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Toxicity and Histopathological Effect of Cypermethrin on Juveniles of *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration between all authors. Author ROO conceived and designed the experiment. Author FPA managed the experiment and wrote the first draft. Author OOA also participated in managing the experiment and its analysis and wrote the final draft. Author RJK managed the experiment and analysis and wrote the first draft. Author RAO participated in the analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To study the toxicity and histopathological effects of Cypermethrin on the gill and liver tissues of the *Clarias gariepinus*.
Place of the Study: It was carried out at the toxicology section of the of Water Resources, Aquaculture and Fisheries Technology Department, School of Agriculture and Agricultural Technology of the Federal university, Minna, Niger State, Nigeria.
Methodology: The fish were exposed to six (6) acute concentrations (0.025mg/l, 0.050mg/l, 0.075mg/l, 0.100mg/l, 0.125mg/l and 0.000mg/l) for 96 hours. The histopathology of the gills and liver were determined and the LC_{50} was determined.
Results: The 96 hours LC_{50} of the toxicant to the test fish was 0.060mg/L. The most common gill changes at all doses of cypermethrin in solution were destruction of gill lamella, epithelial hyperplasia and epithelial hypertrophy. Hepatic lesions in the liver tissues of fish exposed to Cypermethrin in solution were characterized by degeneration of

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hepatocyte, vascuolization of cell cytoplasm, fatty degeneration and hypertrophy of hepatocytes. Histological comparison of tissues indicated that most damage occurred in the gill rather than in the liver.

Conclusion: Juveniles of *Clarias gariepinus* exposed to various concentration of Cypermethrin showed that this synthetic pyrethroid was highly toxic to the *Clarias gariepinus* fingerlings with lethal concentration (L_{C50}) of 0.060mg/l. Evidences of toxicity manifested significantly in the damages caused to the gills and liver studied. The severity of these damages to some selected organs in the body of the fish is proportional to the concentration of the pesticides.

Keywords: Histopathology; Clarias gariepinus; toxicity; cypermethrin; LC_{50}.

1. INTRODUCTION

Water pollution is any chemical, physical or biological changes in the quality of water that has a harmful effect on any living thing that drinks, uses or lives in [1]. However, there is overwhelming evidence that agricultural use of pesticides has a major impact on water quality and leads to serious environmental consequences. Appreciation of fisheries and aquatic systems has been accompanied by increasing concern about the effects of growing human populations and human activities on aquatic life and water quality. Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality [2]. Fish and other aquatic biota may be harmed by pesticides-contaminated water [3]. Pesticides surface runoffs into the rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream [4]. Application of herbicides to bodies of water can cause fish kills; when dead plants rot and use up the water's oxygen, it suffocates fish [3]. Some herbicides, such as Copper sulphite that are applied to waters to kill plants are toxic to fish and other aquatic animals at concentration similar to those used to kill plants [3].

The term pesticide is composite term that includes all chemicals that are used to kill or control pest. In agriculture, these include herbicides (weed), insecticides (insects), fungicides (fungi), nematocides (nematode), and rodenticides (vertebrate poison). While agricultural use of chemicals is restricted to a limited number of compounds, agriculture is one of the few activities where chemicals are intentionally released into the environment to eliminate agricultural pests. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species both on land and in water [5]. Pesticides contaminates land and water when its escape from production sites and storage tanks, when it runs off from field, when discarded, when sprayed aerially and when sprayed into water to kill algae [6]. Some pesticides contribute to global warming and depletion of the ozone layer [7].

Most pesticides related effect on fish goes unreported and, in documented cases, the number of fish killed is often underestimated. The usefulness of the pesticide has always masked its toxic effects on the aquatic environment [8,9]. They are also considered to be non-persistent. However, considering the toxicity and use of various pyrethroids as major insecticides in agricultural practices, special attention has to be paid to the effect of these pesticides on aquatic life such as fish, with more attention on commercially important species like *Clarias gariepinus* juveniles.

Cypermethrin is a synthetic pyrethroid used for the control of ectoparasites which infest cattle, sheep, poultry and some companion animals. Recently, the compound has been used as a chemotherapeutic agent for the control of ectoparasite infestations (*Lepeophtheirus salmonis* and *Caligus elongatus*) in marine cage culture of Atlantic salmon, *Salmo salar* [10,11,12,13,14].

Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds. The half-lives for elimination of several pyrethroids by rainbow trout are all longer than 48 h, while elimination half-lives for birds and mammals range from 6 to 12 h [15].

The present study aimed to determine the effect of acute toxicity and histopathological effect of Cypermethrin on juveniles of *Clarias gariepinus* in a static bioassay in order to establish the toxic effect of this pyrethroid.

2. MATERIALS AND METHODS

180 healthy juveniles of *Clarias gariepinus* of the same cohort with average weight (16.62±4.36)g, standard length (12.64±1.03) cm and total length (14.97±8.94) cm were sourced from the hatchery unit of the Federal college of Freshwater Fisheries Technology, Baga Maiduguri, Borno State. They were acclimatized for seven (7) days during which they were fed 5% of their body weight with commercial Coppens (2 mm). Feeding was stopped 24hrs prior to the commencement of the toxicity test experiment.

2.1 Toxicity Test

A preliminary range finding test was carried out based on the concentration of the active ingredient in the test chemical. The range finding was done using the following concentrations; 0.1mg/l,10mg/l and100mg/l of Cypermethrin for 24hrs in triplicates.

The result obtained from the range finding test provided a guide for the definitive test. Following this, the definitive test was carried out using; 0.025mg/l, 0.050mg/l, 0.075mg/l, 0.100mg/l, 0.125mg/l and 0.000mg/l of Cypermethrin. The result obtained was used to determine the median lethal concentration (LC_{50}) using Probit analysis.

2.2 Experimental Procedure

A total of eighteen (18) glass aquaria were used for the definitive toxicity test. Ten juveniles of *Clarias gariepinus* were introduced into each aquarium with 20 litres of water containing; 0.025mg/l, 0.050mg/l, 0.075mg/l, 0.100mg/l, 0.125mg/l and 0.000mg/l concentration of Cypermethrin at the same time. Each of the toxicant concentration was replicated three times. The experiment was carried out using a static non-renewal bioassay for 96 hours. Mortality and general behavior of fish were noted 24 hourly.

2.3 Histopathological Examination

The histology of the gills and liver of *Clarias gariepinus* was carried out after 96hours exposure period to the various concentration of the toxicant (Cypermethrin).

2.4 Tissue Processing

Organs of gills and liver were collected and fixed in 10% formal saline for one week. Then they were processed for routine paraffin histological sectioning. The tissues were dehydrated through graded concentration of ethanol (30%, 50%, 70%, and 90%), absolute ethanol and cleared in xylene. The tissues were pre-impregnated in xylene paraffin wax in the oven and embedded in pure paraffin wax. The organs were sectioned at 7µm thickness and tissues were stained with Haematoxylin and Eosin (H&E) for light microscopic examinations [16].

3. RESULTS AND DISCUSSION

3.1 Fish Behaviour and Mortality

During exposure, the test fish exhibited the following behavioural patterns before death occurred; restlessness, respiratory distress, loss of balance, gasping for air, vertical movement, excessive accumulation of mucus on the skin and death. The reaction to the toxicant was more pronounced in the aquaria containing the highest three (3) of 0.075mg/l, 0.1mg/l and 0.125mg/l concentrations of the toxicant. The mean cumulative mortality value, log 10 values of the concentrations, the total numbers of exposed fish and the probit kills' values are presented on Table 1. The observation made with control group were however different, they had stable swimming and no respiratory stress.

3.2 Lethal Concentration (LC_{50})

The result obtained from this research work showed that the 96 hour LC_{50} was determined to be 0.060mg/l by plotting the graph of probit kill values against log of concentration and finding the values of 50% probit kill as against the antilog value on log concentration value (Fig. 1).

The stressful behaviour exhibited by the fish may be attributed to the effects of the toxicant on the gill. This was clearly noted from the result of histopathological examination of the gill. The observed increase in erratic swimming, instability and subsequent immobilization before death which is directly proportional to the concentration of the toxicant could be attributed to the respiratory impairment of the gills filaments by the insecticide. This agrees with the reports of several authors who had reported work on similar patterns of abnormal behavioural responses in fish exposed to toxicant [17,18]. Previous histopathological studies of fish exposed to pollutant have shown that fish gills are efficient indicators of water quality. Fish gills are vulnerable to pollutants in water because of their large surface area and external location. However, the gills perform numerous functions, which include respiration, excretion of nitrogenous waste products and acid-base balance.

Table 4.1. Mortality rate, percentage mortality and Probit value of *Clarias gariepinus* juveniles exposed to acute dose of Cypermethrin for 96 hour in a static non-renewal bioassay

Conc. Mg/l	Log_{10} of conc.	Total numb. of exposed fish	Numb. of dead fish	% mortality	Probit kill value
0.000	0.00	10	0.0	0.0	00000
0.025	- 1.60	10	1.3	13	3.8736
0.050	- 1.30	10	2.7	27	4.3872
0.075	- 1.12	10	3.3	33	4.5603
0.100	- 1.00	10	5.0	50	5.0000
0.125	- 0.90	10	7.3	73	5.6128

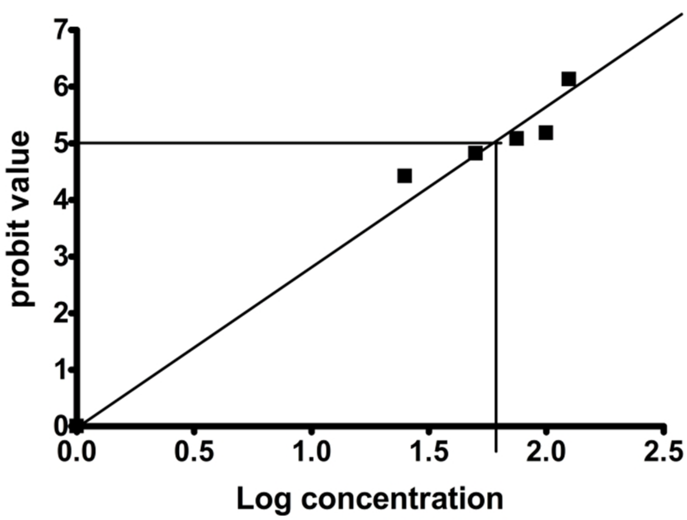

Fig. 1. 96 hour LC_{50} of *Clarias gariepinus* exposed to different concentration of cypermethrin in static system

3.3 Liver Tissue

The liver tissue (plate I) of fish in the control set up showed a normal hepatocyte radiating from the central vein and sinusoids. It exhibited the typical parenchymatous appearance. The histomorphology of liver specimens exposed to cypermethrin insectides showed a significant variation from the control group. Liver specimens (plate II) exposed to 0.025mg/l of cypermethrin shows a wide spread sinusoids haemorrhage, mild area of congestion, necrosis and vacuolation. A focal area of mononuclear cell infiltration was also evident. Specimen of liver exposed to 0.05mg/l (plate III) of cypermethrin showed an enlarged vascular channel of congestion, cloudy swelling of hepatocyte and a focal area of inflammatory cells. Liver of fish exposed to 0.075mg/l (plate IV) showed a wide spread of fatty tissue degeneration thus, leading to a fatty tissue, cloudy swelling of hepatocyte with a severe thick fibrous connective tissue observed in the liver of fish exposed to 0.1mg/l (plate V) of Cypermethrin. Plate VI indicates inflammatory cells, vacuolation enlarge fatty tissue.

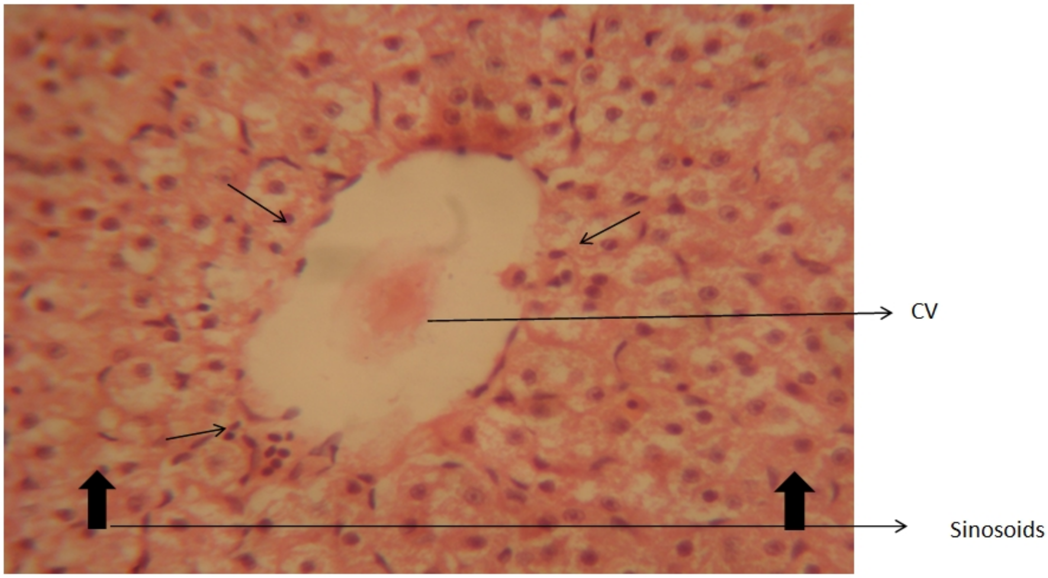

Plate I. Photomicrograph of fish liver of control showing normal hepatocytes (arrows), radiating away from central vein (CV) and sinusoids (arrow head) H&E x400

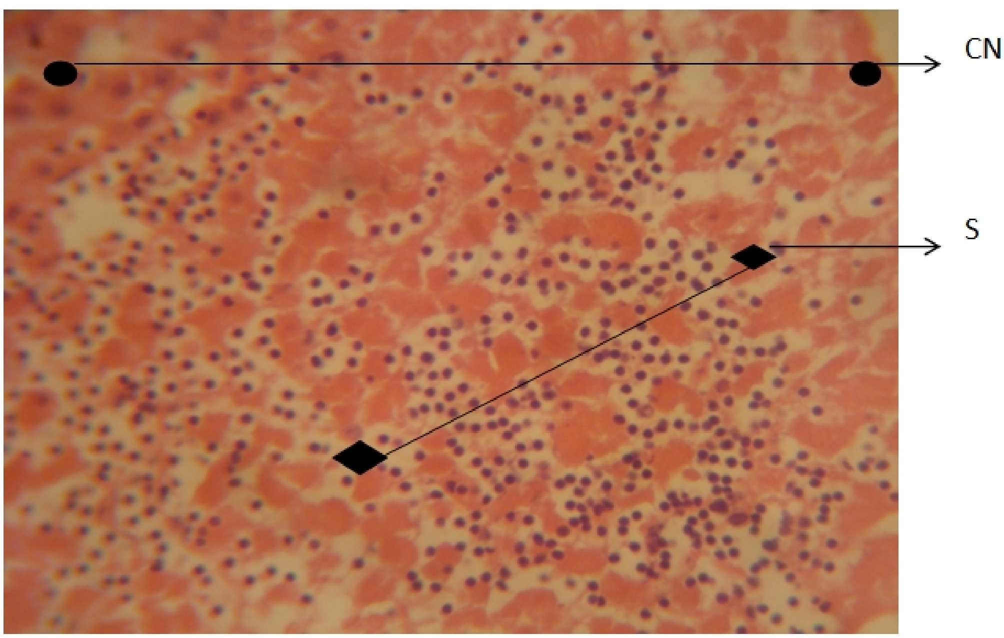

Plate II. Photomicrograph of fish liver treated with 0.025mg/L of Cypermethrin showing wide spread sinusoidal haemorrhages (S) and mild areas of coagulative necrosis (CN) H&E x400.

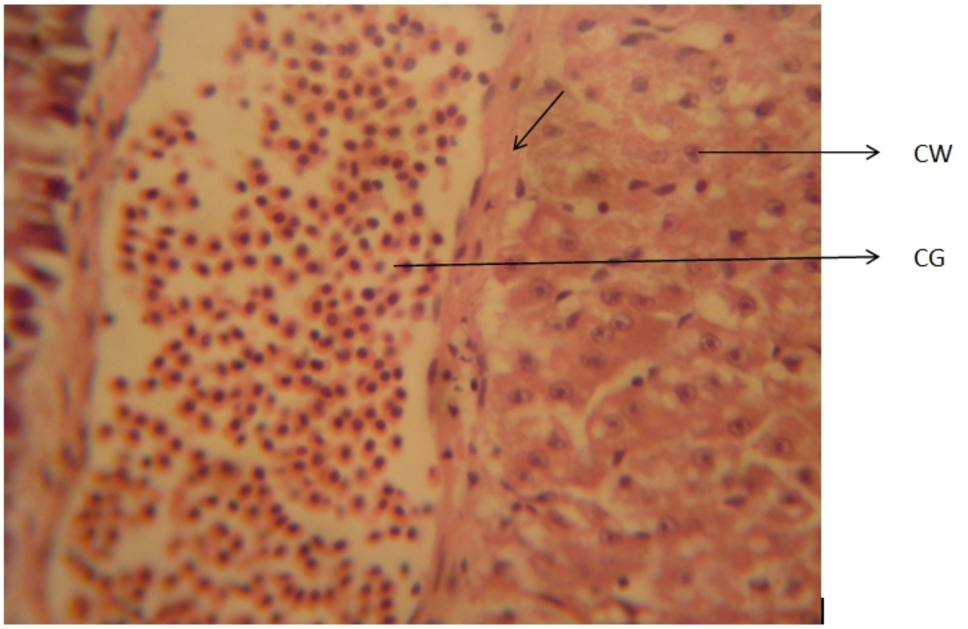

Plate III. Photomicrograph of fish liver treated with 0.050mg/L of Cypermethrin showing enlarged vascular channel (arrow), congestion (CG) and cloudy swelling hepatocytes (CW) H&E x400.

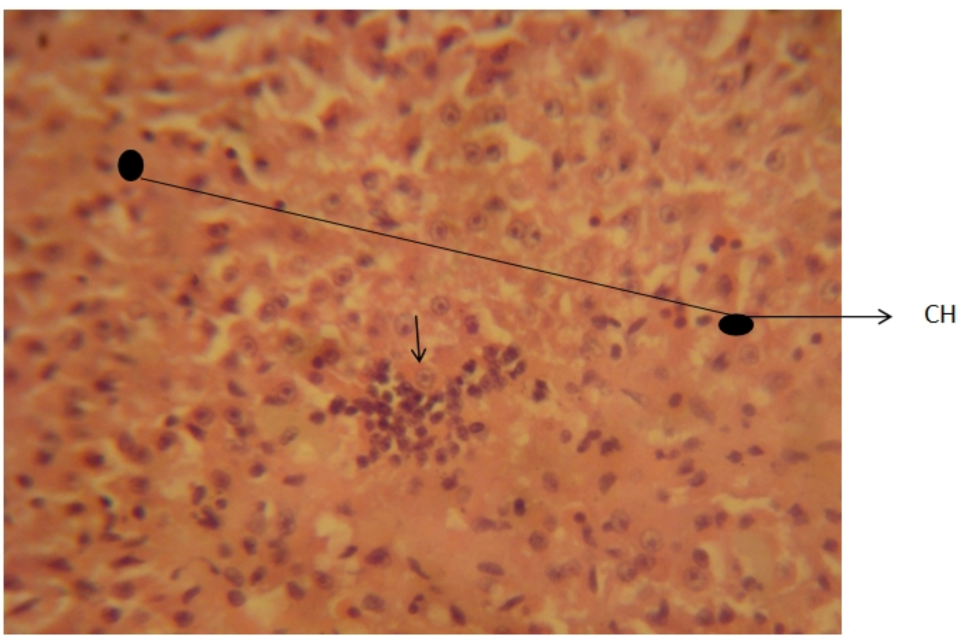

Plate IV. Photomicrograph of fish liver treated with 0.075mg/L of Cypermethrin showing wide spread cloudy swelling of hepatocytes (CH) and focal area of inflammatory cells (arrow) H&E x400

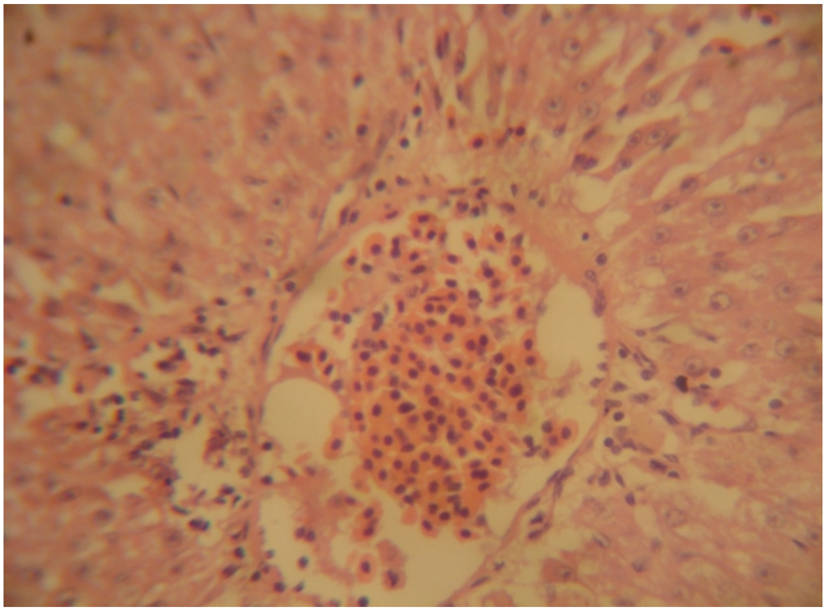

Plate V. Photomicrograph of fish liver treated with 0.10mg/L of Cypermetrin showing enlarged vascular channel (arrow), congestion (CG) and cloudy swelling (CW) H&E x400

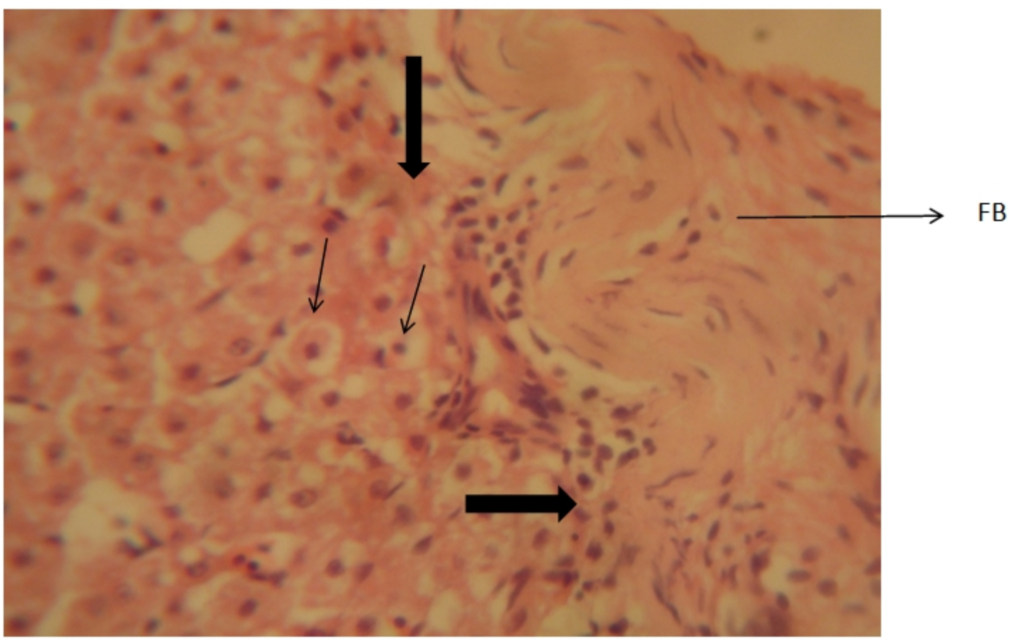

Plate VI. Photomicrograph of fish liver treated with 0.125mg/L of Cypermethrin showing thick fibrous connective tissue (FB), inflammatory cells (thick arrows) and vacuolation (arrows) H&E x400

3.4 Gill Tissue

The degree of damages and variation noticed are shown in photomicrograph. Photomicrograph of fish gill treated with 0.025mg/l of cypermethrin shows gill necrosis, congestion, cartilage and interstitial haemorrhages (Plate VII). This was more evident in higher concentration of the insecticide. Gills exposed to0.05mg/l showed a multi focal area of interstitial haemorrhages. The gills exhibits a marked alteration in their epithelia (plate VIII). Plate VIII further shows a moderate gill necrosis with multi focal haemorrhages. Plate IX showed the gills of fish treated with 0.075mg/l of cypermethrin with severe gill necrosis. (Plate X) also showed gills of fish exposed to 0.100mg/l of cypermethrin with extensive vacuolation within the epithelia of the gills. Infiltration, cartilage and interstitial haemorrhages were variation noticed in the gills of fish treated with 0.125mg/l of cypermethrin (Plate X). The gill arches of *Clarias gariepinus* in the control fish showed normal arrangement pattern. The arches contain primary lamella (Plate XI) projecting on the lateral sides of the primary and secondary lamella (respiratory lamella). This ultimately showed a normal gill without any alteration or damages.

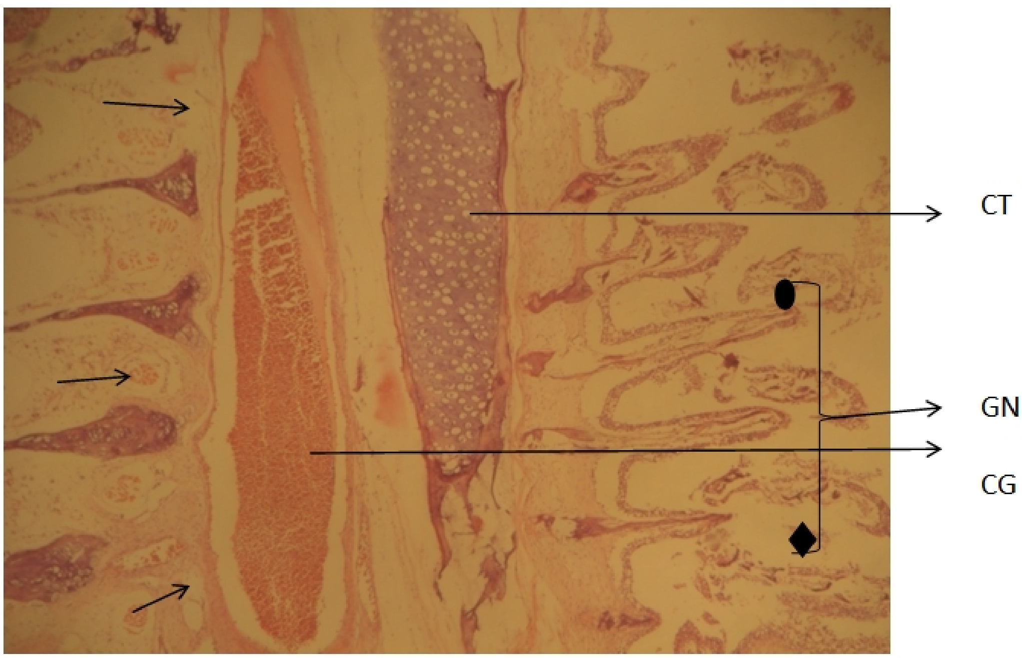

Plate VII. Photomicrograph of fish gills treated with 0.025mg/L of Cypermethrin showing gill necrosis (GN), congestion (CG), cartilage (CT) and interstitial haemorrhage (arrows) H&E x40

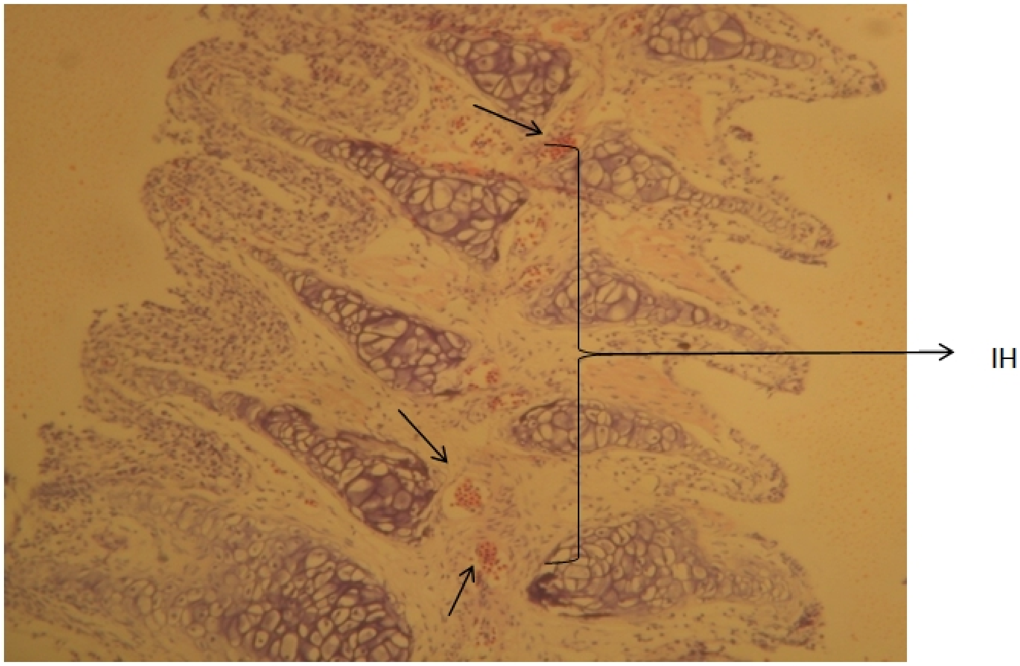

Plate VIII. Photomicrograph of fish gills treated with 0.050mg/L of Cypermethrin showing (IH) interstitial haemorrhage (arrows) H&E x400

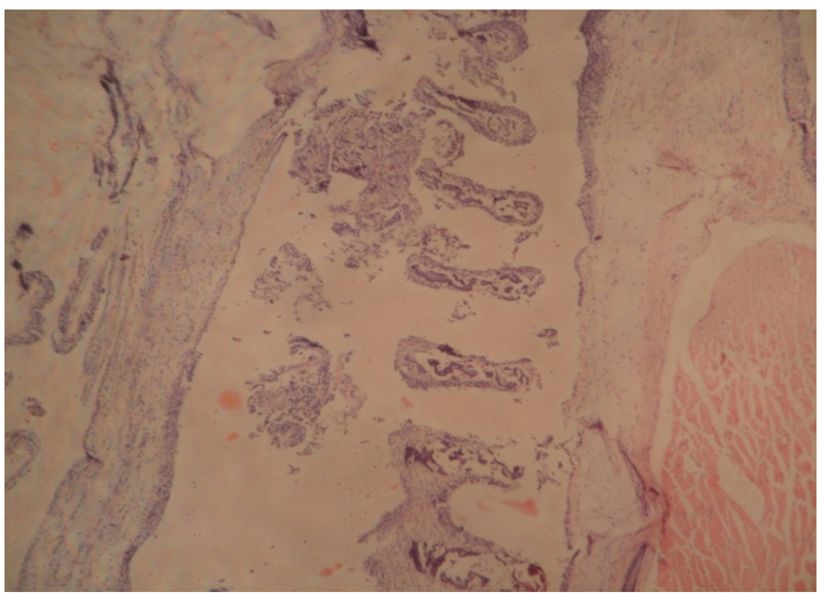

Plate IX. Photomicrograph of fish gills treated with 0.075mg/L of Cypermethrin showing severe gill necrosis H&E x400

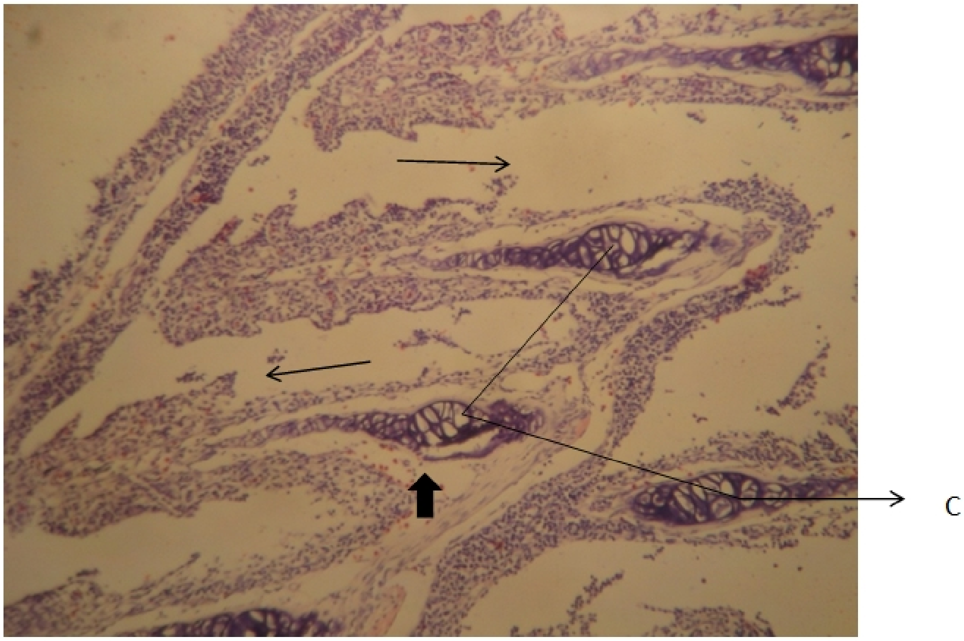

Plate X. Photomicrograph of fish gill treated with 0.100mg/L of Cypermethrin showing necrosis of the gill filament (thin arrows), interstitial haemorrhage (thick arrows) cartilage (C) H&E x400

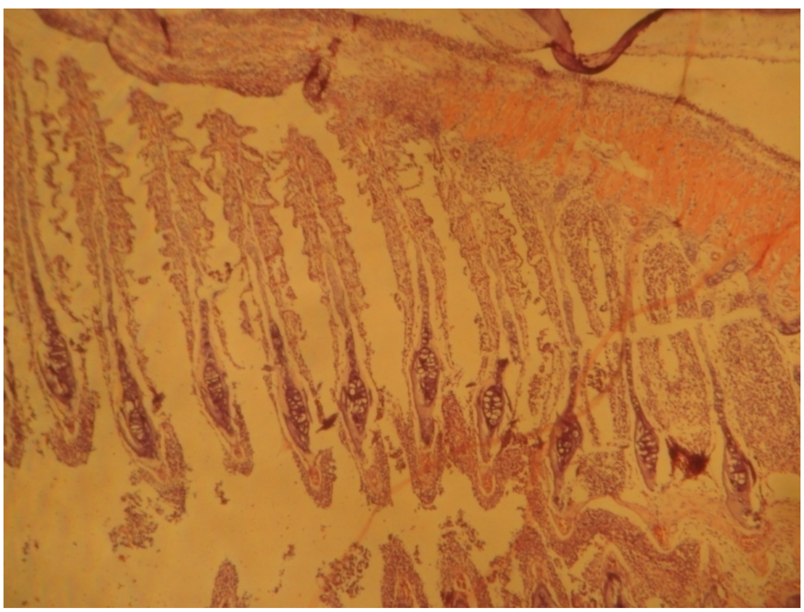

Plate XI. Photomicrograph of control fish gills showing normal gills H&E x400

4. DISCUSSION

Clarias gariepinus juveniles were stressed progressively with time before death. The stressful behaviour of respiratory impairment due to the toxic effect of cypermethrin on the gills was similar with the reports of [19] and [20] that pesticide impairs respiratory organs. Death could therefore have occurred either by direct poisoning or indirectly by making the medium unconducive or even by both, whichever is the case, the source of death was cypermethrin. Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercula activities, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of [21,22,20]. The stressful and erratic behaviour of Clarias gariepinus juvenile in the experiment indicates respiratory impairment; probably due to the effect of the toxicant cypermethrin on the gills. The fishes became inactive at higher concentrations with increased time of exposure to toxicant.

The result of the light microscopic studies showed that the morphologic changes were more evidence in the liver of exposed fish as changes were not observed in the control fish. The liver of the exposed fish compared to the control showed varying degree of degeneration of cells, which include hypertrophy of hepatocytes, Fatty degeneration, vascular channel congestion and vascuolization of cell cytoplasm; these alterations were dose-dependent. [23] observed similar histological alterations when varied concentrations of thiodanR were applied to mosquito fish (*Gambusia affinis*). [24] reported congestion, cloudy swelling of hepatocyte and focal necrosis in liver of *Cirrhinus mrigala* exposed to fenvalerate. Similar result was reported by [25], when Oreochromis niloticus was exposed to solution of Portland cement. Changes such as hyperplasia, disintegration of hepatic mass and focal coagulative necrosis were found in Labeo rohita exposed to cypermethrin [26]. Liver is the detoxification place of toxicants. The hepatic changes suggested mobilization of same kind of defensive mechanism in an endeavour to detoxify the toxicant-cypermethrin.

5. CONCLUSION

Juveniles of *Clarias gariepinus* exposed to various concentration of Cypermethrin showed that this synthetic pyrethroid was highly toxic to the *Clarias gariepinus* fingerlings with lethal concentration (L_{C50}) of 0.060mg/l. Evidences of toxicity manifested significantly in the damages caused to the gills and liver studied. The severity of these damages to some selected organs in the body of the fish is proportional to the concentration of the pesticides.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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