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

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ABSTRACT

Indiscriminate use of herbicides may pose serious health challenges to the aquatic biota due to runoff from treated fields. The study evaluated the effect of acute exposure to glyphosate using changes in behaviour and haematological parameters under the static bioassay method in *Clarias gariepinus*. Test specimens were exposed to acute concentrations of glyphosate (0.00 mg/l (control), 0.72, 1.44, 2.16, and 2.88 mg/l) for 96 h duration. Significant ($P < .05$) dose-dependent behavioural and morphological changes of respiratory disturbance, erratic swimming, loss of equilibrium, mucous secretion, and mortality were observed in the surviving fish. There were significant ($P < .05$) concentration decreases in erythrocyte (RBC), haemoglobin (Hb), pack cell volume (PCV), and a significant ($P < .05$) increase in the leukocytes (WBC) in the treated fish compared to the control. Insignificant decreases ($P > .05$) in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were observed in the treated fish compared to the control. Glyphosate also elicits insignificant dose-dependent changes in the counts of neutrophils, monocytes, basophils, and eosinophils. The changes observed in this study showed that glyphosate negatively affected the health of the fish. Educating farmers on recommended modes for the administration of glyphosate-herbicides on farmlands should be monitored.

Keywords: glyphosate; acute toxicity; Clarias gariepinus; behaviour; Haematology.

1. INTRODUCTION

Increased and extensive use of herbicides has raised concerns in crop production [1]. Pollutants accumulate in aquatic organisms directly from contaminated water and indirectly via 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. Herbicides are widely used to control water plants, which may impede flow during the summer when sudden heavy rain can cause flooding [3]. In addition, it is recorded that these compounds may cause inheritable changes in the genetic material without these changes being expressed immediately [4].

Glyphosate is a broad-spectrum herbicide used primarily in agricultural applications to control a variety of annual, biennial, and perennial grasses, sedges, broad-leaved weeds, and woody shrubs. It is also used in fruit orchards, vineyards, conifer plantations, and many plantation crops [5]. It is also used for aquatic weed control in fish ponds, lakes, canals, and slow-running water [6]. The use of glyphosate continues to grow, from 16 million kilograms spread worldwide in 1994 to 79 million in 2014, including 15% in the United States alone [7]. The herbicide glyphosate, N-(phosphonomethyl) glycine, is a biocide with broad-spectrum activity that was introduced to control weeds in agricultural production fields in 1974 [7]. Glyphosate is a systemic organophosphorus compound 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. Chekan et al. [8] found that glyphosate is quite resistant to degradation because of the inert C-P linkages in the molecule. Modern pesticides are more target-specific and generally have higher acute toxicity, allowing for low-dose applications. One disadvantage of many modern pesticides, like organophosphates, is that they bind less easily to soil particles and can be transported 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]. Pesticides sprayed over arable land are partly retained and degraded by soil microorganisms. Still, some are leaked to the surrounding land, transported to the atmosphere, and enter ground waters through volatilization, wind drift, surface, and runoff, thus causing havoc to the aquatic ecosystem [11]. In addition, the loss of herbicides from arable land can adversely affect marine life, as pesticides can affect non-target organisms [2,12]. Toxicity testing of chemicals on aquatic animals has been used for decades to test the potential hazards posed to a broader population, and invariably on humans [13]. In addition, an aquatic bioassay is necessary for water pollution control to ascertain if a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentrations and their effect on aquatic animals [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]. The African catfish (*Clarias gariepinus*) was used 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].

Since African catfish local availability is threatened not only by their habitat degradation and food availability but also by various environmental stressors [17]. The focus of this current study was to determine the acute toxicity of commercially formulated glyphosate and its effects on behaviour and haematological profile in light of the excessive use of glyphosate in agriculture.

2. MATERIALS AND METHOD

2.1 Experimental Fish Collection and Maintenance

Male and female two-month-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. The juveniles were transported in clean-aerated freshwater to the laboratory with care to lessen stress. *Clarias gariepinus* juvenile's mean weight was 11.4 ± 1.1g and 10.2 ± 0.9 cm in length. They were allocated to aquaria randomly and left to be acclimatized under laboratory conditions for two weeks before running the static bioassay. The average values of water quality were (temperature 27 ± 1.0˚C, pH 7.3 ± 0.2, dissolved oxygen 9.50 ± 2. .04 mg/l, and salinity 140.4 ± 1.7 mg/l). The light and dark cycle of 12 h: 12 h was maintained throughout the whole study duration. Fish were fed twice daily (9:00-17:00) with a commercial diet (Aleraqua International) containing 35% crude protein once daily. Faecal matter was siphoned off every day from the water in the experimental tanks to avoid fouling.

2.2 Chemicals

The commercial formulation of glyphosate-based herbicide (360 g/l) with the trade name "Forceup." manufactured by Zhejiang Xian Chem. Group co., Ltd China, was purchased from a local market in Awka, Anambra State, and was used to prepare the different acute concentrations (0.72, 1.44, 2.16, and 2.88 mg /l), including a control group to which no glyphosate was added.

2.3 Experimental Procedures and Design

A static renewal bioassay technique was adopted following the methods of Sprague [18] and APHA-AWWA-WPCF [19]. A total of 150 juveniles were randomly distributed into 15 experimental glass tanks (46 x 31.5 x 25 cm; n = 10 fish per aquarium). 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 with no glyphosate. All treatments were replicated three times and filled with 20 litres of dechlorinated water. The stock solution was measured and introduced into the experimental tanks. The solutions were stirred for homogenous mixing before each aquarium was randomly stocked with the fish. Feeding was discontinued 24 h before the start of the bioassay to avoid interference with faeces, as Reish and Oshida [20] recommended. Survival and mortality were recorded for 24, 48, 72, and 96 hours. Fish were considered dead when the opercula movement ceased and there was no response to gentle prodding. Dead fish was removed with the aid of forceps, and the LC_{50} values of glyphosate for the fish at 24, 48, 72, and 96 h of exposure were determined by probit analysis [21].

2.4 Behavioural/Morphological Assessment

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Observations of behavioural/morphological responses of *C. gariepinus* juveniles exposed to glyphosate herbicide were conducted at 24, 48, 72, and 96 h during the acute toxicity tests. The methods developed by Drummond et al. [22] were used for this study. Controls without toxicant exposure and acute concentrations were monitored to offer a reference for assessing any behavioural and morphological changes. Responses were recorded if they differed from the control group or if they occurred in 10% of the fish within each test tank. The changes included: loss of equilibrium, general activity, startle response, erratic swimming, deformity, haemorrhage, and respiratory disturbance. Each test chamber was observed for 5–10 min. Startle responses were monitored by lightly touching the fish with a plastic applicator stick (tactile stimulus). For the experiment, death was defined as the permanent cessation of spontaneous movement and failure to respond to mild stimuli.

2.5 Blood Collection and Haematological Analysis

The caudal artery was severed at 2 cm from the caudal peduncle of three fish per treatment, as described by Blaxhall and Diasley [23]. Fish specimens were anesthetized with tricaine methane sulfonate (MS-222; 50.0 mg/l) to minimize stress and enable the accessible collection of blood samples. Blood collection was done at the end of 96 h of exposure from the surviving fish of the various treatments and transferred immediately (10–20 min) to the laboratory 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].

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

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

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

where 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

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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 different 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, exposed fish to glyphosate exhibited various 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 secretions, and erosion of fins. These behavioural changes were dose-dependent as shown in Table 1.

3.2 Fish Mortality Rate

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.

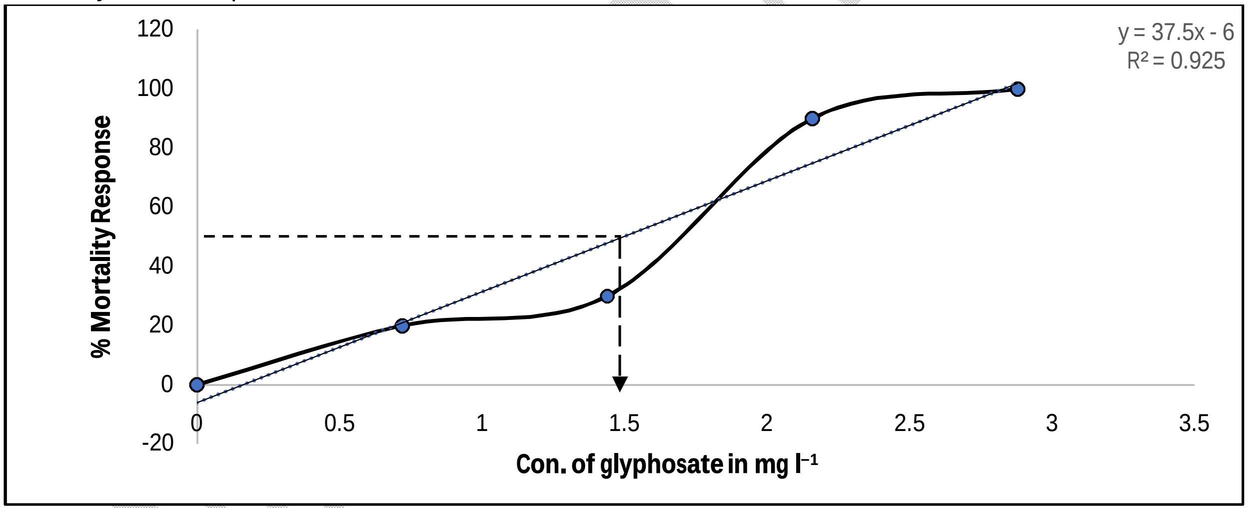

Figure 1: The relationship between glyphosate concentrations and mortality percentages.

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.

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At 96 hours, the highest mortality rate 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 in Table 3 showed that glyphosate exposure affected the Red Blood Cell (RBC) count of the treated fish when compared to the control group, which was a statistically significant difference (*P* < .05) in the blood samples of fish from the various acute concentrations of glyphosate (0.00, 0.72, 1.44, 2.16, and 2.88 mg/l) after 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.

		Concentration(mg/l)									
Exposure Period (hours)	**Number exposed**	**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 level 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

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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, undefined enzymatic defence response to glyphosate toxicity, and the duration of the exposure [12].

Generally, the toxicity of xenobiotics to organisms in aquatic ecosystems, which can cause detrimental effects to some organisms even at low concentrations, may be more or less toxic to some other organisms at the higher or same concentration, which has been reported to be affected by exposure concentration, duration of exposure, bioaccumulation, sex, the strain of species, temperature, pH, dissolved oxygen, the composition of the chemical, 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. The anaemic effect could be due to erythrocyte production and haemodilution inhibition. Erythropenia (deficiency in the number of red blood cells) was reflected by the reduced haemoglobin content and hematocrit value as well as erythrocyte sedimentation rate (ESR) [2]. Haematocrit determined the blood's ratio of plasma to corpuscles and oxygen-carrying capacity.

This study's significant decrease in the packed cell volume (PCV) could be attributed to gill damage and impaired osmoregulation, causing anaemia and haemodilution due to toxicity. This observation aligns with the work of Amaeze et

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al. [17], who reported that when exposed to unfavourable conditions induced by stressors, fish tend to improve their oxygen-binding efficiency and carrying capacity by stabilizing their haemoglobin, hematocrit, and red blood cell concentrations. Maurya et al. [42] also reported a similar decrease in haemoglobin (Hb), hematocrit (Ht), mean cellular volume (MCV), and leukocyte count (WBC) in juveniles of

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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 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 from the fish's innate immunity trying 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 and other harmful chemicals. Hence, protecting not only life forms like fish from extinction, but also ensures biological diversity and stability for healthier ecosystems.

ETHICAL APPROVAL

All 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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