Histopathological Alterations in some Tissues of *Heteroclarias* Juveniles Exposed to Lethal and Sub-lethal Concentrations of Chlorpyrifos and Ameliorative Potentials of Vitamin E to the Toxicity of the Pesticide

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Abstract

The use of pesticides on farmlands to reduce pest and increase crop yields could have detrimental effect on fish when eroded into water bodies. Histopathological effect of lethal and sub-lethal concentrations of Chlorpyrifos (CPF) on Heteroclarias juveniles (Heterobranchus and Clarias) and the ameliorative potentials of vitamin E to the pesticide was investigated. Juveniles of Heteroclarias were assessed in a static renewal bioassay for the period of 96 h, the fish were exposed to various concentrations (0.0 ppm, 1.0 ppm, 2.0 ppm, 3.0 ppm, 4.0 ppm and 5.0 ppm), 0.24 ppm for 28 days and fed on varying concentrations (50 mg, 250 mg, 500 mg and 1000 mg) of vitamin E per kg of diet. The 96 hours LC_{50} values were calculated to be 2.4 ppm. During the experiments, uncoordinated behaviours such as erratic swimming and sudden quick movements were observed. At the end of the experiments, the fish were sacrificed, with the gills and liver obtained for histopathological assay. The result revealed varying types of alterations, like disruption of cartilaginous core, ruptured epithelia, epithelia necrosis, aneurysm, cellular degeneration, lamella degeneration, lamella fusion and hyperplasia in the gills of the exposed fish compared to control, while nuclear alterations, nuclear vacuolation, sinusoidal disruption, fatty degeneration, focal fibrosis and necrosis were recorded in the liver of CPF-exposed fish compared to control. However, the severity of damage reduced as the concentration of vitamin E in the diet increased except in the group fed on the highest concentration of vitamin E. The result of this study indicated that both acute and chronic concentrations of Chlorpyrifos caused various pathological alterations in Heteroclarias juveniles, but, vitamin E is capable of neutralizing the toxic effect of Chlorpyrifos and reduce the pathological lesions in Heteroclarias juveniles.

Keywords: Lethal, Sub-lethal, Chlorpyrifos, Histopathology, Heteroclarias, Vitamin E

Introduction

Increased human population growth and advances in agricultural practices in the use of modern chemicals have caused great decline in fish population. Among the chemical pollutants that contaminate water bodies are pesticides, the use of pesticides in the control of pests that attack food crops has tremendously reduced cost, improved the quality and quantity of food products. However, continuous use of these pesticides could pose potential health hazards on aquatic organisms, particularly fish, when the residues of the pesticides are eroded or leached into the water bodies through runoff during rainfall, which may in turns have adverse effect on man through food chain. Ability of pesticides to persist in the environment and accumulate in the organs of fish could cause pathological alterations in these organs that may eventually cause stress and fish death (Arcury *et al.*, 2007). Fish constitute one of the major sources of animal protein that contribute to a nation's economy through foreign exchange and employment (FAO, 2000). Among the

commonly used pesticides is Chlorpyrifos (CPF)(O,O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate), a broad spectrum organophosphate insecticide in the control of home pests and sub-terrestrial insects such as termites that affect agricultural crops (Rao *et al.*, 2005) and Fountain *et al.*, 2007).

The osmoregulatory and excretory roles of both the gills and the liver exposed them to adverse effect of toxicants in water (Camargo and Martinez, 2007). Several reports have been documented on the effects of different organophosphate pesticides on the tissues of different fish species, such as effect of Chlorpyrifos on guppies (*Poecilia reticulata*) (De Silva and Samayawardhena, 2002); *Oreochromis mossambicus* (Kunjamma *et al.*, 2008); *Oreochromis niloticus* (Kunjamma, 2008); *Channa punctatus* (Devi and Mishra. 2013); *Channa punctatus* (Raibeemol and Chitra, 2015); monocrotophos on *Anabas testudineus* (Santhakumar *et al.*, 2001); methyl parathion on *Corydoras paleatus* (Fanta *et al.*, 2003); deltamethrin on *Gambusia affinis* (Cengiz and Unlu, 2006) and dichlorvos on *Cirrhinus mrigala* (Velmurugan *et al.*, 2009). Among these research works, information on the toxicity effect of Chlorpyrifos on *Heteroclarias* and ameliorative potential of vitamin E is sparse. Vitamin E is a fat-soluble organic compound that has antioxidant effect (Brigelius-Flohe and Traber, 1999). It helps to stop the production of reactive oxygen species (ROS) by donating hydrogen atom to a free radical (Packer, *et al.*, 2001 and Traber and Stevens, 2011). The use of vitamin E as an ameliorative agent has been carried out by many researchers (Isa *et al.*, 2011, Ibrahim and Banaee, 2014 and Olsvik *et al.*, 2015), but none of these has used Heteroclarias as test organism. There is therefore the need to investigate the ameliorative potentials of vitamin E on this fish. This research work estimated the ameliorative potentials of vitamin E on *Heteroclarias* exposed to Chlorpyrifos.

Materials and Methods

Experiment set up

Juveniles of *Heteroclarias* with average weight 21.4 ± 1.3 g and average length 11.12 ± 0.8 cm were purchased from TJ Farm Ogidi, Ilorin, Kwara State, Nigeria. The fish were transported in a well aerated plastic container to the laboratory of the Department of Zoology University of Ilorin, Kwara State, Nigeria. They were left unfed throughout the day so as to avoid mortality that may result from indigestion as a result of stress. The fish were kept in large bowls of 40 litre capacity and were acclimatized for 14 days under a temperature of 24-25◦C. During this period, they were fed twice daily with commercial feeds (Durante-35% crude protein) at 4% body weight (Meyer *et al.*, 1993) and water was changed every two days to prevent pollution (FAO, 1986). The fish were observed for behavioural changes (USEPA, 1996). Feeding was suspended 24 h before the commencement of the experiment (USEPA, 1996). The pesticide used was purchased from a commercial agro-chemical store in Ilorin, Kwara State It is a chemical formulation of O, O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate and is also known as Dursban or Lorsban.

Range finding test

Range finding test (Abdulkareem *et al.* 2019) was carried out to determine the concentration of chlorpyrifos to be used during the acute toxicity test. This test was carried out by dissolving varying concentrations of chlorpyrifos in 100 ml of distilled water to form the stock solution and monitored for 24 hours to determine concentration of toxicant to be used for the experiment.

Acute toxicity test

Acute static toxicity tests were carried out according to USEPA, (1996). Based on the results from the range finding test, two (2 ml) of Chlorpyrifos was introduced into 100 ml distilled water and mixed thoroughly to make the stock solution from which varying concentrations of 1.0 ppm, 2.0 ppm, 3.0 ppm, 4.0 ppm and 5.0 ppm each were measured into into10 litres of water in plastic aquarium with ten fish each in each concentration. The fish were not fed 24 h before the experiment and during the experiment (USEPA, 1996). The experiment lasted for 96 hours and a constant renewal method was used to maintain the concentration of the toxicant during the test period. The fish behaviour and

mortalities with the physicochemical parameters were also observed in the test accordingly. Fish were considered dead when the opercula movement ceased and they stopped responding to gentle poking. Lethal concentration at which 50% of the *Heteroclarias* juveniles die (LC_{50}) was calculated after 96 hours of exposure using Arithmetic method of Kaber (Dede, 1992, using Arithmetic method of kaber for calculating LC_{50} (adopted by Dede 1992),

$$\sum Lc_{50} = L_{c100} - \frac{\sum mean\ death X concentration\ difference}{Number\ of\ \ fish\ per\ group}$$

Ameliorative experiment (chronic)

Ameliorative experiment was carried out by exposing the fish to sub-lethal concentrations of Chlorpyrifos for 28 days based on the LC_{50} (2.40 ppm) value obtained in the acute toxicity test. The fish were selected randomly and distributed into six groups (A,B,C,D,E&F) of ten fish each in plastic aquaria containing chlorine-free bore-hole water. The fish exposed to 0.24 ppm (1/10 LC_{50} of Chlorpyrifos) for 28 days (Narra *et al.,* 2011) and the experiment was carried out in triplicate (Pathirantine and Athauda 1999). Group A served as the control and was fed on basal diet without toxicant; Group B was exposed to 0.24 ppm Chlorpyrifos and fed on basal diet; while group C was exposed to 0.24 ppm Chlorpyrifos and fed on 50 mg vitamin E/kg of diet; and group D was exposed to 0.24 ppm Chlorpyrifos and fed on 250 mg of vitamin E/kg of diet. The other two groups E & F were exposed to 0.24 ppm chlorpyrifos and fed on 500 mg & 1000 mg of vitamin E/kg of diet respectively. Renewal of corresponding concentration was done every day with freshly prepared chlorpyrifos to maintain the Chlorpyrifos concentration (Vutukuru, 2007). During the chronic phase, the fish were fed twice daily (9:00 a.m. and 4:00 p.m.) on basal diets formulated with fish meal (30%), soya bean (20%), maize corn (30%), groundnut cake (18%), bone meal (0.4%), methionine (0.4%), lysine (0.4%), vitamin C (0.2%), salt (0.2%), premix (0.2%) which formed 100% balanced diet. The diets were supplemented with graded levels (0, 50, 250, 500 and 1000 mg) of vitamin E kg^{-1} and fed to six groups of ten (10) fish each for 28 days. After the experimental period, five fish were randomly picked and sacrificed for histopathological analysis.

Water quality parameters

Some water quality parameters such as: pH, temperature and dissolved oxygen (DO) were measured by the use of PH meter, thermometer DO meter respectively Standard procedure in the methods describe by the American Public Health Association (APHA, 1995).

Histopathological assay

At the end of the exposure periods, the fish were sacrificed to remove gills and liver for histological examination. The tissues were placed in 10% formalin to prevent autolytic and bacterial spoilage. The fixed tissues were dehydrated in alcohol and dealcoholized with clearing agent, embedded in molten paraffin wax (56-58°C) and sectioned at 7 µm using a microtome. The sections were oven-dried and stained with haematoxylin and eosin. Stained tissues were dehydrated, rinsed to remove excess stain and allowed to dry, after which microscopic examination was carried out and photomicrographs were taken through photographic attachment to OLYMPUS microscope (Hinto *et al.,* 1992).

Results

Behavioural responses

The various abnormal behaviours observed in the exposed fish included erratic swimming, air-gasping attitude (respiratory distress), leaning behaviour and mucus discharge. Exposed fish showed morphological and behavioural alterations with varying concentrations of Chlorpyrifos before death. These changes include white coloration of fins, slow response to touch, lateral bending of the body, erratic and spiral swimming, sudden quick movement, body colour change and swollen abdomen. Extent of damage increases with increase in concentrations of pesticide. The percentage mortality increases with increase in concentration of Chlorpyrifos. Highest percentage mortality (100%) was recorded at 5.0 ppm while the lowest per cent mortality (10%) was recorded at 1.0 ppm. The lethal concentration of Chlorpyrifos that caused

50% mortality in *Heteroclarias* juveniles was 2.4 ppm (Table 1).

Table 1: Lethal concentration (LC_{50}) value of chlorpyrifos on *Heteroclarias* for 96 hr

Concentration (ppm)	Concentration difference	∑ Real & replicate mean alive	∑ Real & replicate mean death	Mean death	Mean death x concentration difference
0.00	1	10	0	0	0
1.00	1	9	1	1	1
2.00	1	8	2	2	2
3.00	1	4	6	6	6
4.00	1	3	7	7	7
5.00	1	0	10	10	10

$$L_{c50} = 5.0 - \frac{26.0}{10}$$

Lc_{50}=2.4ppm

Histopathological alteration

The gills of fish in the control group recorded normal cellular orientation, while the gills of the groups exposed to different concentrations of chlorpyrifos for 96 h showed general cellular disorientation such as lamellar fusion, oedema and degenerative gill filament, disruption of cartilaginous core, aneurysm, lamellar disorganization and necrosis (Fig. 1a-e). Several histopathological alterations were also recorded in the liver of *Heteroclarias* exposed to different concentrations of chlorpyrifos after 96 h were cytoplasmic degeneration, Fatty degeneration, sinusoidal distortion, focal fibrosis and necrosis compared to the control group with normal cellular structure (Fig. 2a-e).

At the end of 28 days of amelioration, the gills of the fish exposed to chlorpyrifos without ameliorative agents showed various types of pathological lesions like epithelial necrosis, cellular degeneration, disruption of cartilaginous core and ruptured epithelial. However, some histopathological alterations were also recorded in the groups of fish ameliorated with varying concentrations of vitamin E, but, the severity of damage decreased as the concentrations of vitamin E increased, except in the group ameliorated with the highest concentration of vitamin E with severe damages such as ruptured epithelia and disruption of cartilaginous core (Fig. 3 a-f) here was no alteration in the liver of the control group fed on basal feed only without toxicant, but the liver of the group exposed to chlorpyrifos without amelioration recorded different types of alterations, among which are nuclear alteration, sinusoidal disruption , fatty degeneration and focal fibrosis. However, the severity of alterations reduced as the concentrations of vitamin E in the diet increased except in group F that were fed on the highest concentration of vitamin E with epithelial necrosis which is a severe damage (Fig. 4 a-f).

Discussion

The abnormal behaviours such as slow response to touch, lateral bending of the body, gasping for air, erratic and spiral swimming and sudden quick movement observed in this experiment could be a response to the toxic effect of Chlorpyrifos. This was similar to the observations of Ali and Kumar, (2012) and Jindal and Kaur (2014) who exposed *Channa punctatus* and *Ctenopharyngodon idellus* to Chlorpyrifos respectively. Gasping for air at the surface of water could be attributed to oxygen depletion as a result of oxidative stress induced by the toxicant (Katja *et al.*, 2005). The abnormal behaviour such as hyperactivity exhibited by the Chlorpyrifos-exposed fish might be attributed to accumulation of acetylcholine in synaptic and neuromuscular junctions of the fish as a result of inhibition of hydrolysis of acetylcholine by enzyme acetylcholinesterase induced by toxic effect of the toxicant (Rao *et al.* 2005)

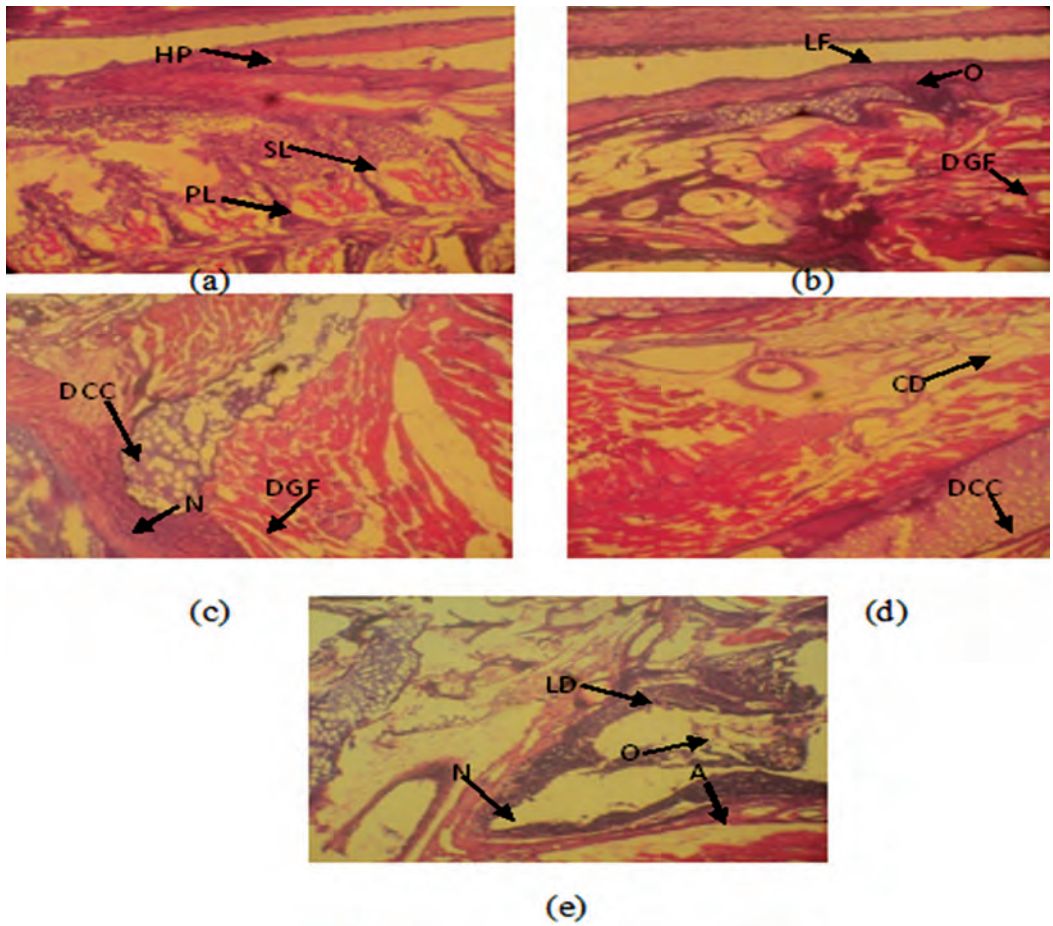

Figure 1: Photomicrograph of gills of *Heteroclaria* exposed to chlopyrifos for 96 hours (H&E x100)

(a) Gills of Heteroclarias in the control group show normal morphology with normal primary and secondary lamellae and hyperplasia (HP);
(b) Lamellar fusion (LF), oedema (O) and degenerative gill filament (DGF) in the gills of *Heteroclarias* exposed to 1.0 ppm chlopyrifos;
(c) Disruption of cartilegenous core (DCC), degenerative gill filament (DGF) and necrosis (N) in the gill of *Heteroclarias* exposed to 2.0 ppm chlopyrifos;
(d) Cellular degeneration (CD) and disruption of cartilegenous core (DCC) in the gill of *Heteroclarias* exposed to 3.0 ppm chlopyrifos and
(e) Lamellar disorganization (LD), necrosis (N), oedema (O) and aneurysm (A) in the gill of *Heteroclarias* exposed to 4.0 ppm chlorpyrifos

and Lakshmaiah, 2016). The increased mucus secretion observed in the Chlorpyrifos-exposed fish was a sign of the inflammatory reaction in response to the toxic effect of the toxicant (Rao, 2006 and Adekunle, 2015). The 96 h LC_{50} value of 2.4 ppm recorded in this study was in contrast with the report of Oruc, (2010) who recorded 98.67µgL as the 96 h LC_{50} values of Chlorpyrifos in juveniles of *Oreochromis niloticus*. Various histopathological lesions recorded in the gills of Heteroclarias exposed to Chlorpyrifos could probably be attributed to the oxidative stress induced by Chlorpyrifos, gill being an organ of excretion, respiration and osmoregulation. Hyperplasia shown in the control gill could be due to the stress incurred during the course of the experiment or as a result of protective and defensive mechanism. This was in accordance with the reports of Kunjamma *et al.* (2008) in the gills of *Oreochromis mossambicus* exposed

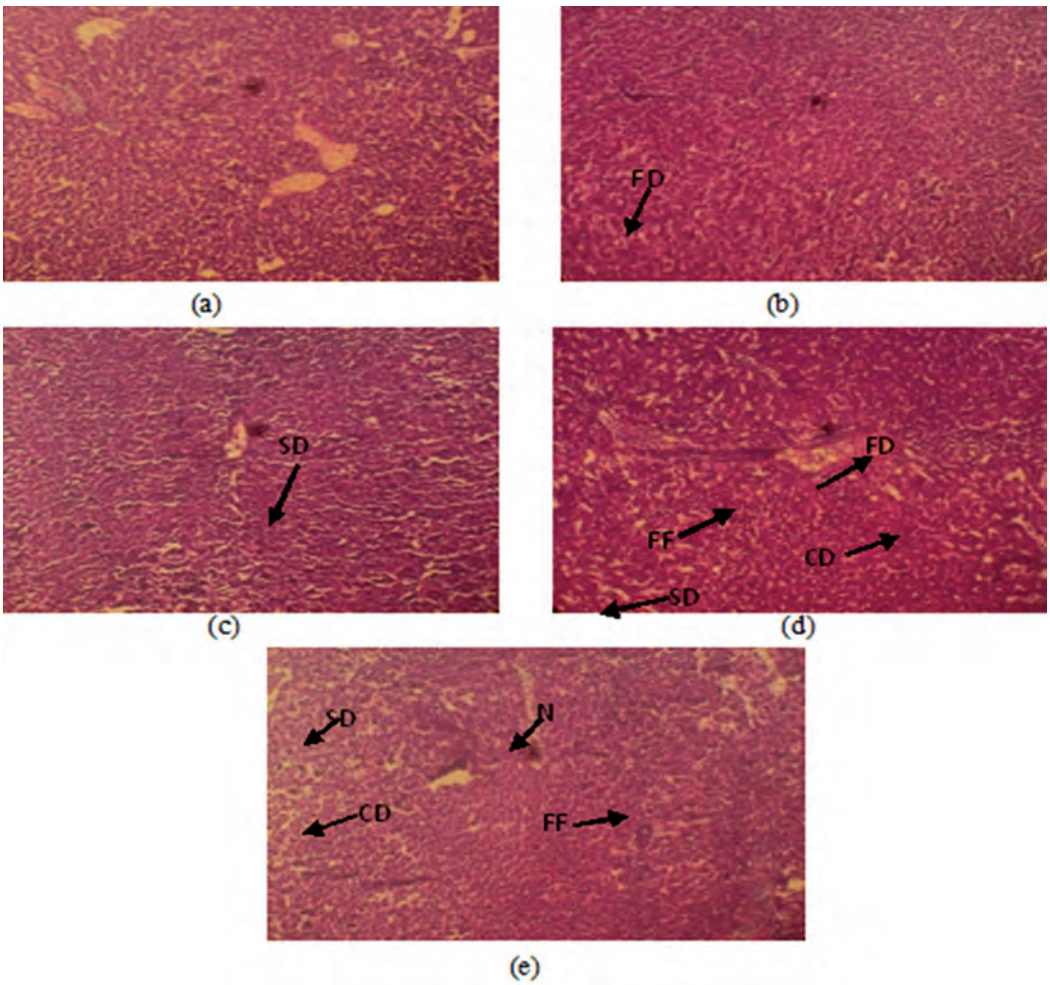

Figure 2: Photomicrograph of liver of Heteroclarias exposed to chlorpyrifos for 96 hours (H&E x100)

(a) Liver of *Heteroclarias* in the control group showed normal hepatocytes;
(b) Fatty degeneration (FD) in the liver of *Heteroclarias* exposed to 1.0 ppm chlorpyrifos;
(c) Sinusoidal distortion in the liver of *Heteroclarias* exposed to 2.0 ppm chlorpyrifos;
(d) Cytoplasmic degeneration (CD), fatty degeneration (FD), sinusoidal distortion (SD) and focal fibrosis (FF) in the liver of Heteroclarias exposed to 3.0 ppm chlorpyrifos and
(e) Sinusoidal distortion (SD), focal fibrosis (FF), cytoplasmic degeneration (CD) and necrosis (N) in the liver of *Heteroclarias* exposed to 4.0 ppm chlorpyrifos.

to Chlorpyrifos, and Velmurugan *et al.* (2009) in the gills of *Cirrhinus mrigala* exposed to Dichlorvos. Lamella fusion, aneurysm, lamella disorganization and cellular degeneration observed in the Chlorpyrifos-exposed fish could be due to damage of the secondary lamellae that lead to rupture of the blood vessels as a result of toxic effect of Chlorpyrifos. This was in agreement with the reports of Jiraungkoorskul *et al.* (2002) in *Oreochromis niloticus* exposed to glyphosate, Lawrence *et al.* (2010) in *Clarias gariepinus* exposed to Gammalin 20 and Devi and Mishra (2013) in the gills of *Channa punctatus* exposed to Chlorpyrifos. Epithelial necrosis observed in the chlorpyrifos-exposed fish could probably be due to damage of the cellular membrane as a result of depletion of oxygen across the gill filaments. This was in accordance with the reports of Juan *et al.* (2003) and Kunjamma, (2008) in the gills of *Oreochromis niloticus* exposed to Chlorpyrifos. Several hepatic damages recorded in the

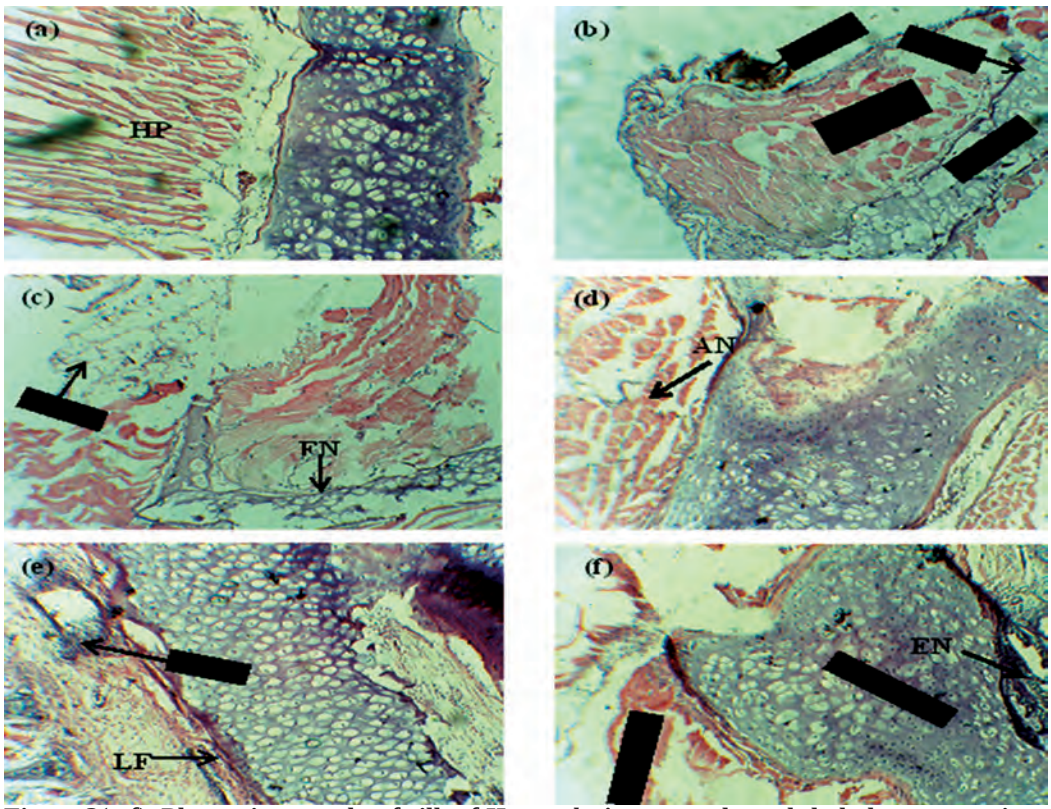

Figure 3(a-f): Photomicrographs of gills of *Heteroclarias* exposed to sub-lethal concentrations of chlorpyrifos and ameliorated with varying concentrations of vitamin E. (H&E.x100)

(a) Gills of control fish fed on basal diet without toxicant show hyperplasia (HP);
(b) Epithelia necrosis (EN), cellular degeneration (CD), disruption of cartilaginous core (DCC) and ruptured epithelia (RE) in the gills of fish exposed to chlorpyrifos and fed on basal diet;
(c) Lamella degeneration (LD) and Epithelia necrosis (EN) in the gills of chlorpyrifos-exposed fish fed on 50 mg vitamin E/kg of diet;
(d) Aneurysm (AN) in the gill tissue of chlorpyrifos-exposed fish fed on 250 mg of vitamin E/kg of diet;
(e) Aneurysm (AN) and lamellar fusion (LF) in chlorpyrifos-exposed fish fed on 500 mg of vitamin E/kg of diet and
(f) Disruption of cartilaginous core (DCC), ruptured epithelia (RE) and Epithelia necrosis (EN) in chlorpyrifos-exposed fish fed on 1000 mg of vitamin E/kg of diet.

liver of *Heteroclarias* juveniles exposed to Chlorpyrifos could be attributed to the roles played as a detoxification organ (Dutta *et al.*, 1993). Histopathological alterations such as cytoplasmic degeneration, fatty degeneration and sinusoidal distortion observed in the liver of Chlorpyrifos-exposed fish could be as a result of accumulation of the toxicant through consumption of Chlorpyrifos contaminated feed. These alterations recorded agreed with the reports of Nassr-Allah (2007) in *Oreochromis niloticus* exposed to phenol, (Devi and Mishra, 2013) in *Channa punctatus*, Raibeemol and Chitra (2015) in the liver of *Etroplus maculatus* exposed to Chlorpyrifos; Ahmadivand *et al.*, (2014) in *Oncorhunchus mykiss* exposed to butchalor. Nuclear alteration, vacuolation and focal fibrosis recorded in this work was similar to the reports of Fanta *et al.*, (2003) in *Corydoras paleatus* exposed to methyl parathion, Cengiz and Unlu (2006) in *Gambusia affinis* exposed to deltamethrin and Velmurugan *et al.*, (2009) in *Cirrhinus mrigala* exposed to Dichlorvos. Reduction in the severity of damage recorded in both the gills and the liver of the groups of fish ameliorated with vitamin E was an indication that vitamin E is capable of playing the role of antioxidant to neutralize the toxic

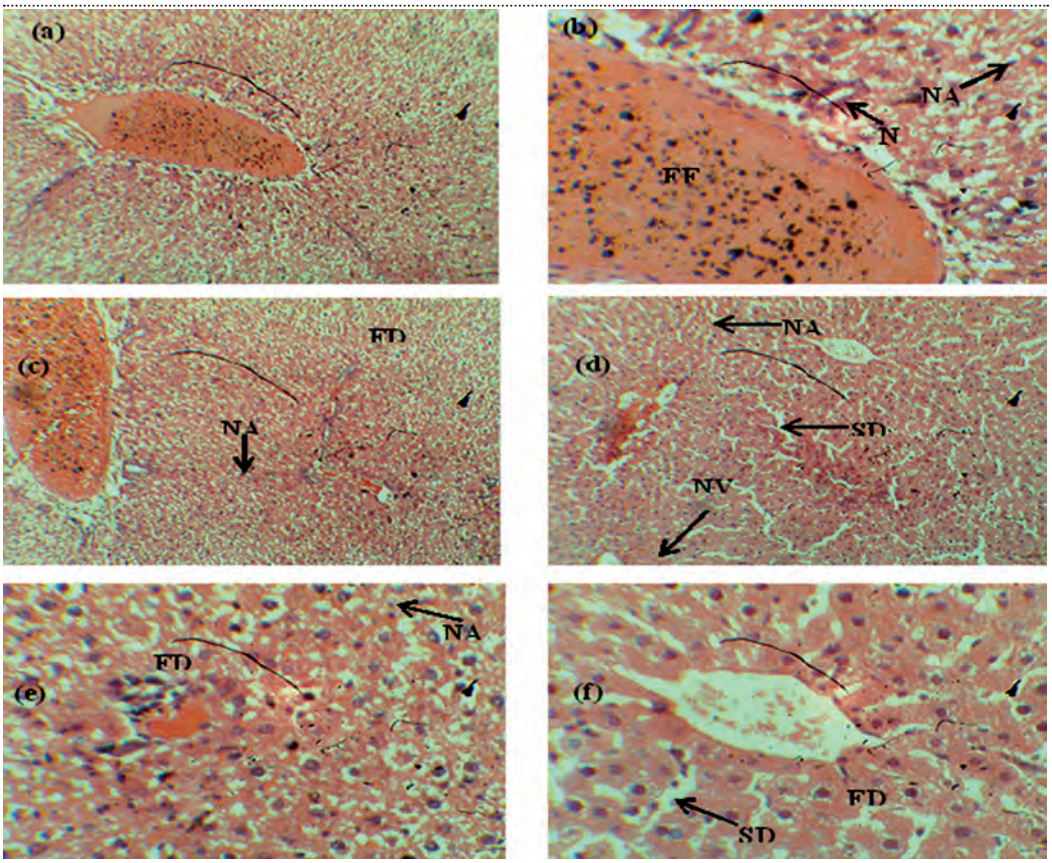

Figure 4 (a-f): Photomicrographs of liver of *Heteroclarias* exposed to sub-lethal concentrations of chlorpyrifos and fed on varying concentrations of vitamin E for 28 days (H&E 100)

(a) Liver of fish fed on basal diet without toxicant;
(b) Focal fibrosis (FF), necrosis (N) and nuclear alteration (NA) in the liver of chlorpyrifos-exposed fish fed on basal diet;
(c) Fatty degeneration (FD) and nuclear alteration (NA) in the liver of chlorpyrifos-exposed fish fed on 50 mg of vitamin E/kg of diet;
(d) Sinusoidal distortion (SD), nuclear vacuolation (NV) and nuclear alteration (NA) in the liver of chlorpyrifos-exposed fish fed on 250 mg of vitamin E/kg of diet;
(e) Fatty degeneration (FD) and nuclear alteration (NA) in the liver of chlorpyrifos-exposed fish fed on 500 mg of vitamin E/kg of diet and
(f) Fatty degeneration and sinusoidal distortion in the liver of chlorpyrifos-exposed fish fed on 1000 mg of vitamin E/kg of diet.

effect induced by Chlorpyrifos in *Heteroclarias* juveniles. Reduction in micronucleus induction and percentage frequencies were also reported in *Heteroclarias* exposed to Chlorpyrifos and fed on feed that was supplemented with Vitamin E (Abdulkareem *et al.,* 2019). Also, the reduction in the pathological lesions recorded in the gills and liver of these fish were similar to those reported in the gills and liver of *Clarias gariepinus* ameliorated with Ackee Apple (*Blighia sapida*) (Iyiola *et al.,* 2018).

Conclusion

This study revealed that chlorpyrifos induced oxidative stress which induced various pathological lesions in the gills and liver of *Heteroclarias* in both the acute and chronic exposure. The mild alterations recorded in vitamin E-fed fish, implies that vitamin E is capable of ameliorating the toxic effect of Chlorpyrifos in *Heteroclarias* juveniles.

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