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Paint wastewater induced histopathological changes in the gill and liver of freshwater African catfish, Clarias gariepinus (Burchell, 1822)

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Abstract

This study investigated the effects of paint wastewater on the histology of gill and liver of *Clarias gariepinus*. Juvenile *C. gariepinus* were exposed to acute (400, 500, 600 and 700 ml) and chronic (100, 150, 200 and 250 ml) concentrations of paint wastewater for 96 hours and 28 days respectively. After the exposure periods, gill and liver were excised for histological analysis. Histological alterations in the gill during both exposures were characterised by moderate to severe hyperplasia, displacement of cartilaginous core, epithelia lifting, epithelia soughing, oedema, lamella fusion and necrosis compared to the control. Liver hepatocytes of fishes in both assays revealed distortions in parenchymal lattice network, hyperplasia, pyknosis, vein degeneration, hypertrophy and hepatic necrosis compared to the control. The severity of these histological changes increased in concentration and time dependent manner. Epithelia lifting, lamella fusion, oedema and hyperplasia are probably adaptive/defensive mechanisms against severe pathological changes induced by the wastewater effluent. In conclusion, this study showed that paint wastewater effluent caused severe pathological damages to the branchial and hepatic tissues of *C. gariepinus* and this may threaten the survival of the fish. Hence, there is the need for proper treatment of paint wastewater before being discharged into water bodies.

Keyword: Paint, Wastewater, Histopathology, Gill, Liver, *Clarias gariepinus*

1. Introduction

Wastewater has been a major environmental concern especially in developing countries of the world. Industries discharge large quantities of wastewater into aquatic environments indiscriminately without treatment in order to maximise profit. Wastewater poses environmental threats to the quality of receiving water bodies by adding various chemical substances such as pesticides, fertilizers, heavy metals, acidic and coloured substances which are deleterious to aquatic environment and its biota.

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These chemical substances are capable of building up in the food chain and cause considerable stress on the ecosystem leading to extensive damage to aquatic life and human that depends on aquatic biota for survival (Farkas *et al*., 2002). Amongst industries, paint industries play a significant role in the pollution of water bodies through direct discharge of wastewater.

In Nigeria, paint industry has been in existence for many years. Presently, local production is on the increase due to government's successful effort to stimulate private and public involvements in paint manufacturing. As a result of this development, several billion litres of paint effluent is being produced and discharged into the aquatic systems every year without pre-treatment. These effluents may elicit physiological, biochemical and pathological disorders in fish. With the increase use of chemicals for various purposes; food, water, air, and soil contamination is of increased concern (Emere and Balogun, 2014). Hence, safety of element of basic human consumption is of critical interest as there is link between the quality of foods and human health.

Fish are important source of food as well as useful in evaluating the health status of aquatic ecosystem. *Clarias gariepinus* is an important food fish widely cultured in Nigeria. It is known for its hardiness, wide tolerance to environmental changes and fast growth. *Clarias gariepinus* subsists on wide varieties of food and it is readily available throughout the year. Histopathological evaluation has widely been used to assess the effect of pollutants in fish. It provides a useful data on structural changes of organ at both cellular and subcellular levels much earlier than obvious external notifications (Nwani *et al*., 2015). According to Fanta, *et al*. (2003), these structural changes are easily identified than functional ones. These changes may also serve as early warning signs of cellular damage in fish. The gill is the main organ of gaseous exchange, ionic regulation and excretion of metabolic wastes. It is the internal environment of fish that has the first contact with materials from external environment, thus predisposing the gill to pollutants in the surrounding water (Liebel *et al*., 2013). The liver is an organ that detoxifies variety of toxicants from the blood (Soufy *et al*., 2007). It is concerned with basic metabolism such as accumulation, biotransformation and excretion of xenobiotics in fish (Chavan and Muley, 2014). The liver is particularly susceptible to damage from numerous toxicants it has contact with, and this may threaten the well-being of fish. Therefore, the lesions from these organs may serve as early signs or good indicators of paint effluent intoxication.

Reports on the effects of industrial effluents on the histopathology of fishes are available (Navaraj and Yasmin, 2012; Saroja *et al*., 2013). These reports showed that exposure of fish to industrial effluents resulted to structural and functional changes in the gill and liver. However, studies dealing with the effects of paint wastewater on the histopathology of freshwater fishes are limited. The present study aimed at investigating the histopathological effects of paint wastewater on the gill and liver of *C. gariepinus* may provide a useful tool for assessing the health status of fish in industrial paint contaminated waters, with the overall goal of developing policies and regulatory framework for the protection of aquatic ecosystems and resident fish species.

2. Materials and Methods

Experimental Fish and Maintenance

Juvenile *Clarias gariepinus* ((average weight: 14.00 ± 0.50g and average length 12.40 ± 1.51cm) were procured from commercial fish farm in Ilorin ($10^0$53'0"N, 4^0 1' 0"E), Nigeria) transported in plastic containers under ideal conditions to the Fisheries and Hydrobiology Research laboratory, Department of Zoology, University of Ilorin, where they were acclimatized to laboratory conditions for 14 days in 500 litres of continuously aerated fresh water. During the period, fish were fed at 3% body weight with commercial fish feed twice daily at 9.00 and 16.00 hours but feeding was terminated 24 hours prior to the commencement of the experiment.

Effluent Collection and Range Finding Test

Raw effluent collected directly from the effluent canal at the point of discharge was brought to the laboratory and kept at 4^oC in refrigerator prior use. Stock solutions of the effluent were prepared by dissolving the effluent in distilled water to a final volume of 1.0 litre. Each treatment solution was prepared after a range-finding test was conducted by diluting the stock solution with water to achieve the appropriate exposure concentrations.

Acute and Chronic Toxicity Bioassays

Acute toxicity bioassay was conducted using the definitive concentrations 400, 500, 600 and 700 ml derived from the range finding tests to which 10 fishes per concentration were randomly

exposed for 96 hours. Another set of 10 fishes were simultaneously maintained in borehole water devoid of the effluent and considered as the control. The experiment was set in triplicates. Based on the LC_{50} value obtained, four chronic concentrations: 100, 150, 200 and 250 ml corresponding to 1/4, 1/3.33, 1/3 and 1/2.8th of the effluent respectively, were prepared by serial dilution of the stock solution. A total of 30 fishes were exposed to each concentration in triplicates of 10 fishes per replicate. The control experiment was maintained in borehole water without the effluent. The exposure lasted 28 days after which the fish were sacrificed for histopathological analysis.

Histopathological Assay

At the end of each treatment duration, the fishes were sacrificed and carefully dissected to remove the gills and the liver. The gills and the liver were fixed in aqueous Bouin's fluid for 24 hours at room temperature. After fixation, the tissues were dehydrated through graded series of alcohol (i.e. 70, 80, 90 and 100 %). Tissues were subsequently cleared in xylene and infiltrated in paraffin wax using a rotary microtome and sections of 4-6 µm were obtained from paraffin-blocks and stained with Haematoxylin-Eosin. Histopathological lesions were examined and photomicrographic impressions taken using Carl Zeiss binocular microscope (Axiophot, Germany).

3. Results and Discussion

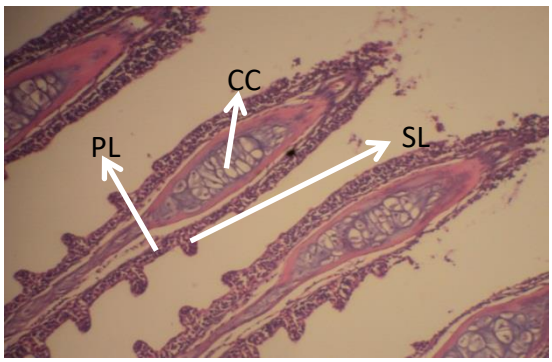
In the control groups of both acute and chronic toxicity tests, the normal histological architectures of primary and secondary lamellae, inter-lamellae spaces and cartilaginous core were preserved (Plates 1a and 2a). The primary gill lamellae exhibited laterally compressed leaf-like structures, comprising a central core of cartilaginous rod, dense mass of red blood cell at its tips and linings of epithelial cells closely applied to the gill ray. Each primary gill lamellae consist of secondary gill lamellae on both sides, which run perpendicularly to its long axis. In the acute test, various histological changes such as hyperplasia, displacement of cartilaginous core, soughing of epithelia tissue, oedema, fusion of primary lamella and necrosis were observed (Plate 1b, c, d, and e). In the chronic test, however, the gill sections revealed distortions which included displacement of cartilaginous core, hyperplasia, severe necrosis, thickening of the tip of the secondary lamellae and cellular infiltration (Plate 2b, c, d and e). In

both assays, the severity and frequency of lesions on the gill were more with increasing concentrations.

Liver histological section shows normal lattice network of parenchymatous cells in the control group (Plates 3a and 4a). In the acute toxicity test, irregular clumps and convergence of sinusoids were observed in the lattice network of parenchymal in the group exposed to 400 ml concentration of paint wastewater (Plate 3b). Moderate to severe necrosis, displacement of cartilaginous core, cellular infiltration and hyperplasia were observed in the groups exposed to 500ml, 600ml and 700 ml concentrations of paint wastewater (Plate 3c, d and e). In the chronic toxicity test, hypertrophy, hyperplasia, pyknosis, necrosis and oedema were various histological abnormalities observed (Plate 4 b, c, d and e). The frequency of these lesions and their severity also increased with increasing concentration of effluent in both assays.

Rapid industrialization across the globe has contributed to the alteration of natural condition of aquatic habitat through introduction of various chemical pollutants (Ibrahem, 2012). Industrial processes have created both localized and regional pollution problems in nearly every country around the world (Adeogun and Chukwuka, 2011). Wastewaters such as those emanating from paints are recognised as serious pollutant of concern. Direct or indirect exposure to such pollutant could manifest its deleterious effects on the structural and functional histology of the tissues of exposed fauna. The gills remain in close contact with the immediate environment and are very sensitive to changes in water quality, thus they are considered as primary target of waterborne contaminants (Fernandez and Mazon, 2003). The liver plays an important role in metabolism and excretion of xenobiotics in the body of organisms (Hinton and Lauren, 1990) and due to its location and proximity to blood supply, it is also affected by water-borne contaminants (Camargo and Martinez, 2007).

The observed histopathological changes in the gill and liver tissues of *Clarias gariepinus* exposed to paint wastewater showed that acute and chronic concentrations of the effluent elicited moderate to severe structural distortions. These histopathological distortions are similar to the observations of Kolawole *et al*. (2011) and Somdare *et al* (2015) who utilised such histopathological distortions in the tissues of *C. gariepinus* as biomarkers of environmental pollution.



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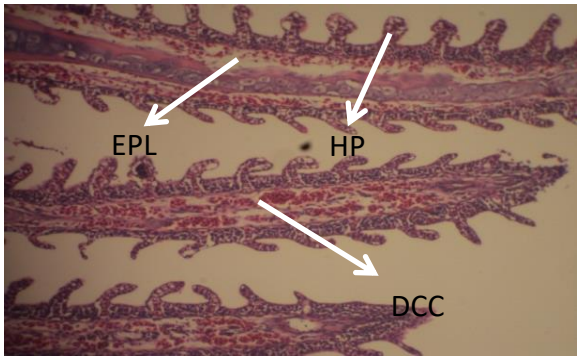

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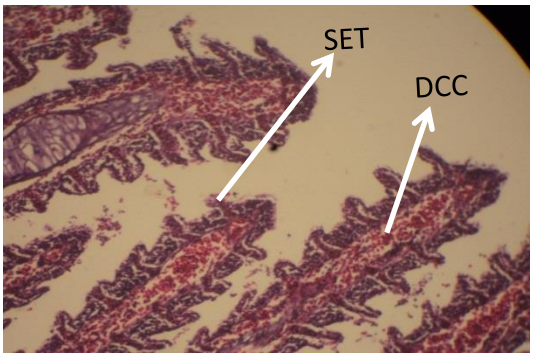

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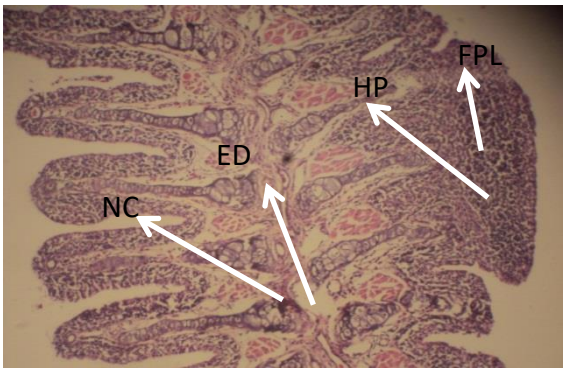

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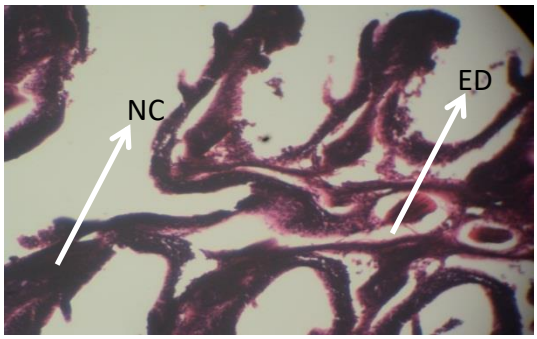
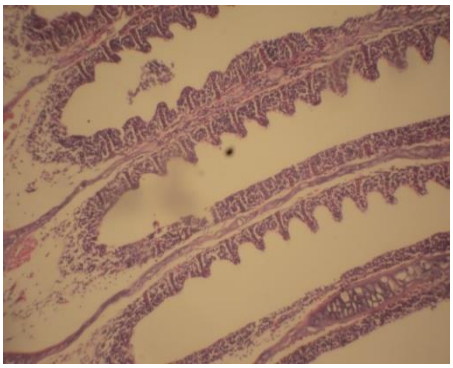


(e)

Plate 1: Histological sections of the gill of *C. gariepinus* exposed to acute concentrations of paint wastewater.

(a) Normal primary lamella (PL), secondary lamella (SL) and cartilaginous core (CC) of gill of fish in control group (b) Hyperplasia (HP), partial epithelia lifting (EPL) and displacement of cartilaginous core (DCC) of gill in fish exposed to 400 ml concentration of paint wastewater (c) Displacement of cartilaginous core (DCC) and soughing of epithelia tissue (SET) of gill in fish exposed to 500 ml concentration of paint wastewater (d) Hyperplasia (HP), oedema (ED), fusion of primary lamella (FPL) and necrosis (NC) of gill in fish exposed to 600 ml concentration of paint wastewater (e) Oedema (ED) and severe necrosis (NC) of gill in fish exposed to 700 ml concentration of paint wastewater



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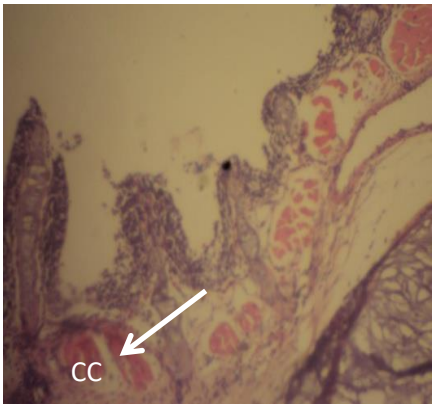

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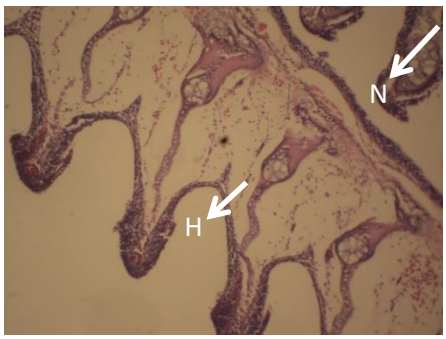

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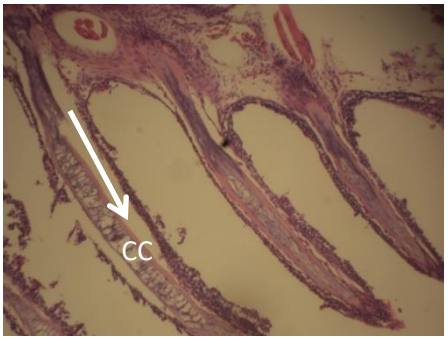

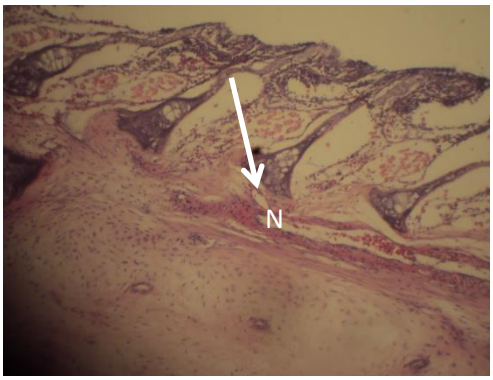

(e)

Plate 2: Histological sections of the gill of *C. gariepinus* exposed to chronic concentrations of paint wastewater

(a) Normal cartilaginous core, primary and secondary lamella of gill of fish in control group (b) Displacement of cartilaginous core and hyperplasia of gill tissue in fish exposed to 100ml concentration of paint wastewater. (c) Displacement of cartilaginous core, necrosis, thickening of the tip of the secondary lamella due to hyperplasia of gill tissue in fish exposed to 150ml concentration of paint wastewater. (d) Displacement of cartilaginous core and secondary lamella, cellular infiltration and hyperplasia of gill tissue in fish exposed to 200ml concentration of paint wastewater. (e) Severe necrosis of gill tissue in fish exposed to 250ml concentration of paint wastewater.

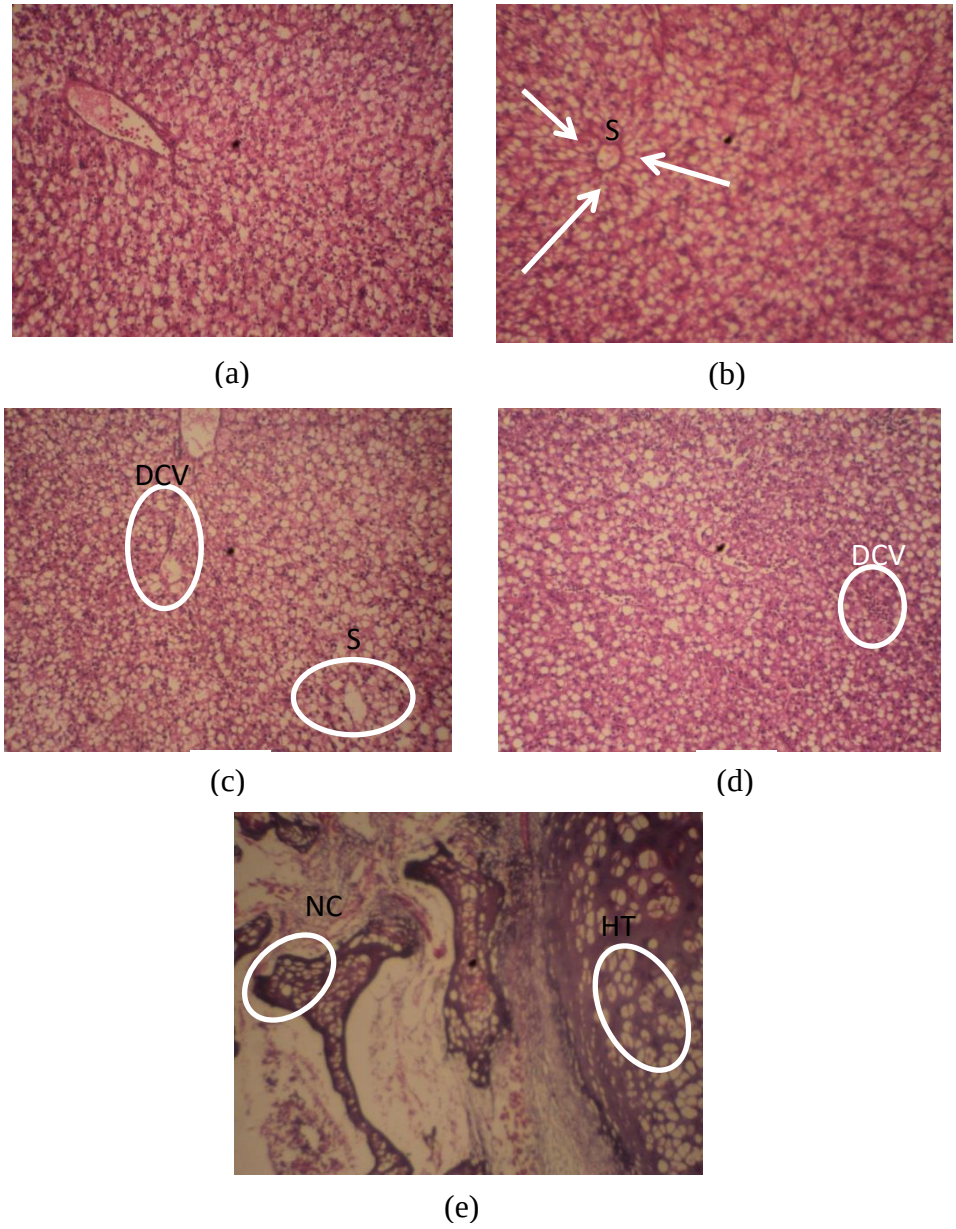

Plate 3: Histological sections of the liver of *C. gariepinus* exposed to acute concentrations of paint wastewater

(a) Normal lattice network of parenchymatous cells of liver of fish in control group (b) Lattice network of parenchymatous cells with formation of irregular clumps and convergence of sinusoids (S) in liver of fish exposed to 400ml concentration of paint wastewater (c) Slight degeneration of central vein (DCV) with mild congestion of nuclei (CN). The sinusoidal network loosed their normal arrangement and appearance with partial degeneration of liver in fish exposed to 500ml concentration of paint wastewater (d) Hyperplasia, pyknosis of liver in fish exposed to 600ml concentration of paint wastewater (e) Hypertrophy and severe necrosis of liver in fish exposed to 700ml concentration of paint wastewater

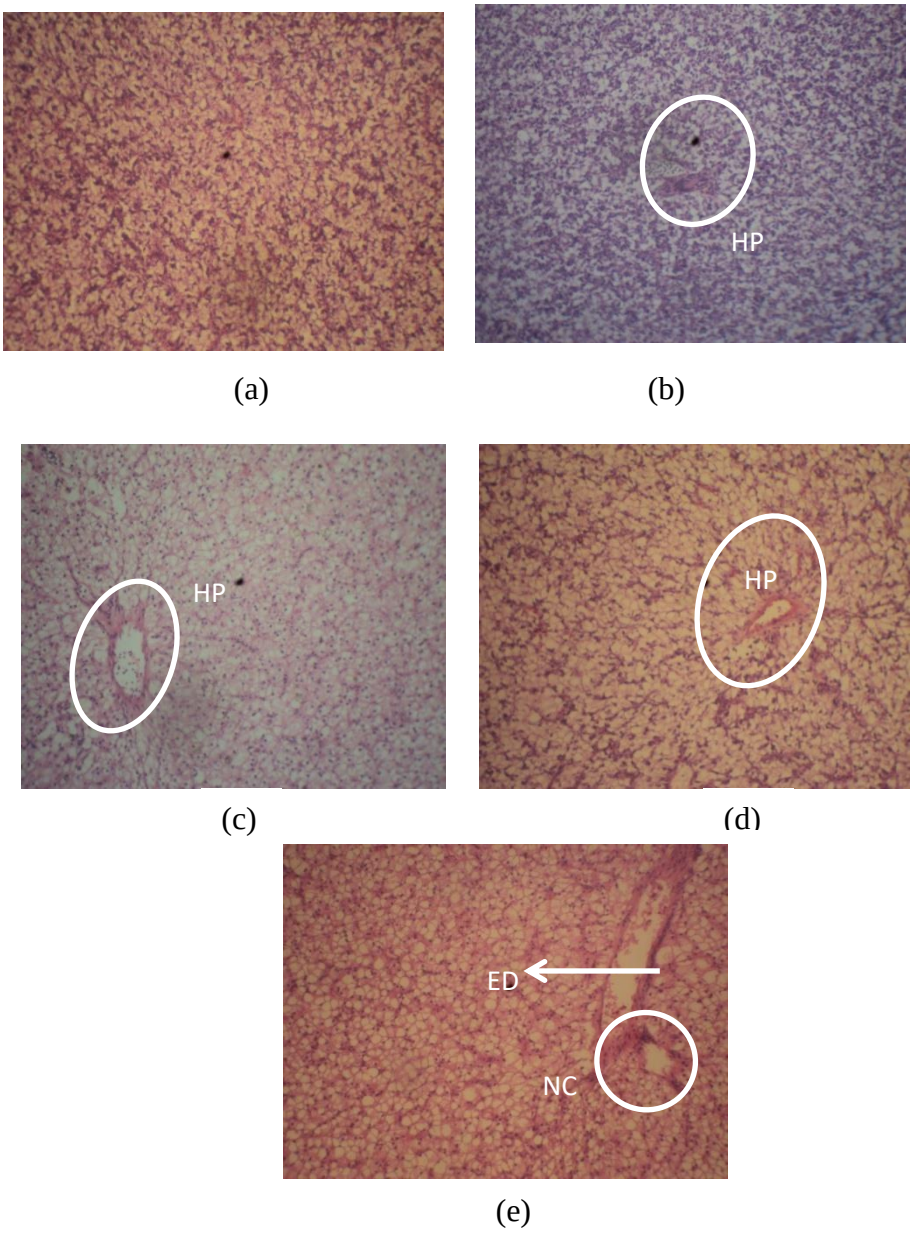

Plate 4: Histological sections of the liver of *C. gariepinus* exposed to chronic concentrations of paint wastewater.

(a) Normal parenchymatous cell with the central vein of liver of fish in control group (b) Hypertrophy (HP) of liver in fish exposed to 100ml concentration of paint wastewater (c) Hyperplasia (HP) of liver in fish exposed to 150ml concentration of paint wastewater (d) Hyperplasia (HP) of the liver in fish exposed to 200ml concentration of paint wastewater (e)Necrosis (NC) and oedema (ED) of the liver in fish exposed to 250ml concentration of paint wastewater

Paint effluents contain heavy metals such as lead, cobalt, copper, chromium, zinc, cadmium, etc. which are mainly used as pigments and additives in the paint industry. These heavy metals are noted for their tendency to build up in the food chain and accumulate in the tissues of fish causing several debilities (Olsson, 1998, Thopon, *et al*. 2003; Thopon, *et al*., 2004; Besirovic *et al*., 2010). Hyperplasia, epithelia lifting, necrosis, lamella fusion and displacement of cartilaginous core observed in the gill tissues of *C. gariepinus* in this study characterised tissue alterations due to metal intoxication in fish (Hanna *et al*., 2005; Figueiredo-Fernandes *et al*., 2007; Besirovic *et al*., 2010; Otludi *et al*., 2017). Parallel to this findings, such histopathlogical alterations have also been reported in the gill of fish exposed to petroleum residues (Engerhardt, *et al*., 1981), sewage effluent (Coutinho and Gokhale, 2000; Fontaínhas-Fernandes *et al*. 2008)), mining effluent (Tkatcheva *et al*. 2004), drugs (Schwaiger *et al*., 2004), tannery wastewater (Navaraj and Yasmin, 2012) and pesticides (Somdare *et al*., 2015). Thus, indicating that these alterations are not specifically related to a particular toxicant and different toxicants may induce similar histopathological alterations in fish; further corroborating the observation of Mallat (1985).

The manifestations of epithelial lifting, hyperplasia and oedema are defensive mechanisms to reduce the gill superficial area in contact with the contaminated water. However, these mechanisms increase the distance between the external environment and the blood stream thereby causing respiratory impairment due to gill dysfunction resulting in reduced oxygen uptake (Kumar *et al*., 2010; Somdare *et al*., 2015). Lamella fusion may also be a protective or adaptive response of the fish to reduced absorption rate in order to prevent the entry of the toxicant into the body (Olurin *et al*., 2006). Soughing of epithelia tissue could be due to epithelial detachment (Machado and Fanta, 2003) and oedema, suggestive of proliferation of lesion in the gill as observed in this study agree with the reports of Navaraj and Yasmin (2012) and Emere and Balogun (2014). Necrosis of gill epithelial cells, uncontrolled regeneration of the primary and secondary lamellae and hyperplasia have been reported by Oguz (2015) in fish migrating from alkaline water to fresh water.

The liver is known to be associated with detoxification and biotransformation processes, and due to these functions combined with its location and access to blood supply, it is one of the organs most affected by water borne contaminants (Camargo and Martinez, 2007). In this study, abnormalities such as irregular clumps and convergence of sinusoids, displacement of cartilaginous core, hepatic hyperplasia and necrosis and vacuolar degeneration are all similar to the reports of Tilak *et al.* (2005) and Younis (2013). These abnormalities may be explained

by the direct toxic effects of the paint wastewater on the hepatocytes of the liver. According to Gingerich, (1982), vacoular degeneration of hepatic cells is indicative of an imbalance between the rates of synthesis of substances in the parenchymal cells and the rate of their release into the circulatory system. Lindstoma-Seppa *et al.* (1981) opined that alterations in liver tissues are probably linked with alterations in gills and kidney. If this assertion holds true, it follows that the disruptions of the branchial tissue of *C. gariepinus* in this study which might have resulted in oxygen deficiency may have caused the degeneration and malfunction of the liver. Oxygen deficiency due to gill degeneration has also been observed as the most common cause of cellular degeneration in fish liver (Younis *et al*., 2013).

4. Conclusion

Conclusively, this study revealed that paint wastewater poses threat to the survival of *Clarias gariepinus*, as evidenced by the various histological changes observed in the branchial and hepatic tissues. Therefore, there is need to enforce stringent laws that will prevent industries from discharging paint wastewater into water bodies without treatment.

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