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Biochemical Effects of Glyphosate on Juvenile African Catfish (*Clarias Gariepinus*)

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ABSTRACT

The present study investigated some biochemical effects of glyphosate on juvenile African catfish - *Clariasgariepinus*. Eighty acclimatized *C. gariepinus* were selected and divided into eight groups in laboratory aquaria containing 10 fish each for acute toxicity test. The groups were exposed tovarious concentrations of glyphosate to obtain the96 h LC_{50}. The 96 h LC_{50} value was 367.133 mg l^{-1}. A total of 36 acclimatized fish were divided into three groups containing 12 fish in each aquarium for biochemical studies.Each group of *C. gariepinus* was exposed to three sub-lethal concentrations of 91.78, 45.89 and 36.71 mg $l^{-1}$of glyphosate, corresponding to the ¼th, $^{1}/_{8th}$and $^{1}/_{10th}$of 96 h $LC_{50,}$ for 16 days. A set of 12 fish was simultaneously maintained in an aquarium to serve as the control. The result from the serum collected every 4 days showed significant (P<0.05) increases in the activities of ALT, AST and ALP; withsignificant (P<0.05) increases in the concentrations of direct bilirubin,total protein, creatinine, urea, sodium and potassium. Also, there weresignificant (P<0.05) decreases in the activities of SOD, catalase, GST and GSR; withsignificant (P<0.05) increase (P<0.05) in the concentration of MDA. The results revealed that the use of herbicides such as glyphosate in the upland can be toxic to aquatic life when washed down from streams into larger water bodies via oxidative stress, liver and kidney damages.

Key words: Glyphosate, fish, *Clarias gariepinus,*herbicide, liver function parameters, antioxidant enzymes, kidney function parameters.

I. INTRODUCTION

The application of herbicides in weed control has been a normal practice for many years. However, this practice could expose non-target aquatic organisms to danger. This is observed in small water bodies close to lands on which herbicides were applied; after being washed into the water bodies by rain as a result of their solubility, or by careless handling such as spillage or washing their containers in such water bodies. High levels of the herbicide scan cause the dead of fishes and other aquatic organisms, thereby producing foul odours that pollute the water bodies and can become a threat to human health.

Glyphosate is non-selective herbicide used in the control of plants in agricultural, industrial, urban, forestry and aquatic landscapes [1]. It is a very important herbicide developed and has been continually used and are even tolerated in plant varieties that are modified genetically.[2].Formulations of glyphosate include an acid, monoammonium salt, diammonium salt, is opropylamine salt, potassium salt, sodium salt, and trimethylsulfonium or trimesium salt [3],[4],[5]. Glyphosate is mainly formulated as “Roundup”, in which

glyphosate is formulated as isopropylamine salt and a surfactant, polyethoxylene amine (POEA), is added to enhance its efficacy [6],[7].

Glyphosate is translocated throughout the plant after its absorption across the stems and leaves[3], [8]. Its area of concentration is in the tissue of the meristem [9]. Malformation of leaf or wrinkling, green colour loss, stunted growth and death of tissue are associated with glyphosate-exposed plants. It takes between 4 to about 20 days for the exposed plant to die[5], [9]. Due to the high solubility of glyphosate in water and its vast usage, especially in or near shallow water systems, the non-target aquatic organisms may be affected [6]. The World Health Organization has indicated low acute toxicity for glyphosate [10]. However, the acutely-toxic commercially formulated glyphosate ishigher than glyphosate [11],[12].

Fishes can be used as bio-markers of environmental pollution and can be necessary in investigating thecontamination risks in water bodies since they are exposed directly to chemicals used from agricultural practicesthrough surface run-off or indirectly through food chain in ecosystem [13]. Herbicides, insecticides and heavy metals are some of the environmental contaminants thatregulate antioxidant defense systems, resulting in the production of reactive oxygen species (ROS) that cause oxidative damage to aquatic organisms.[14], [15], [16], [17].

Clariasgariepinus, also knownas African catfish, belongs to the air-breathing catfishesofthe family *Clariidae.* They are very much available inAfrica, as well asin the Middle East. They live in rivers, freshwater lakes, swamps, evenman-made aquaria, such as ponds,including urban sewage systems.They migrate within streams and rivers [18].*C.gariepinus* live in different freshwater habitats, including ponds, lakesand pools. Also, they areabundantaround damsand in flowing rivers. They can survive in extremeconditions of environmentand in waterswith pH range of6.5to8.0. They canthrive in turbid waters and temperatures of 8 - 35 ^{0}C. Their optimal growth temperature is 28 – 30 ^{0}C [18]. They are bottom-water dwellers and most of their feedings take place there. They can spend some time on the water surface, making them obligate air breathers. This species can live in very poorly oxygenated waters and is one of the last species to live in such an uninhabitable place [19]. They secrete mucus that prevent them from drying and can burrow in the muddy substrate of a drying body of water [20].
*C. gariepinus*have elongated shape with fairly long anal and dorsal fins. The dorsal fin has 61-80 soft rays and the anal fin has 45-65 soft rays. They have strong pectoral fins with spines that are serrated on the outer side [18].*C. gariepinus* specie can attain up to 1.7 meters in length, with weight up to 59 kg when fully grown. They possess nasal and maxillary barbels and somewhat smallish eyes. They have dark grey or black colour dorsally and ventrallycream colour. Dark-longitudinal lines are found on either side of the head in adult, which is not present in young fish. Adult's heads are coarsely granulated, while the head is smooth in the young. They have large, depressed, and heavily boned head; with large and sub-terminal mouth[20].*C.gariepinus*cansurviveinboth poorly and well oxygenated waters; thereby making it very hardy.Hence, theyare mostly used for fish farming in Nigeria and are normally used as samples in aquatic toxicity studies, due to their easy management when compared to some other fish species.

II. MATERIALS AND METHODS

Materials: Glyphosate herbicide (glyphosatetested as 360 g isopropylamine (IPA) salt per litre) was purchased at Abakpa Market, Abakaliki, Ebonyi State; while one hundred and fifty (150) juvenile *Clariasgariepinus* (African catfish)were procured from Chi-boy Farms, Abakaliki, Ebonyi State, and were acclimatized in the laboratory aquaria for two weeks and were fed with fish feed before exposure to the toxicant.

Equipment: Spectrophotometer, oven, weighing balance, measuring cylinder, glass wares (pyrex), centrifuge (Binatone), refrigerator, sample containers.

Chemicals/Reagents: All the chemicals and reagents used in this research were of the purest analytical grade commercially available. The assay kits were products of Randox Laboratories Limited, BT29 4QY, United Kingdom.

Methods

Acute Toxicity Test

Acute toxicity test to determine the 24, 48, 78 and 96 hour LC_{50} values of glyphosate on *C. gariepinus*(weighing between 240.00 ± 20.00 g and 180.00 ± 60.00 g with length 30.80 cm to 25.50 cm) was conducted in a semi – static system in the laboratory according to the OECD guideline NO 23 [21]. The water with the glyphosate concentrations was changed every 24 hours with freshwater and glyphosate in order to counter-balance their decreasing concentrations. Eighty (80) fish were selected and divided into eight groups containing ten (10) fish in each aquarium for acute toxicity testing. The groups were respectively exposed to 3600, 1800, 900, 450, 360, 270, 180 and 0 mg l^{-1} of glyphosate, to obtain their 24 h, 48 h, 72 h and 96 h LC_{50}. The experiment was conducted in aquaria containing 40 litres of aerated tap water. The percentage survival and mortality were calculated, while the LC_{50} was determined following the Probit analysis [22].

Sub-acute Toxicity Test

The 96 h LC_{50} value Glyphosate on *C. gariepinus* (367.133 mg l^{-1}) was used for the sub-acute toxicity (biochemical studies). A total of 36 acclimatized fish were divided into three groups containing twelve (12) fish in each aquarium. The two groups were exposed to 91.78, 45.89 and 36.71 mg l^{-1} of glyphosate respectively, corresponding to their $^1/_{4th}$ LC_{50}, $^1/_{8th}$ LC_{50} and $^1/_{10th}$ $LC_{50,}$ for sixteen days. A set of 12 fish was simultaneously maintained in an aquarium to serve as the control.

Sample Collection

At the end of every 4 days, three fish were taken from each aquarium and their blood sample collected from the head and caudal fin region in an anticoagulant free sample containers and allowed to clot. The blood samples were centrifuged at 3000 ppm for 30 minutes. The serum collected was used for assaying the various biochemical parameters.

Determination of Biochemical Parameters

Standard methods were used for all the biochemical analysis. For liver function parameters;serum alanine aminotransferase (ALT) andaspartate aminotransferase (AST) were determined by the method of Reitman and Frankel [23]. Alkaline phosphatase (ALP) activity was determined by the method of Kind and King [24]. Direct Bilirubin concentration was determined by the method described by Jendrassik and Grof [25], while total protein concentration wasdetermined by Tietz method[26]. In the case of kidney function parameters; serum urea and creatinineconcentrations were determined by the method of Tietz [27], while serum sodium and potassium concentrations were determined by flame photometry method [28]. For oxidative stress parameters;superoxide dismutase (SOD) and catalase (CAT) activities weredetermined using the methods of Xin *et al.* [29] andAebi [30], respectively. Serumglutathione transferase (GST) and glutathione reductase(GSR) activities were determined according to the method ofHabig *et al.*, [31].Lipid peroxidation was determinedaccording to the method of Wallin *et al.* [31].

Statistical Analysis: The percentage survival andmortality were calculated, while the LC50 was determinedfollowing the Probit analysis [22]. Statisticalanalysis was performed using one-way Analysis ofVariance (ANOVA) followed by Ducan's multiple rangetest procedure of SAS software version 9.1. All the resultsobtained were expressed as mean ± Standard Deviation(S.D.) of three replicates of each sample and thedifferences between means were regarded significant at $P<0.05$.

III. RESULTS

Acute Toxicity effect of Glyphosate on *Clariasgariepinus*

The acute toxicity effect of *Clariasgariepinus* juvenile exposed to glyphosatefor 96 hours is shown Table 1. The result showed that there was no death (100 % survival/0 % mortality) recorded on the fish exposed to glyphosate from 0 to 270 mgl^{-1}. A total of 4 fish died in the aquaria exposed to 360 mgl^{-1} (60 % survival/40 % mortality) and 8 fish died in the aquaria containing 450 mgl^{-1}, (20 % survival/80 % mortality) of glyphosate; whereas all the fish died in the aquaria containing 900 to 3600 mgl^{-1} (0 % survival/100 % mortality) within 96 hours. The various mean lethal concentrations (LC_{50}) of glyphosate are shown.

Table 1. Acute toxicity test of *Clariasgariepinus* juvenile exposed to Glyphosate

Glyphosate Conc.(mgl^{-1})	Number of fish exposed	Number of deaths 24hrs	48hrs	72hrs	96hrs	% mortality 24hrs	48hrs	72hrs	96hrs
00	10	00	00	00	00	00	00	00	00
180	10	00	00	00	00	00	00	00	00
270	10	00	00	00	00	00	00	00	00
360	10	00	02	04	04	00	20	40	40
450	10	06	08	08	08	60	80	80	80
900	10	08	10	10	10	80	100	100	100
1800	10	10	10	10	10	100	100	100	100
3600	10	10	10	10	10	100	100	100	100

The mean lethal concentrations (LC_{50}) expressed in mg l^{-1} at various exposure times (95% confidence intervals) are shown below.
24 h LC_{50} = 556.557 (344.389 – 1926.439)
48 h LC_{50} = 402.692 (361.525 – 452.442)
72 h LC_{50} = 384.745 (339.743 – 435.011)
96 h LC_{50} = 367.133 (320.566 – 413.040)

Sub-lethal Toxicity/Biochemical Parameters

Liver Function Parameters: The result of the effects of sub-lethal concentrations of glyphosate on some serum liver function parametersof *Clariasgariepinus* is shown in Figure 2.The result showed that there were significant increases ($P<0.05$) in the activities of ALT, AST and ALP, with concomitant significant increases ($P<0.05$) in the concentrations of direct bilirubin and total protein in a dose and time dependent manner, when compared with that of the normal control. The highest effects were observed in the group exposed to $^1/_{4th}$ LC_{50} of glyphosate, while the lowest effects were observed in those exposed to $^1/_{10th}$ LC_{50}. For example, ALT activity increased from 20 % on the 4th day (13.56 ± 0.25) in the $^1/_{4th}$ $LC_{50}$to 84 % on the 16th day (65.50 ± 0.17).

Table 2. Effects of sub-lethal concentrations of glyphosate on some serum liver function parameters of *C. gariepinnus*

Days/Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Direct Bilirubin (µmol/l)	Total Protein (g/l)
4th Day					
Control	44.56 ± 0.69[a]	10.78 ± 0.25[a]	13.12 ± 0.24[a]	19.84 ± 0.38[a]	37.85 ± 0.41[a]
1/10th LC_{50}	48.33 ± 0.33[b]	10.72 ± 0.25[a]	14.39 ± 0.24[b]	22.88 ± 0.25[b]	43.71 ± 0.40[b]
1/8th LC_{50}	50.89 ± 0.38[c]	11.39 ± 0.25[b]	15.87 ± 0.32[c]	25.83 ± 0.25[c]	48.77 ± 0.20[d]
1/4th LC_{50}	55.33 ± 0.33[d]	13.56 ± 0.25[d]	19.37 ± 0.16[e]	29.36 ± 0.14[e]	53.83 ± 0.20[f]
8th Day					
Control	45.11 ± 0.84[a]	11.01 ± 0.35[a]	13.04 ± 0.37[a]	19.88 ± 0.25[a]	37.96 ± 0.55[a]
1/10th LC_{50}	59.44 ± 0.51[b]	23.33 ± 0.33[b]	24.20 ± 0.18[b]	32.80 ± 0.38[b]	54.90 ± 0.55[b]
1/8th LC_{50}	65.12 ± 0.39[c]	33.89 ± 0.25[d]	25.09 ± 0.18[c]	35.59 ± 0.38[c]	58.29 ± 0.42[c]
1/4th LC_{50}	73.33 ± 0.33[e]	37.89 ± 0.25[e]	28.17 ± 0.10[d]	41.57 ± 0.25[e]	66.03 ± 0.36[e]
12th Day					
Control	44.89 ± 0.84[a]	10.89 ± 0.44[a]	13.20 ± 0.47[a]	20.12 ± 0.25[a]	37.26 ± 0.46[a]
1/10th LC_{50}	67.22 ± 0.84[b]	39.06 ± 0.25[c]	25.22 ± 0.29[b]	41.25 ± 0.14[b]	60.87 ± 0.46[c]
1/8th LC_{50}	71.78 ± 0.51[c]	38.28 ± 0.25[b]	26.97 ± 0.19[c]	45.18 ± 0.38[c]	64.23 ± 0.70[d]

1/4th LC_{50}	88.78 ± 0.19[e]	42.39 ± 0.10[d]	34.71 ± 0.29[f]	50.68 ± 0.25[f]	73.38 ± 0.70[e]
16th Day					
Control	45.21 ± 0.85[a]	11.10 ± 0.29[a]	13.24 ± 0.39[a]	20.11 ± 0.25[a]	38.33 ± 0.36[a]
1/10th LC_{50}	82.00 ± 0.33[b]	57.78 ± 0.25[b]	33.87 ± 0.50[b]	52.73 ± 0.38[b]	72.71 ± 0.21[b]
1/8th LC_{50}	87.44 ± 0.51[c]	60.78 ± 0.25[c]	35.07 ± 0.32[c]	59.53 ± 0.25[c]	78.00 ± 0.36[d]
1/4th LC_{50}	97.33 ± 0.33[e]	65.50 ± 0.17[e]	40.15 ± 0.50[e]	76.51 ± 0.25[f]	88.28 ± 0.21[f]

Data are presented as mean ± Standard deviation of 3 fish in each group. Values with different alphabet superscript differ significantly ($p < 0.05$) between durations within concentration.
AST = Aspartate Aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline Phosphatase

Kidney Function Parameters: The result of the effects of sub-lethal concentrations of glyphosate on some serum kidney function parametersof *Clariasgariepinus* is shown in Figure 3. The result showed significant increases ($P < 0.05$) in the concentrations of urea, creatinine, sodium and potassium in a dose and time dependent manner, when compared with that of the normal control.The highest increaseswere observed in the group exposed to $^{1}/_{4th}$ LC_{50} of the toxicants while the lowest level was observed in those exposed to $^{1}/_{10th}$ LC_{50}. For example, the urea concentration increased from 46 % on the 4th day (11.48 ± 0.08) in the $^{1}/_{4th}$ $LC_{50}$to 75 % on the 16th day (21.85 ± 0.13).

Table 3. Effects of sub-lethal concentrations of glyphosate on some serum kidney function parameters of *C.gariepinnus*

Days/Groups	Creatinine (mg/dl)	Urea (mg/dl)	Sodium (Na^+) (mEq/l)	Potassium (K+) (mEq/l)
4th Day				
Control	5.55 ± 0.31[a]	7.49 ± 0.08[a]	14.30 ± 0.35[a]	3.80 ± 0.01[a]
1/10th LC_{50}	7.17 ± 0.23[b]	10.13 ± 0.00[b]	19.07 ± 0.53[b]	4.41 ± 0.01[b]
1/8th LC_{50}	7.65 ± 0.12[c]	10.83 ± 0.13[c]	20.00 ± 0.53[c]	5.31 ± 0.01[c]
1/4th LC_{50}	9.74 ± 0.20[d]	11.48 ± 0.08[d]	22.33 ± 0.35[d]	6.14 ± 0.01[d]
8th Day				
Control	5.48 ± 0.27[a]	7.55 ± 0.17[a]	14.32 ± 0.17[a]	3.79 ± 0.01[a]
1/10th LC_{50}	9.99 ± 0.27[b]	11.25 ± 0.06[b]	21.00 ± 0.53[b]	6.28 ± 0.00[b]
1/8th LC_{50}	10.25 ± 0.09[c]	13.26 ± 0.06[c]	21.93 ± 0.35[c]	6.83 ± 0.00[bc]
1/4th LC_{50}	12.23 ± 0.24[d]	14.84 ± 0.11[d]	24.71 ± 0.35[d]	8.36 ± 0.01[d]
12th Day				
Control	5.53 ± 0.38[a]	7.78 ± 0.00[a]	14.26 ± 0.41[a]	3.82 ± 0.06[a]
1/10th LC_{50}	11.39 ± 0.13[b]	13.94 ± 0.05[b]	23.71 ± 0.62[b]	7.79 ± 0.02[b]
1/8th LC_{50}	12.29 ± 0.05[c]	14.84 ± 0.10[c]	26.02 ± 0.81[c]	8.70 ± 0.06[c]
1/4th LC_{50}	14.58 ± 0.05[e]	16.09 ± 0.09[d]	27.64 ± 0.41[d]	10.35 ± 0.06[d]
16th Day				
Control	5.50 ± 0.24[a]	7.51 ± 0.20[a]	14.31 ± 0.23[a]	3.86 ± 0.01[a]
1/10th LC_{50}	13.56 ± 0.18[b]	18.32 ± 0.15[b]	26.77 ± 0.23[b]	10.75 ± 0.01[b]
1/8th LC_{50}	14.29 ± 0.15[c]	19.74 ± 0.13[c]	27.85 ± 0.13[c]	10.86 ± 0.01[c]
1/4th LC_{50}	16.16 ± 0.30[d]	21.85 ± 0.13[d]	30.54 ± 0.27[d]	12.94 ± 0.00[d]

Data are presented as mean ± Standard deviation of 3 fish in each group. Values with different alphabet superscript differ significantly ($p < 0.05$) between durations within concentration.

Oxidative Stress Parameters: The result of the effects of sub-lethal concentrations of glyphosate on some serum oxidative stress parametersof *Clariasgariepinus* is shown in Figure 4. The result showed significant ($P<0.05$) decreases in the activities of SOD, CAT, GST and GSR, with a significant ($P<0.05$) increasein the concentration of MDA in a dose and time dependent manner, when compared with that of the normal control. The highest effects were observed in the group exposed to $^{1}/_{4th}$ LC_{50} of glyphosate, while the lowest effects were observed in those exposed to $^{1}/_{10th}$ LC_{50}.

Table 4. Effects of sub-lethal concentrations of glyphosate on some serum oxidative stress parameters of *Clariasgariepinnus*

Days/Groups	SOD (IU/g)	CAT (IU/g)	GST (M/min)	GSR (Umol/ml)	MDA (mg/ml)
4th Day					
Control	6.22 ± 0.01^{d}	14.28 ± 0.01^{d}	2.50 ± 0.01^{d}	0.37 ± 0.01^{d}	5.64 ± 0.02^{a}
1/10th LC_{50}	6.18 ± 0.01^{c}	13.99 ± 0.01^{c}	2.29 ± 0.01^{c}	0.34 ± 0.00^{c}	5.88 ± 0.01^{b}
1/8th LC_{50}	5.73 ± 0.00^{b}	13.51 ± 0.00^{b}	2.12 ± 0.01^{b}	0.28 ± 0.00^{b}	6.24 ± 0.00^{c}
1/4th LC_{50}	5.31 ± 0.00^{a}	13.09 ± 0.00^{a}	1.71 ± 0.01^{a}	0.25 ± 0.01^{a}	7.01 ± 0.00^{d}
8th Day					
Control	6.15 ± 0.01^{d}	14.22 ± 0.00^{d}	2.46 ± 0.03^{d}	0.37 ± 0.01^{d}	5.66 ± 0.01^{a}
1/10th LC_{50}	5.09 ± 0.00^{c}	12.79 ± 0.00^{c}	1.88 ± 0.01^{c}	0.28 ± 0.00^{c}	7.51 ± 0.00^{b}
1/8th LC_{50}	4.87 ± 0.00^{b}	12.63 ± 0.05^{b}	1.76 ± 0.01^{b}	0.25 ± 0.00^{b}	7.85 ± 0.01^{c}
1/4th LC_{50}	4.29 ± 0.00^{a}	12.02 ± 0.00^{a}	1.44 ± 0.02^{a}	0.22 ± 0.00^{a}	8.36 ± 0.00^{d}
12th Day					
Control	6.20 ± 0.01^{d}	14.13 ± 0.01^{d}	2.48 ± 0.01^{d}	0.37 ± 0.01^{d}	5.63 ± 0.01^{a}
1/10th LC_{50}	4.30 ± 0.00^{c}	11.64 ± 0.00^{c}	1.72 ± 0.02^{c}	0.24 ± 0.00^{c}	8.82 ± 0.00^{b}
1/8th LC_{50}	4.29 ± 0.00^{b}	11.50 ± 0.00^{b}	1.57 ± 0.02^{b}	0.22 ± 0.00^{b}	9.32 ± 0.00^{c}
1/4th LC_{50}	4.09 ± 0.00^{a}	11.11 ± 0.00^{a}	1.53 ± 0.01^{a}	0.20 ± 0.00^{a}	9.89 ± 0.00^{d}
16th Day					
Control	6.12 ± 0.01^{d}	14.17 ± 0.01^{d}	2.51 ± 0.01^{d}	0.37 ± 0.01^{d}	5.65 ± 0.01^{a}
1/10th LC_{50}	4.04 ± 0.00^{c}	10.94 ± 0.00^{c}	1.54 ± 0.01^{c}	0.21 ± 0.00^{c}	9.99 ± 0.01^{b}
1/8th LC_{50}	3.62 ± 0.00^{b}	10.63 ± 0.00^{b}	1.48 ± 0.02^{b}	0.20 ± 0.00^{b}	10.54 ± 0.00^{c}
1/4th LC_{50}	3.53 ± 0.00^{a}	10.16 ± 0.00^{a}	1.31 ± 0.01^{a}	0.18 ± 0.01^{a}	10.66 ± 0.00^{d}

Data are presented as mean ± Standard deviation of 3 fish in each group. Values with different alphabet superscript differ significantly ($p < 0.05$) between durations within concentration.
SOD = Superoxide Dismutase, CAT = Catalase, GST = Glutathione S-transferase, GSR = Glutathione reductase, MDA = Malondialdehyde

IV. DISCUSSION

Acute Toxicity: During acute toxicity testing, the fish in the aquaria containing high concentrations (450 to 3600 mg l^{-1}) of glyphosate displayed erratic swimming, quick and sudden movement, slow movement, settled in the bottom and dorsal swimming/floating before they finally died. The fish in the aquaria with lower concentrations of glyphosate displayed less erratic and quick movements but were weak after 96 hours. The normal control fish (0.00 mg l^{-1}) displayed normal movements after the 96 hours. The 24 h, 48 h, 72 h and 96 h LC_{50} of glyphosate were 556.557, 402.692, 384.745, and 367.133 mg l^{-1} respectively (Table 1). Neskovic*et al.* carried out acute toxicity test of glyphosate with carp (*Cyprinus carpio*) and found the 96 h LC_{50} of glyphosate to be fairly high (620 mg l^{-1}) [33]. However, considering the formulated product Roundup, 96 h-LC_{50}could vary, depending on the fish species, life stage and test conditions [34].

Liver Function: Our results showed that serum ALT, AST and ALP activities, and MDA, bilirubin and total protein levels in *Clariasgariepinus*exposed to glyphosate showed significant ($P<0.05$) increasescompared to the normal control, in a time and concentration dependent manner from the $^{1}/_{10th}$ to $^{1}/_{4th}$ LC_{50} of the fourth day to sixteenth day of the study. The most significant effects were observed in the ¼$_{th}$ LC_{50} of the 16th day, whereas the least effects were observed in the$^{1}/_{10th}$ LC_{50} of the 4th day.

The increases in the activities of ALT (20 % - 84 %), AST (8 % - 54 %), ALP (9 % - 67 %); the increased levels of bilirubin (13 % - 75 %) and total protein (13 % - 57%) in our study (Table 2), indicated that high doses of glyphosate can cause liver damage in fish. This result is in agreement with the report that the liver of *C. gariepinus* exposed to glyphosate for 96 hours

showed fatty degeneration, severe fat vacuolation, diffuse hepatic necrosis and darkly stained specks of necrotic nuclei and infiltration of leukocytes [35].
Serum ALT and AST are necessarymarkers for identifying liver inflammation and necrosis [36]. The highest concentration of ALT is found in the liver. Lesser activity of the enzyme is found in the kidney and skeletal muscles. ALT activity is a more specific measurement for liver damage than AST. Also, ALT activity is usually higher than that of AST at early or acute hepatocelluar disease [37]. However, in chronic liver diseases such as cirrhosis, AST seems to be released more thanALT [37]. Liver, bone, placenta and intestine are clinically important sources of the plasma activity of ALP. Thisenzyme activity increases in many clinical states; mostly arein bone and liver diseases. Hence, serum ALP activity is very useful in diagnosis, screening and follow up for cholestatic hepatobiliary lesions [38]. Cholestasis is the main, if not the only liver disease responsible for increased plasma alkaline phosphatase activity. Therefore, a normal ALP activity, in the presence of abnormal levels of other liver function parameters, may be suggestive of liver pathology other than obstruction [36].

An increase in tissue or serum bilirubin concentration results in jaundice and it occurs in toxic or infectious disease of the liver e.g., hepatitis or bile obstruction [39]. Elevated bilirubin is an indication of liver cell impairment. Bilirubin measurement is also a useful index of determining the excretory function of the liver and assessment of haemolyticaneamia. In the liver, bilirubin is conjugated with glucoronic acid in a reaction catalysed by bilirubin –UDP-glucuronyltransferase which renders it soluble and subsequently excreted into the bile [40]. Increased plasma total protein concentration observed in the current work at high doses may be due to dehydration and/or increased plasma immunoglobulin concentration due to infection [40].

Kidney Function: From our results, there was gradual increases in the levels of serum creatinine (23 % to 66 %), urea (46 % to 75 %), sodium (25 % to 53 %) and potassium (14 % to 71 %)of *C. gariepinus* exposed to glyphosate (Table 3). The increases in the kidney function parameters observed in this study could be as a result of kidney damage in the fish exposed to glyphosate. Our finding is in agreement with a previous study that reported that the kidney tissue from *C. gariepinus*exposed to different concentrations of glyphosate showed necrosis, degenerated kidney tubules pyknosis, exfoliated and swollen with pyknotic nuclei [35].

Creatinine is the major catabolic products of the muscle and it is excreted in the kidneys. High creatinine levels are used as indicator of renal failure [41]. The increased level of urea observed is an indication of azotaemia. High blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea [42]. The levels of sodium(Na^+) and potassium(K^+)in glyphosate-exposed fishincrease significantlyin a dose and time dependent manner. The increase in levels of theseelectrolytes could be attributed to renal impairment.

Oxidative Stress: Antioxidants represent the cellular defense against oxidative stress caused by free radicals. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GSR or GR) and the non-enzymatic reduced glutathione (GSH) always mediate in oxidative crisis in the cells in a synergic manner. Hence, the activities of these enzymes are used as parameters for oxidative stress.

From the results of our study, there was a gradual decrease ($p > 0.05$) in the serum activities of SOD (1 % to 43 %), CAT (7 % to 40 %), GST (8 % to 29 %) and GSR (11 % to 53 %), while the

level of malondialdehyde (MDA) gradually increased ($p < 0.05$) from 4 % to 47 % in *C. gariepinus* exposed to glyphosate within 16 days. The decrease in the activities SOD, CAT, GST, GSR and increased MDA level observed in this study is in agreement with the findings of Lushchak*et al.* [43] which reported that Roundup (a glyphosate herbicide) exposed to goldfish generally suppressed superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione reductase (GSR) activities,and caused an increase in catalase (CAT) activity; while lipid peroxides (LOOH) concentration increased by 3.2 fold in the brain and liver of goldfish. However, Modesto and Martinez [44] reported a transient decrease in the activities of SOD and catalase in fish after exposure to Roundup Transorb in 6 hours while it inhibited glutathione S-transferase activity after 6 and 24 hours. Another herbicide, butachlor, induced lipid peroxidation (increased MDA concentration) and decreased GST activity in *C. gariepinus*[45]. The alterations in the activities of SOD, CAT, GST, and GSR observed in this study could be due to inefficient scavenging of reactive oxygen species (ROS) by the body antioxidants, which might be due to oxidative inactivation of the enzymes [46]; while the increasedlevel of malondialdehyde (MDA) is suggestive that glyphosate caused lipid peroxidation in the fish.

Superoxide dismutase catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2, and catalase reduces H_2O_2 to $2H_2O$ [47]. Reduced activity of SOD could lead to immune system damage [48]. Catalase is a ubiquitous enzyme present in cells of aerobic organism. It converts hydrogen peroxide to molecular oxygen and two molecules of water [49]. The decrease in catalase activity observed in this study could be due to the flux of superoxide radicals, which have been reported to inhibit CAT activity [50].

Glutathione peroxidase (GPx) plays a role in defending cells against oxidative damage and this in turn involves GSH as its cofactor. GPxcatalyses the oxidation of GSH to GSSG (oxidized glutathione or glutathione disulfide) at the expense of H_2O_2[51]. Glutathione reductase (GSR) mediates in the glutathione disulfide (GSSG) reduction to form two molecules ofGSH, which is necessaryfor combatingoxidative stress, including the maintenance of the reductive state of the cell [52]. Glutathione transferase (GST) catalyzes the conjugation of GSH via a sulfhydryl group to electrophilic centers on various substrates to make the compounds more soluble [53]. The decreased activities of GSR and GSTs observed in this study could be likened to decreased activity of glutathione peroxidase (GPx) since they function in 'synergy' to combat reactive oxygen species that cause oxidative stress.

High level of MDA is an indication of lipid peroxidation as a result of free radicals [54], [55]. Lipid peroxidation can occur in the cell if the rate of ROSproduction is higher than that of the antioxidants synthesis/activities. MDA levels in the test samples were significantly elevated compared to those in normal controls. This indicates that free radical-mediated injury occurred in the exposed animals leading to lipid peroxidation.

V. CONCLUSION

Glyphosate, a commercially available herbicide, is toxic to fish such as *Clariasgariepinus*. Therefore, indiscriminate spilling of glyphosate on or near water bodies should be avoided since it is harmful to aquatic lives and could lead to environmental toxicity.

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