

HISTOPATHOLOGICAL PROFILE OF FRESHWATER TELEOST, *Channa punctatus* EXPOSED TO ARSENIC

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ABSTRACT

Channa punctatus is a common freshwater fish of India and is regularly consumed because of its high nutritional value. The release of pollutants, especially arsenic, into the aquatic environment is known to cause detrimental effects as this fresh water fish is continuously exposed to arsenic toxicity. Fresh water *C.punctatus* were exposed to sublethal concentrations of sodium arsenite for varied span of time in controlled laboratory condition to measure histopathological responses and to establish the parameter as indicator of the health of the larger population and community.

Keywords:-*Channa punctatus*, arsenic, histopathology

Arsenic, a sulphydryl reactive metalloid is one of the most important and concerned global environment toxicants. It is wide spread in the aquatic environment as a result of both geogenic processes and anthropogenic disturbances (Bear *et al.*, 2006). In aquatic environment several species of microorganisms make arsenic biologically available to organisms including fish (Duker *et al.*, 2005). Fish appear to be particularly susceptible to arsenic toxicity as they are continually exposed to it through gills and intake of arsenic contaminated food (Ahmed *et al.*, 2008). *C.punctatus* were exposed to different sub lethal concentrations of sodium arsenite for varied span of time in controlled laboratory condition to measure the histopathological profile of gill, liver and kidney. Data is indicative to marked pathological changes in presence of metalloid toxicity.

MATERIALS AND METHODS

Collection & acclimatization of fish

The small size freshwater fish, *C.punctatus*, weighting 15±2 gram and measuring 11±2 cm were collected with the help of local fisherman from water bodies located in the subregion of Coochbehar. The fish was properly washed in tap water and treated with 0.02% KMnO₄ and 0.004% formalin solution to remove external infection of algae, fungi etc. Fishes were separately maintained at temperature ranging between 14°C-30°C in aquarium of 20 liter capacity with continuously aerated and dechlorinated tapwater (pH 7.2-7.4; hardness 185-200 mg/l as CaCO₃; alkalinity 170-175 mg/l as CaCO₃) for 15 days before taken for experimentation. The animals were fed with boiled eggs and earthworms (Jayanthi and Selvakumar, 2011). Water was renewed periodically so as to maintain the dissolved oxygen. The specimens were devoid of feeding prior to the test

period to reduce the quantum of excretory products in the aquarium to avoid vomiting of the fish.

Determination of LC₅₀

Prior to treatment, LC₅₀ value of sodium arsenite for *C.punctatus* was calculated following Trimmed Spearman Karber Method (Hamilton *et al.*, 1977). During determination of the median lethal concentration (LC₅₀) of sodium arsenite to *C.punctatus*, the fishes were divided into five equal groups consisting of 10 each and each group was transferred separately to glass aquaria of 20 liter volume. The groups I fish were maintained as control without any treatment, the group II, III, IV and V fishes were exposed to sublethal concentrations of sodium arsenite for four days to determine the median lethal concentration (LC₅₀) for selection of sublethal dose.

Experimental design

The experiment was conducted in a static system in glass aquaria of 10 litre capacity. The acclimatized fishes were grouped into four experimental groups each consisting of five fishes. The experimental groups were categorized based on the LC₅₀ value and from the reports of highest level of arsenic contamination of natural freshwater bodies.

Group1: Fish subjected to zero arsenic level (control).

Group2: Fish subjected to 3.2 mg/L of sodium arsenite.

Group3: Fish subjected to 2.4 mg/L of sodium arsenite.

Group4: Fish subjected to 1.8 mg/L of sodium arsenite.

The fish were exposed to sublethal concentrations of arsenic for 2, 4 and 7 days. Tissues like gill, liver and kidney were isolated from control and arsenic exposed fish for histopathological study.

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Histopathology

Tissues like gill, liver and kidney were isolated from normal and experimental fish. Physiological saline solution (0.58% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hr processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections were cut at 6 μm thickness with the help of 820-Spencer rotatory microtome, stained with hematoxylineosin and were mounted in canada balsam.

RESULTS

Gill

The primary gill lamellae are flat leaf like structure with a central rod like supporting axis and a row of secondary gill lamellae an each side of it. They are situated laterally on either side of interbranchial septum. The primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The secondary gill lamellae are also known as respiratory lamellae. The surface is covered with simple squamous epithelial cells separated by mucous cells. Numerous blood vessels are extended into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have a single nucleus which is flat in appearance. The region between the two adjacent secondary gill lamellae is known as interlamellar region(Figure 1).



X400

Figure1: Histopathological section of gill of *Channa punctatus* exhibiting gill lamellae (gl) and distinct water channel (wc)

Arsenic treatment has induced marked pathological changes in the gills (Figure2).The changes included the bulging of tip of primary gill filaments with distortion of the shape of secondary filaments. A number of cuts were also observed in the gill lamellae. The pillar cell nucleus showed

necrosis and developed vacuoles in the secondary gill epithelium. There is tendency of fusion of disorganized secondary gill filaments. Similar changes were also observed by Waneeet al.(2002) and Bradbury and Coats(1989).

Liver

The surface of the liver is covered with serous membrane and some connective tissue extends inward into the parenchyma (Figure 3).

It is composed of parenchymal cells(hepatic cells) and lattice fibreswhich support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as hepatic cell cords. In fish,these structures are generally obscure. Bile canaliculus is centrally located in each cord. Arsenic toxicity has induced discrete pathological changes in the liver tissue of fish, *C. punctatus* (Figure 4). These changes include degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords. These changes were also reported by Maline(1980) in liver diseases of bottom dwelling fishes due to high concentrations of sediment bound polynuclear hydrocarbons.

Kidney

Teleostean kidney consists of head and body kidneys (Figure 5). Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. Body kidney is composed of many nephrons and intestinal lymphoid tissue. The interstitial tissue is the major haematopoietic tissue in the body. Each nephron consists of two parts, the glomerulus and the urinary tubule. The Bowman's capsule consists of an inner and outer layer of single flattened epithelia. Renal tubule consists of single layer of epithelial cells. Mesangium fills the spaces between the loops of glomerular capillaries. The renal tissue of *C.punctatus* under sublethal toxicity of arsenic showed marked pathological changes (Figure 6).

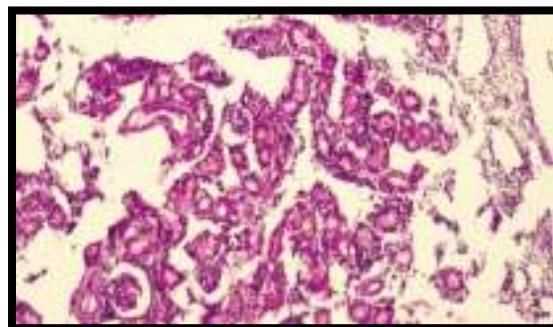
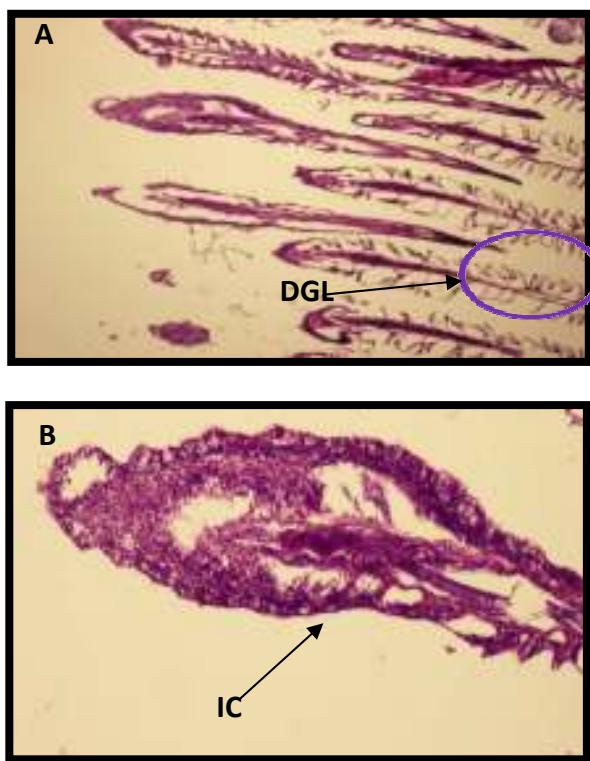


Figure 6: Histopathological section of kidney of *Channa punctatus* exposed to sodiumarsenite (3.2mg/L/ 7days) exhibiting disrupted collecting tubule (dct)

Highly degenerative changes in hematopoietic tissue which include severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm were evident. The epithelial cells of the distal convoluted tubules decreased in size. The interstitial renal tissue showed formation of vacuoles and cellular contours were not clearly distinguished. The effect of sublethal concentration of arsenic showed vacuolization of tubular epithelium, enlargement of nuclei and degeneration of the kidney. The study is similar to the observations of Ravindrakumar(2000).

DISCUSSION

Fish serve as vital indicators of arsenic toxicity as they are continuously exposed to arsenic through gill respiration and ingestion of arsenic contaminated food (Hameid, 2009). Arsenic has induced marked pathological changes in the gills. The marked pathological changes include the bulging of tip of primary gill filaments with distortion of the shape of secondary filaments (Figure 2).



X1000

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Figure 2: Histopathological section of gill of *Channa punctatus* exposed to sodium arsenite (3.2mg/L/ 7days). (A). Gill lamellae showing disrupted gill lamellae (dgl). (B). Gill lamellae exhibiting cellular infiltration (ic)

There is tendency of fusion of disorganized secondary gill filaments (Vijaya Lakshmi and Tilak, 1996). Histological damage to gill surface in the present study may be attributed to high accumulations of metalloid toxin in gills, irritation due to elevated mucous secretion, increased ventilation volume and decreased gill oxygen uptake efficiency as reported by Ravindrakumar (2000). The fish liver is one of the sensitive organ in which various metabolic pathways take place. Therefore the effects of a chemical usually appear primarily in the liver (Bhatnagar and Bana, 1993). The histopathological section of liver under sodium arsenite induced toxicity exhibit swollen central vein, degeneration of hepatic cell (Figure 4).

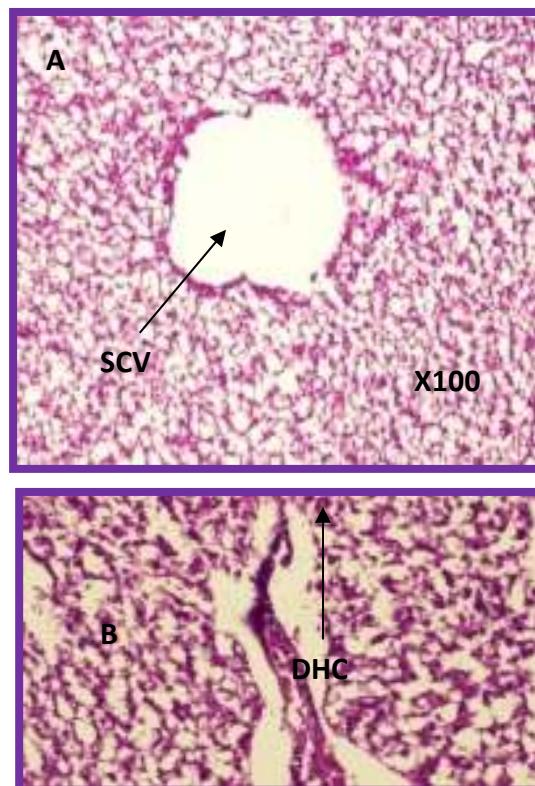
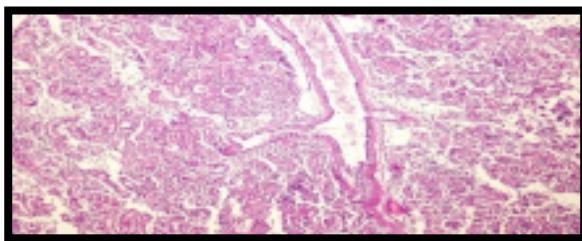


Figure 4: Histopathological section of liver of *Channa punctatus* exposed to sodium arsenite (3.2 mg/l / 7days). (A). Hepatic tissue exhibiting swollen central vein (scv).(B). Liver showing disrupted hepatic cell (dhc)

Teleostean kidney under sublethal exposure of sodium arsenite showed marked

pathological changes. The renal tissue showed formation of vacuoles, degeneration of collecting tubule and cellular contours were not clearly distinguished (Figure 5). The pathological changes in the gill, kidney and liver in the present study are in the agreement with reports of Tilak *et al.* (2001, 2005). Damage to gill tissue may result in long-term consequences particularly if the tissue damage is not repairable. The end result may be reduced

Figure 5: Histopathological section of kidney of *Channa punctatus* exhibiting Bowman's capsule



(bc) and collecting tubule (ct)

flow of oxygen-enriched water to lamellar tissues and ultimately a reduction in fish's performance capacity. The findings of the present study reflect that arsenic exposure of *C. punctatus* exhibit marked histopathological alteration in

Figure 3: Histopathological section of liver of *Channapunctatus* showing central vein (cv)

Various tissues. This parameter would be effectively used as potential biomarker of arsenic toxicity to the freshwater fish in the field of environmental biomonitoring. The measurement of histopathological alterations of individual fish in



respect to xenobiotic toxicity indicates the health of the larger population and community, contributing a lot in the subject of biomarker research.

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