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Effect of *Euphorbia hirta* leaf extracts on histopathology of juvenile *Clarias gariepinus*

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Abstract

This study investigated the effect of Euphorbia hirta leaf extracts on the gill and liver tissues of Clarias gariepinus juveniles. The experiment was carried out at the University Fish Farm, Federal University of Agriculture, Abeokuta. The fishes were acclimatized for one week and were fed twice daily at the rate of 3% body weight and water was siphoned on daily basis. A total of 300 C. gariepinus juveniles were used for the study. The experiment was carried out using 5 treatments at concentrations of 0g/l, 1.25g/l, 2.5g/l, 3.75g/l and 5g/l; and in replicates. The fishes were exposed to the leaf extract for a period of 96 hours. The result showed significant difference ($p<0.05$) *in mortality rate as it decreases with decreasing extract concentration. The histological result revealed severe lesion on the gills of fish exposed to varying concentrations of the leaves extract. However, severity decreases with lower concentration of the leave extract. More so, in the liver, severe fatty degeneration was observed and the severity of this degeneration decreasing with decrease in the extract concentration. The result suggested that E. hirta have adverse effect on the juvenile Clarias gariepinus and should be disposed carefully.*

Key words: *Euphorbia hirta*, Lesion, Concentration.

Description of Problem

Clarias gariepinus is indigenous to Africa where they inhabit tropical swamps, lakes, rivers, and floodplains some of which are subjected to seasonal drying. The fish is hardy in nature, due to the accessory breathing organs it possesses. It can survive in burrows beneath pond beds between seasons and it is commonly cultured in several tropical countries of the world and the commercial value of catfish has increased pressure on their population which might result in a decline in catches from their natural habitats if the fishery is left unmanaged. Thus, this makes it the best species for this experiment.

The use of plants has long been considered as valuable sources of medicines for treating variety of diseases and ailments (1). In many developing countries, a large number of civilizations depend heavily on traditional practitioners and medicinal plants to meet their primary health needs. Some of these plants includes *Balanites aegyptiaca, Croton tiglium* (2;3) can be toxic to aquatic animals and can be used as piscicides during pond preparation to control unwanted aquatic animal such as leeches, crayfish, snails, tadpoles, frogs and fish which are known to drastically reduce aquacultural yields by competing with stocked fish for space and feed. Unlike the use of synthetic chemicals, extract from plants can be used as piscicides which are more environmental friendly (4).

Euphorbia hirta belongs to the plant

family *Euphorbiaceae* and genus *Euphorbia*. It is a small annual herb common in tropical countries. It is frequently seen occupying open waste spaces, banks of water courses, grasslands, roadsides, and pathways (**4**). This plant extract exhibited an antimicrobial activity against *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus (3). E. hirta* is primarily used as a medicinal herb due to its unique chemical structure and powerful effects on the body. (1) reported that the plant has been used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.) and in conjunctivitis. Its aqueous extract also exhibits anxiolytic, analgesic, antipyretic and anti-inflammatory activities. It has been used traditionally for thousands of years, and modern research is still being conducted to fully understand all the chemical pathways that this herb can affect. The test for toxicity effect of *E. hirta* leaves extract on *C. gariepinus* can be of economic importance as a way of sustaining fish in the wild. Thus the present study was aimed to examine the toxicity effect of *E. hirta* leaves extract on *C. gariepinus* juveniles.

Materials and Methods

Experimental Site

The experimental site was at the University Fish Farm (Hatchery Section) of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. Nigeria.

Collection and Identification of Plant Materials

Matured *E. hirta* leaves were collected in front of the College of Environmental Resources Management (Phase II) of FUNAAB and the plants was authenticated at the Department of Forestry and Wildlife Management, FUNAAB with code number RFK 4112. Phytochemical analysis of the extract was conducted in the Biology Laboratory of FUNAAB.

Collection of *Clarias gariepinus* Juveniles

Three hundred (300) juveniles of *C. gariepinus* of ten weeks old with weight ranging from 8.3-10g and length between 6.6-7.8cm were collected from a farm at Fatola, Abeokuta, Ogun State, Nigeria. Fish were transported in a 25 litres plastic container half-filled with water to the hatchery section in the farm of the Federal University of Agriculture Abeokuta, Ogun State Nigeria.

Acclimatization of the *C. gariepinus*

The juveniles *Clarias gariepinus* were acclimatized under normal condition for two weeks and fed with 2mm Coppens^{R} feed at the rate of 3% body weight during the period. Feeding was stopped 48hours before the commencement of the experiment. Unconsumed feed and wastes were removed and water replenished daily.

Preparation of *E. hirta* Leaf Extract

The extract was removed from the plant by blending the fresh plant with a little quantity of water using blending machine and kept for 24hours for stability The leaves were washed in clean water and one kilogramme (1kg) of the fresh leaf was macerated in one litre of deionized water to homogenize the leaves, the broth was sieved out into a bowl using muslin cloth to collect the filtrate and used as stock solution. Serial dilutions were made from the stock solution, and the extract was introduced to the treatments at different concentrations of 0, 1.25, 2.5, 3.75 and 5g/L.

Phytochemical Screening

The extract was subjected to various phytochemical tests to identify and determine the chemical constituents present quantitatively. This was carried out at the Biology Laboratory, FUNAAB. Phytochemical screening of *E. hirta* water extract was carried

out on fresh extract. Both qualitative and quantitative assessment was carried out to determine the level of inclusion of the plant extract following the standard procedures as described by (6) for the presence of phenols, tannins, alkaloids, saponins, flavonoid, steroid, oxalate, phytate, glycoside and anthraquinone,.

Bioassay Test

The bioassay test to determine the 48-h acute toxicity of *E. hirta* leaf extracts on juvenile *C. gariepinus* was conducted following static bioassay procedures described by (7). Clean water was used as the control at 0.0g/L. Based on the result from the range finding test (lethal toxicity) described above 96-h, definitive (sub lethal) test following static bioassay procedure described by (7) was carried out using 10 juveniles *C. gariepinus* at different concentrations of *E. hirta*. Five test solutions were prepared by dissolving 0, 1.25, 2.5, 3.75 and 5g/L of *E. hirta* represented as T_1 (control), T_2, T_3, T_4 and T_5 replicated thrice for each treatment in a transparent plastic container. Feeding was stopped 48 hours before the commencement of the experiment to avoid pollution. The test fishes were fed twice throughout the 96-hour test. The mortality of the samples in each tank was monitored and recorded every 24hours until 96hours. Dead fish were removed immediately with scoop net to avoid contamination due to rotting.

Histopathological Test

Histopathological analysis of experimental fishes were carried out at Soar Research and Diagnostic Laboratory by removing the gill and liver of the fish exposed to *E. hirta* extracts after 96-h using Haematoxylin and Eosin techniques as described by (9).

Statistical Analysis

One-way ANOVA was used to validate the test concentration and Duncan Multiple Range Test was used to separate the means. The lethal concentration that caused 50% mortality (96-h LC50) was determined using the probit analysis. The indices of toxicity and their 95% confidence limits were derived from a computer statistical program SPSS 17 (14).

Results

In the qualitative analysis of *E. hirta* leaves extract, tannin, saponin, alkaloid, flavonoid, phenol, steroid, oxalate, phytate, glycoside, anthraquinone were detected and the level of toxicity were determined

In the quantitative analysis of the fresh extract tannin was detected to be 12.18%, saponin was 21.67%, alkaloid was 6.89%, flavonoid was 18.11%, phenol was 0.41%, steroid was 1.26%, oxalate was 32.14%, phytate was 4.62%, glycoside was 2.15% and antraquinone was 0.92%, as shown in Table 1:

Table 1: Qualitative and Quantitative analysis of *E. hirta* leaf extract

S/N	Chemicals	Qualitative	Quantitative (%)
1	Tannin	++	12.18
2.	Saponin	+++	21.67
3.	Alkaloid	+	6.89
4.	Flavonoid	+++	18.11
5.	Phenol	-	0.41
6.	Steroid	+	1.26
7.	Oxalate	++	32.14
8.	Phytate	+	4.62
9.	Glycoside	+	2.15
10.	Antraquinone	+	0.92

Key: - Not present + Slight++ Mild +++ Strong

Record of Water Analysis

The water temperature was fairly constant at the highest concentration of the extract administration (Table 2). The dissolved oxygen increased with reduction in the concentration of extract, vice versa. The pH increased at lower concentration of extract while the conductivity and total dissolved oxygen reduced at lower concentration of the extract.

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Table 2: Analysis of water media in juvenile *Clarias gariepinus* exposed to Euphobia hirta leaf extract

Concentration (g/L)	Temperature (^{0}C)	DO (g/L)	pH	Conductivity (µm)	TDS (g/L)
T_5	20.0±0.5^a	5.21±1.37^b	6.8±0.7^a	125±15^a	78±10.5^a
T_4	19.0±0.5^b	5.1±1.42^a	6.9±0.66^{ab}	118±12^b	75±10.2^b
T_3	19.5±0.5^b	6.3±1.31^c	7.0±0.58^b	110±14^d	74.4±10.1^{bc}
T_2	20.5±0.5^a	6.1±1.44^b	7.1±0.64^c	115±15^c	71±10.5^d
T_1	20.0±0.5^a	6.2±1.33^{bc}	7.3±0.6^d	102±10^e	64.5±7.5^e

Mean value with the same superscript in each column are not significantly ($p>0.05$) different; where DO – Dissolved oxygen, TDS – Total dissolved solids

Table 3: Mean mortality of *C. gariepinus* juvenile exposed to *E. hirta* leaf extract

Conc (g/L)	24hours	48hours	72hours	96hours
T_5	6	2	1	-
T_4	3	2	1	-
T_3	2	1	2	-
T_2	2	1	1	1
T_1	0	0	0	0

Table 4: Percentage mortality of *C. gariepinus* juvenile exposed at different concentration of *E. hirta*

Conc (g/l)	Conc log	% mortality	Probit mortality
T_5	0.699	94	6.55
T_4	0.398	80	5.84
T_3	0.097	50	5.0
T_2	-0.204	33	4.56
T_1	0	0	0

Mean Lethal Concentration

The median lethal concentration of various acute concentrations of *E. hirta* leaf extract exposed to *C. gariepinus* during the 96 hours period was 0.4g/l with the best line fit equation at Y = 4.997621 + 2.086379 X as represented in Fig 1.

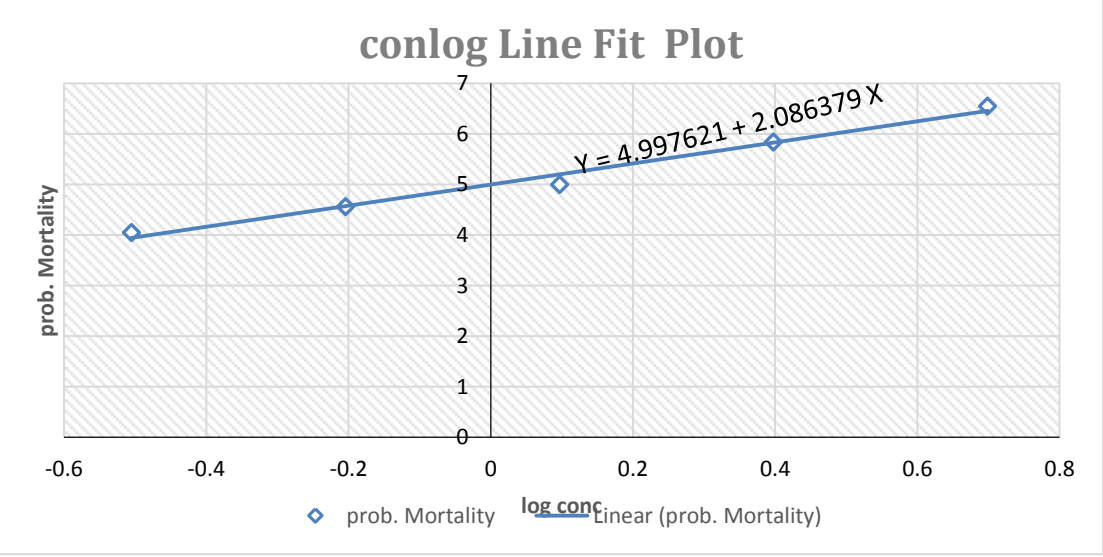

Figure 1: Linear relationship between probit mortality and log concentration of *C. gariepinus* juvenile exposed to leaf extract of *E. hirta*

Histopathological changes in the gills of *C. gariepinus* juveniles

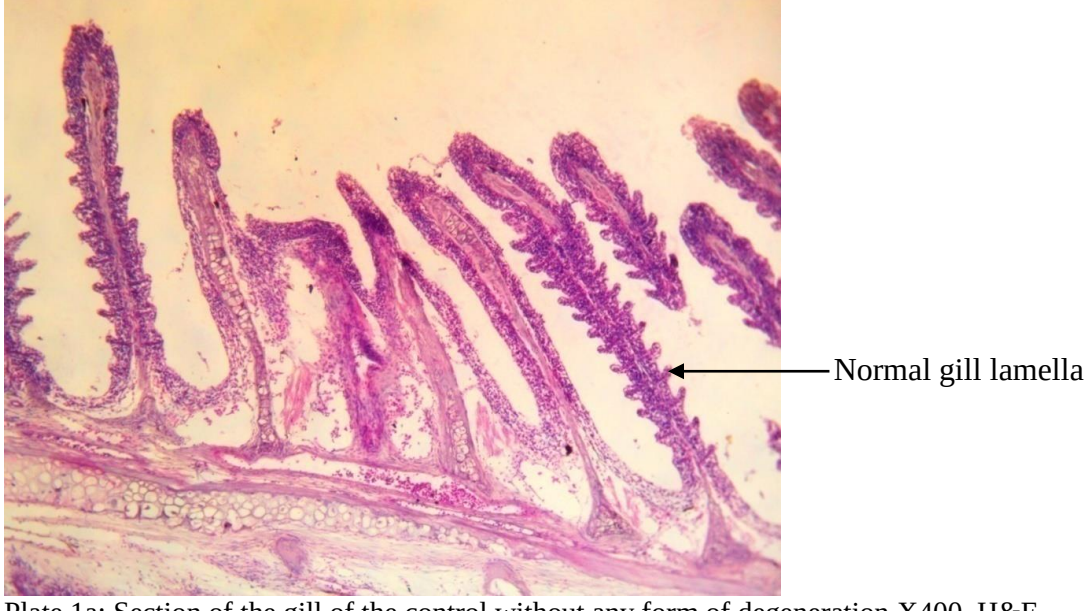

Plate 1a: Section of the gill of the control without any form of degeneration X400, H&E

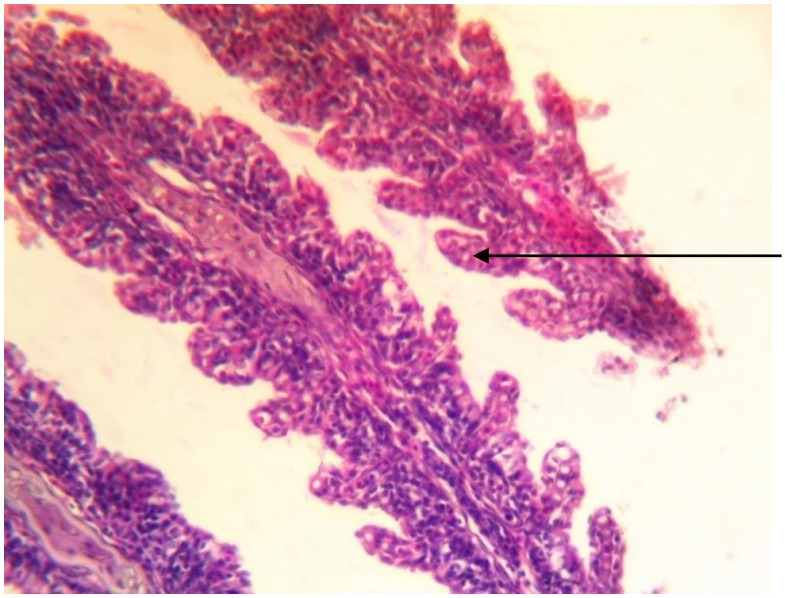

Plate 1b: Section of the gill tissue exposed to 1.25g/L of the extract showing moderate vacuolar degeneration of the epithelial cells of the lamella and dilatation of blood vessel. X400, H&E

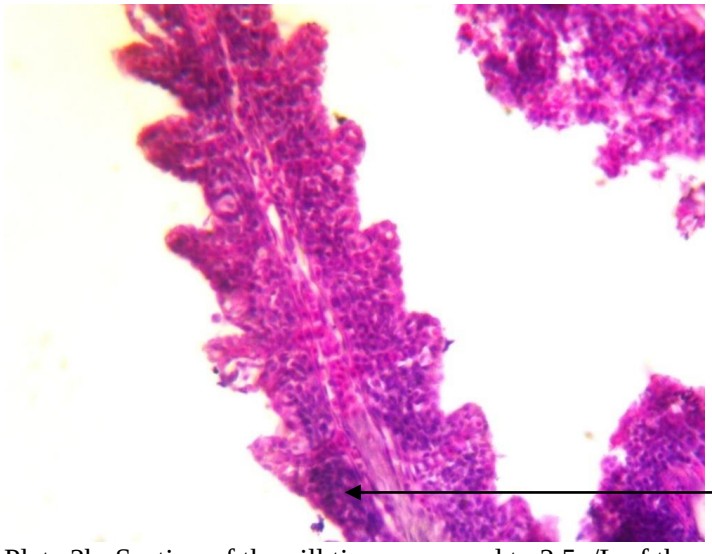

Plate 2b: Section of the gill tissue exposed to 2.5g/L of the extract showing severe vacuolar degeneration of epithelial cells of the lamella. X400, H&E

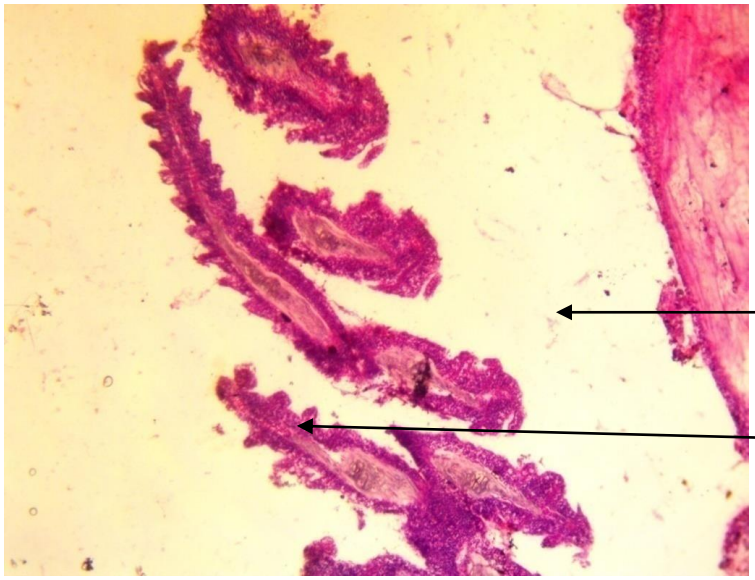

Plate 3b: Section of the gill tissue exposed to 3.75g/L of the extract showing moderate vacuolar degeneration of epithelial cells of the lamella X400, H&E

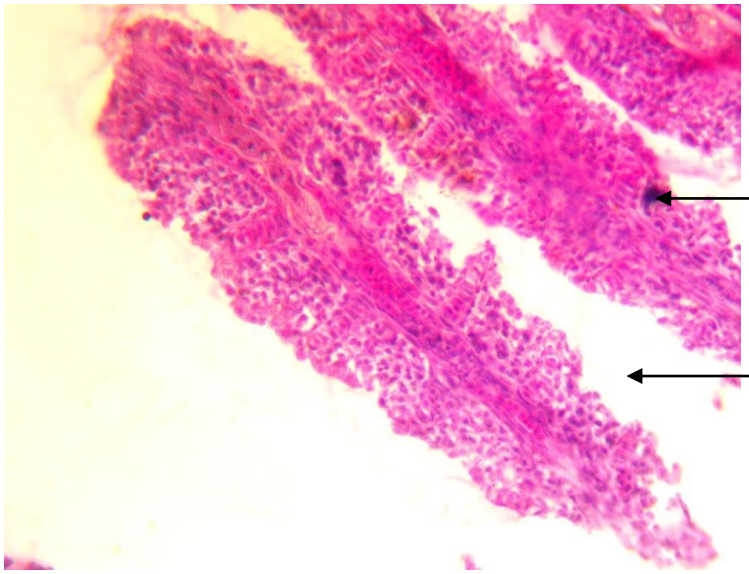

Plate 4b: Section of the gill tissue exposed to 5g/L of the extract showing mild degeneration of epithelial cells of the lamella X400, H&E

Histopathological changes in the liver of *C. gariepinus* juveniles

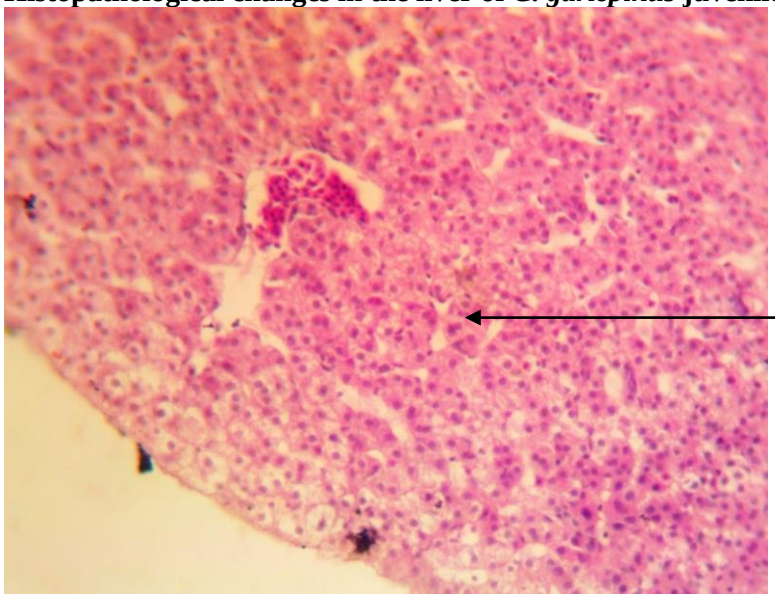

Plate 5a: Section of the liver of the control without fatty degeneration X400, H&E

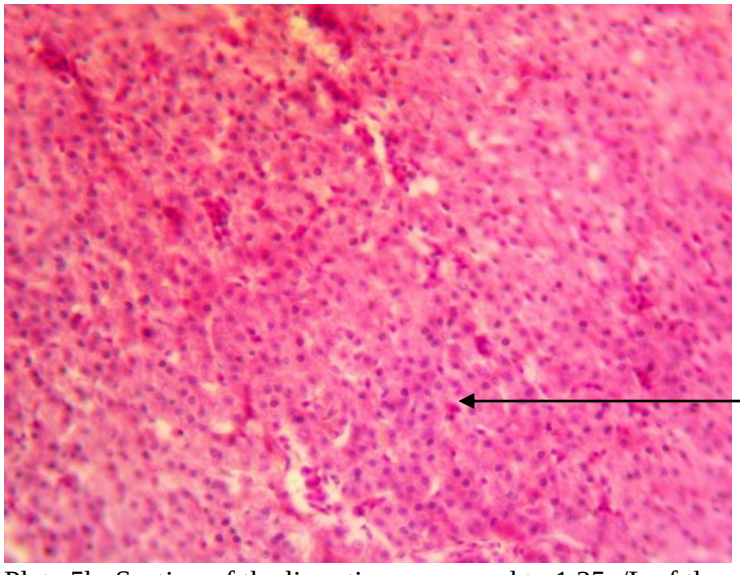

Plate 5b: Section of the liver tissue exposed to 1.25g/L of the extract showing severe fatty degeneration of hepatocytes X400, H&E

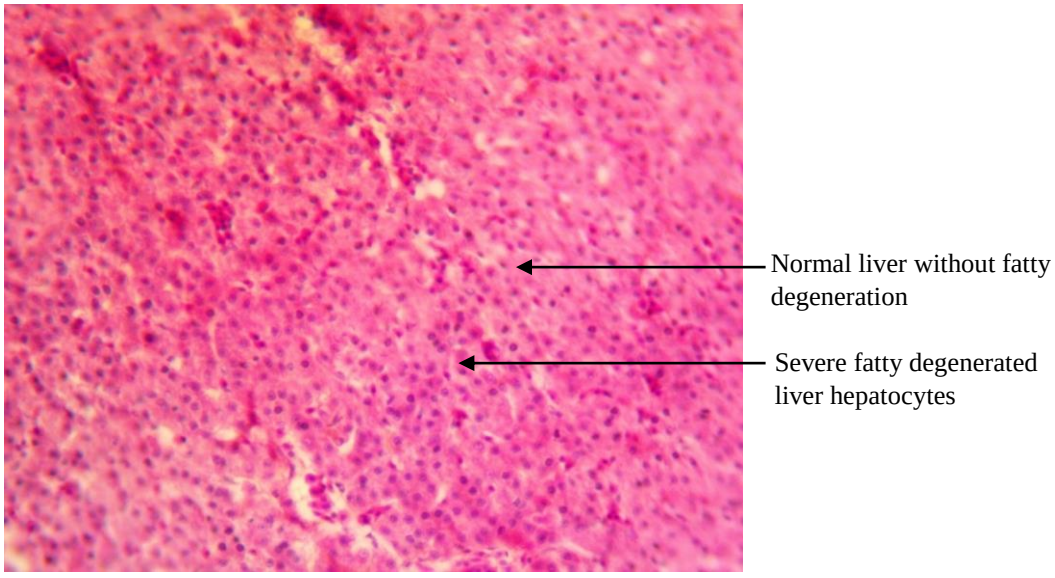

Plate 6b: Section of the liver tissue exposed to 2.5g/L showing severe fatty degeneration of the hepatocytes X400, H&E

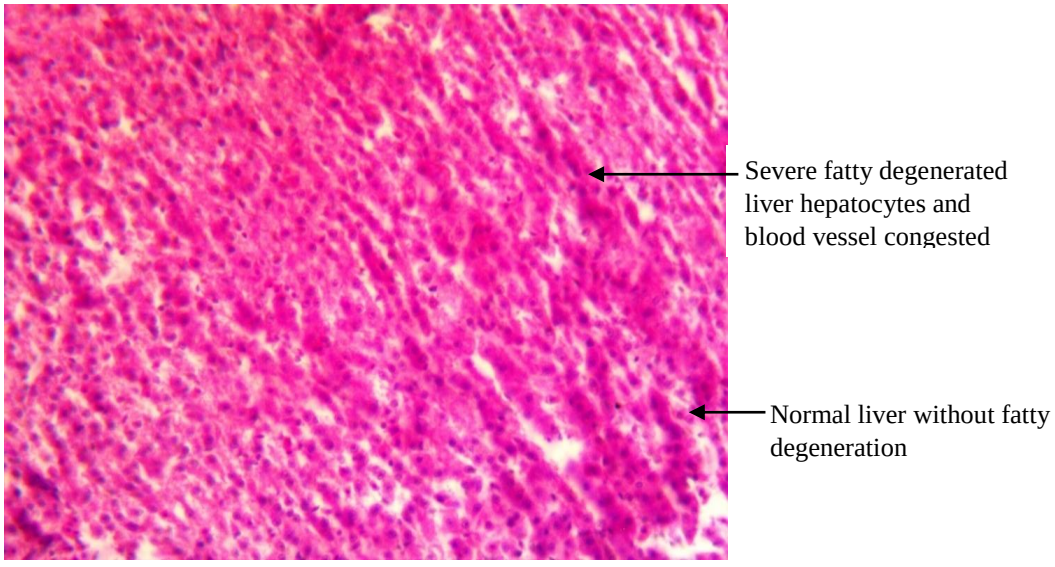

Plate 7b: Section of the liver tissue exposed to 3.75g/L of the extract showing severe fatty degeneration of hepatocytes and congestion of blood vessel. X400, H&E

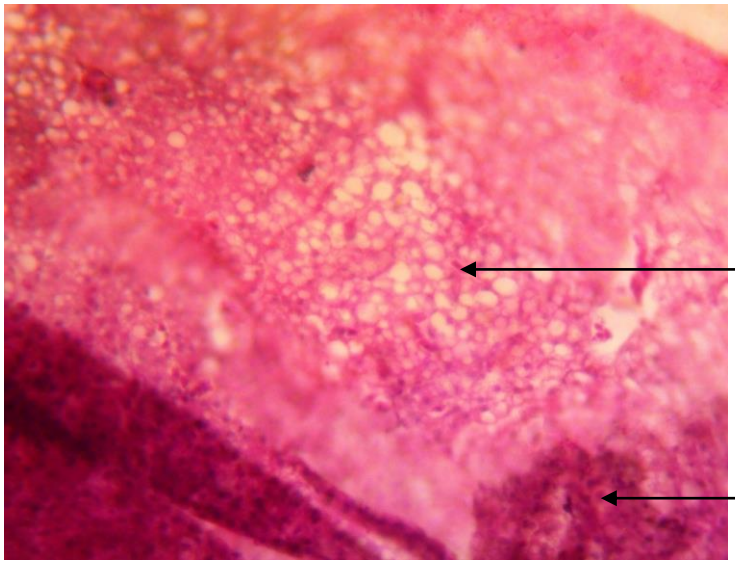

Plate 8b: Section of the liver tissue exposed to 5g/L of the extract showing fatty degeneration and necrosis of hepatocytes and severe inflammatory cells X400, H&E

Discussion

Water quality is of importance for the healthy living and biological sustainability of aquatic organisms and hydrology. This becomes more pertinent for fish species as they are in close association with the aquatic environment. Thus, any slight disturbance of the hitherto stable environment would be immediately reflected in the biological stability of the species. Result of the water analysis shows no obvious deviation from regulatory standards. However, though still within normal permissible range, dissolved oxygen concentration decreases as the concentration of the *E. hirta* extract increases. This could be as a result of the introduction of the extract to the treatments in increasing concentration, thereby affecting the oxygen stability of the water environment. Several researchers have reported that water quality is often affected by toxicants, thus bringing about physiological and behavioral changes in swimming activity of fish (10; 11; 12; 13).

This study revealed the presence of some bioactive chemicals such as alkanoid, flavonoids, tannins, saponins and phenol in the aqueous leaf extract of *E. hirta* in different quantities. The acute exposure of *C. gariepinus* to varying level of *E. hirta* obviously led to progressive changes in the histological formations of the liver and the gills. This is in consonance with observation of several researchers on fish exposed to plant extracts; *Raphia hookeri* (14), *Adenium obesum* (15), *Luffa cylindrica* (16) and *Vernonia amygdalina* (17), among others. (18) reported that potent bioactive substances in plants are numerous and diverse in structural compositions, and this is responsible for the varying composition of these substances in plants. However, phytochemical constituents of plants vary with geographical location (19). As observed in the present study, fresh leaves of *E. hirta* contained high content of some bioactive compounds such as tannin, saponin, alkaloid, flavonoid, phenol, steroid, oxalate, phytate, glycoside, anthraquinone. This is similar to evidence from previous studies which also affirmed that *E. hirta* extract has phenol, sugars, flavonoid, quercetine etc. as active ingredients (20; 21 and22).

The morphological functionalities of the gill of fish place it as an essential organ for biological homeostasis in its aquatic environment. Their roles, especially in respiration, remain highly critical to the fish due to its close association with its immediate aquatic environment and the presence of an extensive respiratory epithelia surface area. This makes it the major organ that is usually vulnerable to unbalanced water quality that may arise from the presence of contaminants or pollutants in the aquatic environment (23; 24; 25 and 26).

In the present study, gill epithelia of the control treatments were similar to that of other teleosts, with no observed tissue alteration or distortion. However *C. gariepinus* exposed to *E. hirta* extract showed several gill histopathological alterations on exposure to varying concentrations of the leaf extract. These includes severe vacuolar degeneration of the epithelial cells of the lamella, moderate vacuolar degeneration of the epithelial cells of the lamella and dilation of blood vessel, and mild degeneration of epithelial cells of the lamella. Generally, the severities of the lesion observed in gill tissues in this study increases with increase in extract concentration. That is, it represents a dose-dependent distortion, especially with obvious severity in those given the higher concentration of the extract (30). The functional implication of these distortions presents could evolve into obstructions of respiration activities of the interlamellar space (water channel) which has a direct effect on gaseous exchange across the lamellar epithelium of the gill (27; 28).

Severe fatty degeneration was observed in liver of *C. gariepinus* exposed to 96 hours acute toxicity in all the treatments exposed to *E. hirta* extract. Tissue distortions in the treatments varied from severe fatty degeneration of hepatocytes and congestion of blood vessel, severe fatty degeneration and necrosis of hepatocytes and severe infiltration by inflammatory cells. The control however showed liver sections appearing intact with no fatty degeneration of the hepatocytes. This observation follows the affirmation of (29) who stated that the liver is the major organs that are quite sensitive to pollutants in the aquatic environment.

The histological distortions readily observed in the liver; ranging from moderate to severe hepatocellular degeneration and necrosis of hepatocytes and severe inflammatory cells of *C. gariepinus* exposed to levels of *E. hirta* is a confirmation of the toxic potential of the plant. Observations showed mortality increasing with increase in concentration of the plant extract. This histological disruption shows direct link to the level of extract concentration. Higher concentrations of the extract of *E. hirta* to *C. gariepinus* juveniles could pose severe tissue functional abilities of the fish (30). These varying distortions to the liver tissue follow similar observations in the work of (31) when *Clarias gariepinus* was exposed to ethanol extract of *Adenium obesum* stem bark and also in line with (32) when *Clarias gariepinus* was exposed to the methanolic extracts of *Raphia hookeri*. Furthermore, (33) reported that exposure of *Clarias gariepinus* to high dosage of methanol extract of *E. hirta* causes some abnormalities in the liver of the fish. (26) posited that histopathological changes have been widely used as indicators in evaluating the health of fish exposed to contaminants.

Conclusion and Applications

1. This study concluded that *Euphorbia hirta* leaves extract has an adverse effect on the internal organs of *Clarias gariepinus* juveniles which leads to mortality of the fish at higher concentrations of the leaves extract.
2. Thus, care should be taken by farmers when disposing remnants or fresh plants of *Euphorbia hirta* leaves into water bodies as this can lead to loss in farmed fish.

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