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Effect of the Glyphosate Herbicide uproot® on Non-functional Blood Plasma Enzymes of Juveniles of Clariasgariepinus

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Abstract— **The effect of the herbicide glyphosate on the plasma enzymes of Clariasgariepinus was investigated in order to gauge the possible effect of its use in the environment. Juveniles of Clariasgariepinus of mean length14.0cm ± 1.2cm and mean weight 8.0gm ± 0.3gm were procured from Ellah Lakes Obrikom, Rivers State.They were transported in plastic containers under cool condition to the Laboratory of the Department of Biological Sciences, Niger Delta University, Amasoma, Bayelsa State.The fish were acclimated in big plastic basins for 7 days and fed pelleted diets at an estimated 3% body weight. Mortality during acclamation did not exceed 3% of total fish population therefore the fish were certified fit for use. A range finder test was conducted prior to the definitive test. This was done to determine the suitable range of concentration for the experimental test. They were exposed to sublethal concentrations of 0.25ml/l, 0.50ml/l and 0.75ml/l. The control tank had no glyphosate. They experimental tanks were exposed for seven (7) days and blood collected from fish in each aquaria tank by cardiac puncture using physical restrain with a 21 gauge hypodermic needle and syringe. Blood samples were taken in triplicates. Blood samples were taken to the laboratory of Federal Medical Centre, Yenagoa. They were analysed for Alanine Amino Transferase (ALT), Aspartic Amino Transferase (AST), Alkaline Phosphatase (ALP) using standard techniques. Data were analysed for mean, standard deviation and analysis of variance (ANOVA) at 95% confidence limit. Duncan Multiple Range Test (DMRT) was employed to compare means. This was done with the aid of the SPSS software. Results indicate that all the enzymes from blood plasma of fish increased with increasing concentrations of glyphosate. This is indicative of either liver, kidney or tissue damage. Therefore, the presence of glyphosate in aquatic ecosystem could be dangerous to fish and subsequently to human health. There is a need to observe restrain in its usage.**

Index Terms— **Amino Transferase (ALT), Alkaline Phosphatase (ALP).**

I. INTRODUCTION

All over the world, weeds constitute perhaps the most important natural barrier to commercial agricultural crop production. The menace of weed leads to poor yields and the loss of enumerable output. The control of weeds mostly in commercial agriculture require a lot of investment in human capital, so much so that if human labour were present to control weed, it will be impossible to find the finance to pay them. Fortunately, herbicides provide an effective and economic weed control mechanism in terms of reduced labour and overall cost. [1].

Herbicides are simply known as weed killers. They are employed for various purposes such as to clear waste grounds, industrial sites, railway embankment forest management and weed control in commercial agriculture. The importance of herbicide in weed control cannot be overemphasized. Sadly, herbicides even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physicochemical properties of water.[2] Residues of herbicides have been detected in phyto-toxic concentrations in ground water, lakes and streams as a result of run-off from treated fields. More disturbing is the fact that herbicides are also widely used for the control of aquatic and terrestrial plants under the formulation of different trade mark names.[3]

Toxicants often contaminate fresh water bodies and affect non-target organisms including fish. The effect of some known herbicides such as Roundup®, Rramoxone® and Rodeo® on aquatic life especially fish, is well documented[4,5]while research in other combination such as Uproot® (Isopropylamine Salt) is scarce.In contrast to humans (a single species), our understanding of biochemical regulatory mechanisms in fish (thousands of species) is still evolving and many significant species differences exist. There are adequate data on salmonids[5], English sole[6], striped mullet and pinfish[7] to make certain presumptive evaluations; however, the same responses may not occur in other species. Consequently, we must expand our database of blood chemistry measurements to other species prior to making any broad generalizations or extrapolations concerning potential toxicant effects. Although there have been numerous studies on the effects of toxicants on the blood chemistry of fish,[7]most studies evaluated general hematology or measured only a single specific indicator.Fish physiology (Biochemical blood parameters and metabolic enzymes) are suitable tools for assessing environmental influences and stress effects of anthropogenic origin on the condition and health of aquatic vertebrates.[8] Since there is close association between the circulatory system of fish and the external environment[9] the effect of external stressors and toxic substances on exposed fish could be made manifest through clinical diagnosis of fish physiology.

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Therefore, enzymatic activities can be suitably used to determine the effect of herbicides on the physiology of fish under sub-lethal condition prior to sudden death of the fish. Non-functional plasma enzymes perform no physiological function in the plasma and mostly arise from destruction of Red Blood Cells (RBCs) Also, tissue damage caused by injury or disease can cause marked increase in their level. Therefore, the measurement of these enzymes level can help in diagnosis and prognosis of diseases. The most important non-functional plasma enzymes are: Transaminases: Two enzymes of this group are considered as non-functional plasma enzymes, Alanine transaminase (ALT or SGPT) and Aspartate transaminase (AST or SGOT).

Fishes are by far the most useful and indeed the most priced aquatic biota to man, as they serve mostly for food among other uses. The necessity of determining the toxicity of substances to commercially important aquatic forms at the lower levels of the food chain has been useful and accepted for water quality management.[10]

However, fishes are widely used to evaluate the health of aquatic ecosystem; and physiological changes serve as biomarkers of environmental pollutio.[11]*C. gariepinus*is most the widely used fish species because it is able to tolerate both well and poorly oxygenated waters and respires bimodally through the use of accessory breathing organs.[12]

As *Clariasgariepinus* represents one of the popular fresh water fish delicacies in Nigeria and occupy flood plains, swamps and pools, it is necessary to measure their response to exposure of this widely used herbicide. In view of the poor knowledge of the aquatic side-effects of this agrochemical, the result will serve useful purposes for the management of our fisheries and the protection of the environment

II. Materials and Methods

A. *Toxicant*

The general herbicide uproot® (Isopropylamine) was procured from an open market chemical store at Ekeki, Yenagoa, Bayelsa State, Nigeria. The chemical composition of the toxicant was noted from the manufacturer's instruction manual leaflet and this information cross checked against the information provided in the referral book Rhone-poutenc Laboratory chemicals and reagents. This chemical is classified in the W.H.O III classification as slightly toxic but highly inflammatory.[13]

B. *Test Organism*

Juveniles of *Clariasgariepinus*of mean length14.0cm ± 1.2cm and mean weight 8.0gm ± 0.3gm were procured from Ellah Lakes Obrikom, Rivers State, Nigeria. They were transported in plastic containers under cool condition to the Laboratory of the Department of Biological Sciences, Niger Delta University, Amassoma, Bayelsa State. Juveniles were chosen due to the more sensitive nature of juveniles than adult for toxicity test.[14, 15, 16]

C. *Acclamatization*

The fish were acclimated in big plastic basins for 7 days and fed pelleted diets at an estimated 3% body weight. Mortality during acclamation did not exceed 3% of total fish population. Therefore the fish stock was assumed to be fit and disease free.

D. *Range Finder Test*

A range finder test was conducted prior to the definitive test. This was done to determine the suitable range of concentration for the experimental test.

During the range finder test, the fish were exposed to different concentrations of the toxicant in increasing log series.The concentration of toxicant that did not result in death of the fish in the range-finder test was taken as the highest concentration in the definitive test.

E. *Definitive Test (Sublethal Toxicity Test)*

Ten (10) fish were put in each aquaria tank containing 20lt of water. A total of four (4) aquaria tanks were used for the experiment. Exposure concentration of 5ml, 10ml, and 15ml of the toxicant were added to 20L of water in each of the aquaria tank. Therefore toxicant concentrations of 0.25ml/l, 0.5ml/l and 0.75ml/l were used. The toxicant concentrations decided were measured into each plastic tank using a calibrated measuring cylinder and marked accordingly. The control tank had no toxicant added to it. Proper mixing of the toxicant with the water was ensured by steering each water tank vigorously for 5minutes with a glass rod. During the test, each tank was also steered every 12hours to ensure proper mixing and proper circulation of oxygen. The fish were fed ad-libitum twice daily. Test solution was change and reconstituted every two (2) days to ensure good water quality and remove fouling materials

F. *Blood Collection Technique*

Blood was collected after one weeks from one fish at a time in each plastic basin by cardiac puncture with physical restraint using a 21 gauge hypodermic needles and syringes and put in previously tagged anticoagulant bottles containing potassium salt of ethylene diamine tetra-acetic acid (EDTA) for laboratory analysis. Blood was collected from 3 fish from each aquaria tank. A total of 12 fish were sampled in study. Freshly collected blood samples were taken immediately to the Haematology laboratory of the Federal Medical Centre YenagoaBayelsa State for analysis of plasma enzymes. Analysis of all blood samples took place less than 4 hours after time of collection.

Fig 1: Blood collection using cardiac puncture

G. Analysis of Blood

Biochemical parameters analyses

The non-functional blood plasma enzymes of Juveniles of *Clariasgariepinus* such as Aspartic Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) were determined using kits of Randox Company Limited.

Alanine Aminotransferase (ALT)

Alanine Aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4dinitrophenyldrazine. Add phosphate buffer 100mmol/l, L-alamine 200mmol/l, and α-oxogluturate to the sample mix and incubate for 30minutes at 37^{o}c then add 2,4 dinitrophenyldrazine mix and allow to stand for 20 minutes at 20 to 25^{o}c read the absorbance of samples against the reagent blank after 5 minutes using a spectrophotometer at a wavelength of 530-550 nm. The absorbance represents the level of ALT.

Aspartate Aminotransferase (AST)

Aspartate Aminotransferase (AST) was measured by monitoring the concentration of oxaloacetate formed with 2,4dinitrophenyldrazine. The absorbance of the complex formed was read after 5 minutes using spectrophotometer at a wavelength between 530- 550 nm and it represents the level of AST.

Alkaline Phosphatase (ALP)

The determination of ALP is based on the enzymatic kinetic method (DGKC and SCE method). The principle is based on the fact that in the presence of mg2+ and diethanolamine as phosphate acceptor, p-nitrophenylphosphate is transformed by alkaline phosphatases into phosphate and p-nitrophenol (yellow compound). The procedure consists of the use of the following reagents: Reagent 1: Diethanolamine, pH 10.2 and magnesium chloride. Reagent 2: p-Nitrophenylphosphate 50mmol/l. Heparinized plasma from freshly collected blood sample was mixed with reagent 1 and after 43 seconds, reagents 2 was added and mixed together. Wait for133 seconds of incubation. The variation of absorbance was measured per minute (A/min).

Data Analysis

Data will be analysed for means and standard deviations. A one-way Analysis of variance (ANOVA) at the 95% probability was conducted using the SPSS statistics tool in order to determine the relation and variation between measured plasma parameters from the different exposure tanks and control. This was followed by the DMRT test in order to compare and separate means. Pearson's correlation was employed to determine the relationship and interconnectivity of the measured enzyme parameters. These test were considered significant if P=0.05.

III. Result

The result of this study is presented in Tables I, II and III and figure 2. Table I reveals that all the plasma enzymes increased in value as the concentration of the toxicant in the test media increased.

Table I: Mean blood plasma Enzymes in *Clariasgariepinus*

S/N		PLASMA ENZYMES (IU/L)		
	Concentration (ml/l)	AST	ALT	ALP
1	0	0.1±0.01^{a}	1.02±0.23^{a}	0.5±0.02^{a}
2	0.25	6.7±1.32^{b}	4.5±0.62^{b}	6.2±0.71^{b}
3	0.50	7.0±1.51^{b}	6.5±1.11^{c}	8.0±1.5^{c}
4	0.75	6.78±1.22^{b}	5.1±1.12^{b}	7.0±2.1^{bc}

Means ± Standard Deviation

Means with the same superscript along the same column are not significantly different (p<0.05).

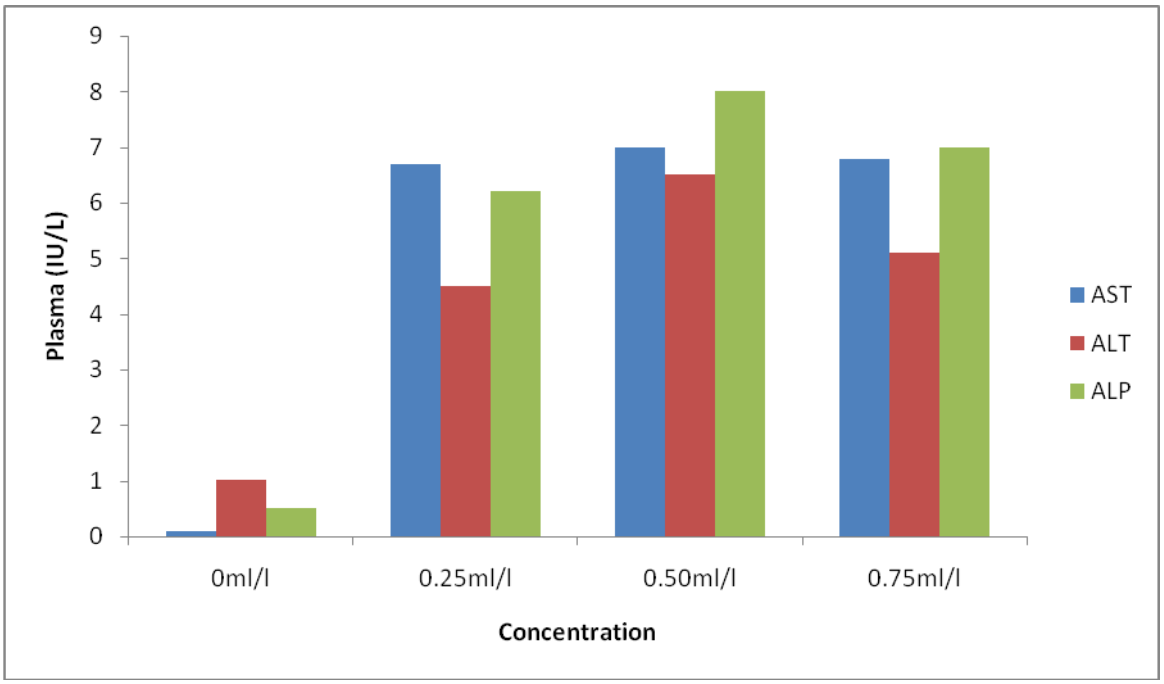

Fig. 2: Mean blood plasma Enzymes in *Clariasgariepinus*

Table II: ANOVA for Blood plasma enzymes in *Clariasgariepinus*

		Sum of Squares	Df	Mean Square	F	Sig.
AST	Between Groups	1.109	10	.111	.347	.880
	Within Groups	.320	1	.320		
	Total	1.429	11			
ALT	Between Groups	.170	10	.017	.294	.905
	Within Groups	.058	1	.058		
	Total	.228	11			
ALP	Between Groups	.006	10	.001	.263	.920
	Within Groups	.002	1	.002		
	Total	.009	11			

Table III: Pearsons Correlation for blood plasma enzymes of *C. gariepinus*

		AST	ALT	ALP
AST	Pearson Correlation	1	.772**	.466
	Sig. (2-tailed)		.003	.127
	N	12	12	12
ALT	Pearson Correlation	.772**	1	.786**
	Sig. (2-tailed)	.003		.002
	N	12	12	12
ALP	Pearson Correlation	.466	.786**	1
	Sig. (2-tailed)	.127	.002	
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

IV. Discussion

The study observed a steady increase of all the measured enzyme levels of ALT, AST and ALP respectively from control to increasing levels of the glyphosate concentration. In liver disease, these enzymes "leak out" of hepatocytes reaching the circulation, leading to marked increase in their concentration in blood. So, increase in these enzymes value in blood indicates liver disease as hepatitis and liver cirrhosis. AST and ALP are non-plasma specific enzymes that are localized in tissue cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the blood may give specific information about organ dysfunction.[17]

This result of this study is in disagreement with the findings of Luskova et al.[18] who observed decrease in the activities of ALT and AST in the fish *Cyprinuscarpio*exposed to diazinon but corroborates with that of Servizi et al,[19]in *Channa punctatus* treated with sublethal levels of alcoholic extracts of *Neriumindicum.*

The increase in ALP in exposed fish in this study may be due to the rise in the rate of synthesis of glycogen resulting from the increased metabolic demands[20] and an increase in metabolic transport.[21, 22] The increase may also indicate that there was slight kidney damage and an increase in the hydrolytic action on a number of phosphor monoesters of organic origin such as glycogen.[22]

The significant differences between the control and experimental groups of *Clariasgariepinus* following the action of the herbicide measured especially in ALT ($p< 0.01$) or even AST ($p< 0.05$), may be considered to be the manifestation of stress.

Also in agreement with our results, Raciket[22] report significant glucose increase in common eel (*Anguilla anguilla*) following a 96 hrs action of sublethal concentrations ofdiazinon. Glucose increase is a general response of fish to acute pollutant effects, including organophosphates.[23, 24. 25, 26]

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