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Histopathological Observations on Liver and Gills of *Clarias gariepinus* Juveniles Treated with Some Prophylactics

Jeremiah Olanipekun Jimoh[*1], Bayode Paul Omobepade[1], Ademola Michael Akinsorotan[1], John Bunmi Olasunkanmi[1], Tolulope Omolayo Ariyomo[1] and Victoria Oluwabunmi Fatoyinbo[1]

[1]Department of Fisheries and Aquaculture, Federal University, Oye Ekiti PMB 373, KM 3, Are/Afao Road, Oye Ekiti, Ekiti State, 370112, Nigeria

*Correspondence :
jeremiah.jimoh@fuoye.edu.ng

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Abstract

This study investigated histopathological changes in the liver and gills of *C. gariepinus* juveniles treated intermittently with different concentrations of prophylactics. Potassium permanganate (T2), Hydrogen peroxide (T3), and Formalin (T4) were used as prophylactics and administered to 15 fish each at concentrations of 2 ppm, 150 ppm, and 100 ppm respectively. The treatments which were in three replicates each with a control setup (T1) were repeated biweekly for 10 weeks. The histomacrographs of the liver and the gills show that there were no visible alterations in the structures of the tissues of fish in the control experiment (T1). However, major histopathological observations for the fish in T2, T3, and T4 were severe degeneration in the gill structure, fusion of the gill rakers, slight changes in the hepatocytes, and diffuse vacuolation of hepatocytes. Among the used chemicals, potassium permanganate appeared to have the least negative effects on the tissues and it is therefore recommended at 2 ppm for prophylactic treatment of fish against diseases.

INTRODUCTION

Disease issues are of great concern in aquaculture production (Idowu *et al.,* 2017). It is a condition in living organisms that causes impairment of physiological functions as a result of alteration in the body systems and is manifested by symptoms (Adedeji *et al.,* 2017). Fish diseases affect the survival and growth rates of fish under culture (Sogbesan and Ugwumba, 2008). Studies showed that almost fifty percent of production loss is because of more severe diseases in developing countries (Assefa and Abunna, 2018). To combat diseases, farmers often resort to the use of synthetic chemicals. These chemicals are important components that have contributed to the success of aquaculture and they have been used in various ways for centuries (Subasinghe *et al*., 1996).

Common chemicals used among Nigerian fish farmers include formalin, hydrogen peroxide, potassium permanganate, common salt copper sulfate, etc. (Awa and Alegbeleye, 2006). The use of these chemicals may be to disinfect, aid spawning, control disease or simply used as prophylactics for disease prevention. However, there is concern over using and

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potentially misusing some of these chemicals. The misuse of approved drugs or chemicals in aquaculture may pose potential hazards to fish. These substances may be toxic, allergenic, or carcinogenic or cause antibiotic resistance in pathogens (Manyi-Loh *et al.*, 2018). Sometimes farmers apply a higher concentration of chemicals than the recommended concentration without taking into consideration the effects the chemical might have on the fish (Singh and Singh, 2018).

Marecaux *et al.* (2005) reported 100 % mortality when a 3.0 mg/l concentration of $KMnO_4$ was used to treat *Poecilia latipinna*. *C. gariepinus* broodstock exposed to 2 ml of 37% formalin were observed to develop disrupted and depleted seminiferous tubules in the testes, while the liver was observed to develop multifocal necrosis of the hepatocytes (Adeyemo *et al.,* 2012). Similarly, a wide range of histopathological alterations such as lamellar necrosis, hyperplasia, lamellar adhesion, and clubbing of gill lamellae was observed in gills tissue of grass carp, *Ceteopharyngodon idella* exposed to copper sulfate and potassium permanganate with the severity of these alterations increasing with increasing of the doses of the prophylactics (Jooyandeh *et al.*, 2016). Reduced growth rate has been reported in fish treated with high doses of hydrogen peroxide (Speare *et al*. 1999).

This study therefore is aimed at examining the histopathological changes on the liver and gills of *C. gariepinus* treated with prophylactics.

METHODOLOGY

Ethical Approval

All procedures were carried out in the analytical techniques laboratory of the Department of Fisheries and Aquaculture in accordance with the ethical standard of the animal ethics committee of the Federal University, Oye Ekiti, Nigeria.

Place and Time

The experiment was conducted at the Department of Fisheries and Aquaculture, Federal University, Oye Ekiti, Nigeria between August and October 2018.

Research Materials

The research materials include 180 fingerlings of *C. gariepinus* (weight, 2.44± 0.05g), plastic aquaria (36 x 25 x 25 cm), potassium permanganate, Hydrogen peroxide, formalin, hematoxylin, eosin, commercial feed (40% CP), electronic weighing balance Kerro BL10001 compact scale, mercury-in-glass, pH meter (Jenway model 9060), oxygen meter (Hanna model H1-9142), and light microscope.

Work Procedure

Fish Rearing

One hundred and eighty fingerlings (2.44±0.05g) were procured from a reputable farm. They were transported to the laboratory in an open plastic bucket containing aerated water. The *C. gariepinus* fingerlings were acclimatized in plastic holding tanks for 21 days**.** Fifteen fish were randomly distributed into 12 plastic aquaria (36 x 25 x 25 cm) in triplicates. They were fed twice daily at 3% of body weight with commercial feed (40% CP). The treatments in the form of short baths were given on the first day of the experiment for 30 minutes each and then repeated biweekly for 10 weeks. All used water was diluted and drained into a septic tank for proper disposal as effluent. Weights of fish were taken fortnightly using an electronic weighing balance Kerro BL10001 compact scale.

Water Quality Management and Measurement

Aquaria water was changed every other day while uneaten feeds were daily siphoned. Water temperature, pH, and dissolved oxygen were monitored daily. Temperature was measured using a mercury-in-glass thermometer, pH was

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measured with a pH meter and the dissolved oxygen was measured with an oxygen meter.

Survival Rate

The record of fish mortality in each treatment was recorded daily from where the survival rate (SR) was calculated.

$$SR = \frac{\text{Total number of fish} - \text{Mortality}}{\text{Total number of fish}} \times 100$$

Histopathological Examinations

At the end of the experiment, four fish were randomly selected from each treatment. The fish were anesthetized, before being dissected to remove the gills and livers. The organs were preserved in 10% formalin to retain the structural integrity of the cells and tissue. The dealcoholized tissues were inserted in molten paraffin wax for 3 hours. The tissues were later embedded using a disposable embedding mold and allowed to cool. Sections of organs were cut at 5µm and stained with hematoxylin and eosin (Bancroft and Layton, 2013). The slides were later viewed under a light microscope with 400x image magnification.

A semi-quantitative histopathological scoring system was developed containing both index and ancillary criteria for use in this study (Table 1). The index criteria were selected as common pathological changes that occur in response to the treatments. The ancillary criteria were selected to reflect the occurrence of mild, medium or severe indicators of gill pathology. The score for the index parameters was based on the presence and extent of the following: lamellar hyperplasia (gill arch enlargement), lamellar fusion, and cellular anomalies (including degeneration of gill rakers, and epithelial cell layer positioning) with a score ranging from 0 to 3 being assigned to each parameter (Mitchell *et al*., 2012).

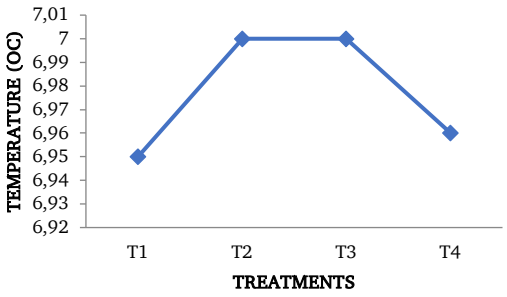
Data Analysis

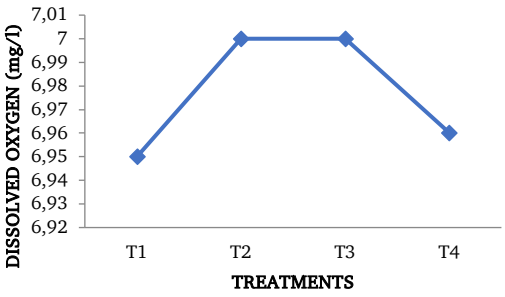
Data from the percentage survival and water quality parameters were subjected to Analysis of variances. Means were separated using the New Duncan Multiple Range Test at a 0.05 significance level.

RESULTS AND DISCUSSION

Water Quality

The results for temperature, dissolved oxygen, and pH of the experimental setup are presented in Figure 1. The results of this study indicated that the mean pH value ranged from 6.92±0.03 to 7.00± 0.06 while the temperature of water is within the range of 22.63±0.33 °C and 22.70± 0.10 °C. Mean dissolved oxygen varied from 9.40±0.00 mg/l to 9.43±0.02 mg/l. There was no significant difference among all the tested water quality parameters in this study.





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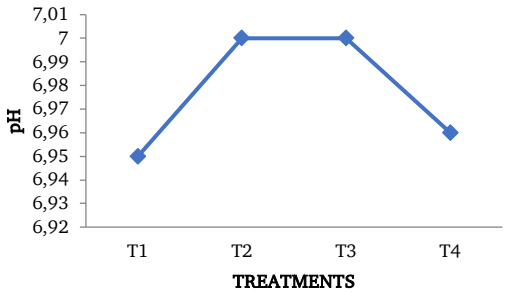

Figure 1. Average water quality parameters during the study.

The water quality parameters recorded were within the range described as optimal by Boyd (2000). This agrees with similar work by Jamabo *et al.* (2015) and Jamabo and Dienye (2017). Therefore, the mortality recorded might not be connected to poor water quality.

Survival Rate

The highest survival rate (72.11± 7.45%) was recorded in the control experiment (T1), followed by treatment 3 (61.91±2.38), treatment 2 (56.00±4.81), and treatment 4 (45.22±10.02) (Figure 2). The poor survival in treatment 4 might be a reason for the severe effect of formalin observed on the gills of the treated *C. gariepinus* (Table 1). A number of critical functions depend on the good working condition of the gills in fish. Apart from respiration, gills also help in osmoregulation, excretion of nitrogenous waste, pH regulation, and hormone production (Herrero *et al.,* 2018). Therefore, a severe alteration in the activities of the gill may lead to mortality (Foyle *et al*., 2020).

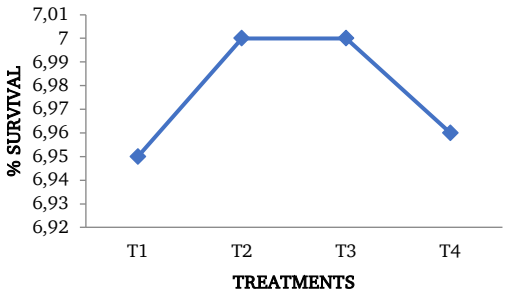

Figure 2. Survival rate of *C. gariepinus*.

Histopathological Examinations

The fish in control treatment had gills with no pathological lesion (Figure 3A), while the gills of fish exposed to 2 ppm of $KMnO_4$, showed severe degeneration in the gill structure with a section showing some eroded gill rakers (Figure 3B). There was a severe degeneration in the gill structure of fish treated in hydrogen peroxide with sections showing that the entire gill rakers have been eroded (Figure 3C). Figure 3D showed a fusion of the gill rakers and an alteration in the gill structure.

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Table 1. Histopathological observations on the gills of *Clarias gariepinus* given prophylactic treatments.

Treatments	Pathology score	Lamellar hyperplasia	Lamella fusion	Cellular anomalies
T1	None (0)	None	None	None
T2	Mild (1)	Mild expansion of the gill arch	None	Slight lifting of epithelial cell layer
T3	Severe (3)	Severe expansion of the gill arch	Severe degeneration of the gill rakers	Extensive lifting of epithelial cell layer. More than 50 % of gill tissues affected
T4	Severe (3)	Severe expansion of the gill arch	Severe fusion of gill rakers	Severe alteration of the entire gill structure.

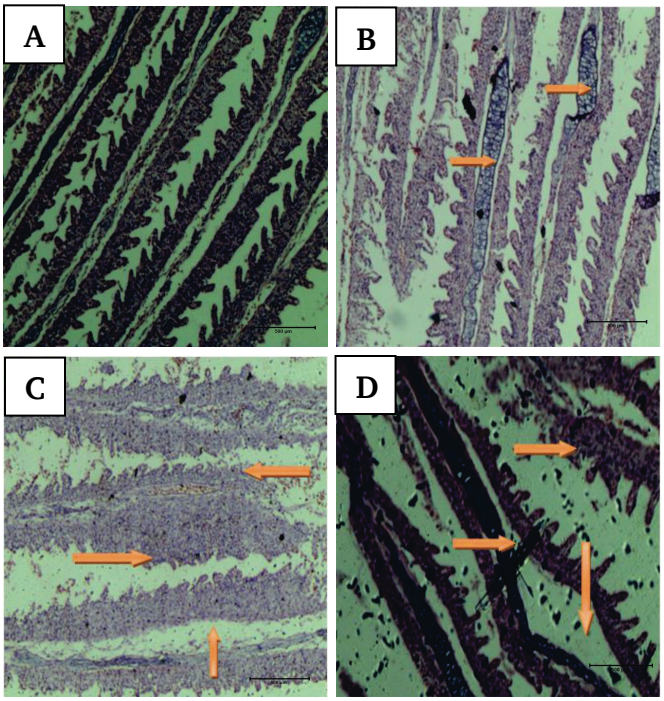

Figure 3. Gill tissues of *C. gariepinus* in each treatment. A. Gills of *C. gariepinus* in T1 with no pathological lesion; B. Gill of *C. gariepinus* in T2 with expansion of the gill arch; C. Gills of *C. gariepinus* in T3 showing the degeneration of the gill rakers; D. Gills of *C. gariepinus* in T4 showing the fusion of the gill rakers and severe alteration of gill structure.

On the liver tissues, the control treatment showed no pathological lesion (Figure 4A). However, the photomicrograph of *C. gariepinus* liver exposed to 0.74ml of potassium permanganate showed slight changes in the hepatocytes (Figure 4B) while the liver of fish treated with 0.34 ml of hydrogen peroxide showed an increase in interstitial cell and vacuolation (Figure 4C). For fish treated with formalin, there was a very mild diffuse vacuolation of hepatocytes on the liver.

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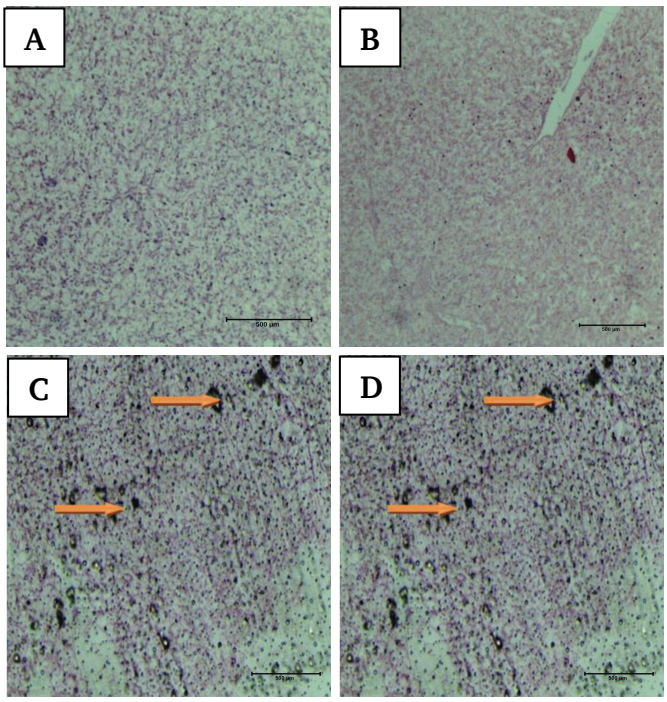

Figure 4. Liver tissues of *C. gariepinus* in each treatment. A. Liver of *C. gariepinus* in T1 showing no pathological lesion; B. Liver of *C. gariepinus* in T1 showing slight changes in the hepatocyte; C. Liver of *C. gariepinus* in T3 showing an increase in the interstitial cell and diffuse vacuolation of hepatocytes; D. Liver of *C. gariepinus* in T4 showing a very mild diffuse vacuolation of hepatocytes.

The histopathological examination of the gill and liver of the exposed fish indicated that the gills and liver were affected by the chemicals. In fish, gills are critical organs for their respiratory, osmoregulatory, and excretory functions (Ezemonye and Ogbomida, 2010). Gills are considered good indicators of water quality, being models for studies of environmental impact, since they are the primary route for the entry of chemicals (Akindele *et al.*, 2015). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply. Damage to these vital organs can cause a chain of destructive events, which will ultimately lead to respiratory distress.

Ezemonye and Ogbomida (2010) reported that if gills were destroyed due to xenobiotic chemicals or the membrane functions were disturbed by a changed permeability, the oxygen uptake rate would be rapidly decreased. Gill exposed to $KMnO_4$, showed slight degeneration in the gill structure with a section showing some eroded gill rakers. There was a severe degeneration in the gill structure with a section showing that all of the gill rakers have been eroded in the fish treated with hydrogen peroxide. Plate 4 showed a fusion of the gill rakers and alteration in the gill structure for fish treated with formalin. This is similar to the observation of Adebayo and Fapohunda (2016). Vascular changes in the gills of exposed fish could be attempts by the fish to supply more blood to the gills, to increase oxygen uptake and supply to the internal organs (DiGuilio and Hinton, 2008).

According to Ezemonye and Ogbomida (2010), the liver is the main organ for detoxification. The liver is the main organ that suffers serious morphological alterations in fish exposed to pesticides and chemicals (Rodrigues and Fanta, 1998). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had slight

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changes in the hepatocytes and vacuolated cells showing evidence of fatty/severe degeneration. This result agrees with those of Ezemonye and Ogbomida (2010), Olukunle (2011), and Jimoh *et al.* (2015).

CONCLUSION

This study has revealed that exposure of *C. gariepinus* to potassium permanganate, hydrogen peroxide, and formalin can cause severe changes in the physiology of *C. gariepinus* as manifested in the histological parameters. Persistent exposure of fish to these chemicals, especially formalin may lead to the mortality of *C. gariepinus* due to disruption of internal physiology. It is recommended that good management practices that would not warrant disease infections in farms should be observed constantly. However, if a farmer must use chemicals to treat fish, in cases of infections, potassium permanganate would be recommended as its effect on fish histology was not as severe as the other two chemicals.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare concerning this study.

AUTHOR CONTRIBUTION

Jeremiah Olanipekun Jimoh wrote the manuscript, Bayode Paul Omobepade, carried out the histological analysis, Ademola Michael Akinsorotan did the proofreading and editing, John Bunmi Olasunkanmi designed and supervised the experiment in conjunction with Tolulope Omolayo Ariyomo while Victoria Oluwabunmi Fatoyinbo monitored the experiment.

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