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Toxicity of Orizo plus (Propanil and 2, 4-Dichlorophynoxyacetic Acid): Effects on the Behavioral, Hematological and Biochemical Parameters of *Clarias gariepinus* (Burchell)

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Abstract: This study was undertaken to investigate the interaction of a chemical herbicide, Orizo plus (Propanil and 2, 4-Dichlorophynoxyacetic acid) with the environment and a local fresh water fish *Clarias gariepinus* (Family: Clarridae). A total of 350 juveniles of the fish weighing 25.6g ± 23.4 and with a mean length of 23.14 ± 16.21 were purchased from a local fish farm and were acclimatized under laboratory conditions for two weeks. After a range finding test, a set of ten acclimated fish specimens were randomly exposed to five static tank each containing (60.00, 80.00, 100.00, 120.00 and 140.00 µg/L) of the herbicide.The experiments were set in triplicates. A control experiment was set up simultaneously. The test media were changed every 48 hours to maintain a more constant concentration of the media and to prevent excessive accumulation of toxic metabolites. The exposure was continued up to 96 hours. A dose dependent significant ($p < 0.05$) increase in mortalitywas recorded and the median lethal concentration (LC_{50}) determined to be 25.12 µg/l. Three test concentrations, *Viz*1 (1/10th Lc_{50}, 2.51 µg/L), II (1/9th of LC_{50}, 2.79 µg/L) and III (1/8th of Lc_{50}, 3.14 µg/L) were determined and a control used in the assay. The water quality was reduced, abnormal behavioral responses which included erratic swimming, loss of equilibrium, gasping for air, convulsion, somersaulting and restlessness were observed in the exposed groups.The red blood cell count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) were reduced while a significant ($p<0.05$) increase in WBC in correlation with the concentration of the herbicide was recorded. The serum glucose was reduced; the small intestine and muscle were damaged. These results indicated that this commonly used herbicide-orizo plus is toxic and must be used with caution to protect the environment especially the non-target organisms particularly fishes to ensure sustainability of the environment and biodiversity.

Key words: Herbicide · Behaviour · Mortality · Hematology and Biochemical Parameters

INTRODUCTION

More than half of global population growth between now and 2050 are expected to occur in Africa [1]. The necessity to increase crop production to meet ever increasing human population especially in the developing countries, has given rise to the increasing use of agrochemicals such as herbicides which saves the farmers' time by replacing laborious weed control [2]. When herbicides are applied in restricted areas, they are washed and carried away by rains and floods to nearby aquatic systems, thereby affecting aquatic biota, especially fish, which serves as a rich source of protein supplement for the teaming population. Aquatic habitats are particularly sensitive because a wide variety of pollutants derived from human activities sooner or later end up being washed to streams and rivers, where they can act synergistically, jeopardizing the quality of these habitats [3]. Chemicals and toxins affect humans directly or bio-accumulate in fish and other organisms consumed by humans causing developmental and neurological damages [4]. The effects of growing human activity on aquatic life and water quality has resulted to increasing concern since the application of chemicals in agriculture, aquaculture, animal husbandry and postharvest technology is a threat to aquatic biota (Ecosystem), public health and welfare of mankind [5]. It has been reported that diseases, pollution and presence of agricultural chemicals in water cause alterations in blood cells of fish resulting in losses in aquaculture [6-10]. Histological

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studies has shown that residual effects of pesticides and herbicides has resulted in the damage of important organs like the kidney, liver, gills, stomach, brain and muscles [11-13].

Orizo plus (Propanil and 2, 4Dichlorophynoxyacetic acid) herbicide is readily available in our local markets and is commonly used by our local farmers to control weeds in their farms. Run-offs of this herbicide to our local aquatic habitats are expected and they pose serious problems not only on the quality of the water but constitute risk to non-target organisms such as fish and the teaming population that use the fish as their source of protein. This study therefore intended to investigate the exposure and effects of this herbicide on the water quality using a freshwater fish *C. gariepinus* as the non-target organism. Fishes are important sources of proteins and lipids for humans and domestic animals, so the health of fishes is very important for human beings [12]. This study investigated the interaction of this commonly used chemical herbicide, Orizo plus (Propanil and 2, 4-Dichlorophynoxyacetic acid.), with emphasis on the behaviour, histology of the intestine, skin, some blood parameters and biochemical properties of a local fresh water fish *C. gariepinus*. The choice of *C. gariepinus* in this study is based on several ecotoxicological characteristics of the species such as wide distribution in the fresh water environment, noninvasiveness, availability throughout the season, ability to acclimatize easily to laboratory conditions and the fact that it is choice fish which is always in high demand by the populace.

MATERIALS AND METHODS

A total of 350 juveniles of a Fresh water fish, *C. gariepinus* (Family: Clarridae) weighing 25.6g ± 23.4, with a mean length of 23.14 ± 16.21were purchased from a local fish farm and were acclimatized under laboratory conditions for two weeks.The specimens were weighed in grams (g) using electronic weighing balance, Model: Scout Pro SPU401 and the length in centimeter (cm) was determined using meter rule.

The herbicide Orizo plus containing 360g of Propanil and 200g 2, 4.Dichlorophynoxyacetic acid (2, 4. D) was procured from the local market and used in the research. The fish samples were fed on a commercial floating pellets diet (3% of the body weight per day). The water was changed every 24 hours to eliminate fecal matter and other waste materials and reduce ammonia content in water. Some of the fish specimen (≤ 3%) died during the acclimation period while the rest survived and were used in the range finding test and the definitive test. Based on the range finding test, five concentrations, (60.00, 80.00, 100.00, 120.00 and 140.00 µg/L,) of the herbicide were prepared in five plastic tanks (20 x 20 x 20 cm) each containing 10 liters of water. The tanks were labeled, A, B, C, D and E in descending order. A set of ten acclimated fish specimens was randomly exposed to each static tank containing the different concentrations of the herbicide. The experiments were set in triplicates to obtain the LC_{50} value of the herbicide. A control experiment was set up simultaneously. The test media were changed every 48hours to maintain a more constant concentration of the test media to which the animals were exposed and to prevent excessive accumulation of toxic metabolites. The exposure was continued up to 96 hrs. During this exposure period the mortality rate of the fish was recorded at 24, 48, 72 and 96 hours. Dead fish were promptly removed to reduce further fowling of the water. A fish was considered dead if it makes no movement when prodded with a glass rod. Mortality rates of the fish population based on concentration and time of exposure were recorded.The median lethal concentration (LC_{50}) of Orizo plus was calculated to be 25.12 µg/l. Based on the 96 hour LC_{50} value, three test concentrations of the herbicide Viz. I(1/10th Lc_{50}), II(1/9th of LC_{50}) and III(1/8th of Lc_{50}) were determined and labeled F, G and H in descending order. Afterward, a set of ten fish were exposed to each of the three concentrations in three triplicates. A control experiment was set up simultaneously. The exposure was continued up to 96 hours.

Some behavioral responses of fish were observed in exposed as well as the control group at 24, 48, 76 and 96 hours of exposure and recorded as suggested by OECD [13]. Some physicochemical parameters of the test water namely, temperature, pH, conductivity, dissolved oxygen and total dissolved solutes were analyzed using the standard methods. Temperature reading was taken using a thermometer and the pH by the pH meter. The total Dissolved oxygen level (DO) of the test water, conductivity and total dissolved solutes (DTS) of the test water were also measured by the use of Hanna digital instruments.

At the end of the 96h experiment, two fish from each tank (Six fish per group) were removed; the blood was collected by puncture of the caudal vein into tubes containing anticoagulant potassium salt of ethylene diamine tetra-acetic acid (EDTA), Sodium fluoride and plain tubes. Each tube was labeled accordingly. The blood in the EDTA tubes was used for hematological analysis; the blood in the fluoride tubes was used for glucose

analysis while the blood samples in the plain tubes were used for biochemical analysis. The fish were then dissected and the small intestine and muscle were excised and preserved with normal saline for histological analysis following the procedure adopted by Tayeb *et al.* [14] with some minor modifications.

Hemotological Analysis: The blood parameters assessed in this study were determined using the method of Ochei and Kolhatkar [15].

The Red blood cells (RBCs) count was determined by adding whole blood (20 µl) to 3.98 ml of diluting fluid (10 % sodium citrate) and mixed thoroughly. After 5 minutes, the first five drops were discarded by holding the pipette vertically. The diluted blood was then introduced into the counting chamber and counted after three minutes with the aid of a compound microscope. Therefore;

$$\text{Total RBCs (mm/l)} = N \times \frac{1}{0.2} \times \frac{1}{0.1} \times 200$$

N= number of cells counted, 0.1 = depth of chamber, 0.2 = area counted, 200 = dilution factor.

To estimate the white blood cell (WBC) count, whole blood (20 µl) was added to 380 µl of diluting fluid (acetic acid, with gentian violet) and mixed. The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leucocyte. The counting chamber was charged with the well mixed diluted blood (After discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes and counted in all the four marked corner squares. Therefore;

$$\text{Total WBCs (mm}^3) = \frac{N}{0.1 \times A} X 20$$

N = numbers of cells counted; 0.1 = depth of the chamber, A = area counted; 20 = dilution factor.

The packed cell volume (PCV) was estimated by centrifuging 1ml of whole blood in heparinized capillary tube using a microhaematocrit centrifuge spunned at 10, 000 rpm for 5 minutes. Spunned tubes were placed into a specially designed scale and the PCV was read as a percentage of the whole blood.

$$\text{PVC \%} = \frac{\text{Packed RBC column height}}{\text{Total blood volume height}} \times 100$$

Hemoglobin (Hb) concentration was determined using cyanomethaglobin technique as outlined by Ochei and Kolhatkar [15]. Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution and mixed very well. This was allowed to stand for 10 minutes at room temperature. Drabkin's solution contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin which is converted to colored cyanmethemogloin by the cyanide and the absorbance read colorimetrically at 540 nm with Drabkin's solution as a blank.

$$\text{Hemoglobin (Hb)} = \frac{\text{Reading of test x conc. standard}}{\text{Reading of standard}}$$

The Wintrobe indices; (i) mean cell corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean cell haemoglobin content (MCH) were derived from these primary indices.

Biochemical Analysis: The activities of liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit [16]. The total protein content was determined using the method of Tietz [17]. Plasma glucose concentration was analyzed using a commercial enzyme kit (Glu L 1000, PLIVA-Lachema, Czech Republic).

Statistical Analysis: The LC_{50}value of the herbicide for juveniles of *C. gariepinus* were determined following the probit analysis method as described by Tilak *et al.* [5], Nwani *et al.* [8], Okogwu *et al.* [10] and OECD [13]. The statistical differences between test groups were estimated with Analysis of variance (ANOVA) using Statistical Programme for Social Sciences (SPSS) software, version 21. P values less than 0.05 ($p < 0.05$) was considered to be statistically significant.

RESULTS

Effect of Orizo plus on the Mortality Rate of *C. gariepinus*: No death was observed in the control group throughout the period of exposure of *C. gariepinus* to Orizo plus. However, different mortality rates were recorded in the various concentrations (Groups). In Group A, 50.00 % mortality was recorded while 60.00 % mortality was recorded in Group B. Mortality rates of 63.33, 80.00

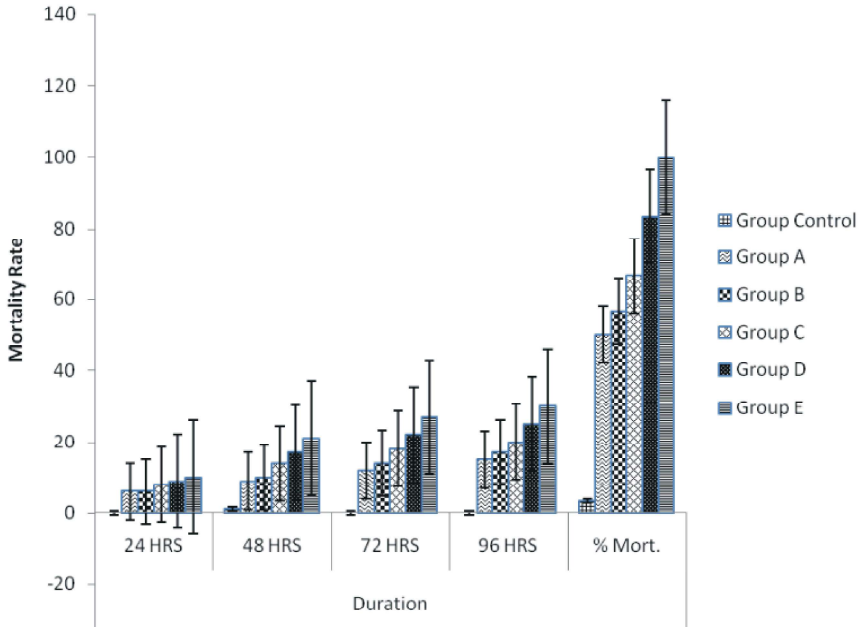

Fig. 1: Mortality rate of *C. gariepinus* during 96 hour exposure to different concentrations of orizoplus. The vertical lines represent standard errors

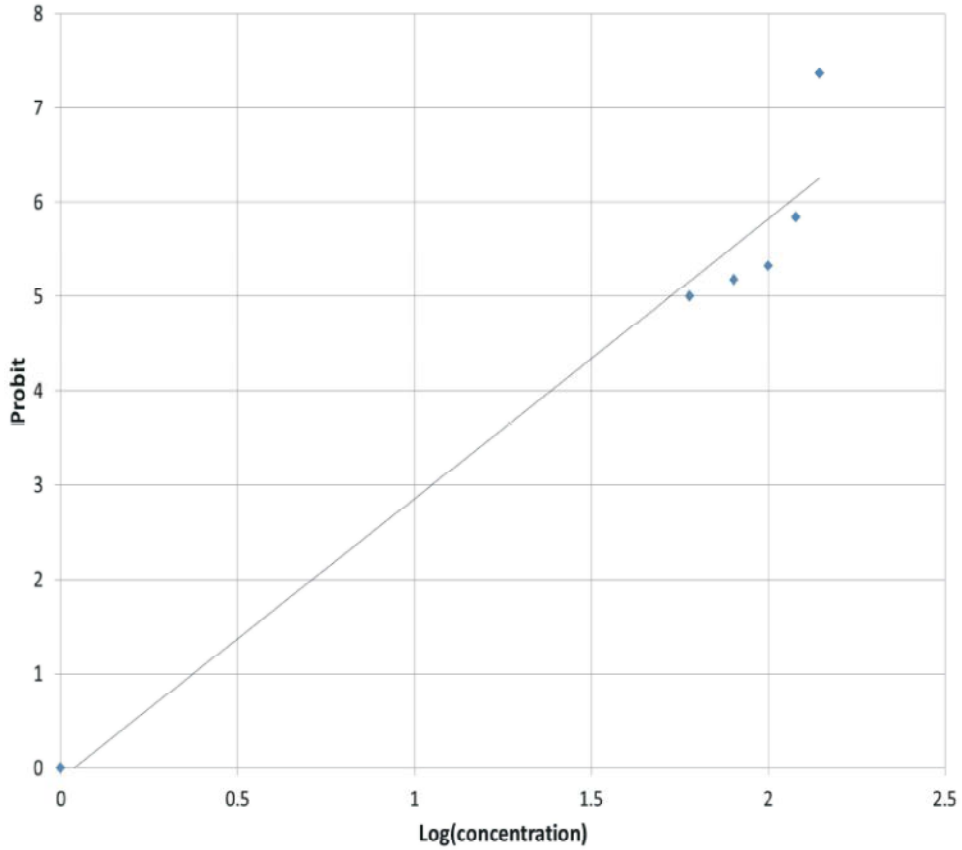

Fig. 2: Probit Method for Estimating Median Lethal Concentration (LC50) of Orizoplus in *C. gariepinus* (LC50 = 25.12 µg/L)

Table 1: Physico-chemical parameters of the treatments during the in on *C. gariepinus* at 96hours

Conc.	Temperature (OC)	pH	DO (mg/L)	Conductivity (µS/cm)	TDS (mg/L)
Control	27.61±0.21a	7.60±0.06a	5.30±0.12a	735.67±2.91a	326.00±3.79a
D	28.30 ± 0.25b	7.30±0.09b	4.77±0.26b	763.33±3.18b	387.33±1.20b
E	28.47±0.28b	7.20 ±0.12b	3.83±0.18b	803.00 ±9.45b	369.33±21.85b
F	28.57±0.12b	6.93±0.09b	2.93±0.24b	832.33±7.31b	436.00±11.64b

and 100 % were recorded in in Group C, D and E respectively. In a similar trend as in the exposure of *C. gariepinus* to primextra, dose and time dependent increase in mortality was observed in the bioassay.

The mortality rate observed during the 96 hour exposure of *C. gariepinus* to Orizo plus is presented in Fig. 1.

Median Lethal Concentration of Orizoplus Exposed to *C. gariepinus*: The median Lethal Concentration which is the concentration of the toxicant that will kill 50% of the test organism, in this bioassay, *C. gariepinus* exposed to orizoplus was determined by Probit method (Graphical method) to be 25.12µg/L as shown in Fig. 2.

Impact of Orizoplus on Some Physico-Chemical Variables of the Treatments) at 96 hours: The impact of Orizo plus on some physico-chemical variables of the Treatments is presented in Table 1. The value of the temperature was 27.30 ± 0.21 in the control. The value increased to 28.30 ± 0.25 in group F, then to 28.47 ± 0.28 in group G and 28.57 ± 0.12 in group H. The pH was 7.60 ± 0.06 in the control. The value was significantly reduced to 7.27 ± 0.09 in group F, later to 7.20 ± 0.12 in group G and finally to 6.93 ±0.09 in group H. The value of DO was 5.30 ± 0.12 in the control group. This value was reduced to 4.77 ± 0.26 in group F, later to 3.83 ± 0.18 in group G and 2.93 ± 0.24 in group H. The value of the conductivity in the control was 735.67 ± 2.91. The value appreciated to 763.33 ± 3.18 in group F, 803.00 ±9.45 in group G and 832.33 ± 7.31 in group H. For TDS in orizoplus, the value was 326.00 ± 3.79 in the control. The value increased to 387.33 ± 1.20 in group F, to 369.33 ± 21.85 in group G and 436.00 ± 11.68 in group H.

Impact of Orizo plus on Some Behavioral Parameters of *C. gariepinus*: There was no abnormal behaviour in the fish in the control group throughout the 96 hours of the bioassay while the fish subjected to different concentrations of the herbicide displayed uncoordinated behaviors at different durations. The fish in group F showed mild hyperactivity, moderate equilibrium status, moderate swimming rate, mild gulping of air, no convulsion nor somersaulting, moderate fin and opercula movement at 24 hours, but at 48 hours the fish exhibited mild hyperactivity, moderate equilibrium status, moderate swimming rate, moderate gulping of air, mild convulsion and somersaulting and moderate fin and opercula movement. At 72 and 96 hours, the fish showed mild hyperactivity, moderate equilibrium status, moderate swimming rate and moderate gulping of air, no convulsion and no somersaulting, moderate fin and opercula movement. The fish in group G showed moderate hyperactivity, moderate swimming rate, mild gulping of air, mild convulsion and somersaulting but moderate fin and opercula movement within 24 and 48 hours of exposure. At 72 and 96 hours this group of fish displayed mild hyperactivity, moderate equilibrium status, moderate swimming rate, mild gulping of air, mild convulsion, no somersaulting, moderate fin and opercula movement. In group H, the fish exhibited moderate hyperactivity, mild equilibrium status, moderate swimming rate, moderate gulping of air, mild convulsion and somersaulting, moderate fin and opercula movement at 24 hours. At 48 hours the fish displayed moderate hyperactivity, mild equilibrium status and moderate swimming rate, moderate gulping of air and convulsion, mild somersaulting, moderate fin and opercula movement. At 72 hours the fish showed moderate hyperactivity, mild equilibrium status, moderate swimming rate and gulping of air, mild convulsion and no somersaulting, moderate fin and opercula movement. At 96 hours the fish displayed moderate hyperactivity, mild equilibrium status, moderate swimming rate, mild gulping of air, no convulsion nor somersaulting and modrate fin and opercula movement (Table 2).

Hematological Effects of Orizo plus on the Peripheral Blood of *C. gariepinus*: The hematological parameters such as RBC, WBC, Hb, PCV, MCH, MCV and MCHC in *C. gariepinus* exposed to oryzoplus are presented in Table 3.

The RBC value in the control group was 10.42 ± 1.45. The value however decreased to 8.12 ± 2.01 in group F, later to 8.09 ± 1.56 and 8.20 ± 1.75 in group G and H respectively. The WBC value in the control group was 9400 ± 10.50. However, the value significantly increased ($p < 0.05$) to 11400 ± 15.05 and 10800 ± 10.02 in the fish in

Table 2: Impact of Orizoplus on the Behavioral parameters of *C. gariepinus* at Various Concentrations and Durations

TC. (µg/L)	Time (h)	Hyperactivity	Equilibrium status	Swimming rate	Gulping of air	Convulsion	Somersaulting activity	Fin movement	Opercula movement
Control	24	-	+++	++	-	-	-	++	++
	48	-	+++	+	-	-	-	++	++
	72	-	+++	++	-	-	-	++	++
	96	-	+++	++	-	-	-	++	++
D	24	+	++	++	+	-	-	++	++
	48	+	++	++	++	+	+	++	++
	72	+	++	++	++	-	-	++	++
	96	+	++	++	++	-	-	++	++
E	24	++	+	++	+	+	+	++	++
	48	++	+	++	+	+	+	++	++
	72	+	++	++	++	+	-	++	++
	96	+	++	++	++	+	-	++	++
F	24	++	+	++	++	+	+	++	++
	48	++	+	++	++	++	+	++	++
	72	++	+	++	++	+	-	++	++
	96	+	+	+	+	-	-	+	+

Keys: - = none, + = Mild; ++ = Moderate; +++ = Strong, TC = Toxicant concentration

Table 3: Hematological parameters (mean ± SE) of juvenile *C. gariepinus* at 96 h exposure to sublethal concentrations of Dragon, Oryzoplus and Primextra.

		Haematological parameters						
Pesticide	Concentration (µg/L)	RBC (×106)	WBC (×103g/L)	Hb (%g dl/L)	PVC (%)	MCH (pgcell/L)	MCV (fl cell/L)	MCHC (g dl/L)
Oryzoplus	Control	10.42±1.45a	9400 ± 10.50a	10.70 ± 1.50a	35.00 ± 2.06a	10.27 ± 1.05a	33.59 ± 1.43a	30.57 ± 1.02a
	D	8.12 ± 2.01a	11400 ± 15.05b	9.00 ± 0.60a	28.00 ± 1.80b	11.08 ± 0.99a	34.48 ± 1.59a	32.14 ± 0.93a
	E	8.09 ± 1.56a	11200 ± 11.15b	8.70 ± 0.80a	32.00 ± 2.50a	10.75 ± 0.89a	39.56 ± 1.88a	27.19 ± 0.85a
	F	8.20 ± 1.75a	10800 ± 10.02b	9.60 ± 0.78a	33. 00 ± 2.60a	11.71 ± 1.02a	40.24 ± 2.03b	29.09 ± 0.87a

Table 4: Biochemical parameters (mean ± SE) of juvenile *C.gariepinus* after 96 h exposure to sublethal concentrations of Dragon, Oryzoplus and Primextra. Values with different alphabetic superscripts differ significantly ($p < 0.05$) between concentrations within the same column

		Biochemical parameters					
Pesticide	Concentration (µg/L)	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	Glucose (mg/dL)	Protein (mg/dL)	Bilirubin (mg/dL)
Orizo plus	Control	40.01 ± 1.01a	68.02 ± 1.38a	64.04 ± 1.10a	80.20 ± 2.33a	8.42 ± 0.96a	4.30 ± 0.17a
	D	38.10 ± 0.98a	75.04 ± 1.79b	64.02 ± 1.23a	61.14 ± 2.17b	5.61 ± 0.88a	4.28 ± 0.25a
	E	36.04 ± 0.87a	70.07 ± 2.30a	66.14 ± 2.04a	68.24 ± 2.30c	5.83 ± 0.75a	3.64 ± 0.23a
	F	40.02 ± 0.15a	78.11 ± 2.01b	58.20 ± 1.09a	60.13 ± 1.08b	6.11 ± 0.77a	4.63 ± 0.21a

Legend: Values with different alphabetic superscripts differ significantly ($p < 0.05$) between concentrations within the same column

group F and group G respectively. For the Hb, the value in the control fish was 10.70 ± 1.50. The value however decreased to 9.00 ± 0.60 and 9.60 ± 0.78 in fish in group F and group G respectively. The PCV value in the control fish was 35.00 ± 2.06. The value decreased to 28.00 ± 1.80 and 33.00 ± 2.60 in fish in group F and group H respectively. The value of MCH in the control group was 10.27 ± 1.05. The value increased to 11.71 ± 1.02 in fish in group H. No significant difference($p>0.05$) was observed between the experimental group and the control. Similar increase in MCV was observed in all the concentrations. However, significant increase ($p < 0.05$) was observed in fish exposed to G and H. For MCHC, no significant differences ($p > 0.05$) were observed between the control and fish exposed to all the concentrations.

Biochemical Effects of the Herbicides on the Peripheral Blood of *C. gariepinus*: The biochemical parameters which include, AST, ALP, ALT, glucose, Protein and bilirubin *of C. gariepinus* exposed orizoplus are presented in Table 4.

The value of AST in the control was 40.01 ± 1.01. The value was altered to 38.10 ± 0.98 in group F, 36.04 ± 0.87 in group G and 40.02 ± 0.15 in group H. However, no significant difference ($p >0.05$) was observed in the groups compared to the control. Similar trend was observed for ALP. The value in the control was 68.02 ± 1.38. The value increased to 75.04 ± 1.79 in group F, 70.07 ± 2.30 in group G and 78.11 ± 2.01 in group H. The value of ALT was 64.04 ± 1.10 in the control and was altered to 64.02 ± 1.23 in group F, 66.14 ± 2.04 in group G and 58.20

± 1.09 in groupH. The glucose value was 80.20 ± 2.33 in the control. The value was reduced to 61.14 ± 2.17 in group F, 68.24 ± 2.30 in group G and 60.13 ± 1.08 in group H. |The value of protein was 8.42 ± 0.96 in the control. The value decreased to 5.61 ± 0.88 in group F, 5.83 ± 0.75 in group G and 6.11 ± 0.77 in group H. Though there were decreases in the values of protein in all the groups, the decrease was not significantly ($p > 0.05$) different compared to the control. The value of was 4.30 ± 0.17 in the control. The value was altered to 4.28 ± 0.25 in group F, 3.64 ± 0.23 in group G and 4.63 ± 0.21 in group H. In all, no significant difference ($p < 0.05$) was evidenced in the values of bilirubin after exposure.

DISCUSSION

Fish are purely aquatic organisms and any substance that has negative impact on the water quality consequently has adverse effects on the fish. The indirect proportional relationship observed in the present study between the DO and concentration of the herbicide is in consonance with the report of many authors [7, 10, 18-21]. The decrease in the DO (Hypoxia) in the higher concentrations could have contributed to the high mortality rate observed in the fish exposed to the higher concentrations of the herbicides. The decline in dissolved oxygen in the treated groups is attributed to asphyxiation resulting to the surfacing and gulping of air, somersaulting, strong swimming rate and strong fin and opercula movement in the fish as attempts to adjust to life in the water that has been contaminated by the herbicides.

Fish are directly exposed to aquatic pollutants and their behavioral responses are the most sensitive indication of potential toxic effects [11]. Abnormal behavioral responses which include, erratic swimming, loss of equilibrium, gasping for air, convulsion, somersaulting, restless opercula and fin movement observed in the present study is in agreement with the report of many authors [10, 19-23]. The abnormal behaviors could be attributed to direct poisoning of the herbicide or deteriorating of the water quality by the polluting effect of the herbicide [19].

Mortality is a decisive criterion in toxicity tests because it is easy to determine and has obvious biological and ecological significance [8, 24]. A dose dependent increase in mortality observed during the bioassay is in agreement with the report of many researchers [8, 9, 19, 25] who have shown that as the concentration of the herbicide increased, fish mortality also increased indicating a direct proportional relationship between mortality and concentration of an herbicide.The median Lethal Concentration (LC50) estimated to be 25.12µg/L in the present study differs from that reported by [22] estimated the LC_{50} of Diethyl Phthalate (DEP) at log toxicant concentration as 2.217, 2.734, 3.435 and 3.931 µg/l at 24, 48, 72, 96 h for *C. Gariepinus*[10] reported LC_{50} of 1.04mg/L for 2, 4-D in Clarias gariepinus [6] reported an LC_{50} value 0.0072 ml/l. at 96 h during their study on the exposure *Clarias gariepinus* to an herbicide (Glyphosate). The differences in the LC_{50} estimated in the present study and those reported by these authors is in line with the report of Neskoviæ *et al*. [25] who have shown that the effects of herbicides depend on fish species, type of compound and its concentrations in water and exposure time.

The reduction in the RBC, Hb and PCV though not significant in the present study might have resulted from the depletion of the oxygen content of the water due to the presence of herbicide in the test media. Dorucu and Girigin [6] reported that Cypermethrin had no effect on the blood indices of Oncorhynchus mykiss while Okogwu *et al*. [10] reported that PVC, RBC and Hb decreased significantly in their study on the exposure of *Clarias gariepinus* to 2, 4 Dichlorophenoxy acetic Acid (2, 4-D).The significant ($p<0.05$) increase inWBC in the present study is in agreement with the report of many authors [10, 20, 26] and could be attributed to protective response of the fish against the effects of the herbicide.

The reduction in the serum glucose in the present study can be attributed to stress caused by the herbicide orizo plus that resulted to excess utilization of the serum glucose.Many researchers' have reported similar reduction in serum glucose which is a sensitive and reliable indicator of pollutants causing environmental stress in fish [27, 28] opined that Stress is as an adaptive mechanism that allows the fish to cope with real or perceived stressors, in order to maintain its normal or homeostatic state, meaning that stress can be considered as a state of threatened homeostasis that is reestablished by a complex suite of adaptive responses.

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [29]. The intestine can be used as a sensitive organ in toxicity studies since it is directly exposed to pollutants via drinking and feeding or indirectly via blood or lymph [9, 30]. The alterations and damage observed in the

intestine of *C. gariepinus* in the present study is in agreement with report of Vidhya and Radhakrishnan [31] who reported degeneration of mucosa and sub mucosa layer, degeneration changes in tips of villi, condensed musculosa, curved vill, necrosis, ruptured villi, completely damaged villi, loss of architecture and degeneration of serosa in their study on the effect of Pyrethroid insecticide, Lambda-Cyhalothrin on the intestine of fish, *Etroplus suratensis*. Samanta *et al.* [32] reported damaged Columnar Epithelial Cells (CEC) and mucosal folds, thinning of the top plate and fused mucosal folds in their work on the gastrointestinal pathology in freshwater fish, *Oreochromis niloticus* under almix exposure. The primary functions of the intestine are digestion and absorption of digested food. Any substance that has the capacity to damage the intestine will have a consequent effect on the fish health since digestion will be impaired and absorption process slowed down or completely stopped.

Distortion, fragmentation and loss of muscular tissue observed in the present study is in agreement with the necrosis of skeletal muscle reported by Omoniyi *et al*. [11] in *Tilapia nilotica* in White Nile most likely be to contamination by pendimethalin a herbicide heavily used for the control of growth of weed in White Nile State. Rahman *et al.* [33] reported swelling and necrosis of muscle on the histopathological study on the effect of rice herbicides on grass carp (*Ctenopharyngodanidella)* fibres. Similar distortions of fish muscles exposed to different xenobiotics have been reported by several authors [9, 34, 35]. Fish muscle is a choice food and delicacy for humans, consequently any substance that has a negative effect on fish muscle can indirect affect man via food chain.

Histopathological alterations of vital organs could affect the survival rate, biological activities, osmoregulation, reproduction, buoyancy, etc., which finally could lead to failures in stock recruitment and population changes [24] and conservation of the affected species.

CONCLUSIONS

The results of this study showed thatthe water quality was reduced, the behaviour of the fish was adversely affected, the hematological and biochemical parameters were altered and the organs examined were damaged. These results indicate that this herbicide is toxic and must be used with caution to protect the environment especially the non-target organisms particularly fishes.

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