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Article

Surfactants involvement in the toxicity of glyphosate-based herbicides on the cerebral of African catfish (*Clarias gariepinus*)

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Abstract: The proliferation of glyphosate-based herbicides is becoming a global menace due to their economic viability, thereby resulting in their indiscriminate usage among the consumers. Consequently, there is an increase in the occurrence of these herbicides in aquatic bodies, posing a threat to aquatic life. The present study sought to investigate the variations in toxicities of glyphosate-based herbicides in African catfish (*Clarias gariepinus*). Evaluation of the response of *Clarias gariepinus* juveniles when exposed to glyphosate-based herbicides, namely Round Up (RU) and Force Up (FU), during a range study, which was between 0.0 and 5000 mg/L in thousand doses, was carried out for 96 h. Thereafter, fish were exposed to lower concentrations of RU and higher concentrations of FU. Additionally, the effects of glyphosate-based herbicides were evaluated in surviving catfish for biochemical indices (enzymatic and redox status). At the expiration of the exposure period, there was total mortality of fish in the group treated with RU, while zero mortality was observed in the FU-treated group. As a result, RU concentration was downscaled to 0.0–200 mg/L in arithmetic progression, while FU was increased to 4800–5800 mg/L. Round Up was observed to have more toxicological effect on the biochemical indices investigated namely; lipid peroxidation, 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging property, ferric radical-reducing antioxidant property (FRAP), glutathione (γ-glutamylcysteinyl glycine (GSH)) levels, and the modulation of activities of redox-sensitive enzymes in the cerebral tissues of exposed catfish than observed with Force Up. Therefore, the variation in toxicological effects of these glyphosate herbicide products confers more or less toxicity on the environment, and this may be a function of the composition of surfactants included in their individual formulations.

Keywords: *Clarias gariepinus*; force up; glyphosate herbicides; mortality; round up; surfactant

1. Introduction

Glyphosate is one of the most common and important organophosphorus herbicides developed for use around the world [1] and has been described as an herbicide of broad-spectrum activity with low cost in ecological restoration programs due to its genetic modifications [2,3]. It has the IUPAC name N-(phosphonomethyl)-glycine and is a non-selective, post-emergent systemic herbicide whose herbicidal activity is expressed through direct contact with the leaves and subsequent translocation throughout the plant. Moreover, the effectiveness of glyphosate has led to its emergence as a major active ingredient in several commercial herbicide formulations and hence a proliferation of its occurrence in aquatic environments [4,5].

Furthermore, glyphosate was previously reported to be environmentally friendly and less toxic [6–8] but it now poses a threat to aquatic biota and other living

organisms in the surrounding area due to its longtime accumulation and existence in the environs prior to decomposition [9]. Also, it significantly altered metabolic, oxidative, and hematological parameters in non-target organisms [10–14]. Alarape et al. [15] reported genotoxicity, hepatoxicity and nephrotoxicity effects on the *Clarias gariepinus* exposed to glyphosate herbicide at sublethal doses, with even more adverse effects at increased concentrations. However, every herbicide contains ingredients other than the active ingredients called surfactants which are required at possibly higher concentrations than necessary for maximum reduction of the spray solution surface tension. This indicates that their mode of action is not limited to increasing the spreading characteristics of the spray droplets [16] but is also involved in increasing permeability of the cuticle, plasma membrane, or both in increasing foliar uptake of glyphosate and promoting phytotoxicity [17]. Such surfactants may be more toxic than glyphosate alone and may synergistically increase glyphosate toxicity, as reports have suggested that the presence of multiple toxicants generally results in greater toxicity than any of the individual components [18–20]. The formulations and surfactants like polyoxyethylene amine (POEA) have been discovered to be highly toxic and consequently responsible for the relatively high toxicity of formulated glyphosate to several freshwater invertebrates and fishes.

However, due to the emergence of different brands of glyphosate, there are variations in their toxicity and behavioral response to organisms. Shiogiri et al. [21] reported that the LC_{50} values when *Cyprinus carpio* and *Palloceros caudimaculatus* were exposed to glyphosate commercial formulation (Rodeo) herbicides are 620 mg/L and 975 mg/L for 96 h, respectively. Also, 211.80 mg/L and 32 mg/L were obtained by Nwani et al. [22] as LC_{50} for Force Up and Round Up, respectively, on exposure of *Tilapia zillii and Clarias gariepinus*. Additionally, Awoke et al. [14] reported 44.67 mg/L as the LC_{50} value for 96 h exposure of glyphosate commercial formulation (Mulsate) to *Clarias gariepinus*. The observation of significant differences in the toxicities of different brands of glyphosate herbicides in non-target organisms, as highlighted above, suggests the possibility that the active ingredient, glyphosate, is not the sole toxic agent present in these formulations. It is also possible that glyphosate interacts in varied dimensions with other constituents, impacting the availability of reactive functional groups or possibly eliciting more reactive functional groups. Essentially, the differential toxicity of different glyphosate brands is likely a function of the chemical nature of other constituents present in individual commercial formulations. Hence, this study was carried out with the aim of providing more information on the chemical basis for the disparity in the toxicities of commercial glyphosate formulations and to underscore the possible involvement of surfactants in RU- and FU-mediated toxicity on the cerebral tissue of African catfish (*Clarias gariepinus*).

2. Materials and methods

2.1. Chemicals

Round Up and Force Up brands of glyphosate herbicide were obtained at Oba's market, Akure, Nigeria, thiobarbituric acid (TBA), and adenosine triphosphate (ATP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 1,1-diphenyl-2-

picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), and all other chemicals used were of analytical grade and were obtained from standard commercial suppliers.

2.2. Materials

From the literature:

Chemical composition of Round Up = glyphosate isopropylamine salt 360 g/L or 480 g/L + POEA + minor additives (water, preservatives, pH stabilizers, antifoam) [23–25].

Chemical composition of Force Up: Glyphosate isopropylamine salt = 360 g/L or 480 g/L.

Potential alternative surfactants in Force Up = alkyl polyglucosides (APGs), cocamidopropyl betane, propoxylated quaternary ammonium compounds and coconut shell extract-based surfactants [26–28].

2.3. Methodology

2.3.1. Fish purchase and acclimatization

Five hundred juveniles of *Clarias gariepinus* with a mean weight of 30 ± 0.3 g and a mean standard length of 15 ± 0.1 cm were used for the experiment and were purchased from Adeoti Farm in Ondo State, Nigeria. Juveniles were used due to their sensitivity to toxicity tests compared to adults [29]. The fish were acclimatized under laboratory conditions in 1000-liter plastic tanks with dimensions of 1400 mm in diameter × 895 mm in height for 7 days and were fed with commercial floating pellets at 10% of their body weight. The pond where the fish were obtained had a temperature of 26.8 ℃, a pH of 7.1, and dissolved oxygen of 62.77 ms/cmand the dechlorinated tap water used for acclimatization had a temperature of 26.0 ± 0.8 ℃, a pH of 7.0, and dissolved oxygen of 6.3 ± 0.1 mg/L.

2.3.2. Toxicity study

A static bioassay technique was adopted, and preliminary screening was carried out to determine the appropriate concentration range for testing chemicals as described by Chinedu et al. [30]. The concentrations, in weight per volume, of RU and FU used for the range test were 0.0, 1000, 2000, 3000, 4000, and 5000 mg/L. Ten *Clarias gariepinus* juveniles per concentration of toxicant were used for 96 h. Based on the result of the first range test, it was further evaluated using RU—0.0, 1, 10, 20, 30, 50, 100, and 200 mg/L; and FU—0.0, 6000, 8000, and 10,000 mg/L. The definitive range of concentrations at which both initiated death was further evaluated.

Fish mortality was daily recorded, removed, and discarded. Fish were considered dead when no movement upon gentle prodding was observed. Also, the mean physicochemical parameters (pH, temperature, and dissolved oxygen) of the test water containing different concentrations of glyphosate were monitored and recorded. The pH of the solutions was measured using a pH meter, temperature using a mercury-in-glass thermometer, and dissolved oxygen with a digital dissolved oxygen meter.

2.3.3. Biochemical assays

(a) Tissue preparation

The cerebral tissue of the surviving catfish from the RU and FU exposure after

4 days was quickly removed, placed on ice, and homogenized in cold 50 mM Tris–HCl at pH 7.4. The homogenate was centrifuged at 4000× g for 10 min to yield the low-speed supernatant (S1) fraction that was used for all biochemical assays. Protein content was determined with bovine serum albumin as the standard using the method modified by Kade and Rocha [31].

(b) Antioxidant status assays

(i) Lipid peroxidation assay: Lipid peroxidation was determined by monitoring the production of thiobarbituric acid reactive substances (TBARS) as described by Kade et al. [32] and Iyanda et al. [8]. The color reaction was developed by adding 300 µL of 8.1% SDS to the medium, followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) and 500 µL of 0.8% TBA. This mixture was incubated at 95 °C for 1 h, and the absorbance values of TBARS produced were measured at 532 nm. The absorbance value was compared to that of a standard curve obtained using malondialdehyde (MDA).

(ii) GSH level estimation: GSH level of brain tissue of fish exposed to RU and FU was determined and was estimated using Ellman's reagent. The brain tissue homogenate was initially deproteinized with TCA (5% in 1 mmol/EDTA) following the method modified by Kade et al. [32] and cited by Iyanda et al. [8]. The absorbance of the yellow color formed in the reaction system was measured at 412 nm.

(iii) DPPH—free radical scavenging ability: The free radical scavenging ability of the cerebral tissues against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals was evaluated according to Kade and Rocha [31], and Iyanda et al. [8]. Brain tissue homogenate was mixed with 600 µL of 0.3 mM methanolic solution, which contains DPPH radicals; the mixture was kept in the dark for 30 min, after which a golden yellow color was formed as product and was measured at an absorbance of 516 nm.

(iv) FRAP—reducing property: Ferric-reducing antioxidant properties: The ferric-reducing antioxidant properties of the compounds against TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) were evaluated as cited by Kade et al. [32] and Iyanda et al. [8]. 300 µL of the protein-free tissue homogenates were mixed with 300 µL and 900 µL of TPTZ solution. The mixture was kept in the dark for 10 min, and absorbance values were measured at 543 nm.

(c) Enzymes activity

(i) Na^+/K^+-ATPase assay: Na^+/K^+-ATPase activity was assayed according to Kade et al. [33] and Iyanda et al. [8]. Briefly, the reaction mixture contained 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, 200 mM sodium azide, 50 mM Tris–HCl at pH 7.4, and 100 µL of brain tissue homogenate, all in a final volume of 500 µL. The reaction was initiated by adding 3.0 mM ATP. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. Na^+/K^+-ATPase activity was estimated by the difference between the two assays. Released inorganic phosphorous (Pi) was measured by the method modified by Kade et al. [33]. Protein was estimated using a modification method of Kade and Rocha [31] using bovine serum albumin.

(ii) Purinergic enzymes: NTPDase assay: NTPDase enzymatic activity in the reaction medium was determined as described by Kade et al. [31] and Iyanda et al. [8]. 50 µL of tissue homogenate was added to the reaction mixture and was pre-

incubated for 10 min at 37 ℃. The reaction was initiated by the addition of 3.0 mM ATP.

5'-Nucleotidase assay: 5'-Nucleotidase activity in a reaction medium was determined as described by Kade et al. [31] and Iyanda et al. [8]. 50 µL of brain tissue homogenate was added to a reaction mixture containing 0.1 M Tris-HCl buffer, pH 7.4, and 30 mM $MgSO_4$ and was pre-incubated for 10 min at 37 ℃. 3.0 mM AMP was added to the mixture to initiate the reaction and incubated for 30 min, after which absorbance was read at 650 nm.

2.4. Statistical analysis

Results were analyzed by appropriate analysis of variance (ANOVA), and this is indicated in the text of the results. Differences between groups were considered to be significant when $P \leq 0.05$.

3. Results

3.1. Physicochemical parameters analysis

The range of values of the physicochemical analyses of diluting test water for glyphosate brands is presented in **Table 1**. These values are within the recommended range for fish rearing.

Table 1. Physicochemical parameters of diluting water monitored during an experiment with glyphosate.

Parameters	Control	RU	FU
Temperature (℃)	25.25 ± 0.03	26.2 ± 1.5	25.8 ± 1.3
pH	7.14 ± 0.04	6.26 ± 0.3	7.06 ± 0.3
Dissolved oxygen (mg/L)	6.16 ± 0.37	5.10 ± 0.3	5.16 ± 0.3

3.2. Mortality/96 h range analysis

This is a comparative experimental study between RU and FU. Firstly, the fish were exposed to both RU and FU at high concentrations in thousands ranging from 1000 to 5000 mg/L, 10 fish in each tank for RU and FU. Total fish mortality was observed in groups treated with RU (**Figure 1**), while the fish exposed to FU all survived (**Figure 2**). Then RU was narrowed down, ranging from a concentration of 200–1000 mg/L, and fish exposed had total mortality (this experiment was a pilot study and was not included due to the complete mortality). Furthermore, fish were exposed to concentrations ranging from 10 to 200 mg/L (**Figure 3**); fish survived within the range of 0 to <20 mg/L, while fish mortality occurred within the range of 20 to 200 mg/L. FU, on the other hand, was increased, ranging from 6000 to 10,000 mg/L, due to a survival rate at 5000 mg/L (**Figure 4**), and complete mortality was recorded. However, it is imperative to further narrow the concentrations of both RU and FU to investigate the mortality between survival and the last smallest concentration for mortality to find the exact concentration range that the herbicide initiated death. Therefore, the concentration range of RU was narrowed between 10 and 18 mg/L (**Figure 5**), and the concentration range of FU was downscale to 4800

mg/L and 5400 mg/L (**Figure 6**), and a fair number of fish were observed to survive between these ranges after 4 days, thereby estimating the lethal concentration at which 50% (LC_{50}) of the fish survived.

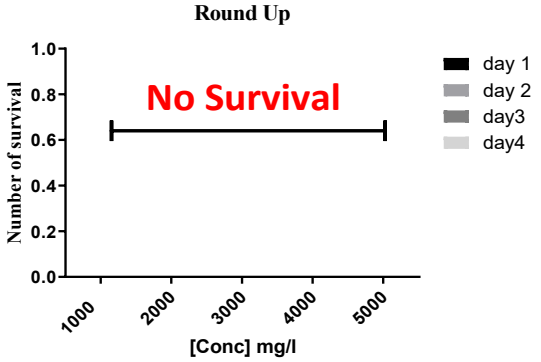

Figure 1. Range-finding test showing the response of catfish to various concentrations of RU.

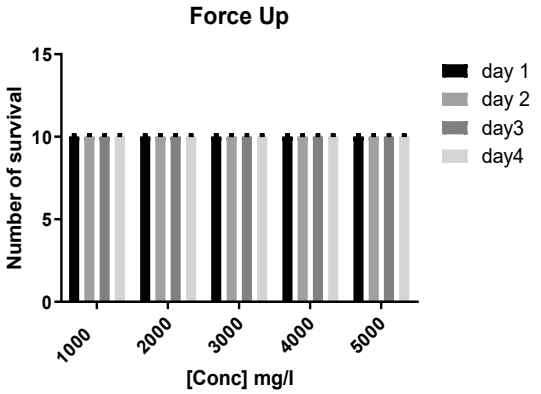

Figure 2. Range-finding test showing the response of catfish to various concentrations of FU.

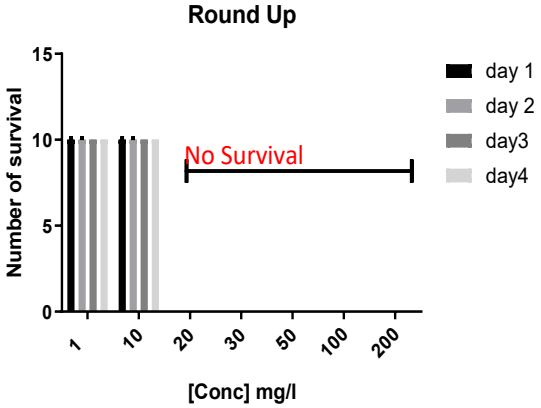

Figure 3. Showing the range of concentrations further analyzed for toxicity of RU in which the experimental catfish were exposed.

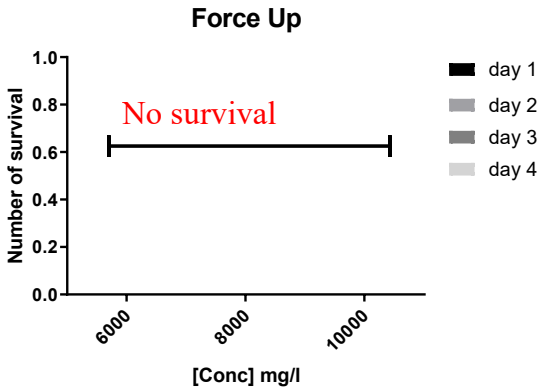

Figure 4. Range concentrations for the determination of LC_{50} of FU.

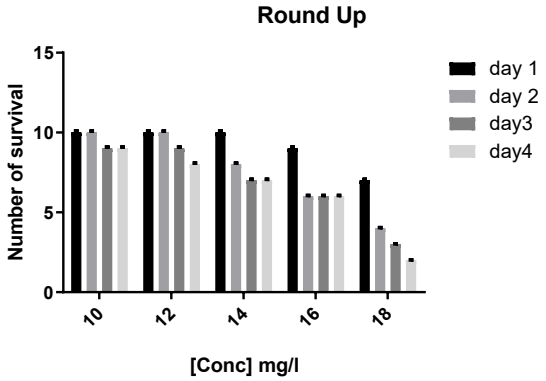

Figure 5. Concentration range used for definitive test for RU.

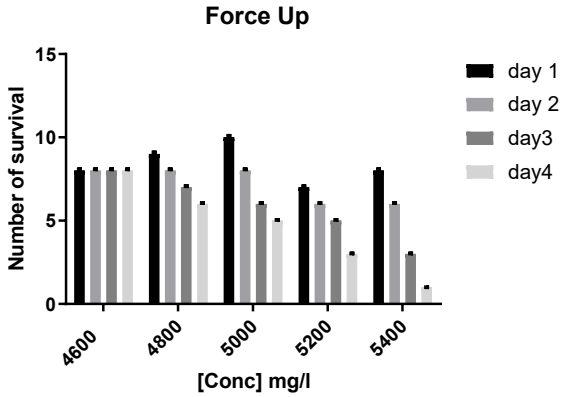

Figure 6. Concentration range used for definitive test for FU.

3.3. Biochemical evaluation of the catfish exposed to RU and FU herbicides

Biochemical indices are necessary to be evaluated to have insight into mechanistic activities in the fish that are probably responsible for the mortality recorded in toxicity studies. The evaluation of these indices was analyzed on the surviving juveniles of *Clarias gariepinus* after 4 days of exposure to definitive concentrations of RU and FU, and the results are presented below:

3.3.1. Effect of RU- and FU-induced lipid peroxidation in brain tissue of exposed catfish

The effect of RU and FU on the level of TBARS in the brain of exposed catfish

was presented in **Table 2**. When compared with the control group, it was observed that the level of TBARS formed in the cerebral homogenate of the exposed catfish increased ($P < 0.05$) as the concentration increased, and the level of TBARS formed was higher in the RU-treated fish than in the FU-treated group, thereby resulting in higher lipid peroxidation in the RU-treated fish than in the FU-treated fish.

3.3.2. Effect of RU and FU on DPPH radical scavenging ability in cerebral tissue of African catfish

The ability of the cerebral tissue homogenates to scavenge DPPH radicals after exposure to RU and FU decreased and was observed to be more pronounced in the group treated with RU than in the group treated with FU as their concentrations increased when compared with the control (**Table 2**).

3.3.3. Effect of RU and FU on the ferric reducing antioxidant power

Table 2 revealed the effect of RU and FU on the ferric reducing power of the cerebral tissue. There was a reduction in the reducing properties of the brains of catfish exposed to RU compared to catfish exposed to FU at an increasing concentration.

3.3.4. Effect of RU and FU on cerebral GSH level in African catfish

The levels of GSH in the brains of exposed catfish are presented in **Table 2**. It was observed to be significantly lower than the catfish in the control group and is more pronounced in the group treated with RU than in the FU-treated group. The decreased GSH level diminishes the antioxidant capacity of the catfish, which eventually induces oxidative stress, thereby exposing the cells to degenerative syndromes [34,35].

3.3.5. Enzyme activity

(i) Effect of glyphosate—based on the activity of Na^+/K^+ ATPase in exposed catfish

The effect of FU and RU on the activity of Na^+/K^+ ATPase in the cerebral homogenate of the exposed experimental catfish was evaluated and presented in **Table 3**. Exposure to RU and FU caused a significant ($P < 0.05$) inhibitory effect on the levels of Na^+/K^+ ATPase in the brain of African catfish in a concentration-dependent manner when compared with the control group.

(ii) Effect of RU and FU on the activity of NTPDase

Table 3 shows the effect of RU and FU on the activity of NTPDase in the cerebral homogenate of the exposed catfish. Exposure to FU and RU solutions caused a significant increase in the levels of NTPDase in the tissues of African catfish in a concentration-dependent manner whereby RU-treated fish had higher NTPDase activity than the FU-treated group.

(iii) Effect of FU and RU on the activity of 5'nucleotidase in exposed catfish

The effect of RU and FU on the 5'nucleotidase activity in the cerebral homogenate of the exposed catfish was evaluated and presented in **Table 3**. Exposure to both RU and FU, when compared with the control group ($P < 0.05$), caused a significant increase in the activity of 5'nucleotidase in which the activity was higher in the group treated with RU than in the group treated with FU in the cerebrum of African catfish in a concentration-dependent manner.

Table 2. Effects of RU and FU on redox status of the brain of exposed African catfish.

Experimental toxicants	Concentrations (mg/L)	Lipid peroxidation	DPPH	FRAP	GSH
RU	Control	197.90 ± 8.9	31.08 ± 1.6	86.21 ± 4.3	0.106 ± 0.005
	10	377.12 ± 13.9	46.10 ± 2.3	61.20 ± 3.0	0.096 ± 0.004
	11.5	389.57 ± 14.5	55.18 ± 2.5	43.16 ± 2.1	0.085 ± 0.004
	13	503.14 ± 15.2*	68.19 ± 2.9*	31.14 ± 1.6*	0.067 ± 0.004*
FU	Control	142.96 ± 8.3	28.50 ± 1.4	84.12 ± 3.7	0.123 ± 0.003
	4600	221.55 ± 10.1	41.72 ± 2.1	71.24 ± 2.6	0.098 ± 0.005
	4800	297.87 ± 10.4	59.73 ± 3.0	65.21 ± 2.0	0.091 ± 0.005
	5000	327.79 ± 11.8*	65.77 ± 3.2*	46.13 ± 1.8*	0.08 ± 0.004*

Data are reported as mean ±SEM for at least ten fish per group,
A) Unit of TBARS is µM malondialdehyde (MDA)/h/g tissues,
B) Unit of DPPH is % free radicals unscavenged properties,
C) Unit of FRAP is % ferric reducing antioxidant properties,
D) Reduced glutathione (GSH) levels are presented as µmol/g tissue,
*Significantly different from the control group (ANOVA/Duncan, $p < 0.05$).

Table 3. Evaluation of sulfhydryl enzymes in catfish exposed to RU and FU (nmolPi/mgProtein/min).

Experimental toxicants	Concentrations (mg/L)	Na^+/K^+-ATPase	NTPDase	5'-Nucleotidase
RU	Control	196.31 ± 9.8	108.59 ± 5.4	11.73 ± 0.6
	10	141.93 ± 7.1	157.46 ± 7.9	34.88 ± 1.7
	11.5	112.01 ± 5.6*	165.61 ± 8.3	48.88 ± 2.4
	13	100.82 ± 5.1*	187.90 ± 9.4*	61.45 ± 3.1*
FU	Control	202.29 ± 10.1	104.32 ± 5.2	15.73 ± 1.1
	4600	147.41 ± 7.4	133.18 ± 6.7	34.88 ± 1.7
	4800	139.69 ± 7.0*	138.46 ± 6.9	46.59 ± 2.3
	5000	115.98 ± 5.8*	168.61 ± 8.4*	55.31 ± 2.8*

Data are expressed as means ± SEM of ten catfish.
*Significantly different from the control group (ANOVA/Duncan, $P < 0.05$).

4. Discussion

Several studies have reported a significant difference in the toxicities of glyphosate standards and different glyphosate-based commercial herbicide formulations. The differential toxicity of commercial glyphosate herbicides and glyphosate standards is indicative of the presence of chemical species with divergent reactivities [16,17]. An understanding of the biochemical mechanisms involved in their toxicities could give substantive insights into the likely intracellular interactions of the constituent chemical species present in individual commercial formulations. This study was carried out to evaluate the toxicity of glyphosate-based herbicides in response to catfish. There was a modulatory effect observed in the glyphosate-based herbicides (RU and FU) in response to the environment and toxicity on the non-target organisms, especially aquatic organisms. RU (**Figure 1**) in this study appears to be toxic at extremely low concentration whereas FU toxicity was at extremely high concentrations. Nwani et al. [22] reported 211.80 mg/L and 32 mg/L as LC_{50} obtained when *Tilapia zillii and Clarias gariepinus* were exposed to FU and RU, respectively.

Also, Ukaegbu et al. [36] reported the LC_{50} value of 0.56 mg/L as the sublethal concentration of Round Up glyphosate in juvenile African catfish. Additionally,

Awoke et al. [14] and Edeh et al. [37] reported 44.67 mg/L and 1.50 mg/L, respectively, as LC_{50} values for 96 h exposure of glyphosate commercial formulation (Mulsate) to *Clarias gariepinus*. This disparity in toxicity of glyphosate-based herbicide may speculatively be due to their industrial formulation process and the composition of surfactants used in the formulation. To corroborate this speculation, since the emergence of many new glyphosate-based herbicides, there has been a modification of different chemistry and surfactant mixtures that contain alkyl polysaccharides or other biodegradable surfactants with less acute toxicity to non-target organisms like fish [16,28,38]. Such surfactant mixtures are (a) alkyl polysaccharide, which is derived from sugars and fatty alcohols and used in herbicides to improve wetting and spreading; (b) cocamidopropyl betaine, which is a surfactant derived from coconut oil, offers effective wetting and foaming properties; (c) propoxylated quaternary ammonium surfactants, which are developed to replace first-generation POEAs; and (d) coconut shell extract-based surfactants, which have improved glyphosate efficacy by 27% over those with POEA, reduce surface tension, and enhance plant uptake [26 – 28,39]. These surfactants cause milder gill alterations, degrade more easily and rapidly exhibiting less toxicity, reduce impact on the environment, and are less disruptive to biological membranes, making them safe for aquatic life. Therefore, the significantly less toxicity mediated by FU may probably be a function of the presence of such lower acutely toxic and environmentally friendly Surfactant mixture. However, the composition of FU is proprietary and not publicly disclosed, probably due to commercial reasons such as protecting proprietary blends from duplication and preserving market advantage.

On the contrary, the speculation in this study is further corroborated by reports that implicate polyoxyethylene amine (POEA) as one of the major surfactants used in RU glyphosate-based commercial herbicide formulations and is more toxic than the active ingredients as well as the formulated product itself [24,25,40–44]. Meanwhile, most herbicides must cross the plasma membrane before reaching their site of action, and several researchers have suggested that the plasma membrane is a limiting barrier to the foliar uptake of glyphosate into the cell [16,23,45,46]. However, surfactant MON 08184 (Monsanto Agricultura Products Co., St. Louis, MO), a polyethoxylated tallow amine (POEA) used mostly in the commercial formulation of RU glyphosate, can penetrate the cuticle and act at the plasma membrane [45,47]. Therefore, the involvement of effective surfactants increases the permeability of the cuticle, plasma membrane, or both in foliar uptake of glyphosate and promotes phytotoxicity [16,17,47,48].

Glyphosate-based herbicides are available in the form of isopropylamine salt, which aids the solubility, stability, and absorption but is not a major driver of toxicity. Apart from the constituent chemical species in commercial herbicide formulations, which were specified in the Section 2.2 of this study, other environmental factors such as the physicochemical properties of the aquatic habitat could impact their toxicity.

The physicochemical properties of aquatic bodies significantly influence the toxicity of xenobiotics on aquatic organisms such as fish [49–51]. In the present study, the ranges of values of the physicochemical analyses of test water for RU and

FU as presented in **Table 1** were reported and were within the recommended range for good culturing and survival of catfish (*Clarias gariepinus*). Hence, they did not significantly influence the toxicity of the chemicals to aquatic organisms. However, investigation of biochemical parameters could give tangible insights into the toxicological mechanism as reactivities of the chemical species present in the individual commercial herbicide formulations.

From the foregoing, this discrepancy in the toxicity of glyphosate herbicides may relatively perturb their toxicological responses to the physiology like redox status and enzyme activities in the tissue homogenate of the exposed organisms. Therefore, it is imperative to ascertain the antioxidant status of the exposed catfish. Glutathione (γ-glutamylcysteinyl glycine, GSH) is a major component of the antioxidant defense system in fish and is an antioxidant that protects against oxidative stress and neutralizes reactive oxygen species (ROS) and their harmful reaction products. It also contributes to a stronger immune system in fish, potentially by increasing the abundance of beneficial intestinal microbiota and reducing the risk of infections. In this study, a depletion in the level of GSH (**Table 2**) was observed in the brain of catfish exposed to both brands of glyphosate but was more pronounced in RU than in the fish exposed to FU, which may be due to the reactivities of surfactants used for the formulation of RU, as earlier speculated. However, depletion in the level of GSH can lead to immune deficiency, increased susceptibility to oxidative stress through increased ROS, potentially damaging the cell membranes like DNA and proteins, resulting in cell death [52] and possibly death of the fish, as recorded in **Figures 1–6**. Another parameter to evaluate the antioxidant level of the exposed catfish is lipid peroxidation, which is a biomarker to measure the ROS damage of lipids in cell membranes. A marked increase was observed in the level of thiobarbituric acid reactive substances (TBARS) formed by the peroxidation of the membrane lipid bilayer of the cerebral tissue of exposed catfish in both groups but was more significant in the RU-treated group than in the FU-treated group (**Table 2**). This corroborates the speculative verdict of surfactants being responsible for alteration in the toxicity of glyphosate herbicide. However, the formation of these products (TBARS) generates a cascade of free-radical reactions that can greatly alter the physicochemical properties and physiological function of biological membranes, resulting in cell death and loss of tissue function [53]. This increased lipid peroxidation is validated by Mansour et al. [54], who reported an increase in lipid peroxidation in the liver and testes of catfish exposed to oxyfluorfen.

Additionally, the inherent antioxidative ability in the cerebral tissue of catfish after exposure to RU and FU was assessed using the DPPH scavenging test, which is a common and reliable way of assessing the antioxidant activity of catfish. This is because catfish like *Clarias gariepinus* and *Pangasius hypopthalmus* possess antioxidant peptides and compounds that can scavenge DPPH radicals, indicating their antioxidant potential [55,56]. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [57] and is used to evaluate antioxidant levels since antioxidants have the ability to readily donate their hydrogen to DPPH. It was discovered that both groups of exposed fish (**Table 2**) displayed a reduction in the ability to scavenge free radicals, though it was more pronounced in the group treated with RU than in the group treated with FU.

The reduced cerebral ability of the catfish to scavenge free radicals implies that more radicals are left unscavenged, building up ROS, a situation that may result in fish being more susceptible to oxidative stress, causing damage to cell membranes and various health problems, including cardiovascular disease, neurodegenerative disorders, effects on growth, reproduction, and immune function [58,59].

Similarly, ferric reducing antioxidant power (FRAP) is another antioxidant status parameter that is used to assess the total antioxidant capacity of catfish. FRAP measures the ability of an organ to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) via the inherent antioxidant capacity in them [60,61]. Hence, higher reducing power indicates better abilities to reduce transition metals and transfer an electron to free radicals to become stable chemical species [62]. In this study, a toxicity of both groups of glyphosate was demonstrated (**Table 2**), with a marked reduction in antioxidant power in the cerebral tissue of exposed catfish having the group treated with RU lower in antioxidant power than the group treated with FU. This is an indication of weaker antioxidant power consequently leading to oxidative stress, which can damage cells and tissues, impair the antioxidant system, and lower FRAP values.

All these antioxidant status parameters had depletion in the antioxidant levels in the exposed catfish, leading to accumulation of free radicals, ultimately resulting in oxidative stress, which can increase inflammation of purinergic enzymes and shutdown of crucial enzymes like ion-transport protein (e.g., sodium/potassium ATPase). Therefore, it is vital to evaluate these enzymes to ascertain the possible toxicological effects of these glyphosate products on the brains of exposed catfish.

The sodium-potassium pump, or Na^+/K^+-ATPase, is a vital transmembrane protein in animal cells, including catfish, that maintains sodium and potassium ion gradients across the cell membrane. This pump helps catfish with osmoregulation—controlling the osmotic pressure and preventing cells from swelling and shrinking—and nerve function, which is essential for the transmission of electrical signals along nerve pathways and muscle contraction by maintaining the proper balance of sodium and potassium ions [63–65]. There is depletion in the activity of Na^+/K^+ ATPase exerted by RU and FU (**Table 3**) in the brain of exposed catfish, which was more prominent in the group treated with RU than in the FU-treated group, which also corroborates the speculation of reactivities of surfactants to the formulation of commercial glyphosate. However, the inhibition or reduction in the activity of the sodium pump leads to several effects, including increased intracellular sodium, altered membrane potential, calcium influx changes, disrupted cellular processes, altered pathological conditions, and environmental toxicants that modulate the sodium pump activity [8,65–67], which may lead to inflammation and increased activities of inflammation biomarkers like purinergic signaling enzymes.

Likewise, the activity of purinergic signaling enzymes (NTPDase and 5’nucleotidase) was evaluated due to their crucial role in physiological processes, including modulation of immune responses, particularly during stress, infection, and anti-inflammatory effects—protecting against tissue damage and promoting healing—and playing a role in handling stress, contributing to the restoration of homeostasis. The enzymes like NTPDase and 5’nucleotidase regulate the breakdown of extracellular nucleotides and nucleosides like ATP, ADP, and AMP, which act as

signaling molecules [68,69]. The brain of African catfish exposed to RU and FU in this study revealed a marked increase in the level of NTPDase and 5'nucleotidase activities in catfish brain exposed to RU (**Table 3**) compared to the FU-treated group. However, these increased activities lead to pro-inflammatory effects, which trigger an inflammatory response in tissues and immune impairment [70]. Overproduction of these enzymes may lead to organ toxicity. Furthermore, the release of endogenous nucleotides represents a critical component that affects different genes and excessive inflammation, which may lead to activation of a signaling cascade (cell death pathways) that is apoptotic [8,71,72], which leads to death, which may contribute to higher mortality observed in RU and FU earlier.

5. Conclusion

The disparity in the toxicity of the commercial-grade glyphosate may depend on surfactants employed in herbicide formulations, fish species, and the test conditions. Moreover, it is clear that the presence of surfactants has a modulatory effect on the oxidative capacity of glyphosate. Also, the glyphosate brands had toxicological effects (even though RU is more than FU), leading to inflammation of purinergic enzymes, shutdown of iron transport in the brain of the exposed catfish, and depletion of the antioxidant status of the fish, leading to reduced fish quality, loss of appetite, neurological changes, inflammation of cells, and eventually death of the fish, as observed and recorded in the definitive studies.

Recommendation

There is a dearth of information available in the literature about the chemical nature and specific surfactants industrially introduced in the various formulated products of glyphosate. This serves as a major limitation to the investigation of these interferences, and therefore, more research still needs to be done to justify and ascertain these.

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