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Toxicological Assessment of *Chromoleana odorata* on *Clarias gariepinus* Juveniles

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Abstract

A study was carried out to investigate the toxicity effects of exposure of aqueous extract of *Chromolaena odorata* leave on gill/liver (histological) of juveniles catfish *Clarias gariepinus*. The leaves were harvested fresh, air dried for 7 days under ambient room temperature. 200 healthy juveniles catfish *Clarias gariepinus* specimen with average initial weight of 12.40 ± 2.46 cm (mean total length) and 8.26 ± 1.25 g (mean body weight) were exposed to aqueous extract of *Chromolaena odorata* leave at the concentration of 50, 100, 150, 200, 250 mg/l respectively. The physical reactions observed in the fish were: erratic swimming, gasping for air, loss of reflex, hyperactivities and skin discolorations which were more pronounced at high concentration and exposure time. The pH and dissolved oxygen significantly ($P < 0.05$) decreased as the concentrations of *C. odorata* leaves extract increased. However, the values of Ammonia in the exposed media significantly ($P < 0.05$) increased as the concentrations of *C. odorata* leaves extract increased, compared to the control test medium. A high mortality rate was recorded, the histological conditions on gill and liver includes the deformation of gill tissue with overlapping of secondary lamella and disintegration of epithelial tissue leading to diffuse mass of the gill lamella due to rapid cell lysis, gill clogging and gill structure disruption. Deformed primary and secondary lamella with disintegrated gill filament, increasing vasculation, total fusion of gill filament lamella and filament length variation. Damage became severe with increasing concentration in *C. odorata* leaves to fish and exposure, while liver of *Clarias gariepinus* shows liver hepatocellular alteration and increase in hepatocyte disintegration, rupture blood cells in the entire cell with hemorrhage of the vessels and blur nature with severe breakage due to rupture.

Keywords

Chromolaena odorata, *Clarias gariepinus*, Hemorrhage, Toxicant

1. Introduction

Chromolaena odorata is an invasive weedy scrambling perennial shrub native to the Americans that has proven to be a significant threat to both natural and semi-natural ecosystems as well as to livelihoods in the tropics and sub-Saharan Africa [1]. *Chromolaena odorata* is considered to be a significant economic and ecological burden to many tropical and sub-tropical regions of the world where it impacts negatively on agriculture, biodiversity and livelihoods [2]. Fish is usually affected by toxicant in aquatic environment. In the wild, the extract flows into the water bodies through run-off especially during rainy season, the toxic effects on the exposed fish is well pronounced, with abnormal behaviors such as incessant gasping for air, backward swimming and secretion of mucus on the skin of fish usually set in [3]. *Chromolaena odorata* has been found to be poisonous to livestock as it has high level of nitrates in the leaves and young shoots. Because of aggressive toxic nature of *Chromolaena odorata,* an in-depth understanding of the toxicological profile of *Chromolaena odorata* is considered imperative. Therefore, this study was aimed at investigating the hepatic effects of *Chromolaena odorata* and its acute toxicity to *Clarias gariepinus* in order to ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystem. Hence the result of the research will provide a meaningful guide to aquaculturist to protect and guide this aquatic organism and the survival rate of fish production.

2. Materials and Method

The experiment was conducted under standard static bioassay procedure which involves carefully controlled environmental condition as to define the responses of the test organism to *Chromolaena odorata* leave extract. A total of two hundred (200) healthy catfish *Clarias gariepinus* juveniles were used for the experiment. The fish were weighed using electronic sensitive weighing balance scale (OHAUS) model (No4002) to determine the average weight of the experimental fish and a meter rule was used to measure the lengths of the fish (with a mean length of (12.40 ± 2.46) and weight of (8.26 ± 1.26)). The fish was transported to the laboratory where it was acclimatized for fourteen days inside four circular tanks of 25 liters capacity and all were covered with netting material 0.2 mesh sizes to prevent escape of fish. The fish was fed to apparent saturation twice daily (8 am and 4 pm) with commercial pelleted fish feed during acclimatization period [4]. Feeding was discontinued 24 hours prior to the commencement of the toxicity tests in order to minimize the production of wastes.

2.1. Preparation of the Aqueous (Plant) Leaves Extract

Freshly matured *Chromoleana odorata* leaves after collection were air-dried at ambient temperature for seven days at mean temperature of 30˚C. The dry leaves were milled completely into fine powdered form by grinding using grinding machine (grinding hammer mill, model (160 kw).

2.2. Extraction Procedure

100 grams of the fine powdered *C. odorata* was weighed using a sensitive balance. The weighed sample was soaked in 1000 ml of distilled water in a 2000 ml conical flask and swirled. After 48 hours, with interval stirring, the mixture was filtered using Whatman No.1 filter paper [5] into a clean beaker, the extract obtained was centrifuged at 10,000 g for 5 minute and the supernatant stored in an air-tight bottle at room temperature. From the stock solution, ten-fold serial dilution was prepared out of a standard concentration.

2.3. Dilution Water

Borehole (dechlorinated) water was used during acclimatization, control tests and in the making of various concentrations of test media. The water was chemically and biologically certified before it was used for toxicity test and the chemical criteria include low or undetectable levels of priority pollutants [6].

2.4. Acute Toxicity Studies

Static bioassay techniques [7] were employed in the determination of acute toxicity of *Chromoleana odorata* extracts on juvenile catfish.

2.5. The Bioassay Experiment (Exploratory Test)

Standard method for bioassay as described by [4] was used. The acute toxicity procedure started with a Range Finding Test, which was conducted for 96-hour period to determine the concentration at which *Chromoleana odorata* extracts was lethal to the fish. Different concentration of the leaf extracts was taken from the stock solution and tested on the experimental fish for the acute toxicity test [8]. The concentration of the exploratory test of *C. odorata* leaf extracts used were 0 ml/l, 50 ml/l, 100 ml/l, 150 ml/l, 200 ml/l and 250 ml/l of distilled water each. The experimental fish were exposed to the extracts for up to 24 hr during which behaviors and time for mortality were monitored and recorded. Dead fish were removed immediately to avoid pollution.

2.6. Experimental Design and Procedure

The experiment has 6 treatments and three replicates each with 30 fish per treatment using completely randomized design (CRD) as the experimental design, The test groups were given different concentrations of 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l and 0.0 mg/l of *chromoleana odorata* leaf extracts as the control. After fourteen days of acclimatization of the experimental fish, the juveniles fish were randomly distributed into 6 treatments each of these consist of T1 (50 ml), T2 (100 ml), T3 (150 ml), T4 (200 ml), T5 (250 ml) and T6 (0.00 ml) (control) for the aqueous extract. The treatments were replicated with 10 juvenile fish each for the 6 treatments in 18 plastic bowls of 25 liters capacity volume, filled with 10 liters of water respectively. In order to maintain a more constant concentration of test media to which test fish were exposed, before the

introduction of the experimental fish to the toxicant for the bioassay test, the same volume of the extracts to be used were removed from the volume of the water and replaced with the extracts. The average weight and length of the experimental fish were taken as 8.26 g and 12.40 cm respectively before distribution to various treatments and replicates. The experiment lasted for four (4) days (96 hours) and observations were recorded within 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively.

2.7. Control Test

Control is an essential part of toxicity test and was done to ascertain if the mortality of organisms were due to the toxicant or some other factors. Control test were typically conducted by placing the organisms in dechlorinated borehole water with no toxicant. As a rule, a toxicity test is valid if control mortality was less than 10% [9].

2.8. Determination of Physico-Chemical Parameters

Comprehensive analyses of the four important physico-chemical parameters (Dissolved oxygen, temperature, pH and ammonia) were carried out using La-Motte® Freshwater Aquaculture Test Kit.

2.9. Statistical Analysis

Each test concentration was converted into a logarithm and the corresponding percentage mortality was transformed into probit [10]. The median lethal toxicity (LC50), were determined according to the method described by [11]. Analysis of Variance (ANOVA) was used to test for significant differences in the number of survivors in different concentrations of the toxicants (*Chromoleana odorata* extracts).

3. Results

3.1. Behavioral Characteristics of the Experimental Fish

The behavioral responses of the tested fish to the toxicant at different concentrations were observed and recorded (Table 1). Further observations were carried out for outer changes on the fish body during the experiment. The fish were

Table 1. Behavioral responses of *Clarias gariepinus* during 96 hrs of exposure to C. odorata.

Behavior	concentration (mg/l)					
Erratic swimming	Control	50	100	150	200	250
Loss of reflex	-	+	+	+	+	+
Hyperventilation	-	+	+	+	+	+
Motionless state	-	+	+	+	+	+
Discoloration	-	+	+	+	+	+

confirmed dead when stimulus could not respond to any touch. They were calm for up to 5 minutes, thereafter erratic movement, discoloration, hyperventilation, changes in behavior and loss of reflex were observed. The tested fish showed increase in weakness, motionless and gasping for air with slow opercula movement as the concentration level of *Chromolaena odorata* extracts increases with duration of exposure. Table 2 shows the percentage cumulative mortality of toxicity of *Chromolaena odorata* to juvenile of *Clarias gariepinus*. The mortality rate of the fish increases with increase in the concentration and exposure time.

3.2. Physico-Chemical Parameters of Water Monitored during the Experiment

Mean values of water quality parameters for the different concentrations of *C. odorata* leaves extract and control media to which the test fish *C. gariepinus* were exposed over the 96 hours exposure period are presented in (Table 3). Mean values of the water temperature were not significantly ($P < 0.05$) affected by the concentrations of *C. odorata* leaves extract. On the other hand, pH and dissolved oxygen significantly ($P < 0.05$) decreased as the concentrations of *C. odorata* leaves extract increased. However, the values of Ammonia in the exposed media significantly ($P < 0.05$) increased as the concentrations of *C. odorata* leaves extract increased, compared to the control test.

3.3. Toxicity Bioassay (Mortality Response)

Mortality in the three replicate of *C. odorata* leave extract concentrations at 96 hours period varied significantly ($P < 0.05$) in all the treatments and increased with increase in concentration. Mucos was copiously observed on the gills of the dead fish in all the treatments except the control which recorded no mortality. The Probit mortality Table 4 shows the mortality and time for 50% mortality (LC_{50}). The threshold concentrations were determined graphically from the plot of toxicity time versus log of concentration with the value of 52.0 mg/l. The save concentration was determined by multiplying LC_{50} with the factor of 0.01 [12], which gave value as 0.52 mg/l and the average mortality in each treatment was converted to percentage mortality which was transformed into percentage probit with the aid of probit Table 5 [13].

Table 2. Mortality record for *C. gariepinus* juvenile exposed to different concentration of *C. odorata* leave extract.

Conc.mg/l	log conc.	mortality in hours				cumulative	mortality%	survival%	probit
		24	48	72	96	Mortality			
0.00	0.000	0	0	0	0	0	0	0	0
50.00	1.699	4	1	0	1	6	60	40	5.2533
100.00	2.000	3	0	4	0	7	70	30	5.5244
150.00	2.176	2	2	0	1	5	50	50	5.0000
200.00	2.301	4	1	0	0	5	50	50	5.0000
250.00	2.398	4	3	0	1	80	20		5.8416

Table 3. Average water quality parameters recorded during the experiment.

Parameter	T0 (control)	T1 (50 ml/l)	T2 (100 ml/l)	T3 (150 ml/l)	T4 (200 ml/l)	T5 (250 ml/l)
Temperature (˚C)	27	27	27	27	27	27
Dissolve oxygen (mg/l)	8.8	8.2	6.3	5.6	5.2	4.9
pH	6.9	6.6	6.3	6.1	6.0	5.7
Ammonia (mg/l)	0.01	0.03	0.06	0.09	1.5	2.2

Table 4. Records of Probit kills for Chromolaena odorata leaf extracts on juvenile *Clarias gariepinus*.

Probit kills					
conc. mg/l	log conc.	24 hrs	48 hrs	72 hrs	96 hrs
50	1.699	4.31	4.97	5.60	6.83
100	2.000	4.32	4.97	5.60	6.84
150	2.176	4.31	4.96	5.59	6.81
200	2.301	4.31	4.96	5.59	6.81
250	2.398	4.32	4.97	5.61	6.85

Table 5. Threshold for C. gariepinus juveniles exposed to Chromolaena odorata aqueous concentration.

Conc.mg/l	Log. Conc.	Time for 50% mortality in (mins)
50	1.699	110
100	2.000	98
150	2.176	82
200	2.301	78
250	2.398	75

3.4. Histological Analyses

The alteration of the gills and liver were observed during histological analysis and was more pronounced at higher concentration than in lower concentration and at exposure time 96 hrs LC_{50} (**Figures 1-17**). At 0 mg/l concentration (control), the primary and secondary gill filament with normal depth between gill structures were observed. At 50 mg/l concentration, deformation of gill tissue with overlapping of secondary lamella (**OSL**) and disintegration of epithelial tissue (**DET**) leading to diffuse mass of the gill lamella due to rapid cell lysis (**RCL**), gill clugging (**GC**) and gill structure disruption (GSD) were observed. Gill with normal primary and deformed Secondary lamella gill tissue (NPDSL), overlapping and deformed primary and secondary gill structure (ODPS) with raptured gill lamella (RGL), chronic deformation (**CD**) and epithelial lifting of the gills and increased vascuolation (ELIV) were observed at 100 mg/l concentration

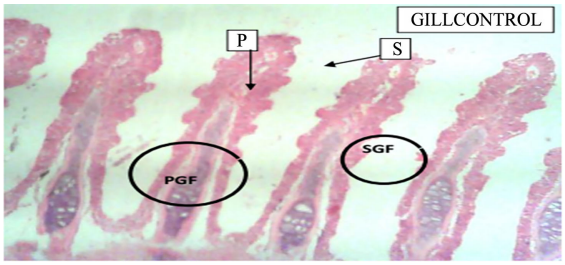

Figure 1. The microphotograph of *Clarias gariepinus* gill showing primary and secondary gill filament with normal depth between gill structure (M ×400).

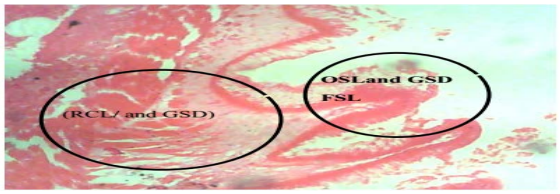

Figure 2. The microphotograph of *Clarias gariepinus* gill at 50 ml/l conc. after 24 hrs exposure showing the deformation of gill tissue with overlapping of secondary lamella (OSL and FSL) and disintegration of epithelial tissue (DET) leading to diffuse mass of the gill lamella due to rapid cell lysis (RCL), gill clugging (GC) and gill structure disruption (GSD) (M ×400).

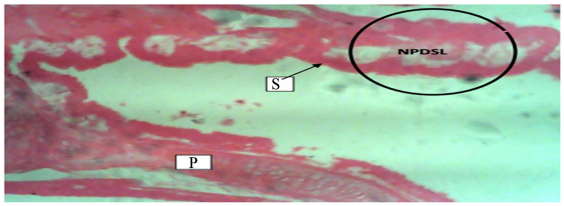

Figure 3. The microphotograph of *Clarias gariepinus* gill showing normal primary and deformed Secondary lamella gill tissue (NPDSL) at 100 ml/l concentration of *C. odorata* after 24 hours (M ×400).

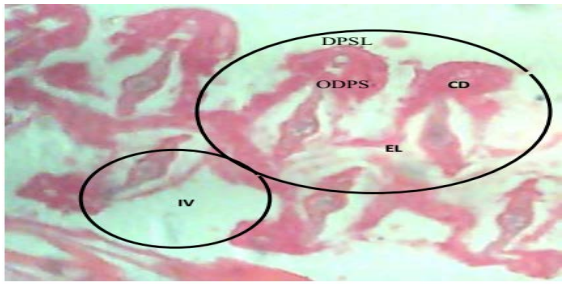

Figure 4. The microphotograph of the fish gill exposed at 100 ml/l concentration of *C. odorata* after 96 hours showing overlapping and deformed primary and secondary gill structure (ODPS) with raptured gill lamella (RGL), chronic deformation (CD) and epithelial lifting and increased vascuolation (ELIV) (M ×400).

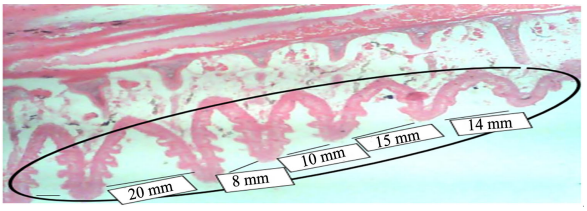

Figure 5. The microphotograph of *Clarias gariepinus* gill exposed at 150 ml/l concentration of *C. odorata* after 24 hours showing gradual deformation of primary and secondary gill filament with varying depth between gill primary filament (M ×400).

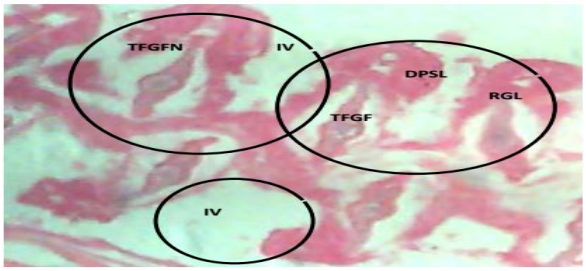

Figure 6. The microphotograph of *Clarias gariepinus* gill exposed at 150 ml/l concentration of *C. odorata* after 96 hours showing deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL) and total disintegrated gill filament (TDGF), total fussion of gill filament lamella (TFGF) and increasing Vasculation (IV) (M ×400).

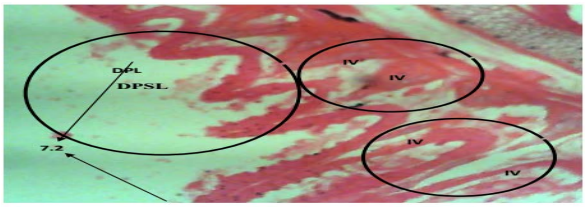

Figure 7. The microphotograph of *Clarias gariepinus* gill exposed at 200 ml/l concentration of *C. odorata* after 24 hours showing deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL) and increasing vasculation (IV) (M ×400).

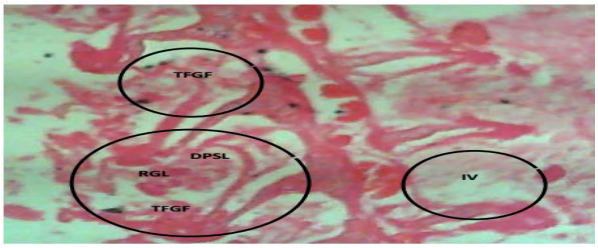

Figure 8. The microphotograph of *Clarias gariepinus* gill exposed at 200 ml/l concentration of *C. odorata* after 96 hours showing deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL) and total disintegrated gill filament (TDGF), total fussion of gill filament lamella (TFGF) and increasing Vasculation (IV) (M ×400).

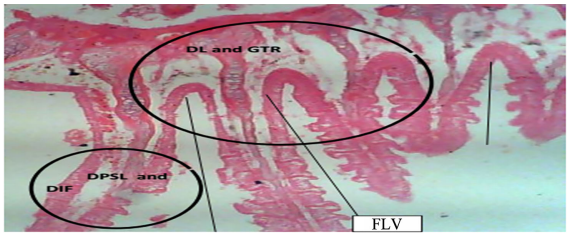

Figure 9. The microphotograph of *Clarias gariepinus* gill exposed at 250 ml/l conc. of *C. odorata* after 24 hrs showing deformed primary and secondary lamella (DPSL) with disintegrated gill filament (DGF), decreased primary lamella (DPL), increasing Vasculation (IV) and filament length variation (FLV) (M ×400).

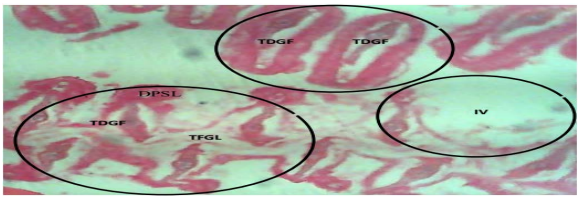

Figure10. The microphotograph of *Clarias gariepinus* gill exposed at 250 ml/l conc. of *C. odorata* after 96 hrs showing deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL) and total disintegrated gill filament (TDGF), total fussion of gill filament lamella (TFGF) and increasing Vasculation (IV) (M ×400).

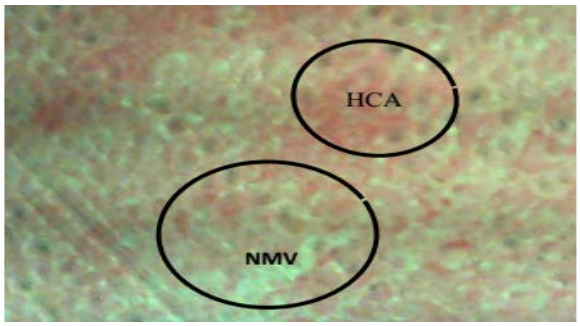

Figure 11. The microphotograph of *Clarias gariepinus* liver exposed at 50 ml/l concentration of *C. odorata* after 24 hrs showing hepatocellular alteration (HCA) and pre-neoplastic lesion due to induced cell alteration as a result of uncontrolled cell division. It's also showing normal or moderate vacuolization (NMV) (M ×400).

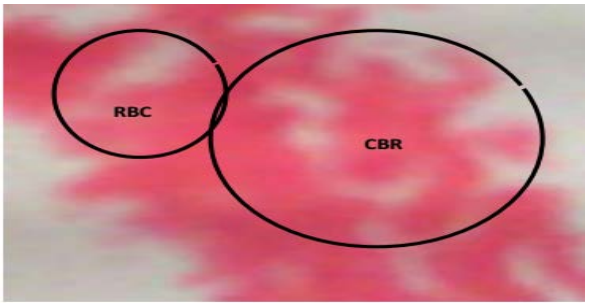

Figure 12. The microphotograph of *Clarias gariepinus* liver exposed at 50 ml/l concentration of *C. odorata* after 96 hrs showing rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and severe breakage due to rupture (CBR) with likely collapse of the blood vessels (M ×400).

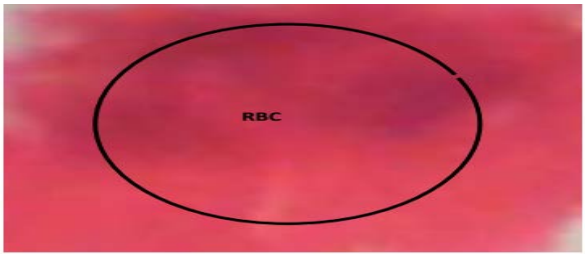

Figure 13. The microphotograph of *Clarias gariepinus* liver exposed at 100 ml/l concentration of *C. odorata* after 96 hrs showing rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and blur nature (M ×400).

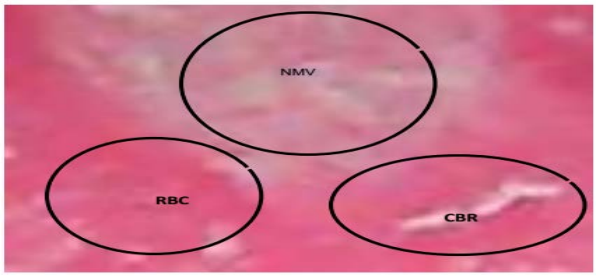

Figure 14. The microphotograph of *Clarias gariepinus* liver exposed at 150 ml/l concentration of *C. odorata* after 24 hrs showing rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and blur nature with severe breakage due to rupture (CBR) (M ×400).

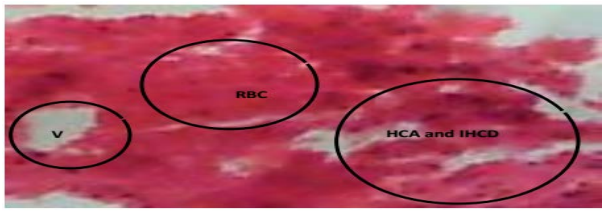

Figure 15. The microphotograph of *Clarias gariepinus* exposed at 150 ml/l concentration of *C. odorata* after 96 hrs showing liver hepatocellular alteration (HCA) and increase in hepatocyte disintegration (IHCD) and vascuolation (V) (M ×400).

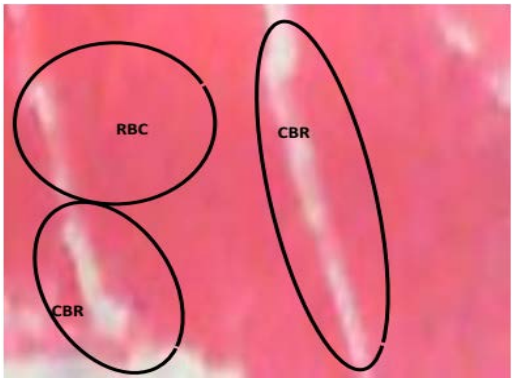

Figure 16. The microphotograph of *Clarias gariepinus* liver exposed at 250 ml/l concentration of *C. odorata* after 24 hrs showing rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and blur nature with severe breakage due to rupture (CBR) (M ×400).

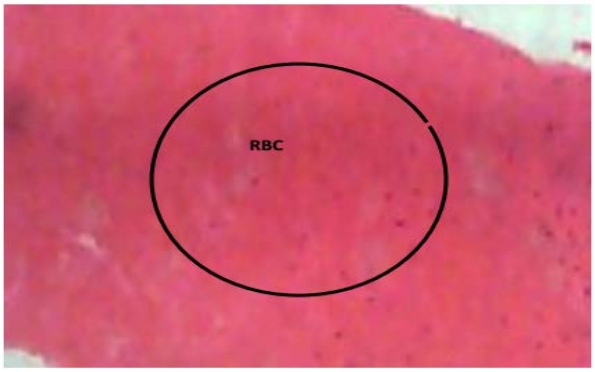

Figure 17. The microphotograph of *Clarias gariepinus* liver exposed at 250 ml/l concentration of *C. odorata* after 96 hours showing rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and blur nature (M ×400).

of 24 hours and 96 hours exposure. However, gill showing gradual deformation of primary and secondary gill filament (GDPSGF) with varying depth between gill primary filaments, deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL), total disintegrated gill filament (TDGF), total fusion gill filament lamella (**TFGF**) and increasing Vasculation (IV) were observed at 150 mg/l concentration of 24 and 96 hours exposure. At 200 and 250 mg/l concentration of 24 and 96 hours exposure, gill showing deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL), decreased primary lamella (DPL), increased Vasculation (IV), deformed primary and secondary lamella (DPSL) with raptured gill lamella (RGL) and total disintegrated gill filament (**TDGF**) were recorded. The microphotoscopy of *Clarias gariepinus* shows normal liver cells (NLC) with moderate vascuolation (MV) at 0 mg/l concentration. But at 50, 100, 150, 200 and 250 ml/l concentration at 24 and 96 hours, the microphotoscopy of *Clarias gariepinus* liver showed hepatocellular alteration (HCA) and pre-neoplastic lesion due to induced cell alteration as a result of uncontrolled cell division, rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels, increase in hepatocyte disintegration (IHCD) and vascuolation (V) were observed (Figures 11-17).

4. Discussion

From the results of this study, it can be deduced that *Chromoleana odorata* has significant toxic effect on the gills and liver of *Clarias gariepinus* juveniles. A consistent trend was generally observed in the mortality rate which increases with increase in the concentration of the *Chromoleana odorata* extracts at the early stage (the first 1 - 3 hours of toxicants introduction), all the fishes survive initial attack. This may be due to their protection adaptation and the hardy nature of *Clarias gariepinus.* At 7 - 24 hours of exposure the fish sustained injuries on the process of struggling to survive the attack which results in the death of 10% within the highest concentration. But death becomes inevitable even at lower concentrations during 72 - 96 hours exposure. As the concentration in-

creases, also the mortality rate becomes the same at 100 - 200 mg/l and increases more at 250 mg/l concentration which further shows that *Clarias gariepinus* has limited tolerance of abnormal pH changes, the dissolved oxygen of the test medium decreased with increase in the concentration of toxicants. The *Chromolaena odorata* leave has a histological effect correlation with exposure time from 24 - 96 hours, even at 50 mg/l concentration were observed to be lethal to the experimental *Clarias gariepinus* juveniles.

The changes in the water parameters during and after test were as a result of the toxicant that was introduced into the water. This is in agreement with the work of [14] who reported that environmental factors, such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity are influenced by the rate of pollutants entering the water, with some lethal effects on the aquatic organisms. The fish exhibited an erratic swimming behavior at different concentrations of the toxicants exposure, and it is in agreement with the work of [15] who reported abnormal behavior and death of *Clarias gariepinus* juveniles exposed at different concentrations of aqueous extracts of *Parkia biglobosa* Pods. At 24 - 96 hours of fish exposure to the toxicants, the microphotoscopy of *Clarias gariepinus* gills and liver revealed rupture blood cells (RBC) in the entire cell with hemorrhage of the vessels and blur nature supporting the work of [16] and [17], showing that histological biomarkers of toxicity in fish organs are useful indicators of environmental pollution.

Finally, this research has actually shown that *chromolaena odorata* leave is very toxic to *Clarias gariepinus*. Therefore, it is advisable for fishermen and aquaculturist who uses *Chromolaena odorata* as feedstuff to stop using *Chromolaena odorata* leave either in catching fish from the wild or eradication of unwanted animal in the pond.

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