, more macromolecules may form intracellular droplets or dense bodies in higher vertebrates (Rollason and Brewer, 1984). During this process the pollutants are excreted through kidney and appear to cause considerable damage. Necrosis and vacuolisation were observed by Dhanapakiam and Premalatha (1994). Sastry and Sharma (1979) observed a number of striking changes in the histological structure of the kidney of *Channa punctatus* exposed to sublethal concentration of endrin for 30 days and found that the shrinkage of glomerulus was the visible sign of intoxication.

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