



Effect of Ascorbic Acid on Glyphosate-Induced Residues in Muscles of Juvenile Catfish (*Clarias gariepinus*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author CFI designed the study, executed the project and prepared the initial manuscript. Author OOI reviewed the manuscript, was responsible for the accuracy and integrity of data including the statistical analysis. Author TOO coordinated the study, read and corrected the manuscript. Author BEO contributed to the study design, ratified the proposal, read the manuscript. All authors read and approved the final manuscript submitted for publication.

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ABSTRACT

Aims: This study focused on acute toxicity of Glyphosate and its residual effect on muscles of juvenile *Clarias gariepinus* fish, as well as on L-ascorbic acid (vitamin C) treatment of induced glyphosate residues in muscles of the fish to prevent bioaccumulation of glyphosate and subsequent toxicity when consumed by humans.

Study Design: Latin square.

Place and Duration of Study: Department of Fisheries and Aquaculture Management, Nnamdi Azikiwe University Awka, Nigeria, between December 2018 and April 2019.

Methodology: Forty-eight hours acute toxicity tests were initially carried out on eight juveniles of *C. gariepinus* of mean weight 41.50 ± 1.35 g and length 20.75 ± 0.43 cm to determine LC50 of both

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glyphosate (Delsate®) and Vitamin C (Kepro®). Thereafter the fish were exposed for 91 days to sub-lethal concentrations of 0.0 (control), 5.0, 10.0 and 15mgL⁻¹ glyphosate; and later treated separately with 50 and 100 mgL⁻¹ of vitamin C for 7 days. A second set of fish was exposed to glyphosate and vitamin C concurrently for 91 days. Glyphosate residues in the muscles of the fish were determined by gas chromatography.

Results: The LC₅₀ of Delsate® and Kepro® were 75 and 175 mgL⁻¹ respectively. There was significant increase ($P < 0.05$) in mean values of glyphosate retained in muscles of fish exposed to glyphosate for 91 days when compared with the control. Seven days post-exposure treatment with vitamin C resulted in significant decrease ($P < 0.05$) in glyphosate residues in fish muscles; with 100mgL⁻¹ of vitamin C being more effective than the 50 mgL⁻¹. The second set-up did not show significant therapeutic effects on the muscles of *C. gariepinus*.

Conclusion: This work has determined the LC₅₀ of glyphosate and Vitamin C. Glyphosate residues in muscles of exposed fish could be rendered safe for human consumption by treatment of exposed fish in water bath containing with Vitamin C. Our on-going research is on histopathological alterations in tissues of glyphosate-exposed *C. gariepinus* juvenile fishes.

Keywords: Glyphosate; bioaccumulation; *clarias gariepinus*; Vitamin C treatment.

1. INTRODUCTION

Water pollution with herbicides is a subject of great concern because of the threat to safe water supply and damage to aquatic life, especially fishes [1]. Aquatic animals in polluted waters tend to accumulate many chemicals in high concentrations [2,3,4,5,6] which is a potentially hazardous situation for the entire food chain [7]. Glyphosate-based herbicides are approved for plant protection and sold in Nigeria with different trade names such as Delsate, Dizensate, Clearweed, Sarosate liquid, Sunate, Roundup liquid, and Glystate [8]. According to [9], glyphosate patented as an antimicrobial and biocide is also a chelator, endocrine-disrupting chemical (EDC), and a class two carcinogen. Several glyphosate based products have been used for research and these have deleterious effect on fish and aquatic animals [9].

The African catfish, *C. gariepinus* is a remarkable fish species in Nigeria where it is a successful aquaculture species and of high consumption demand [10]. Nigeria has been reported as the largest producer of catfish [11] and *C. gariepinus* is commonly found in the Nigerian inland waters [12]. Contamination of inland waters by pesticides can lead to fish morbidity and mortality, reduced fish productivity and elevated concentration of undesirable chemicals in edible fish tissue which can affect public health [13]. Many authors [4,14,15,16,17,18,19 and 20] have reported potential health risks from consumption of contaminated fisheries [21] and aquaculture products.

The presence of chemical anthropogenic stressors like glyphosate herbicides in the water

can alter physiological endpoints critical to maintaining normal functions and cause adverse effects ranging from the cellular to the population level [22]. Occupying higher trophic levels, fish are also susceptible to indirect effects via contaminated food sources such as algae, invertebrates or other prey fish species [23].

Vitamin C is a reducing agent and antioxidant which typically reacts with oxidants of the reactive oxygen species which are detrimental to animals and plants at the molecular level due to their possible reaction with nucleic acids, proteins and lipids. It has been reported [24] that high levels of vitamin C are efficient in reducing toxicity, preventing disease that enhancing fish tolerance to environmental stress. Due to the lack of gulonolactone oxidase, an enzyme responsible for the synthesis of vitamin C in teleost fishes, it becomes necessary to supplement vitamin C in the diet or water of fish for optimum performance [25].

The effects of glyphosate-based herbicide formulations have been studied in a wide variety of aquatic organisms including fish using standard toxicity bioassays [22,26 and 27] which allow the determination under specific and controlled conditions, the physical and or the chemical effects of a substance on a test organism. A classic study to characterize the potential toxicity of such substance is the acute lethal toxicity test in which the lethal concentration (LC₅₀) value is determined. This is the concentration of a substance that causes the death of 50% population of the test organisms in stipulated time which could be 24, 48, or 96 hours. The present study focused on acute toxicity of Glyphosate and its residual effect on

muscles of juvenile *C. gariepinus* fish, as well as on the use of L-ascorbic acid (vitamin C) in the treatment of glyphosate-residues in fish muscles to ensure its safety for human consumption.

2. MATERIAL AND METHODS

2.1 Study Juvenile Fish

The study was carried out with 300 juveniles of *C. gariepinus*, of mean weight 41.50 ± 1.35 g and length 20.75 ± 0.43 cm, procured from a farm in Ibadan and transported to Onitsha in a 50L capacity cylinder containing 30L of borehole water. The juveniles were acclimated in an 32 cm x 50 cm x 33 cm aquarium and fed with 3mm Skretting fish pellets at 3% biomass half at 9.00am and 5.00pm daily for 14 days.

2.2 Study Chemicals

The glyphosate preparation used in the study was Delsate® manufactured in India for CANDEL Company Limited Ikoyi Lagos Nigeria, while the ascorbic acid was Kepro® vitamin C manufactured by Kepro B.V. Holland for Kepro Nigeria Limited Lagos Nigeria.

2.2.1 Determination of Lethal Concentration (LC₅₀) of glyphosate and vitamin c

Determination of the mean lethal concentrations (LC₅₀) of Delsate® and Kepro vitamin C in juvenile *Clarias gariepinus* fish were carried out according [28]. Acute toxicity of glyphosate in 4 juveniles (mean weight 43.20 ± 1.41 g, and mean length 20.75 ± 1.96 cm) was determined as shown in Table 1 while that of Vitamin C in another 4 *C. gariepinus* juveniles (mean weight 39.8 ± 1.51 g, and mean length 20.10 ± 1.41 cm) was determined as shown in Table 2.

$$\text{Lethal dose} = \text{LC}_{50} = \frac{[M_0 + M_1]}{2},$$

where

MO = highest dose of test substance that recorded no mortality, and

M₁ = lowest dose of test substance that recorded mortality [28].

$$\text{LC}_{50} \text{ of Delsate}^{\circledR} = \frac{[100+50]}{2} = \frac{150}{2} = 75\text{mgL}^{-1}$$

Therefore Sub-lethal concentrations of 0.0, 5, 10, and 15 mgL⁻¹ of glyphosate were respectively

chosen for the chronic toxicity studies of on the *C. gariepinus*.

$$\text{Lethal dose} = \text{LC}_{50} = \frac{[M_0 + M_1]}{2},$$

MO = highest dose of test substance that recorded no mortality, and

M₁ = lowest dose of test substance that recorded mortality [28].

$$\text{LC}_{50} \text{ of Vitamin C (Kepro}^{\circledR}) = \frac{[200+150]}{2} = \frac{350}{2} = 175\text{mgL}^{-1}$$

Therefore doses of 50 and 100mgL⁻¹ Kepro® were respectively used for the assessment of therapeutic effects of vitamin C on the pathology induced by glyphosate on *C. gariepinus* after chronic exposure, for 91 days.

2.2.2 Toxicity bioassays of Delsate® and Kepro® in *C. gariepinus*

In Latin Square Design adopted for this study, 300 juveniles of the catfish (*C. gariepinus*) were initially divided into 3 major experimental groups (Table 3) GP1 (n= 12), GP2 (n=144), and GP3 (n=144).

Juveniles in GP1 were sub-divided into 4 experimental groups A1, B1, C1, and D1 with each having 3 juveniles (Table 4). Group A1 acted as control and was not exposed to glyphosate. Groups B1, C1, and D1 were respectively exposed to 5, 10, and 15.mgL⁻¹ Delsate in 3 replicates of 1 per replicate at Day 0. Muscle samples were excised from muscles ventral to the dorsal midline of the juveniles and the levels of glyphosate residues in them were determined by gas chromatography.

Juveniles in GP2 were sub-divided into 4 experimental groups A2, B2, C2, and D2, with 36 juveniles in each (Table 5). Group A2 was set up as Control while groups B2, C2 and D2 were respectively exposed to 5, 10 and 15mgL⁻¹ of Delsate® for 91days in 3 replicates of 12 per replicate.

At the end of 91 days glyphosate exposure, juveniles in GP2 were divided into 2 sub-groups GPA and GPB with 72 juveniles in each (Table 6). The sub-groups A, B, C, and D were respectively divided into (A₁, B₁, C₁, D₁) and (A₂, B₂, C₂, D₂) with 18 juveniles in each. Muscle samples were excised from muscles ventral to

the dorsal midline of juveniles from A₁, B₁, C₁, and D₁ in GPA. The levels of glyphosate residues in muscles were determined by gas chromatography.

The 72 juveniles in GPB were then sub-divided into 2 groups GB1 and GB2 with 36 juveniles in each (Table 7). The groups in GB1 were A5, B5, C5, and D5 with 9 juveniles in each, and were respectively treated with 50mgL⁻¹ of vitamin C for 7 days, in 3 replicates, at 3 per replicate. The components of GB2 were A6, B6, C6, and D6 with 9 juveniles in each, and were similarly treated with 100mgL⁻¹ of vitamin C for 7 days, in 3 replicates of 3 per replicate. At the end of the 7 days post-exposure treatments with vitamin C, muscle samples from juveniles of both groups were tested for levels of glyphosate residues.

The 144 juveniles in the major group GP3 (see Table 3) were divided into 2 sub-groups GP3A and GP3B with 72 juveniles in each (Table 8). Juveniles in GP3A were divided into A7, B7, C7, and D7 with 18 juveniles in each, and were respectively and concurrently exposed to 0, 5, 10 and 15mgL⁻¹ of glyphosate and 50mgL⁻¹ of vitamin C in 3 replicates of 6 per replicate for 91 days. The other 72 juveniles in GP3B were also divided into A8, B8, C8, and D8 with 18 juveniles in each, and were respectively and concurrently exposed to 0, 5, 10 and 15mgL⁻¹ of glyphosate and 100mgL⁻¹ of vitamin C in 3 replicates of 6 per replicate for 91 days. Experiments with GP3A were inconclusive due to circumstances beyond human control. Only glyphosate residues in muscles of *C. gariepinus* juveniles in GP3B were therefore determined by gas chromatography.

Table 1. Determination of LC₅₀ of Delsate[®] in *Clarias gariepinus* juveniles

| Tank | Dose (mgL ⁻¹) | No. of fish | Observation after 48 hours |
|------|---------------------------|-------------|----------------------------|
| 1 | 10 | 1 | Alive |
| 2 | 50 | 1 | Alive |
| 3 | 100 | 1 | Dead |
| 4 | 300 | 1 | Dead |

Table 2. Acute toxicity of vitamin C (Kepro[®]) in *Clarias gariepinus* juveniles

| Tank | Dose (mgL ⁻¹) | No. of fish | Observation after 48 hours |
|------|---------------------------|-------------|----------------------------|
| 1 | 100 | 1 | Alive |
| 2 | 150 | 1 | Alive |
| 3 | 200 | 1 | Dead |
| 4 | 200 | 1 | Dead |

Table 3. Major experimental groups of juvenile catfish *Clarias gariepinus* studied

| The major Experimental groups of juvenile catfish <i>C. gariepinus</i> | | | | Total number of juveniles |
|--|---------------|---------------|---------------|---------------------------|
| Major groups | Group 1 (GP1) | Group 2 (GP2) | Group 3 (GP3) | |
| Number of juveniles | 12 | 144 | 144 | 300 |

Table 4. Experimental groups exposed to varying concentrations of glyphosate at D₀

| Group GP1 → | A1 | B1 | C1 | D1 |
|------------------|------------------------------|----------------------------|-----------------------------|-----------------------------|
| Day 0 exposure → | 0.0mgL ⁻¹ Delsate | 5mgL ⁻¹ Delsate | 10mgL ⁻¹ Delsate | 15mgL ⁻¹ Delsate |
| Replicate 1 | 1 juveniles | 1 juveniles | 1 juveniles | 1 juveniles |
| Replicate 2 | 1 juveniles | 1 juveniles | 1 juveniles | 1 juveniles |
| Replicate 3 | 1 juveniles | 1 juveniles | 1 juveniles | 1 juveniles |
| Total (n= 12) | 3 | 3 | 3 | 3 |

Table 5. Experimental groups exposed to varying concentrations of glyphosate for 91 days

| Group GP2 → | A2 | B2 | C2 | D2 |
|--------------------|------------------------------|----------------------------|-----------------------------|-----------------------------|
| 91 days exposure → | 0.0mgL ⁻¹ Delsate | 5mgL ⁻¹ Delsate | 10mgL ⁻¹ Delsate | 15mgL ⁻¹ Delsate |
| Replicate 1 | 12 juveniles | 12 juveniles | 12 juveniles | 12 juveniles |
| Replicate 2 | 12 juveniles | 12 juveniles | 12 juveniles | 12 juveniles |
| Replicate 3 | 12 juveniles | 12 juveniles | 12 juveniles | 12 juveniles |
| Total (n= 144) | 36 | 36 | 36 | 36 |

Table 6. Experimental groups for the determination of glyphosate-induced residue in muscles

| GP2 | A2 | B2 | C2 | D2 | Total juveniles |
|-----------------|-----------|-----------|-----------|-----------|-----------------|
| GPA → | A3 (n=18) | B3 (n=18) | C3 (n=18) | D3 (n=18) | 72 |
| GPB → | A4 (n=18) | B4 (n=18) | C4 (n=18) | D4 (n=18) | 72 |
| Total juveniles | 36 | 36 | 36 | 36 | 144 |

Table 7. Groups treated with vitamin C for 7 days after 91 days exposure to glyphosate

| GB1 | 7 days treatment with 50mgL ⁻¹ of Vit. C | A5 | B5 | C5 | D5 |
|--|--|-----------------------|---------------------|---------------------|---------------------|
| | Replicate 1 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Replicate 2 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Replicate 3 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Total no. of juveniles (n= 36) | 9 | 9 | 9 | 9 |
| GB2 | 7 days treatment with 100mgL ⁻¹ of Vit. C | A6 | B6 | C6 | D6 |
| | Replicate 1 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Replicate 2 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Replicate 3 | 3 juveniles | 3 juveniles | 3 juveniles | 3 juveniles |
| | Total no. of juveniles (n= 36) | 9 | 9 | 9 | 9 |
| Concentrations of glyphosate used for exposure | | 0.0 mgL ⁻¹ | 15mgL ⁻¹ | 10mgL ⁻¹ | 15mgL ⁻¹ |

Table 8. Experimental groups of *C. gariepinus* concurrently exposed to varying concentrations of glyphosate and vitamin C for 91 days

| GP3A | 91 days concurrent treatment with Glyphosate and 50mgL ⁻¹ of Vitamin C | A7 | B7 | C7 | D7 |
|--|--|----------------------|---------------------|---------------------|---------------------|
| | Replicate 1 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Replicate 2 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Replicate 3 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Total number of juveniles (n= 72) | 18 | 18 | 18 | 18 |
| GP3B | 91 days concurrent treatment with Glyphosate and 100mgL ⁻¹ of Vitamin C | A8 | B8 | C8 | D8 |
| | Replicate 1 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Replicate 2 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Replicate 3 | 6 juveniles | 6 juveniles | 6 juveniles | 6 juveniles |
| | Total number of juveniles (n= 72) | 18 | 18 | 18 | 18 |
| Concentrations of glyphosate used for exposure | | 0.0mgL ⁻¹ | 15mgL ⁻¹ | 10mgL ⁻¹ | 15mgL ⁻¹ |

During the experiments, water was renewed twice weekly to maintain glyphosate and vitamin C strengths. Results were presented as means and standard error of the mean. Error bars in bar charts produced with Microsoft Excel version 2010 also revealed significant differences ($P < .05$) among the variables.

3. RESULTS

The LC₅₀ of glyphosate and vitamin C in *C. gariepinus* had been determined as 75mgL⁻¹ and 175 mgL⁻¹ respectively. Mean values of glyphosate residues in muscles of *C. gariepinus* were shown in Table 9.

It would be clearly observed in Fig. 1. that in GP1 (Day 0) there was no significant difference ($P < .05$) in mean glyphosate but in GP2A (91 days

exposure to Glyphosate) there was highly significant increase ($P > .05$) in the mean values of glyphosate retained in the muscles of *C. gariepinus* after 91 days exposure with varying concentrations of glyphosate in groups B, C and D when compared with group A in a dose-dependent manner. Also in GP2A (91 days exposure) the highest concentration of glyphosate was retained in muscles of fish in group 1D and the least significant differences of means showed that glyphosate was significantly deposited ($P > .05$) in the muscles of the fish in groups B, C and D when compared to A, the baseline.

It could be observed that 7 days after 91days exposure to glyphosate, there were significant reductions ($P > .05$) of glyphosate concentrations in muscle of the fishes in GB1 treated with

Table 9. Means and SE of glyphosate ($\mu\text{g mL}^{-1}$) detected in muscles of *Clarias gariepinus* juveniles from groups exposed to glyphosate only; groups exposed to glyphosate and later treated with vitamin C; and groups concurrently exposed to glyphosate and vitamin C

| | GP1(n=12) | | GP2 (n=144) | | GP3 (n=144) | |
|------------------------------------|---|---|---|--|---|--|
| | | GP2A | GP2B | | GP3A | GP3B |
| | | | GB1 | GB2 | | |
| Glyphosate (mg L^{-1})↓ | 12 juveniles exposed to glyphosate at Day 0 | 72 juveniles exposed to glyphosate for 91days | 36 juveniles exposed to glyphosate for 91days followed by 7days treatment with 50mg L^{-1} vitamin C | 36 juveniles exposed to glyphosate for 91days followed by 7days treatment with 100mg L^{-1} vitamin C | 72 juveniles Concurrently exposed to glyphosate and 50mg L^{-1} vitamin C for 91days | 72 juveniles Concurrently exposed to glyphosate and 100mg L^{-1} vitamin C for 91days |
| A = 0.0 | 1.034±1.1 ^a | 1.129±1.1 ^a | 0.900±2.48 ^a | 0.732±0.29 ^a | No result due to natural hazard | 1.034±0.94 ^a |
| B = 5.0 | 1.100±0.7 ^a | 36.288±0.18 ^b | 5.432±1.06 ^b | 0.751±0.19 ^a | | 27.441±1.41 ^b |
| C = 10.0 | 0.984±0.0 ^a | 39.421±0.07 ^c | 7.561±0.35 ^b | 1.129±0.03 ^a | | 28.311±0.71 ^b |
| D = 15.0 | 1.134±0.0 ^a | 42.664±0.71 ^c | 9.545±0.42 ^b | 1.779±0.01 ^a | | 30.247±1.96 ^c |

Values with same superscripts in same column were not significantly different.

Values with different superscripts in same column were significantly different

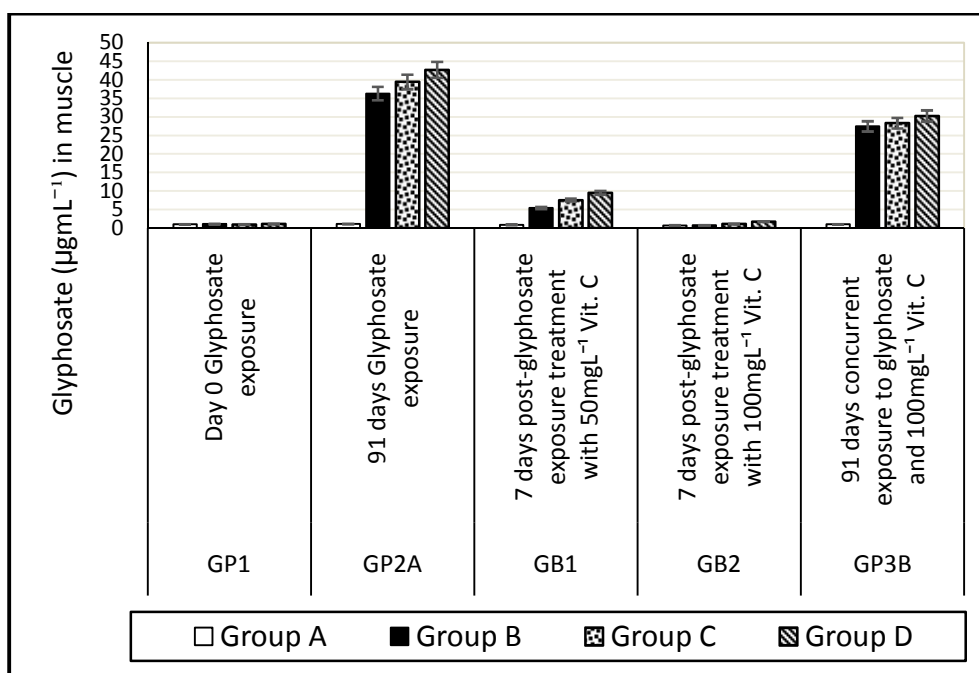


Fig. 1. Glyphosate residues ($\mu\text{g mL}^{-1}$) in muscles of *C. gariepinus* juveniles exposed to varying concentrations of glyphosate and those treated with 50 and 100mg L^{-1} of Vitamin C

*GP1 (Groups exposed to Glyphosate at Day 0); GP2A (Groups exposed to Glyphosate for 91 days); GB1 (Group treated with 50mg L^{-1} Vitamin C for 7 days post-glyphosate exposure); GB2 (Group treated with 100mg L^{-1} Vitamin C for 7 days post-glyphosate exposure); GP3B (Group exposed concurrently to glyphosate and 100mg L^{-1} Vitamin C for 91 days)

50mg L^{-1} Vitamin C and fishes in GB2 treated with 100mg L^{-1} Vitamin C.

The glyphosate concentration in the muscles of *C. gariepinus* in GP3B exposed concurrently to

glyphosate and 100mg L^{-1} Vitamin C for 91 days indicated significant increases ($P > .05$) in the mean muscle glyphosate concentration of fishes in groups B, C, and D when compared to A (control or baseline mean).

4. DISCUSSION

There are numerous studies regarding the acute toxic effects of glyphosate-based commercial formulations on aquatic animals [29] and LC₅₀ values of glyphosate vary widely depending on fish species, test conditions and glyphosate formulations [9]. For example, the acute toxicity results for several teleost from several studies ranged from 7 to 4000 mgL⁻¹. Specifically, values for *Oncorhynchus mykiss* a species of high sensitivity was 140mgL⁻¹ [26], *Poecilia reticulata* >400mgL⁻¹ [30], *Cyprinus carpio*, a highly tolerant species, 620mgL⁻¹ [31]. *Clarias gariepinus* fingerlings using Sunsate[®] herbicide, a glyphosate formulation gave 18.33mgL⁻¹ [32]. In the present study, LC₅₀ obtained for glyphosate Delsate[®] compared favorably with the LC₅₀ of fully formulated glyphosate preparation of 76.8mgL⁻¹ in rainbow trout *Oncorhynchus mykiss* [33]. It could be justifiable to say that LC₅₀ of 75mgL⁻¹ obtained in this study was within the expected range but there was a significant increase in the level of glyphosate in the muscle of the exposed groups when compared with the control (baseline).

The potential for bioaccumulation of glyphosate has been observed in water hyacinth (*Eichornia crassipes*) exposed to pure glyphosate, and also in Carp (*Cyprinus carpio*) and Tilapia (*Oreochromis mossambicus*) exposed to environmentally relevant concentrations [34]. Glyphosate bioaccumulation was also observed in terrestrial snails (*Helix aspersa*) fed a diet contaminated with glyphosate [35]. These reports support the possibility of food chain contamination. The water environment which is constantly in contact with aquatic animal must have an acceptable quality in terms of its biological, chemical and physical attributes for it to be tolerated by aquatic animals [36].

Many xenobiotics such as glyphosate induce reactive oxygen species (ROS) thus causing oxidative damage [27]. Oxidative stress is caused by an imbalance between the production of ROS and ability of an organism to detoxify them or repair the resulting damage. Free radicals like hydroxyl radical (OH[•]), superoxide radical (O²⁻), and Hydrogen peroxide (H₂O₂) can be formed as a result, thus causing DNA damage.

Vitamin C can protect the cells leading to prevention of DNA damage [37]. It has been reported that high levels of Vitamin C are efficient

in reducing toxicity, preventing disease and enhancing fish tolerance to environmental stress [24]. The lack of gulonolactone oxidase, which is responsible for the synthesis of vitamin C in liver and kidney of many fish demands that vitamin C be supplemented in the diet to meet the nutritional requirement for optimum performance of the developing fish. Cypermethrin-induced histopathological and biochemical changes in Nile Tilapia (*Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid (vitamin C) has been reported [25].

Although there was a significant decrease in glyphosate accumulated in fish muscle after treatment of the glyphosate-exposed group with Vitamin C, the 100 mgL⁻¹ vitamin C proved to be more effective in reducing the glyphosate level in the fish muscle as indicated in 1Gp2 (Fig. 1). Decreased cellular damages caused by destructive lipids peroxidation induced by diazinon had been reported by [38] while examining the effects of Vitamin C on oxidative stress parameters in the fish rainbow trout exposed to diazinon.

5. CONCLUSION

This work has determined the LC₅₀ of glyphosate and Vitamin C. Also, it clearly demonstrated that glyphosate residues in muscles of exposed fish could be rendered safe for human consumption by treatment with Vitamin C. There is an on-going research by the present authors on "Histopathological alterations in tissues of glyphosate-exposed *C. gariepinus* juvenile fishes".

ETHICAL APPROVAL

Ethical approval numbered NAU /CEC/STU/INT/ 046 for this study was granted by the Research and Ethics Committee of Nnamdi Azikiwe University in September 2018.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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