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Toxicity of Herbicide Primextra on Juveniles of *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

The contamination of aquatic ecosystem has become a matter of great concern. The use of Primextra chemical constitute potential hazards to the environment resulting in extensive damage to fish. The aim of the study was to evaluate the toxicity of primextra on the juveniles of *Clarias gariepinus.* Mixed sexes of *Clarias gariepinus* juveniles were obtained from school of Agriculture, Mando, Kaduna, and transported in 50-liter capacity plastic container to the Zoological Garden, Biological Sciences Department, Kaduna State University. "Primextra Gold" containing atrazine (370g/l) and S-metolachlor (290g/l) were purchased from Central Market Kaduna. Fish were acclimatized for two weeks and fed with 2mm "Ala Aqua". Stock solution was prepared by adding 1ml of the herbicide obtained in concentration 50g/ml to 99ml of water and used to prepare 0, 10, 13, 16, 19 and 22.1mg/l concentrations. Fish were randomly selected, weighed and distributed into eighteen (18) plastic aquarium tanks with each containing 10 juveniles for the acute toxicity and sub-lethal toxicity bioassay. Behavioural responses and mortality rate were observed. The results of the acute toxicity bioassay, the 96-hours LC_{50} was calculated. Haematological profile of *Clarias gariepinus* include the total erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (WBC) and the differential leukocyte count (DLC) were determined. Length and weight were measured and used to determine the specific Growth Rate and feed conversion ratio. The fish showed abnormal swimming movements including loss of buoyancy, loss of orientation, spasm and death which is more pronounced

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in tanks with higher concentrations of primextra. The first mortality was observed at 24 hours at 10mg/L, 13mg/L, 16mg/L, 19mg/L and 22mg/L concentrations while the RBC count was 2.24±0.00 x10^6 cell/ml while at 10, 13, 16, 19 and 22 mg/L concentration. Results of the histopathological studies of the liver of *C. gariepinus* exposed to acute and sub-lethal of primextra revealed prominent changes in the liver such as perivascular cuffing around the central vein and mild congestion of the sinusoids. The results of the present study revealed that primextra was toxic to *Clarias gariepinus* with LC_{50} value of 12.66mg/L.

Keywords: Toxicity, Herbicide, Primextra, Juveniles, Clariid fish.

INTRODUCTION

In efforts to meet up with food demand and sustainability due to increase in world population, warrant the use of modern Agricultural method to improve agricultural yield. In a way to increase agricultural productivity, people rely heavily on the use of pesticides to protect crops from pests, right from pre-planting, planting and post harvesting to prevent weed and other pests on the farm. Herbicides are very important in modern agriculture as they are predominantly applied to eliminate weed [1]. The contamination of aquatic ecosystem with a wide range of pollutants has become a matter of great concern, not only because of the threat to public water supplies, but also with the damage caused to the aquatic life [2]. There is a growing awareness of the effect of herbicides on aquatic organisms particularly the fish. Many studies have shown that most chemicals including agrochemicals affect several physiological and biochemical functions in an animal [3].

The presence of Primextra chemical in the environment has caused significant social and scientific developmental anxiety worldwide, as their extensive use all over the world can constitute potential hazards to the environment and human health and easily pollute water bodies thereby resulting in extensive damage to non-target species, including fish. As a result of continuous application of Primextra, water is contaminated through direct application into the aquatic system, spray drifts, atmospheric fallout as rain and dust, soil erosion, sewage, industrial effluent and occasionally by spillage [4].

The aquatic body is particularly one susceptible area as it is the final recipient of pollutants due to runoff. The surface waters have been known to receive a wide range of pollutants, which may be introduced to them directly or indirectly. The unselective use of chemicals has resulted in large scale reduction in aquatic productivity. Pesticides have different diverse impacts on aquatic animals especially fishes which are of economic importance and high value from the point of biological conservation [5]. Environmental pollution by pesticides has become a serious problem in terms of global conservation in animals and human health. Besides overexploitation and habitat loss, pollution is ranked third on the list of main causes of fish species loss [6].

Herbicides originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface run-off or leaching and frequently accumulate in soft-bottom sediments and aquatic organisms [7]. Biochemical changes induced by herbicides strain cause disturbance in the metabolism and also cause inhibition of some important enzymes, retardation of growth and reduction in longevity of the

organs. Persistence of these toxic chemicals in aquatic environment is dangerous for the survival of fish and their food organisms [8].

The aim of this study was to evaluate the toxicity of primextra on the juveniles of *Clarias gariepinus.*

Source of Experimental Fish and Primextra

Mixed sexes of *Clarias gariepinus* juveniles were obtained from school of Agriculture, Mando, Kaduna, Kaduna State, Nigeria, and transported in 50 liter capacity plastic container to the outdoor holding ponds situated in the Zoological Garden, Department of Biological Sciences, Kaduna State University, Kaduna [9]. Commercial formulations of primextra with trade name ‘‘Primextra Gold’’ (CAS No 59316-87-9, Syngenta Crop Protection Canada Inc., Guelph, ON, Canada) containing atrazine (370gL1) and S-metolachlor (290gL1) as the active ingredients were purchased from Central Market Kaduna North, Kaduna State, Nigeria.

Acclimatization

Fish were acclimatized in the pond for a period of two (2) weeks in dechlorinated tap water under prevailing weather conditions and natural photoperiods. Water was changed daily in order to avoid contamination throughout the acclimatization period [10].

Feeding of Experimental Fish

During acclimatization, fish were fed with a commercial feed (Ala Aqua 2mm) at 5% body weight daily in two rations that is morning and evening. Twenty-four hours prior to the commencement of the experiment the fish were starved [9].

Preparation of Test Solution

Primextra were obtained at a concentration of 500g/L in a one-liter container. From the 500g/L, a stock solution was prepared by adding 1ml of the herbicide to 99ml of water. The stock solution was used to prepare different nominal concentrations of 0, 10, 13, 16, 19 and 22.1mg/l of the toxicant with dechlorinated tap water. The solution with only dechlorinated tap water without the toxicant was used as control as adopted by [11].

Experimental Design

Healthy fish were randomly selected, weighed and distributed into eighteen (18) plastic aquarium tanks (44 × 29.5 × 24cm) containing 20 liters of dechlorinated water. Ten juveniles of *Clarias gariepinus* regardless of sex were placed in each container making a total of one hundred and eighty (180) fishes for the acute toxicity test that is six (6) treatments, including control in three (3) replications. Similarly, 10 fish were randomly placed in each test tank for the sub-lethal toxicity bioassay consisting of four treatments together with a control in three replications of one hundred and twenty (120) fish. The

experiments were set up in 6×3 factorial in a completely randomized design for the acute toxicity test and 4×3 in the sub-lethal bioassay. During sub-lethal toxicity bioassay, fish were fed at 3% body weight daily. Daily ration was divided into two and fish were fed twice daily around 9:00am and 5:00pm.

Exposure of Fish to Primextra

Acute toxicity Bioassay

A 4-day static renewal toxicity bioassay was carried out in the laboratory to determine the toxicity of primextra to juveniles of *Clarias gariepinus*. Concentrations of 0, 10, 13, 16, 19 and 22.1mg/l were selected and used for the acute toxicity. These concentrations were distributed using a syringe into each aquarium tank and allowed to stay for period of 10 minutes in order for the toxicant to be evenly distributed by dilution. The control is made up of only dechlorinated tap water. 10 fish specimens were randomly selected and kept in each aquarium tank. These were replicated three times for each concentration. Temperature, pH, total dissolved solids, electrical conductivity and dissolved oxygen (Water quality properties parameter) of the test solution and the control were monitored every 24 hours. During this experiment the fish were not fed [11].

Behavioural Studies

After exposure to various concentrations, observations on behavioural responses were made at 12, 24, 48, 72 and 96 hours. The toxicant exposed fish were monitored and compared with the control so as to provide a reference point for determining changes in morphological or behavioural features such as erratic swimming, loss of equilibrium, lethargy, increased excitability, mortality, vertical suspension, mucus secretion, sluggishness, starlet response, deformities and haemorrhage. Differences in toxicant-exposed fish and compared with that of the control were recorded [12].

Opercular Ventilation/Tail Fin Movement Rates

The opercular ventilation frequency and tail fin movement were observed as beats per minute using a stopwatch. The opercular ventilation frequency and tail fin movement were determined twice daily at predetermined time intervals. Observations were made for three fishes in each aquarium and the mean was recorded [13].

Mortality Rates

After the exposure, examination of the experimental set-up for fish mortality rate was made at the end of 1, 2, 4, 6 and 12 hours and then twice daily before termination of the experiment. Juveniles of *Clarias gariepinus* were certified dead when no movement in opercular and a gentle prodding elicited no response. The number of dead fishes was recorded against the time of death in a tabular form in each treatment. Dead fish were immediately removed to avoid dissolved oxygen depletion [14].

Sub-lethal toxicity Bioassay

Based on the results of the acute toxicity bioassay, the 96-hours LC_{50} was calculated and fractions of 1/10, 1/15, 1/20 of the 96-hour LC_{50} were used as the concentrations of the toxicant used during the sub-lethal toxicity bioassay. Following the same pattern of randomization as in the acute toxicity test, fresh specimens were exposed to concentrations of (0, 0.06, 0.08, 0.1mg/l) of the toxicant in triplicates, at a stocking density of 10 fish per aquarium tank. A total of one hundred and twenty fishes were used i.e. four treatments (including a control) in three replications. 12 plastic aquaria were used for the sub-lethal experiment which lasted for eight (8) weeks. Fish were fed with pelleted commercial feed at 3% body weight daily, which was split into two rations. Water quality properties (temperature, pH, electrical conductivity and dissolved oxygen) were monitored on a weekly basis throughout the period of the experiment following methods described by [12].

Haematological Analysis

Blood sampling

At the end of both acute and sub-lethal toxicity bioassays, blood samples were collected from fish using 2ml syringes from the dorsal blood vessel lying below the vertebral column. The blood was then quickly transferred into EDTA bottles to avoid clotting. From each replicate, composite samples were obtained from 3-5 fishes to get sufficient blood for haematological analysis. Indices used to evaluate the haematological profile of *Clarias gariepinus* from control and toxicant exposed groups include the total erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (WBC) and the differential leukocyte count (DLC) [15].

Packed cell volume, PCV (Haematocrit)

PCV of fish exposed to the toxicant as well as that from the control in both tests was determined by the Unified Methods for Haematological Examination of Fish. Blood sample was drawn into micro-capillary tubes. The tubes were then centrifuged for five minutes and the reading taken with the aid of a haematocrit reader and expressed as the volume of erythrocytes per $100cm^3$ [16].

Total Leucocyte count (TLC)

The number of leucocytes of the fish was determined using Shaw's solution which is made up of two diluting fluids A (neutral red 25mg; sodium chloride 0.9g; distilled water 100mls) and B (crystal violet 12mg; sodium citrate 3.8g; formaldehyde 0.4mls; distilled water 100mls). Blood was drawn to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn until the bulb of the pipette was filled halfway and filled with solution B to the 101 mark and shaken. Few drops were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted with the microscope at

×40. The number of cells counted was multiplied by 500 to obtain the total number of leucocytes per cubic millimetre (mm^3) of blood [17].

Red Blood Cells Count (RBC)

For erythrocytes count, Hendricks diluting solution was used. The standard RBC diluting pipette was used. This was carried out by drawing blood up to the 0.5 mark and then filling the pipette up to the 101 mark with the diluting fluid. The pipette was shaken to ensure thorough mixing. The diluted suspension of cells was drawn into the counting chamber of the improved Neubauer's haemocytometer by capillary action. The haemocytometer was placed under the microscope and the number of cells was counted and multiplied by 10 which gave the total number of cells per cubic millimeter of blood [18].

Differential leucocyte Count (DLC)

The slide method was used to determine the Differential leucocyte Count (DLC) of the fish by employing Giemsa staining technique. 2 drops of the blood sample were placed on a slide and made into a thin-film smear. After drying, the smear was fixed in absolute methanol, stained with Giemsa stain and rinsed with buffered distilled water. The slide was allowed to stand for 30 minutes, rinsed again with buffered distilled water and allowed to air-dry. After drying, a microscope was used to count the number of cells [19].

Determination of absolute values

The absolute values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were obtained from the results of the RBCC, WBCC and PCV using the formulae by Dacie and Lewis (1991) as expressed below:

$$\text{MCV } (\mu m^3) = \frac{\text{Ht}(\%) \times 10}{\text{RBCC (cells/mm}^3} \quad \text{as adopted by [20].}$$

$$\text{MCH (Pg/cell)} = \frac{\text{Hb (g/100ml)} \times 10}{\text{RBCC (cells/mm}^3} \quad \text{as adopted by [20].}$$

$$\text{MCHC (g/100ml)} = \frac{\text{Hb (g/100ml)} \times 100}{\text{Ht (\%)}} \quad \text{as adopted by [21].}$$

Where Ht is the haematocrit or PCV value; RBCC is the erythrocyte or red blood cell count; Hb is the haemoglobin value and pg = picogram.

Growth and Nutrient Utilization of Experimental Fish

The total length, standard length as well as weight of fish were measured at the start of the experiment and same after exposure to sub-lethal concentrations of the toxicant. The data was used to analyze fish growth and nutrient utilization using the appropriate indices [22].

Fish weight Determination

Fish in each tank were brought out using a hand net and weighed. The average weight of fish in each tank was obtained by dividing the total weight of all the fishes by their number. Mean and percentage cumulative weights were calculated at the end of the experiment. The standard and total lengths of fish were also taken using a measuring board. This was done by gently placing the fish on the board and the corresponding length on the scaled rule [9].

Weight gain (WG)

Fish weight gain was calculated as the difference between the final weight of fish at the end of the experiment and the initial weight in grams.

$WG\ (g) = W_t - W_0$ as adopted by [23].

Where: $W$is the final weight and W_0 the initial weight of fish.

Percentage live weight gain (%LWG)

The percentage live weight gain (**%**LWG) was calculated as the difference between the initial and final fish weight of fish divided by the initial weight expressed as a percentage [24].

$$\%LWG = \frac{W_t - W_0}{W_0} \times 100$$

Where: W_0 = Initial Fish weight (g)

= Weight at time 't' days (g)

Specific Growth Rate (SGR)

The SGR was calculated using the formula below as adopted by [9].

$$SGR = \frac{l_n W_t - l_n W_0}{D}$$

Where: W_t = Weight at time of observation (g)

W_0 = Initial weight

D = the period under study in days

l_n = The natural logarithm

Feed Conversion Ratio (FCR)

The Feed Conversion ratio, (FCR), was calculated as the dry weight of feed offered divided by the net weight gain of fish [25].

$$FCR = \frac{\text{Weight of feed offerd (g)}}{\text{Net weight gain of fish (g)}}$$

Gross Feed Conversion Efficiency (GFCE)

This was calculated as the reciprocal of the FCR expressed as a percentage [26].

$$GFCE = \frac{1}{FCR} \times 100$$

Feed Efficiency

Feed Efficiency was computed as the ratio of the fish weight gain to the quantity of feed as reported by [27].

$$FE = \frac{\text{Weight gain (g)}}{\text{Feed fed (g)}}$$

Nitrogen Metabolism

Nitrogen metabolism of the experimental fish was calculated using the formular described by [28].

$$NM = \frac{(0.549)(\text{b}-\text{a})\text{h}}{2}$$ where:

a = initial weight of fish (g)

b = final weight of fish (g)

h = period of experiment (days)

Data Analyses

At the end of the acute toxicity test, regression coefficient between the probit kills and log of concentration of the herbicide was determined by the probit model developed by Finney (2000), using Minitab software version 17. Mean values of haematological parameters, opercular and tail fin beats, growth parameters, biochemical activities and physico-chemical properties of the test water were analyzed for any significant difference using Analysis of Variance (ANOVA). Differences between means were partitioned using the Duncan's Multiple Range Test (Duncan, 2005). Statistical Package for Social Sciences (SPSS) version 16 was used.

RESULTS

Acute Toxicity Test

Behavioural and Morphological Responses of *C.gariepinus* Juveniles Exposed to Acute Concentrations of Primextra

The behavioural reactions on juveniles of *Clarias gariepinus* immediately introduced into the tanks containing the different treatments (10,13,16,19 and 22mg/L) of primextra herbicide in the order of their appearance were, agitated swimming, loss of equilibrium, opercular ventilation count, tailfin movement and period of quiescence. Fish were observed to frequently move to the surface of water and occasionally tried to jump out. As the activities decreased, the fishes became sluggish and lethargic. As the time of exposure increased fish stood in vertical position with their heads above the water surface. The fish showed abnormal swimming movements including loss of buoyancy, loss of orientation and spasm before death occurred. These reactions to the toxicants were more pronounced in tanks with higher concentrations of primextra. The fishes in the control tanks were swimming freely at the middle of the test tanks. The result showed that the symptoms were dose dependent for each tank containing the toxicant. The fish certified dead had swollen abdomen and their mouth wide opened, and there was also copious accumulation of mucus observed in the gill's filaments and body surface

Table 1: Behaviour of *C. gariepinus* Exposed to Varying Concentrations of Primextra

Behaviour Activity	**24 h …… Primextra (mg/ l) ……**						**48 h …… Primextra (mg/ l) ……**						**72h …… Primextra (mg/ l) ……**						**96 h …… Primextra (mg/ l) ……**					
Pattern	0.0	10	13	16	19	22	0.0	10	13	16	19	22	0.0	10	13	16	19	22	0.0	10	13	16	19	22
Erratic Swimming	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Rapid Opercula Movement	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Surfacing	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+
Loss of equilibrium	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	+
Change in Pigmentation	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	+

Opercular Ventilation and Tail fin Movement of *C. gariepinus* exposed to acute concentrations of primextra.

Opercular Ventilation

The result of opercular ventilation counts is presented in figure 1. The mean values show that the opercular ventilation of the exposed groups were significantly high (<0.05) in the 12th and 24th hours of exposure than that of the control. At 48, 72 and 96 hour, the opercular ventilation of the control group were significantly high (<0.05) than those of the exposed groups.

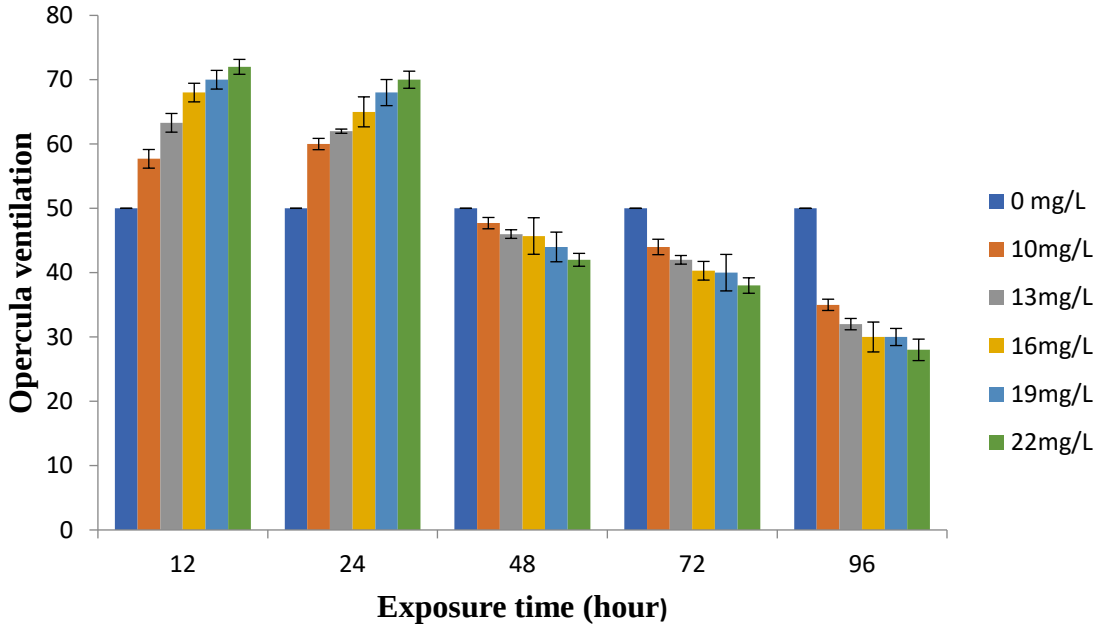

Figure 1: Opercular Ventilation (per minute) of *C. gariepanus* Exposed to Acute Concentration of Primextra

Tail fin Movement

The results of the tailfin beat are presented in figure 2 and exhibited a similar pattern to that of the ventricular beats. The mean values showed that the tail fin beat rate per minute were significantly high ($p< 0.05$). At the 12th and 24th hour of observation, significantly higher rates of tail fin movements were observed in juveniles of *C. gariepinus*. However, decreased tail fin movements were recorded in the exposed groups between 48th and 96th hour of exposure compared to that of the control. However there were no significant differences in the tail fin beat frequency of *C. gariepinus* between the treatment groups. Tail fin movement was dose-dependent and also time-dependent.

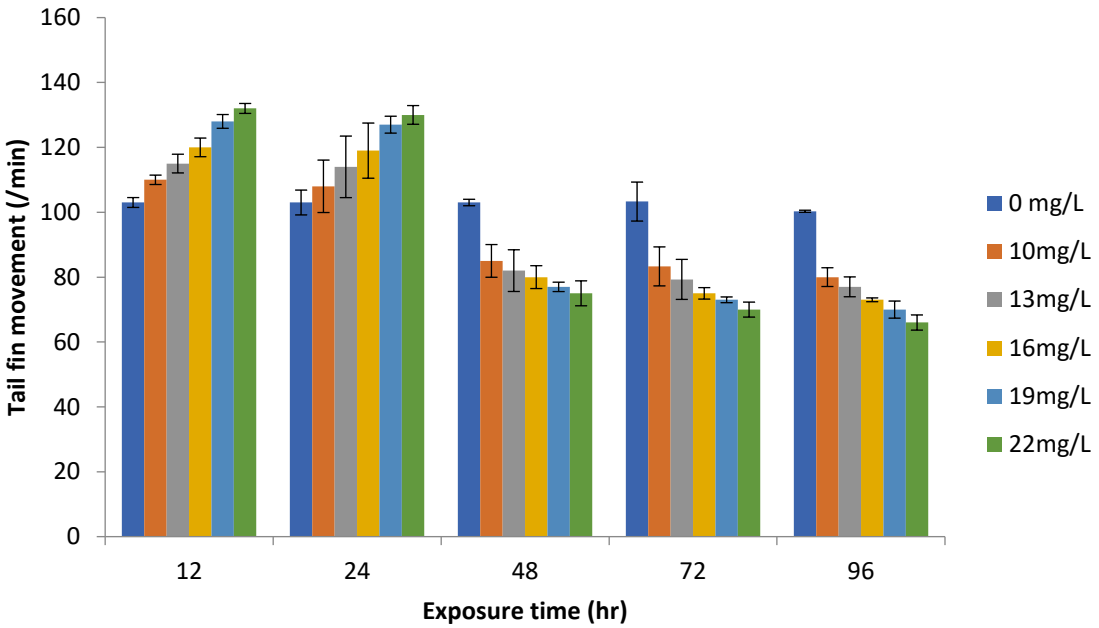

Figure 2: Tail Fin Beat (per minute) of *C. gariepanus* Exposed to Acute Concentration of Primextra

Mortality rates and Log of concentration in *Clarias gariepinus* exposed to acute concentration of Primextra

Fish mortality was observed in all the tanks except in the control tanks as represented in Table 4.3. The first mortality was observed at 24 hours at 10mg/L, 13mg/L, 16mg/L, 19mg/L and 22mg/L of Primextra. By the 48th hour more mortality was observed at the various concentrations except in the control tank. But at 72 and 96 hours, mortality was observed in all concentrations except the control. The highest mortality value of 21 was observed at 22mg/L concentrations while lowest value of 3 for the lowest concentration of 10mg/L. This clearly shows that mortality was dose-dependent. Similarly, mortality rates and Log of concentration in *Clarias gariepinus* exposed to acute concentration of Primextra as shown in Table 4.3 indicates that the concentration 22mg/L as having the highest number of mortality rate (21) and Log of concentration value (-1.363611) while concentration 10mg/L as having the lowest number of mortality rate (3) and Log of concentration value (-0.995635) with the control having the value 0.0000 respectively. The LC_{50} of 96hrs of Primextra on *Clarias gariepinus* is 12.66mg/L as shown in Figure 3

Table 2: Mortality Rates and Log of Concentration in *Clarias gariepinus* Exposed to Acute Concentration of Primextra

Concentration in mg/L	Log. of Conc.	No. exposed	Mortality rates	% mortality	Probit kill
0.00	0.00000	30	0	0	0
10	-0.995635	30	6	10	3.72
13	-1.120573	30	12	16	4.00
16	-1.217483	30	14	46	4.91
19	-1.296666	30	15	53	5.08
22	-1.363611	30	17	70	5.52

Values representing mortality rates and values of log conc.at different concentration.

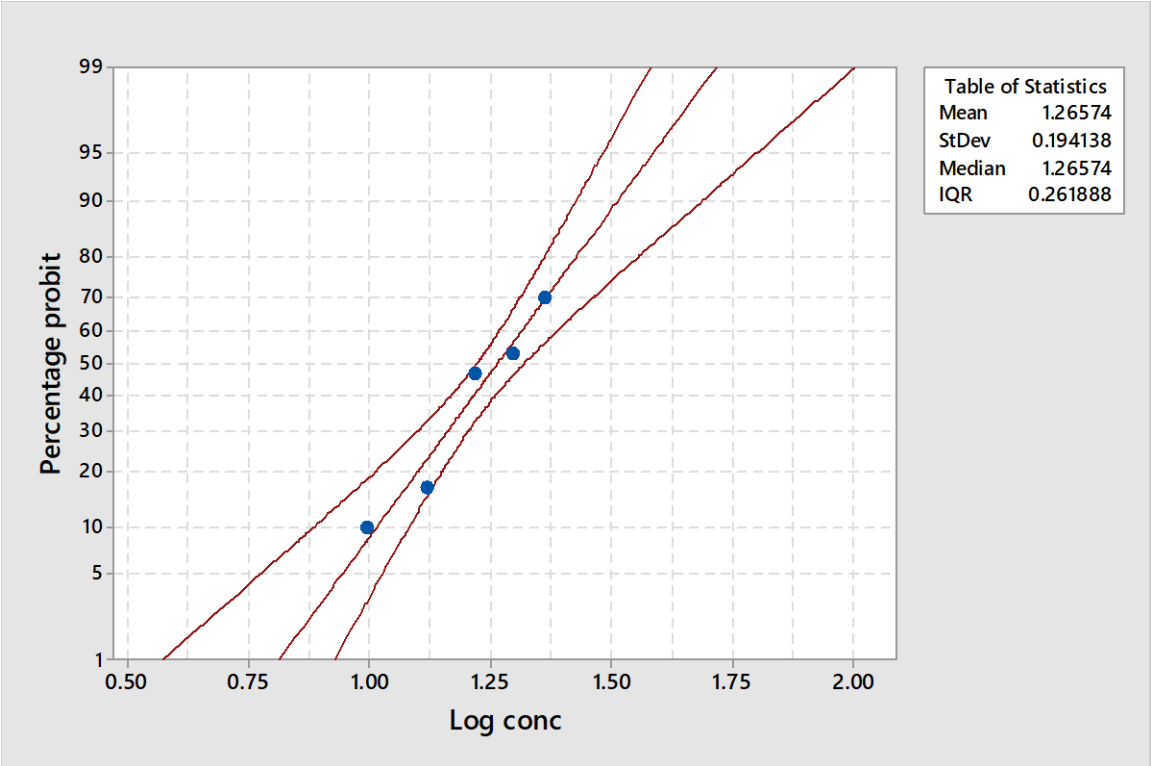

Figure 3: The 96 Hour Median Lethal Concentration (LC_{50}) of *C. gariepinus* Exposed to Primextra

Haematological Parameters of *Clarias gariepinus* exposed to Acute and Sub-lethal Concentration of Primextra

Red Blood Cell (RBC) of *Clarias gariepinus* exposed to Acute and Sub-lethal Concentration of Primextra

As the concentration of the herbicide increased, a corresponding decrease in red blood cell count was also observed. In the control group (0 mg/L of Primextra) the RBC count was 2.24±0.00 $x10^6$ cell/ml while at 10, 13, 16, 19 and 22 mg/L concentration of the herbicide the RBC count was 2.21±0.01, 2.02±0.01, 1.94±0.02, 1.88±0.01 and 1.78±0.05 $x10^6$ cell/mm^3, respectively. The results indicated that the RBC count of the control was significantly higher ($p< 0.05$) than those exposed to 10, 13, 16, 19 and 22 mg/L of the herbicide (Table 3). The RBC counts of Juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra were significantly lower than the counts in the control group, from week 2 to week 8. Furthermore, the decrease in RBC count was dose-dependent with increasing concentrations of the herbicide associated with decreasing RBC count.

White Blood Cell (WBC)

The result of the WBC count from the acute toxicity study showed that there was statistically significant difference ($p< 0.05$) in the WBC count between that of the control and the treated groups, with the mean WBC count of the control group being lower than those of the treated groups. Furthermore, the elevation in WBC count was dose-dependent. At 0 mg/L of herbicide the WBC was 125.0±0.00, while at 10, 13, 16, 19 and 22 mg/L of the herbicide the respective WBC counts were 194.0±2.08, 212.3±0.88, 226.0±1.15, 240.7±1.76, and 263.0±6.51 x 500/mm^3 as seen in Table 4.4. The WBC counts of respectively of *C. gariepinus* exposed to sub-lethal concentrations of Primextra were significantly higher than the count in the control group at each week of observation. Furthermore, the increase in WBC count was observed to be dose-dependent with increasing concentrations of the herbicide associated with increasing WBC count. The highest increase in WBC was recorded in the 0.10 mg/L treated group during the week 8 of observation, while the least recorded WBC was at week 2 in the 0.06 mg/L treated group

Packed Cell Volume (PCV)

The values show a significant difference in PCV ($P<0.05$) values of the exposed fish compare with the control as shown in Table 4.4. This also indicates that the PCV of the exposed fish decreased with increase in concentration. The control has highest of (30.9±0.00) value while 22mg/L has the lowest value (20±0.15) respectively. PCV counts of juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra. The PCV increases with increase in the toxicant concentration. It was observed in 0.06mg/L, 0.08mg/L and 0.10mg/L and as the exposure period (2-8 weeks) there was decrease in PCV in the exposed tanks and comparing this with the control tanks it showed significant difference ($p<0.05$).

Hemoglobin Concentration (Hb)

The Hb concentration of the control group (11.9±00 g/dL) was significantly higher than the values of Hb in the herbicide exposed groups with decreasing Hb values recorded in the treated groups. Furthermore, the decrease in Hb in the treated groups was dose-dependent. The haemoglobin concentrations in the 10, 13, 16, 19 and 22 mg/L herbicide treated groups were 10.9±0.04, 10.2±0.15, 9.06±0.07, 7.02±0.22 and 5.06±0.07 respectively as shown in Table 3. Hb counts of juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra The data for Hb revealed that with high concentration of the toxicant there is decrease in the Hb of the exposed fish compared to the control as observed in week 2- week 8, with increase in the exposure time, Hb decreases which showed a significant difference ($p<0.05$).

Mean Corpuscular Volume (MCV)

The mean corpuscular volume of the control group was significantly higher than those of the herbicide treated group. Furthermore, increased concentration of the herbicide correlated inversely with the mean MCV values. The mean MCV for the 10, 13, 16, 19 and 22 mg/l herbicide treated groups were 126.5±0.56, 123.8±0.30, 110.4±0.07, 110.0±0.12, and 108.2±0.19 μm^3, respectively, with the 22mg/l herbicide treated group having the least MCV value and the 10mg/l group with the highest (Table 4.4). MCV counts of Juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra shows that control have the highest values followed by 0.06mg/L while for 0.08mg/L and 0.10mg/L, respectively, there was significant difference ($P<0.05$)

Mean Corpuscular Hemoglobin (MCH)

There was statistically significant difference ($p< 0.05$) in MCH between control group and the groups of fish exposed of increasing concentration of the herbicide. Furthermore, the differences in mean MCH values of the herbicide treated groups were statistically significant ($p < 0.05$). At 10mg/L of the herbicide, MCH was 49.3±0.20 with a marginal increase observed at 13mg/L (51.6±0.33); between 16 and 22mg/L concentration of the herbicide, the MCH decreased with increasing concentrations of the herbicide. The mean MCH values for 10, 13, 16, 19 and 22 mg/L herbicide exposed groups were 49.3±0.20, 51.6±0.33, 40.3±0.27, 36.4±0.23, and 27.1±0.32 respectively (Table 4.4). MCH counts of Juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra For week 2, there was significant difference in MCH as the values shows that 0.00mg/L having the highest value followed by 0.06mg/L and 0.08mg/L while there was highly significant difference in MCH for 0.10mg/L. For week 4-8, there was significant difference in MCH as the values indicate that control having the highest value followed by 0.06mg/L, 0.08mg/L and 0.10mg/L.

Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC of the control (38.5±0.00) and the 10mg/L Primextra group (38.5±0.36) did not differ significantly ($p < 0.05$) from each other. However, there were significant elevations in MCHC values (Table 4.4) in the groups exposed to 13mg/L (41.6±0.30) and 16mg/L (40.1±0.42) of the herbicide when compared with the control group. Exposure to 19mg/L (34.2±0.44) and 22mg/L (25.6±0.20) of the herbicide, respectively, resulted in progressive decline in decline in MCHC values (Table 4.4). MCHC

counts of Juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra for week 2-6, there was no significant difference in MCHC as the values indicate for control, 0.06mg/L, 0.08mg/L and 0.10mg/L respectively. For week 8, there was significant difference in MCHC have different value with control having the highest value followed by 0.06mg/L, 0.08mg/L and 0.10mg/L

Neutrophil Count

There was significant difference ($p< 0.05$) in the Neutrophil counts between the control and the groups exposed to acute concentrations of Primextra, with the count in the control group (14.5±0.00 %) being higher than the treated groups. The decrease in neutrophil count in the herbicide exposed groups was dose-dependent; at 10, 13, 16, 19 and 22 mg/L concentrations of the herbicide, the neutrophil counts were 14.3±0.15, 13.4±0.23, 13.0±0.24, 12.0±0.24, and 11.6±0.28 percent, respectively (Table 3). Neutrophil counts in Juveniles of *Clarias gariepinus* exposed to sub-lethal concentrations of Primextra were significantly lower than those of the Juveniles in the control group at each week of observation. Decrease in neutrophil count was most pronounced in the 0.10 mg/L herbicide exposed group at week 2 (10.1±0.13) and week 8 (10.3±0.34), while the least effect was seen in the 0.06 mg/L herbicide exposed group

Lymphocyte count

On the other hand, lymphocyte counts were significantly elevated in the treated groups when compared to the control. Furthermore, the increase in lymphocyte counts in the herbicide exposed group was concentration dependent with the least increase in count recorded in the 10mg/L group and the highest in the 22mg/L treatment group (Table 3). It was observed that, no values detected for Basophils, Eosinophils and Monocytes respectively. The lymphocyte counts of Juveniles of *C. gariepinus* exposed to sub-lethal concentrations of Primextra were significantly higher than the counts in the control group, from week to week. Furthermore, the increase in lymphocyte count was concentration dependent with increasing concentrations of the herbicide associated with increasing count. In week 2, the lymphocyte count for 0, 0.06, 0.08, and 0.10 mg/L herbicide exposed groups were 194.7±0.00, 199.7±0.22, 209.4±0.31, and 223.9±0.66, respectively; in week 4 the respective values were 94.4±0.00, 96.6±0.60, 97.6±0.29, and 98.7±0.18; by the sixth week, the values were 95.0±0.00, 95.5±0.44, 97.6±0.23, and 98.9±0.32, respectively; and in week 8, the respective values were 94.0±0.00, 95.0±0.15, 96.5±0.35, and 98.4±0.38. The lymphocyte counts in week 2 for each treatment group was significantly higher ($p< 0.05$) than those recorded between weeks 4 and 8.

Table 3: Acute Effects of Primextra on Some Haematological Parameters of *Clarias gariepinus*

Parameters	**Concentration (mg/L)**					
	0	10	13	16	19	22
RBC ($x10^6$/ml)	2.24 ± 0.00^{d}	2.21 ± 0.01^{d}	2.02 ± 0.01^{c}	1.94 ± 0.02^{cb}	1.88 ± 0.01^{b}	1.78 ± 0.05^{a}
WBC (x500/ml)	125.0 ± 0.00^{a}	194.0 ± 2.08^{b}	212.3 ± 0.88^{c}	226.0 ± 1.15^{d}	240.7 ± 1.76^{e}	263.0 ± 6.51^{f}
PCV (%)	30.9 ± 0.00^{f}	28.1 ± 0.15^{e}	24.9 ± 0.07^{d}	22.1 ± 0.19^{c}	20.7 ± 0.41^{b}	20.1 ± 0.15^{a}
Hb (g/dL)	11.9 ± 0.00^{f}	10.9 ± 0.04^{e}	10.2 ± 0.15^{d}	9.06 ± 0.07^{c}	7.02 ± 0.22^{b}	5.06 ± 0.07^{a}
MCV (μm^3)	137.9 ± 0.00^{e}	126.5 ± 0.56^{d}	123.8 ± 0.30^{c}	110.4 ± 0.07^{b}	110.0 ± 0.12^{b}	108.2 ± 0.19^{a}
MCH (pg/cell)	53.1 ± 0.00^{f}	49.3 ± 0.20^{d}	51.6 ± 0.33^{e}	40.3 ± 0.27^{c}	36.4 ± 0.23^{b}	27.1 ± 0.32^{a}
MCHC (g/100ml)	38.5 ± 0.00^{c}	38.5 ± 0.36^{c}	41.6 ± 0.30^{e}	40.1 ± 0.42^{d}	34.2 ± 0.44^{b}	25.6 ± 0.20^{a}
Neutrophils (%)	14.5 ± 0.00^{c}	14.3 ± 0.15^{c}	13.4 ± 0.23^{b}	13.0 ± 0.24^{b}	12.0 ± 0.23^{a}	11.6 ± 0.28^{a}
Lymphocytes (%)	94.5 ± 0.00^{a}	95.2 ± 0.31^{ab}	95.6 ± 0.23^{ab}	96.3 ± 0.35^{b}	95.9 ± 0.58^{b}	97.8 ± 0.58^{c}
Monocytes (%)	ND	ND	ND	ND	ND	ND
Eosinophils (%)	ND	ND	ND	ND	ND	ND
Basophils (%)	ND	ND	ND	ND	ND	ND

Values are given as mean ± standard error of mean (SEM). In each row, values with different superscripts have statistically significant difference ($p< 0.05$).

KEY: Red Blood Cell (RBC), White Blood Cell Count (WBC), Packed Cell Volume (PCV), Haemoglobin (Hb) Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Not Detected (ND)

Histopathology of liver of C. gariepinus Exposed to Acute and Sub-lethal Concentrations of Primextra

Results of the histopathological studies of the liver of *C. gariepinus* exposed to acute and sub-lethal of primextra revealed prominent changes in the liver such as perivascular cuffing around the central vein and mild congestion of the sinusoids (Plate 2); pyknosis of the nuclei and mild necrotic damage (Plate 3); congestion of the central vein as well as mild coagulative necrosis (Plate 4).

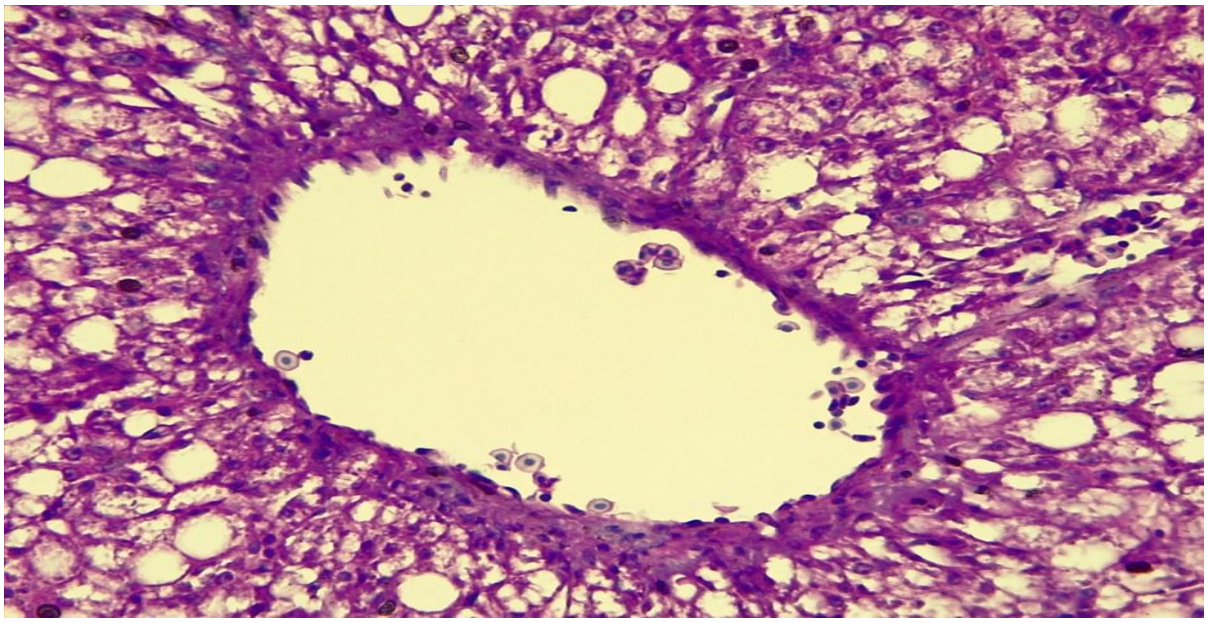

Plate 1: Control 0.00mg/l Photomicrograph of normal liver mg X400

Note the periportal vein PV with some blood cells. Well defined nucleus with chromatin materials. Well outlined nucleoplasmic architecture.

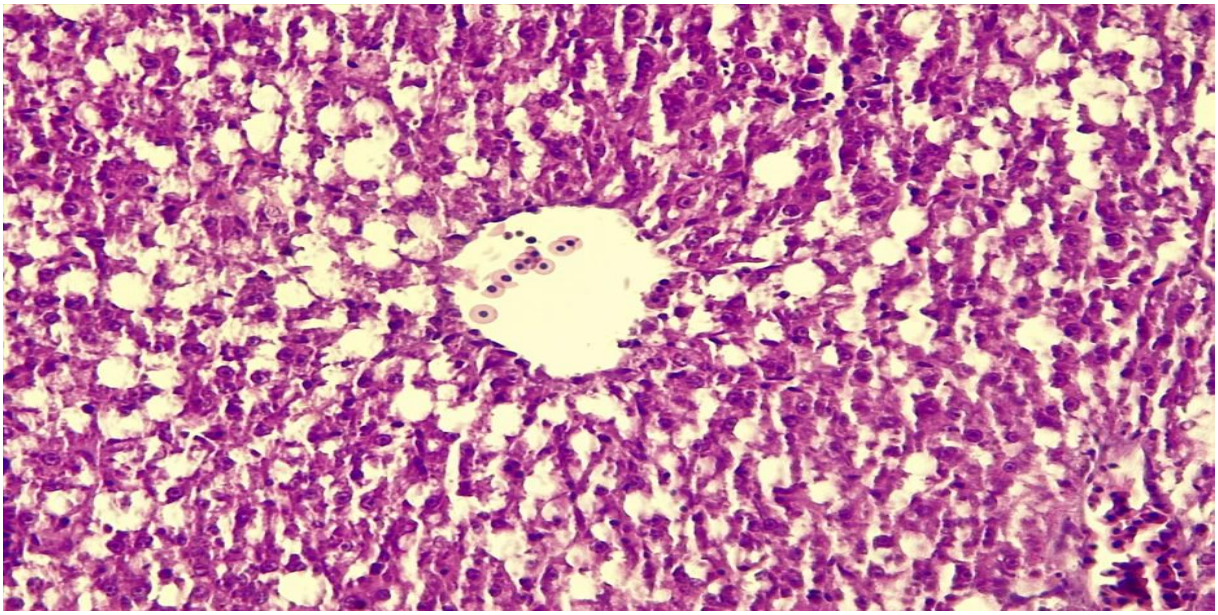

Plate 2: 0.06mg/l concentration sublethal dose. Mg X 400.

Photomicrograph showing the liver, good nuclear architecture (N) but with thickened cell membrane (CM), periportal vein (PV) remain intact with visible blood cells.

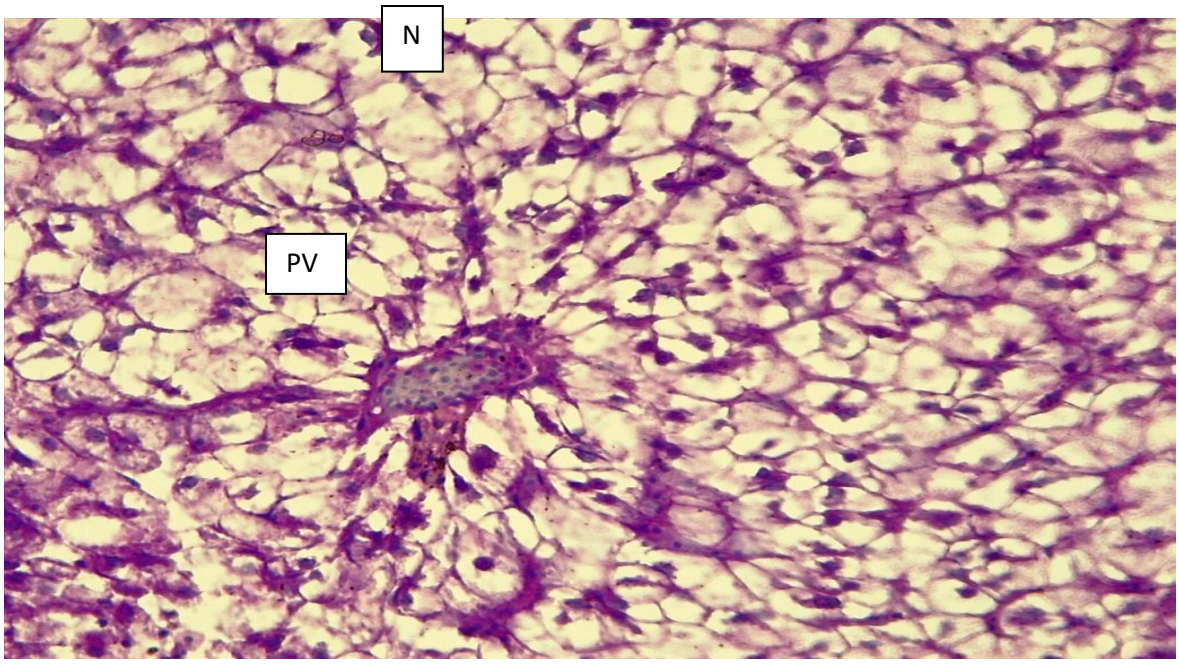

Plate 3: 0.08.mg/l concentration sublethal dose. Mg X 400.

Photomicrograph showing the liver, poor nuclear architecture (N) with increase in the number of nucleus, which is a sign of inflammatory reactions, periportal vein (PV) shows distorted outline with visible blood cells.

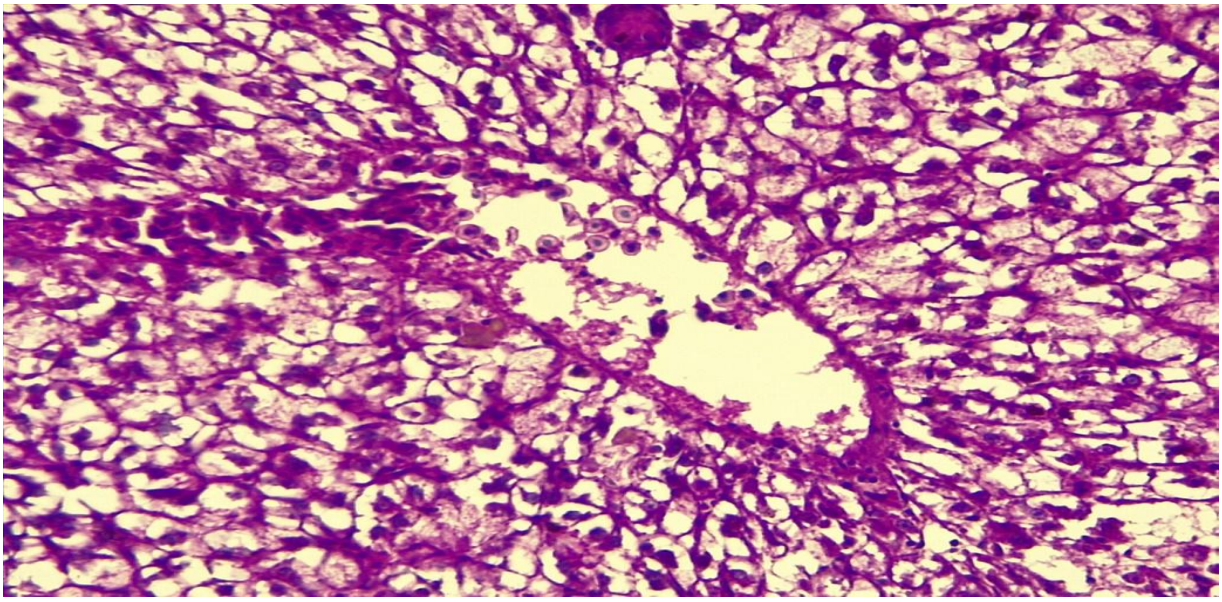

Plate 4:0.1mg/l concentration sublethal dose.Mg X 400.

Photomicrograph showing the liver, compressed nuclear architecture (CN) which are often found outside the cytoplasm and very few, there is evidence of enucleated cytoplasm reflecting necrosis. periportal vein (PV) shows distorted outline with no visible blood cells.

Effect of sub-lethal Concentration of primextra on the Growth of Clarias gariepinus.

Table 4 shows result of growth of *Clarias gariepinus* after exposure to sublethal concentrations of primextra for 8 weeks. The results of weight gain and SGR clearly showed that the best growth performance was recorded in the control group (% weight gained was 72.29% and SGR 0.059g) respectively. For the exposed fish, the best performance was recorded in concentration 0.06mg/L (% weight gained 27.82% and SGR 0.027g). The poorest growth performance was recorded in the highest concentration of 0.1mg/L. The growth performance was dose dependant, showing decrease in growth with increase in concentration.

Sub- lethal Effect of Primextra Nutrient Utilization of Clarias gariepinus in 8 weeks

The nutrient utilization indices of *C. gariepinus* exposed to sub-lethal dose of primextra for 8weeks presented in Table 5 showed a significant reduction in GFCE, FE, PER and NM in the fish exposed to the toxicant compared to that of the control. The best food utilization was obtained in the control tanks with FCR 2.98, PER 0.67g and NM 220.87, while the poorest were obtained in the tanks containing the highest concentration of the toxicant with FCR 4.1, PER 0.05g and NM 39.53g.

Table 4: Growth of *Clarias gariepinus* Exposed to Sub-lethal Concentrations of Primextra for 8 Weeks.

	Concentrations (mg/L)			
Parameters	0	0.06	0.08	0.1
Average initial (g)	38.54	38.03	37.12	37.64
Average final (g)	66.4	48.61	40.66	39.80
weight gain (g)	27.86	10.58	3.54	2.16
% weight gain (g)	72.29%	27.82%	9.54%	5.74%
SGR	0.059	0.027	0.01	0.006

SGR = Specific Growth Rate

Table 5: Nutrient Utilization of *Clarias gariepinus* Exposed to Sub-lethal Concentration of Primextra for 8 Weeks

Concentrations (mg/L)				
Parameters	0	0.06	0.08	0.1
FCR (g)	1.08	1.8	2.09	2.4
GFCE (g)	33.56	28.41	25.71	24.39
FE (g)	0.18	0.11	0.06	0.01
PER (g)	0.67	0.27	0.13	0.05
NM (g)	220.87	156.47	66.2	39.53

FCR= Feed Conversion Ratio

GFCE= Gross Feed Conversion Efficiency

FE= Feed Efficiency

PER= Protein Efficiency Ratio

NM= Nitrogen Metabolism

DISCUSSION

The results of the *Clarias gariepinus* juveniles exposed to acute concentration of primextra showed abnormal behaviours, most notable behaviours were erratic swimming, gulping of air, restlessness, loss of balance, excessive secretion of mucus and finally death. Similar observations were reported by [12] who reported that hyperactivity of fish on introduction to an unfavourable environment as the primary and principal sign of system failure due to pesticide poisoning which affect the physiological and enzyme activities. These behavioural alterations may occur as a result of nervous impairment due to blockage of nervous transmission among the system and various effector sites [29].When the fish were exposed to acute concentrations of different toxicants. The fish were stressed progressively with time before eventually dying. The stressful ailment of respiratory impairment due to the toxic effect of primextra on the gills [30]. The observed increasing state of inactivity with both increasing concentrations and exposure period [31]. The accumulation of the mucus might have resulted from an increase in the activity of the mucus cells following exposure to the toxicant. Accumulation of mucus on the gills reduces respiratory activities in the fish and lack of ability of the gill surface to actively carry out gaseous exchange and this might be responsible for the observed mortalities [32].

The mortality was observed to be concentration and time dependent in the present experiment and indicated that all substance could be poison when exposed to organisms beyond certain concentrations

and time limit. Toxicity of any poison is species and environmental factors related. Hence, it is expected that toxicity of toxicants may vary based on the above factors and different species may have differing values of LC_{50} [33].

The opercular ventilation and tail fin beats of *Clarias gariepinus* exposed to the toxicant showing increase in tail fin beat may be associated with sudden response of the fish to the shock of exposure to the toxicant [34]. This might be due to hyperventilation to cope at the initial period but become weak with increase in exposure, hence subsequent drop in opercular and tail fin beat and finally death due to suffocation [35].

The decrease in the value of HGb with increase in concentration is an indication of severe anaemia caused by the toxicant on the exposed fish. The anaemia condition could be due to an inhibition in erythrocyte production and destruction of intestinal cells by the toxicant [36]. Erythropenia (deficiency in the number of red blood cells) was reflected by the reduced haemoglobin content and haematocrit value as well as erythrocyte sedimentation rate (ESR) (Eisler, 1967). Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen carrying capacity in the blood. The decrease with increasing concentrations of RBC and MCV values could be attributed to the inhibition of the erythrocyte production [37].

The significant decrease in the packed cell volume (PCV) in this study could be attributed to gill damage and impaired osmoregulation causing anaemia and haemodilution in the fish. The findings were similar with anaemia associated with erythropenia that was reported by [16].

The fluctuations in MCH from the present study show that, a decrease with increasing concentration of the toxicant may have increased the number of to compensate for low load of haemoglobin per cell. Haemoglobin is very important in the tissue of vertebrates because it serves as an oxygen carrier. Therefore, tissue respiration and metabolism induce morbidity and mortality. White blood cells (WBC) increase with increase in the concentration of the toxicant, which is likely to be associated with an increase in antibody production which help in survival and recovery in the fish exposed to sub-lethal concentration of the toxicant [34].

The liver also plays a central role in osmoregulation. Hence, attempt in detoxification of toxicants may compromise its integrity and affect its functions. Changes observed in the liver of exposed fish. In this present study, the liver of juveniles of *Clarias gariepinus* exposed to different sub-lethal concentrations of primextra showed compressed nuclear architecture, necrosis and periportal vein shows distorted outline with no visible blood cells. There was pathological lesion observed on the fish in the control tank [38].

Necrosis of some portions of the liver tissue that were observed may result from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, similar observations were reported by [39]. [40] also observed that the volume of the nuclei and nucleoli increased and led to necrosis of the liver cells due to disturbance in cellular and osmotic regulation power of cellular and biological membranes.

In this study the observation in the growth data showed a significant decrease ($P<0.05$) in weight gain (WG) of the exposed fish to the toxicant compared to that of the control. This showed that growth rate was reduced as both the concentration of the toxicant and exposure period increased. The suppressed reduction in growth was the effect of the toxicant in the water which resulted in physiological dysfunction in the fish, also resulting in poor feeding [41]. Similar findings by [42], reported that *Dattura inoxia* leaf at sub-lethal concentration on fingerlings of *Clarias gariepinus* impaired the growth showing significant difference between the control and that of the concentrations of toxicant. [43] reported that thiobencarb exposed on *Oreochromis niloticus* for 8 weeks revealed a reduction in body weight gain compared to the control group.

the reduction in food utilization could be due to the effect of the toxicant of the fish or energy content of the diet and environmental factors. Fishes are noted to increase their metabolic activities and excretion of toxicant, hence making more energy available for homeostatic maintenance than storage which could be used for growth [44].

Generally, it is probable that increase in concentration of the toxicant led to an increase in oxygen tension which reduced metabolic activity such as synthesis of cellular material, ATP,etc. This also affects synthesis of new cells which in turn leads to reduced or no growth at all. Reduced growth hence will therefore translate to reduced or no increase in weight of the fish [45].

CONCLUSIONS

The results of the present study revealed that primextra was toxic to *Clarias gariepinus* with LC_{50} value of 12.66mg/L. In the acute toxicity bioassay, Primextra significantly reduced packed cell volume (35.29%), red blood cells count (20.54%) and haemoglobin (57.48%) which are clearly signs of anaemia, whereas total white blood cells increased with (52.47%). Similarly, reductions were recorded in sub-lethal toxicity of packed cell volume (11.25%), red blood cells count (40.35%) and haemoglobin (15.46%). Histopathology of liver in *Clarias gariepinus* compressed nuclear architecture, evidence of enucleated cytoplasm reflecting necrosis and distortion of periportal vein was observed in the liver. The herbicide have a negative effect on the growth of juvenile of *Clarias gariepinus*, at 0.06mg/L it was 10.58g while at 0.1mg/L it was 2.16g. The result showed that there was decrease with increase in concentration of the toxicant. The control had the highest weight gain of 27.86g and lowest value of 2.16g was recorded in concentration 0.1mg/L

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