

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

**ABSTRACT**

**Aims:** The study was to determine the effects of sub-lethal concentrations of glyphosate-based herbicide (Delsate<sup>®</sup>) on blood parameters, serum enzymes and urea of *Clarias gariepinus* juveniles as well as therapeutic effect of Vitamin C (Kepro<sup>®</sup>) 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<sub>50</sub> of Delsate<sup>®</sup> and Kepro<sup>®</sup> using 8 *C. gariepinus* juveniles of mean weight 41.50±1.35g and mean length 20.75±0.43cm. Thereafter one group of *C. gariepinus* juveniles (n=144) was exposed to 0, 5, 10 and 15mgL<sup>-1</sup> sub-lethal concentrations of Delsate<sup>®</sup> for 91 days followed by different treatments with 50mgL<sup>-1</sup> and 100mg L<sup>-1</sup> 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<sub>50</sub> of Delsate<sup>®</sup> was 75mgL<sup>-1</sup> and Kepro<sup>®</sup> 175mgL<sup>-1</sup>. 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<sup>-1</sup> 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 100mgL<sup>-1</sup> produced better therapeutic benefit than 50mgL<sup>-1</sup> 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<sub>50</sub> of Delsate<sup>®</sup> and Kepro<sup>®</sup> for catfish have been determined. Delsate<sup>®</sup> toxicity induced perturbations in some haematological and biochemical parameters in fish. The level of ascorbic acid (100mgL<sup>-1</sup> Kepro<sup>®</sup>) used in this study enhances catfish tolerance to environmental stress and could reduce Delsate<sup>®</sup> 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. MATERIAL AND METHODS

### Study animals

Three hundred and forty juveniles of *C. gariepinus* (mean weight=40.60±1.48g; mean length=17.60±1.51cm) 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 3mm Skretting fish pellets at 3% biomass half at 9.00am and 5.00pm daily. 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.

### 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<sub>50</sub>) of Delsate® and Kepro®

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

**Table 1:** Determination of LC<sub>50</sub> of Delsate® in *C. gariepinus* juveniles

Tank	Dose (mgL <sup>-1</sup> )	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 =  $LC_{50} = \frac{[M_0 + M_1]}{2}$ , where  $M_0$  = highest dose of test substance that recorded no mortality, and  $M_1$  = lowest dose of test substance that recorded mortality [13].

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

**Table 2:** Acute toxicity of Kepro<sup>®</sup> in *C. gariepinus* juveniles

Tank	Dose (mgL <sup>-1</sup> )	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 =  $LC_{50} = \frac{[M_0 + M_1]}{2}$ , where  $M_0$  = highest dose of test substance that recorded no mortality, and  $M_1$  = lowest dose of test substance that recorded mortality [13].

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

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

#### Toxicity bioassays of glyphosate (Delsate<sup>®</sup>) and Vitamin C (Kepro<sup>®</sup>) in *C. gariepinus*

The study design was Latin Square. Sub-lethal concentrations of 0, 5, 10 and 15mgL<sup>-1</sup> of Delsate<sup>®</sup> were employed in the experiment in 3 replicates of 12 juveniles per replicate, labeled as 1A, 1B, 1C and 1D respectively while 50mgL<sup>-1</sup> and 100mgL<sup>-1</sup> of Kepro<sup>®</sup> 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<sub>1</sub>, 1B<sub>1</sub>, 1C<sub>1</sub> and 1D<sub>1</sub> were treated with 50mgL<sup>-1</sup> vitamin C while Groups 1A<sub>2</sub>, 1B<sub>2</sub>, 1C<sub>2</sub> and 1D<sub>2</sub> were treated with 100mg/l vitamin C respectively for 7 days.

Another 144 juveniles of *C. gariepinus* were concurrently-exposed to Delsate<sup>®</sup> and Kepro<sup>®</sup> for 91 days. A total of 72 *C. gariepinus* exposed to 0, 5, 10 and 15mgL<sup>-1</sup> Delsate<sup>®</sup> and treated with 50mgL<sup>-1</sup> Kepro<sup>®</sup> were labeled as 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub> and 2D<sub>1</sub> respectively. The other 72 were exposed to 0, 5, 10 and 15mgL<sup>-1</sup> Delsate<sup>®</sup> and treated with 100mg/l Kepro<sup>®</sup> and labeled as 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub> and 2D<sub>2</sub> respectively.

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

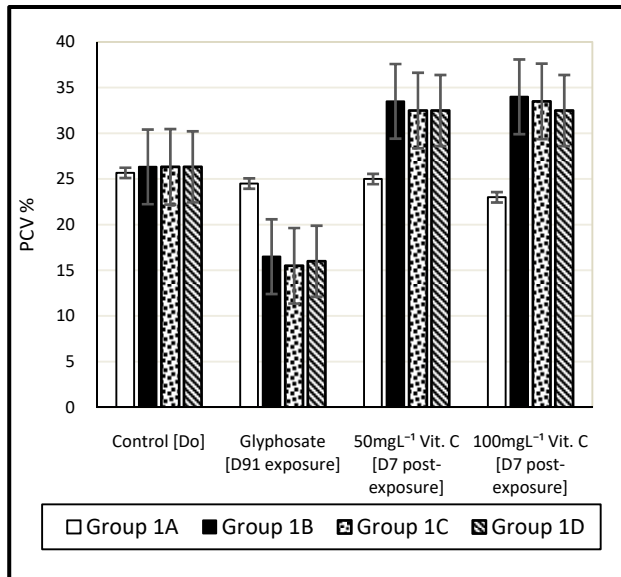
### 3. RESULTS

#### Lethal concentrations (LC<sub>50</sub>) of the glyphosate Delsate® and Vitamin C Kepro®

The LC<sub>50</sub> of the glyphosate Delsate® and the vitamin C Kepro® were determined as 75mgL<sup>-1</sup> and 175mgL<sup>-1</sup>, respectively.

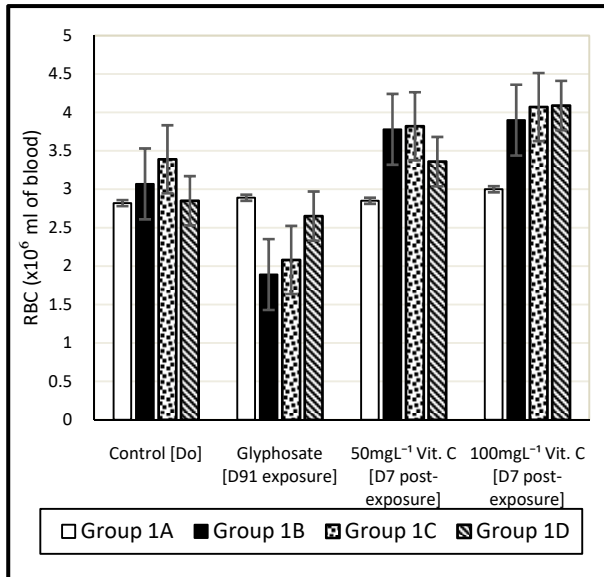
#### Effects on blood parameters (PCV, RBC, Hb, TWBC and DWBC) of *C. gariepinus* due to 91-days exposure to Delsate® and subsequent Day-7 post-exposure treatment with Kepro®

This study revealed a significant decrease in the mean PCV values of *C. gariepinus* juveniles exposed to glyphosate for 91 days, with group 1C showing the highest decrease in Figure 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 50mgL<sup>-1</sup> Vitamin C group and 100mgL<sup>-1</sup> Vitamin C group.



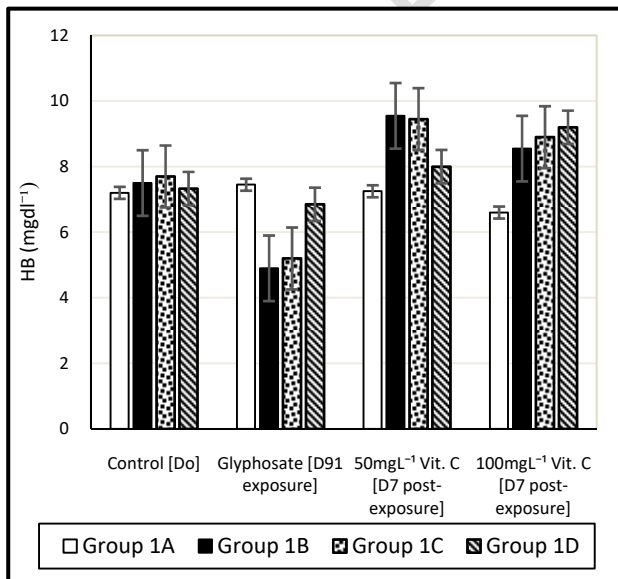
**Figure 1:** Mean PCV of *C. gariepinus* treated respectively with 50 and 100mgL<sup>-1</sup> vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days.

There was a significant decrease in the mean RBC of fish in groups 1B, 1C, and not 1D, when compared to 1A in Figure 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 50mgL<sup>-1</sup> and 100mgL<sup>-1</sup> Vitamin C, respectively.



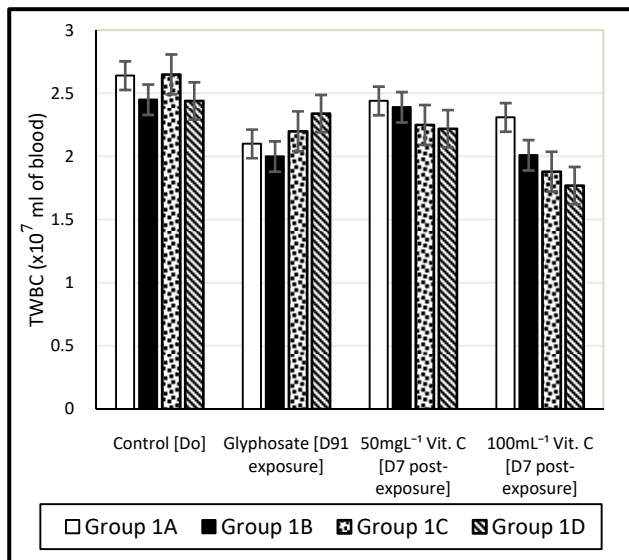
**Figure 2:** Mean RBC of *C. gariepinus* treated respectively with 50 and 100mgL<sup>-1</sup> vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

Figure 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 $\pm$  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 50mgL<sup>-1</sup> and 100mgL<sup>-1</sup> Vitamin C, respectively. The observed effects of the glyphosate exposure and the vitamin C treatments in Figure 3 followed the same trends already observed for Hb in Figure 2



**Figure 3:** Mean Hb of *C. gariepinus* treated respectively with 50 and 100mgL<sup>-1</sup> vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

The TWBC counts were shown in Figure 4. There was no significant difference ( $P < .05$ ) between the mean TWBC values of fish in groups 1A, 1B, 1C and 1D (Figure 4). The  $SE \pm$  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  $100\text{mgL}^{-1}$  of vitamin C when compared to groups treated with  $50\text{mgL}^{-1}$  of vitamin C as clearly shown in Figure 4.



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

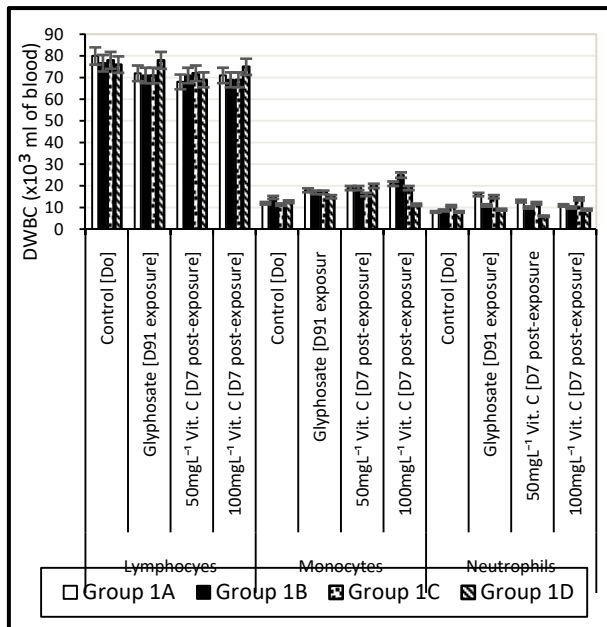
The DWBC count (Figure 5) indicated no significant difference ( $P < .05$ ) in the mean lymphocyte count of fish in groups 1B, 1C and 1D when compared to group 1A. The  $SE \pm$  showed that the glyphosate had no significant effect on the mean lymphocyte count of fish in groups 1B, 1C and 1D when compared 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  $SE \pm$  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,  $SE \pm$  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.

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  $100\text{mgL}^{-1}$  of vitamin C when compared to group  $1D_1$  treated with  $50\text{mgL}^{-1}$  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 monocyte count of fish in groups  $1A_1$ ,  $1B_1$ , and  $1D_1$  (group treated with  $50\text{mgL}^{-1}$  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  $100\text{mg/l}$  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  $5\text{mgL}^{-1}$  of glyphosate and treated with  $100\text{mgL}^{-1}$  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<sub>1</sub> when compared to 1B<sub>2</sub>. A significant difference also occurred between the mean monocyte counts ( $P < .05$ ) of group 1D<sub>1</sub> when compared to 1D<sub>2</sub>. No significant difference ( $P < .05$ ) occurred between 1A<sub>1</sub> and 1A<sub>2</sub> and between 1C<sub>1</sub> and 1C<sub>2</sub>.

Figure 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<sub>1</sub>. The least significant differences between means showed a significant difference ( $P > .05$ ) between the fish in groups 1C<sub>1</sub> and 1C<sub>2</sub>; and between the fish in groups 1D<sub>1</sub> and 1D<sub>2</sub>.



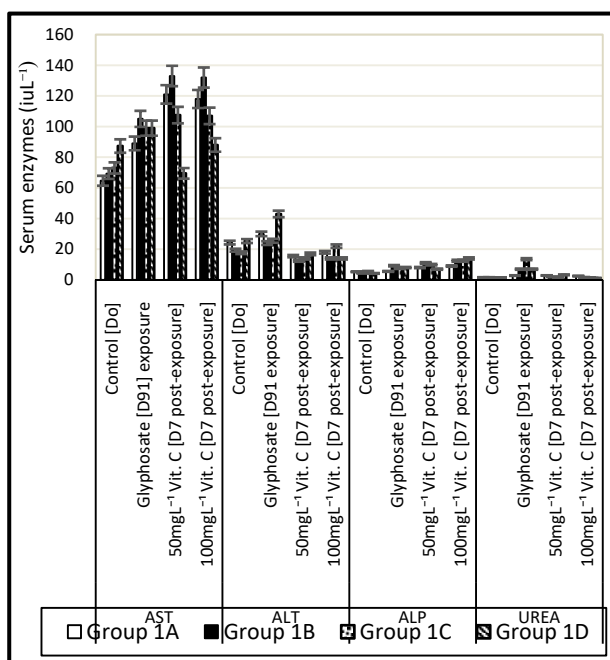
**Figure 5:** Mean DWBC counts of *C. gariepinus* treated respectively with 50 and 100mgL<sup>-1</sup> vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

#### 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 Figure 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<sup>-1</sup> and 100mgL<sup>-1</sup> vitamin C for 7 days were also shown in Figure 6. There was a significant decrease ( $P > .05$ ) in the mean AST of fish in groups 1A<sub>1</sub>, 1B<sub>1</sub>, 1C<sub>1</sub> and 1D<sub>1</sub> and 1A<sub>2</sub>, 1B<sub>2</sub>, 1C<sub>2</sub> and 1D<sub>2</sub> 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<sub>1</sub> and 1D<sub>2</sub> 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<sub>1</sub>, 1B<sub>1</sub>, 1C<sub>1</sub> and 1D<sub>1</sub> and 1A<sub>2</sub>, 1B<sub>2</sub>, 1C<sub>2</sub> and 1D<sub>2</sub> 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 50mgL<sup>-1</sup> vitamin C. The result showed a significant increase ( $P > .05$ ) in mean ALP values of 50mgL<sup>-1</sup> vitamin C and 100mgL<sup>-1</sup> vitamin C treated groups when compared to the control and baseline groups. The least significant difference between means showed a significant difference between 1B<sub>1</sub>, 1C<sub>1</sub> and 1D<sub>1</sub> and 1B<sub>2</sub>, 1C<sub>2</sub> and 1D<sub>2</sub> when compared to control. Figure 6 also showed a significant decrease ( $p > 0.05$ ) in the mean urea values of 1A<sub>1</sub>, 1B<sub>1</sub>, 1C<sub>1</sub> and 1D<sub>1</sub> and 1A<sub>2</sub>, 1B<sub>2</sub>, 1C<sub>2</sub> and 1D<sub>2</sub> 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<sub>2</sub>.



**Figure 6:** Means of serum enzymes (AST, ALT and ALP) and Urea of *C. gariepinus* treated respectively with 50 and 100mgL<sup>-1</sup> vitamin C on day-7 (D7) post-exposure to varying concentrations of glyphosate for 91 days

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

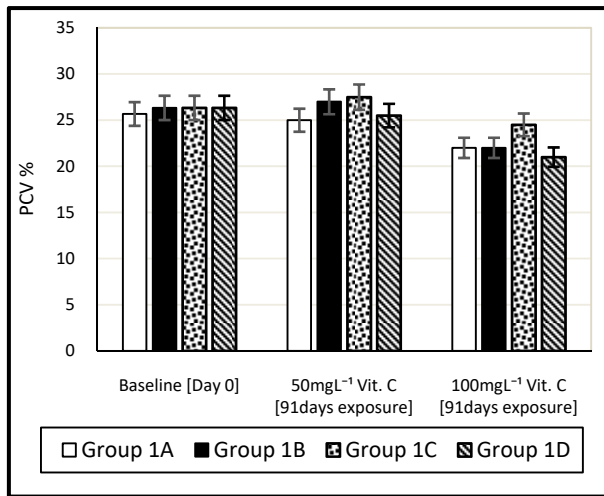
The mean PCV values of *Clarias gariepinus* treated concurrently with glyphosate and vitamin C in varying concentrations are shown in Figure 7. There was a significant difference ( $P > .05$ ) between the mean PCV of fish in groups 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub> and 2D<sub>1</sub> when compared to 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub> and 2D<sub>2</sub> and baseline groups. The highest means PCV occurred in group 2C<sub>1</sub> while the least mean PCV occurred in group 2D<sub>2</sub>.

There was a significant increase ( $P > .05$ ) in mean RBC of fish in groups 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub> and 2D<sub>1</sub> when compared to baseline (Figure 8). There was no significant difference ( $P < .05$ ) between the mean RBC of 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub> and 2D<sub>1</sub> when compared to 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub> and 2D<sub>2</sub>. However 2C<sub>1</sub> had highest mean RBC while the least occurred in 2D<sub>2</sub>.

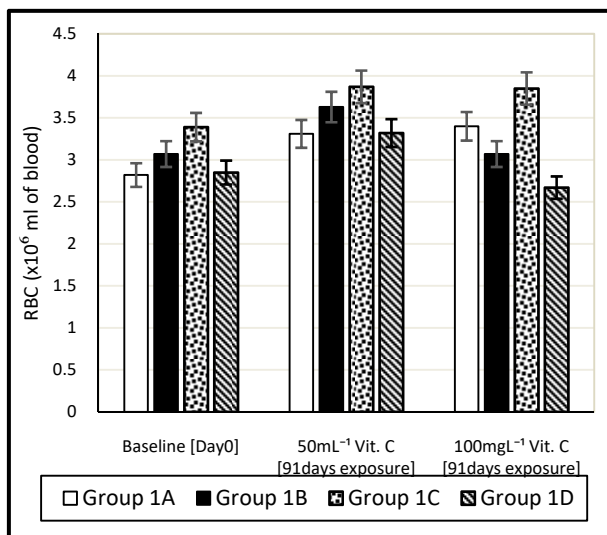
There was also no significant difference ( $P < .05$ ) between the mean Hb values of 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub> and 2D<sub>1</sub> when compared to 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub> and 2D<sub>2</sub> and baseline groups (Figure 9). The highest mean Hb value was in group 2C<sub>1</sub> while the least mean Hb value was in group



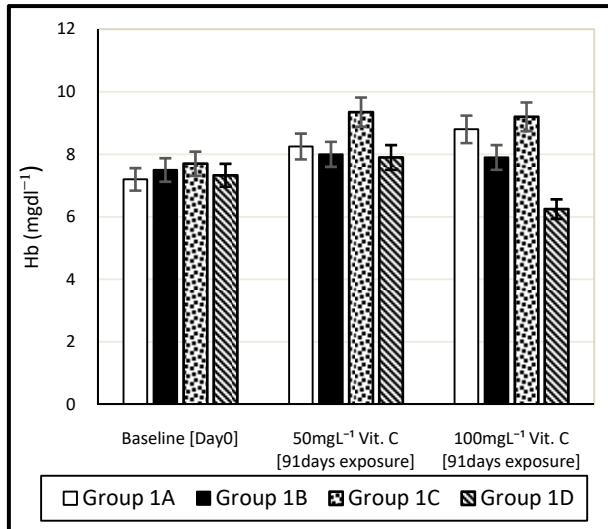
2D<sub>2</sub>. The observation on Hb (Figure 9) followed the same trend already observed for RBC in Figure 8.



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

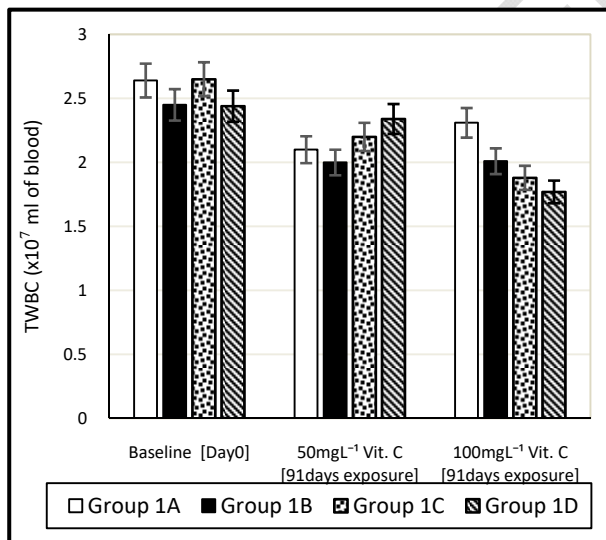


**Figure 8:** Mean RBC of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days.



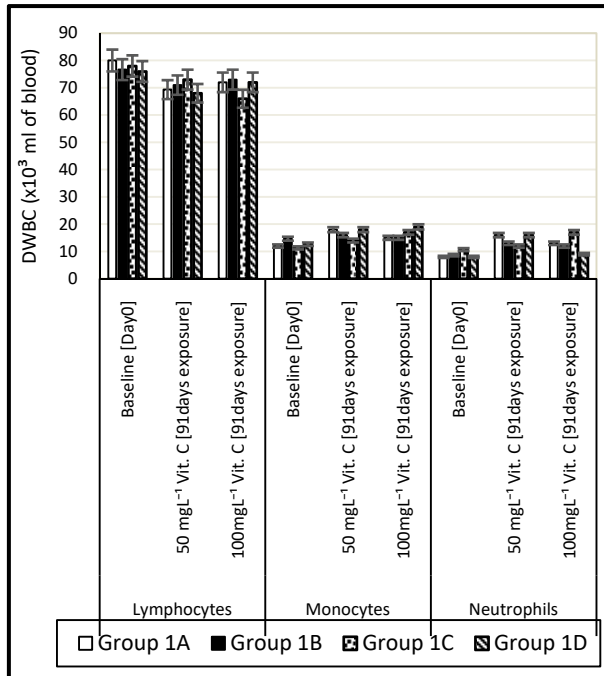
**Figure 9:** Mean Hb of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

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 Figure 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<sub>1</sub> while the least occurred in group 2B<sub>2</sub>.



**Figure 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 Figure 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<sub>2</sub>. There was no significant difference ( $P > .05$ ) between the mean lymphocyte count of 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub> and 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, 2D<sub>2</sub>.

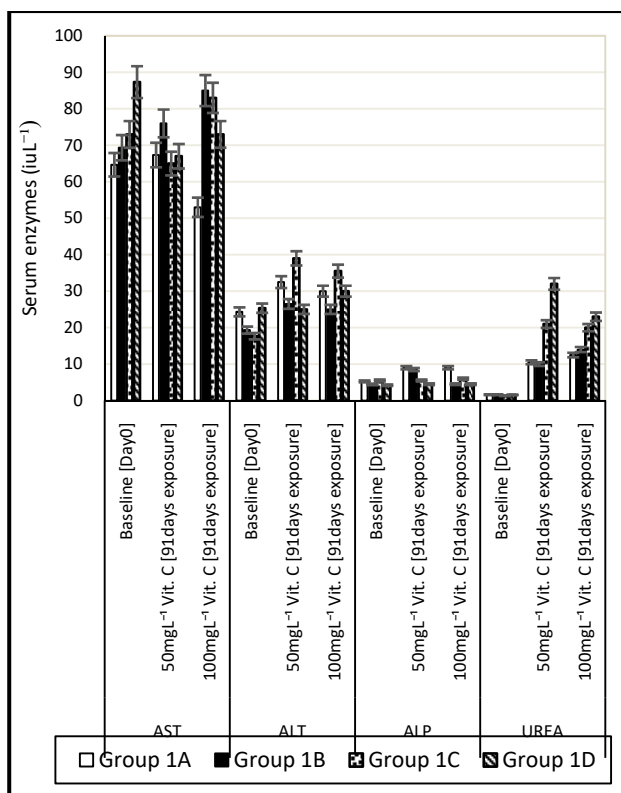


**Figure 11:** Mean DWBC of *C. gariepinus* concurrently-exposed to glyphosate and vitamin C for 91 days

There was an increase in the mean monocyte count of baseline and experimental groups with the highest mean monocyte value occurring in 2D<sub>2</sub> 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<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub> and 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, 2D<sub>2</sub>. 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<sub>2</sub> while the least neutrophil count occurred in group 2D<sub>2</sub>. There was no significant difference ( $P < .05$ ) between the mean neutrophil count of groups 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub>, and 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, 2D<sub>2</sub>.

#### **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 Figure 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<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub> and 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, 2D<sub>2</sub>. The results showed increase in the mean ALP values of the baseline when compared with 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub> but there was no significant difference ( $P < .05$ ) in the mean ALP values of 2A<sub>1</sub>, 2B<sub>1</sub>, 2C<sub>1</sub>, 2D<sub>1</sub> when compared with 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, 2D<sub>2</sub>. Standard error bars showed no significant difference ( $P < .05$ ) between the mean ALP values of the baseline group when compared to 2A<sub>2</sub>, 2B<sub>2</sub>, 2C<sub>2</sub>, and 2D<sub>2</sub>. Figure 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.



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

#### 4. DISCUSSIONS

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 and 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 0.50µg/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 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 hepatocellular 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 50mgL<sup>-1</sup> vitamin C and 100mgL<sup>-1</sup> 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. Cypermethrin-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<sub>50</sub> 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 (100mgL<sup>-1</sup> 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*.”

## REFERENCES

1. Christopher DN, Naresh SN, Ravindra K, Basedo K, Pavan K, Wazir, SI. Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to fresh water air breathing fish. *Channa punctatus* (Bloch.) International Aquatic Research. 2010; 3:29-44.
2. WHO. Glyphosate. Environmental Health Criteria Publication No. 159, the World Health Organization. Geneva Switzerland.1994.
3. Pérez GL, Torremorell A, Mugni H, Rodríguez P, Vera MS, De Nascimento M, Allende L, Bustingorry J, Escaray R, Ferraro M, Izaguirre I, Pizarro H, Bonetto C, Morris DP, Zagarese H. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecol. Appl.*, 2011; 17:2310–2322.
4. Woodburn AT. Glyphosate: production, pricing and use worldwide. *Pest Manage., Sci.* 2000; 56:309-312.
5. Okomoda VT, Ataguba GA, Ayuba VO. Hematological response of *Clarias gariepinus* fingerlings exposed to acute concentrations of Sunsate®. *Journal of Stress Physiology and Biochemistry*, 2013; 9(2):271-278.
6. Haux C, Larson A, Lithner G, Sjobeck M. A field study of physiological effects on fish in lead- contaminated lakes. *Environmental Toxicology and Chemistry*, 1986; 5:283-288.
7. Hemming JM, Waller WT, Chow MC, Denslow ND, Venables B. Assessment of the estrogenicity and toxicity of a domestic waste water effluent flowing through a constructed wetland system using biomarkers in male fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, 2001; 20:2268-2275.
8. Maheswaran R, Devapaul A, Velmurugan B, Ignacimuthu S. Haematological studies of freshwater fish *Clarias batrachus* exposed to mercuric chloride. *International Journal of Integrated Biology*, 2008; 2(1):49-54.
9. Udem SC, Asogwa O. Effects on haematological and biochemical parameters in albino mice fed *Ipomoea batatas* leaf aqueous extract. *Comprehensive Clinical Pathology*, 2011; 20:475-479.
10. Tavares-Dias M, Barcellos JFM. Peripheral blood cells of the armored catfish *Hoplosternum littorale* (Hancock, 1828); a morphological and cytochemical study. *Brazilian Journal of Morphological Science*, 2005; 22:215-220.
11. Winsto, GW, Di Giulio RT. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat Toxicol.* 1991; 19:137–161.
12. Chatterjee IB, Majunder AK, Nandi BK, Subramadian N. “Synthesis and some major functions of vitamin C in animals” *Ann .NY Acad. Sci.*, 1995; 258:24-47.
13. Enevide C, Arome C, Solomon A. A new method for determining acute toxicity in animal models. *Toxicology International*, 2013; 20(3):224-226.
14. Thrall MA, Weiser MG. Hematology in Hendrix. C.M editor Laboratory procedures for Veterinary Technicians. 4<sup>th</sup> edition Missouri: Mosby Inc: P 29-74. 2002
15. Higgins T, Beutler E, Doumas B. Measurement of haemoglobin in blood in Tietz fundamentals of Clinical chemistry, 6<sup>th</sup> edition CA. Burtis, ER Ashwood and DE Bruns. Saint Louis: M.O Sanders Elsevier. S24-52S. 2008.
16. Coles EH. Veterinary Clinical Pathology (4<sup>th</sup> edition). Saunders, W.B. Company Philadelphia. 110-115. 1986.
17. Bush BM. Interpretation of laboratory results for small animal clinician Blackwell Scientific London.1991.
18. Okomoda J, Ayuba VO, Omeji S. Hematological changes of *Clarias gariepinus* fingerlings exposed to acute toxicity of Formalin. Publication of Nasarawa State University, Keffi. PAT Patnsuk Journal.net. 2010.

19. Aderolu AZ, Ayoola SO, Otitolaju AA. Effects of acute and sublethal concentrations of Actellic on weight changes and haematology parameters of *Clarias gariepinus*. World Journal of Biological Research, 2010; 3:30-39.
20. Gluszcak L, Miron DS, Crestani M, Dafonseca MB, Pedron FDA, Duarte MF, Vieira VUP. Effects of glyphosate herbicide on acetylcholinesterase activity and metabolic and haematological parameters in Piava (*Leporinus obtusidens*). Ecotoxicol. Environ. Safety, 2006; 65:237-241.
21. Adeyemo OK. Haematological and Histopathological effects of cassava mill effluent in *Clarias gariepinus*. African Journal of Biomedical Research, 2005; 8:179-183.
22. Kori-Siakpere O, Ogbe MG, Ikomi RB. Haematological response of the African catfish *Clarias gariepinus* to sublethal concentrations of Potassium permanganate, Scientific Research and Essay, 2009; 4(5):457-466.
23. Ayuba VO, Ofojekwu PC, Francis A. Growth and haematological changes in *C. gariepinus* fingerlings exposed to *Datura imoxia* seed extract for 12 weeks on weight gain in the African catfish, Annals of Research in Nigeria, 2008; 6:46-55.
24. Joshi P, Deep H. Effect of Lindane and malathion exposure to certain blood parameters in a freshwater teleost fish *Clarias batrachus*. Poel. Res., 2002; 21:55-57.
25. Neelima P, Sunitha K, Gopala RN, Rao JCS. Haematological alterations in *Cyprinus carpio* as biomarkers of cypermethrin toxicity. Int. J. Curr. Res., 2015; 7(8):18864-18870
26. Khartun MA, Rahman MR, Islam MS. Effects of cypermethrin and diazinon on haematology of *Labeo rohita*. Int. J. Develop. Res., 2014; 4(5):953-957.
27. Akinrotimi OA, Gabriel UU, Ariweriokuma SV. Haematotoxicity of cypermethrin to African catfish *Clarias gariepinus* under laboratory conditions. Journal of Environmental Engineering and Technology, 2012; 1(2): 20-25.
28. Jasper R, Locatelli GO, Pilati C, Locatelli C. Evaluation of biochemical, haematological and oxidative parameters in mice exposed to the herbicide, glyphosate- Round up®. Interdisciplinary Toxicology, 2012; 5(3):133-140. doi:10.2478/V10102-012-0022-5.
29. Alfredo S, Roberto S. Gendusa C. *In vitro* evaluation of genomic damage induced by glyphosate on human lymphocytes. Environmental Science and Pollution Research, 2018; 25: 34693-34700.
30. Kondera E, Teodorczuk B, Lugowska K, Witeska M. Effect of glyphosate –based herbicide on haematological and hemopoietic parameters in common carp *Cyprinus carpio*. Fish Physiology and Biochemistry, 2018; 44(3):1011-1018.
31. Prasad G, Charles S. Haematology and leucocyte enzyme cytochemistry of a threatened yellow catfish *Horabagrus brachysoma*. Fish Physiology and Biochemistry, 2010; 36(3):435-443.
32. Cazenave J, Wunderlin DA, Hued AC, Bistoni MDA. Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae) captured from pristine and polluted water. Hydrobiologia, 2005; 537:25–33.
33. Clair A, Mesnage R, Travert C, Seralini GEA. Glyphosate based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro* and testosterone decrease at lower levels. Toxicology in vitro, 2012; 26(2):269-279.
34. Jiraungkoorskul W, Uptham ES, Kruatrachue M, Sahaphong S Vichasrigrams, Okethitiyook. Biochemical and Histopathological effects of glyphosate herbicide on Nile tilapia *Oreochromis niloticus*. Environ.Toxicol., 2003; 8(4):260-267.
35. Jyothi B. Narayan G. Certain pesticide–induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus*. Food Chem. Toxicol., 1999; 37:417-42.
36. Udeh GN, Oti EE. Behavioural and some physico-chemical assessments of the freshwater catfish, *Clarias gariepinus* exposed to acute concentrations of Delsate® herbicide (Glyphosate). Journal of Aquatic Sciences, 2014; 29(2A):275-283.
37. Attia Hala. Hepatoprotective effect of *Rubus idaeus* leaves against CCL<sub>4</sub> – induced liver injury via antioxidant,anti-inflammatory and antipoptotic mechanism. European Journal of Medicinal Plants, 2016; 14(1):1-16.

38. Sanchez-Moreno C, Larraun JA. Main methods used in lipid oxidation determination. Food Science and Technology International, 1998; 4:391-399.
39. Adel ME Shalaby, Abo-Hammad Abbassa. The opposing effect of ascorbic acid (vitamin C) on ochratoxin toxicity in Nile tilapia. (*Oreochromis niloticus*). Acta Polonica, 2009; 2:18-22.
- [40]. Korkmaz N, Cengiz EI, Unlu E, Uysal E, Yanar M. Cypermethrin-induced Histopathological and biochemical changes in Nile Tilapia (*Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid. Environmental Toxicology and Pharmacology, 2009; 28:198-205.
41. Youness ER, Agha FE, El-Toukhy ES, El-Naggars MM, Selim AAI, Ibrahim, AMM. The protective effect of orange juice on glyphosate toxicity in adult male mice. Journal of Chemical and Pharmaceutical Research, 2016; 8(2):13-28.

UNDER PEER REVIEW