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Tissue-Specific Genotoxic Effects of Low-Dose Glyphosate-Based Herbicide (*Roundup)* Exposure in Juvenile *Clarias gariepinus*

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ABSTRACT: Glyphosate is the most widely used herbicide in the world today and has increased in use as weed resistance has developed. Due to its effectiveness and affordability, "Roundup", a commercialized formulation of glyphosate, is today the second most widely used herbicide in urban areas and in agriculture. The increased use of glyphosate and glyphosate-based herbicides has led to adverse toxic effects on the environment, animals, and humans, even at low concentrations. In this study, the genotoxic effects of very low concentrations of the glyphosate-based herbicide ("Roundup") on the gills, gonads, and liver of juvenile *Clarias gariepinus* were assessed. The different concentrations of "Roundup" used in this study were 0.03 mL/L, 0.06 mL/L, 0.10 mL/L, and 0.13 mL/L, respectively. An acute toxicity study carried out revealed mortality in some of the treated groups, especially in the group administered the highest concentration of "Roundup," where one hundred percent (100 %) mortality was observed after 96 hours. Behavioural changes such as erratic movements, bubble production from the mouth, discoloration, restlessness, and gasping for air were also observed across the various "Roundup" concentrations. Probit analysis revealed that the LC_{50} of "Roundup" in this study was 0.04 mL/L. Genotoxicity assessment using the comet assay revealed significant ($p < 0.01$) damage to genetic material (DNA) in the gills, gonads, and liver of juvenile *Clarias gariepinus* when treatments were compared with the control. The findings from this study indicate that even at very low concentrations, significant genotoxic damage was observed in the gills, gonads, and liver of *Clarias gariepinus*. The results underscore the importance of stricter regulation and monitoring of glyphosate use, as well as the need to explore safer alternatives to protect aquatic biodiversity and human health.

Keywords: Glyphosate, Roundup, Catfish, Genotoxicity, Comet assay

Introduction

Glyphosate-based herbicides (GBHs), primarily formulated around N-(phosphonomethyl) glycine, represent the most widely deployed class of non-selective, post-emergence herbicides in modern agriculture (Zanardi *et al.*, 2024). Since their commercialization in 1974 under the trade name "Roundup", GBHs have been applied to control more than 150 plant species (Matozzo *et al.*, 2020; Rahmani *et al.*, 2023), with usage intensifying following the advent of glyphosate-resistant crops in the late 1980s (Werner *et al.*, 2022). Today, glyphosate-containing formulations account for approximately 60% of the global non-selective herbicide market (Berestetskiy, 2023), underscoring their central role in crop management strategies. Despite initial assumptions about safety (based on glyphosate's inhibition of the plant-specific enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is absent in mammals), growing evidence shows that glyphosate and its commercial formulations have toxic effects beyond their intended targets. Residues of glyphosate are now routinely found in soil, water, vegetation, food products, and even human biological samples (Ojelade *et al*., 2022), raising concerns about long-term exposure. Additionally, co-formulants and adjuvants, such as polyoxyethylene amine (POEA), increase the biological activity and toxicity of GBHs (Novotny, 2022), leading to negative outcomes like cytotoxicity, endocrine disruption, neurotoxicity, and cancer risk (Mazuryk *et al*., 2023). Notably, the toxicity of commercial formulations often surpasses that of glyphosate alone (Martins-Gomes *et al*., 2022), emphasizing the need for comprehensive risk assessments that evaluate entire product mixtures instead of just active ingredients.

Aquatic ecosystems are particularly vulnerable to glyphosate contamination due to agricultural runoff. Sentinel species such as *Clarias gariepinus* (African catfish) provide a robust model for evaluating aquatic toxicology. This species is widely distributed, resilient under high-density aquaculture conditions, and increasingly employed in biomarker-based studies of environmental pollutants, including pesticides, heavy metals, fertilizers, and microplastics (Ayanda *et al*., 2021; Sorichetti *et al*., 2022; Mohammed *et al*., 2024). Its physiological and ecological attributes (including rapid growth, adaptability, and the presence of an epibranchial organ) make it an ideal organism for assessing genotoxic and systemic impacts of GBHs in aquatic environments (Abdallah *et al*., 2024; Beebe, 2025).

Given the ubiquity of glyphosate residues and the growing evidence of their biological effects, it is imperative to investigate their potential genotoxicity in non-target organisms. The present study, therefore, aimed to evaluate the acute toxicity of "Roundup" and also determine the genotoxic effects of environmentally relevant, low concentrations of GBHs on critical organs such as the gills, gonads, and liver of juvenile *C. gariepinus*. This study provides mechanistic insights into glyphosate-induced toxicity and contributes to the broader understanding of ecological and public health risks associated with GBH exposure.

Materials and methods

Chemicals: The glyphosate-based herbicide employed in this study was "*Roundup Turbo"*, manufactured by Monsanto Europe N.V., Belgium. Each litre of the formulation contains 450 g of glyphosate acid as the active ingredient. The product was procured from an authorized distributor in Benin City, Nigeria (Ring Road).

Fish collection and acclimatization: A total of ninety (90) juvenile *C. gariepinus,* with an average length of 15 ± 1.0 cm and a mean weight of 10.5 ± 0.5 g, were sourced from the Faculty of Agriculture Fish Farm, University of Benin, Nigeria. Fish were transported in aerated aquarium tanks to the Departmental wet laboratory, where they were maintained under controlled conditions. Acclimatization was conducted for 7 days in plastic tanks (30 × 36 × 52 cm), and fish were fed twice daily at 3% of body weight using commercial fishmeal. To minimize the accumulation of metabolic waste, water was replaced twice during acclimatization. Feeding was discontinued 24 hours before experimental exposure.

*Preparation of test concentrations of "*roundup": Four test concentrations of *Roundup* were prepared: 0.03 mL/L, 0.06 mL/L, 0.10 mL/L, and 0.13 mL/L. For each concentration, the required volume of herbicide was measured using a calibrated syringe, diluted with distilled water to 1000 mL in a measuring cylinder, and transferred into plastic bowls. Each concentration was prepared in duplicate, yielding eight experimental units. A control unit containing 2000 mL of distilled water was maintained under identical conditions.

Experimental procedure for acute toxicity: Following acclimatization, ten (10) juveniles were introduced into each bowl containing the respective concentrations of "Roundup". A control group of ten fish was maintained in distilled water. Fish were monitored for behavioral abnormalities, external morphological changes, and mortality at 24, 48, 72, and 96 hours. Dead specimens were promptly removed. To reduce waste accumulation, water was replaced daily. The bioassay lasted 96 hours, and the median lethal concentration (LC_{50}) was calculated using probit analysis. Surviving juveniles were subsequently transferred into labeled perforated containers and transported within 24 hours to the Cell Biology and Genetics Laboratory, University of Lagos, Nigeria, for comet assay analysis.

Comet assay procedure: The comet assay was performed following the alkaline single-cell gel electrophoresis protocol described by Singh *et al*. (1998). After the acute toxicity test, gills, gonads, and liver were excised from both treated and control organisms, placed in individually labelled Eppendorf tubes, and stored in boxes to ensure proper identification and prevent mix-ups. The tissues were digested with trypsin to release cellular material and DNA for analysis. Microscope slides were marked with diamond pens for accurate sample tracking, and a lysing solution containing sodium chloride, disodium EDTA, dimethyl sulfoxide (DMSO), Triton X-100, and deionized water was prepared to disrupt cell membranes and expose DNA. A base layer of normal agarose was applied to the slides, followed by embedding the digested cells in 0.5% low-melting-point agarose. Cover slips were used to create a uniform thin film, and once solidified, a final protective layer of 1% agarose was added to prevent contamination. The slides were then immersed in the lysing solution for 24 hours to allow nucleoids to form. For electrophoresis, a buffer was prepared by mixing 30 mL NaOH solution and 5 mL EDTA solution, diluted with deionized water to a final volume of 1 L. This buffer was applied to the slides to maintain conductivity and pH during electrophoresis. The slides were run in a dark room to minimize background light, enabling clearer visualization of DNA migration. Damaged DNA fragments moved away from the nucleoids, forming characteristic comet tails. After electrophoresis, slides were neutralized with Tris buffer (pH 7.4), fixed briefly in cold ethanol, and stored at −20 °C for preservation. Staining was performed with Giemsa to enhance contrast, allowing comet structures to be observed under a compound light microscope. DNA damage was quantified by assessing the extent of migration from the nucleus, and images were captured using ImageJ software. At least 50 comets per slide were recorded, ensuring that a minimum of 100 comets were analyzed for each sample.

Scoring and statistical analysis: Comet images were analyzed using ImageJ v1.3.1 software. Data were exported to Excel, cleaned, and subjected to statistical analysis. Two-way ANOVA was performed using GraphPad Prism 8.0.2, followed by Tukey's multiple comparison test to determine significant differences among treatment groups. Probit analysis, a regression-based statistical method, was employed to estimate the LC_{50} values on SPSS 22 for windows 10/11. This approach linearizes the sigmoidal dose–response curve, enabling regression analysis of binomial response variables.

Results

Acute toxicity test: The mortality responses after a 96-hour exposure to "Roundup" are shown in Tables 1 and 2.

Table 1: Results showing the mortality rate of *Clarias garienpinus* in different concentrations of "Roundup" for Exposure 1

Conc (mL/L	Mortality					Number of Mortality	Percentage Mortality (%)
	12 hours	24 hours	48 hours	72 hours	96 hours		
Control	0	0	0	0	0	0/10	0
0.03	0	0	1	2	1	4/10	40
0.06	0	0	1	1	3	5/10	50
0.10	0	1	1	2	4	8/10	80
0.13	1	1	3	2	3	10/10	100

Table 2: Results showing mortality rate of *Clarias garienpinus* in different concentrations of "Roundup" for Exposure 2.

CONC. (mL/L)	Mortality					Number of Mortality	Percentage Mortality (%)
	12 hours	24 hours	48 hours	72 hours	96 hours		
Control	0	0	0	0	0	0/10	0
0.03	0	1	1	1	2	5/10	50
0.06	0	0	2	1	4	7/10	70
0.10	1	1	2	2	3	9/10	90
0.13	0	1	3	4	2	10/10	100

Dose-response relationship of Roundup in Clarias gariepinus: To establish the toxicological profile of Roundup in *Clarias gariepinus*, probit analysis was employed to determine the median lethal concentration (LC_{50}). The analysis revealed LC_{50} values of 0.04 mL/L in the first exposure and 0.03 mL/L in the second exposure. The mean LC_{50} at 96 hours was estimated at 0.04 mL/L, with confidence limits ranging from 0.01 mL/L to 0.05 mL/L. Regression equations derived from the probit model were $Y = 3.872 + 2.859x$ ($R^2 = 0.873$) for experiment 1 and $Y = 4.304 + 2.904x$ ($R^2 = 0.968$) for experiment 2, where Y represents probit kill. These regression outputs demonstrate strong linear associations between Roundup concentration and fish mortality, confirming the robustness and reliability of the dose–response relationship observed in this study.

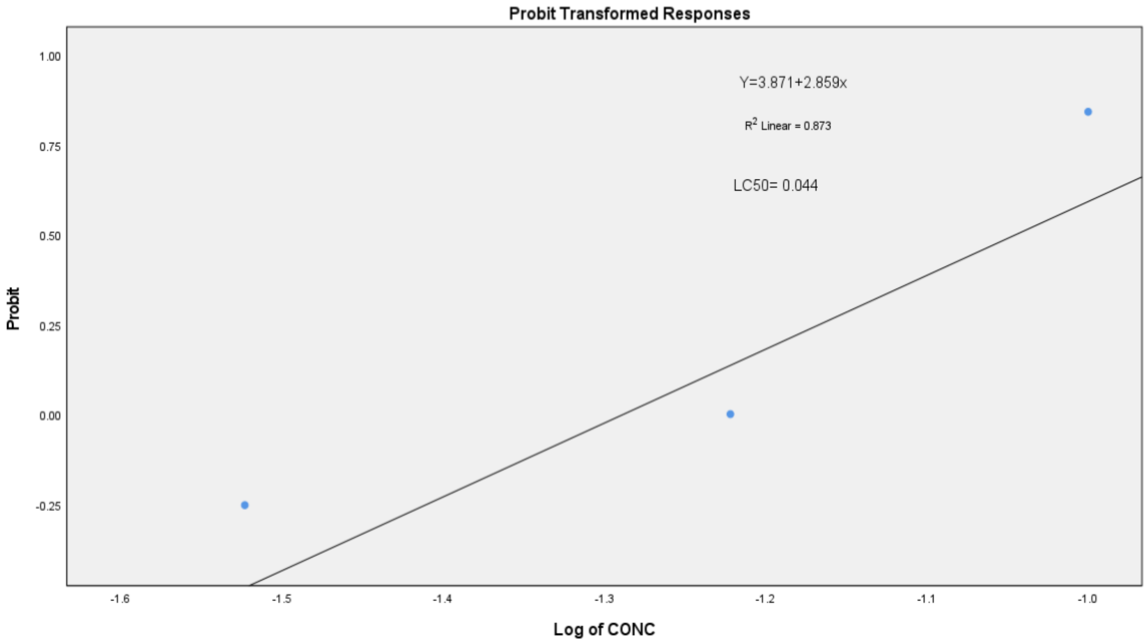


Figure 1: Linear relationship between probit mortality and log concentration of *Clarias gariepinus* juveniles exposed to “Roundup” for 96 hours in Exposure 1.

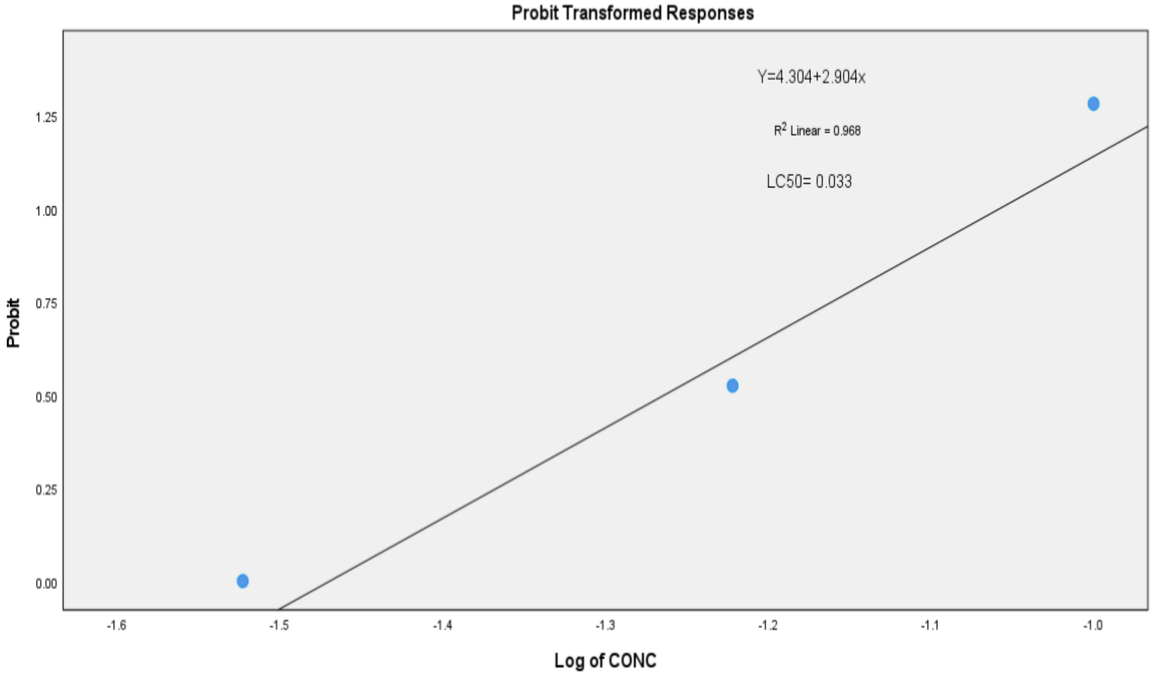
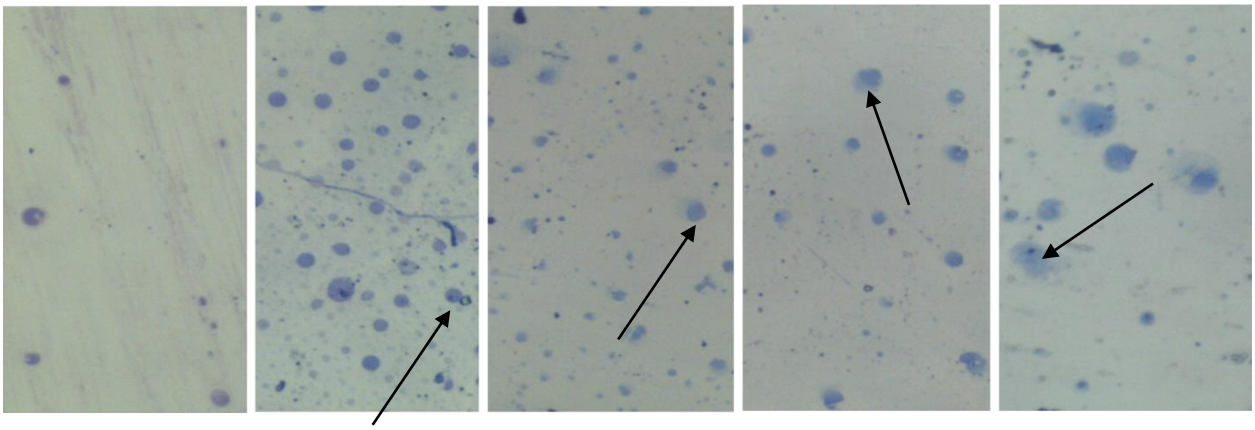


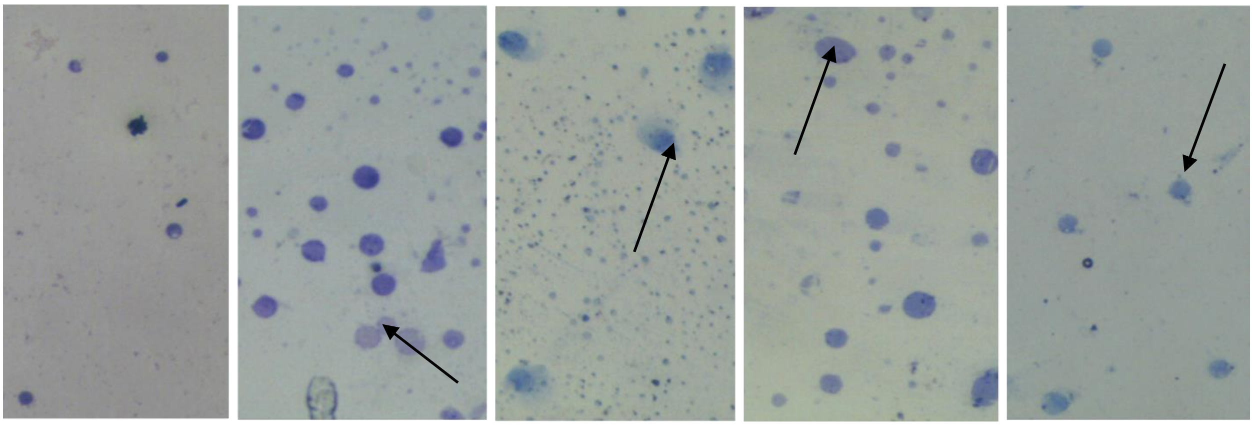
Figure 2: Linear relationship between probit mortality and log concentration of *Clarias gariepinus* juveniles exposed to “Roundup” for 96 hours in Exposure 2.

DNA damage assessment in Clarias gariepinus: Results from the Comet assay evaluation demonstrated that exposure to Roundup induced markedly higher levels of DNA damage in the gills, gonads, and liver of *Clarias gariepinus* compared with the negative control, as shown in Figures 2 and 3.



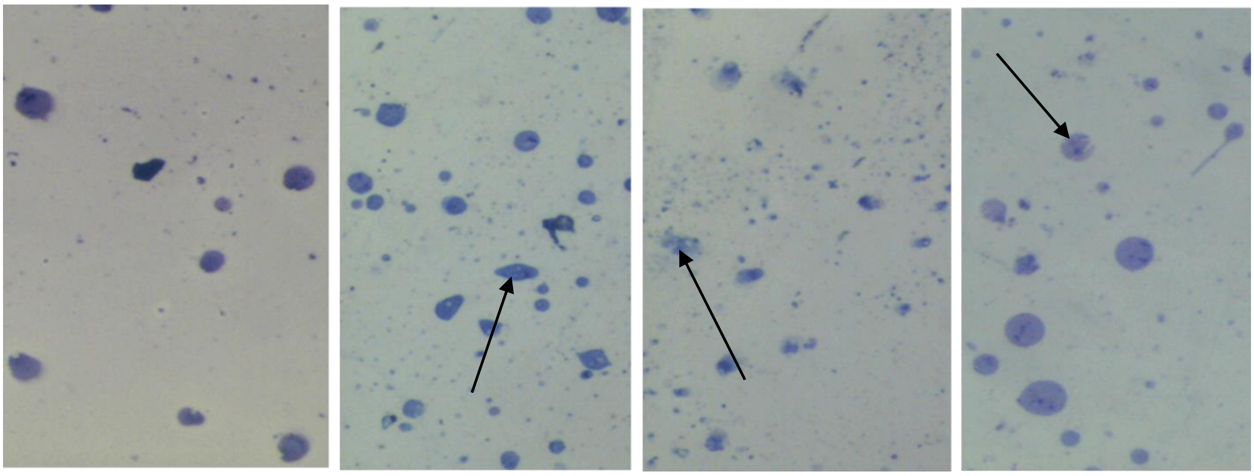
Control **0.03 mL/L** **0.06 mL/L** **0.10 mL/L** **0.13 mL/L**

Plate 1: Micrographs of DNA damage showed by gills of *Clarias garienpinus* exposed to "Roundup" at concentrations 0 mL/L (Control), 0.03 mL/L, 0.06 mL/L, 0.10 mL/L, and 0.13 mL/L. Arrows indicate comets formed.



Control **0.03 mL/L** **0.06 mL/L** **0.10 mL/L** **0.13 mL/L**

Plate 2: Micrographs of DNA damage in the gonads of *Clarias garienpinus* exposed to "Roundup" at concentrations 0 mL/L (Control), 0.03 mL/L, 0.06 mL/L, 0.10 mL/L, and 0.13 mL/L. Arrows indicate the comet formation.



Control **0.03 mL/L** **0.06 mL/L** **0.13 mL/L**

Plate 3: Micrographs of DNA damage showed by liver of *Clarias garienpinus* exposed to "Roundup" at concentrations 0 mL/L (control), 0.03 mL/L, 0.06 mL/L, and 0.13 mL/L. Arrows indicate comets formed.

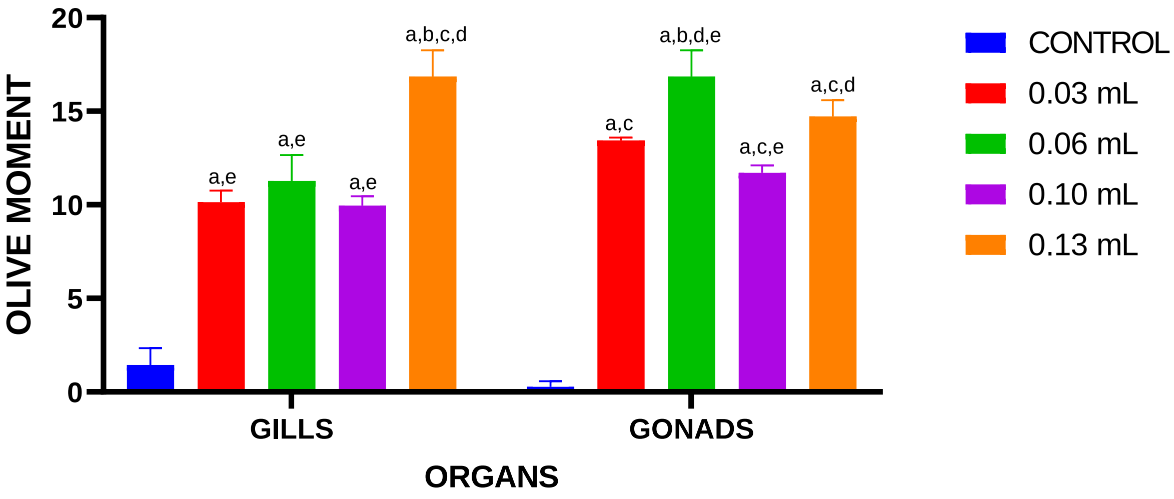


Figure 3: Effects of "Roundup" on the gills and gonads of *C. garienpinus. Each bar represents the mean ± SEM (n=4). SEM- Standard error of the mean. a, b, c, d, e- Significance as compared with control; p < 0.01*

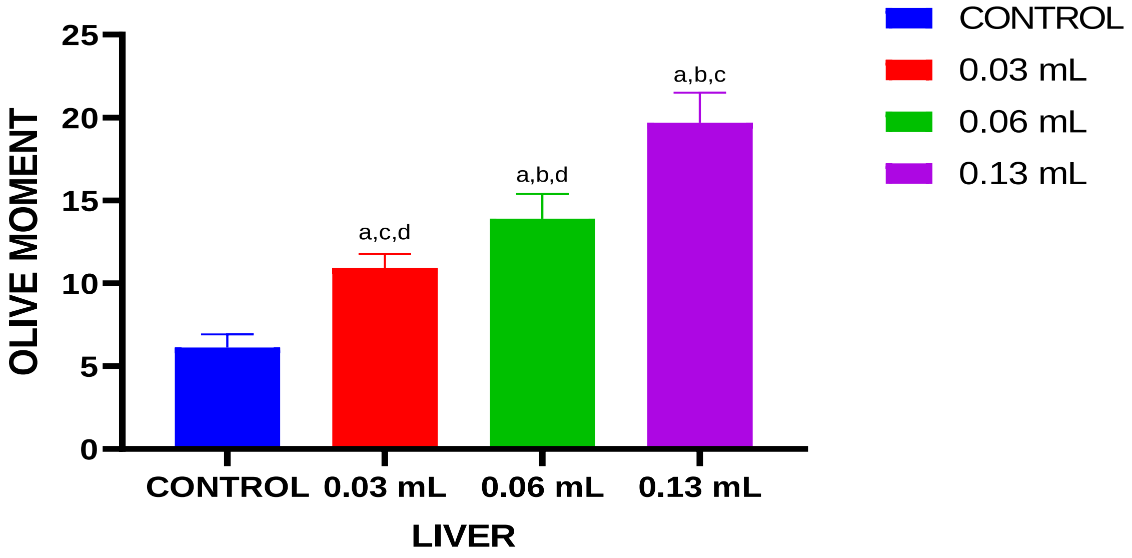


Figure 4: Effects of "Roundup" on the liver of *Clarias garienpinus*. *Each bar represents the mean ± SEM (n=4) SEM- Standard error of Mean. a, b, c, d, e- Significance as compared with control; p<0.01*

Discussion

This study investigated the acute toxicity and genotoxic effects of glyphosate-based herbicide ("Roundup") at very low concentrations on the gills, gonads, and liver of *Clarias gariepinus*. Glyphosate has historically been considered relatively safe because it was believed to act specifically on the shikimate pathway (an enzyme system absent in mammals) and to degrade rapidly into carbon dioxide (CO_2). Furthermore, additives in commercial formulations were often labeled as "inert," thereby minimizing perceived risks (Ahuja *et al.*, 2024). However, recent evidence has challenged these assumptions, demonstrating that glyphosate and glyphosate-based herbicides (GBHs) exert toxicological effects across multiple taxa, including aquatic organisms (Ojelade *et al.*, 2022).

In the present study, fish exposed to "Roundup" exhibited abnormal behavioral responses such as erratic swimming, bubble release from the Mouth, discoloration, restlessness, and gasping for air. These behavioral alterations were absent in the control group, which displayed normal activity. Such behavioral endpoints are consistent with previous findings by Ajibare and Ayeku (2024), who reported similar stress responses in fish exposed to glyphosate formulations. Likewise, Ofor *et al.* (2025) documented comparable behavioral disruptions in *Tilapia zillii* exposed to “Forceup,” another glyphosate-based herbicide. Erratic swimming and respiratory distress are indicative of systemic toxicity, likely linked to disruptions in metabolic and physiological processes (Chukwuka *et al*., 2022).

Mortality data further confirmed the acute toxicity of “Roundup”. In exposure 1, mortality rates increased progressively with concentration, ranging from 40% at 0.03 mL/L to 100% at 0.13 mL/L after 96 hours (Table 1). Experiment 2 showed slightly higher mortality, with 50% at 0.03 mL/L and complete mortality at 0.13 mL/L (Table 2). These findings demonstrate a clear concentration-dependent lethality, with significant mortality observed even at the lowest tested dose. The LC_{50} values obtained (0.03–0.04 mL/L) highlight the sensitivity of *C. gariepinus* to glyphosate-based herbicides and confirm that “Roundup” poses a substantial risk to aquatic organisms at environmentally relevant concentrations. The observation of 100% mortality at 0.13 mL/L underscores the potency of glyphosate formulation, aligning with broader toxicological evidence that GBHs are not biologically inert but instead exert profound effects on aquatic biota.

Probit analysis revealed that the median lethal concentration (LC_{50}) of “Roundup” for *Clarias gariepinus* was 0.04 mL/L in exposure 1 and 0.03 mL/L in exposure 2. The mean LC_{50} at 96 hours was calculated as 0.04 mL/L, with confidence limits ranging between 0.01 mL/L and 0.05 mL/L. The regression equations derived from the probit analysis were: experiment 1, $Y = 3.872 + 2.859x$ ($R^2 = 0.873$), and experiment 2, $Y = 4.304 + 2.904x$ ($R^2 = 0.968$), where Y represents probit kill. These regression values indicate strong linear relationships between concentration and mortality, confirming the reliability of the dose–response model. The exposure of fish to *Roundup* demonstrated a clear linear toxicity pattern (Figures 1 and 2), with mortality increasing proportionally to herbicide concentration. At 0.04 mL/L, approximately 50% of the fish population was predicted to die within 96 hours, establishing this concentration as a critical threshold for population survival. Such findings highlight the ecological significance of glyphosate contamination in aquatic environments, as even relatively low concentrations can compromise population fitness. The implications of these results extend beyond acute toxicity. At concentrations near the LC_{50}, fish populations would experience significant reductions in survival, thereby affecting recruitment, reproductive success, and long-term sustainability. As mortality continues to rise with increasing concentrations, the resilience of fish populations in contaminated ecosystems would be severely diminished. This observation is consistent with previous ecotoxicological studies, which emphasize that glyphosate-based herbicides pose substantial risks to aquatic biodiversity and ecosystem stability (Cunillera-Montcusí *et al*., 2022).

It has been demonstrated that the comet assay (Single-cell Gel Electrophoresis) test is a useful technique for determining the relationship between DNA damage and aquatic animals' exposure to genotoxic pollutants (Jiang, *et al*., 2023). The comet assay test was used to analyze the level of DNA damage (if any) in the tissues extracted from the gills, gonads, and liver of *clarias garienpinus* induced by different concentrations of “Roundup”.

The gills of fish represent one of the most vulnerable organs to environmental pollutants due to their continuous and direct contact with waterborne contaminants (Xu *et al*., 2020). In the present study, comet assay analysis revealed significantly higher levels of DNA damage in the gills of *Clarias gariepinus* exposed to *Roundup* compared with the negative control (Plate 1). This observation is consistent with the findings of Noor and Rahman (2025), who reported increased DNA damage in the gill cells of *Poecilia reticulata* following exposure to glyphosate-based herbicides.

Comparative analysis of DNA damage across exposure concentrations in the gills, revealed that the 0.03 mL/L group did not differ significantly ($p < 0.01$) from the 0.06 mL/L and 0.10 mL/L groups, while the 0.06 mL/L group was likewise not significantly different from the 0.10 mL/L group. Notably, the 0.13 mL/L treatment produced the highest level of DNA damage, followed by 0.06 mL/L, 0.03 mL/L, and 0.10 mL/L (Figure 3). These findings indicate that Roundup-induced genotoxicity in gill tissues did not conform to a strictly dose-dependent pattern, underscoring the complex nature of DNA damage responses in aquatic organisms. However, there were significant differences between all the treatment groups when compared with the control group.

Gonadal tissues, which are regulated by steroid hormones essential for spermatogenesis and oogenesis (Li *et al*., 2021), also exhibited elevated DNA damage in exposed groups compared with the control (Plate 2). Statistical evaluation showed that the 0.03 mL/L group was not significantly different ($p < 0.01$) from the 0.10 mL/L and 0.13 mL/L groups. Among all treatments, the 0.06 mL/L group displayed the greatest DNA damage, followed by 0.13 mL/L, 0.03 mL/L, and 0.10 mL/L (Figure 3). Similar to the gills, these results suggest that DNA damage in gonadal tissues was not strictly dose-dependent.

Previous research has documented pesticide-induced genotoxicity in fish reproductive organs, such as diazinon-related damage (Barbosa *et al*., 2025). However, relatively few studies have examined glyphosate’s specific impact on fish reproductive systems (Barbhuiya and Baruah, 2025). The present findings therefore provide important evidence that glyphosate-based herbicides compromise reproductive integrity in fish, with potential consequences for population sustainability and ecosystem health.

The liver is a central organ in xenobiotic metabolism and detoxification, making it highly susceptible to chemical insults (Song *et al*., 2023). Pesticides, including glyphosate-based herbicides, undergo biotransformation in hepatic tissue, where they can generate reactive metabolites that contribute to oxidative stress, lipid peroxidation, and genotoxicity (Jin *et al*., 2014). In the present study, comet assay analysis revealed statistically significant DNA damage in the liver of *Clarias gariepinus* across all concentrations of *Roundup* compared with the negative control, underscoring the vulnerability of hepatic tissue to glyphosate-induced toxicity. These findings are consistent with Gupta and Verma (2022), who reported DNA strand breaks in the hepatic tissue of *Corydoras paleatus* following short-term exposure to environmentally relevant concentrations of *Roundup*. Similarly, Ayanda *et al*. (2021) demonstrated comparable genotoxic responses in fish exposed to glyphosate, even in the absence of commercial adjuvants, suggesting that both the active ingredient and formulation additives contribute to hepatotoxicity. Comparable studies in other teleosts, such as *Oreochromis niloticus* and *Danio rerio*, have also documented glyphosate-induced oxidative stress and DNA damage, reinforcing the generalizability of these findings across species (e.g., de Souza *et al*., 2019; Lushchak *et al*., 2021).

The dose-dependent relationship observed in this study further supports the mechanistic link between glyphosate exposure and hepatic genotoxicity. Higher concentrations of *Roundup* were associated with increased DNA fragmentation, indicating that the extent of damage correlates with exposure intensity. This pattern aligns with broader ecotoxicological evidence that pesticide-induced genotoxicity is concentration-dependent, with potential consequences for liver function, metabolic regulation, and overall organismal health (Cunillera-Montcusí *et al*., 2022).

Taken together, these results highlight the hepatotoxic potential of glyphosate-based herbicides in aquatic organisms. The consistency of findings across multiple studies suggests that glyphosate exposure compromises hepatic genomic integrity, thereby impairing detoxification processes and increasing susceptibility to secondary stressors (Wang *et al*., 2022; Qi *et al*., 2023). Given the ecological importance of fish as both bioindicators and integral components of aquatic food webs, these findings underscore the need for stricter regulation of glyphosate formulations to mitigate risks to aquatic ecosystems and public health.

Conclusion

This study demonstrates that *Roundup* exerts significant toxicological effects on *Clarias gariepinus*, even at very low concentrations. The herbicide induced DNA damage in critical organs, including the gills, gonads, and liver, while also causing high mortality rates. These findings highlight the dual threat of acute toxicity and genotoxicity, confirming that glyphosate-based herbicides compromise both organismal survival and genomic integrity. Given the ecological role of fish at the base of aquatic food chains, such toxic effects have broader implications for ecosystem stability and food safety. The results underscore the importance of stricter regulation and monitoring of glyphosate use, as well as the need to explore safer alternatives to protect aquatic biodiversity and human health.

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