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Histopathological Alterations of Gills, Liver and Kidney of Freshwater Fish, *Oreochromis niloticus*, Exposed to Cypermethrin

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ABSTRACT

Cypermethrin, a fourth-generation synthetic pyrethroid pesticide, is a common pollutant of the freshwater ecosystem in tropical countries due to its mass-scale use in agriculture and subsequent release into different freshwater ecosystems. This study was conducted to evaluate the histopathological effects of sub-lethal concentrations of cypermethrin (0.0, 1.25 and 2.5µg/L) on gills, liver and kidney tissues of the freshwater fish Nile tilapia, *Oreochromis niloticus*. Cypermethrin exposure caused epithelial hyperplasia on secondary gill lamellae, necrosis on primary gill lamellae, clumped epithelial cells at the base of secondary lamellae and haemorrhages. Hepatocytic vacuolation and necrosis in the nuclear membrane were observed in the treated fish liver. Glomerular shrinkage, thickening of Bowman's capsule, and necrosis of renal tubule were found within the kidney of *O. niloticus* exposed to 1.25 and 2.5 µg/L cypermethrin. Histopathological disorders increased with the increase in the concentration of cypermethrin. It is concluded that studies on histopathological disorders of gills, liver and kidney tissues of *O. niloticus* serve as a useful tool in determining ecologically safe concentrations of cypermethrin and managing natural water bodies and the safety of non-target aquatic organisms.

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1. Introduction

The use of chemical pesticides in agriculture and households has increased exponentially during the last few decades worldwide. As a result, the natural freshwater bodies, including rivers, lakes and ponds, continuously receive run-offs containing residue of pesticides from these sources, rendering fish and other aquatic organisms susceptible to the toxic effects of these pesticides. Cypermethrin is a type II synthetic pyrethroid pesticide and is extensively used in agricultural fields in India (Kaviraj and Gupta, 2014). The concentrations at which this pesticide is detected in different water bodies, including rivers and streams, soil and even in rainwater, are toxic to freshwater fish and other non-target organisms (Prusty *et al.*, 2015; Majumder and Kaviraj, 2015, 2017; Ullah *et al.*, 2018).

Histopathological changes in different fish tissues have long been recognized as a valuable tool to understand both adaptive processes and detrimental effects in fish induced by environmental pollutants (Cenzig and Unlu, 2006; Korkmaz *et al.*, 2009). Lesions in the gills of fish can be considered as the primary markers for aquatic pollution as gill filaments come in direct contact with contaminated water during respiration (Velmurugan *et al.*, 2018; Alalibo *et al.*, 2019). Lipophilicity of the pyrethroid pesticides makes them attracted to the non-water soluble components of the cells of gills. Pyrethroid pesticides are thus strongly absorbed by the gills (Smith and Stratton, 1986) and produce respiratory troubles in fish. In addition, the liver in fish is involved in biotransformation and excretion of pesticides and the kidney maintains electrolyte and water balance and excretes out nitrogenous waste products. Histopathological changes in these organs also serve as effective biomarkers of aquatic pollution (Velmurugan *et al.*, 2009).

Several histopathological changes have been recorded in the gills of fish exposed to cypermethrin. These include epithelial hypertrophy, epithelial lifting and oedema, hyperplasia of primary epithelial cells, fusion of secondary lamellae, necrosis and desquamation (Velmurugan *et al.*, 2009), telangiectasia in the secondary lamellae of gills and damage of pillar cells (Velisek *et al.*, 2006; Velmurugan *et al.*, 2009; Korkmaz *et al.*, 2009). Similar histopathological disorders have been recorded in the gills of fish when they were exposed to sub-lethal concentrations of other pyrethroids like deltamethrin (Cenzig and Unlu, 2006), fenvalerate (Prusty *et al.*, 2015) and alpha-cyhalothrin (Alalibo *et al.*, 2019). Damage to hepatocytes is the common effect of cypermethrin on the liver of fish (Velisek *et al.*, 2006; Velmurugan *et al.*, 2009). Mass lesions and focal coagulative necrosis was reported in the liver of *Labeo rohita* due to cypermethrin exposure (Sarkar *et al.*, 2005). Cypermethrin has also been found to induce atrophy in the glomerulus and broadening of Bowman's capsule in the kidney tissue of fish (Velmurugan *et al.*, 2009).

However, the intensity of histopathological effects of pyrethroids on different tissues of fish varies with the species of fish due to variation in tolerance to pollutants as well as due to variation in structural organization of the tissue concerned. *Oreochromis niloticus* is an exotic freshwater fish cultured widely in ponds and tanks in India. The fish can survive in a wide range of aquatic habitats and serve as an excellent model to study the effects of pesticides, including cypermethrin (Majumder and Kaviraj,

2017, 2020). The objectives of the present study were to evaluate histopathological changes in gills, liver and kidney tissues of *O. niloticus* exposed to sub-lethal concentrations of cypermethrin and verify if these changes could be established as biomarkers of cypermethrin pollution in freshwater bodies.

2. Materials and Methods

Healthy specimens of *O. niloticus* (mean length 4.77±0.25cm. and mean weight 2.74±0.45g) were collected from a local fish hatchery (Naihati, W.B., India). The fish were acclimatized to the test conditions for 96h before using in the bioassay. A balanced diet containing 30% crude protein was supplied to the fish during acclimatization. Cypermethrin (10 % EC) under the brand name Ustaad® was obtained from the United Phosphorus Ltd., Vapi-396195, Gujarat.

Bioassays were made in 15 liters glass aquaria, each holding 10 liters of deep tube well water stored in an overhead tank (temperature 30 ± 3^{0}C, pH 7.1 ± 0.1; free CO_2 3.42 ± 0.31 mg/L; dissolved oxygen 6.7 ± 0.2 mg/L; total alkalinity 125.75 ± 3.27 mg/L as $CaCO_3$; total hardness 142.33 ± 9.01 mg/L as $CaCO_3$) and five fish. The aquaria were arranged as per randomized block design so that there were three replicates for each of three treatments of cypermethrin (10% EC): 0, 1.25 and 2.5 µg/L representing approximately 0, 25 and 50 % of the 96h LC_{50} values of nominal concentrations of cypermethrin (10% EC) to *O. niloticus* (4.85 µg/L) respectively (Majumder and Kaviraj, 2017). Fish excreta were removed every 24h interval during the 28-day experiment to avoid interference with the test chemical. Besides, 20% of the test medium was renewed at every 7-day interval with a pulse treatment of cypermethrin at 20% of the initial nominal concentration.

For histological studies, gill, liver and kidney tissues were collected from treated and control fish after completion of 28 days and fixed in aqueous Bouin's fixative overnight. Gill tissues were decalcified before fixation using 5% HNO_3 in 70% alcohol. After fixation, the tissues were washed in distilled water, dehydrated through graded ethanol series, cleared in xylene and finally stubbed in paraffin blocks. Tissue sections of 5µ were cut out from the paraffin blocks using a rotary microtome and stretched on slide smeared with Mayer's albumin. Sections were deparaffinized in xylene, hydrated through graded ethanol series and stained with Haematoxylin and Eosin (Bradbury, 1969). Tissue sections were mounted with DPX and observed under a light microscope.

3. Results

3.1. Histopathological changes in gill

Histopathological observations on the gill tissues of different cypermethrin exposed groups of *O. niloticus* have been presented in Fig.1 (A-C). Each gill consists of primary lamellae with secondary lamellae on either side. In control fish, the primary gill filament showed epithelial cell linings and blood vessels, while secondary gill lamellae contain blood vessels and thin epithelial cell linings with distinct pillar cells. Interlamellar spaces are found to be normal (Fig. 1A). Fish showed varying degrees of histological changes in gill structure depending upon the concentrations of cypermethrin exposure. At a low concentration of cypermethrin (1.25 µg/L), the primary gill lamellae show increased intralamellar space. There are clumped epithelial cells at the base of the secondary lamellae, while necrosis is noted on its two sides. The higher concentration of cypermethrin (2.50 µg/L) reveals shortening of secondary lamellae, necrosis within primary and secondary gill lamellae, epithelial hyperplasia at the base of secondary lamellae and desquamation of the epithelium of the secondary lamellae in addition to increased intralamellar space on primary gill lamellae.

3.2. Histopathological changes in liver

Histopathological alterations on the hepatic tissues of control and cypermethrin exposed *O. niloticus* are briefly presented in Fig. 2 (A-C). Hepatocytes are seen as polygonal in shape with homogenous cytoplasm and a central spherical nucleus in the control fish. Sinusoids are found to be irregularly distributed between hepatocytes. At a low concentration of cypermethrin (1.25 µg/L), vacuolation of hepatocytes and deformed nuclear membrane are noted (Fig. 2B). At higher concentration (2.50 µg/L), the hepatocytes show an extensive reduction of glycogen leading to vacuolation and pycnosis (Fig. 2C).

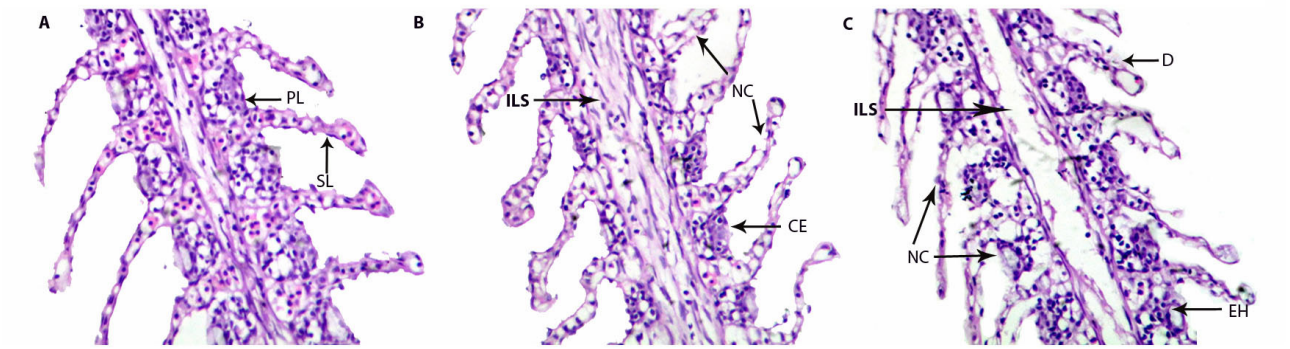

Fig. 1. Histology of gills of *Oreochromis niloticus* exposed to cypermethrin

(A. Gill of control *O. niloticus* showing normal primary lamellae (PL) and secondary lamellae (SL). B. Gill of *O. niloticus* exposed to 1.25 µg/L cypermethrin for 28 days showing increased intra lamellar space (ILS) on primary lamellae, clumped epithelial cells (CE) at the base of secondary lamellae and necrosis (NC) on secondary lamellae. C. Gill of *O. niloticus* exposed to 2.5µg/L cypermethrin for 28 days showing increased epithelial hyperplasia (EH), necrosis (NC) and desquamation (D) on secondary lamellae. H & E; X 400)

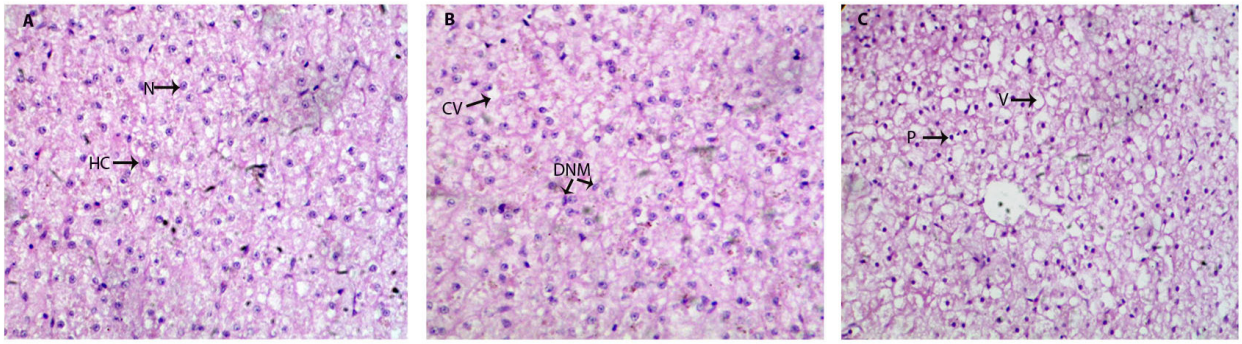

Fig. 2. Histology of liver of *Oreochromis niloticus* exposed to cypermethrin

(A. Liver of control *O. niloticus* showing normal polygonal hepatocytes (HC) with distinct spherical nucleus (N), cytoplasmic glycogen and fat droplets . B. Liver of *O. niloticus* exposed to 1.25 µg/L cypermethrin for 28 days showing vacuolation of hepatocytes (CV) and deformed nuclear membrane (DNM). C. Liver of *O. niloticus* exposed to 2.5 µg/L cypermethrin for 28 days showing reduced glycogen granules, vacuolation (V) and pycnosis (P). H & E; X 400)

3.3. Histopathological changes in kidney

The kidney of the control fish consists of many renal tubules and well-developed glomeruli within the bowman's capsule. The proximal renal tubules have a brush boarder in their columnar epithelial cells, whereas distal tubules and collecting ducts lack the brush boarders (Fig. 3A). At a lower concentration of cypermethrin (1.25 µg/L), glomerulus shrinkage, resulting in expanded space between glomerulus and bowman's capsule and hyaline degeneration in the renal tubular epithelium (Fig. 3B). In addition to these effects the higher concentration of cypermethrin (2.50 µg/L) produced intracytoplasmic vacuolation in epithelium of renal tubule, hyaline degeneration of tubular epithelium and dilation of the tubular lumen (Fig 3C).

4. Discussion

The present study showed that cypermethrin exposure caused epithelial hyperplasia, epithelial clump at the base of secondary lamellae, necrosis on primary and secondary gill lamellae, and oedematous separation epithelium from secondary lamellae and shortening of secondary lamellae. These are common effects of cypermethrin on fish gills (Ayoola and Ajani, 2008; Korkmaz *et al.*, 2009; Olufayo and Alade, 2012; Velmurugan *et al.*, 2014; Wei and Yang, 2015; Arslan *et al.*, 2017). Results of the present study also revealed that the damage to the gill epithelium increased with the increase in the concentration of cypermethrin. The implication is that if these damages to the gill epithelium persist, it may result in respiratory trouble and other physiological disorders of the fish, which are typically not easily detected. Therefore, the histology of gills may serve as a valuable biomarker to assess water pollution by cypermethrin.

The liver is the primary metabolic organ responsible for the detoxification of xenobiotics. However, elevated concentrations of such toxicants could alter the gross histology of the organ. Histopathological alterations in the form of irregular shape, vacuolation, pycnotic nuclei and focal necrosis of hepatocytes, ruptured sinusoids with haemorrhages, perivascular fibrosis of liver cells, disposition of yellow-brown grains on hepatic tissues were found in cypermethrin treated different fish species: *O. niloticus* (present study), *Heteropneustes fossilis* (Joshi *et al.*, 2007), *Clarias gariepinus* (Velmurugan *et al.*, 2009), *Heterobranchus bidorsalis* (Olufayo and Alade, 2012), *Cyprinus carpio* (Arslan *et al.*, 2017) and *Catla catla* (Sharma and Jindal, 2020). These disorders indicate that the liver attempt to detoxify cypermethrin and, in turn, suffers cellular damage. These damages could be recovered

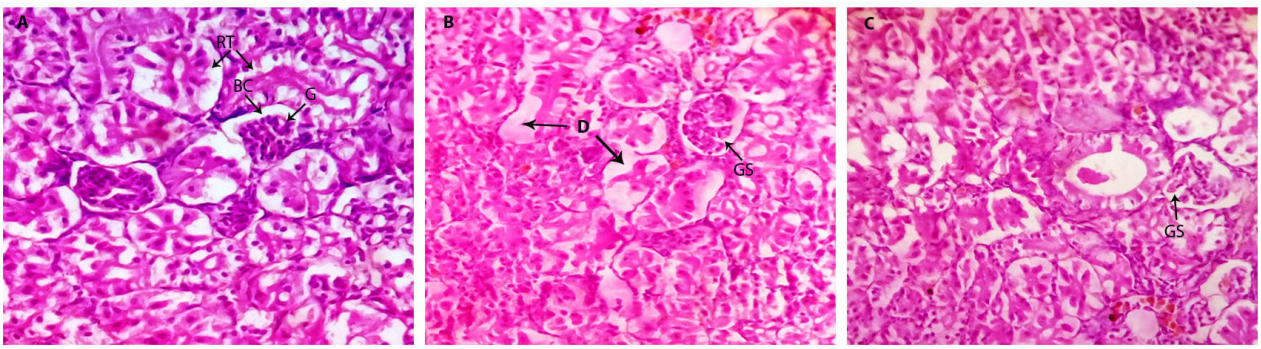

Fig. 3. Histology of kidney of *Oreochromis niloticus* exposed to cypermethrin

(A. Kidney of control *O. niloticus* showing normal Bowman's capsule (BC), glomerulus (G) and renal tubules (RT). B. Kidney of *O. niloticus* exposed to 1.25µg/L cypermethrin for 28 days showing shrinkage of glomerulus (GS) and hyaline degeneration in the renal tubular epithelium (D). C. Kidney of *O. niloticus* exposed to 2.5µg/L cypermethrin for 28 days showing glomerular shrinkage (GS), intracytoplasmic vacuoles and hyaline degeneration (D) in the renal tubular epithelium (black arrow). H & E, X 400)

if cypermethrin exposure was at a low magnitude and for a short period. However, the results of the present study indicate that persistent exposure (pulse treatment in this study) to a high concentration of cypermethrin may result in severe damage to the liver rendering the fish incapable of detoxifying cypermethrin in its body.

The cypermethrin treated *O. niloticus* also exhibited glomerular shrinkage and expansion of the space between glomerulus and Bowman's capsule. Moreover, intracytoplasmic vacuolation in the renal tubular epithelium, hyaline degeneration of tubular epithelium and dilation of tubular lumen were also noted due to cypermethrin exposure. Similarly, Olufayo and Alade (2012) observed cypermethrin exposure to cause karyolysis of nucleic material, vacuole formation in epithelial cells of renal tubule, necrosis and dilation of renal tubules in *Heterobranchus bidorsalis*. Velmurugan *et al.* (2009) described the effects of cypermethrin exposure on the kidney tissues of *C. gariepinus* in the form of glomerular atrophy, broader Bowman's capsule, narrow tubular lumen, epithelial necrosis of renal tubule and pyknosis in the hematopoietic tissues. Haque *et al.* (2017) observed vacuolation, necrosis, cellular degeneration, karyolysis and rupturing of renal tubules as histopathological changes within the kidney tissues of *Mystus tengara* due to cypermethrin assault.

5. Conclusion

It is concluded from the present study that cypermethrin, even at a level equivalent to 25% of its lethal value to *O. niloticus,* may cause severe damage to the gill, liver and kidney, which may potentially result in respiratory trouble and other physiological disorders of the fish. Cypermethrin at this dose may render the fish incapable of detoxifying the pesticide in its body. Therefore, the histopathological study of gill, liver and kidney tissues of fish serves as a useful tool in determining ecologically safe concentrations of cypermethrin and managing natural bodies of water and safety of the non-target aquatic organisms.

Ethical standards

We declare that the experiments were conducted as per the guidelines of the institutional animals ethics committee.

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