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Histopathological changes in *Channa punctaus* (Bloch.) induced to ammonium sulphate

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Abstract
The present investigation undertaken was the snakehead fish, *Channa punctatus* exposed to sublethal concentrations (16.5 mg/L) of inorganic fertilizer, ammonium sulphate for 30 days showed histological alteration. The exposed fish showed very significant histopathological changes like vacuolar degenerative changes, deterioration of secondary lamellae and gill arch, desquamation in epithelium cells at the apex of the villi and necrosis etc. found in gill, liver, intestine and kidney. It has been analyzed that dysfunction of organs of exposed fish, *C. punctatus* was in response to ammonium sulphate toxicity effect. The above finding suggested that contamination of fertilizer, ammonium sulphate concentration of less than 16.5 mg/L in water may be more appropriate condition to culture in fish culture pond for optimum growth performance.

Keywords: *Channa punctatus*, ammonium sulphate, nitrogenous fertilizer, histopathology, agrochemicals, kidney histology

Introduction
The agrochemical waste causes water pollution has recently become a subject of biological interest because of their hazardous potential, low concentration with growing intensive application of chemical fertilizers in field as well as aquaculture causes contamination to water is increasing day by day.
Channa punctatus, locally known as "Garai" is an edible fish. This fish is found breed in stagnant water body of this region and most important to paddy cum fish culture practices. The ammonia toxicity from nitrogenous fertilizers occurrence became severe during the indiscriminate use of fertilizers in field.
When ammonia present in higher concentrations in water bodies is toxic to fish and produces several biochemical and physiological alterations at cellular level (Narayana, *et al.* 2017) [11]. Fish are useful model for toxicological investigation of aquatic ecosystem. The vital organs of fish induced to different toxicants were used to investigate contamination in aquatic systems and severe affect of water pollutants on organisms. Histopathological alterations reveal the physiological status of animals in stress condition due to toxic effects of pollutants.
Hence, in the current study an attempt has been made to understand the changes in histology of vital organs of snakehead fish, *Channa punctatus* to induce by chemical fertilizer, ammonium sulphate.

Materials and Methods
Experimental fish
The healthy mixed sex, air-breathing, snakehead fish, *Channa punctatus* was taken as experimental fish. The specimens have average length (8–10 cm) and weight (30–34 g) belonging to single population was procured from the local fish dealer at Darbhanga and brought to the laboratory into an aerated plastic container. The 70 healthy fish were given dip treatment of 0.4% $KMnO_4$ solution to avoid dermal infection. The acclimatization of fish in aquaria for 15 days to laboratory conditions was carried out follows the methods of APHA (1985) [1]. During the whole period of experiment fish were fed the commercial feed (28% crude protein) with daily ration rate 3% of body weight in the morning (10.00 AM) and ensured routine cleaning of the aquaria, leaving no faecal matter or unconsumed food or dead fish (if any) as well as change water.

Experiment designed

The determination of LC_{50} values acute bioassays of ammonium sulphate, the mortality was recorded after 24, 48, 72 and 96 hour and the Finney method (1978) used for calculation. The LC_{50} values analyzed for these periods were concentration of ammonium sulphate 318 mg/l, 242 mg/l, 198 mg/l and 165 mg/l respectively. Prior to commencement of experiment sublethal concentration were taken as 1/10th value of the LC_{50} value for 96 hr (Sprague, 1971). 10 fish were exposed to a sub-lethal concentration (16.5 mg) of ammonium sulphate while simultaneously the control group of 10 fish were maintained for 30 days without the addition of ammonium sulphate. The physico- chemical parameters were used to characterization of the water quality considered essential. On 30th day of exposure fish were sacrificed by sudden spinal dislocation and the gill, liver, kidney and intestine were dissected out.

For the histopathological analysis small pieces were made of different dissected organs. There after microtome was used to made thin sections (5-7 µm) of paraffin embedded tissue blocks followed standard Luna method (1968) [8]. The sections were mounted on individual microscope slides and stained with hematoxylin and eosin and finally viewed under OLYMPUS microscope (X40) and microphotograph taken.

Results

The effects of fertilizer, ammonium sulphate were studied in the present experiment. The fish, *Channa punctatus* induced to ammonium sulphate (16.5 mg/l) sublethal concentration for 30 days showed alteration in histopathology.

Gill

Gills are prime site of toxicant action. All metabolic pathways depend upon the status of gills. The histology of gills showed primary and secondary gill filaments and gill arch were arranged and well established in control fish (Figure:-1). The sublethal 16.5 mg/l concentrations of ammonium sulphate after 30 days treated fish gill were observed lamellar fusion, deterioration of secondary lamellae and gill arch. Damage of lamellae was observed in treated group, gill filament and test buds apparently changed, hemorrhage and destruction of gill arches (Figure:-2).

Liver

In the present histological observation the liver of control fish showed systematically arranged sinusoids and central vein (Figure:-3). After 30 days of the exposure of fish to 16.5mg/l ammonium sulphate resulted changes in the liver tissue like hyperemia, vacuolar degeneration, necrosis and mononuclear cells filtration in portal regions (Figure:- 4).

Intestine

The histology of intestine in the control fish showed systematically arranged muscularis, mucosa; sub mucosa and serosa's membrane (Figure:-5). After 30 days of the exposure of fish to 16.5mg/l ammonium sulphate were resulting changes in the intestine tissue of *Channa punctatus* like hydropic degeneration, necrosis, desquamation in epithelium cells at the apex of the villi and mononuclear cell infiltration in the lamina propria was slightly observed. In the treated group also observed desquamation mononuclear cell infiltration (MHI) in connective tissue (Figure:-6).

Kidney

The fish kidney consists of head and body kidneys (Figure:-7). Head kidney is called anterior portion of the kidney and composed of lymphoid tissue. The part of body kidney is made of many nephrons and intestitial lymphoid tissue. The interstitial tissues are the major hematopoietic tissue in the body. Each nephron composed of two parts, the urinary tubule and the glomerulus. Renal tubules composed of single layer of epithelial cells. The Bowman's capsule composed of outer and an inner layer of single flat epithelia.

After 30 days of exposure of fish to 16.5mg/l ammonium sulphate were resulting changes in the glomerular tufts became highly melanized. The proximal and distal tubules showed cellular shrinkage and nuclear pycnosis. The hematopoietic tissues were enlarged and occupied wide area between the tubules. The vacuolar degenerative changes in the tubular epithelium and slight congestion were observed (Figure:-8).

Fig 1: Histology photomicrograph of normal gill of *Channa punctatus*. GL= Gill Lamellae, GA= Gill Arch, TB= Test buds. X40.

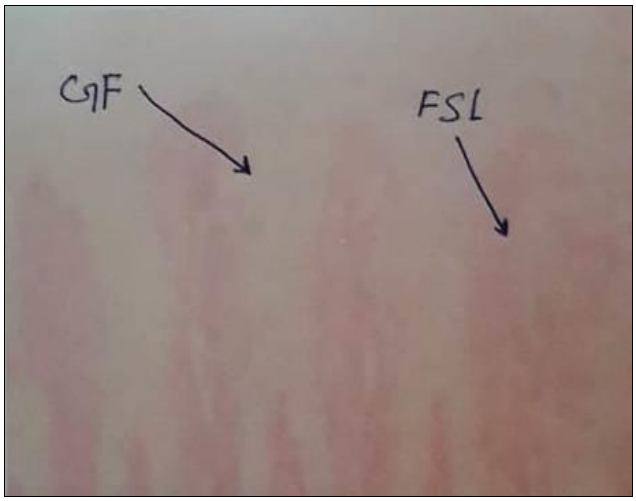

Fig 2: Histology photomicrograph of 16.5mg/l ammonium sulphate for 30 days treated gill of *Channa punctatus* showed Fusion of Secondary Lamellae (FSL), Damage of Gill Lamellae (DGL), Gill Filament (GF) and Damage of Gill Arch (DGA).X 40

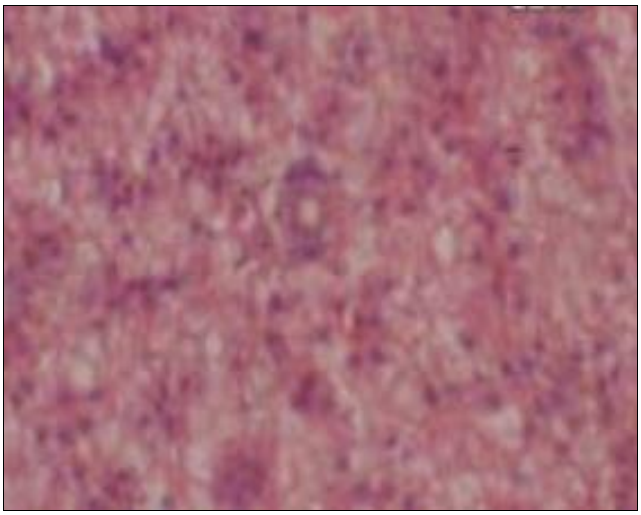

Fig 3: Histology photomicrograph of normal liver of *Channa punctatus* showed sinusoids (S), Blood vessels (BV). X40.

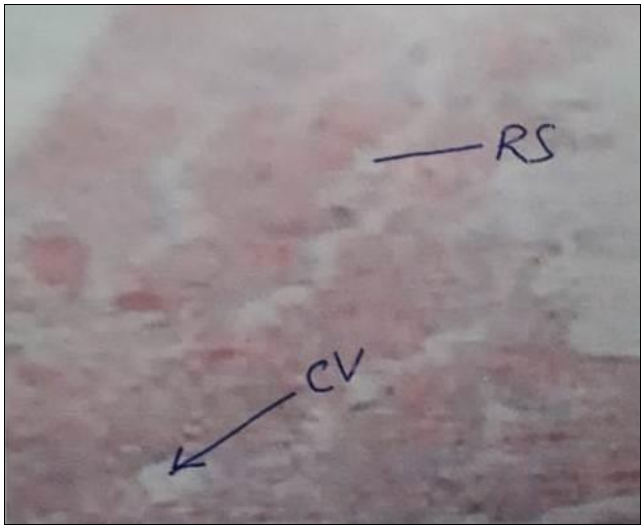

Fig 4: Histology photomicrograph of 16.5mg/l ammonium sulphate for 30 days treated liver of *Channa punctatus* showed rupture of sinusoids (RS), Bile Duct (BD) and Central vessel (CV). X40

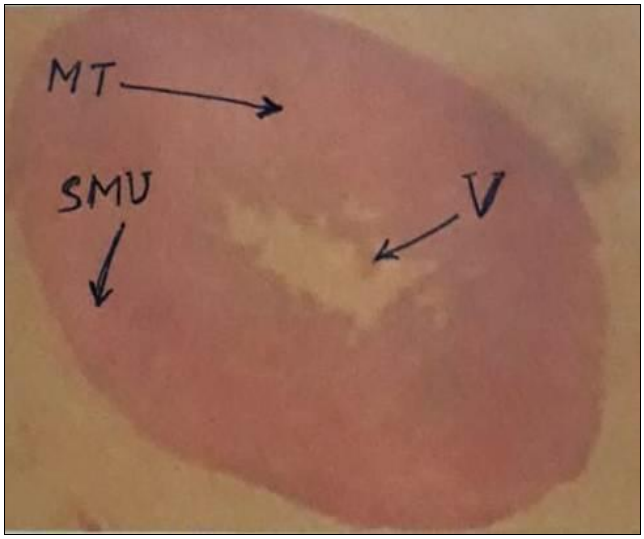

Fig 5: Histology photomicrograph of normal intestine of *Channa punctatus* showed muscular tissue (MT), sub-mucosa (SM) and villi (V). X 40.

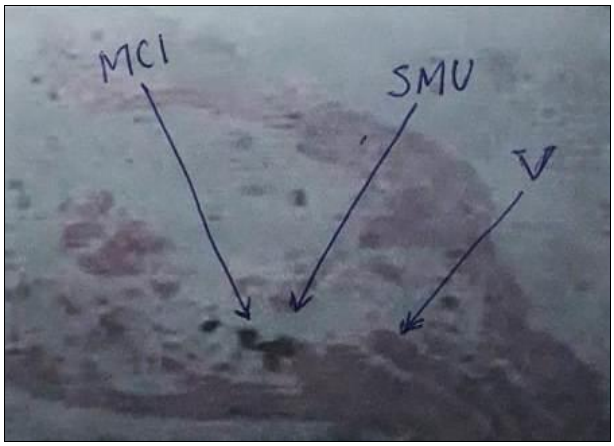

Fig 6: Histology photomicrograph of 16.5mg/l ammonium sulphate for 30 days treated intestine of *Channa punctatus* showed Desquamation mononuclear cell infiltration (MCI), degeneration and desquamation in villi (V). X40

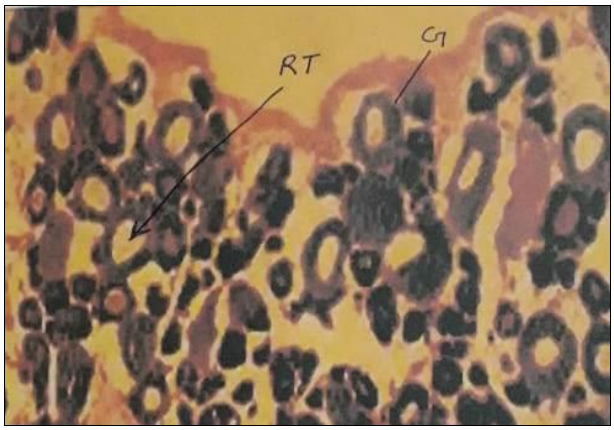

Fig 7: Histology photomicrograph of normal kidney of *Channa punctatus* showed renal tubule (RT), glomerulus (G) and Bowman's capsule (BC). X40.

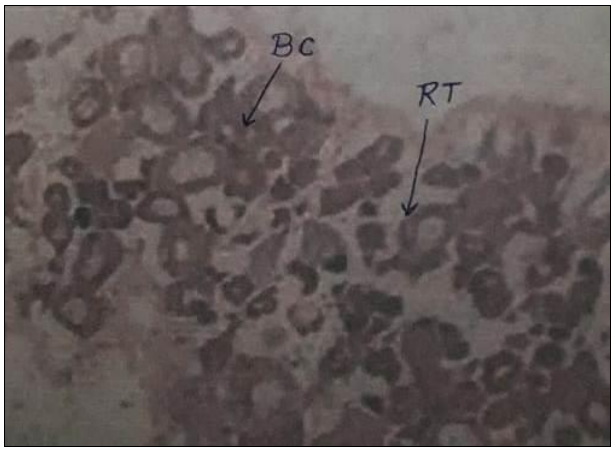

Fig 8: Histology photomicrograph of 16.5mg/l ammonium sulphate for 30 days treated intestine of *Channa punctatus* showed renal tubule (RT), glomerulus (G) and Bowman's capsule (BC). X40

Discussion

The present histopathological investigation is the fish, *Channa punctatus* induced to fertilizer, ammonium sulphate (16.5 mg/l) sublethal concentration for 30 days showed alteration in histology of gill, liver, intestine and kidney. Here we discuss the present findings and similarity of earlier works

of others. Yeldrimet *et al,* (2005) [18] described the route of introduce pesticides inside the body through the experiment and gills were the main target tissue induced by Carbofuran. Velmurugan, (2009) [17] reported that *Cirrhinus mrigala* exposed to various concentrations of dichlovos and organophosphate pesticide showed remarkable histopathological alterations in the liver tissues i.e. hyperplasia, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and in the gill necrosis of epithelial, oedema, lameller fusion, collapsed secondary lamellae and curling of secondary lamellae. In another study, Cattaneo *et al*, (2008) [3] also suggested that pesticide exposed silver catfish showed remarkable histological alteration like fragmentation and rupture in cell membrane, vacuoles in sinusoids of the liver tissues. Recently, similar histological changes in liver tissues reported by Cengiz (2006) [4] histopathology in the freshwater fish (*Cyprinus carpio*) after acute exposure to deltamethrin; Matos *et al*, (2007) and Sepici-Dincel *et al*, (2009) [14] when *O. niloticus* and *C. carpio* exposed to sub-lethal concentrations of carbaryl and cyfluthrin. The present findings has similarity with above studies.

Ullah, *et al*. (2015) [16] during experiment with pesticides found that detoxification of the cypermethrin in liver causes necrosis, it could be due to the extra work load on hepatocyte. Recently, Mishra & Poddar (2016) [10] suggested that liver plays as major metabolic site and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish.

The affected kidney showed degeneration of renal tubule epithelia, hyaline droplet degeneration, eventually may induce renal failure (Nayan, 2012) [12]. Boran *et al*, (2010) [2] reported that induced juvenile rainbow trout, *Oncorhynchus mykiss* to maneb and carbarys revealed the acute toxicity resulted remarkable histopathological changes like lamellar fusion, abnormality of lamellae, fusion of lamellae, necrosis in epithelial cell of the treated fishes. The insecticides were causes almost similar in histological changes in the gills, liver and kidney of exposed fishes.

Recently, Velmurugan *et al*, (2009) [17] reported that *Clarias gariepinus* exposed with various concentrations of cypermethrin resulted histopathological changes in histology like tubular fusion, epithelial hypertrophy, glomerular condensation, hemorrhage and necrosis of Bowman's capsule were found in kidney tissues and in gills fusion of secondary lamellae, necrosis, while in liver swelling of hepatocytes, necrosis, pycnosis and vacuoles. The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Sastri and Gupta (1978) [13] in *Channa punctatus.* Very recently, Kumari, *et al.*, (2020) [7] reported that histopathological alteration in liver, kidney and intestine by fertilizer, ammonium chloride on fish *Clarias batrachus*. The above reporting is similar to the present findings.

Conclusion

In present study conclude that the pathological changes in gills, liver, intestine and kidney were associated with fertilizer ammonium sulphate in water. *Channa punctatus* with average weight 30.0± 4.0 g, were more suitable to fish culture at water fertilizer, ammonium sulphate concentration of < 16.5 mg/l for survival rate than other water conditions. The present study will help in formulating guidelines for the use of this chemical in agricultural farms without any major setback to the surrounding ecosystems and their inhabitants.

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