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Full Length Research Paper

Histological changes in liver, gills and kidney of catfish (*Heterobranchus bidorsalis) e*xposed to *c*ypermethrin concentration

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Fingerlings of *Heterobranchus bidorsalis* (mean weight 11.88 g) were exposed to various sub-lethal concentrations of cypermethrin (0.032, 0.034, 0.036, 0.038 and 0.040 ml/L), a toxicant, and the histological effects were recorded. The 96 h bioassays were also conducted to determine the lethal concentration (LC_{50}) of cypermethrin on the test fish. The 96 h LC_{50} of *H. bidorsalis* exposed to Cypermethrin was 0.036 ml/L. The physical reactions observed in the fish were: discolorations of the skin, erratic swimming, loss of reflex, hyperactivities, surfacing, and these effects increased with increasing concentration of the toxicants and duration of exposure. The pH and dissolved oxygen of the test media showed slight decrease from lowest concentration (0.032 ml/L) to the highest concentration (0.040 ml/L) while the temperature increased slightly with increasing concentration (0.032, 0.034, 0.036, 0.038 and 0.040 ml/L). The histological examination of the gills, kidney, livers of the fish after 96 h showed pathological changes and alterations such as gills infiltration, inflammation of the livers, vacuolation and necrosis. There was excessive necrosis degeneration in higher concentrations (0.038 and 0.040 ml/L) and there was increase in mortality as the concentrations increased.

Key words: Histology, cypermethrin, xenobiotic, *Heterobranchus bidorsalis,* acute toxicity, LC_{50}.

INTRODUCTION

An indiscriminate use of pesticides in agriculture, animal husbandry, post-harvest technology is a treat to the natural water system, public health and welfare of mankind (Tilak et al., 2007). Pesticides applied directly to the soil are carried away by rains and floods as runoff to the water bodies and this alters the physico-chemical properties of water (Richardson, 1988).

Exposure of organisms to xenobiotics such pesticides, insecticides, herbicides and other synthetic materials is a serious matter in environmental and toxicological chemistry. Cypermethrin is highly toxic to fish and aquatic invertebrates (Asztalos et al., 1990), in an ecosystem, intricate relationships exist between the organisms and their surroundings, exposure of an ecosystem to such toxicant may result in loss of species diversity, which is an important characteristic of healthy ecosystems. Synthetic pyrethroid insecticides are extensively used in place of organ chlorine, organ phosphorus insecticides and carbonates to control various types of pests and increase agricultural production (Wengatz et al., 1996).

These chemicals are potentially more toxic to fish and other aquatic organisms, and are least toxic to mammals. Owing to the excessive use of synthetic pyrethroids, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly (Hill, 1989).

Fishes exposed to toxicants undergo stress, which is a state of re-established homeostasis, a complex suite of mal-adaptive responses (Chrouses, 1998). Under stress,

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Table 1. Behavioral responses of *H. bidorsalis* during 96 h exposure to cypermethrin.

Behavior	Concentration (ml/L)					
	Control	0.032	0.034	0.036	0.038	0.040
Erratic swimming	-	+	+	+	+	+
Loss of Reflex	-	+	+	+	+	+
Hyperventilation	-	+	+	+	+	+
Motionless State	-	+	+	+	+	+
Discoloration	-	+	+	+	+	+

- absent + present.

physiological and biochemical responses may be compromised, becoming detrimental to the fish's health and well being at which point the fish is termed distressed (Barton and Iwama, 1991). Fishes in a contaminated environment show some altered behavioral patterns which may include avoidance, locomotive activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition (Gormley and Teather, 2003; Morgan et al., 1991). Xenobiotics generally contaminate freshwater bodies, various researchers have reported on the effects of different pesticides on aquatic organisms. Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity are influenced by the rate of pollutants entering the water or lethal effects on the aquatic organisms (Fagbenro, 2002; Olufayo, 2009). Therefore, there is a justifiable need to establish reference histological and toxicity values of *H. bidorsalis* juveniles exposed to xenobiotic (*cypermethrin)*.

In the present study, an attempt was made to examine the sub-lethal toxic effects of different concentrations of *cypermethrin* on the gills, liver, kidney of the test fish and as well determine the 96 h LC_{50} of the *Heterobranchus bidorsalis* exposed to the toxicant.

MATERIALS AND METHODS

180 healthy fish of catfish fingerlings (*H. bidorsalis)* of mean weight 11.88 g were purchased from a fish farm at Akure, and transported in un-aerated plastic containers to Fisheries and Aquaculture Technology Laboratory of the Federal University of Technology, Akure. The fishes were acclimatized for 48 h in plastic tanks prior to the toxicity test. The fish were not fed prior to the test in order to minimize the production of waste thereby reducing ammonia production from the wastes.

Cypermethrin (Cypercot agricultural insecticides) with BATCH NO-ISA-286 NAFDAC was purchased from an agro-chemical shop in Akure, Ondo State, Registration number: 04-8709. This was used as toxicant on the experimental fish.

A static bioassay (APHA, 1995) was done after carrying out a range finding test to obtain five graded concentrations (Ayotunde and Ofem, 2008) of *Cypermethrin.* 0.02 ml of *Cypermethrin* was diluted into 1000 ml of distilled water, five varying concentrations of Cypermethrin (0.032, 0.034, 0.036, 0.038 and 0.040 ml/L) representing ten treatments and a control containing no extracts were used on test fish *H. bidorsalis* fingerlings. These varying concentrations were prepared arithmetically. Ten fish were introduced into each aquarium with varying concentrations of *Cypermethrin per* 10 L of water which had been allowed to mix properly for about 30 min (Usman et al., 2005). The responses of the fish to slight stimuli was used as an index for toxicity or death. LC_{50} of *Cypermethrin* on the test fish was determined using probit analysis.

The pH, dissolved oxygen concentration, temperature, salinity, turbidity, conductivity tests were conducted during the experiment using a digital JENWAY 3150 Dual-purpose meter by inserting the electrode into the sample bottle containing sample water. Water quality monitoring was done prior to the experiment, during the experiment and after the experiment.

After the 96 h experiment, gills, liver and kidney specimens were exercised from fish alive and were preserved in 10% formalin and processed for histological examination using standard histological techniques (Avwioro, 2002). All data obtained in both tests were analyzed using probit method and the graphical method (Finney, 1971) and multiple range test using Statistical Package for Social Sciences (SPSS version 14.0).

RESULTS AND DISCUSSION

Fish were observed to show erratic swimming, loss of reflex, discoloration, hyperventilation, changes in behavior and increasing opercula ventilation and movement. As the duration of the experiment increased the test fish showed increase in weakness, motionless and gasp for air with slow opercula movement (Table 1). The 96 h LC_{50} was value was observed to be was 0.036 ml/L (Figure 1) with the lowest and highest mortality in 0.032 and 0.040 ml respectively. The histological alteration of the gills, liver and kidney were observed and this was more pronounced in higher concentrations (0.038 and 0.040 ml/L) and exposure time. The gills showed lamella hypertrophy, some hyperplasia at the base of the secondary lamellae and desquamation of the epithelial lining and telangiectasia of the secondary lamellae at lower concentrations – 0.032 and 0.034 ml/L (Figure 2- Plates 1 and 2). The toxicity rate of each organism increased with increase in the concentration. Similar findings were reported by Edwards et al. (1986) that rainbow trout exposed to 10 µg/L cypermethrin exhibited toxin signs of gill failing and hyperactivity,

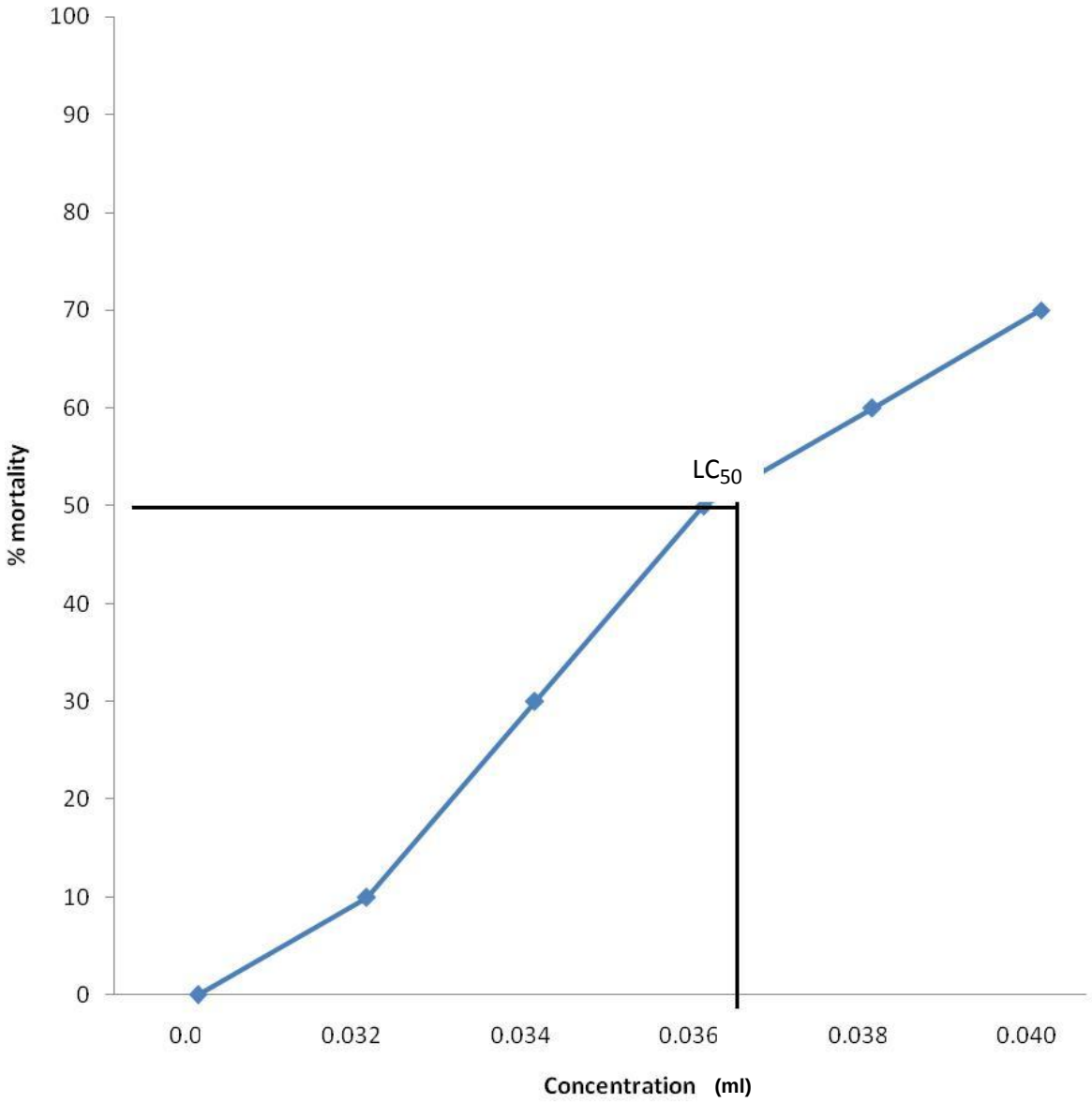

Figure 1. 96 h LC_{50} of *H. bidorsalis* fingerlings exposed to different concentrations of cypermethrin.

followed by loss of buoyancy and trim control while Koprucu et al. (2006) also reported that European catfish (*Silurus glanis*) exposed to acute concentrations of deltamethrin higher than 0.50 µg/L showed abnormal behavior such as loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming, swimming at the water surface, air gulping and lighting in color of fingerlings. Changes in behavioral patterns exhibited by fish were possibly to counteract aquatic hypoxia condition possibly caused by the agrochemical (Kind et al., 2002). When there is impossibility of escape from hypoxic stress, physiological alterations may be evoked to compensate for low oxygen supply (Graham, 2003) Observations of mortality rate of *H. bidorsalis* were made at 24, 48, 72 and 96 h. It also shows difference in mortality rate at different concentrations, Sub – lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. They have significant effects which can result in several physiological changes in the fish (Olufayo, 2009).

Histological examination of *H. bidorsalis* gave significant indication of toxicity of Cypermethrin (Figure 2-Plates 1 to 18). The effects include gill alteration such as desquamation of the epithelial lining, telangiectasia, haemoragic and hyperplasia at the secondary lamellae (Figure 2-Plates 2, 3, 4, 5 and 6) while there was no pathological lesion in the control experiment (Figure 2-

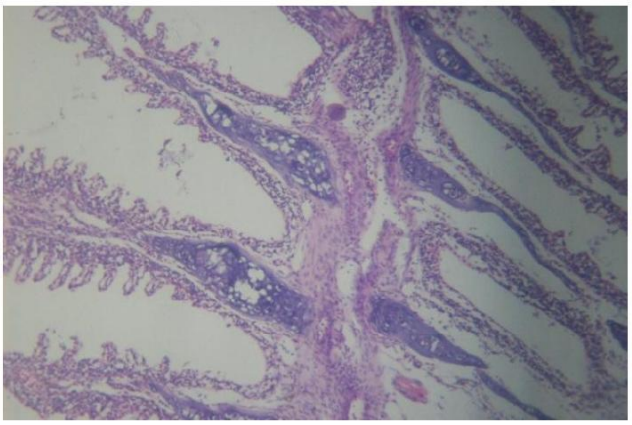

Plate l : Gills of *H. bidorsalis* in the control tank shows no pathological lesion.×400

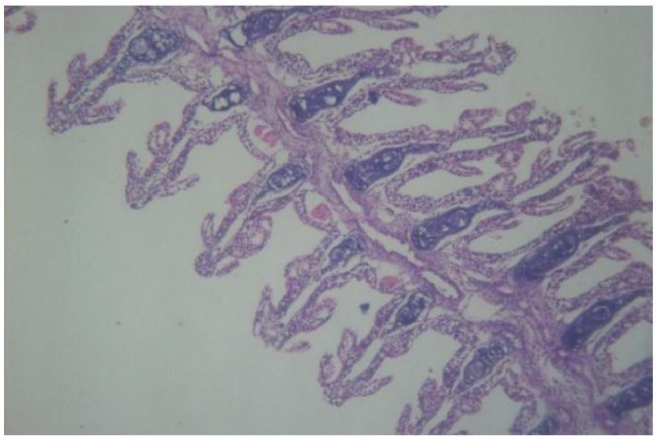

Plate 2: Gills of *H. bidorsalis* exposed to 0.032 ml of Cypermethrin shows desquamation of the epithelial lining and telangiectasia of the secondary lamellae. .×400

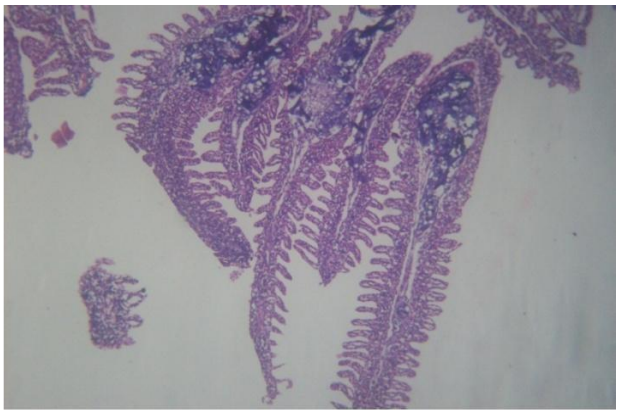

Plate 3: Glls of *H. bidorsalis* exposed to 0.034 ml of Cypermethrin shows deaquamation of the epithelial lining of secondary lamellae . ×400

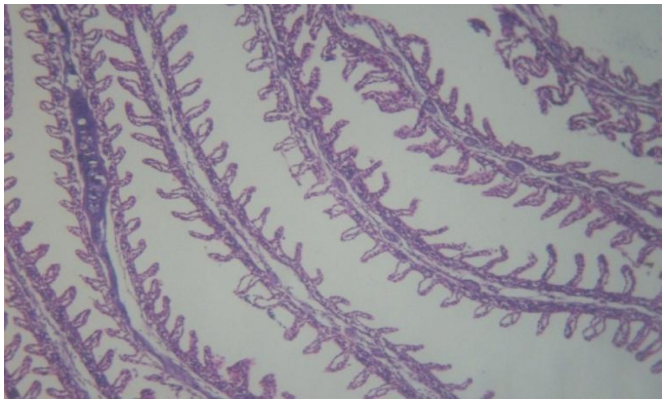

Plate 4: Gills of *H. bidorsalis* exposed to 0.036 ml of Cypermethrin shows haemoragic of the secondary lamellae .×400

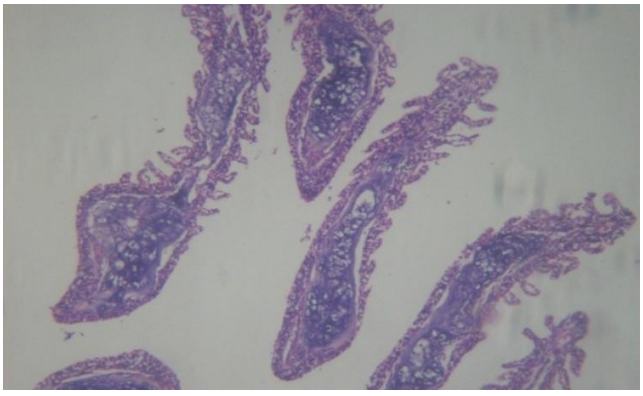

Plate 5: Gills of *H. bidorsalis* exposed to 0.038 ml of Cypermethrin shows telangiectatic of secondary lamellae of the gill showing lamellar hypertrophy and some hyperplasia at the base of the secondary lamellae. ×400

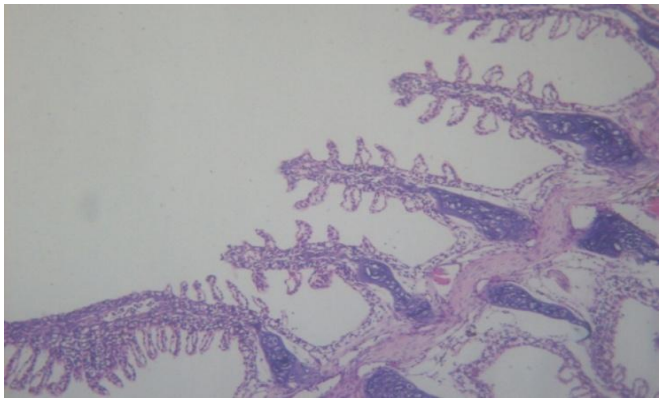

Plate 6: Gills of *H. bidorsalis* exposed to 0.040 ml of Cypermethrin shows desquamation and hyperplasia at the secondary lamellae. ×400

Figuer 2. Histology of *H. bidorsalis* exposed to different concentrations of Cypermethrin and susceptibility of fish.

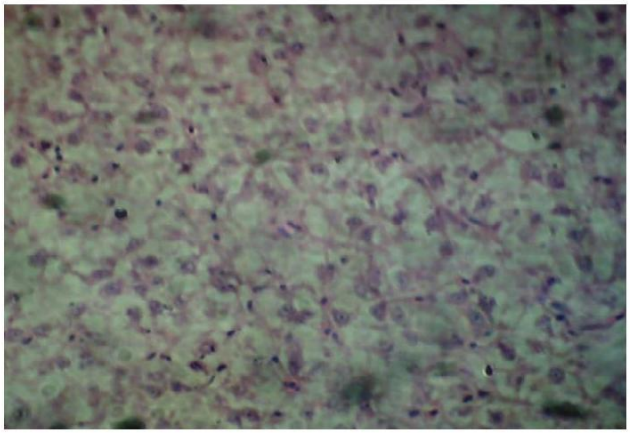

Plate 7::Liver of *H. bidorsalis* in the control tank shows no pathological lesion. ×400

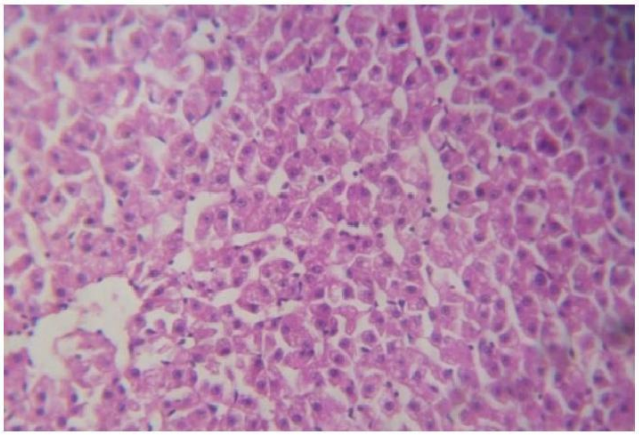

Plate 8: Liver of *H. bidorsalis* exposed to 0.032 mlof Cypermethrin shows Inflammation of the liver cells. ×400

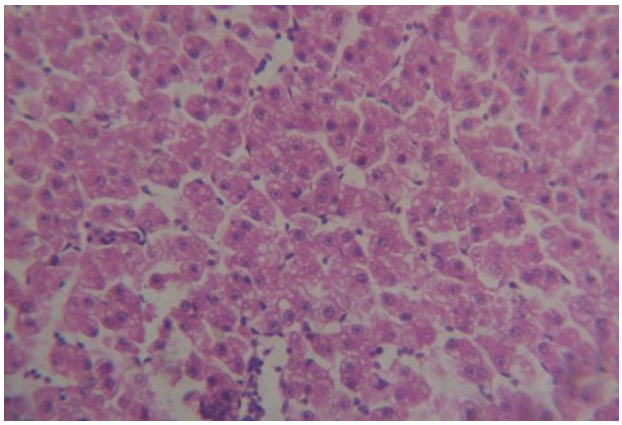

Plate 9: Liver of *H. bidorsalis* exposed to 0.034 ml of Cypermethrin shows mild diffuse vacuolation of hepatocytes . ×400

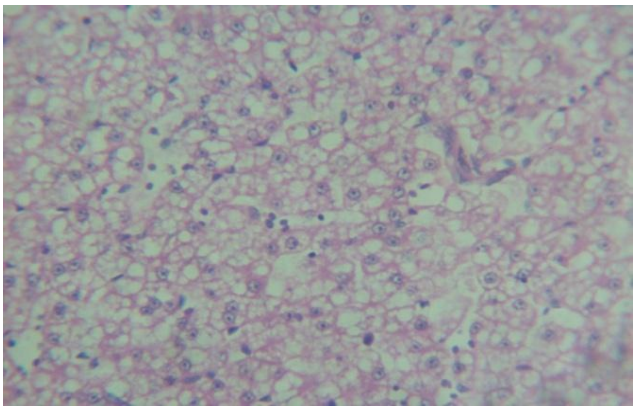

Plate 10: Liver of *H. bidorsalis* exposed to 0.036 ml of Cypermethrin shows very severe diffuse vacuolation of hepatocytes. ×400

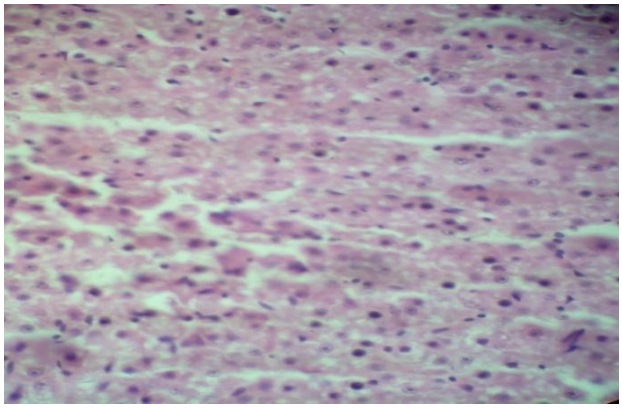

Plate 11: Liver of *H. bidorsalis* exposed to 0.038ml of Cypermethrin shows Necrosis of the hepatic cells rupture of sinusoids with hemorrhages at several points. ×400

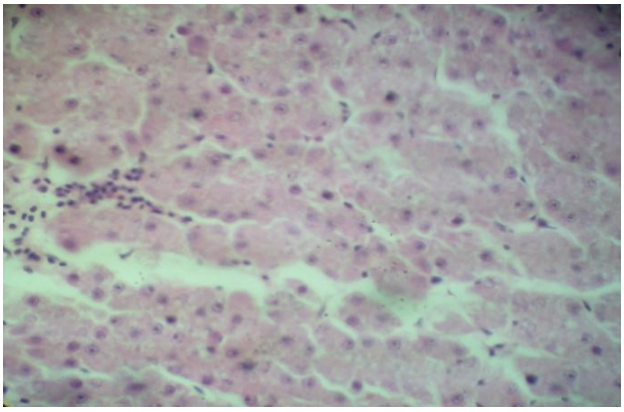

Plate 12: Liver of *H. bidorsalis* exposed to 0.040ml of Cypermethrin shows fatty infiltration and vacuole formation . ×400

Figuer 2. Contd.

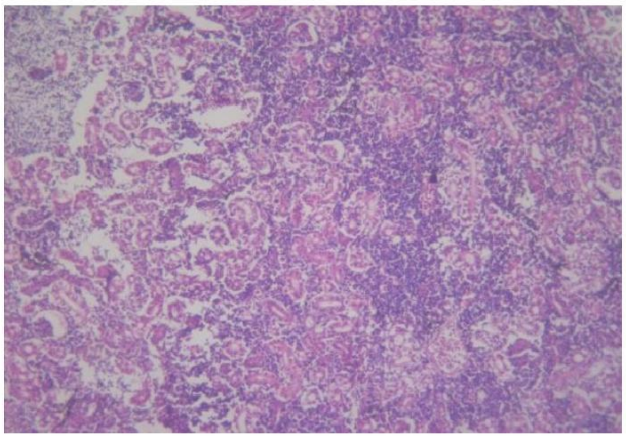

Plate 13: Kidney of *H. bidorsalis* in the control tank shows no pathological lesion. ×400

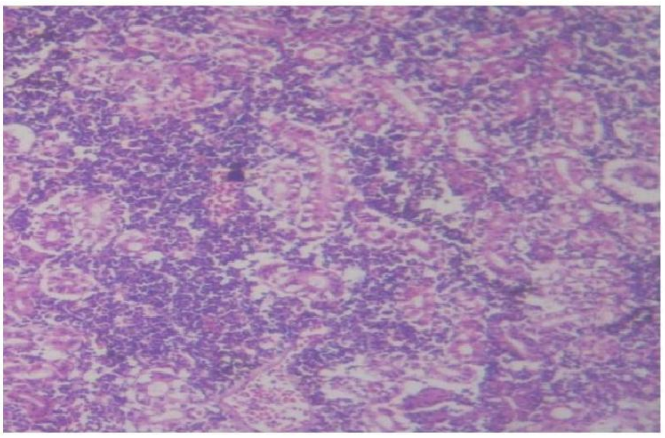

Plate 14: Kidney of *H. bidorsalis* exposed to 0.032 ml of Cypermethrin shows Karyolysis of nucleic material . ×400

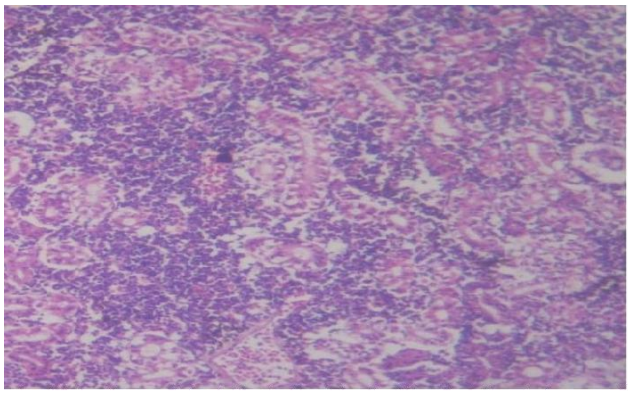

Plate 15: Kidney of *H. bidorsalis* exposed to 0.034ml of Cypermethrin shows karyolysis of nucleic material. ×400

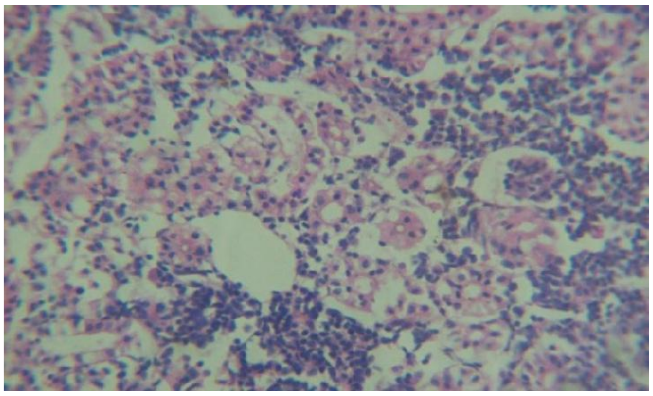

Plate 16: Kidney of *H. bidorsalis* exposed to 0.036 ml .of Cypermethrin shows vacuole formation of the tubular epithelial cells. ×400

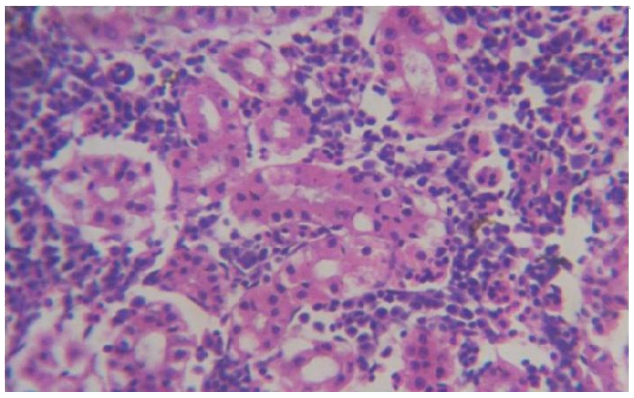

Plate 17: Kidney of *H. bidorsalis* exposed to 0.038 ml of Cypermethrin shows Evidence of tubular necrosis shown, glomerulus was also with blood stain. ×400

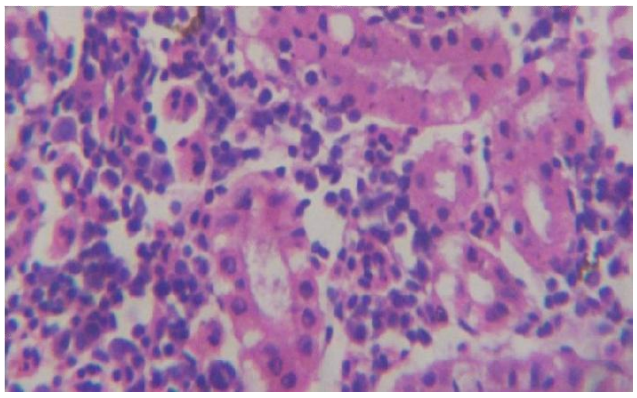

Plate 18: Kidney of *H. bidorsalis* exposed to 0.040 ml of Cypermethrin shows that glomerulus is observed containing blood stain , tubular necrosis is equally visible. The renal tubules were dilated. ×400

Figuer 2. Contd.

Plate 1). In higher concentration 0.036 ml, diffused vacuolation of hepatocytes in the liver was observed (Figure 2-plate 10). *H. bidorsalis* exposed to different concentrations of cypermethrin (0.038 and 0.040 ml/L) showed necrosis of the hepatic cells rupture of sinusoids with hemorrhages at several points (Figure 2-Plates 11 and 12). Some changes such as karyolysis of nucleic material, vacuole formation of the tubular epithelial cells were also observed (Figure 2-Plates 14.15 and16). Oulmi et al. (1995) studied the effects of linuron herbicide on the rainbow trout (*Oncorhynchus mykiss*) and stated that small cytoplasmic vacuoles, nuclear deformation in the epithelium of the first and second segments of the proximal tubule were observed. The kidney cells were observed to have been massively destroyed (Figure 2-Plates 17 and 18). The renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions, these findings agreed with Olufayo (2009).

The liver of the exposed fish had slightly vacuolated hepatocytes showing evidence of fatty degeneration, necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver .The inability of fish to regenerate new liver cells may also have led to necrosis of hepatic cells of sinusoids. Histological effects of xenobiotic on organs of fish have been studied by several authors, in view of the studies cited above, it is apparent that in the present investigation, cypermethrin at lethal and sub lethal concentrations caused considerable histological damages to the organs studied. It is concluded that more or less similar pathological changes are induced in the kidney of different fishes by different toxicants but the extent of damage varies depending upon the dose of toxicants, duration damage varies depending upon the dose of chemical, duration of exposure, toxicity of chemical and susceptibility of the fish.

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