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Ascorbic Acid Effect on Glyphosate-induced Haematological and Serological Pathology in Juveniles of the Catfish *Clarias gariepinus* (Pisces: Clariidae) Burchell, 1822

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Authors' contributions

This work was carried out in collaboration among all authors. Author CFI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOI and TOO managed the analyses of the study. Author BEO managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The study was to determine the effects of sub-lethal concentrations of glyphosate-based herbicide (Delsate®) on blood parameters, serum enzymes and urea of *Clarias gariepinus* juveniles as well as therapeutic effect of Vitamin C(Kepro®)on the glyphosate-induced pathology.
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: A 48 hours-acute toxicity tests were initially done to determine the respective LC_{50} of Delsate® and Kepro® using 8 *C. gariepinus* juveniles of mean weight 41.50±1.35 g and mean length

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20.75±0.43 cm. Thereafter one group of *C. gariepinus* juveniles (n=144) was exposed to 0, 5, 10 and 15mgL^{-1} sub-lethal concentrations of Delsate® for 91 days followed by different treatments with 50mgL^{-1} and 100mg L^{-1} of the vitamin C after 7 days post exposure to glyphosate. Another group of *C. gariepinus* juveniles (n=144) were exposed concurrently to glyphosate and vitamin C for 91 days.
Results: The LC_{50} of Delsate® was 75 mgL^{-1} and Kepro® 175 mgL^{-1}. There was significant decrease (P<.05) in PCV, Hb, RBC and AST of glyphosate-exposed groups when compared with Control. No significant difference occurred between TWBC, DWBC and ALP of exposed and control groups, except in neutrophils where significant increase occurred in ALT and urea. Treatment with 50 and 100mgL^{-1} vitamin C in glyphosate-exposed groups showed significant increase in PCV, Hb, RBC and ALP with a decrease in mean AST, ALT and Urea The 100 mgL^{-1} produced better therapeutic benefit than 50 mgL^{-1} vitamin C. However, concurrent exposure to glyphosate and vitamin C indicated no significant therapeutic effect on the tested blood and serum parameters.
Conclusion: The LC_{50} of Delsate® and Kepro® for catfish have been determined. Delsate® toxicity induced perturbations in some haematological and biochemical parameters in fish. The level of ascorbic acid (100 mgL^{-1} Kepro®) used in this study enhances catfish tolerance to environmental stress and could reduce Delsate® toxicity.

Keywords: Catfish; glyphosate-toxicity; haematological and biochemical perturbations; vitamin C-treatment.

1. INTRODUCTION

The use of chemical herbicides for weed control in agriculture has been recognized worldwide [1]. Plant-protection chemicals include several formulations containing antibiotic glyphosate used in producing genetically modified soybeans and corn used in animal feeds [2]. Glyphosate-based herbicides are the world's leading post-emergent, organophosphonate systemic, broad-spectrum and non-selective herbicides for weeds control [3]. Glyphosate (Roundup®) was introduced by Monsanto Company in 1970 and was registered as a broad spectrum non selective herbicide [4]. Glyphosate-based herbicides are known to be persistent and mobile in soil and water, and are among the most common terrestrial and aquatic contaminants [5].

Haematology has been performed in aquatic toxicology to assess the effects of metal pollution [6] and pulp mill effluent [7] on the blood parameters of fish. Blood parameters are considered pathophysiological biomarkers of the whole body and are important in diagnosing the anatomical and physiological status of fish exposed to toxicants [8]. An important step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. The activity of these liver enzymes (AST, ALT and ALP) is normally used to evaluate liver function [9]. These enzymes are biomarkers used to identify possible environmental contaminations before the health of aquatic organisms is adversely affected. This approach has been accepted as an early indication of potential damage in stressed fish tissues [10].

Since toxicity of any chemical alters the physiological state of animals thereby impairing their metabolic activities, it becomes necessary to understand how glyphosate causes damaging effects on the blood system. Herbicides such as glyphosate can produce reactive oxygen species (ROS) through various mechanisms such as interference in electron transport in the mitochondrial membrane and subsequent accumulation of reactive intermediates, inactivation of antioxidants enzymes, depletion of non-enzymatic antioxidants and membrane lipid peroxidation [11]. The resultant free radicals are damaging to animals at the molecular level due to their interaction with nucleic acids, proteins and lipids, where they initiate chain reactions by electron transfer. L-ascorbic acid (Vitamin C) is a powerful reducing agent and has been shown with *in vitro* studies to scavenge a number of reactive oxygen species [12]. This study was focused on the chronic effects of glyphosate on blood and serum chemistry of *C. gariepinus* and the ability of L-ascorbic acid to ameliorate the damaging effects of glyphosate herbicide on blood parameters.

2. MATERIALS AND METHODS

2.1 Study Animals

Three hundred and forty juveniles of *C. gariepinus* (mean weight=40.60±1.48 g; mean

length=17.60±1.51 cm) were procured from CHI Farms Ibadan and conveyed in a 50 litre-capacity plastic container, with 30 litres of borehole water to Flourish Farms Onitsha for the study. While the juveniles were acclimatizing for 14 days, they were fed 3 mm Skretting fish pellets at 3% biomass half at 9.00 am and 5.00 pm daily.

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. Determination of Lethal concentration (LC_{50}) of Delsate® and Kepro®

Eight *C. gariepinus* juveniles were used to determine the median lethal concentrations LC_{50} of both Delsate® and Kepro® vitamin C according to [13]. The LC_{50} of Delsate® in four juveniles (mean weight=43.20±1.41 g; mean length =20.75±1.96 cm) was determined (Table 1) while the LC_{50} of Kepro® in another set of four *C. gariepinus* juveniles (mean weight = 39.8±1.51 g; mean length = 20.10±1.41 cm) was also determined (Table 2).

Therefore doses of 50 and 100 mgL^{-1} were respectively used for the assessment of therapeutic effects on pathophysiological changes on *C. gariepinus* caused by 91 day exposure to glyphosate.

2.2.1 Toxicity bioassays of glyphosate (Delsate®) and Vitamin C (Kepro®) in *C. gariepinus*

The study design was Latin Square. Sub-lethal concentrations of 0, 5, 10 and 15 mgL^{-1} of Delsate® were employed in the experiment in 3 replicates of 12 juveniles per replicate, labeled as 1A, 1B, 1C and 1D respectively while 50 mgL^{-1} and 100 mgL^{-1} of Kepro® were employed as therapeutic doses after exposing the 144 juveniles of *C. gariepinus* to glyphosate for 91 days. At the end of 91 day-exposure, the juveniles were divided into 2 groups of 72*C. gariepinus* juveniles each. Groups $1A_1$, $1B_1$, $1C_1$ and $1D_1$ were treated with 50 mgL^{-1} vitamin C while Groups $1A_2$, $1B_2$, $1C_2$ and $1D_2$ were treated with 100 mg/l vitamin C respectively for 7 days.

Another 144 juveniles of *C. gariepinus* were concurrently-exposed to Delsate® and Kepro® for 91 days. A total of 72 *C. gariepinus* exposed to 0, 5, 10 and 15 mgL^{-1} Delsate® and treated with 50 mgL^{-1} Kepro® were labeled as $2A_1$, $2B_1$, $2C_1$ and $2D_1$ respectively. The other 72 were exposed to 0, 5, 10 and 15 mgL^{-1} Delsate® and treated with 100 mg/l Kepro® and labeled as $2A_2$, $2B_2$, $2C_2$ and $2D_2$ respectively.

Table 1. Determination of LC_{50} of Delsate® in *C. gariepinus* juveniles

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

Lethal dose = LC_{50} = $\frac{[Mo+M1]}{2}$, where MO = highest dose of test substance that recorded no mortality, and M_1 = lowest dose of test substance that recorded mortality [13].

LC_{50} of Delsate® = $\frac{[50+100]}{2} = \frac{150}{2}$ = 75mgL $^{-1}$

Table 2. Acute toxicity of Kepro® in *C. 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

Lethal dose = LC_{50} = $\frac{[Mo+M1]}{2}$, where MO = highest dose of test substance that recorded no mortality, and M_1 = lowest dose of test substance that recorded mortality [13].

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

Blood and serum samples were collected by cardiac puncture on day 0 and day 91 to determine the effects of Delsate®. Blood and serum samples were also collected after the 7 day- Kepro® treatment to determine the effects of Kepro® on the Delsate®-induced haemo-pathology. The Packed cell volume (PCV %), Red blood cell (RBC x10^6 ml blood), Total white blood cell (TWBC x10^7 ml blood) count and Differential white blood cell (DWBC x10^3 ml blood) count were determined according to [14] while Haemoglobin (Hb mgdl^{-1} blood) concentration was determined by the cyanomethaemoglobin method of [15].

Standard serum biochemistry procedures for determination of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Urea (Carbamide) were carried out with Randox Test Kits from Randox Laboratories United Kingdom. Water quality parameters: temperature, pH and dissolved oxygen were monitored during the experiments. Water was changed twice weekly with the renewal glyphosate and vitamin C strength.

Results were presented as means and standard error of the mean while the error bars in bar charts produced with Microsoft Excel version 2010 revealed significant differences (P< .05) between the variables.

3. RESULTS

3.1 Lethal Concentrations (LC_{50}) of the Glyphosate Delsate® and Vitamin C Kepro®

The LC_{50} of the glyphosate Delsate® and the vitamin C Kepro® were determined as 75 mgL^{-1} and 175 mgL^{-1}, respectively.

3.1.1 Effects on blood parameters (PCV, RBC, Hb, TWBC and DWBC) of *C. gariepinus* due to 91-days exposure to Delsate® and subsequent Day-7 post-exposure treatment with Kepro®

This study revealed a significant decrease in the mean PCV values of *C. gariepinus* juveniles exposed to glyphosate for 91days, with group 1C showing the highest decrease in Fig. 1. The standard error of means (SE±) showed that glyphosate exposure on the fish had a significant effect on the PCV of groups 1B, 1C and 1D when compared with 1A (P>.05). After Day 7 post-exposure treatment with Vitamin C, a significant increase (P>.05) was observed in the mean PCV values of groups 1B, 1C and 1D as compared to 1A but the error bars indicated no significant difference (P< .05) between the mean PCV of 50 mg/l Vitamin C group and 100 mgL^{-1} Vitamin C group.

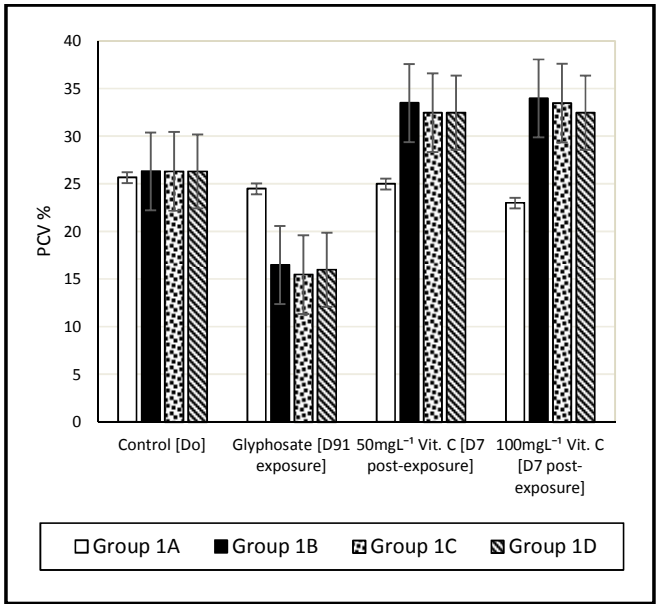

Fig. 1. Mean PCV of *C. gariepinus* treated respectively with 50 and 100 mgL^{-1} vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

There was a significant decrease in the mean RBC of fish in groups 1B, 1C, and not 1D, when compared to 1A in Fig. 2. The SE± showed that glyphosate exposure had a significant effect on the mean RBC of the fish in groups 1B and 1C (*P*>.05) when compared to group 1A while 1D showed no significant difference (*P*<.05). After the Day 7 post-exposure treatment with Vitamin C, the error bars indicated significant difference (*P*>.05) between the mean RBC values of the groups treated with 50 mgL^{-1} and 100 mgL^{-1} Vitamin C, respectively.

Fig. 3 revealed significant decrease in the mean Hb content of the blood of the fish in groups 1B and 1C when compared to group 1A. There was no significant difference (*P*<.05) in the mean haemoglobin content of group 1D when compared to group 1A. The SE± showed that the glyphosate exposure had a significant effect on the mean haemoglobin of the fish in groups 1B and 1C when compared to 1A (*P*>.05) but not on the mean haemoglobin of the fish in group 1D (*P*<.05). The error bars indicated significant differences (*P*>.05) in the mean Hb values of the groups treated with 50 mgL^{-1} and 100 mgL^{-1} Vitamin C, respectively. The observed effects of the glyphosate exposure and the vitamin C treatments in Fig. 3 followed the same trends already observed for Hb in Fig. 2.

The TWBC counts were shown in Fig. 4. There was no significant difference (*P*<.05) between the mean TWBC values of fish in groups 1A, 1B, 1C and 1D (Fig. 4). The SE± showed that glyphosate had no significant effect on the mean TWBC of fish in groups 1A, 1B, 1C and 1D. On vitamin C treatment, there was a significant difference (*P*>.05) between the mean TWBC count of the fish in groups with 100 mgL^{-1} of vitamin C when compared to groups treated with 50 mgL^{-1} of vitamin C as clearly shown in Fig. 4.

The DWBC count (Fig. 5) indicated no significant difference (*P*<.05) in the mean lymphocyte count of fish in groups 1B, 1C and 1D when compared to group 1A. The SE± showed that the glyphosate had no significant effect on the mean lymphocyte count of fish in groups 1B, 1C and 1D when compared to group 1A. There was slight increase in the mean monocyte count of fish in groups 1A, 1B, 1C and 1D when compared to baseline group but SE± indicated no significant differences of means (*P*<.05) showing that glyphosate had no significant effect on the mean monocyte count of fish in groups 1A, 1B, 1C and 1D when compared to baseline group but had the highest significant increase (*P*<.05) on the mean monocyte count of fish in group 1D. However there was significant increase in the mean neutrophil count of fish in groups 1A, 1B, and 1C when compared with baseline (*P*>.05). However, SE± indicated that glyphosate exposure on the fish had no significant effect (*P*<.05) on the mean neutrophil count of fish in groups 1D when compared to 1A.

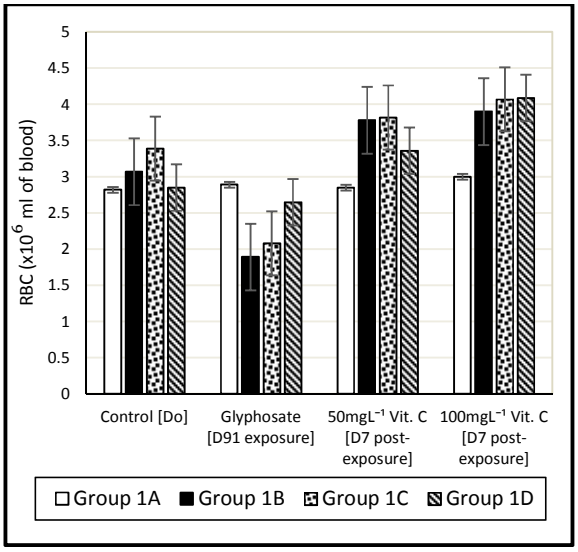

Fig. 2. Mean RBC of *C. gariepinus* treated respectively with 50 and 100mgL⁻¹ vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

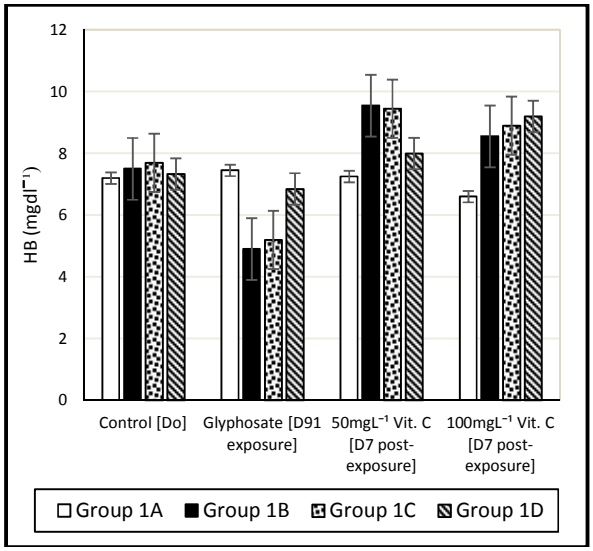

Fig. 3. Mean Hb of *C. gariepinus* treated respectively with 50 and 100mgL^{-1} vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

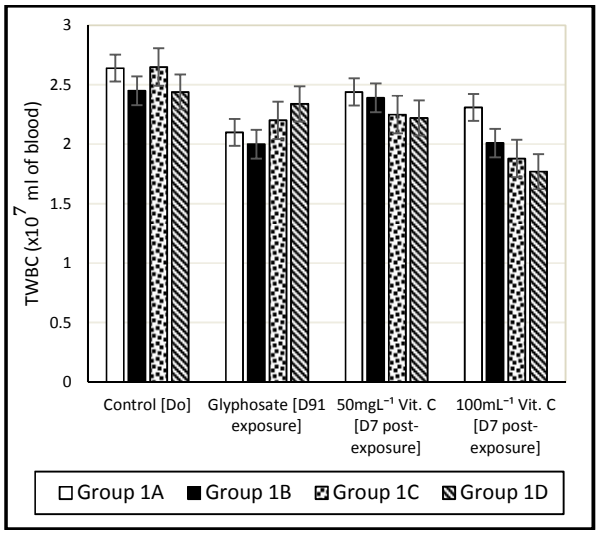

Fig. 4. Mean TWBC counts of *C. gariepinus* treated respectively with 50 and 100mgL^{-1} vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

On treatment with vitamin C, there was a significant increase (*P*>.05) in the mean lymphocyte count of the fish in group $1D_2$ with 100mgL^{-1} of vitamin C when compared to group $1D_1$ treated with 50 mgL^{-1} of vitamin C. The least significant difference between means showed a significant difference (*P*>.05) in lymphocyte counts between the baseline and group $1D_1$. There was no significant difference (*P*<.05) between the mean lymphocyte counts of the baseline group and the group $1D_2$.

There was also a significant increase (*P*>.05) in the mean monocytecount of fish in groups $1A_1$, $1B_1$, and $1D_1$ (group treated with 50 mgL^{-1} vitamin C) except in group $1C_1$, where there was a decrease in the mean monocyte count, though not significant. Significant increase was observed in the mean monocyte count of groups $1A_2$, $1B_2$, and $1C_2$. (Groups treated with 100 mg/l vitamin C), except in $1D_2$ where there was a significant decrease (*P*>.05). The highest mean monocyte count was in group $1A_2$, which was exposed to 5 mgL^{-1} of glyphosate and treated with 100 mgL^{-1}

of vitamin C. The least significant differences between means showed that a significant difference (*P*>.05) occurred between the mean monocyte counts of group $1B_1$ when compared to $1B_2$. A significant difference also occurred between the mean monocyte counts (*P*<.05) of group $1D_1$ when compared to $1D_2$. No significant difference (*P*<.05) occurred between $1A_1$ and $1A_2$ and between $1C_1$ and $1C_2$.

Fig. 5 also indicated a significant decrease (*P*>.05) in the mean neutrophil counts of fish in the vitamin C treated-groups when compared with groups exposed to glyphosate, with the least effect shown in group $1D_1$. The least significant differences between means showed a significant difference (*P*>.05) between the fish in groups $1C_1$ and $1C_2$; and between the fish in groups $1D_1$ and $1D_2$.

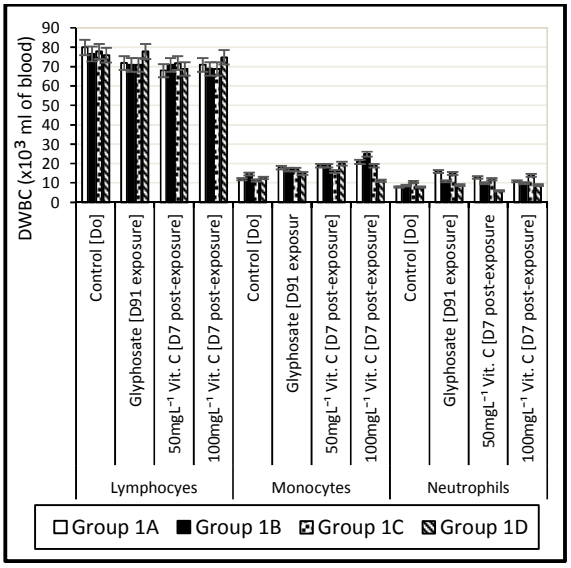

Fig. 5. Mean DWBC counts of *C. gariepinus* treated respectively with 50 and 100mgL^{-1} vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

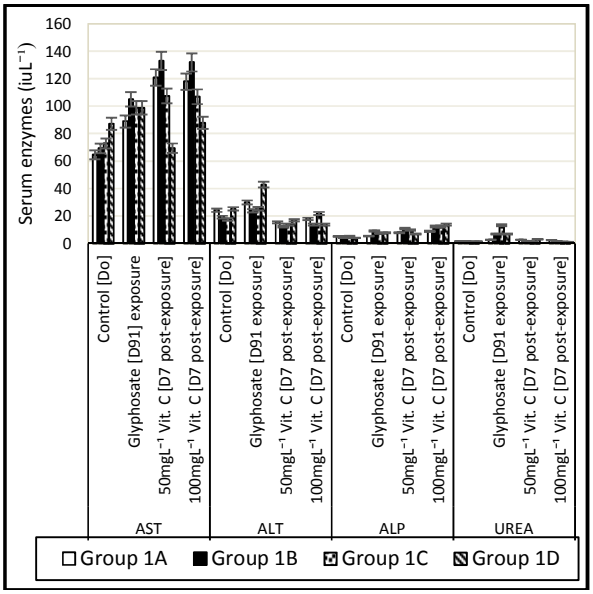

Fig. 6. Means of serum enzymes (AST, ALT and ALP) and Urea of *C. gariepinus* treated respectively with 50 and 100 mgL^{-1} vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

3.1.2 Effects on serum enzymes (AST, ALT, and ALP) and Urea of *C. gariepinus* due to 91 days exposure to glyphosate and subsequent 7 Days post-exposure treatment with Vitamin C

The mean values of serum enzymes and urea were as presented in Fig. 6. There was a significant decrease (*P*>.05) in the mean values of AST of fish in groups 1B, 1C and 1D when compared to 1A which showed that glyphosate had an effect on the mean AST values of fish in groups 1B, 1C and 1D when compared with 1A. The increase in the mean values of ALT of fish in groups 1B, 1C and 1D when compared with 1A were not significant, except for 1D (*P*>.05). Mean error also showed no significant increase (*P*<.05) in the mean values of ALP of fish in groups 1B, 1C and 1D when compared with 1A; an indication that glyphosate had minimal effect on the mean ALP values of fish in groups 1B, 1C and 1D when compared with 1A. Though there was observable increase in mean values of urea, the error bars showed that glyphosate had only significant effect (*P*>.05) on the mean urea values of fish in groups 1C and 1D when compared to 1A.

Values for the serum enzymes of *C. gariepinus* exposed to varying concentrations of glyphosate for 91 days and treated with 50mgL^{-1} and 100mgL^{-1} vitamin C for 7 days were also shown in Fig. 6. There was a significant decrease (*P*>.05) in the mean AST of fish in groups $1A_1$, $1B_1$, $1C_1$ and $1D_1$ and $1A_2$, $1B_2$, $1C_2$ and $1D_2$ when compared with 1A, 1B, 1C and 1D. Standard error of means showed significant difference (*P*>.05) between the mean AST value of fish in groups $1D_1$ and $1D_2$ when compared with 1A,1B,1C and 1D and the baseline group. The results showed that a significant decrease (*P*>.05) occurred in the mean ALT values of fish in groups $1A_1$, $1B_1$, $1C_1$ and $1D_1$ and $1A_2$, $1B_2$, $1C_2$ and $1D_2$ when compared with 1A, 1B, 1C and 1D, and the baseline means. The least significant differences of means showed that there was no difference (*P*<.05) between the groups treated with 50 mgL^{-1} vitamin C. the result showed a significant increase (*P*>.05) in mean ALP values of 50 mgL^{-1} vitamin C and 100 mgL^{-1} vitamin C treated groups when compared to the control and baseline groups. The least significant difference between means showed a significant difference between $1B_1$, $1C_1$ and $1D_1$ and $1B_2$, $1C_2$ and $1D_2$ when compared to control. Fig. 6 also showed a significant decrease (p> 0.05) in the mean urea values of $1A_1$, $1B_1$, $1C_1$ and $1D_1$ and $1A_2$, $1B_2$, $1C_2$ and $1D_2$ when compared to the baseline groups. The least significant difference between means showed a significant difference the glyphosate exposed groups and the vitamin C treated groups with the least significant mean urea value occurred in group $1D_2$.

3.1.3 Effects on blood parameters (PCV, RBC, Hb, TWBC and DWBC) of *C. gariepinus* concurrently-exposed to varying concentrations of glyphosate and vitamin C

The mean PCV values of *Clarias gariepinus* treated concurrently with glyphosate and vitamin C in varying concentrations are shown in Fig. 7. There was a significant difference (*P*>.05) between the mean PCV of fish in groups $2A_1$, $2B_1$, $2C_1$ and $2D_1$ when compared to $2A_2$, $2B_2$, $2C_2$ and $2D_2$ and baseline groups. The highest means PCV occurred in group $2C_1$ while the least mean PCV occurred in group $2D_2$.

There was a significant increase (*P*>.05) in mean RBC of fish in groups $2A_1$, $2B_1$, $2C_1$ and $2D_1$ when compared to baseline (Fig. 8). There was no significant difference (*P*<.05) between the mean RBC of $2A_1$, $2B_1$, $2C_1$ and $2D_1$ when compared to $2A_2$, $2B_2$, $2C_2$ and $2D_2$. However $2C_1$ had highest mean RBC while the least occurred in $2D_2$.

There was also no significant difference (*P*<.05) between the mean Hb values of $2A_1$, $2B_1$, $2C_1$ and $2D_1$ when compared to $2A_2$, 2B2, 2C2 and $2D_2$ and baseline groups (Fig. 9). The highest mean Hb value was in group $2C_1$ while the least mean Hb value was in group $2D_2$. The observation on Hb (Fig. 9) followed the same trend already observed for RBC in Fig. 8.

Effects of Glyphosate and Vitamin C on Total White Blood Cells (TWBC) counts of *Clarias gariepinus* juveniles concurrently exposed to glyphosate and vitamin C for 91 days were indicated in Fig. 10. There was significant differences (*P*>.05) in mean TWBC between the baseline group and the experimental groups. The highest mean TWBC occurred in $2C_1$ while the least occurred in group $2B_2$.

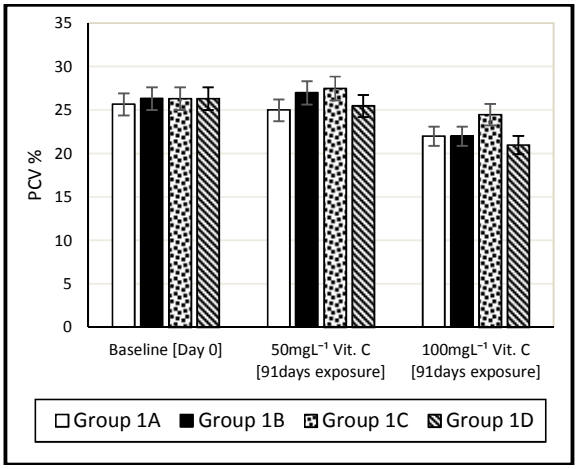

Fig. 7. Mean PCV of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

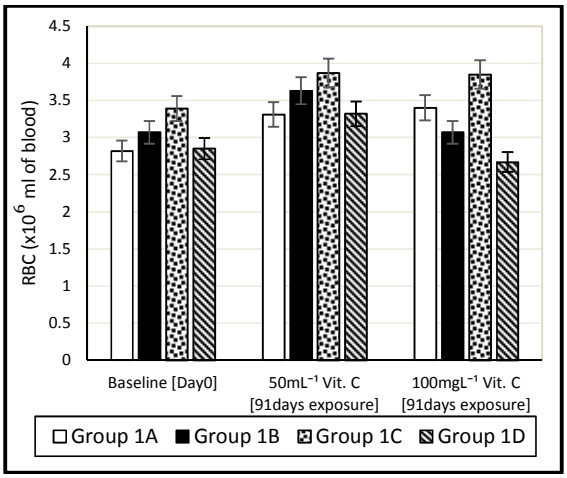

Fig. 8. Mean RBC of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

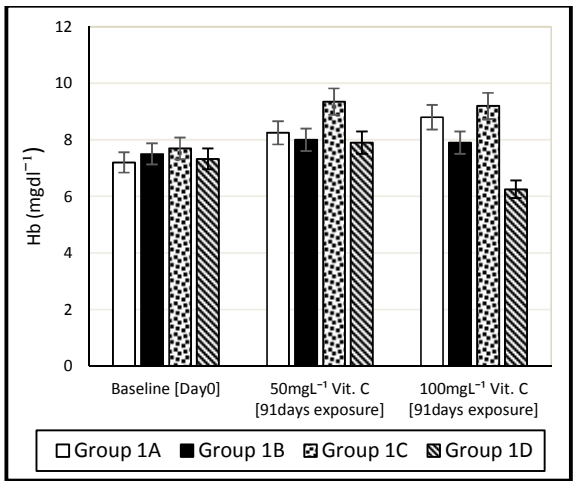

Fig. 9. Mean Hb of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

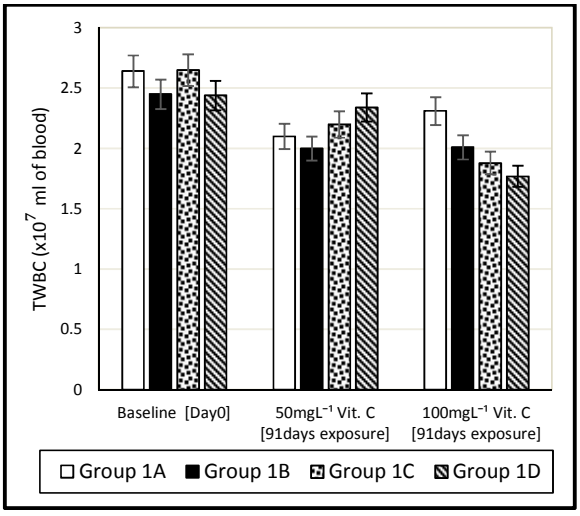

Fig. 10. Mean TWBC of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

Effects of the Glyphosate and the Vitamin C on Differential White Blood Cells (DWBC) counts of *Clarias gariepinus* juveniles concurrently exposed to glyphosate and vitamin C for 91 days were shown in Fig. 11. There was a significant decrease (*P*>.05) between the mean lymphocyte values of fish in the baseline group and the experimental groups, with the highest mean lymphocyte value occurring in group $2C_2$. There was no significant difference (*P*>.05) between the mean lymphocyte count of $2A_1$, $2B_1$, $2C_1$, $2D_1$ and $2A_2$, $2B_2$, $2C_2$, $2D_2$.

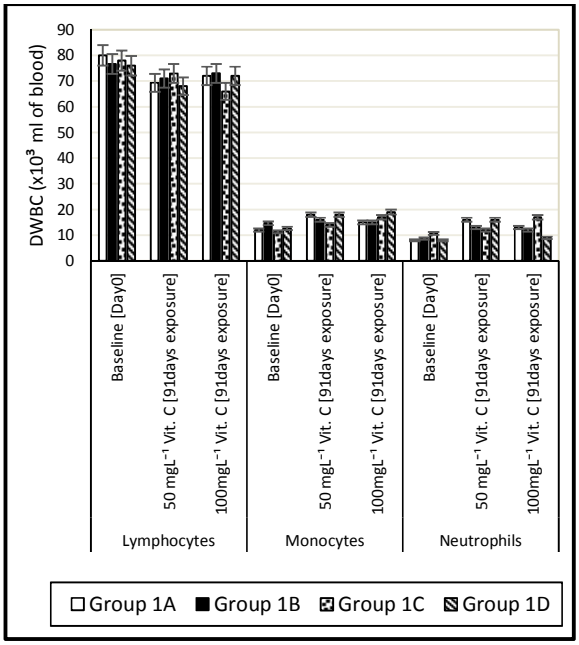

Fig. 11. Mean DWBC of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

There was an increase in the mean monocyte count of baseline and experimental groups with the highest mean monocyte value occurring in $2D_2$ while the least mean monocyte value occurred in 1C, though not statistically different (P<.05). There was no significant difference (P<.05) between the mean monocyte count of $2A_1$, $2B_1$, $2C_1$, $2D_1$ and $2A_2$, $2B_2$, $2C_2$, $2D_2$. There was significant increase (P>.05) in the mean neutrophil counts of the fish in the baseline and experimental groups, with the highest neutrophil count in group $2C_2$ while the least neutrophil count occurred in group $2D_2$. There was no significant difference (P<.05) between the mean neutrophil count of groups $2A_1$, $2B_1$, $2C_1$, $2D_1$, and $2A_2$, $2B_2$, $2C_2$, $2D_2$.

3.1.4 Effects on serum enzymes (AST, ALT, ALP) and Urea of *C. gariepinus* concurrently-exposed to varying concentrations of glyphosate and vitamin C

The mean serum enzymes and urea values of *C. gariepinus* concurrently exposed to glyphosate and vitamin C for 91 days are presented in Fig. 12. There was significant difference (P>.05) between the mean AST values of the baseline and the experimental groups. There was a significant increase (P>.05) in the mean ALT values of experimental groups when compared to the baseline mean ALT values. There was also significant difference (P>.05) in the mean ALT values between groups $2A_1$, $2B_1$, $2C_1$, $2D_1$ and $2A_2$, $2B_2$, $2C_2$, $2D_2$. The results showed increase in the mean ALP values of the baseline when compared with $2A_1$, $2B_1$, $2C_1$, $2D_1$ but there was no significant difference (P<.05) in the mean ALP values of $2A_1$, $2B_1$, $2C_1$, $2D_1$ when compared with $2A_2$, $2B_2$, $2C_2$, $2D_2$. Standard error bars showed no significant difference (P<.05) between the mean ALP values of the baseline group when compared to $2A_2$, $2B_2$, $2C_2$, and $2D_2$. Fig. 12 also showed a significant increase (P>.05) in the mean urea level of the fish in the experimental groups when compared to the mean urea level of the baseline.

4. DISCUSSION

Blood, being an important medium for assessing the health status of animals, the physiological and pathological conditions of animals can be assessed by the evaluation of haematological and biochemical analyses of blood [16,17]. In this study in which haematological responses of the juvenile catfish exhibited significant decrease in RBC, Hb and PCV when compared to the control group was in line with the reports of [5,18,19,20, and 21]. The significant reduction in these parameters was an indication of severe anaemia caused by destruction of erythrocytes [22], haemo-dilution [21,23] resulting from impaired osmoregulation across the gill epithelium. According to [18], this could be as a result of the destruction of intestinal cells, leading to impaired intestinal absorption of iron as has been suggested [24]. The decreased trend in RBC, Hb and PCV in the present study was also in accordance with earlier findings on different fish species [25, 26, 27,28].

The significant increase in TWBC, lymphocytes, monocytes and neutrophils observed in the fish exposed to chronic glyphosate when compared to the control and baseline group agreed with the report [29] that glyphosate-based herbicides are genotoxic and cytotoxic to human white blood cells (lymphocytes) at low concentration doses under the acceptable daily intake of 0.50 µg/ml. Genotoxicity is described [29] as the property of chemical agent that damages the genetic information within a cell causing mutations which may lead to cancer. There was also a report [30] on reduction in WBC which concluded that sub-lethal concentrations of glyphosate in polluted natural waters caused minor anaemia and significant immunosuppressive response in juveniles of the common carp. According to [31], white blood cells are key components in specific (adaptive) and non-specific (innate) immune responses and this is supported by the fact that an increased WBC is indicative of damage due to infection of body tissues, severe physical stress and even leukaemia (Singh et al., 2008). According to [25], the progressive increase in TWBC, termed leukocytosis, is as a result of the body trying to fight the foreign body being the toxicant, glyphosate. Increase in leucocytes numbers suggests that defense mechanisms in response to toxicant exposure were activated in the fish [32].

The Liver markers AST, ALP and ALT did not vary at day zero of the chronic experiment, showing that all the fish used in this study could be assumed to be of the same health status. It has been revealed by [33] that glyphosate caused significant increase in liver enzymes in *Oreochromis niloticus*. Significant increase in ALT and ALP in this study could be an indication of liver damage. The ALT is a specific enzyme marker produced by the liver and it only increases when the hepatocytes are

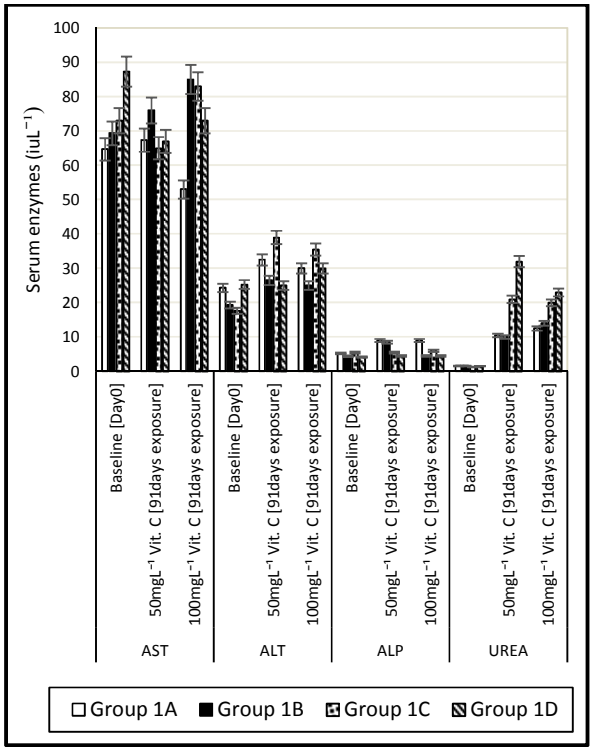

Fig. 12. Means of serum enzymes (AST, ALT and ALP) and Urea of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

damaged. Significant increases in AST, ALT and ALP in *Oreochromis niloticus* exposed to sub lethal concentrations of Roundup® for 3 months has also been reported [34]. These biochemical alterations are indicative of increased metabolism of amino acids in response to elevated energy during periods of physiological stress and cellular damage [35]. Given that the liver is the primary site of xenobiotic detoxification, significant alterations to structure or normal function will likely have deleterious effects on animal health and performance.

Blood Urea Nitrogen (BUN) measures the amount of urea nitrogen present in the blood after it breaks down proteins used by the cells, and is used as an assessment of kidney function. The BUN helps to eliminate toxic substances and is formed in the liver, and carried through the blood stream to the kidneys to be eliminated. The significant high levels of urea in the fish exposed to glyphosate, known as azotemia, is an indicator of poor renal function resulting from electrolyte imbalance. There was an earlier report [36] on increased levels of urea in *C. gariepinus* exposed to acute concentrations of Delsate. According to [37], elevations of liver enzymes are the most useful indicators of hepatic dysfunction and hepato-cellular damage. Moreover, it had been observed [38] that sub chronic exposure to glyphosate caused liver damage.

In the present study, treatment of glyphosate-stressed catfish with 50 mgL^{-1} vitamin C and 100 mgL^{-1} vitamin C which resulted in significant increase in the mean RBC, Hb, and PCV values, close to those of control fish, was similar to the report [39] that vitamin C enhanced the blood parameters in Nile tilapia (*Oreochromis niloticus*) exposed to ochratoxin toxicity which had caused significant decrease in the RBC, Hb and PCV of the fish. Cypermetrin-induced histopathological and biochemical changes in Nile Tilapia *(Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid (vitamin C) has also been reported [40]

Treatment of the glyphosate-exposed group with vitamin C also showed significant decrease in the elevated AST, ALT and urea in the glyphosate-exposed group. Liver damage in mice previously exposed to glyphosate for four weeks was also shown to be ameliorated by orange juice which also increased serum urea [41].

5. CONCLUSION

The LC_{50} of the glyphosate Delsate® and ascorbic acid Kepro® has been determined. Delsate® toxicity induced perturbations in some haematological and biochemical parameters in catfish. Vitamin C can play protective and therapeutic roles in glyphosate-toxicity in the African catfish. We recommend that the level of ascorbic acid (100 mgL^{-1} vitamin C Kepro®) used in this study could enhance catfish tolerance to environmental stress and reduce glyphosate Delsate® toxicity. Work is in progress on the "Effect of Ascorbic acid on Glyphosate-induced residues in the muscles of *Clarias gariepinus."*

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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