

The effect of cypermethrin (25% ec) on histology of kidney of *Ophiocephalus striatus*

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Abstract

The present investigation was aimed to observe the histological changes in the kidney of *Ophiocephalus striatus* exposed to LC₅₀ dose ($0.72 \pm 0.007 \mu\text{l lit}^{-1}$) of Cypermethrin (25% EC) for 96 hours. Kidneys were dissected out to study the histological alterations. Light microscopic studies showed the many his to pahological changes in the kidney. Kidneys were significantly affected showing degenerative and necrotic changes. Vacuolation, glomerular shrinkage, disorganized renal tubules, damage to haemopoietic tissue and blood vessels was observed. Loss of integrity of tubular epithelial cell and interstitial tissue, as well as nuclear pkynosis and karyolysis in renal tubules was prominent in the kidney.

Keywords: *Ophiocephalus striatus*, Kidney, Cypermethrin, LC₅₀

1. Introduction

Pesticides are widely used in agriculture to prevent crop damage and thus protecting the crop yield and increasing world food supply. Indiscriminate use of various pesticides in agriculture has been increasing especially in developing countries (Santhakumar and Balaji, 2000). These pesticides through surface run off reach, fresh water bodies like ponds and rivers and affects the physico-chemical properties of water and in turn affecting aquatic organisms (Kamble and Muley 2000) ^[10]. Pesticides and related chemicals destroy the delicate balance between species and a functioning ecosystem (Khan and Francis, 2005) ^[11].

Use of various types of pesticides in agriculture has disturbed the ecological balance by killing many of the 'non-target' organisms like insect, crustaceans, zooplanktons and benthic organisms even at (non-lethal) concentration, which are integral part of food chain thus adversely affects the secondary and tertiary productivity of freshwater ecosystem. Toxic substances like pesticides come in contact with the fish through the gills and act very rapidly to enter the bloodstream of fish very directly altering the behaviour and physiology of fish.

Cypermethrin is a pyrethroid insecticide which is highly toxic to fish that interferes with the transmission of nerve impulses by inhibiting Ca⁺ and Mg-ATP_{ase} by blocking GABA receptors and sodium ion channels on neuronal membrane, leading to prolonged openings of these channels (Narahashi, 1996; Vijverberg and Vandenbercken, 1990) ^[20]. In fishes, these toxicants enter through the gills and alters the normal histology of the vital organs like liver, kidney, gonads, gills etc. (Camargo and Martinez, 2007) ^[4] that disturbs the normal physiology and weakens the fish to make it susceptible to various diseases. Thus, present investigation was done to assess the histological alterations in the kidney of fish *Ophiocephalus striatus* after exposed to sublethal dose (LC₅₀) of cypermethrin (25%EC) for 96 hours.

2. Materials and Methods

For experiment, *Ophiocephalus striatus* (average length 15 ± 1.5 cm, average weight 100 ± 14.75 g) were obtained

from the local fish market of Skandera, Nagpur. All the specimens were brought to the laboratory and length and weight of each specimen was measured with the help of electronic balance. Fishes were acclimatized for one week to the laboratory conditions in a rectangular glass aquarium of thirty litre capacity containing clear unchlorinated aerated well water. The water of aquaria was changed after every 24 hours followed by fresh introduction of LC₅₀ concentration of cypermethrin (25% EC). The freshly prepared stock solution was used during entire experiment. The Cypermethrin 25% (EC) insecticide Shoot supplied by Yawalkar Agro Industries Corporation Limited, Nagpur, was used for the present toxicity experiments. Stock solution is prepared by dissolving the pesticide in acetone. Acetone equal to the concentrations were mixed in the aquarium of the control fishes. During acclimatization fishes were fed twice a day in the morning and at evening with Red sea freeze-dried blood worms (containing $60 \pm 5\%$ crude protein, $8 \pm 5\%$ crude fat, $7 \pm 5\%$ crude fiber, $12 \pm 5\%$ Crude ash and $5 \pm 2\%$ moisture packed by- Insha Products, Mumbai- 16). In each aquarium 10 fishes were kept in 15 litres of water. The acclimatized fishes were not given feed a 24 hours prior to the start of experiment.

After 96 hours, control and cyprmethrin (25% EC) exposed fishes were randomly selected and removed from aquarium. Fishes were anaesthetised in the solution of paraaldehyde. Fishes were sacrificed and dissected to remove the kidney. Kidneys were cleaned in physiological saline solution (0.75% NaCl) and fixed in bouins fluid for 24 hrs for fixation. The fixed preserved tissues were transferred in 70% alcohol and were used for the block making. Dehydration was carried out with increasing grade of alcohol. Clearing of the tissue was done by using xylene. Cleared tissues were processed for infiltration in premelt paraffin wax at 62°C for about 1-2 hours. After infiltration embedding was done in paraffin wax. Tissues were cut at 5-6 microns thickness with the help of Leica Rotary Microtome. The ribbon of the sections was spreads on glass slides which is already coated by Mayer's albumin. The standard Haematoxyline-Eosine double staining technique method was followed for histological staining of kidney

(Humason, 1979) [6].

Physico-chemical characteristics of water

The present study was conducted in controlled physico-chemical characteristics of water. The physico-chemical parameters analysed were temperature, pH, dissolved oxygen (Wrinkler’s method), dissolved carbondioxide (Titration method), alkalinity (Methyl Orange titration method), hardness (EDTA titration method), Na⁺ (Flame photometry) and K⁺ (Flame photometry). The analysis of various physic-chemical parameters of water samples were carried out by following the standard methods (APHA,2005; Saxena, 1994) [1, 18]. Triplicates of each analysis were performed and mean values were used for calculation.

3. Results

Physico-chemical characterisation of water

Physico-chemical characteristics of water used in aquarium for experimental purpose for determining LC₅₀ in *Ophiocephalus striatus* was analyzed as per the standard methods (APHA, 1998) [1]. The water quality parameters like temperature (22 ± 1°C), pH (7.33), dissolved oxygen (6.4 mg Lit⁻¹), dissolved carbon dioxide (1.99 mg Lit⁻¹), total alkalinity (168 mg Lit⁻¹), total hardness (148 mg Lit⁻¹), Na⁺ (5 ppm) and K⁺ (1ppm).

Histological structure of Kidney in control *Ophiocephalus striatus*

In control fish, kidney shows normal histological structure. Kidney of control fishes consisted of head and body kidneys. Head kidney comprises of lymphoid tissue. Body kidney composed of glomerular nephrons and interstitial lymphoid tissues. Glomerular nephron consists of renal corpuscle and renal tubule. Renal corpuscle consists of highly vascular glomerulus enclosed in Bowman’s capsule (Figs. 1, 2, 3). Bowman’s capsule possesses outer parietal epithelium and inner visceral epithelium. The space in between these two epithelia called "Bowman's space" which separates glomerulus from rest of the kidney. Renal tubules are proximal and distal renal tubules. Renal tubules consisted of single layer of epithelial cells. Bowman’s capsule is in close vicinity of the renal tubules. Glomeruli and renal tubules are intact.

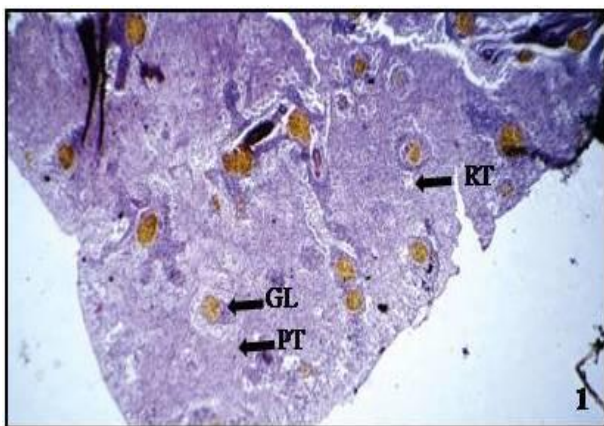


Fig 1: Section of kidney of control fish, *Ophiocephalus striatus* showing normal bowman's capsule, glomerulus and proximal and distal tubules (H & E × 100).

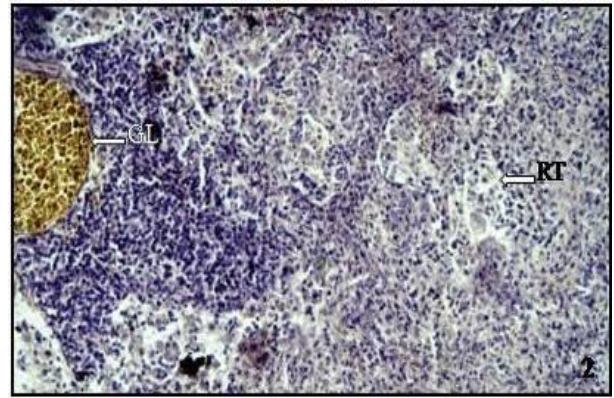


Fig 2: Section of kidney of control fish, *Ophiocephalus striatus* showing Bowman's capsule, Renal tubules and Interstitial tissue and Lymphoid tissue, (H & E × 400).

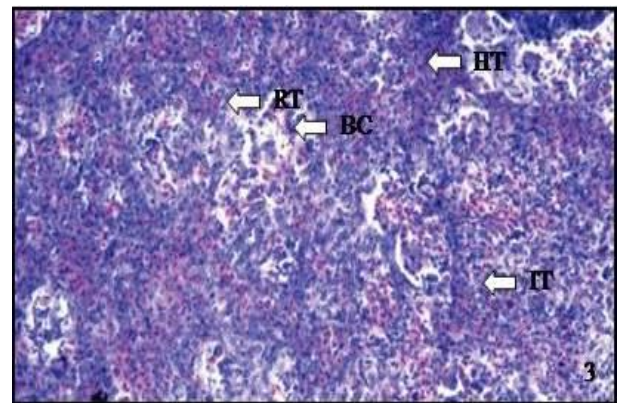


Fig 3: Section of kidney of control fish, *Ophiocephalus striatus* showing normal bowman's capsule, glomerulus and proximal and distal tubules (H & E × 100).

Abbreviations: GL- glomerulus, LC- lymphoid cells, IT- Interstitial tissue, BC- Bowman's capsule, BV- Blood vessel, DT- Distal tubule, PT- Proximal tubule, RT- Renal tubule, HT- Hemopoietic tissue

Histological Changes in Kidney of *Ophiocephalus striatus* exposed to Cypermethrin (25% EC)

Ophiocephalus striatus exposed to LC₅₀ dose of (0.72µl/lit) Cypermethrin for 96 hours shows significant changes in the histological structure of the kidney. Degenerative changes were observed in the renal corpuscle and renal tubules (Fig. 4). Glomerular shrinkage was observed which results in increase in space between glomerulus and Bowman's capsule (Fig. 5). Renal tubules shows the tubular degeneration and necrosis (Fig. 6). Nuclear pyknosis and karyolysis along with cytoplasmic degeneration in renal tubules occurred, resulting in the disorganized and disintegrated tubules (fat vacuoles). Loss of cellular integrity, connective tissue disorganization, degeneration of interstitial tissue, hypertrophy of nuclei of renal tubule was observed (Figs. 7, 8, 9). Due to loss of cellular integrity and degeneration, renal tubules of varying diameters were seen (Fig.10). Hydropic swelling is observed due to edematous fluid between renal tubules. Vacuolation in tubular epithelial cells of renal tubule and damage in the blood vessels was noted (Fig.11).

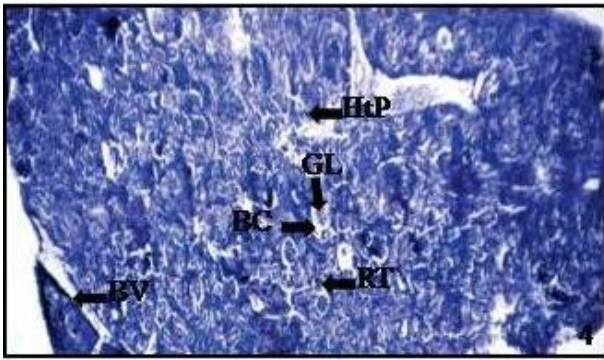


Fig 4: Section of kidney of fish, *Ophiocephalus striatus* exposed to cypermethrin (0.72µl/litre) showing vacuolation and disorganized tubules (fat vacuoles), damaged blood vessel, Glomerular shrinkage, increase in space between glomerulus and bowman's capsule. (H & E ×100).

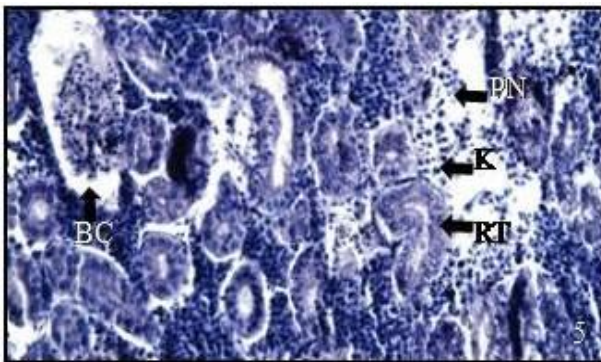


Fig 5: Section of kidney of fish, *Ophiocephalus striatus* exposed to cypermethrin (0.72µl/litre) showing (HS) Hydropic swelling, Renal tubular degeneration and necrosis. Vacuole formations in tubular epithelial cell.

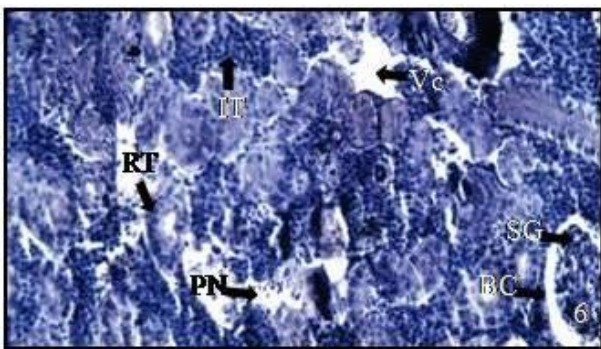


Fig 6: showing loss of integrity and interstitial tissue, vacuolization, shrunken glomeruli, nuclear pyknosis and karyolysis in renal tubules.

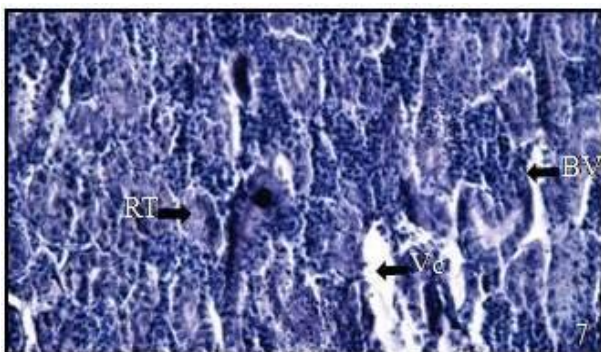


Fig 7: Showing loss of integrity and interstitial tissue, vacuolization and degenerating renal tubules.

Abbreviations: GL- Glomerulus, LC- lymphoid cells, IT- Interstitial tissue, BC- Bowman's capsule, BV- Blood vessel, DT-Distal tubule, PT- Proximal tubule, RT- Renal tubule, HtP- Heterogenous parenchyma, PN- Pyknotic Nuclei, Vc- Vacuolization, SG- Shrinked glomeruli.

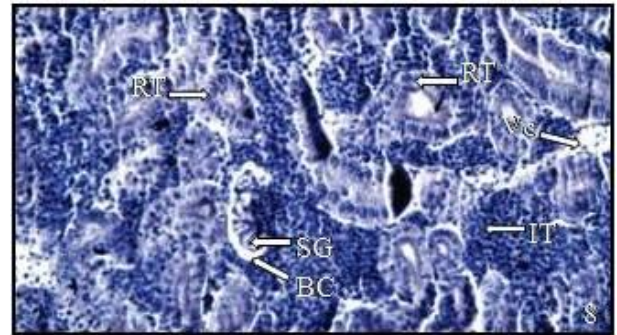


Fig 8: Showing shrinkage of glomeruli, Disintegrating renal tubule, Karyolysis and loss of cellular integrity.

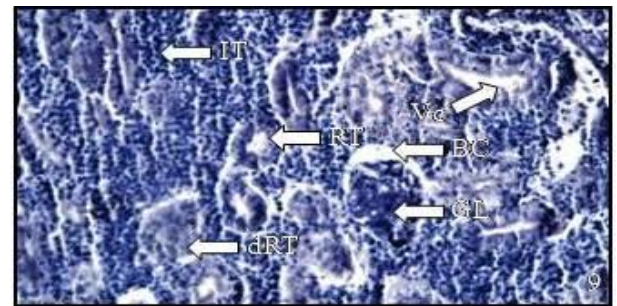


Fig 9: Showing complete disintegration of renal tubule, tubular hypertrophy, degeneration of interstitial tissue and vacuolization, irregular diameters and renal tubules.

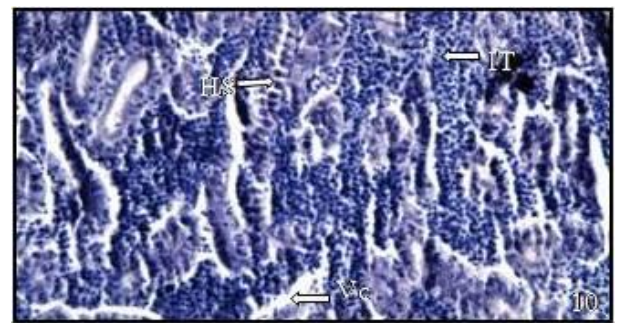


Fig 10: Showing degeneration of renal tubules and interstitial tissue, karyolysis in renal tubules, Hydropic swelling is clearly observed due to edematous fluid between renal tubules (H & E × 400).

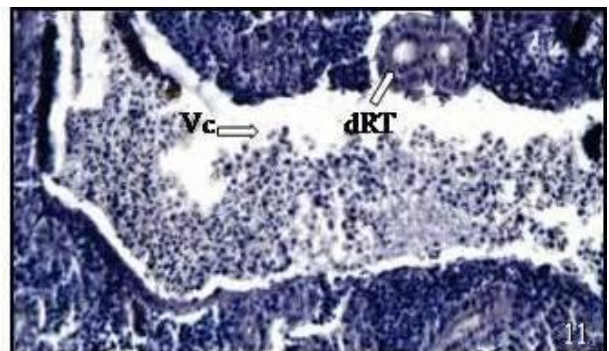


Fig 11. Showing vacuolization, degeneration of interstitial tissue and loss of cellular integrity and renal tubules.

Abbreviation: GL- Glomerulus, LC- lymphoid cells, IT- Interstitial tissue, BC- Bowman's capsule, BV- Blood vessel, DT-Distal tubule, PT- Proximal tubule, RT- Renal tubule, HtP- Heterogenous parenchyma, PN- Pyknotic Nuclei, Vc- Vacuolization, SG- Shrunk glomeruli, dRT- Disintegrating renal tubules, HS- Hydropic swelling

4. Discussion

Kidney is an important vital organ in fish which act as excretory organ and helps in maintaining osmotic balance. Head kidney is mainly involved in Erythropoiesis (Iqbal *et al.*, 2004) ^[9]. In control fishes, the histology of kidney reveals the normal Bowman's capsule, glomeruli and proximal and distal renal tubules. However, when *Ophiocephalus striatus* exposed to LC₅₀ dose of (0.72µl/lit) Cypermethrin for 96 hours result in the significant changes in the histology of the kidney. Degenerative changes were reported in the renal corpuscle and renal tubules. Vacuolation, karyolysis, pyknosis, necrosis, Hydropic swelling, cytoplasmic degeneration was noted in the renal tubules. Glomerular shrinkage was observed in Bowman's capsule. Similar observations have been reported in *Mystus tengara* when exposed to various sublethal doses of cypermethrin such as 0.026 ppm (20% of 96 h LC₅₀), 0.052 ppm (40% of 96 h LC₅₀) and 0.104 ppm (80% of 96 h LC₅₀) (Haque *et al.*, 2017) ^[5], in Silver barb (*Barbonymus gonionotus*) exposed to an organophosphorous Quinalphos 25 EC (Mostakim *et al.*, 2014), in Nile tilapia (*Oreochromis niloticus*) exposed to an organophosphorous Kinalux 25 EC (Nannu, 2014) ^[13], in *Heterobranchus bidorsalis* exposed to different doses of cypermethrin (Olufayo and Alade, 2012), *Cirrhinus mrigala* exposed to lethal (5.13 µg/l) and sublethal (1.026 µg/l) doses of cypermethrin (Prashanth, 2011), in the kidney of *Clarias gariepinus* exposed to diethyl phthalate (Ikele *et al.*, 2011) ^[7], in *Labeo rohita* exposed to organochlorine endosulfan (Indirabai *et al.*, 2010) ^[8], in *Channa punctatus* exposed to alachlor for 10 days (Butchiram *et al.*, 2009) ^[3], in African catfish (*Clarias gariepinus*) exposed to Cypermethrin (Ayoola and Ajani, 2008) ^[2]. in Mugil species *Cyprinus carpio* and *Barbus* species exposed to lindane (Ortiz, 2003) ^[16]. Shrinkage in the glomeruli and widening of the space between Bowman's capsule and glomerulus was also observed (Ortiz, 2003) ^[16]. Tilak *et al.* (2005) ^[19] had reported the decrease in size of distal convoluted tubules and vacuolations after exposure to chlorpyrifos in *Catla catla*.

5. Conclusion

The present investigation revealed that the pesticide Cypermethrin (25% EC) is toxic to aquatic organisms. It significantly causes the histological changes in the vital organs like kidney and thus alters the physiology and behaviour in *Ophiocephalus striatus*. Minimising the use of pesticides in the agriculture will control the indiscriminate killing of beneficial organism as well as the economically important fishes.

6. References

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