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HISTOLOGICAL CHANGES IN LIVER AND KIDNEY OF CAT FISH, *HETEROPNEUSTES FOSSILIS*, EXPOSED TO PENTACHLOROPHENOL (PCP)

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Abstract

Present investigation deals with the effect of Pentachlorophenol (PCP) on the histo-architectural changes in kidney and liver of a fresh water fish *Heteropneustes fossilis*. Fish was exposed to low and high doses of sub lethal concentration (6µg and 32µg/l/day) of PCP for a period of 21 days. Histological observation exhibited progressive degenerative changes in kidney which are marked by shrinkage of glomeruli, increase in Bowman's capsule space, damage and necrosis in tubular cells. Liver showed degeneration and disintegration in most cytoplasmic content, lymphatic aggregation, necrosis and rupture of hepatocytes.

Key words : *Heteropneustes fossilis*, cytoplasmic content, necrosis, hepatocytes.

Introduction

Endocrine-disrupting chemicals (EDCs) include synthetic and naturally occurring chemicals that affect the balance of normal function in animals (Razia *et al*., 2006). It has been found that exposure to natural and synthetic estrogenic chemicals may adversely affect wildlife and human health (Colborn *et al*., 1993). *In vitro* exposures (Soto *et al*., 1992; Soto *et al*., 1994; Toomey *et al*., 1999) have confirmed the effects of EDCs on tissue structure and cellular processes. PCP is used globally in the production of plastics, pesticides, wood preservatives, herbicides and cleaning products and are present in sewage effluents around the world (Talmage, 1994). It has been reported that PCP is the most important degradation products of phenolic compounds because of its enhanced resistance towards biodegradation, toxicity, ability to bio accumulate in aquatic organisms, and estrogenicity (Ahel *et al*., 1994). PCP is found in surface waters, aquatic sediments and groundwater (Bennie, 1999 and Talmage, 1994).

The application of environmental toxicological studies on non-mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become valuable indicator for the evaluation of the effects of noxious compounds (Khidr and Mekkawy, 2008). Histology and histopathology can be used as bio monitoring tools for health in toxicity studies (Meyers and Hendricks, 1985). Histopathology, the study of lesions or abnormalities on cellular and tissue levels is useful tool for assessing the degree of pollution, particularly for sub lethal and chronic effects.

In trout species, Pentachlorophenol was found to accumulate in the liver, gill, gut, fat, and kidney tissue (Ahel *et al*., 1993). So that PCP may affects those organs in corresponding with its impacts on reproductive organs. Most of chlorophenol studies revealed severe effect on the liver and gonads of fish tissues (Christtiansen *et al*., 1998; Jobling *et al*., 1996 and Lech *et al*., 1996) and the corresponding metabolism. Many studies demonstrated that increased concentrations of different pollutants including several heavy metals seriously damage the gills of teleostean fish (Dutta *et al*., 1996 and Wendelaar Bonga, 1997).

Catfish (*H. fossilis*), an carnivore freshwater fish is a popular delicacy relished throughout India, due to fast growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion. This catfish, a native of India, Burma, Sri Lanka, is a seasonal breeder. Its annual gonadal cycle in the vicinity of Delhi (lat.$28^0$35'N', long. $77^0$12'E) comprises the preparatory (February to April), the pre spawning (May to June), the spawning (July to

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August), and the post spawning (September to January) periods. Two consecutive sets of physiologic events intimately interwoven with environmental changes are involved in the completion of the circannual reproductive cycle in the catfish. The first set of events leads to the gradual enlargement of the gonads with concomitant vitellogenesis or spermatogenesis during the late preparatory pre-spawning period, when in nature both the daily photoperiod and mean environmental temperature increase progressively. The second set of physiologic events, involving ovulation and spawning of oocytes or spermiation, seems to be triggered by a consortium of environmental factors prevailing during the monsoon season (July to August), the prime time for breeding in the natural environment.

Materials and Methods

Chemicals

PCP (Crystalline, 99% pure) was purchased from Acros organics (Geel, Belgium). All other chemicals were of analytical grade and purchased locally. PCP was dissolved in ethanol and then diluted with water to obtain the required concentrations 6µ/l(1/50 of LC_{50}) and 32µ/l(1/10 of LC_{50})

Animal collection and maintenance

The experiments were performed in accordance with local/national guidelines for experimentation in animals and all care was taken to prevent cruelty of any kind. Mature catfish *H. fossilis* (35-45g) were purchased from local fish market in the preparatory phase (first week of April) of the annual reproductive cycle. They were maintained in the laboratory under normal photo period (13.0L:11.0D) and temperature (25±2^{0}C) during experiments. The fish were fed goat liver daily ad libitum.

Experimental setup

The adapted adult fish classified into three groups (15 fish per each): first group control, second group PCP treated (21 days with 6 µg/l/ day), and third group PCP - treated (21 days with 32 µg/l/day). In the present study, the range of PCP exposures was 6 µg-32µg/l/day and the exposure concentrations were determined by performing LC50 experiment. The fish were acclimatized for 10 days. The tap water and concentrations of PCP were changed every day.

Hematoxylin-Eosin (H&E) histological preparations

For microscopic preparations, after 21 days, 4 surviving fish of each group were removed and dissected. Small pieces of the liver and kidneys were taken and immediately fixed in Bouin's fluid. Fixed tissues were embedded in paraffin wax and sectioned at 7µ in thickness with the help of microtome and then stained with Harris' hematoxylin and eosin stain (H & E) according to Bancroft and Steven, (1982) and mounted in DPX. Sections were visualized and studied using Lac Zene microscope model HL-23 (Lac Zene Biosciences, India).

Results

Histological changes in liver

The liver of the control fish *H. fossilis* appears as a continuous mass of hepatic cells. Hepatocytes shows cord like pattern interrupted by blood vessels and sinusoids. The cords of hepatocytes are arranged around the central vein. The hepatocytes are large in size, polygonal in shape with centrally located nuclei and homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells and Kuffer cells. Examination of liver sections after exposure to 6 µg/l/day of PCP for 21 days showed degeneration in the form of disintegration in most cytoplasmic contents. Lymphatic aggregation and necrosis were observed along with Pyknosis and rupture of hepatocytes. Severe damage occurred in liver sections after exposure to 32 µg/l/day of PCP for 21 days.

Histological changes in the kidney

Histological structure of kidney of *H. fossilis* contains nephrons, which are composed of renal corpuscles and renal tubules. The renal corpuscle of nephron consisted of glomerulus and Bowman's capsule followed by a tubular neck. Other regions of the renal tubule were proximal distal and collecting tubules. The interstices of the tubules were enriched with hematopoietic tissue, which contained round to polygonal cells.

Histological studies made in the kidney of fishes exposed to 6 µg/l /day PCP showed elongation of tubules, damage of hematopoietic tissue and disintegration of glomeruli at the end of the exposure for 21 days. Vacuolation of proximal tubules and enlargement of its basement membrane were also observed. Shrinkage of glomerular structure and increase in Bowman's space were seen after exposure to 32 µg/l/day of PCP for 21days

Discussion

It has been reported that Organochlorine compounds are sterogenic and effects the histology of immune and endocrine organs (Yokota *et al*., 2001 and Razia *et al*., 2006). Skin and gills are highly sensitive to pollutants due to their direct contact to aquatic environment (Pottinger

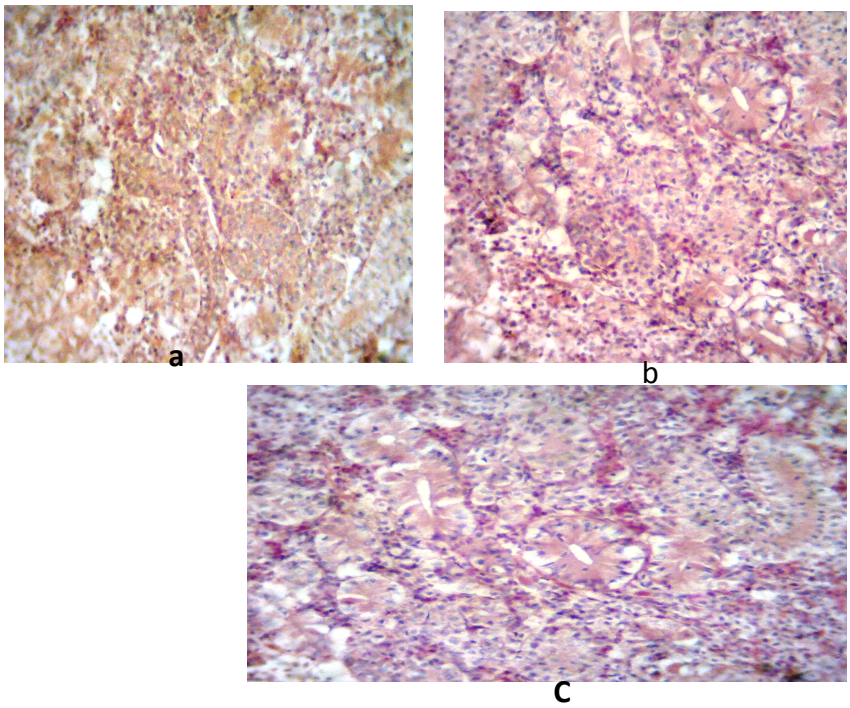

Fig. 1 : Section of catfish *H. fossilis* kidney treated with **a**. control, **b**. 6 µg/l and **c**. 32 µg/l showing histological changes during 21 days exposure periods

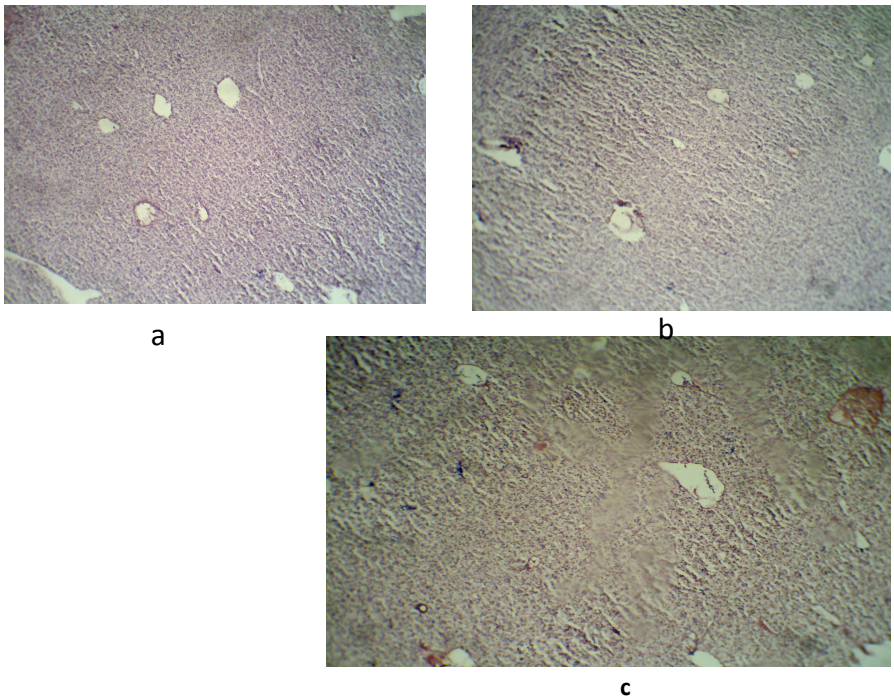

Fig. 2 : Section of catfish *H. fossilis* liver treated with **a**. control, **b**. 6 µg/l and **c**. 32 µg/l showing histological changes during 21 days exposure periods.

and Pickering, 1985; Holm, 2000). The effect of organophosphorus pesticide on hepato-renal and reproductive organ of *H. fossilis* showed adverse effect on growth and maturation (Sanjoy Deka and Rita Mahanta, 2012). For the first time effect of pentachlorophenol was studied on liver and kidney of Indian catfish *H. fossilis*.

The primary function of the kidney tubules is to remove excess of water, salts, waste material and foreign substances from the blood. The blood is filtered through the glomerulus which is composed of a leaky capillary

endothelium, a fine porous basement membrane and the epithelium of the Bowman's capsule. During ultra filtration inorganic molecules, glucose ,urea, amino acids are filtered in to the tubule. But the fish which live in toxicant contaminated water filter out the toxicant from blood. These filtered out chemicals cause immense damage to the structure and function of kidney. In the present study, the damage has been observed histologically as elongation of tubules, damage of haemopoietic tissue, disintegration of glomeruli, enlargement and increase in bowman's space.

Significant changes in histology of liver was also noticed during exposure periods in both the concentration groups in comparison to control. Acute and extensive necrosis of liver cells was observed and density of the connective tissue increased markedly, leading to more congestion, usually located in the victinity of hepatic arteries and bile duct. At prolonged exposure of high dose for 21 days of treated specimen showed varied degree of hepatic destruction within and around the hepatic parenchyma. The liver of *H. fossilis* clearly showed that the parenchymal architecture of the liver is disturbed and hepatocyte showed dissociation and granular appearance.

The present results exhibited severe damage in liver and kidney tissues of *H. fossilis* exposed to PCP. The lesions detected in tissues or organ represent an integration of cumulative effect of physiological and biochemical stressors and therefore, can be link to the exposure and subsequent metabolism of chemical contaminant.

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