Behavioural and Haematological Alterations in the African Catfish (*Clarias gariepinus*) Exposed to Varying Concentrations of Glyphosate

ABSTRACT

Extensive use of herbicides poses a serious threat to aquatic life due to runoff from treated fields. A static bioassay method was used to evaluate the toxicity of acute exposure to glyphosate on *Clarias gariepinus* behaviour and haematological parameters. Glyphosate was tested at varying concentrations (0.72, 1.44, 2.16, and 2.88 mg/l (control), 0.72, 1.44, 2.16, and 2.88 mg/l) for 96 hours in the laboratory. Significant ($P < .05$) dose-dependent behavioural and morphological changes of respiratory disturbance, erratic swimming, loss of equilibrium, mucous secretion, and mortality were recorded in the surviving fish. Erythrocyte (RBC), haemoglobin (Hb), packed cell volume (PCV) and leukocyte (WBC) values decreased significantly ($P < .05$) in treated fish as compared with controls. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were not significantly different ($P >.05$) between the treated and control fish. Insignificant dose-dependent changes in the counts of neutrophils, monocytes, basophils, and eosinophils were also induced by glyphosate. The variations seen in this study demonstrated that glyphosate had a detrimental effect on the health of the fish. Educating farmers on recommended modes for the administration of glyphosate-herbicides on farmlands should be implemented and enhanced.

Keywords: Glyphosate; Acute Toxicity; *Clarias gariepinus*; Behaviour; Haematology.

1. INTRODUCTION

Increased and extensive use of herbicides has raised concerns in crop production [1]. "Aquatic organisms absorb pollutants both directly from contaminated water and indirectly through the food chain, which may pose a significant hazard to aquatic biodiversity and human health" [2]. Herbicide-based pesticides are seen in vast arrays of complex agricultural, domestic, and industrial effluent. Water plants can obstruct flow during the summer when sudden heavy rain can cause flooding, so herbicides are frequently used to control them. [3]. In addition, it is recorded that these compounds may cause heritable changes in the genetic material without these changes being expressed immediately [4].

A wide range of annual, biennial, and perennial grasses, sedges, broad-leaved weeds, and woody shrubs are all controlled by the broad-spectrum herbicide glyphosate, which is primarily used in agricultural applications. Additionally, it is utilized in vineyards, conifer plantations, and orchards that grow fruit [5]. Additionally, it is employed in slow-moving water, lakes, canals, and fish ponds to control aquatic weeds. [6]. In 2014, there were 79 million kilograms of glyphosate used globally, up from 16 million kilograms in 1994, with 15% of that use occurring in the United States alone [7]. The herbicide N-(phosphonomethyl) glycine (glyphosate) is a broad-spectrum biocide that was first used in 1974 to control weeds in agricultural production fields [7]. The systemic organophosphorus compound glyphosate, is effectively used globally for weed control [2].

In addition to being used intensively, glyphosate leaves an increasing amount of residue in the environment and on plants. Due to the inert C-P linkages in the molecule, glyphosate is quite resistant to degradation, according to Chekan et al [8]. Modern pesticides allow for low-dose applications because they are more target-specific and typically have higher acute toxicity. Many contemporary pesticides, such as organophosphates, have the drawback of binding to soil particles less readily and moving relatively quickly through soils to groundwater and surface waters [9]. Thus, several compounds dispersed in the environment may represent a danger to aquatic life, biodiversity, and human health, since they potentially induce mutations [10]. Spraying pesticides over arable land results in some of them being retained and degraded by soil microorganisms. However, some are released into the environment, moved to the atmosphere, and enter groundwaters via volatilization, wind drift, surface runoff, and runoff, impacting negatively the aquatic ecosystem [11]. Additionally, just as pesticides can harm non-target organisms, the loss of

herbicides from arable land can have a negative impact on marine life [2,12]. Chemical toxicity testing on aquatic animals has been used for years to evaluate the risks that may be posed to a larger population, almost always including humans [13]. In order to determine whether a potential toxicant is harmful to aquatic life, there is a need to determine the relationship between toxicant concentrations and their impact on aquatic animals, an aquatic bioassay is also required for water pollution control [2]. Thus, toxicological studies have the potential to identify possible risks in the environment since contamination mutagens recorded in aquatic organisms may directly or indirectly affect the health of the entire ecosystem, including humans [14]. *Clarias gariepinus*, an African catfish, served as a biological model for this toxicological investigation due to the intrinsic potential of its respiratory structure and tolerance for polluted waters. Moreover, it is common in Nigeria's water bodies [15]. Hence, they are used as biological indicators for ecotoxicological studies as they closely relate to their aquatic habitat and can biotransform xenobiotics within their water environment [16].

A number of anthropogenic and environmental stressors, including the degradation of their habitat and the availability of food, pose a threat to the local availability of healthy African catfish [17]. The primary goal of this study was to evaluate the acute toxicity of glyphosate in commercial formulations and its effects on behaviour and haematological profile in light of glyphosate's widespread overuse in agriculture.

2. MATERIALS AND METHOD

2.1 Experimental Fish Collection and Maintenance

Male and female 56-day-old African Catfish; *Clarias gariepinus* juveniles of relatively uniform weight (11.4 ± 1.1g) and standard length (10.2 ± 0.9 cm) were procured from a local fish farmer in Anambra state, Nigeria. In order to reduce stress, the juveniles were carefully transported in clean, aerated freshwater to the lab. They were acclimatized in the laboratory for two weeks before the static bioassay was conducted after being randomly assigned to aquaria. Salinity 140.4 1.7 mg/l, pH 7.3 0.2, dissolved oxygen 9.50 2.04 mg/l, and temperature 27 1.0 C were the average values for water quality. Over the course of the entire study period, the 12 h: 12 h light/dark cycle was maintained. A commercial diet containing 35% crude protein was fed to fish twice daily (9 a.m.–1 p.m.). In order to prevent fouling, faeces were removed from the water in the experimental tanks each day.

2.2 Chemicals

Glyphosate (herbicide) is the chemical evaluated for its probable toxicity effect on African catfish. The commercial grade, which goes by the trade name "Forceup," is made in China by Zhejiang Xian Chem Group co., Ltd. It was bought at a local market in the Anambra State. It has a 360 g/l stock solution that was used to make the different concentrations (0.72, 1.44, 2.16, and 2.88 mg/l), as well as a control group to which glyphosate wasn't added.

2.3 Experimental Procedures and Design

The Sprague [18] and APHA-AWWA-WPCF [19] methods were followed in the adoption of a static renewal bioassay technique. A total of 15 experimental glass aquariums (46 x 31.5 x 25 cm; n = 10 fish per aquarium) were filled with a total of 150 juveniles. The glyphosate-based herbicide was used to formulate five concentrations for the bioassay: 0.72, 1.44, 2.16, and 2.88 mg l−1 and a control group without glyphosate. Twenty litres (20) of dechlorinated water were used in each treatment, which was repeated three times. After being measured, the stock solution was added to the test tanks. The solutions were stirred for homogenous mixing before each aquarium was randomly stocked with the fish. In accordance with the guidelines of Reish and Oshida [20], feeding was stopped 24 hours before the start of the bioassay to prevent interference from faeces. The 24, 48, 72, and 96-hour survival and mortality rates were noted. Forceps were used to remove the dead fish, and probit analysis was used to calculate the glyphosate LC50 values for the fish at 96 h of exposure [21].

2.4 Behavioural/Morphological Assessment

At 24, 48, 72, and 96 hours into the acute toxicity tests, observations of the behavioural and morphological responses of *C. gariepinus* juveniles exposed to glyphosate herbicide were made. For this study, the techniques created by Drummond et al. [22] were applied. To provide a baseline for evaluating any behavioural and

morphological changes, controls without toxicant exposure and acute concentrations were observed. Responses were noted if they were different from the control group or if 10% of the fish in each test tank experienced them. Loss of equilibrium, general activity, startle response, erratic swimming, deformity, haemorrhage, and respiratory disturbance were among the changes. For 5–10 minutes, each test chamber was monitored. By lightly touching the fish with a plastic applicator stick, startle reactions were observed (tactile stimulus). For the purposes of the experiment, death was defined as the end of spontaneous movement and the inability to react to mild stimuli.

2.5 Blood Collection and Haematological Analysis

Blood samples were taken from the fish that survived in the various treatments for assay by severing the artery at the caudal region at 2 cm from the caudal peduncle of three fish per treatment, as described by Blaxhall and Diasley [23]. Fish specimens were anaesthetized with tricaine methane sulfonate (MS-222; 50.0 mg/l) to reduce stress to enable the accessible collection of the blood samples. Blood collection was done at the end of 96 h of exposure from the surviving fish of the various treatments for haematological analysis. The Red Blood Cell was estimated using Neubauer's as blood was pipetted from the blood sample and added to 4 ml of the RBC diluting fluid (Toisson's solution). described by Hesser [24]. Briefly, 0.02 ml of This was done to make a 1:200 dilution of the blood sample in a fresh test tube. The mixed blood sample was loaded onto a Neubauer counting chamber and all RBCs in the central area of the Neubauer improved cell counting chamber were counted using a light microscope. at 40 × objectives. The number of cells counted for each sample was multiplied by 10 000 to obtain the RBC count per ml of blood. The packed cell volume (PCV) was determined by the micro-Westergren method as described in Blaxhall and Daisley [23]. The well-mixed sampled blood from the heparinized was drawn into a microhematocrit tube, 75 mm long and 1.1–1.2 mm internal diameter. The tubes were centrifuged for 5 minutes. The reading is made with a micro-hematocrit reader and expressed as the volume of the erythrocytes per 100 cm3. The haemoglobin content (Hb) of blood samples was determined using the cynomethaemoglobin method described by Briggs and Bain [25], using a Drabkins reagent that converts the haemoglobin and carboxyhaematoglobin to cynomethaemoglobin. The White Blood Cell (WBC) was estimated after diluting the blood with WBC diluting fluid (1:20 v/v) as described by Houston [26]. A total of 0.02 ml of the blood was drawn up to the 0.5 mark on the stem of the white cell blood and pipetted into a small test tube, and 0.38 ml of the dilution fluid was added. A few drops of the diluted blood were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted using a 4 mm objective lens at 40 x magnification. The number of cells was multiplied by 10 x to obtain the total number of leucocytes per cubic millimetre (mm3) of blood [26]. While counting, the method of Hibiya [27] and Chinabut et al. [28] was used for identifying the numbers of the different classes of leukocytes (neutrophils, monocytes, lymphocytes, eosinophils, and basophils) in the blood smears. The number of each type of leukocyte was calculated as a percentage. Erythrocyte indices, such as MCHC, MCH, and MCV, were determined from the outcome of the RBC count, and Hb and PCV were estimated using the unified method of Dacie and Lewis [29].

$$\text{MCV } (fl) = \frac{\text{Ht\% x 10}}{\text{RBC (cells mm3)}}$$

$$\text{MCH } (Pg\ cell) = \frac{\text{Hb (g/100ml) x 10}}{\text{RBC (cells mm3)}}$$

$$\text{MCHC } (g/100ml) = \frac{\text{Hb (g/100ml) x 100}}{\text{PCV\%}}$$

MCV is Mean Corpuscular Volume, MCH is the Mean Corpuscular Haemoglobin and MCHC is Mean Corpuscular Haemoglobin Concentration.

2.6 Data Analysis

The data generated from the study were presented as mean and standard deviation (SD) and were analyzed using the SPSS IBM version 25.0 computer program (SPSS Inc., Chicago, Illinois, USA). Variations in the means were subjected to a one-way analysis of variance (ANOVA) to test the significant differences between the treatments. Statistical significance was declared at $P \leq 0.05$.

3. RESULT

The 96-h LC_{50} of glyphosate upon exposure of African catfish (*Clatrias gariepinus),* to varying glyphosate concentrations (0.00, 0.72, 1.44, 2.16, 2.88 mg/l) are presented in Figure 1. The LC_{50} was determined to be 1.50 mg/l, using the probit analysis method.

3.1 Behavioural and Morphological Changes in Fish

The behavioural responses of the test fish were observed at 24 to 96 h durations of exposure. The control group exhibited normal gill pattern, active swimming, static equilibrium, natural skin colouration, and no mortality throughout the bioassay as compared to the treated fish. However, fish exposed to the varying concentrations of glyphosate exhibited diverse abnormal behavioural responses, and morphological changes such as hyperactivity, jerky movements, loss of equilibrium and change of skin colouration from shining dark to dull ash, irregular fin movements, increased mucus secret, and erosion of fins. These behavioural changes were dose-dependent as shown in Table 1.

3.2 Fish Mortality

Fish mortality was observed in all the experimental aquaria tanks except in the control group. The result of the acute toxicity test showed mortality of the fish at various concentrations of glyphosate at varying durations (24, 48, 72, 96h), as presented in Table 2. The first mortality was observed at 12 hours for the exposed groups. By the 24, 48, and 72 hours, more mortality was observed in the various concentrations except in the control.

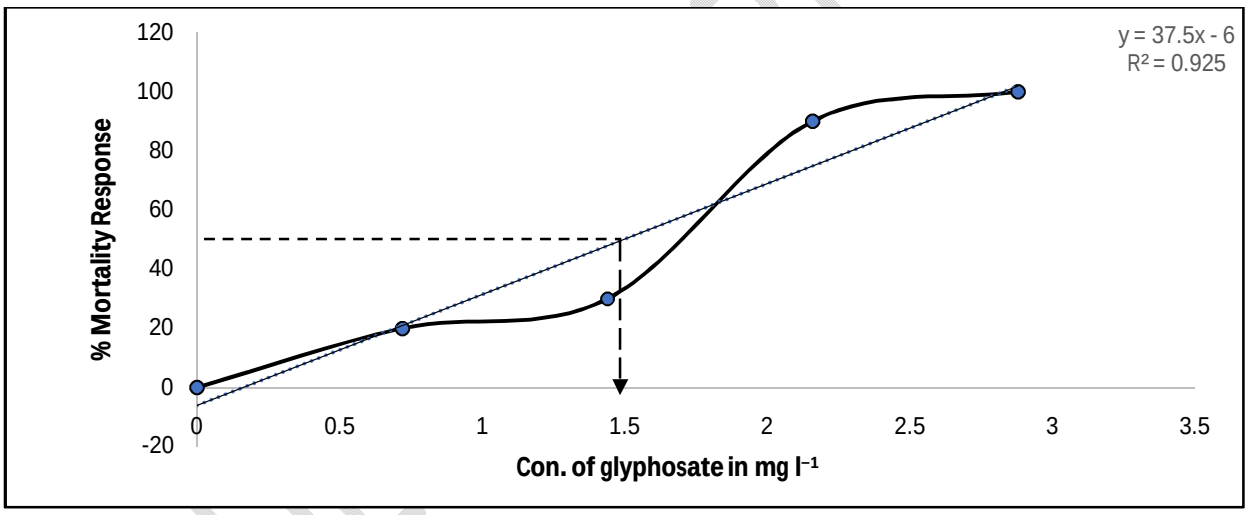

Figure 1: The relationship between glyphosate concentrations and mortality percentages.

Table 1: Behavioral and morphological characteristics of *Clarias gariepinus* exposed to different concentrations of glyphosate.

Concentration (mg /l)	Jerky movement	Hyper activity	Vertical Swimming	Gasping for air	Loss of equilibrium	Erosion of Skin	Mucus secretion
24 h							
Control	-	-	-	-	-	-	-
0.72	++	+	+	++	+	+	+
1.44	++	+	++	++	++	++	++
2.16	++	++	+++	+++	++	+++	++
2.88	++	+++	+++	+++	+++	+++	+++
48 h							
Control	-	-	-	-	-	-	-
0.72	++	++	++	+	+	++	+
1.44	++	++	+++	++	++	+++	++
2.16	+++	++	+++	+++	+++	+++	+++
2.88	+++	+	+++	+++	+++	+++	+++
72 h							
Control	-	-	-	-	-	-	-
0.72	+++	++	+	+	+	+	+
1.44	++	++	++	++	++	++	++
2.16	+	+	+++	+++	+++	+++	++
2.88	-	-	-	+++	-	-	-
96 h							
Control	-	-	-	-	-	-	-
0.72	++	++	+	++	++	+	+++
1.44	++	++	++	++	++	++	+++
2.16	++	++	+++	+++	+++	+++	+++
2.88	-	-	-	-	-	-	-

- None; + mild; ++moderate; +++ strong.

At 96 hours, the highest mortality of 80% and 100% were observed at 2.16 mg /l and 2.88 mg /l concentrations, respectively, while the lowest rate of 4% was recorded in the lowest concentration of 0.72 mg /l, with the control recording no death.

3.3 Haematological Parameters of *Clarias gariepinus* Exposed to Glyphosate

The result of the study presented in Table 3 showed that glyphosate exposure significantly ($P < .05$) affected the Red Blood Cell (RBC) count of the treated fish when compared to the control group, at the end of the 96h exposure period. The mean values of the RBC also showed the 0.00mg/l (control) having 28.2±2.14 x106 mm-3 which decreased significantly with an increase in glyphosate concentration as recorded in 2.16mg/l (5.3±0.35 x106 mm-3), an indication of dose-dependent toxicity. Similarly, the white blood cell (WBC) profile of the treated fish samples significantly increased ($P<0.05$) with an increase in glyphosate concentration in the various treatments after the 96h period of exposure. The result of the variations recorded in the WBC count among the various treatments suggested that the increased WBC count of the exposed fish is a form of an adaptive defence mechanism in response to acute glyphosate exposure. The Packed Cell Volume (PCV) showed a significant ($P<0.05$) dose-dependent decrease in the mean values with (36 ± 1.16 x 106 mm^{-3}) in the control to (28 ± 1.16 x 106 mm^{-3}) in the 2.16mg/l treatment group. The mean values of the haemoglobin (Hb) content recorded in Table 3 also showed a significant ($P<.05$) dose-dependent decrease, from (11.90 ± 0.15 g d/l) in the control group to (8.6 ± 0.26 g d/l) in the 2.16mg/l treatment.

Table 2: Cumulative mortality rate of *Clarias gariepinus* exposed to acute concentrations of glyphosate at different time intervals.

Exposure Period (hours)	Number exposed	Concentration(mg/l) Control		0.72		1.44		2.16		2.88	
		No dead	%	No dead	%	No dead	%	No dead	%	No dead	%
12	30	-	-	-	-	-	-	3	10	15	50
24	30	-	-	3	10	6	20	13	45	21	70
48	30	-	-	3	10	6	20	16	55	27	90
72	30	-	-	3	10	7	25	21	70	30	100
96	30	-	-	6	20	9	30	24	80	30	100

%= percentage mortality, No mortality (-).

Conc (mg/L)	RBC (x106 mm^{-3})	WBC (x106 mm^{-3})	PCV (%)	Hb (g d/L)	MCV (X106 Pgcell)	MCH (x106 Pgcell)	MCHC (g/100ml)
Control)	28.2±2.14a	4933.33±6.67a	36±1.16c	11.90±0.15c	36.96±1.59 a	12.14±5.22 a	33.10±0.78 a
0.72	6.6±0.23b	6233.33±2.40b	34.67±0.88bc	11.63±0.27bc	52.63±1.96 a	17.66±0.69 a	33.56±0.12 a
1.44	6.0±0.12b	5666.67±6.6b	33±0.58b	10.57±0.29b	55.00±0.10 a	17.64±0.75 a	32.06±1.32 a
2.16	5.3±0.35b	6333.33±2.43b	28±1.16a	8.6±0.26 a	53.05±2.13 a	16.36±1.12 a	30.77±0.93 a
2.88	nr	nr	nr	nr	Nr	nr	nr

Table 3: Effect of acute concentrations of glyphosate on haematological parameters of *Clarias gariepinus* after 96 hours of exposure.

Columns indicate the variation in the mean values ± with different superscripts P < .05. nr-no representative. RBC= Red blood cell; WBC= White blood cell; PCV=Packed cell volume; Hb= Haemoglobin; MCV= Mean corpuscular volume; MCH-= Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration.

The result indicated that the haemoglobin concentration of the exposed fish decreased with an increase in concentration which is a biomarker of distress in response to the acute concentrations of glyphosate after the exposure period. Moreso, the results of the erythrocyte indices (MCHC, MCH, and MCV) of the treated fish, also recorded in Table 3, showed an insignificant variation ($P > .05$) when compared to the control group after the 96h acute glyphosate exposure period. The mean values of the neutrophils, lymphocytes, eosinophils, and monocytes recorded in Table 4 showed an insignificant variation ($P > .05$) in the mean values in the various treatments compared to the control group. Basophils were not detected in all the treatment groups.

4. DISCUSSION

The 96 h LC_{50} of glyphosate obtained in this study was 1.50 mg /l. This mortality threshold indicates that glyphosate is highly toxic to *Clarias gariepinus* juveniles. The observed mortality was dose-dependent, which showed that as the glyphosate exposure duration increased from 24 to 96 h, the median lethal concentration required to kill the fish was reduced. However, following exposure to

glyphosate, Thanomsit et al. [30] reported 96h LC_{50} 0f 0.76 mg /L for Asian Sea Bass (*Lates calcarifer*), Nwani et al. [31] recorded the 96h LC_{50} for *Tilapia zilli* at 211.80mg/l, and 86 mg/l for the common carp, *Cyprinus carpio* [32]. The interspecies differences observed in the literature may be connected to the heterogeneous metabolism of individual fish species, which can be attributed to the fish age, species tested, hardiness, physicochemical parameters of experimental water, and inert immunity responses [12].

"Moreso, the effect of pollutants on organisms in aquatic ecosystems, can cause detrimental effects to some organisms at low concentrations and may be less toxic to some other organisms at the higher or same concentration, which has been reported to be affected by the duration of exposure, bioaccumulation, sex, the strain of species, biotransformation, feeding habit and excretion" [33,13,34]. In this study, *Clarias gariepinus* exposed to acute concentrations of 0.72, 1.44, 2.16, and 2.88mg/l of glyphosate for 96hours manifested various behavioural, and morphological stress-related symptoms, which are; restlessness as the fish was seen gasping for air, mucus secretion, jerky swimming movement, loss of equilibrium, degeneration, as well as erosion of fins and outer epithelial cells at higher acute concentrations when compared to the control group. All symptoms occurred before mortality due to the physiological reaction emanating from acute glyphosate toxicity. The behavioural changes reported in this study are similar to the observations of Nwani et al. [31], Ayanda et al. [35], and Lanzarin et al. [36], who exposed *Tilapia zilli*, *Clarias gariepinus*, and Zebra fish models respectively to acute glyphosate toxicity. The mucus secretion noted in the skin and gills of fish has a protective function, but the mucus in the gills may also predispose the fish to respiratory impairments. Mucus cells contain mucins and polyanions made up of glycoprotein that traps toxicants and bar the entry of toxicants into the gill epithelium [37]. "However, the problem associated with the increased mucus cells is an extension in the distance for gas exchange along the secondary lamellae, consequently reducing the efficiency of gas exchange and thereby inducing hypoxic conditions" [38]. "The observed degeneration and erosion of fins and outer epithelial cells (hydroedema) of *C. gariepinus* in higher acute concentrations of glyphosate may be because of COX-1 inhibition that also results in the significant release of endothelin-1, which is a very potent vasoconstrictor that may have caused the degeneration and erosion of epithelial cells. Several authors reported similar findings on exposed fish models to xenobiotics" [2,10,31,37,39]. "The alteration in behaviour can limit the chances of survival of a fish in the wild. The decreased activity could affect the ability of the fish to forage, migrate and avoid natural enemies, as well as their reproductive potential" (40). "Increased swimming activity might benefit the wild if fish can prevent the low-pH water and find refuge in more favourable environments. However, if no escape is possible, the extra energy spent on swimming will reduce the condition of the fish and may impair their capacity to face additional stressors" [41].

Blood is an essential medium in assessing the health status of animals. Haematological parameters act as physiological indicators of a changing environment due to their relationship with energy, respiration, and defence mechanisms [13]. In this study, the haematological responses of RBC, Hb, and PCV during acute exposures decreased. At the same time, the WBC increased in all glyphosate-treated fish compared to the control. This decrease indicates deteriorative effects on the fish's immune system with the consequent release of lymphocytes from lymphomyeloid tissues. The acute toxicity tests showed haematological changes, indicating toxicity in the treated fish [42]. The anaemic effect could be due to glyphosate toxicity. Haematocrit determined the blood's ratio of plasma to corpuscles and oxygen-carrying capacity.

The significant decrease observed in the packed cell volume (PCV) could be attributed to gill damage and impaired osmoregulation, and haemodilution due to toxicity. This observation aligns with the work of Maurya et al. [42] who reported a similar decrease in haemoglobin (Hb), hematocrit (Ht), mean cellular volume (MCV), and leukocyte count (WBC) in juveniles of

Table 4: Mean(±SE) of *Clarias gariepinus* exposed to acute concentrations of glyphosate after 96 hours on some leucocytes differential count

Conc.(mg/)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
0.00(control)	53.67±2.03a	42.67±2.33 a	2.33±0.33 a	1.00±0.0 a	Nd
0.72	55.33±1.77 a	40.33±1.86 a	2.33±0.67 a	2.00±0.58 a	nd
1.44	54.67±2.40 a	41.67±2.19 a	2.00±0.00 a	1.67±0.33 a	nd
2.16	50.33±0.88 a	47.33±1.46 a	1.33±0.33 a	1.00±0.00 a	nd
2.88	Nr	nr	nr	nr	nr

Mean values + S.E with the same superscripts are not significantly different (P > .05). nd- not detected, nr-no representative.

Heteropneustes fossilis exposed to pesticides from industrial wastewater. This result of the study also aligns with the findings of Odo et al. [37], which reported a significant increase in the WBC in *Clarias gariepinus* exposed to Cyperdicot. The increase in the WBC results is the fish's physiological response to fight the xenobiotics. Acute exposure of *Clarias gariepinus* to glyphosate also resulted in a non-significant increase in MCHC. The reduction in MCV, MCH, and MCHC is a positive indication of defective Hb biosynthesis in the fish. Similar decreases in MCV, MCH, and MCHC have been reported in fish exposed to varying concentrations of pesticides [37, 43].

4. CONCLUSION

The study has shown the toxic impacts of glyphosate on fish behaviour and haematological profile. Therefore, we recommend regulating glyphosate usage in/or near aquatic environments and the importance of establishing environmental monitoring commission guidelines to regulate the use of glyphosate to ensure the availability of healthy fish for human consumption.

ETHICAL APPROVAL

The authors hereby declare that the experimental procedures were approved by the institutional ethics clearance committee (Ref: NAU/AREC/2021/00030) and performed in compliance with the standards described by the institution of animal welfare act in line with the National Environmental Standard Regulations Enforcement Agency (NESREA) Act of Nigeria on the protection of animals against cruelty.

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