

# Effects of Acute Concentrations of Vestaline® (pendimethalin) Herbicide on Histopathology of Liver and Gills of Exposed *Clarias gariepinus* Juveniles

Odo Joel Inya<sup>1,\*</sup>, Manyi Manasseh Msugh-Ter<sup>2</sup>, Ebah Esther Eneyi<sup>3</sup>

<sup>1</sup>Department of Fisheries and Aquaculture University of Agriculture, Makurdi, Nigeria

<sup>2</sup>Department of Zoology, University of Agriculture, Makurdi, Nigeria

<sup>3</sup>Department of Microbiology, University of Agriculture, Makurdi, Nigeria

## Email address:

odojoel@gmail.com (O. J. Inya)

\*Corresponding author

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**Abstract:** This study was carried out to determine histopathological damages of liver and gills caused by acute concentrations of vestaline® (pendimethalin) herbicide on *Clarias gariepinus* using 96-hour bioassay test. The toxicity was carried out using 180 healthy fish of mean weight  $27.97 \pm 0.03$ g which were divided into six treatments with each treatment having ten fish and the setup was in triplicate. The concentrations of Vestaline® (pendimethalin) herbicide used were (0.0mg/l, 33mg/l, 82.5mg/l, 115.5mg/l, 148.5mg/l and 181.5mg/l). After the 96-hours, both gills and liver of the fish were removed from dead fish. Photomicrographs of the histological slides were taken. Histology of the liver in control sample showed normal hepatic tissue (Hepatocytes with granular cytoplasm) and round nucleus. Histology of the gills in control sample showed normal structure of the filaments and lamellae. The histology of the exposed liver showed vacuolation of cytoplasm, rupture of hepatocytes, bile pigment disintegration, necrosis and cytoplasmic degeneration while the histology of gills exposed to different concentrations of vestaline® (pendimethalin) herbicide showed marked distortion of the gills lamellae, hyperplasia of lamellar epithelium and necrosis. The acute concentrations confirmed that vestaline® (pendimethalin) herbicide is highly toxic to *Clarias gariepinus* and showed heightened damages at higher concentrations.

**Keywords:** *Clarias gariepinus*, Vestaline® (pendimethalin), Histopathology, Acute

## 1. Introduction

Vestaline® herbicide belongs to the group of dinitroaniline and its active ingredient is Pendimethalin. It is used in premergence and postmergence applications for the control of grasses. [6]

Pendimethalin (Dinitroaniline) herbicides act by inhibiting cell division (mitosis), specifically; they inhibit microtubulin synthesis necessary in the formation of cell walls and in chromosome movement to daughter cells during mitosis [5]. The cell does not complete division and affected cells remain as single cells with multiple nuclear chromosomes. The herbicides kill susceptible plants by inhibiting cell

division in root cells, which arrests normal root growth. This inhibition leads to plant dehydration due to severely restricting the root system size and function.

Histopathological studies have been conducted to help establish relationship between contaminated exposure and various biological responses. The advantage of histopathology as a biomarker lies in its intermediate location with regards to the level of biological organization [2].

Histological changes appear as a medium-term response to stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs [7].

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out in the Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Benue State Nigeria.

### 2.2. Sample Collection

A total of two hundred and fifty (250) Healthy juveniles of African catfish *Clarias gariepinus* were collected from University of Agriculture Makurdi fish farm for the study. The fish were transported in aerated containers to the laboratory. The fish were acclimatized for two weeks during which they were fed with commercial floating feed (coppens) at 5% body weight. Unconsumed feeds were removed and water replenished twice a week as recommended by [13]

### 2.3. Experimental Procedure

Static renewal bioassay technique was used by which the test media was renewed. Preliminary test was carried out to determine the appropriate concentration range for testing chemicals [15].

A total of eighteen (18) glass aquaria were used for the study. Ten juveniles of *Clarias gariepinus* were introduced into each aquarium containing 20 liters of water into which the concentrations (33mg/l, 82.5mg/l, 115.5mg/l, 148.5mg/l and 181.5mg/l) of Vestaline® were introduced. The experiment was carried out for a period of 96 hours. Dead fishes were removed and organs (gills and livers) removed into 10% formaldehyde.

### 2.4. Histological Assessment

This assessment was carried out at the Anatomy Laboratory, Department of Veterinary Medicine, University of Agriculture, Makurdi; Benue state. The methods of acute toxicity tests as described by [4] were employed.

The gills and liver of fish exposed to lethal concentrations of herbicide were obtained by dissection. Fish that died from the exposure were immediately removed and dissected to obtain gills and liver.

The gills and liver extracted from the fish were immediately fixed in 10% formaldehyde to prevent spoilage. Dehydration was done by putting the fixed liver and gills in various grades of alcohol over different periods ranging from 70% alcohol for 3-8 hours; 90% alcohol for 16 hours; absolute alcohol 1 for 2-3 hours; absolute alcohol 11 for 3 hours; to absolute alcohol 111 for 3 hours and finally into xylene for 17 hours. This process ensures hardening of the tissue and impregnation with wax 1 and wax 11. Embedding was done using wax and embedment mould. After solidifying, the samples were trimmed and mounted on wooden block to fit in microtome. The microtome was used to section samples into 5µm for all histological samples. The samples were further floated with warm water in a floating out bath to unfold the tissue. The tissue were picked, put on a slide and dried on a hot plate and were stained with

hematoxylin and eosin. Finally, the samples were read under a microscope at different magnifications for various changes in the architectural make up of the organs and then followed by microphotographs.

## 3. Result

The Histopathology of *Clarias gariepinus* liver and gills exposed to acute concentrations of Vestaline® (pendimethalin) herbicide are presented in the plates below. From the study, No histopathological changes were observed in the liver and gills of the control fish (Plate 1 and Plate 7) respectively. The histology of the liver of the control fish showed normal hepatic tissue, hepatocytes with granular cytoplasm and round nucleus while the gills consisted of a primary filament, secondary lamellae and epithelial cells. Plate 6 which is Section of the liver exposed to the highest concentration (181.5mg/L) of Vestaline® (pendimethalin) Showed vacuolation of cytoplasm, rupture of hepatocytes, bile pigment disintegration and necrosis while Plate 2 (33.0mg/L) showed vacuolation and cytoplasmic degeneration. Plate 12 (181.5mg/L) showed marked distortion of the gills lamellae, hyperplasia of lamellar epithelium and necrosis. Changes in the structure of Liver and gills are dose dependant.

## 4. Discussion

Histopathology is widely accepted as a useful method for the assessment of injury in fish to the adverse short term and chronic effect of pesticides. Several live lesions have been established as tissue bio-makers consistent with the exposure of fish pesticides.

### 4.1. Gills

Gills can be used as models for studies of environmental impact and can be generally considered as good indicator of water quality [8, 16].

From the present study, No histopathological changes were observed in the gills of the control fish. Each gill consisted of a primary filament, secondary lamellae and epithelial cells. At different concentrations of the herbicide there were cellular infiltration, swollen tip of the gill filaments, distortion of gill filaments and epithelium rupture, Necrosis of secondary lamellae, blanketing of primary lamellae, destruction of secondary lamellae, hyperplasia of lamellar epithelium and necrosis. The finding was similar to that of [3, 10, 11].

Exposure of Pendimethalin Herbicide on Fish (*Tilapia nilotica*) Skeletal Muscles, Gills and its Influence on Human resulted in the bulging and fusion of secondary lamellae, lifting of epithelial cells [1].

### 4.2. Liver

The liver is the organ known for detoxification which can

suffer serious morphological alteration in fish when exposed to pesticides [14]. The histology of control fish liver shows normal hepatic tissue, hepatocytes with granular cytoplasm and round nucleus. The results of the histopathological analysis of the liver shows vacuolation of hepatocytes, blood congestion, Infiltration of Inflammatory Cells, hepatic tissue showing focal necrosis, nuclear and cytoplasmic degeneration and deformed atrophied hepatocytes. This is similar [9] who studied the assessment of Stomp® (pendimethalin) toxicity on *Oreochromis niloticus* and observed vacuolation of hepatocytes, cytoplasmic degeneration and congested blood vessels. This is also similar to the observation of [3].

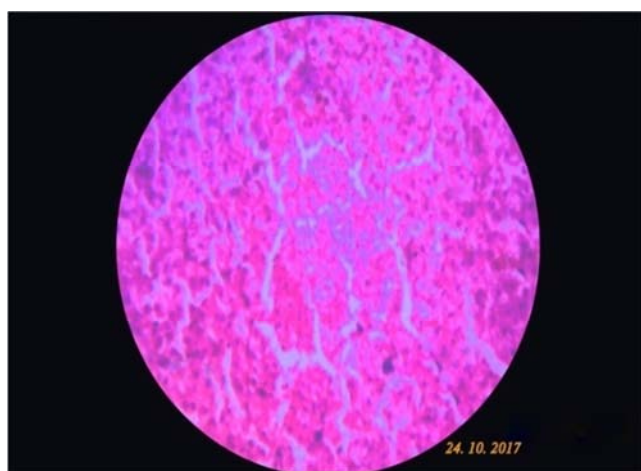
Liver vacuolation in *O niloticus* exposed to Actellic 25EC has been Reported [12].

Necrosis as observed in this work might have resulted from excessive work required by the fish to get rid of the toxicants from its body during the process of detoxification or necrosis became evident as the concentration increases due to the inability of fish to regenerate new liver cells.

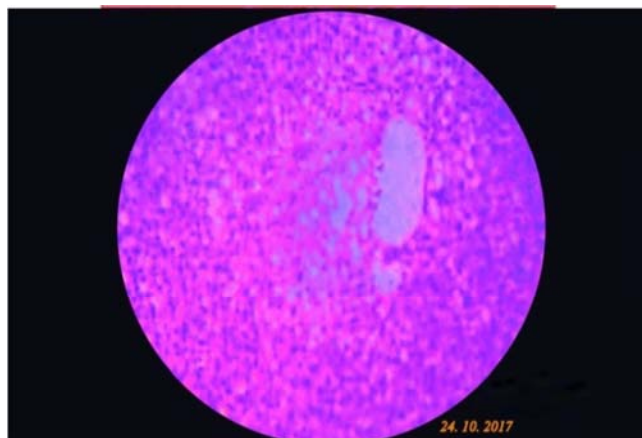
Cellular vacuolation and infiltration may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the toxicants. Therefore, the histological changes observed in the liver of the *C. gariepinus* in the present study indicate that histopathological alterations are good biomarkers for both field and laboratory assessment.

## 5. Conclusions

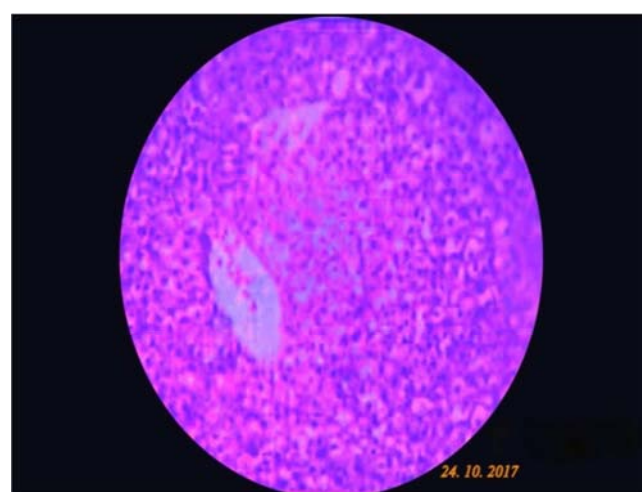
The result of this study shows that Vestaline® (pendimethalin) Herbicide exhibited high level toxicity towards *Clarias gariepinus* Juveniles. The effects of the toxicity of the Herbicide increased with increase in concentration. At lower concentrations there were minimal histopathological alterations but at high concentration more serious histopathological damages observed. Use of this herbicide around aquatic environment should be discouraged.



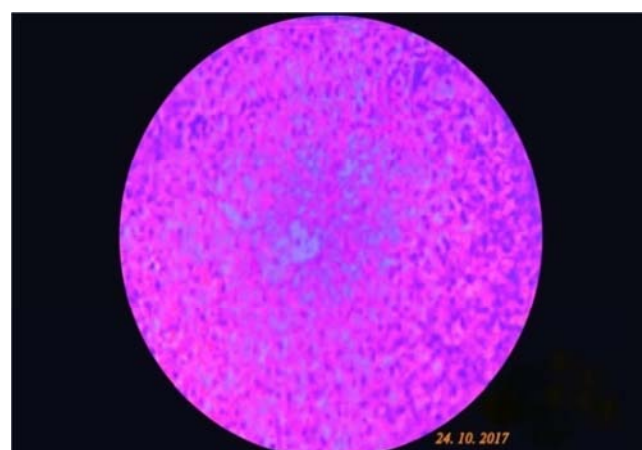
**Figure 1.** Photomicrograph of liver cells of *Clarias gariepinus* juveniles at control (0.0mg/L) treatment, showing normal hepatic tissue. (Hepatocytes with granular cytoplasm) and round nucleus.



**Figure 2.** Photomicrograph of *Clarias gariepinus* liver cells exposed to Vestaline® (pendimethalin) herbicide at (2.0mg/L). Figure 2 shows vacuolation, and cytoplasmic degeneration.

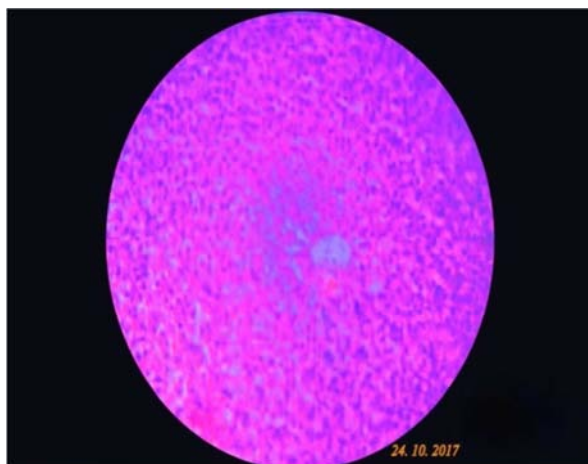


**Figure 3.** Photomicrograph of *Clarias gariepinus* liver cells exposed to Vestaline® (pendimethalin) herbicide at (5.0mg/L). Figure 3 shows vacuolation, necrosis, nuclear degeneration and cytoplasmic degeneration.



**Figure 4.** Photomicrograph of *Clarias gariepinus* liver cells exposed to Vestaline® (pendimethalin) herbicide at (7.0mg/L). Figure 4 shows alterations in the arrangements of hepatic cords, deformed hepatocytes and vacuolation of hepatocytes.

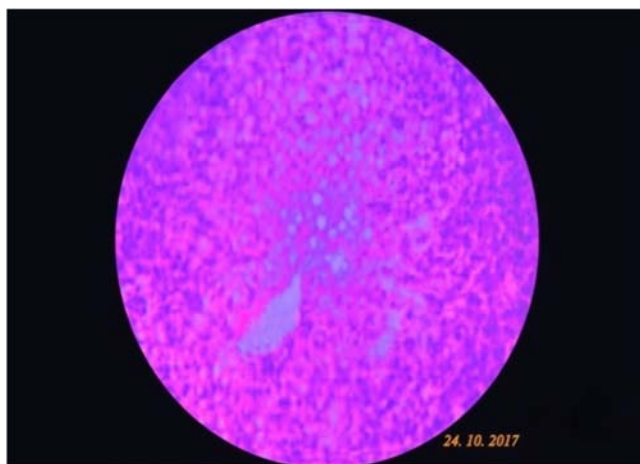




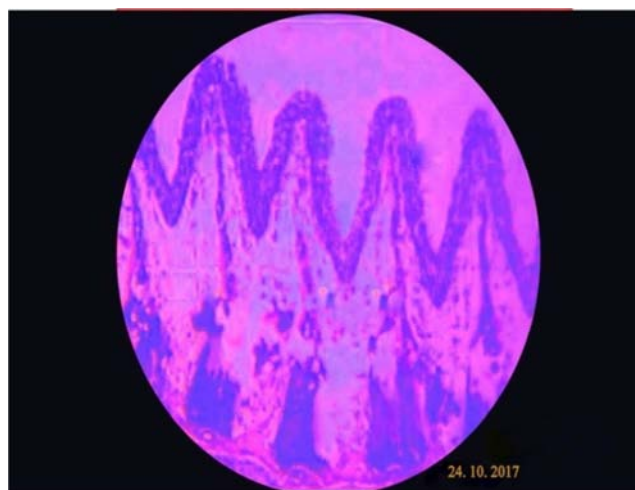
**Figure 5.** Photomicrograph of *Clarias gariepinus* liver cells exposed to Vestaline® (pendimethalin) herbicide at (9.0mg/L) Figure 5 shows congestion of blood in the blood vessels, fibrosis and haemorrhages.



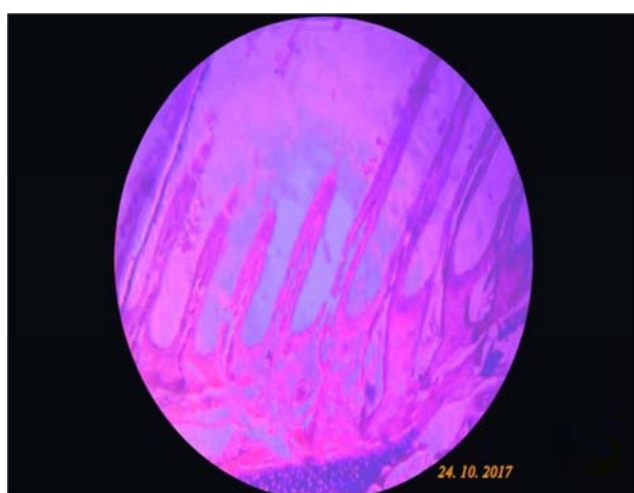
**Figure 8.** Photomicrograph of *Clarias gariepinus* gill cells exposed to Vestaline® (pendimethalin) herbicide at (2.0mg/L) Figure 8 Shows distortion of gill filaments.



**Figure 6.** Photomicrograph of *Clarias gariepinus* liver cells exposed to Vestaline® (pendimethalin) herbicide at (11.0mg/L) Figure 6 shows vacuolation of cytoplasm, rupture of hepatocytes and bile pigment disintegration.



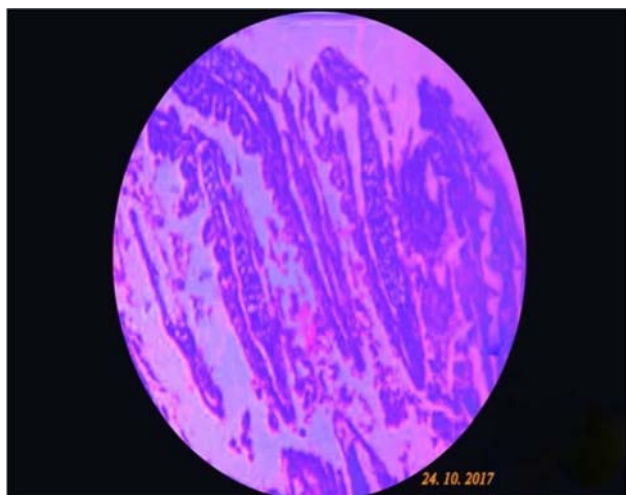
**Figure 9.** Photomicrograph of *Clarias gariepinus* gill cells exposed to Vestaline® (pendimethalin) herbicide at (5.0mg/L) Figure 9 shows distortion of gill filaments and epithelial erosion.



**Figure 7.** Photomicrograph of gill cells of *Clarias gariepinus* juveniles at control (0.0mg/L) treatment 7 shows normal structure of the filaments and lamellae.



**Figure 10.** Photomicrograph of *Clarias gariepinus* gill cells exposed to Vestaline® (pendimethalin) herbicide at (7.0mg/L) Figure 10 showing Necrosis of lamellae and blood congestion.



**Figure 11.** Photomicrograph of *Clarias gariepinus* gill cells exposed to Vestaline® (pendimethalin) herbicide at (9.0mg/L) Figure 11 showing destruction of secondary lamellae and blood congestion in the primary filament.



**Figure 12.** Photomicrograph of *Clarias gariepinus* gill cells exposed to Vestaline® (pendimethalin) herbicide at (11.0mg/L) Figure 12 shows destruction of the lamellae, hyperplasia of lamellar epithelium.

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