HISTOLOGICAL STUDIES ON THE EFFECT OF PHOSTOXIN ON AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES

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ABSTRACT
The toxic effects of phostoxin on juveniles of African catfish (Clarias gariepinus) were investigated. One hundred and twenty (120) juveniles of Clarias gariepinus (wt. 25 ± 0.25 g) were allotted into twelve (12) glass tanks (60 cm x 40cm x 40 cm) at ten (10) fish per tank/treatments (A - F) in two replicates based on different concentrations of Phostoxin (0.01, 0.02, 0.03, 0.04 and 0.05 mg/l) used while treatment A serves as control (0.0 mg/l) tank.. The test fish exhibited some behavioural abnormalities such as; loss of reflex, restlessness, colour change, high rate of opercula movement, abnormal swimming and mortality. The histology of the test fish revealed changes in the gills which included erosion of the secondary lamellae, loss of epithelia cells and degeneration of lamellae, alterations observed in the liver tissues fish were visible lesion, necrosis and mild degeneration while the kidneys showed mild degeneration of renal tubules, interstitial congestion, and diffuse tubular degeneration at high concentrations of phostoxin thereby leading to physiological dysfunctions of the test organs. The 96 h LC_{50} value was observed at 0.025 mg/L. Hence, discharges of phostoxin into the aquatic environment should be restricted.

INTRODUCTION

According to Olurin *et al.,* (2006), fish are sensitive to a wide variety of chemicals and toxic conditions in the aquatic environment which may cause decreased growth, alterations in physiological processes and ultimately death. Pest mitigation agents are typically classified into insecticides, rodenticides, fungicides, herbicides and fumigants (Akan *et al.*, 2013) depending on the type of organisms they are used for. Pesticides are used nearly on daily basis due to their unique activity in controlling pests. Typically, pesticides are recalcitrant to degradation. They may remain in the environment for long period of time which later results in fish mortality and morbidity.

Fish and other aquatic biota may be harmed by pesticides contaminated water. In recent times, pesticides have been widely deployed for the control of weevils in grains especially maize and in terrestrial habitats. The use of herbicides for the control of weeds especially in areas and situations were manual labor is inadequate could also contaminate water leading to bioaccumulation of their compounds in the water bodies. Ladipo *et al.,* (2011) reported that herbicides can accumulate in aquatic ecosystem and have adverse impact on zooplankton community which is a major food source for young fish. Pollution of aquatic ecosystem by natural and anthropogenic activities is a major challenge affecting its sustainability (Ubong *et al.,* 2015; Ogundiran *et al.,* 2010). Improper wastes disposal from industrial facilities could contaminate the environment. In a developing country like Nigeria, surveillance of industrial chemical wastes which are washed off by erosion as runoff into the aquatic environment is poor. Akinsorotan (2014) stated that most Nigerian aquatic ecosystems are experiencing neglect and misuse due to non-enforcement of laws regulating their use. The effect of agricultural chemicals use and their residue on non- target organisms have not been seriously considered in Nigeria (Ayoola, 2007). The indiscriminate use of pesticides, careless handling, accidental spillage or discharges of untreated effluents into natural waterways have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment (Akhtar, 1986). Water pollution by pesticides is a serious problem to all aquatic fauna, flora and man (Ayoola, 2008). In the aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes (Vasanthi *et al*., 1989).

The African catfish (*C.gariepinus*) is the most cultu red fish in Nigeria (Omitoyin, 2004). *C.gariepinus* tolerates both well and poorly oxygenated waters and is widely cultivated and thus used as a biological indicator in ecotoxicology studies (Ayoola, 2008).It is common preliminary practice in fish culture operations to use synthetic toxins including chlorinated hydrocarbons and organophosphates to eradicate predators and competing fish from nursery, rearing and production ponds prior to the stocking of preferred commercial fish species.

Phostoxin which is either in form of tablets or pellets contains 55% aluminum phosphide as its active ingredient and 45 % inert agents which is used as the carrier of the active ingredient. On exposure to water it reacts with it to liberate phosphine gas (Gordon 1972). Phosphine gas is by far the dominant means of controlling insect pests in stored grain and many other stored commodities (Nath *et al.,* 2011). The phosphine gas emitted is the poison used to kill insect pests in grains without affecting grain viability (Atta *et al.,* 2009). Because phosphine gas is highly toxic to aerobic organisms, it could therefore be of considerable health risk to aquatic life. The phosphine gas is colorless and odorless in

its pure form but, due to the presence of substituted phosphines and diphosphines, it has a foul odor resembling that of decaying fish (Chugh 1992).

Fish histology is a very interesting model for the study of interactions between environmental factors and hepatic structures and functions of the organs (Brusle and Anadon 1996). Specifically, in clinical medicine, histology refers to the examination of a biospsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides. Research on fish histology is important, especially in the field of problems induced by aquaculture conditions, aquatic pollution and diseases (Gochfeld *et al.,* 2003). This is the most important tool of the anatomical pathologist in routine clinical diagnosis of cancer and other diseases (Gochfeld *et al.,* 2003).

MATERIALS AND METHODS

One hundred and twenty (120) apparently healthy *C. gariepinus* juveniles (wt. 25 ± 0.25g) were purchased from the Teaching and Research Farm of Fisheries and Aquaculture Department, The Federal University of Technology, Akure and transported to the Limnology laboratory of the Department of Fisheries and Aquaculture Technology for the experiment. The *C. gariepinus* juveniles were weighed using an electronic scale (Mettler Tolebo PB8001) and were distributed uniformly into the twelve (12) experimental tanks (60 cm x 40 cm x 40 cm) at ten (10) fish per tank (10L). Six (6) treatments were used (A, B, C, D, E, F), each treatment replicated twice .The fish were fed to satiation during the acclimatization period (5 days) with commercial feed (2mm of Rannan fish feed) and feeding stopped 24 hours prior to the commencement of the study to minimize the production of waste materials and reduce ammonia build up in the container. The water in the tank was renewed every 24 hours during the feeding period as recommended by Oyelese and Faturoti (1995). Water quality parameters such as temperature, dissolved oxygen, pH and conductivity of the experimental setup were measured and monitored using standard methods APHA (2005).1g of phostoxin was dissolved in 1000 ml of distilled water to form stock solution of 1000 mg/l. From the stock solution, five (5) varying concentrations (0.01 ,0.02, 0.03, 0.04 and 0.05 mg/l) with a control (0.0) were prepared. The pre-determined amounts of phostoxin solution were measured out using 5ml pipette into aquaria tanks containing 10 litres of water. This was prepared 24 hours before the experiment so that the toxicant will be thoroughly dissolved in water. The median lethal concentration (LC_{50}) was determined using probit analysis and graphical method (Finney, 1971). The responses of the fish to slight stimuli were used as an index for toxicity or death. After the 96 h experiment, gills, liver and kidney specimens were excised from fish alive and were preserved in 10% neutral buffered formalin for three days to preserve the structure of the organs and their molecular composition from tissue digestion (autolysis) using standard histological techniques (Avwioro, 2002). All data obtained were presented as mean ± standard error using One Way Analysis of Variance (ANOVA). All statistical analysis were performed using Statistical Package for Social Sciences (SPSS version 20.0 by Sheridan 2012)

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}value was observed at 0.025 mg/L (Figure 1).

Table 1: Behavioral responses of *C. gariepinus* during 96 h exposure to phostoxin.

	24 HOURS						48 HOURS						72 HOURS						96 HOURS					
CONCENTRATION (MGL)	0.0	0.01	0.02	0.03	0.04	0.05	0.00	0.01	0.02	0.03	0.04	0.05	0.0	0.01	0.02	0.03	0.04	0.05	0.00	0.01	0.02	0.03	0.04	0.05
ERRATIC SWIMMING	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
LOSS OF REFLEX	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
HYPER VENTILATION	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+
CHANGES IN BEHAVIOUR	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+
DISCOLOURATION	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+

+ : Presence of specific observation

- : Absence of specific observation

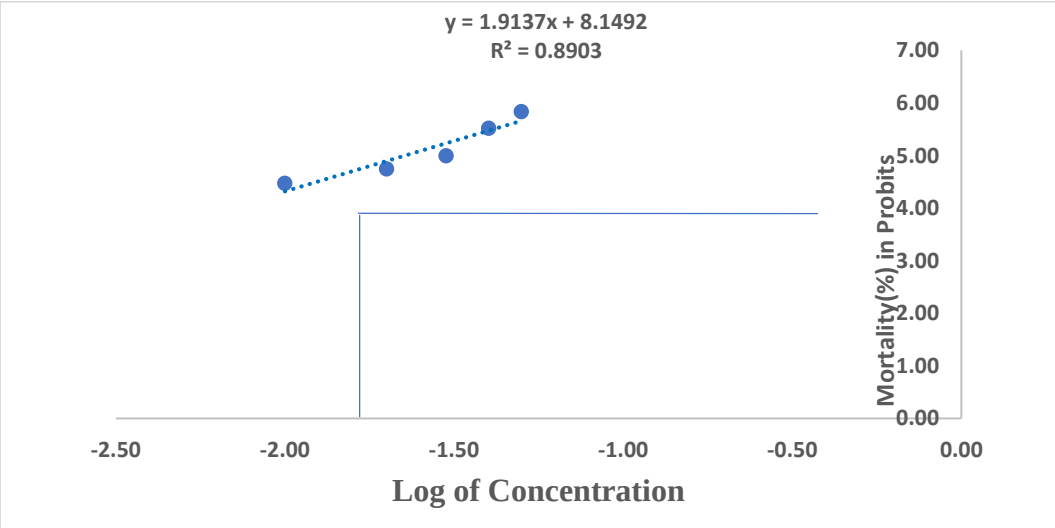
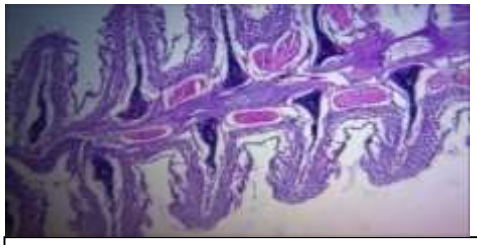


Figure 1: Median Lethal Concentration (LC_{50}) of phostoxin on *C. gariepinus* juveniles.

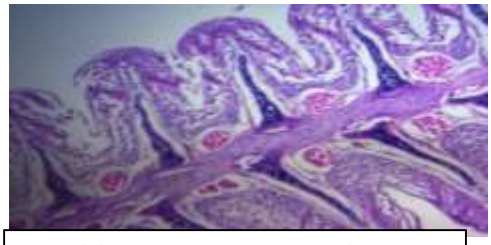
Changes in the gills, liver and kidney of fish were observed to occur as a result of exposure to environmental stressors such as toxicants (Adebayo and Fapohunda 2016). Fish gill is an organ with a large surface area, highly sensitive to the effect of toxicant (Reiser *et al*., 2010). The gills of fish exposed to varying concentrations of phostoxin showed severe erosion in the gill filament and degeneration of the gill lamellae with increasing toxicant concentration as well as loss of epithelial cells. These findings agreed with the reports of Olufayo and Alade (2012) who reported same in *Heterobranchus bidorsalis* exposed to acute toxicity of varying concentrations of Cypermethrin. The liver is prone to damage by toxic chemicals due to its role as a vital organ in breaking down chemicals (Ladipo *et al*., 2011). Liver histology is highly sensitive and is an accurate way to assess the effect of any pollutant on fish. Length of exposure of toxicant to an organism determines the severity of injury on the organs of the fish (Ferguson, 2006). Normal liver architecture was seen in liver of control fish (0.0mg/l), while some pathological alterations, vacuolations of the hepatocytes were recorded in liver of fish exposed to phostoxin concentations. The vacuolar degeneration of hepatocytes and disintegration of the sinusoids that were observed in fish liver in this study was as a result of liver sensitivity to toxicant. The histological section of the liver of *C. gariepinus* juvenile exposed to sub-lethal concentration of phostoxin for 96 h in this study showed abnormal pattern when qualitatively compared with the control group. Ladipo *et al.*, (2011) reported similar result in *C. gariepinus* juveniles exposed to acute toxicity of paraquat dichloride. However, there was evidence of advancing phase of hepatocyte necrosis in *C. gariepinus* juveniles exposed to phostoxin in this

study. Exposure of *C. gariepinus* juveniles to sub-lethal concentrations of phostoxin beyond 96 hours may therefore inflict high noticeable pathological changes in the hepatocyte. The kidney which serves as the major osmoregulatory organ can also be used as pathological indicator of environmental pollution because the largest proportion of post-branchial blood goes to fish kidneys (Cengiz, 2006). Several studies have revealed histological characteristics of kidney as an indicator of pollution (Ortiz *et al.*, 2003; Cengiz, 2006). The kidney of the fish used in this study showed degeneration as the concentration of phostoxin increased. Changes noticed in the kidney of *C. gariepinus* used for this study showed moderate tubular degradation, mild degeneration of renal tubules, shrunken tubules and severe interstitial congestion. Similar results were reported in the kidneys of fishes exposed to different toxicants but the extent of damage varies depending on the dose of toxicants used, duration of exposure, toxicity of chemical and susceptibility of the fish. This also agreed with the findings of Olufayo and Alade (2012) who exposed *Heterobranchus bidorsalis* to cypermethrin concentration and reported that the kidney cells were massively destroyed and causing consequent obstruction to their physiological functions.

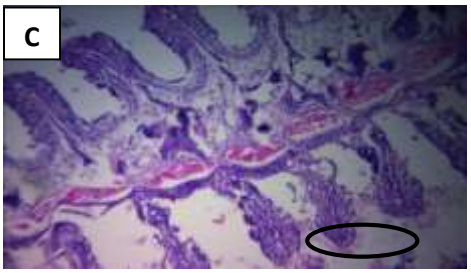
Plate 1: Photomicrograph of Gills of *C. gariepinus* exposed to varying concentrations of phostoxin.



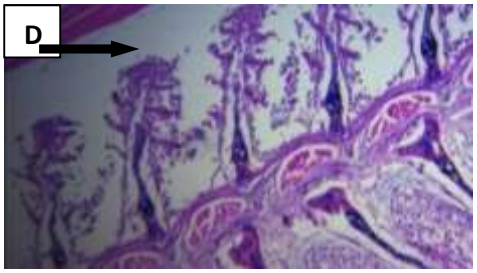
Gill of *C. gariepinus* exposed to 0.01mg/l of Phostoxin shows a moderate to severe erosion of the secondary lamellae (arrow).



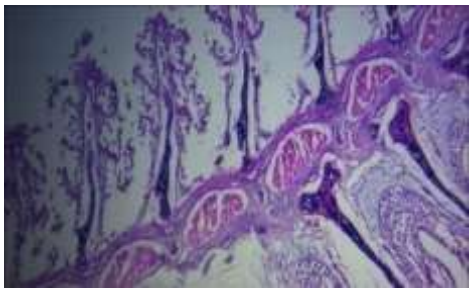
Gill of fish exposed to 0.05mg/l of Phostoxin shows complete degeneration of lamellae and necrosis.



Gill of *C. gariepinus* exposed to 0.02mg/l phostoxin shows a severe erosion with stunting of lamellae (circle) seen in the gill of fish.



Gill of fish exposed to 0.03mg/l of Phostoxin shows severe vacuolization of the gill architecture and alteration of the primary structure of the gill filament (arrow).



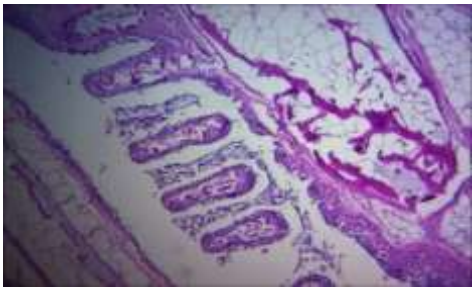
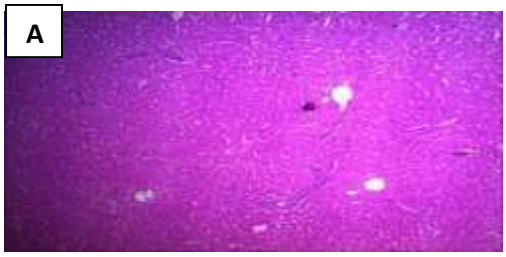
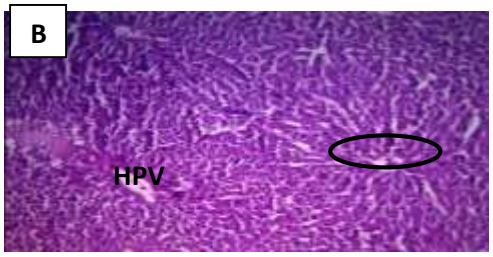


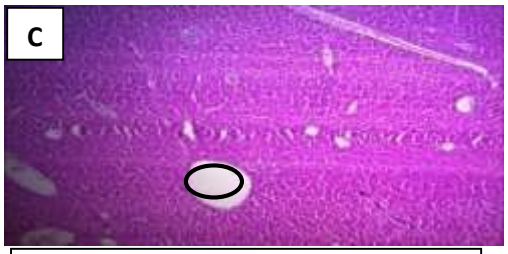
Plate 2: Photomicrograph of Liver of *C. gariepinus* exposed to varying concentrations of phostoxin.



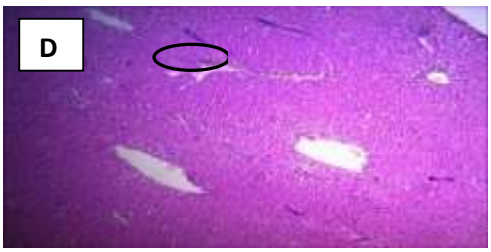
Section of liver of fish exposed to 0.0mg/l of phostoxin (Control)shows no lesion observed in the liver cells.



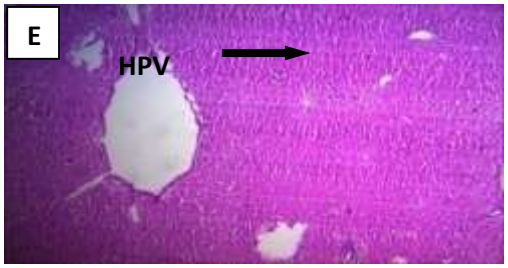
Liver of the fish exposed to 0.01mg/l of phostoxin shows pyknotic nuclei with slight degeneration and vacuolation in the hepatocytes (circle).



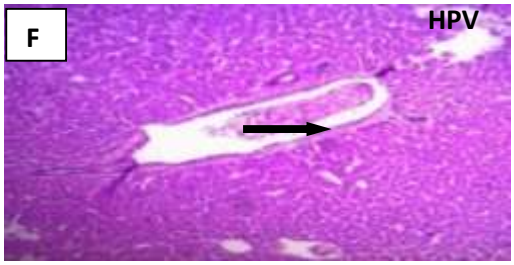
Liver of fish exposed to 0.02mg/l of phostoxin shows a diffuse severe vacuolation of thehepatocytes (circle) and there was disintegration of the sinusoids.



Liver of fish exposed to 0.03mg/l of phostoxin shows moderate to severe periportal vacuolar degeneration of hepatocytes (circle), the lesions is focally diffuse in some other sections.

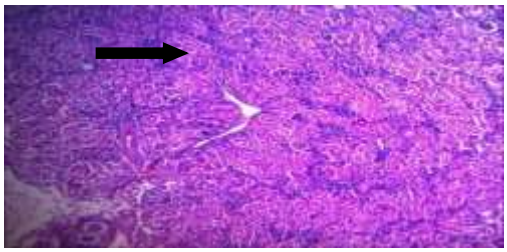


Liver of the fish exposed 0.04mg/l of phostoxin shows alteration in the hepatocites and disintegration of the sinusoids (arrow) with diffuse vacuolation of hepatocytes.

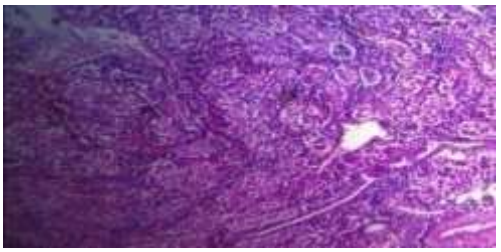


Liver of the fish exposed to 0.05mg/l of phostoxin shows destruction of the normal architecture of the liver cells, destruction of sinusoids (arrow) and centre of necrosis in the parenchyma of the liver cells.

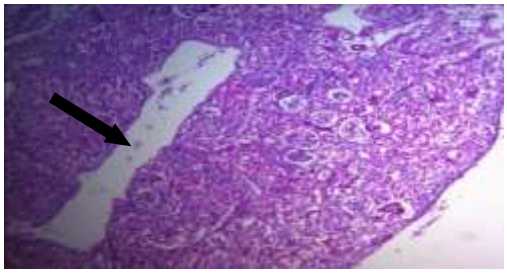
Plate 3: Photomicrograph of Kidney of *C. gariepinus* exposed to varying concentrations of phostoxin.



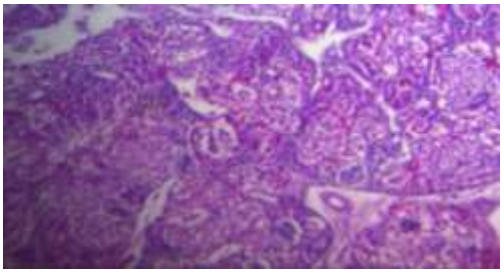
Section of kidney of *C. gariepinus* exposed to 0.0mg/l of phostoxin shows no visible lesions seen.



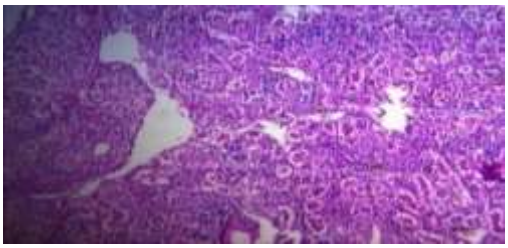
Kidney of *C. gariepinus* exposed to 0.01mg/l of phostoxin shows moderate tubular degradation (arrow).

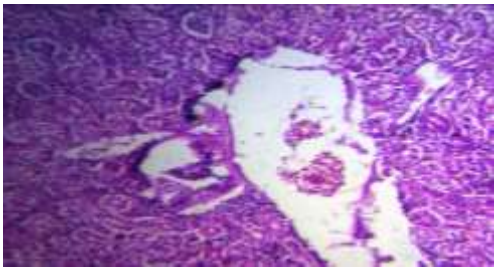


Kidney of *C. gariepinus* exposed to 0.05mg/l of phostoxin showing mild tubular degradation (arrows). There is severe interstitial congestion and oedema (circle).



Kidney shows a very mild interstitial congestion (arrow) with tubular degeneration in fish exposed to 0.03mg/l of phostoxin.





CONCLUSION

The result obtained from this study revealed that phostoxin is highly toxic to the *Clarias gariepinus* juveniles. The 96 h LC_{50} value was observed at 0.025 mg/L. It has the potential to damage the physiology of fish leading to changes in their behavioural pattern and eventually mortality. Histological results of the study clearly indicated that sub-lethal concentrations of phostoxin have destructive effects on the gills, liver and kidney tissues of the test *Clarias gariepinus* juveniles.Toxicity of phostoxin on *C. gariepinus* increased as phostoxin concentrations increased. In the aquatic environment, this can negatively affect the ecosystem depending on the concentrations used. Although, the aquatic environment is not the target of such pesticide but its widespread use has led to some serious problems when washed into water body. Hence, discharges of this pesticide (phostoxin) into the aquatic environment should be restricted.

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