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Research Article

Haematology, Oxidative Stress and Micronuclei Frequency of Clarias Gariepinus Exposed to Glyphosate based Herbicide Glycot® GBHG

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Abstract Glyphosate (N-phosphonomethyl glycine) is a broad-spectrum systemic herbicide used extensively in weed control in Nigeria. The present study was designed to evaluate the toxicity effects of glyphosate-Glycot® on post juveniles (50±1.96g; 22±1.2cm n=300) of *Clarias gariepinus* exposed to triplicate acute doses: 31, 33, 35, 37 and 39 mg glycot L^{-1} of clean water and to 8 day sub-acute doses: 3.39, 6, 78 and 16.95mgL^{-1} of the same, corresponding to $^{1}/_{10}$ LC_{50}=3.39 mgl^{-1}, $^{1}/_{5}$ LC_{50}=6.78 mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1}, A set of fish were also maintained simultaneously in water as the control (0.0 mg glycot /L of clean water), during the exposure periods. The 96 hours LC_{50} of the herbicide to the fish was determined at 33.39 mgL^{-1} which corresponds to 96h safety dose of $^{1}/_{100}$ LC_{50} = 0.35 mg/L. Blood samples were taken at intervals of day 1, 4 and 8 for assessment of micronucleus frequency, hematology and antioxidant enzymes. The hematological parameters were significantly reduced in the treated values of PCV and ranged from 20.00±0.19 – 23.33±0.55 %) below the value of 28.33±0.61% recorded in the control. Similarly, the red blood cells RBC, and haemoglobin HB recorded inhibited ranges of 8.58±0.21 - 9.10±0.03 x 10^{6} mm^{3} below their elevated respective controls of 10.06±0.03 x 10^{6} mm^{3} and 8.73±0.05 g/dL. However, the white blood cells WBC and platelets PL recorded elevated ranges of 12934.00±544.68 - 13700.00±485.72 x 10^{3} mm^{3} and 15933.33±322.79 above their respective control values of 9466.66 0.09±96.86 x 10^{3} mm^{3} and 15633.33±181.89. Catalase CAT was significantly ($p<0.05$) inhibited in treatments compared to control, and it ranged from the highest value of 0.74 umol mm^{-1} mg $protein^{-1}$ in control to the lowest value of 0.21 umol mm^{-1} mg $protein^{-1}$ in exposed group to 16.95 mg/L on day 4 but returned to control value on day 8 in all the treatments. Superoxide dismutase SOD was significantly ($p<0.05$) inhibited among exposed fish compared to control on days 1-4. A range of 11.60 U mg $protein^{-1}$ in control on day 1 to lowest value of 6.50 U mg $protein^{-1}$ returned to control value on day 8. Similarly, GPX was significantly inhibited to a lowest value of 4.25 in fish exposed to 3.39 mg/L compared to the highest value of 9.36 in control fish on day 4 but returned to control value with a high value of 9.25 $umol^{-1}$ min $protein^{-1}$. Similarly, Lipid peroxidation LPO ranged from the highest value of 6.8±0.00058 mMole/TBARS/ mg protein in 16.95mg/L to a lowest value of 6.41 mMole/TBARS/ mg protein in control on day 4, returned to the control value on day 8. The mean values of micronuclei frequency in the treatments were significantly ($p<0.05$) elevated above the control value of 8.50±0.93 to the highest value of 406.66±15.03. This finding indicated that GBHG impaired haematology, antioxidative stress enzymes and cytogenic

potential of *C. gariepinus* and could serve as an early warning signs toward the avoidance of its ecotoxicological hazards in aquatic ecosystems in Nigeria.
Keywords *Ecotoxicological hazards; Micronucleus frequency; Haematology; Anti-oxidative stress enzymes; Catfish*

1. Introduction

Glyphosate based herbicide Glycot® is a commercial formulation of glyphosate (N-phosphonomethyl glycine) based herbicide, whose main surfactant is 15% polyethoxylated tallow amine POEA without the listing of other ingredients (Cox and Surgan, 2006). It was launched in India but has wide application in Nigeria as a broad spectrum in the control of broad-leaved weeds, grasses and sedges in the cultivation of cassava, sugar cane, yam, and potatoes. Its main route into the aquatic environment is indirect from runoff of agricultural fields along waterways occasioned by extensive and unregulated usage, although Tsui and Chu (2008) reported direct use of a related glyphosate herbicide, Roundup in the control of aquatic weeds in fish ponds, lakes, canals and slow running water. Surfactants play the key useful roles and support the adhesion, wetting, spreading and uptake through the leaf in plants but many unspecified members have been reported on related herbicides to be very toxic compared to the original glyphosate and other glyphosate based herbicides GBH (Williams et al., 2000; Santos et al., 2005; Modesto and Martinez, 2010). The toxicity of GBHG is however very scarce.

The study of the alteration in fish blood by herbicides and pesticides has been widely used by several workers to evaluate fish health status (Banaee et al., 2011; Ezike et al., 2017). The use of such haematological parameters such as packed cell volume PCV, numbers of red blood cells RBC and white blood cells WBC, haemoglobin HB and platelets PL has been noted to be indicators of toxicity with wide application in environmental monitoring and aquatic animal toxicological studies (Bacellos, 2003).

Pollutants and several xenobiotics have been implicated to induce reactive oxygen species ROS including hydrogen peroxide H_2O_2, Superoxide anion and hydroxyl radical which often due to their high reactivity result to lipid, protein and carbohydrate damages. Many enzyme activities that act as antioxidants can be used as a biomarker for assessment of pesticide contamination in water (Chandrasekara and Pathirantne, 2005). Biochemical markers, like lipid peroxidation (LPO) and antioxidant enzymes such as catalase CAT, Superoxide dismutase SOD and glutathione peroxidase GPX are widely used to assess the toxic stress, integrity of the immune system and tissue damage in different organisms (Ansari et al., 2011; Dabas et al., 2012). They have the advantage of being sensitive, highly conserved between species and often easier to measure as stress indices (Agrahari et al., 2007). The antioxidant enzymes have been shown to work in synergistic manner to protect against oxidative stress and tissue specific damage. Oxidative stress develops when there is imbalance between pro-oxidants and anti-oxidants ratio, leading to the generation ROS and consequently to membrane LPO (Scandalios, 2005; Modesto and Martinez, 2010; Jaqueline and Biller, 2017) and impaired chromosomal damage (Fenech, 2011).

Micronucleus are essentially lagging whole chromosomes or its fragments which failed to correctly attach to spindle during segregation of chromosomes in anaphase in a mitotic cell division of animals, often without a true nucleus but are enclosed with a nuclear membrane, structurally similar to but smaller in size compared to conventional nucleus. Fenech et al. (2011) reported that micronucleus assay systems are very economical, require much less skill in scoring than conventional metaphase tests, and are much faster than these conventional tests. Since micronucleus assays reflect chromosomal aberrations reliably and rapidly, they are extremely useful for a quick assessment of chromosomal damage. Marked increase in the number of cells with micronuclei can be concluded that the chemical induces structural and/or numerical chromosomal damage. Since micronucleus tests

must be performed on actively dividing RBC produced from bone marrow of animals are ideal candidates for the test.

Sancho et al. (2000) noted that teleost fish have proved to be good models to evaluate the toxicity and effects of contaminants on animals since their biochemical responses are similar to those of mammals. The African catfish *C. gariepinus* is an important economic fish found in most African rivers and is considered as a potential bioindicator species (Shagbanmu et al., 2018).

2. Materials and Methods

2.1. Experimental Fish, Herbicide and Range Finding Test

The test herbicide GBHG is a commercial formulation of glyphosate 41% SL with a trade name Glycot, was produced by Sabero Organics Ltd., Gujrat, India and supplied and distributed by Afcott Nigeria PLC. The herbicide was purchased from Ogbete main market, Enugu North Enugu, Nigeria. Three hundred **(**300) post Juveniles of African catfish, *Clarias gariepinus* (Burchell, 1822) of about 8–12 weeks old with an average weight of 50g±1.96g and 22cm±1.2cm of length were sourced from Nature Blend Remedio Farms, a commercial farm in Onuagu Amon, Onyeama hills Ngwo, along Enugu-Onitsha expressway, Enugu. The fish were transported in FAO aerated fish transit tanks to Heldin Fisheries Laboratory Emene, Enugu where they were acclimatized under laboratory conditions for 2 weeks (14 days) in four plastic aquaria tanks of 300L capacity, containing dechlorinated and aerated tap water. They were fed twice daily at 3% body weight with Copens feed (3.2 mm) at 08.00hrs and 17.00 hours and water changed daily during the acclimatization period. Feeding was however terminated 24 hours before the commencement of the experimental study to empty their stomach and avoid pollution of the water with their feces. Permission from the Committee for the protection and care of animals Enugu State University of Science and Technology Enugu was observed prior to and throughout the research. A 24h range finding test was first carried out prior to the toxicity test to ascertain the concentrations of the test solution for definitive test.

2.2. Acute Toxicity

Acute Toxicity of GBHG was conducted following OECD (1992), guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200 L capacity glass aquaria. Eighteen (18) fish per treatment were randomly exposed to 5 experimental treatments (31, 33, 35, 37 and 39) and a control (0.0) mgL^{-1} of GBHG. Mortality and Survival rate were monitored and recorded at 24, 48, 72 and 96h intervals respectively. The 96h median lethal concentration (LC_{50}) value was determined from the bioassay results using the Probit analysis method described by Finney (1971). The LC_{16}, LC_{50}, and LC_{84} values were read off from the Probit versus log concentration line graph. Feeding was suspended 24h prior to and during acute test. The slope function (S) and F constant were determined by the formula:

$S = \frac{1}{2} (LC_{84}) + (LC_{50})$, F = antilog (2.77log S)

LC_{50} LC_{16} $\sqrt{N}$

S = Slope; F = factor Constant and N = Number of fishes tested at concentrations whose effects are between 16% and 84% mortality (Lichfield and Wilcoxon, 1949).

2.3. Subacute Toxicity

Sub-Acute Toxicity Fish to GBHG were exposed to four (3) sub lethal treatments and a control (0.00mg/L) for 192 hours (8 days). The resultant concentrations of the herbicide were ($^{1}/_{10}$

LC_{50}=3.39mgL^{-1}, $^1/_5$ LC_{50}=6.78mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1} and control 0.0 mgL^{-}1 of GBHG. The test solution was changed and re-treated every 48h to counter-balance the decreasing pesticide concentration due to its hydrolysis in water. Samples for the haematology, oxidative stress and micronuclei frequency were taken in triplicates at 24h (day 1), 96h (day 4) and 192h (day 8) to investigate the hematology and micronuclei effect of glyphosate in the peripheral blood cells and antioxidant enzyme activity and lipid peroxidization in liver during the sub-lethal test. Feeding was served at 5% of body weight once every day using a commercial diet.

2.4. Haematology Assay

Blood samples were collected by incising the caudal vein of the caudal peduncle with a heparinized syringe & EDTA vials and was used to evaluate the effects of glyphosate in the fish blood by estimating according to Ochei and Kolhatkar (2000), these hematological parameters; Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Hemoglobin (Hb/dl), Platelets and total white blood cell count (WBC).

Red Blood Cells (RBC) Count

The red blood cells count was determined by the method of Ochei and Kolhatkar (2000) using a Microscope, Haemocytometer, RBC pipette and RBC diluting fluid (Sodium citrate). The blood specimen was diluted at 1:200 with RBC diluting fluid and cells were counted under (40×) magnification by using a Neuber counting chamber. The number of cells were calculated and reported as the number of red cells/cu.mm of whole blood.

Total RBC (mm^3) = N x 1 x 1 x 200

0.2 0.1

N = Numbers of cells counted; 0.1 = depth of the chamber; 0.2 = area counted; 200 = dilution factor.

The white blood cell count was determined following the method described by Ochei and Kolhatkar (2008). The glacial acetic acid lyses the red cells while gentian violet slightly stains the nuclei of the leucocytes. The blood specimen was diluted1:20 in a WBC pipette with the diluting fluid and the cells were counted under low power microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cell/cu.mm of the whole blood.

Total WBC (mm^3) = N × 20

0.1 × A

N = Number of cells counted; 0.1 = depth of the chamber; A = area counted; 20= dilution factor.

Packed cell volume (PCV) was estimated as described by Ochei and Kolhatkar (2000). Blood sample was taken with a heparinized capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhematocrit centrifuge and spun at 10,000 rpm for 5 minutes. Spunned tubes were placed into a specially designed scale and PCV was read as a percentage.

PCV% = Packed RBC column height × 100

Total Blood Column Height

Hemoglobin (Hb/dl) concentration was determined using cyanomethaglobin technique as outlined by Ochei and Kolhatkar (2000). 4ml of Drabkin's solution which contains potassium ferricyanide,

potassium cyanide and potassium dihydrogen phosphate was well mixed with the haemoglobin in a test tube and allowed to stand for 10mins at room temperature. The ferricyanide formed methemoglobin which was converted to a colored cyanmethemoglobin by the cyanide. The absorbance was measured colorimetrically at 540nm with Drabkin's solution as a blank.

2.5. Oxidative Stress

Antioxidative Stress Enzyme Assay

Fish livers were weighed and homogenized in 0.1M potassium phosphate at 15000g, 4^0C for 20minutes. The supernatant was collected for biochemical parameter of the antioxidant enzymes and lipid peroxidation. The catalase (CAT) in the blood was determined according to the method of Takahara et al. (1960) which involved H_2O_2 breakdown, and was measured spectrophotometrically at 240 nm. Enzyme activity was expressed as nanomoles of H_2O_2 decomposed min/L mg/L protein. Superoxide dismutase (SOD) activity were determined using the method of Misra and Fridovich (1972), based on the oxidation of epinephrine-adrenochrome transition by the enzymes. Superoxide dismutase activity was assed spectrophotometrically at 420 nm and expressed as the amount of enzyme mg/L of protein required to give 50% inhibition of epinephrine auto-oxidation. Glutathione peroxidase (GPX) activities was assayed according to Paglia and Valentine (1967) which was based on the oxidation of glutathione in the presence of NaN_3.

Lipid Peroxidation

Lipid peroxidase LPOX in the liver tissue was determined by estimation of thiobarbituric acid reactive substances (TBARS), according to Sharma and Krishna-Murti (1968). TBARS concentration was measured spectrophotometrically at 535 nm at molar extinction coefficient of 156 Nm cm/L in mMole/TBARS/ mg protein.

2.6. Micro Nucleus Frequency

The genotoxicity assessment/potential of the pesticide was assessed by micronuclei assay. Peripheral blood samples were collected from the caudal vein and smeared on clean, grease-free, frosted glass slides. The slides were fixed in methanol for 10mins and left to air dry at room temperature and finally stained with 6% Geimsa in Sorenson buffer (pH 6.9) for 20mins. After dehydration through graded alcohol and clearing in Xylene, slides were mounted in a mixture of Distyrene (Polystyrene), Plasticizer (tricresyl phosphate) and Xylene. From each slide, 1000 erythrocyte cells were scored under light microscope under 100 magnifications. Non refractive circular or ovoid chromatin bodies smaller than one third of the main nucleus and displaying same staining and focusing patterns as the main nucleus were scored as the micronucleus (Carrasco et al., 1990; Al-Sabti and Metcalfe, 1995; Nwani et al., 2013). The micronuclei frequency was calculated as:

MN (%) = Number of cells containing micronuclei × 100 / Total number of cells counted 1

2.7. Water Quality Parameters

The physico-chemical parameters of the test water were analyzed daily, using standard methods (APHA, 2005, AOAC, 2005) and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1}, temperature 27.75 ± 0.5^0C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}).

2.8. Statistical Analysis

The data obtained were analyzed using statistical package SPSS (Version 22). These data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences among

treatments at 5%. The obtained results and mean values (±SEM) of the toxicity experiment was analyzed using the statistical package SPSS (Version 22). Significantly different results were compared by Duncan's multiple range test with accepted level of significance at $p<0.05$ (Duncan, 1955).

3. Results

Table 1: *The cumulative percentage mortality rate and Probit mortality of Clarias gariepinus exposed to different concentrations of GBHG for 96 hours*

Conc. (mg/L)	Log Conc. (mg/L)	Fish exposed	Cumulative mortality				% Survival	% Mortality	Probit mortality
			24	48	72	96			
Control 0.0	0	10	-	-	-	-	100	0	-
31	1.50	10	-	1	1	1	70	30	4.48
33	1.52	10	-	2	1	1	60	40	4.75
35	1.54	10	1	1	1	2	50	50	5.00
37	1.56	10	1	1	2	3	30	70	5.52
39	1.59	10	1	2	2	3	0	90	6.28

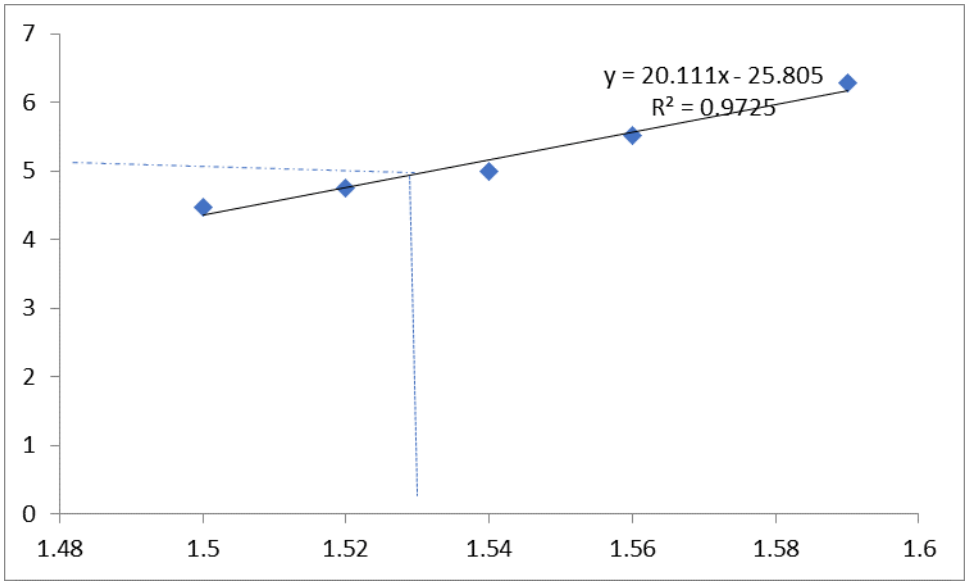


Figure 1: *Logarithmic Probit line to determine 96 h LC_{50}*

3.1. Acute Toxicity

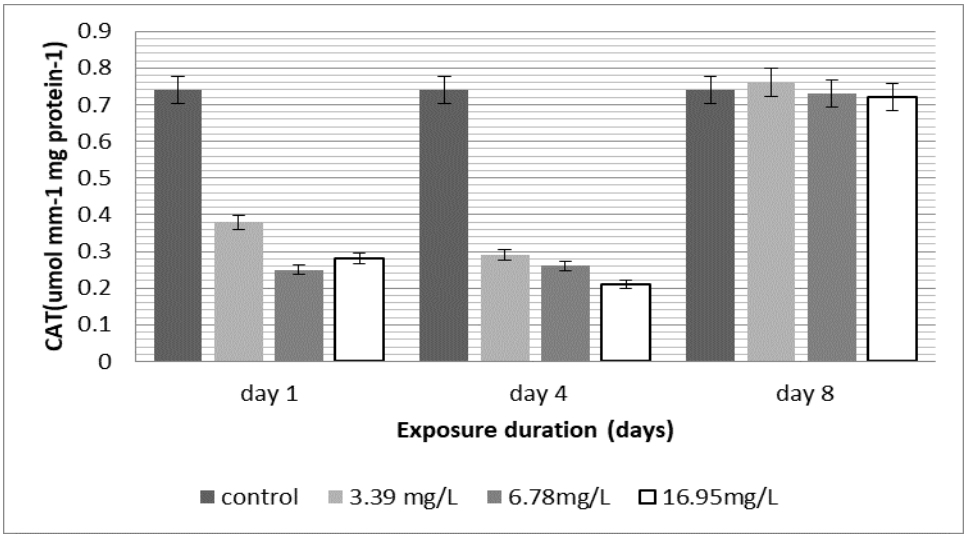
Fish were exposed to acute doses: 31, 33, 35, 37, 39 and control (0.00) mg glycot L^{-1} of clean water and obtained 96h LC_{50} value of 33.9 mgL^{-1} and a logarithmic Probit line y = 20.11x - 25.80, R^2 = 0.972. Sub -acute doses: 3.39, 6.78, and 16.95mgL^{-1} of the same, corresponding to $^1/_{10}$ LC_{50}=3.39 mgL^{-1}, $^1/_5$ LC_{50}=6.78 mgL^{-1}, ½ LC_{50}=16.95 mgL^{-1}, were generated. The 96 h LC_{50} of the herbicide to the fish corresponds to 96h safety dose of $^1/_{100}$ LC_{50} = 0.339 mgL^{-1}.

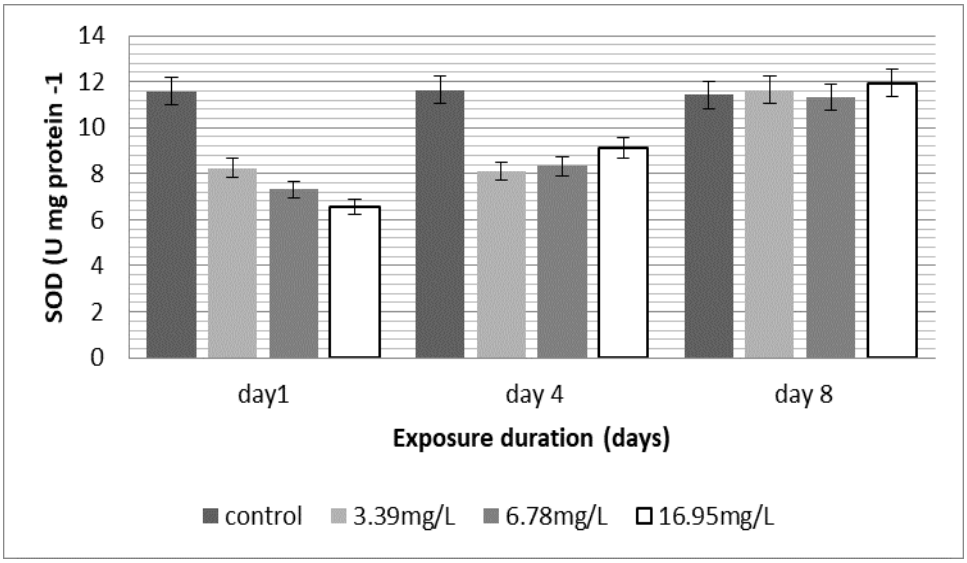
Table 2: *Hematological parameter of Clarias gariepinus exposed to different concentrations of GBHG for 8 days*

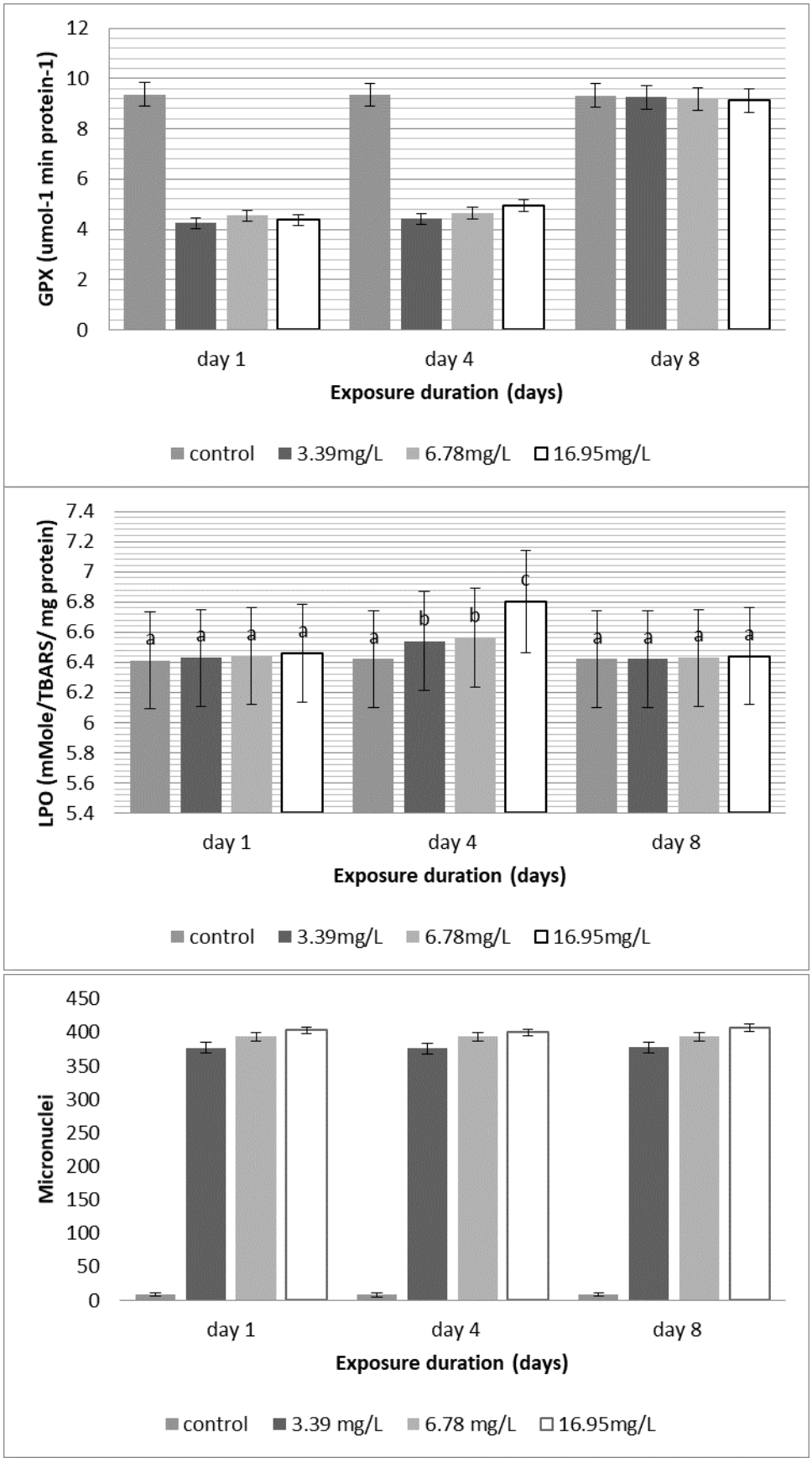
Parameters	Concentrations (mg/L)	Exposure days		
		1	4	8
PCV	Control	28.00±0.38 [a]	27.33±0.40 [a]	28.33±0.61 [a]
	3.39	23.00±0.33 [b]	23.00±0.19 [b]	23.33±0.55 [b]

	6.78	21.33±0.29 ^{b}	20.33±0,29 ^{b}	21.33±0.11 ^{b}
	16.95	21.33±0.58 ^{b}	21.00±0.66 ^{b}	20.00±0.19 ^{b}
RBC	Control	10.05±0.03 ^{a1}	10.06±0.03 ^{a1}	10.05±0.03 ^{a1}
	3.39	8.60±0.06 ^{b1}	8.61±0.06 ^{b1}	8.61±0.06 ^{b1}
	6.78	9.09±0.12 ^{a2}	9.10±0.12 ^{a2}	9.10±0.12 ^{a2}
	16.95	8.58±0.21 ^{b1}	8.58±0.21 ^{b1}	8.59±0.21 ^{b1}
WBC	Control	9433.33±125.21 ^{a1}	9400.00±153.96 ^{a1}	9466.66±96.86 ^{a1}
	3.39	13033.33±544.44 ^{a2}	12934.00±544.66 ^{a2}	13133.66±544.72 ^{a2}
	6.78	13333.33±357.66 ^{a2}	13233.33±363.28 ^{a2}	13400.00±360.55 ^{a2}
	16.95	13633.33±481.25 ^{b1}	13566.66±477.00 ^{a2}	13700.00±485.72 ^{b1}
Hb/dl	Control	8.73±0.05 ^{a1}	8.63±0.07 ^{a1}	8.73±0.05 ^{a1}
	3.39	7.43±0.09 ^{b1}	7.56±0.09 ^{b1}	7.50±0.12 ^{b1}
	6.78	7.70±0.12 ^{a2}	7.73±0.13 ^{a2}	7.70±0.11 ^{a2}
	16.95	7.86±0.11 ^{a2}	7.73±0.12 ^{a2}	0.96±0.10 ^{a2}
Platelets	Control	15600.00±203.67 ^{a}	15600.00±220.94 ^{a}	15633.33±181.89 ^{a}
	3.39	13900.00±472.58 ^{a}	13866.66±449.41 ^{a}	13933.33±496.40 ^{a}
	6.78	15866.66±327.91 ^{a}	15533.33±298.96 ^{a}	15933.33±322.79 ^{a}
	16.95	15633.33±401.07 ^{a}	15600.00±397.67 ^{a}	15600.00±384.41 ^{a}

Mean Values with different alphabetic superscripts (a1, a2, b1 and b2) differ significantly ($p<0.05$) between concentrations in each parameter.







Different alphabetic superscripts differ significantly ($p<0.05$) between concentrations in each parameter.

Figure 2: *Mean of CAT, SOD, GPX and LPO C. gariepinus to GBHG for 8 days*

3.2. Haematology

The hematological parameters were significantly reduced in the treated values of PCV and ranged from 20.00±0.19 – 23.33±0.55 %) below the value of 28.33±0.61% recorded in the control (table 2). Similarly, the red blood cells RBC, and haemoglobin HB recorded inhibited ranges of 8.58±0.21 - 9.10±0.03 x 10^6 mm^3 below their elevated respective controls of 10.06±0.03 x 10^6 mm^3 and 8.73±0.05. However, the white blood cells WBC and platelets PL recorded elevated ranges of 12934.00±544.68 - 13700.00±485.72 x 10^3 mm^3 and 15933.33±322.79 above their respective control values of 9466.66 0.09±96.86 x 10^3 mm^3 and 15633.33±181.89.

3.3. Antioxidative Enzymes and Lipid Peroxidation

Catalase CAT was significantly ($p<0.05$) inhibited in treatments compared to control, and it ranged from the highest value of 0.74 umol mm^{-1} mg $protein^{-1}$ in control to the lowest value of 0.21 umol mm^{-1} mg $protein^{-1}$ in exposed group to 16.95 mg/L on day 4 but returned to control value on day 8 in all the treatments (figure 2). Superoxide dismutase SOD was significantly ($p<0.05$) inhibited among exposed fish compared to control on days 1-4. A range of 11.60 U mg protein $^{-1}$ in control on day 1 to lowest value of 6.50 U mg protein $^{-1}$ returned to control value on day 8 (Figure 2). Similarly, GPX (Figure 2) was significantly inhibited to a lowest value of 4.25 in fish exposed to 3.5mg/L compared to the highest value of 9.36 in control fish on day 4 but returned to control value with a high value of 9.25 $umol^{-1}$ min $protein^{-1}$. Similarly, Lipid peroxidation LPO (Figure 2) ranged from the highest value of 6.8 mMole/TBARS/ mg protein in 16.95mg/L to a lowest value of 6.41 mMole/TBARS/ mg protein in control on day 4, returned to the control value on day 8.

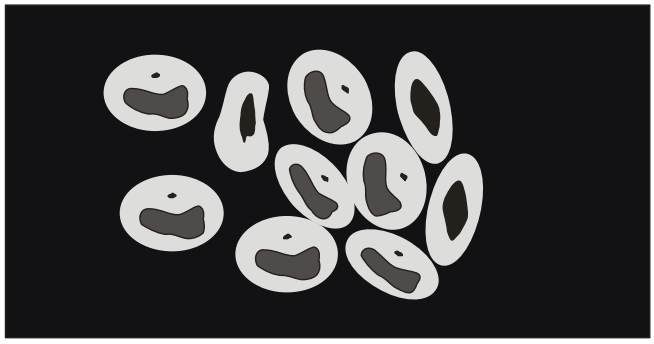


Plate 1: `*Photomicrograph of micronucleated erythrocytes in C. gariepinus exposed to GBHG*

Table 3: *Micronuclei frequencies in erythrocytes of Clarias gariepinus exposed to sub-lethal concentrations of GBHG for 8 days*

Parameters	Concentrations (mg/L)	Exposure days		
		1	4	8
Micronucleus	**Control**	8.50±0.93[a]	8.50±1.00[a]	8.50±0.88[a]
	3.39	376.66±8.01[b]	375.66±8.11[b]	377.66±7.92[b]
	6.78	393.33±6.75[b]	393.33±7.06[b]	393.33±10.45[b]
	16.95	403.33±4.84[b]	400.00±4.91[b]	406.66±15.03[b]

Mean values with different alphabetic superscripts differ significantly ($p<0.05$) between concentrations in each parameter.

3.4. Micronuclei Frequency

The mean values of micronuclei frequency in the treatments were significantly ($p<0.05$) elevated above the control value 8.50±0.93 to the highest value of 406.66±15.03 was about 48 times higher than the control (Table 3, Plate 1).

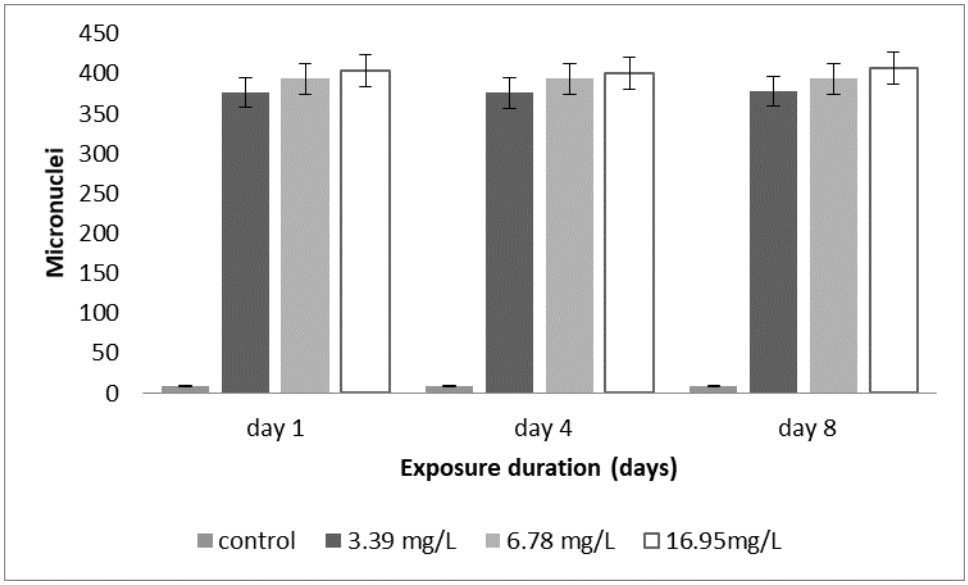


Figure 3: *Micronuclei aberration of C. gariepinus to GBHG for 8 days*

3.5. Water Quality Parameters

The physiochemical parameter of the test water in various treatment levels ranged from pH reading of 8.40 ± 0.1 – 9.8 ± 0.60; temperature reading vales of 25 ± 0.5 – 25.81 ± 0.05 and dissolved oxygen values of 5.0 ± 0.00 – 5.5 ± 0.01 respectively. These ranges of water quality were within the same standard for aquaculture.

4. Discussion

Acute of Glyphosate Toxicity based Herbicide Glycot to Exposed Fish

Substantial reports on the first and second generation groups of the original glyphosate Roundup and several other group of glyphosate based herbicides have been reported (Monsanto, 1995; Glusczak et al., 2009; Modesto and Matinez, 2010; Modesto and Martinez, 2010), but the toxicity effects of GBHG is almost unavailable in literature except maybe a lowered value of 24.6 mgL^{-1} 96h LC_{50} reported by Ani et al. (2017) on the same species of fish juveniles compared to ours of 33.39 mgL^{-1}. The present value also proved to be higher than respective values of 1.05mgL^{-1} and 13.6 mgL^{-1} by Ayoola (2008) and Langiano and Martinez (2008) when they exposed *Oreochromis niloticus and Prochilodus lineatus* to glyphosate and glyphosate-based herbicides respectively. However, the reported value of 108mgL^{-1} of glyphosate to tadpole's juveniles by Clements et al. (1997) is somewhat higher than the present, as well as respective higher values of 620 mgL^{-1} and 975 mgL^{-1} 96h LC_{50} reported by Shiogiri et al. (2012) when *Cyprinus carpio* and *Palloceroscaudi maculatus* were exposed to glyphosate and glyphosate commercial formulation (Rodeo) herbicides probably due to species and formulation variations. The present study indicated that GBHG formulation was less toxic compared with other reported cases which could be an indication of toxicity improvement on the mixture of POEA surfactants and other unspecified inert adjuvants and preservative substances. It has been shown that many manufacturers regard as a trade secret and seldom disclose (Cox and Surgan, 2006; Mesnage et al., 2013), and have been implicated to be responsible for the high level of toxicity reported on glyphosate based commercial formulations and roundup in general. Mesnage et al. (2019) noted that POEA talloamine present in GBH was more toxic compared to the original glyphosate in Roundup.

Haematological effects of glyphosate-based herbicides and indeed other pollutants have been used as a health status biomarker indicator of stressed animals (Modesto and Martinez, 2010). The present indication of inhibited PCV, RBC and HB by subacute doses of GBHG and elevated WBC and PL compared to the control corroborated with report of Glusczak et al. (2006) on the same active principle glyphosate but of different species and formulations on Leporinus obtusidens, suggest to the fact that there was a lowering in the production level from the haemopoietic areas in the exposed group because of hemodilution (Modesto and Martinez 2010) or it may have been hampered by some of unspecified inert substances in the formulation and rendered inefficient to produce sufficient parameters below the control. In order to respond to the forgoing, the fish elevated its WBC and PL to counter the effect of the herbicide and restore normalcy of the blood demand of the fish. However, it disagreed with the reported elevations on PCV, RBC and HB and lowered WBC and PL in fish exposed to a glyphosate based herbicide at short duration (Svodova, 1994). On the contrary, increased total number of leukocytes and platelets could be a defense response in the presence of surfactants and other inert portion of herbicide into the blood stream or as the organism's adaptive response ploy for towards effective immune defense (Barreto-Medeiros et al., 2005; cazenave et al., 2005; Dong et al., 2017; Li et al., 2017).

Anti-oxidative Stress Enzyme Activity Effect of GBHG

Antioxidants are substances that significantly delay or prevent the oxidation of oxidizable substrate (Franco and Martenez-Pinilla, 2017). The antioxidants produced by the body of animals act enzymatically to decrease the excess of free radicals through enzymatic components such as SOD, CAT and GPX (Halliwel et al., 1995). In our finding, inhibition of all three antioxidants CAT, SOD and GPX below their respective controls occurred on day 1 but there was further inhibition of CAT on day 4 followed by an elevation to control level on day 8 compared with elevation in both SOD and GPX on day 4, followed by further elevation to control level on day 8. Voet and Voet (1990) noted that antioxidants work in tandem to dismutase oxygen radicals in which SOD converts superoxide anion to hydrogen peroxide which is broken down to oxygen and water by catalase. The SOD– CAT system has been noted to be the first line of defense against oxygen toxicity, due to the inhibitory effects on the formation of oxygen radicals (Pandey et al., 2003), and these enzymes were frequently used as biomarkers, that indicated the production of reactive oxygen species (ROS) (Monteiro et al., 2006). The reduction in SOD activity after day 1 of exposure to the herbicide may be related to the production of oxidants. An excess of hydrogen peroxide may have reduced SOD activity, while the superoxide anion may be responsible for further decrease in CAT activity on day 4 (Bagnyukova et al., 2006; Scandalios, 2005). Thus, it may be reasonable to assume that hydrogen peroxide was responsible for the reduction observed in SOD activity while the reduction of CAT activity was due probably to accumulated superoxide anions not sufficiently neutralized by SOD. The activities of enzymes involved in animal's antioxidant system have been known to be a complex pathway of interactions among enzymes, because the activity and substrate product of one enzyme may influence the other. In the present work, the inhibition of CAT and SOD limited the antioxidant defenses of the fish during the first 4 days of exposure to the herbicide. But was restored on day 8 when the activities of the antioxidants approached the control level. Although GPX has been noted to function principally in the removal of organic peroxides, Maran et al. (2009) reported its involvement in the metabolism of hydrogen peroxide. The significant increased activity of GPX in the fish after day 4 to the herbicide indicated that the antioxidant pathway was stimulated, probably due to the increased production of peroxides. Thus, the activation of GPX may be an indication of adaptive response to compensate the inhibition of CAT at the period of exposure.

Reactive oxygen species left un-neutralized reacted with membrane lipids which produced lipid peroxidation, considered as one of the main consequences of oxidative stress (Ahmad et al., 2000; Ansari et al., 2011; Nwani et al., 2013). In this work, the occurrence of lipid peroxidation was indicated by a transient increase in LPO in fish to 17.5mgL^{-1} to the herbicide on day 4. However, LPO levels returned to control levels after day 4. Thus, it can be inferred that the antioxidant defense before day

8 of exposure was insufficient due to significant decreases in SOD, CAT and GPX activities which led to increased lipid peroxidation as a function of the presence of GBHG. However, these defenses returned to basal levels on day 8 and then were enough to combat the ROS, which prevented incidence of oxidative damage. Lushchak et al. (2009) using a similar method to quantify lipid peroxidation found that the herbicide Roundup original also did not affect lipid peroxidation in the liver of the goldfish after 96 h of exposure.

The significant increase in the number of micronuclei of exposed fish to GBHG, which was initiated on the first day of exposure, progressed to the 4th and 8th day of exposure (Cavas and Ergene-Gozukara, 2005). They may have been elicited by increased LPO that caused oxidative damage at the onset up to day 4. The restored LPO to control level on day 8 by increased activities of antioxidant enzymes of SOD and GPX could not however revert the micronucleus to basal level after day 8 (Cavas and Konen, 2007).

5. Conclusion

The results obtained in the present study may allow us to conclude that the commercial formulation of GBHG promoted alterations in hematologic and biochemical parameters of the experimental fish which were more evident in fish to the higher concentration of the herbicide. Hematologic changes occurred in decreased RBC, PVC and HB and increased WBC and PL, which probably represented the adaptive responses that assisted the organism to counteract the herbicide effects. Exposed fish showed reduction in SOD, CAT and GPX on day 1-4 which elicited LPO, however it returned to control levels after 8 days exposure to GBHG, when fish showed an increased activity of SOD and GPX apparently enough that combated ROS and prevented oxidative damage but could not restore elevated micronucleus frequency elicited at the unset of LPO, to control level.

References

Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M. and Raisudin, S. 2000. Induction of hepatic antioxidants in freshwater catfish (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure. *Biochim. Biophys. Acta.*, 1523, pp.37–48.

Agrahari, S., Pandey, K.C. and Gopal, K. 2007. Biochemical alteration in induced by monocrotophos in the blood plasma of fish Chanapunctatus (Bloch). *Pectic Biochem. Physiol.*, 88, pp.268-272.

Al- Sabti, K. and Metcalfe, C.D. 1995. Fish micronuclei for assessing genotoxicity in water. *Mtat. Res.*, 343, pp.121-135.

Ani, L.C., Nwamba, H.O., Ejilibe, C.O. and Nwani, C.D. 2017. Acute toxicity of glyphosate-based herbicide glycot on Juvenile African catfish *Clarias gariepinus* (Burchell 1822). *J Fish. Livest. Prod*., 5, p.252.

Ansari, R.A., Rahman, A., Kaur, M., Anjum, S. and Raisuddin, A. 2011. Invivo cytogenic and oxidative stress inducting effects of cypremethrin in freshwater fish, *Chana punctatus*. *Ecotoxicol. Environ. Saf.*, 74, pp.150-156.

AOAC, 2005. Association of Official Analytical Chemists: Official methods of water analysis 20th ed. Washington DC, USA. pp.452-456.

APHA (American Public Health Association, American works Association and water Environmental Federation) 2005. Standard Methods for the Examination of Water and Wastewater 21st ed. APHA, Washington, DC, pp.20001-23710.

Ayoola, S.O. 2008. Toxicity of glyphosate herbicide on Nile Tilapia (*Oreochromis niloticus)* juvenile. *Afri. J. Agric. Res.*, 3, pp.825-834.

Banaee, M., Sureda, A.R. and Ahmadi, K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). *Pest. Biochem. Physiol.*, 99, pp.1-6.

Bagnyukova, T.V., Chahrak, O.I. and Lushchak, V.I. 2006. Coordinated response of goldfish antioxidant defenses to environmental stress. *Aquat. Toxicol.*, 78, pp.325–331.

Barcellos, L.J.G., Kreutz, L.C., Rodrigues, L.B., Fioreze, I., Quevedo, R.M., Cericato, L., Conrad, J., Soso, A.B., Fagundes, M., Lacerda, L.A. and Terra, S. 2003. Haematological and biochemical characteristics of male jundiá (Rhamdia Quelen, Quoy & GaimaRDT, Pimelodidae): changes after acute stress. *Aquacul. Res.*, 34, pp.1465-1469.

Carrasco, K., Tilbury, K.L. and Myers, M.S. 1990. Assessment of the piscine micronucleus test as an in-situ biological indicator of chemical contaminant effects. *Can. J. Fish Aquat. Sci.*, 47, pp.2123–2136.

Chandrasekara, H.U. and Pathirantne, A., 2005. Influence of low concentrations of trichlorfon on haematological parameters and brain acetylcholinesterase activity in common carp *Cyprinus carpio*. *Aquaculture Res.*, 36, pp.144–149.

Clements, C., Ralph, S. and Pertas, M. 1997. Genotoxicity of selected herbicides in Rana catesbeiana tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. *Environ. Mol. Mutagen.*, 29, pp.277–288.

Cox, C. and Surgan, M. 2006. Unidentified inert ingredients in pesticides: Implications for human and environmental health. *Environ. Health Pers.*, 114 (12), pp.1803-1806.

Dabas, A., Nagpure, N.S., Kumar, R., Kushwaha, B. and Pavan Kumar Lakra, W.S. 2012. Assemment of tissue-specific effect of cadmium on antioxidant defense system and lipidbperoxidation in freshwater murrel, *Channa punctatus*. *Fish Physiol. Biochem*., 38, pp.468–482.

Cavas, T. and Ergene-Gozukara, S. 2005. Micronucleus test in fish cells: a bioassay for in situ monitoring of genotoxic pollution in the marine environment. *Environ. Mol. Mutagen.*, 46, pp.64–70.

Cavas, T. and Konen, S. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22(4), pp.263–268.

Cazenave, J., Wunderlin, D.A., Hued, A.C. and Bistoni, M.D.A. 2005. Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. *Hydrobiologia*, 537, pp.25–33.

Duncan, A.C. 1955. Multiple range test finding: determination of confidence level differences among significant treatment means. Root Foundation Academy Press Oxford University, London. pp.24-32.

Dong, M., Liang, Y, Ramalingam, R., Tang, S.W., Shen, W., Ye, R., Gopalakrishnan, S., Au, D.W.T. and Lam, Y.W. 2017. Proteomic characterization of the interactions between fish serum proteins and waterborne bacteria reveals the suppression of antioxidative defense as a serum-mediated antimicrobial mechanism. *Fish Shellfish Immunol.*, 62, pp.96-106.

Ezike, C.O., Echor, F.O., Malachy, N.O.A. and Vera, L.M. 2017. Butrylacetycholinesterase activites in liver and plasma, liver glycogen and plasma glucose content, haematology and behaviour of clariid catfish *Clarias gariepinus* to Dichlorvos. *International Journal of Advanced Fisheries and Aquatic Science*, 3(1), pp.90-105.

Fenech, M., Kirsch-Volders, M., Natarajan, A.T., Surralles, J., Crott, J.W., Parry, J., Norppa, H., Eastmond, D.A. and Tucker, J.D. 2011. Molecular mechanisms of nucleoplsmic bridges and nuclear bud in mammalian and human cells. *Mutagenesis*, 26(1), pp.126-13.

Finney, D.J. 1971. Probit Analysis. The University press, Cambridge. p.333.

Franco, R. and Martínez-Pinilla, E. 2017. Chemical rules on the assessment of antioxidant potential in food and food additives aimed at reducing oxidative stress and neurodegeneration. *Food Chem.*, 235, pp.318-323.

Halliwel, l.B. 2009. The wanderings of a free radical. *Free Radic. Biol. Med.*, 46, pp.531-542.

Glusczak, L., Miron, D.D.S., Crestani, M., Da Fonseca, M.B., Pedron, F.D.A., Duarte, M.F. and Vieira, V.U.P. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in Piava (*Leporinus obtusidens*). *Ecotoxicol. Environ. Saf.*, 65, pp.237–241.

Jaqueline, D. Biller, A.N.D., Leonardo, S. and Takahashi Ortes-Silva, R. 2017. A new approach to feed frequency studies and protein intake regulation in juvenile pirarucu. *An Acad Bras Cienc.*, 89, pp.1243-1250.

Glusczak, L., Moraes, B.S., Simões, R.R., Schetinger, M.R.C., Morsch, V.M. and Loro, V.L. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comp. Biochem. Physiol.*, 146 C, pp.519-524.

Langiano, V.C. and Martinez, C.B. 2008. Toxicity and effects of glyphosatebased herbicide on the Neotropical fish *Prochilodus lineatus*. *Comp. Biochem. Physiol.*, 147, pp.222-231.

Lichfield, J.T. and Wicoxon, T. 1949. A simplified method of evaluating dose effect of pharmacological experiments. *J Pharmacol Exp Ther.*, 96, pp.99-115.

Li, H., Jiang, W., Liu, Y., Jiang, J., Zhang, Y., Wu, P., Zhao, J., Duan, X, Zhou, X. and Feng, L., 2016. The metabolites of glutamine prevent hydroxyl radical-induced apoptosis through inhibiting mitochondria and calcium ion involved pathways in fish erythrocytes. *Free Rad. Biol. Med.*, 92, pp.126-140.

Luschak, O.V., Kubrak, O.I. and Storey, J.M. 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissue. *Chemosphere*, 76, pp.932-937.

Maran, E., Fernández, M., Barbieri, P., Font, G. and Ruiz, M.J. 2009. Effects of four carbamate compounds on antioxidant parameters. *Ecotoxicol. Environ. Saf.*, 72, pp.922–930.

Mesnage, R., Benbrook, C. and Antoniou, M.N., 2019. Insight into the confusion over surfactant co-formulants in glysophate-based herbicides. *Food Chem. Toxicol.*, 128, pp.137-145.

Mesnage, R., Bemay, B. and Seralini, G.E. 2013. Ethoxylated adjuvents of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 16, 313 (2-3), pp.122-128.

Misra, H.P. and Fridovich, L. 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247, pp.3170-3175.

Modesto, K.A. and Martinez, C.B.R. 2010. Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*, 81, pp.781–787.

Modesto, K.A. and Martinez, C.B.R. 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinestrase in muscle and brain of the fish Ponchilodus lineatus. *Chemophere*, 78, pp.294-299.

Monsanto, C.O. 1995. Backgrounder- Glyphosate and environmental fate studies. St. Louis, Missouri, Monsanto. pp.1-4.

Monteiro, D.A., Almeida, J.A., Rantin, F.T. and Kalinin, A.L. 2006. Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). *Comp. Biochem. Physiol.*, 143C, pp.141–149.

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B. and Lakra, W.S. 2013. DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish *Channa punctatus*. *Environmental Toxicology Pharmacology*, 36, pp.539-547.

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S. 2013. Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine*. J. Drug Chem. Toxicol.*, pp.1-8.

Ochei, J. and Kolhatkar, A. 2000. Medical Laboratory Science: Theory and Practice. TetaMcGraw-Hill Publishing Company Limited, New Delhi, pp.165-166.

OECD, 1992. Organization for Economic Co-operation and Development (OECD). Guideline 203. OECD Guideline for Testing of Chemicals: Fish, Acute Toxicity Test.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B. and Raisuddin, S. 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). *Sci. Total Environ.*, 309, pp.105–115.

Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and the qualitative characterization of erythrocyte gluthathione peroxidase. *J. Lab. Clin. Med.*, 70, pp.158-169.

Sancho, E., Cerón, J.J. and Ferrando, M.D. 2000. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in Anguilla anguilla. *Ecotoxicol. Environ.*, Saf. 46, pp.81–86.

Santos, J.B., Ferreira, E.A., Kasuya, M.C.M., Silva, A.A. and Procópio, S.O. 2005. Tolerance of Bradyrhizobium strains to glyphosate formulations. *Crop Protect*, 24, pp.543–547.

Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res.*, 38, pp.995–1014.

Shagbanmu, T.O., Oshiona, O.A., Ogutunde, O.A. and Otitoloju, A.A. 2018. Biomarkers of toxicity in *Clarias gariepinus* exposed to sublethal concentration of PCH. *African Journal of Aquatic Sciences*, 43 (3), pp.281-292.

Sharma, S.K. and Krishna-Muri, C.R. 1968. Production of lipid peroxides by brain. *J. Neurochem.*, 17, p.147.

Shiogiri, N.S, Paulino, M.G., Carraschi, S.P., Baraldi, F.G. and Da Cruz, C. 2012. Acute exposure to glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus*. *Environmental Toxicology Pharmacology*, 34, pp.388-396.

Svodova, Z., Vykusova, B. and Machova, J. 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. In: Müller, R., Lloyd, R. (Eds.), Sublethal and Chronic Effects of Pollutants on Freshwater Fish. FAO Fishing News Books, Great Britain, pp 39–52.

Takahara, S., Hamilton, H.B., Neel, J.V., Kobara, T.Y., Ogura, Y. and Nishimura, E.T. 1960. Hypocatalasemia, a new genetic carrier state. *J. Clin. Invest.*, 39, pp.610–619.

Tsui, M.K. and Chu, L.M. 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere*, 52, pp.1189–1197.

Voet, D. and Voet, J.G. 1990. Biochemistry. John Willy and Sons, New York. pp.10-11.

Williams, G.M., Kroes, R. and Munro, I.C. 2000. Safely evaluation and risk management of the Herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharm.*, 31, pp.117-165.