Histological and Haematological Effects of Glyphosate to Juvenile of the African Catfish

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Abstract- ***Ultimately, the toxicity of glyphosate can result in serious health implication in human through the food chain and bioaccumulation. The short term exposure to herbicide glyphosate on Clarias gariepinus juveniles was evaluated using standard method that assess changes in fish behavior, mortality and histological analysis. The juvenile of the African catfish was exposed to varying acute concentration of glyphosate. The experiment was carried out on 40 Clarias gariepinus juvenile, averaging 31.93 ± 4.38cm standard length and body weight of 18.08 ± 0.98g, that were randomly selected and grouped into three replicates: The treatment was exposed 60, 70, 80, 90, 100, 110 and 120 of Glyphosate concentrations for 96hrs. Fish exhibited some behavioral abnormalities such as hyperactivity, restlessness, loss of equilibrium and hemorrhage, hitting of tail against the wall of the holding medium and abnormal swimming. The herbicides caused mortality of fish depending on the concentration and duration of exposure to acute concentrations of the herbicide. The LC50 value for Glyphosate was 103.368 mg/L. Histological analysis of the fish organs examined revealed varying degrees of pathological alterations/degerations to the gills and kidney in the short term study. The results of the present study revealed that Glyphosate were found to be toxic to juveniles of Clarias gariepinus.***

Indexed Terms- ***Acute Toxicity, Glyphosate, Clarias Gariepinus, Histology, LC50***

I. INTRODUCTION

Glyphosate is a non-selective post-emergence herbicide that is commonly applied in agriculture and forestry for the control or destruction of herbaceous plants in fish-ponds, lakes, canals, slow running water, etc. This herbicide due to the changes of metabolic, oxidative and haematological parameters, may alter the ecological balance causing damage to non-targeted organisms (Ayanda *et al.,* 2017). Glyphosate-based herbicides are among the most widely used broad-spectrum herbicides in the world because they are highly efficacious, cost effective, and degrade readily in the environment (Giesy *et al*., 2000; Williams *et al*., 2000). Glyphosate is soluble in water and tends to bind tightly to sediment, suspended particulates, organic matter and soil, becoming essentially unavailable to plants or other aquatic organisms. Some pesticides bio-accumulate, affecting fish, birds, other animals and in human food sources. Depending on the local cultivation practices, a water body may receive a single pesticide or a varying cocktail of compounds (Ravindran *et al.,* 2017; Fernández-Alba *et al*., 2000). *Clarias gariepinus* is a genus of clariid (order Siluriformes) of the family Clariidae, the air breathing catfish (Froese *et al*., 2011). It is a popular species in warm water aquaculture and it is indigenous to Africa. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, tolerance to poor water quality parameters, omnivorous in its feeding habit, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available (Michael, 2018; Osman *et al.,*2006). *C. gariepinus* is an important fish for aquaculture in Nigeria because it partly meets up the increasing demand for proteins. It has been artificially reproduced and cultured under various Nigerian aquaculture systems (Akeem *et al.,* Ayoola, 2008; Omitoyin *et al*. 2006). This research was designed to assess the histological and haematological effects of glyphosate to juvenile of the African catfish (*clarias gariepinus)*

II. METHODS

- *Collection and maintenance of fish culture*

Juveniles of *Clarias gariepinus* were purchased from a fish farm in Offa 8.1393° N, 4.7174° E, Kwara State, Nigeria. On the average, the juveniles of *C. gariepinus*

were 31.93 ± 4.38cm in standard length and body weight of 18.08 ± 0.98g. The fish were conveyed in a well aerated container from the fish farm to the holding units in the laboratory of Animal and Environmental Biology, Federal University Oye-Ekiti, Nigeria.

- *Acclimatization and Feeding of Fish*

The fish were acclimatized for two weeks in borehole water. During this period, the fish were fed with pelleted diet containing 35% crude protein twice per day, while the water was renewed daily. Feeding was discontinued 24h prior to commencement of the toxicant exposure experiment.

- *Physico-Chemical Parameters of Water*

Borehole water was used for the experiment and the physico-chemical parameter such as temperature, pH, alkalinity, total hardness, conductivity, total dissolved solid and dissolved oxygen were determined.

- *Preparation of test solutions and exposure of fish*

The herbicides (glyphosate) was purchased from a commercial outlet in Offa, Kwara state, Nigeria. These range of concentrations were arrived at due to the fact that it enables the researcher to ascertain the survival and mortality of the fish used for the exposure during the range finding and final exposure. Concentrations for Glyphosate includes 60mg/L, 70mg/L, 80mg/L, 90mg/L, 100mg/L,110mg/L, 120mg/L were all used for the range finding test. Mortalities was monitored and recorded every hour and every 24 hours for the range finding test. The test concentration was fine-tuned until a range of test concentrations in which no fish died in the lowest concentration and all fishes died in the highest concentration in one treatment was obtained. Acute bioassay was conducted in the laboratory following Organization for Economic Cooperation and Development (OECD) guidelines No. 203 to determine the toxicity of Glyphosate to *C. gariepinus*. Four concentrations each of Glyphosate were prepared in addition to the control group. Eight (8) fish juveniles were randomly distributed into each test tank and replicated 3 times. The physicochemical parameters of the diluting water (temperature, pH, dissolved oxygen, total hardness, total alkalinity and conductivity) during the acute test period were recorded. The control solutions were made up of only de-chlorinated water. Each of the four concentrations of the herbicide was administered to fish in the exposure vessels while the responses were monitored until 96hr.

- *Behavioral Studies*

Each fish population was exposed to the different concentrations of the toxicants and assessed after 01hr, 12hr, 24hr, 48hr, 72hr, and 96hr for behavioral and morphological changes (Drummond *et al*., 1986). Simultaneously, the control population was monitored alongside the exposed fish, to establish a reference for any behavioral and morphological changes. The behavioral and morphological responses that were monitored include loss of equilibrium, startle responses, hyperactivity, abnormal swimming, hemorrhage and general restlessness.

- *Haematological Analysis*

This was done according to using standard laboratory procedure described by Jaime (2016). Total Red Blood Count (RBC X 10^{12}/l), Packed Cell Volume, PCV Estimation % (Hematocrit Hct L/L), Haemoglobin Estimation (Hb g/dl), Mean corpuscular volume (MCV fl), Mean Corpuscular Haemoglobin (MCH pg), Mean Cell Haemoglobin Concentration (MCHC g/l), Total White Blood Cell Count (WBC X 10^{9}/L) were determined.

- *Histological Analysis /Tissue Processing*

The fish were dissected and fresh specimen of the livers and gills were collected from both control and treatment groups, while the organs were preserved in 10% formalin in an EDTA bottle for Histological study. The preserved tissues were taken to Histopathology Units of University College Hospital (UCH) Ibadan for the tissue processing. The fish tissues including Gills and liver were examined grossly and were trimmed into small pieces of not more than 4mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 hours to fix. These tissues were processed automatically using automatic tissue processor (Leica TP 1020). The tissues were allowed to pass through various reagents including; stations 1 & 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass

through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours, tissues stayed in each station for 1hour. Each processed tissue was given a solid support medium (paraffin wax) and this is done using a semi-automatic tissue embedding center (LEICA). The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labeled cassette was placed on this and was transferred to a cold plate to solidify. The tissue block formed was separated from the mold. The blocks were trimmed to expose the tissue surface using a rotary microtome at 6 micrometers. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 micrometers (ribbon section). The sections were floated on water bath (Raymond lamb) set at 55^0C and these were picked using clean slides. The slides were labeled and subsequently dried on a hotplate (Raymond lamb) set at 55^0c for 1hour.

III. DATA ANALYSIS

The statistical software programme SPSS (Version 15) was used to compare differences among the test groups. The experimental design was a Complete Randomized Design. A one-way Multivariate Analysis of Variance (MANOVA) was employed to determine whether there were significant differences in the variables measured among the experimental groups when a difference is detected at P<0.05 level of significance, Tukey HSD Test was applied.

IV. RESULTS AND DISCUSSION

Table 1: Physicochemical Parameters of Water Used in Acute Toxicity Test for Glyphosate Concentration

Parameters	Range	Mean ± SE
Temperature (˚C)	24.60 – 25.80	25.50 ± 0.8
pH	5.64 – 6.20	5.84 ± 0.31
Dissolved Oxygen (mg/l)	6.80 – 7.36	7.06 ± 0.28
0Conductivity (μscm^{-1})	160 – 168	163.30 ± 4.16
Hardness (mg/l $CaCO_3$)	144 – 150	146.60 ± 3.03
Alkalinity (ml^{-1})	142 – 156	149.30 ± 6.98
Total Dissolve Solid (ppm)	81 – 85	83.00 ± 2.00

Source: Author computation, 2021.

Table 2: Haematological parameters of juvenile *Clarias gariepinus* exposed to Glyphosate

Parameter	0.0mg/l	80mg/l	90mg/l	100mg/l	110mg/l
Hb (g/dl)	8.17± 3.64	13.60 ±0.21	11.10 ±1.50	12.67 ±1.71	13.47 ±1.54
PCV (%)	25.33 ±11.20	40.67 ±0.67	33.67 ±4.91	38.67 ±4.98	40.67 ±4.81
RBC (mm^3)	1.62± 0.32*	4.03± 0.58	3.38± 0.29	4.00± 0.42*	4.00± 0.25*
WBC (mm^3)	0.70± 0.06	4.93± 2.21	1.17± 0,87	6.10± 3.29	2.08± 1.08
MCV (fl)	186.53±96.54	107.30±15.91	100.67±17.01	97.43 ±11.93	103.43±16.82
MCH (pg)	60.30 ±31.57	26.17 ±9.10	32.97 ±5.47	31.87 ±4.11	34.10 ±5.41
MCHC (%)	21.77 ±9.25	33.43 ±0.13	32.97 ±0.52	22.93 ±9.82	23.00 ±9.80

Based on observed Mean: (*) The mean difference is significant at the 0.05 level

- *Haemoglobin (g/dl)*

The value ranges from 8.17±3.64, 13.60±0.21, 11.10±1.50, 12.67±1.71 and 13.47±1.54g/dl from 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l concentration respectively. There was no significant difference between the control group and those with glyphosate concentration (P>0.05).

- *Packed Cell Volume (%)*

The values of 25.33±11.20, 40.67±0.67, 33.67±4.91, 38.67±4.98, 40.67±4.81% were recorded for treatments from 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l respectively. There was no significant difference (P>0.05) between the catfish in control solution (0.0mg/l) and those with different Glyphosate concentration.

- *Red Blood Cell (mm3)*

The values ranged from 1.62±0.32, 4.03±0.58, 3.38±0.29, 4.00±0.42 and 4.00±0.25mm^3 in 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l glyphosate concentration respectively. There was a significant difference between 0.0mg/l, 100mg/l and 110mg/l ($p<0.05$).

- *White Blood Cell (mm^3)*

The mean value ranged from 0.70±0.06, 4.93±2.21, 1.17±0,87, 6.10±3.29 and 2.08±1.08mm^3 was recorded from 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l glyphosate concentration respectively. There were no significant differences (P>0.05) among the treatments.

- *Mean Cell Volume (fl)*

The mean cell volumes are 186.53±96.54, 107.30±15.91, 100.67±17.01, 97.43±11.93 and 103.43±16.82fl from 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l concentration respectively. There was no significant difference (P>0.05) among the treatments.

- *Mean Cell Haemoglobin (pg)*

The values of Mean Cell Haemoglobin ranges from 60.30±31.57, 26.17±9.10, 32.97±5.47, 31.87±4.11 and 34.10±5.41pg in0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l glyphosate concentration respectively. There was no significant difference (P>0.05) between the catfish in control solution (0.0mg/l) and those with different glyphosate concentration

- *Mean Cell Haemoglobin Concentration (%)*

The values were 21.77±9.25, 33.43±0.13, 32.97±0.52, 22.93±9.82 and 23.00±9.80% for catfish juvenile treat with 0.0mg/l, 80mg/l, 90mg/l, 100mg/l, 110mg/l glyphosate concentration respectively. There was no significant difference (P>0.05) between the catfish in control solution (0.0mg/l) and those with different glyphosate concentration.

- Mortality/96-hr LC50 Values

Mortality was observed to increase with increasing concentrations for each of the herbicides tested

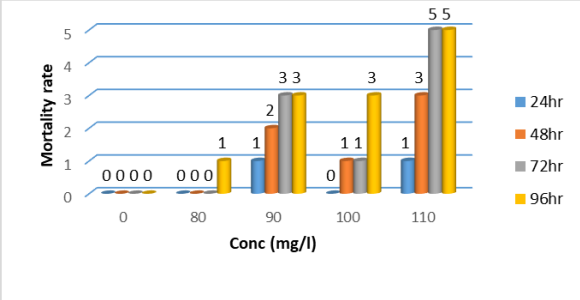
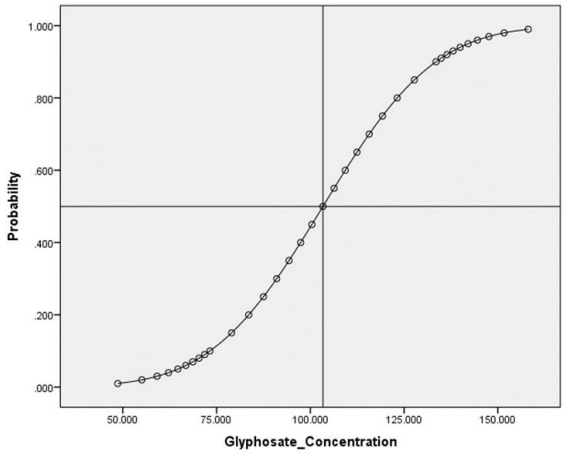


Figure 2: Mortality (total) of juvenile catfish exposed to Glyphosate

Table 3: Mortality and Probit values of *Clarias gariepinus* exposed to Acute concentrations of Glyphosate for 96 hrs.

	Number	Glyphosate Concentration	Number of Subjects	Observed Responses (mortality)	Expected Responses	Residual	Probability
PROBIT	1	1.903	8	1	1.222	-.222	.153
	2	1.954	8	3	2.334	.666	.292
	3	2.000	8	3	3.610	-.610	.451
	4	2.041	8	5	4.830	.170	.604

Source: Authors Computation, 2021.



LC50 = 103.368

Figure 3: Linear relationship between Probability response and log concentration of Glyphosate on juvenile *Clarias gariepinus*

V. HISTOLOGICAL ANALYSIS OF GILLS AND LIVER TISSUES EXPOSED TO ACUTE CONCENTRATIONS OF GLYPHOSATE.

- Gills

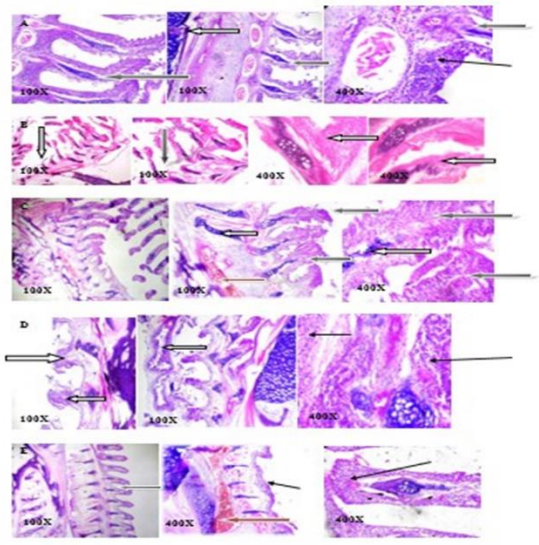


PLATE 1: (A) Photomicrograph of a fish Gill in Control group (0.0mg/L), section stained by Haematoxylin & Eosin, the (black arrow) showing normal gill arch and the Lamellae or filament containing normal pilaster cells, the (slender arrow) indicate chloride cells as well as normal epithelium and red cells and the (white arrow) show Normal cartilage of the arch. (B) Photomicrograph of a fish Gill exposed to 80mg/L of Glyphosate concentration, gill section stained by Haematoxylin & Eosin, the (black arrow) showing gill arch and filament with mild sloughing and the (white arrow) indicate Connective tissues surrounding the cartilage with necrosis. (C) Photomicrograph of a fish Gill exposed to 90mg/L of Glyphosate concentration, section stained by Haematoxylin & Eosin, the (black arrow) showing gill arch with Lamellae exhibiting epithelial hyperplasia, the (white arrow) show the cartilage of the arch is atrophic and the (red arrow) show mild vascular congestion. (D) Photomicrograph of a fish gill exposed to 100mg/L of Glyphosate concentration, section stained by Haematoxylin & Eosin, the (black arrow) showing moderately inflamed gill arch, the (slender arrow) show the Lamellae or filament is moderately infiltrated by inflammatory cells and the (white arrow) show the secondary filament with severe epithelial hyperplasia. (E) Photomicrograph of a fish Gill exposed to 110mg/L of Glyphosate concentration, section stained by Haematoxylin & Eosin, the (black arrow) showing gill arch with normal primary filament, the (red arrow) indicate there is mild vascular congestion and the (slender arrow) indicate the secondary filament is mildly hemorrhagic and show mild to moderate hyperplasia.

- Liver

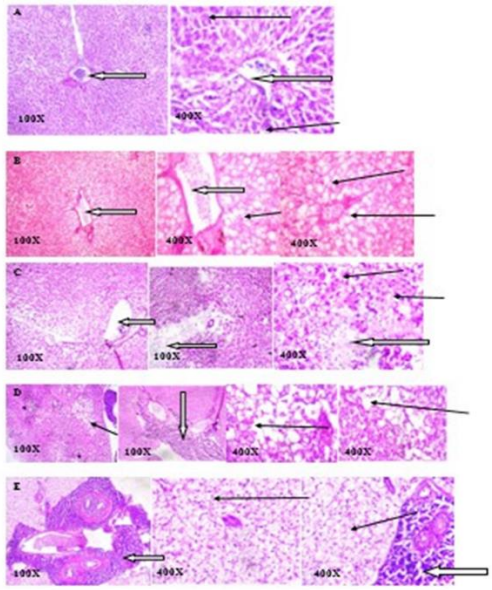


PLATE 2: (A) Photomicrograph of a fish Liver in Control group (0.0mg/L), section stained by H&E, the white arrow showed normal venule without congestion, the slender arrow showed the Hepatocytes which appear normal and the sinusoids are not infiltrated, (B) Photomicrograph of a fish Liver exposed to 80mg/L of Glyphosate concentration, section stained by H&E, the white arrow showed normal uncongested venule, the slender arrow showed the liver hepatocytes with severe degeneration by fat infiltration and the cytoplasm also appear ballooning, (C) Photomicrograph of a fish Liver exposed to 90mg/L of Glyphosate concentration, section stained by H&E, the slender arrow showed hepatocytes with cytoplasmic fat infiltration and the white arrow indicates there is severe liver plate degeneration, (D) Photomicrograph of a fish Liver exposed to 100mg/L of Glyphosate concentration, section stained by H&E, the white arrow showed moderate portal triaditis, the white arrow indicates the portal tracts showed peripheral infiltration of inflammatory cells and the slender arrow indicates the liver hepatocytes showed moderate to severe degeneration by fat infiltration, (E) Photomicrograph of a fish Liver exposed to 110mg/L of Glyphosate concentration, section stained by H&E, the white arrow showed severe portal triaditis; the portal tracts is severely infiltrated by inflammatory cells, and the slender arrow indicates the liver hepatocytes that showed severe degeneration by fat infiltration.

CONCLUSION

Findings in this present study showed glyphosate herbicide toxicity to African catfish (*Clarias gariepinus*). The Glyphosate (at 103.368) was found to be toxic to the gills and liver of the juveniles of *Clarias gariepinus*. Therefore, there is a tendency that these substances may affect the human population due to bioaccumulation and bio magnifications across the food chain even at the concentrations tested.

RECOMMENDATIONS

The study therefore recommends the following:

i. Government should come up with regulations to minimize the use of herbicides in aquatic environment.
ii. Regulatory authority should create awareness on the use of herbicides by farmers on farm lands because of widespread deleterious health effects on aquatic biota.
iii. In view of the toxicity of herbicides to fishes, it is important to consider distance to natural water bodies when situating farmlands in order to minimize the risk of intoxication of fishes and other aquatic organism by herbicides through surface runoffs and atmospheric depositions.

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