EFFECTS OF ACUTE TOXICITY OF ANALYTICAL GRADE GLYPHOSATE (AGG) HERBICIDE ON BIOCHEMICAL METABOLIC INDICES OF AFRICA CATFISH (CLARIAS GARIEPINUS)

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ABSTRACT

Glyphosate is a widely-used herbicide mostly employed primarily in farming settings to control weed. Despite this usefulness, this chemical compound has been described as toxic to other non-targeted living organisms. This study investigated the effect of acute pure analytical grade (AGG) glyphosate concentration on *Clarias gariepinus* biochemical indices. An initial experiment was conducted for glyphosate lethal concentration (LC50) determination using a biostatic assay technique where juvenile African catfish were exposed to varying herbicide concentrations for 96 hours. The 0.0, 100, 125, 150, 175 and 200 mg/L concentrations determined from this pilot experiment were employed for acute toxicity study involving 10 juvenile fishes in each experimental group. After exposure time, the animals' blood, liver tissues as well as kidney tissues were collected for biochemical analysis. Exposure to AGG significantly ($p<0.05$) decreased δ-ALAD and GSH but increased NTPDase and 5' Nucleotidase as well as TBARS activities or levels in the animal tissues. AGG also increased plasma Aspartate (AST) and Alanine Transaminase (ALT), ALP, bilirubin, as well as urea and creatinine in the exposed animals group when compared with control group. This observed alteration in functional biomarkers in treated animals could be associated with the increased oxidative imbalance mediated by herbicide exposure. In conclusion, glyphosate is lethal to aquatic organisms and its exposure may alter or shut down the organism's biochemical, physiological and morphological systems.

Keywords: AGG herbicide, Catfish, LC50, antioxidants, aquatic organisms

1. | Introduction

Glyphosate herbicide is an important organophosphorus herbicide ever developed and mostly used around the world (WHO, 1994, Iyanda *et al.,* 2023). It is a broadly known herbicide that is mainly used in agriculture for controlling diverse grasses, sedges, weeds and shrubs as well as aquatic weed in ponds, lakes and slow running water (Tsui and Chu 2003).

Generally, it is believed via several reports that glyphosate is highly toxic to organisms (both target and non-target organisms) and environment probably due to the adjuvants added to formulate the commercial - based glyphosate herbicides. Glyphosate parent acid (analytical grade) is slightly altered to improve the handling, stability and efficiency of the herbicide formulation. The formulation is done by replacing the acidic carboxyl hydrogen either with the desired ions from a salt or reacting it with an alcohol to form an ester (Nordby and Hage, 2011). Analytical grade glyphosate (AGG) is reported to be less toxic with LC_{50} reported values of 55 mg/L and also discovered in some aquatic species with mild toxicity (Cox, 2000). Additionally, NRAAVC, (1997) revealed an annual report of AGG on bluegill sunfish and rainbow trout to have a 96 hour LC_{50} of 120 mg/L and 86 mg/L respectively. Also, epidemiological and animal feeding studies were performed by IARC using analytical grade glyphosate. Based on research survey, a lot of studies had been reported on the glyphosate – based commercially formulated herbicides while few had been reported on the acute toxicity test of analytical grade.

Therefore, more studies are still needed to validate the su:osed slight toxicity of AGG and establish the mechanisms that may be involved in the possible alteration of the physiology of the non-target organisms like fish. It is in the light of this that this study is carried out to provide more information on the acute toxicity of analytical grade glyphosate (AGG) herbicide and its' possible toxicological effects on enzymic and non enzymic metabolic indices of Africa Catfish (*Clarias gariepinus*).

2 | Materials and Methods

The experiment made use of five hundred species of Juveniles *Clarias gariepinus* having the mean weight and length of 30 ± 0.3g and 15 ± 0.1cm respectively. Experimental fish were obtained from a reliable fish farm in Akure, Ondo State, Nigeria. The fish were subjected to acclimatization for seven days in a big non-metallic tank and were fed with commercially formulated floating pellets using 10% of their body weight prior to the commencement of the study. Clean and tap water with temperature 26.05 ± 0.80^{0}C, pH 7.10 and dissolved oxygen 6.31 ± 0.10 mg/L was used.

2.1 | Determination of Acute Toxicity:

A static bioassay technique was adopted and initial screening test to determine the exact and suitable concentration range for experimental chemical was carried out as described by Chinedu *et al.,* (2013) and Iyanda *et al.,* (2025).The following concentration of analytical grade glyphosate 0.0, 100, 125, 150, 175 and 200 mg /L were prepared and tested on Ten acclimated *C. gariepinus* juvenile for 96h definitive test.

Fish mortality was recorded, removed and discarded daily. Fish were considered dead when no movement upon gentle prodding was observed. Also, the mean physicochemical parameters (pH, temperature, turbidity and dissolved oxygen of the test water containing different concentrations of glyphosate were monitored and recorded daily. The exposure lasted for 96hours and the lethal concentration (LC_{50}) at 96 hour was computed via the probit analysis and analysis of variance (ANOVA).

2.2 | Biochemical Assays

2.2.1 | Tissue preparation

Tissue samples (liver and kidney) from survived exposed catfish were extracted, placed inside ice and homogenized in chilled Tris – HCl (50mM at pH 7.4). The homogenate was then centrifuged at 4000rpm for 8-10 min so as to yield the supernatant (S1) used for all biochemical assays.

2.2.2 | Evaluation of Antioxidant status of the exposed catfish

2.2.2a | Lipid Peroxidation assay on the prepared tissue sample

Production of TBARS after exposure to AGG was determined on the tissue samples prepared in section 2.2.1 using a method quoted by Omotayo *et al.,* (2015) and the color reaction was developed using a method quoted and described by Iyanda *et al.,* (2023). The TBARS used were read at 532nm on UV – Visible Spectrophotometer (General Scientific Model).

2.2.3b Estimation of GSH concentration

The GSH content in hepatic as well as renal tissues of AGG - exposed fish were evaluated using TCA (5% in 1 mmol/EDTA) to deproteinize the tissues and adding Ellman's reagent thereafter following the method described by Kade (2016). The yellow coloration formed was read at 412 nm on UV – Visible spectrophotometer (General Scientific Model).

2.2.3c Evaluation of Radical Scavenging Capacity

The ability of the exposed tissues were evaluated against D:H (1, 1-diphenyl-2 picrylhydrazyl) free radicals through a method cited by Iyanda *et al.,* (2023). 600 μL of 0.3 mM methanolic solution which contained D:H radicals was added to deproteinized tissue homogenates was left in the dark for 30 min and golden yellow product was formed. The color was read at 516 nm on UV – Visible spectrophotometer (General Scientific Model).

2.2.3d Evaluation of Ferric reducing antioxidant ability

Ferric reducing antioxidant ability of catfish exposed to AGG was evaluated. 300μL of the deproteinized tissue homogenates was added to 900 μL of TPTZ (2, 4, 6 -Tri (2-pyridyl)-s-triazine solution according to method quoted by Iyanda *et al.,* (2023). The whole mixture was placed in the dark for approximately 10 minutes, and the readings were taken at 543 nm on UV – Visible spectrophotometer (General Scientific Model).

2.2.3 Evaluation of the enzymes activities levels of the exposed catfish

2.2.3a ð-Aminolevulinate dehydratase (ð-ALA-D) activity

Effect of AGG on the ð- ALA D activities in the liver and kidney were assayed based on the technique described by Kade and Rocha, (2013). Potassium phosphate buffer (1M, pH 6.8) and ALA (2.4mM) were added to tissue homogenate. The mixture was incubated for 2 hours at 37°C, then, the reaction product was evaluated adding Ehrlich's reagent and the absorbance readings were taken at 555 nm.

2.2.3b Purinergic Enzymes

(i) NTPDase Assay

The level of NTPDase activity of exposed catfish tissues was determined as described by Iyanda *et al.,* (2023). 100 μL of supernatant was added and preincubated for 10 min at 37^0C. Then, ATP (3.0 mM) was added to initiate reaction within the medium. The reaction end-product was determined at 650 nm. The inorganic phosphorous (Pi) released and Protein was determined using the cited technique of Omotayo *et al.,* (2015).

(ii) 5'-Nucleotidase Assay

The level of 5'-Nucleotidase activity in catfish tissues exposed to AGG was determined using method described by Kade *et al.,* (2014). 100 μL of protein was added to a medium and preincubated for 10 min at 37^0C after which 2mM of AMP was added and incubated for 20 min. The end-product was determined at 650 nm on UV – Visible spectrophotometer (General Scientific Model).

2.2.4 Liver and Kidney Tests

The levels of AST, ALT, ALP, bilirubin, albumin as well as total protein were measured for the functionality of liver while creatinine and urea were measured for kidney functionality using commercial kits sourced from Randox Laboratories. Blood samples analysed were obtained through the dorsal vein of the exposed catfish using 5 mm syringe into EDTA bottles containing anticoagulant to avoid coagulation of the blood samples. The blood samples were centrifuged at 4000 rpm for about 10 min. The plasma was decanted into another clear bottle while the serum was discarded. The plasma samples were stored in the refrigerator prior to analysis.

2.3 | Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the results. Group differences were considered to be significant when $P < 0.05$.

3 | Results and Discussion

Evaluation of the acute toxicity of analytical grade glyphosate (AGG) on juvenile of *Clarias gariepinus* was assessed and the possible harmful impact on physiology of the catfish was established in this study. The values obtained for the physicochemical analyses of AGG - diluted water alongside the comparison with recommended values is presented in **Table 1** and the values were within the permissible range for good culturing and survival of catfish (*Clarias gariepinus*).

Table 1 | Physicochemical Properties of AGG contaminated Water

Parameters	Control	AGG	Recommended Values
Temperature (0C)	25.25±1.3	25.5±1.3	23.0 – 32.0
pH	7.14±0.4	6.59±0.4	7.0 – 8.0
Dissolved Oxygen (mg/L)	6.16±0.37	5.15±0.3	5.0 – 5.5
Total Hardness (mg/L)	40.5 ±2.0	41.0±2.1	25 - 100
Conductivity (µS/cm)	194.5±9.7	394± 19.7	100 - 500
Alkalinity (mg/L)	38.0±1.9	37.0±1.9	25 - 100

Mortality of Fish was observed at each concentration of analytical grade glyphosate herbicide. Mortality increased with an increased concentration of the AGG. Rate of death, in accordance to each concentration of the herbicide, and the percentage mortality are in Table 2 & 3. The result of toxicity test at 96h estimated showed that the LC_{50} value for AGG is 131.12mg/L. The LC_{50} values gotten is higher than the recorded 96h LC_{50} of 44.67mg/L for mulsate glyphosate herbicide in African Catfish and 96h LC_{50} of 1.50mg/L in *C. gariepinus* recorded by Awoke *et al.,* (2023)and Edeh *et al.,* (2023) respectively. Moreover, the effect of pollutants on organisms in aquatic ecosystems has been reported to be affected

Table 2 | Mortality rate of AGG on African Catfish at 96 hours exposure

Concentration (mg/L)	Number of fish	Number of Mortalities Day 1	Number of Mortality Day 2	Number of Mortality Day 3	Number of Mortalities Day 4	Total Mortality	% mortality
100	10	0	0	0	1	1	10
125	10	0	1	1	1	3	30
150	10	0	3	2	2	7	70
175	10	1	3	4	1	9	90
200	10	1	2	4	3	10	100

Table 3 | Mortality and probit values of *Clarias gariepinus* exposed to acute concentrations of glyphosate for 96 h.

Concentration (mg/l)	Log $_{10}$ conc.	Total Number of fish exposed	Total Mortality	%Mortality	Probit value
100	2.000	10	1	10%	3.72
125	2.097	10	3	30%	4.48
150	2.176	10	7	70%	5.52
175	2.243	10	9	90%	6.28
200	2.301	10	10	100%	8.09

by the exposure, period, bioaccumulation, sex, the strain of species, biotransformation, feeding habit and excretion (Iheanacho and Odo, 2020; Faria *et al.,* 2021; Iyanda *et al.,* 2025).

Investigation of the toxic effect of AGG on the redox status of membrane lipid bilayer of the cell of exposed fish was examined through lipid peroxidation assay and measured as an amount of thiobarbituric acid reactive substances (Catala, 2006; Awoke *et al.,* 2023). In this study, the markedly elevated level of TBARS produced (Table 4) in liver and kidney revealed that AGG increases the production of TBARS at increased concentration. The result is in line with the result recorded by Ayanda *et al.,* (2018) who recorded that glyphosate herbicide increased lipid peroxidation at concentration dependent manner in *Clarias gariepinus*. Also, lipid peroxidation increased in liver and testes of catfish exposed to oxyfluorfen (Abdallah *et al.,* 2023).

The inherent antioxidative capability of antioxidants of the catfish after exposure to AGG was also estimated with D:H which is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. From this study, the ability of liver and kidney of catfish to scavenge D:H radicals after exposure to AGG mildly reduced when compared with control (Table 4). Report presented by Iyanda *et al* (2023) also revealed a reduction in the scavenging activity of D:H in *C. gariepinus* exposed to Round Up with Cadmium as co- contaminant. Likewise, reducing power activity (FRAP) is another measures of assessing antioxidative capability of exposed catfish tissues and is referred to an ability to convert iron (III) to iron (II) because of the in-built antioxidant capacity in them. From this study as presented in Table 4, it was observed that there was a reduction in the FRAP of liver and kidney of catfish exposed to AGG when compared with control group. Similar report was presented by Iyanda *et al* (2025) who revealed a reduction in FRAP in exposed *Clarias gariepinus* to Round Up and Force Up.

Also, glutathione (γ-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) and a major component of the antioxidant defence system in fish. The results obtained revealed a depletion in the level of GSH in comparison to control group (Table 4). This can lead to immune deficiency and may possibly damage the cell membranes like DNA and proteins, resulting in cell death (Zengin, 2021) and possibly death of the fish (Iyanda *et al.,* 2025) as earlier recorded in Table 2 and 3.

δ-ALA D is an enzyme that contain sulfhydryl group and plays an important role in most living organisms by being involved in the biosynthesis of heme, chlorophyll and corrin (Kade and Rocha, 2013; Iyanda *et al.,* 2023). From this study, the influence of AGG on level of activity of δ-ALA D in the liver

Table 4 | Redox Status of Exposed Tissues of Catfish (*Clarias gariepinus*)

Parameters	Control	AGG(100mg/L)	AGG(125mg/L)	AGG(150mg/L)
A) TBARS				
Liver	107.05± 7.35	154.96 ± 7.7*	160.62 ± 8.0*	186.63 ± 9.3*
Kidney	219.44 ±11.0	276.00 ± 13.8*	285.04 ± 14.3*	285.04 ± 14.3*
B) D:H				
Liver	31.07 ± 1.5	47.54 ± 2.4*	50.60± 2.5*	61.86 ± 3.1*
Kidney	40.97 ± 2.1	48.19± 2.4	57.76 ± 2.9*	62.85 ±3.2*
C) FRAP				
Liver	86.23 ± 4.3	60.62 ± 3.0*	44.96 ± 2.7*	37.05 ± 1.8*
Kidney	71.38 ± 3.5	50.04 ± 2.5*	46.00 ± 2.3*	39.44 ±1.9*
D) GSH				
Liver	0.12±0.006	0.09 ± 0.005	0.08 ± 0.004	0.07 ± 0.004*
Kidney	0.10 ± 0.005	0.08 ± 0.004	0.08 ± 0.004	0.04 ± 0.002*

A Unit of TBARS: µM malondialdehyde (MDA)h-1g tissues-1
B Unit of D:H: % free radicals unscavenged properties
C Unit of FRAP: % ferric reducing antioxidant properties
D Reduced glutathione (GSH) levels: µmol/g tissue
* signifies significant different from the control group ($p<0.05$).

and kidney of exposed catfish when compared with control revealed a reduction in the activity of the enzyme as concentration increases (Table 5). The result obtained is in line with the result obtained by Santos *et al.,*(2016) who reported an decrease in the activity of ALA-D in the blood and liver of Nile tilapia exposed to chronic lead concentration. Also, a reduction in ALA-D activity was recorded in the liver and kidney of African cattish exposed to Round up with Cadmium as co-contaminants (Iyanda *et al.,* 2023).

Extracellular adenine nucleotides are controlled by the action of ectonucleotidases, including NTPDase and ecto-5'nucleotidase (Robson *et al.,* 2006, Yegutkin, 2008; Iyanda *et al.,* 2025). The levels of NTPDase and 5' nucleotidase enzymes activities evaluated on liver and kidney of the experimental African catfish in this study revealed a marked increase in the level of NTPDase and 5' nucleotidase activities in liver and kidney of an exposed catfish when compared with control (Table 5). Generally, an overproduction of these enzymes leads to organ toxicity.

Liver Function Tests (LFTs) is a common parameters used for assessing the extent of liver damage. Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST) are enzymes inside the hepatocyte which leak directly into the blood stream when liver cells are damaged consequently leading to elevated level in the blood of the organism (Kade *et al.,* 2010). The increase in the activities of AST and ALT in the exposed catfish (Table 6) signaled a possible increase in the level of metabolites during herbicidal stress resulting in elevation of activities

Table 5 | Activities of sulphydryl and purinergic enzymes in AGG - exposed catfish tissues

Biomarkers	Control	AGG (100mg/L)	AGG(125mg/L)	AGG150mg/L)
A) ð ALA D[b]				
Liver	215.63 ± 10.8	196.41 ± 9.8	171.60 ± 8.6	108.35* ± 5.4
Kidney	219.87± 11.0	164.20 ±8.2	151.93 ±7.6	136.67*±6.8
B) NTPDase[a]				
Liver	112.61±5.6	129.47±6.5	137.05±6.9	187.48*±9.4
Kidney	106.61±5.3	114.6±5.7	153.49±7.7	197.80*±9.9
C) 5' Nucleotidase[a]				
Liver	14.61±0.7	29.47±1.5	37.05±1.9	51.48*±2.6
Kidney	17.61±0.9	34.62±1.7	53.49±2.7	59.80*±3.0

Results are expressed as means ± SEM of ten catfish
[a] Activities of NTPDase and 5' Nucleotidase are presented as nmolPi/mgProtein/minute
[b] Activities of ð ALA D activities are presented as nmol of PBG/μg protein/hr
[*] signifies significant different from the control group ($p<0.05$).

Table 6 | Biochemical parameters of AGG - exposed catfish.

Biomarkers	Control	AGG (100mg/L)	AGG (125mg/L)	AGG (150mg/L)
A) Hepatic markers				
ALT[a]	8.20±0.4	10.00±0.5	11.00±0.5	14.00*±0.7
AST[a]	12.00± 0.6	14.00± 0.7	16.00± 0.8	17.00* ±0.9
ALP[a]	23.00 ±1.3	27.00 ±1.4	28.00±1.4	34.00* ±1.7
Bilirubin[b]	1.00±0.2	2.00±0.1	2.50±0.1	2.80*±0.1
Albumin[b]	31.00±1.6	26.70±1.3	26.00±1.3	21.00*±1.1
B) Renal markers				
Urea[b]	1.80±0.1	2.40±0.1	2.50±0.1	2.80±0.1
Creatinine[b]	44.00± 2.2	49.00± 2.5	54.00± 2.7	60.00* ± 3.0

Results are expressed as means ± SEM of ten catfish.
[b] Results of levels of AST, Bilirubin, Albumin, and Renal markers are presented as mmol/L.
[a] Results of ALT and ALP are presented as U/L.
[*] signifies significantly different from the control group ($p < 0.05$).

of transaminases. Moreover, stress generally induces elevation in the transamination pathway (Ayanda *et al.,* 2018; Iyanda *et al.,* 2023). This is validated by the report on the effect of glyphosate on *Heterobranchus bidosalis* (Inyang *et al.,* 2016). Also, Alanine phosphatase is a brush border enzyme which splits various phosphate esters at an alkaline pH and mediates membrane transport (Bhattacharya and Anilava, 2009). Increase in the level of activity of Alanine phosphatase (ALP) observed in this study may be due to cellular damage in liver system (Ayanda *et al.,* 2015).

An elevated level of bilirubin observed in this study may be due to liver impairment caused by the AGG on the exposed catfish (Table 6). Also, albumin was evaluated and is a major predominant antioxidant in plasma (Roche *et al.,* 2008). A decrease in the plasma level of albumin in catfish exposed to AGG (Table 6) as observed in this study may lead to impairment of vascular transport for nutrients and reduced antioxidant capacity. The result of albumin obtained in this study is in accordance with previous studies where a decrease in the albumin level of catfish exposed to glyphosate with metals was observed (Iyanda *et al.,* 2023; Oyelakin and Aiyesanmi, 2024). The plasma level of total protein in the catfish exposed to AGG (Table 6) revealed an elevation and an increased level of serum total protein may be attributed to liver dysfunctions and disturbance in protein biosynthesis (Abdel-Tawab *et al.,* 2011).

The proper functioning of kidney on the AGG in exposed catfish (Table 6) clearly indicate that AGG had a significant increase in the plasma levels of urea and creatinine at increased concentration when compared with control**.** The observed elevation of serum urea may be attributed to glomerular dysfunction (Kamal and Khalid, 2012). Also, in kidney dysfunction, serum creatinine concentration increases (Tortora and Derrickson, 2006). The injury to kidney by AGG could be obstructive or may induce apoptosis on renal epithelial cells (Fang *et al.,* 2007; Kade and Rocha, 2010).

4 | Conclusion

Results of the present study indicates that AGG herbicide alter biochemical, physiology and morphology systems of organisms in an aquatic environment which is contrary to the earlier reported claimed of its mild toxicity. Likely mechanisms of toxicity include disruption of the enzyme biosynthetic pathway, reduction of antioxidant capacity of the catfish and alteration of some biochemical indices which causes impairment to some vital organs. However, more studies are still required to establish the deleterious effect of analytical grade glyphosate on aquatic organisms and continuous monitoring of toxicity of the herbicide on both water and soil environment.

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