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EFFECTS OF AMMONIUM CHLORIDE ON SOME VITAL ORGANS OF SNAKEHEAD FISH, *Channa punctatus* (BLOCH.)

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ABSTRACT

The present study investigated the effects of chronic exposure to a sublethal concentration (20 mg/L) of the inorganic fertilizer ammonium chloride for 30 days on the snakehead fish *Channa punctatus*. Histopathological alterations were observed in several vital organs of the treated fish. Significant pathological changes were evident in the gills, liver, intestine, and kidney compared to the control group. The gills of ammonium chloride–exposed fish exhibited lamellar fusion, deterioration of secondary lamellae, haemorrhage, and destruction of gill arches. The liver showed marked pathological changes, including hyperaemia, vacuolar degeneration, necrosis, and mononuclear cell infiltration in the portal regions. In the intestine, distinct alterations such as hydropic degeneration, necrosis, desquamation of epithelial cells at the apex of the villi, and mononuclear cell infiltration in the lamina propria were observed. The kidney of treated fish revealed cellular shrinkage in the proximal and distal tubules, nuclear pyknosis, and vacuolar degenerative changes in the tubular epithelium. The findings of the present investigation clearly demonstrate that chronic exposure to ammonium chloride induces severe histopathological damage and dysfunction in vital organs of *C. punctatus*. The study suggests that contamination of fish culture pond water with ammonium chloride fertilizer should be strictly avoided to ensure sustainable fish production and aquatic health.

Key words: *Channa punctatus*, ammonium chloride, nitrogenous fertilizer, histopathology, agrochemical wastes.

INTRODUCTION

Pollutants originating from agricultural activities have caused significant losses in biological diversity and represent one of the most serious global environmental problems. Such anthropogenic activities are now considered a major threat to many species, pushing them toward the verge of global extinction (Díaz *et al.*,

2020). The indiscriminate use of fertilizers in agricultural fields is a major source of surface water pollution due to runoff. Water contaminated with these toxicants can cause mortality in aquatic organisms, particularly fish. Fish are highly susceptible to such pollutants because, once their habitats become contaminated, they are often unable to escape the harmful effects of these substances (Gbaruko and Friday, 2007).

Ammonium chloride is a nitrogenous fertilizer widely used in agricultural practices to enhance soil fertility (Naidu *et al.*, 2017). It is highly soluble in water and releases ammonia, the primary inorganic form of nitrogen, upon dissolution (Clarkson *et al.*, 1986). Aquatic ecosystems may develop ammonium concentrations high enough to adversely affect fish as a result of fertilizer runoff or other anthropogenic sources. Ammonia (NH_3) is a natural byproduct of amino acid metabolism and, due to its basic nature, readily accepts a hydrogen ion to form ammonium (NH_4^+) at physiological pH (Cameron & Heisler, 1983). Both ammonia and ammonium are toxic when accumulated in the body; therefore, an efficient excretory mechanism is essential for maintaining cellular homeostasis. Exposure to ammonium-contaminated water can result in reduced growth, altered behavior, and increased susceptibility to diseases in fish (Thurston *et al.*, 1984).

Hence, the present study was undertaken to investigate the histopathological changes in some vital organs of the snakehead fish, *Channa punctatus*, following exposure to the chemical fertilizer ammonium chloride.

MATERIALS AND METHODS :

The air-breathing snakehead fish, *Channa punctatus*, with an average length of 8–10 cm and body weight of 30–34 g, were procured from the local fish market in Darbhanga and transported to the laboratory in aerated plastic containers. Healthy fish were washed with a 0.4% potassium permanganate ($KMnO_4$) solution to prevent external infections. Under laboratory conditions, the fish were acclimatized for 15 days. During the acclimatization period, the fish were fed commercial feed containing 28% crude protein at a daily ration of 3% of their body weight, administered once per day. Physicochemical parameters of the water were maintained according to standard methods described by APHA (2005).

Acute toxicity bioassays were conducted to determine the LC_{50} values of ammonium chloride. Mortality was recorded after 24, 48, 72, and 96 hours of exposure, and the corresponding LC_{50} values were found to be 300, 260, 225, and 200 mg/L, respectively. The LC_{50} values were calculated using Finney's probit analysis method (1978). The sublethal concentration was determined following Sprague's method (1971), where one-tenth of the 96-hour LC_{50} value was selected. Accordingly, a concentration of 20 mg/L ammonium chloride was used for chronic exposure. Twenty fish were exposed to the sublethal concentration for 30 days, while an equal number of fish were maintained simultaneously as a control group under identical conditions without ammonium chloride exposure. At the end of the 30-day exposure period, the fish were sacrificed, and vital

organs including the gills, liver, kidney, and intestine were carefully dissected. The dissected organs from both control and treated fish were cut into small pieces and processed for histological examination. Tissue blocks were prepared using paraffin wax following the standard Luna method (1968). Thin sections of 5–7 µm thickness were obtained using a rotary microtome. The sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). The prepared slides were examined under an OLYMPUS light microscope at 40× magnification, and microphotographs were taken for histopathological analysis.

RESULTS:

Histopathological alterations were observed in the gills and liver of the snakehead fish, *Channa punctatus*, following chronic exposure to a sublethal concentration of ammonium chloride (20 mg/L) for 30 days.

Gill: The gills of control *C. punctatus* exhibited a normal histological architecture characteristic of the teleostean gill. Each gill consisted of primary and secondary lamellae supported by a cartilaginous skeletal framework, multilayered epithelium, and an extensive vascular system. The primary gill lamellae appeared as flat, leaf-like structures arranged in double rows, from which numerous alternately arranged secondary lamellae projected laterally. These secondary lamellae were lined by squamous epithelium and supported by pillar cells (Figure -1).

In contrast, the gills of fish exposed to a sublethal concentration (20 mg/L) of ammonium chloride for 30 days exhibited marked histopathological alterations. These changes included fusion of secondary lamellae, deterioration and distortion of lamellar architecture, and damage to gill filaments and gill arches. Additionally, haemorrhage, degeneration of lamellae, and destruction of gill arches were clearly evident in the treated group (Figure- 2).

Liver: Histological examination of the liver of control fish revealed a normal hepatic architecture. Hepatocytes were polygonal in shape with granular cytoplasm and centrally located nuclei containing densely stained nucleoli. The cells were arranged in cords separated by narrow, well-organized blood sinusoids, and the liver tissue was richly supplied with blood vessels (Figure 3).

Following 30 days of exposure to ammonium chloride (20 mg/L), pronounced pathological changes were observed in the liver tissue. These alterations included hyperemia, vacuolar degeneration of hepatocytes, focal necrosis, and mononuclear cell infiltration, particularly in the portal regions, indicating hepatic tissue damage and inflammatory response (Figure- 4).

Intestine: The intestine of control *Channa punctatus* exhibited a normal histological organization consisting of four distinct layers: mucosa, submucosa, muscularis, and serosa. The innermost mucosal layer was composed of columnar epithelial cells forming numerous finger-like folds known as villi. These villi

contained distinct mucus-secreting goblet cells and brush border cells. The submucosa consisted of loose connective tissue forming the lamina propria of the villi and was granular in nature with a rich vascular supply. The muscularis layer comprised an inner thick circular muscle layer and an outer thin longitudinal muscle layer. The outermost serosal layer consisted of a thin, vascularized covering (Figure- 5).

In contrast, the intestine of fish exposed to a sublethal concentration of ammonium chloride (20 mg/L) for 30 days showed marked histopathological alterations. These changes included hydropic degeneration, necrosis, and desquamation of epithelial cells at the apex of the villi. Additionally, pronounced mononuclear cell infiltration was observed in the lamina propria and connective tissue, indicating inflammatory responses and tissue damage (Figure- 6).

Kidney: The kidney of control *C. punctatus* exhibited a typical teleostean organization and consisted of two main regions: the head kidney and the body kidney (Figure -7). The head kidney was located anteriorly and was primarily composed of lymphoid tissue. The body kidney consisted mainly of nephrons embedded within interstitial hematopoietic tissue. Each nephron was composed of a glomerulus and a renal tubule. The renal tubules were lined by a single layer of epithelial cells, while Bowman's capsule was formed by an inner layer of flattened epithelial cells, reflecting normal renal histoarchitecture.

The kidney of *Channa punctatus* exposed to a sublethal concentration of ammonium chloride (20 mg/L) for 30 days exhibited pronounced histopathological alterations. The glomerular tufts showed marked melanization, while the proximal and distal renal tubules displayed cellular shrinkage and nuclear pyknosis. Additionally, enlargement of hematopoietic tissue was observed, occupying extensive areas between the renal tubules. Vacuolar degenerative changes in the tubular epithelium and mild congestion were also evident in the treated fish group (Figure -8).

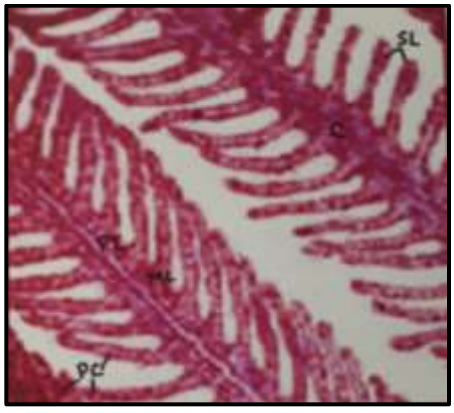

Figure:1. Photomicrograph of normal gill of *Channa punctatus*. PL= Primary gill lamellae, SL=Secondary gill lamellae, PC= Pillar cells, MC= Mucous cells. X40.

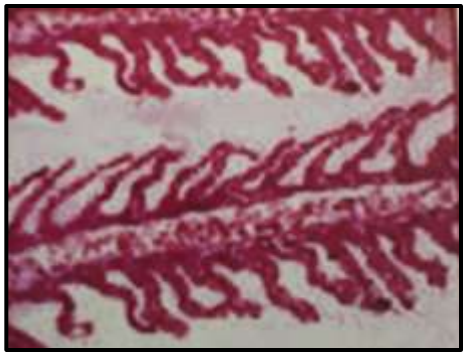

Figure:2. Photomicrograph of gill of *Channa punctatus* exposed to 20 mg/l ammonium chloride for 30 days treated showed Fusion of Secondary Lamellae (FSL), Damage of Gill Lamellae (DGL), Gill Filament (GF) and Damage of Gill Arch (DGA).X 40

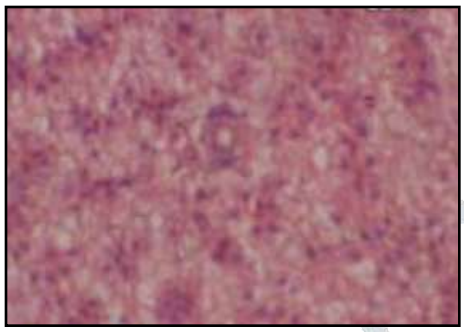

Figure:3. Photomicrograph of normal of liver histology of *Channa punctatus* showed sinusoids (S), Blood vessels (BV). X40.

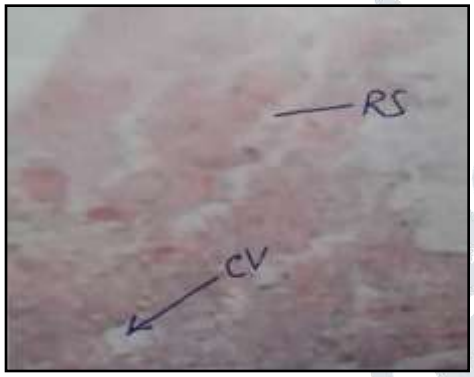

Figure:4. Photomicrograph of liver of *Channa punctatus* exposed to 20 mg/l ammonium chloride for 30 days showed rupture of sinusoids (RS), Bile Duct (BD) and Central vessel (CV). X40

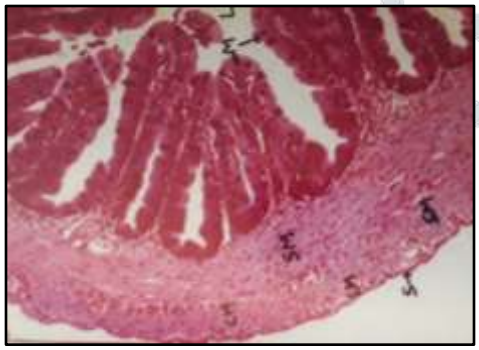

Figure:5. Photomicrograph of normal intestine of *Channa punctatus* showed Circular muscular fibres (CM), sub-mucosa (SM), Mucosa (M), Lumen (L) and villi (V). X 40.

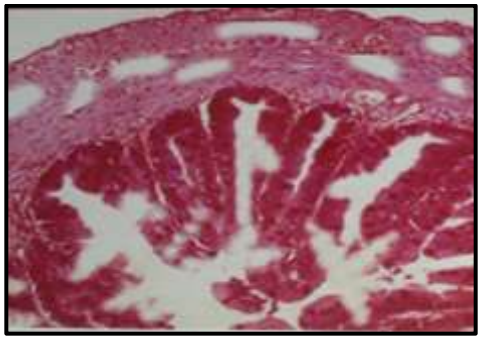

Figure:6. Photomicrograph of intestine of *Channa punctatus* exposed to 20 mg/l ammonium chloride for 30 days showed mononuclear cell infiltration (MCI), degeneration and desquamation in villi (V). X40

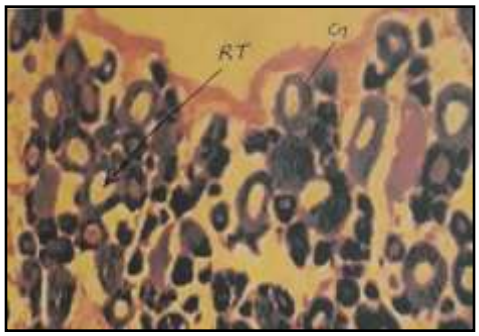

Figure:7. Photomicrograph of normal kidney of *Channa punctatus* showed renal tubule (RT), glomerulus (G) and Bowman's capsule (BC). X40.

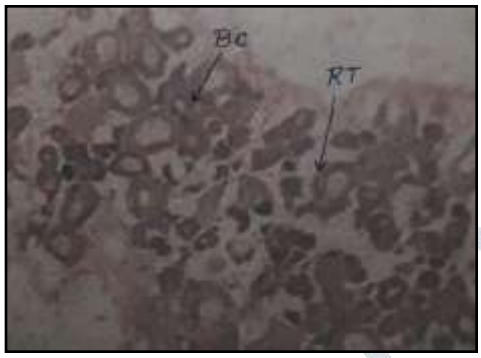

Figure:8. Photomicrograph of intestine of *Channa punctatus* induced to 20 mg/l ammonium chloride for 30 days showed renal tubule (RT), glomerulus (G) and Bowman's capsule (BC). X40

DISCUSSION:

The present investigation was undertaken to evaluate the histopathological effects of chronic exposure to a sublethal concentration (20 mg/L) of the fertilizer ammonium chloride on the snakehead fish, *Channa punctatus*, over a period of 30 days. The results revealed marked histopathological alterations in four vital organs, namely the gills, liver, intestine, and kidney. The findings are discussed below in relation to earlier reports by researchers in the relevant field.

The gills are the first organ to come into direct contact with waterborne contaminants and therefore serve as the primary site for toxicant action. The histological condition of the gills reflects the susceptibility of fish to environmental pollutants. Yeldrim *et al*. (2005) reported significant histological alterations in the gills of fish exposed to the pesticide carbofuran. Similarly, Velmurugan (2009) observed severe gill damage in *Cirrhinus mrigala* exposed to various concentrations of the organophosphate pesticide dichlorvos, including epithelial necrosis, edema, lamellar fusion, collapse of secondary lamellae, and curling of secondary lamellae. The gill alterations observed in the present study, such as lamellar fusion, degeneration, and hemorrhage, are consistent with these earlier findings, indicating impaired respiratory efficiency due to ammonium chloride toxicity.

The liver, being the major metabolic and detoxification organ, is highly susceptible to chemical-induced damage. Cengiz (2006) reported histopathological alterations in the liver of *Cyprinus carpio* exposed to the pesticide deltamethrin. Matos *et al*. (2007) observed hepatic tissue damage in Nile tilapia (*Oreochromis niloticus*) exposed to carbaryl. Likewise, Cattaneo *et al*. (2008) documented fragmentation and rupture of cell

membranes and vacuolization of sinusoids in the liver tissues of silver catfish exposed to pesticides. Sepici-Dinçel *et al*. (2009) also reported histopathological liver changes in *O. niloticus* and *C. carpio* exposed to sublethal concentrations of carbaryl and cyfluthrin. Velmurugan (2009) noted severe hepatic alterations in *C. mrigala* following dichlorvos exposure, including hyperplasia, congestion, vacuolar degeneration, karyolysis, karyorrhexis, and dilation of sinusoids.

Ullah *et al*. (2015) suggested that hepatic necrosis may result from increased detoxification demands placed on hepatocytes during pesticide exposure, leading to excessive metabolic stress. The liver is considered a central metabolic organ, and damage to this tissue can result in severe physiological disturbances and ultimately fish mortality, as suggested by Mishra and Poddar (2016). The hepatic alterations observed in the present study, such as hyperaemia, vacuolar degeneration, necrosis, and mononuclear cell infiltration, closely resemble those reported in previous studies, thereby confirming the toxic impact of ammonium chloride on liver function.

Several earlier studies support the present observations on intestinal histopathology. Sastri and Gupta (1978) reported that *Channa punctatus* exposed to the heavy metal mercuric chloride exhibited proliferation, necrosis of the serosa and mucosa, and rupture of intestinal villi. More recently, Islam *et al*. (2019) documented histopathological alterations in the intestine of *Heteropneustes fossilis* exposed to diazinon (25.0 mg/L), including swelling of the muscularis layer, slight damage to the serosa, fusion or rupture of villi, and disintegration of the submucosa. Kumari *et al*. (2020) also reported significant histopathological changes in the intestine of *Clarias batrachus* exposed to the fertilizer ammonium chloride. These findings are in close agreement with the intestinal alterations observed in the present study, such as villar degeneration, epithelial desquamation, necrosis, and mononuclear cell infiltration.

Similarly, several investigations corroborate the present findings on renal histopathology. Rand and Petrocelli (1985) observed tubular necrosis, pyknosis, and karyorrhexis in the kidney tissues of salmon exposed to 100 ppm amitrole for 144 hours. Rahman *et al*. (2002) reported degenerative changes, necrosis, and hemorrhage in the kidney tissues of *Anabas testudineus, Channa punctatus*, and *Barbodes gonionotus* following exposure to diazinon 60 EC. Velmurugan *et al*. (2009) also observed tubular fusion, epithelial hypertrophy, glomerular condensation, haemorrhage, and necrosis of Bowman's capsule in the kidney of *Clarias gariepinus* exposed to varying concentrations of the pesticide cypermethrin. Boran *et al*. (2010) reported severe epithelial necrosis in the kidneys of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to maneb and carbaryl. Furthermore, Nayan *et al*. (2012) documented renal tubular epithelial degeneration, hyaline droplet degeneration, and renal failure in *Cyprinus carpio* exposed to dimethoate (EC 30%).

The renal alterations observed in the present study, including glomerular melanization, tubular degeneration, nuclear pyknosis, vacuolar changes, and congestion, closely resemble these previously reported findings. This

similarity strongly supports the conclusion that chronic exposure to ammonium chloride induces severe renal dysfunction in *C. punctatus*.

CONCLUSION:

The present study concludes that *Channa punctatus* with an average body weight of 30.0 ± 4.0 g is highly susceptible to the fertilizer ammonium chloride, even at sublethal concentrations (< 20 mg/L). Chronic exposure to ammonium chloride induced pronounced histopathological alterations in vital organs, including the gills, liver, intestine, and kidney. These tissue-level changes clearly indicate the toxic effects of ammonium chloride and its potential to impair essential physiological functions in fish.

The findings of this study emphasize that contamination of fish culture pond water with ammonium chloride fertilizer poses a serious risk to fish health and productivity. Therefore, the use and runoff of ammonium chloride in and around aquaculture systems should be strictly controlled to ensure sustainable fish production and to protect aquatic ecosystems.

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