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Electrolyte And Metabolite Assessment In The Muscle And Blood Of *Clarias gariepinus* Induced With 360 G/L Of Glyphosate Ammonium (SL)

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ABSTRACT
Glyphosate and other pesticides in general are toxic and exerts serious biocidal effects on both target and non-target organisms, thus present a serious risk to humans and other bio-life forms in the environment. Eighteen (18) juvenile *Clarias gariepinus* (African catfish) were used to test the toxicity of Glyphosate Ammonium SL. Experimental fishes where orally administered with 2.00 ppm, 4.00 ppm and 6.00 ppm of toxicant for 21 days. On termination of the experiment, muscle from experimental fishes were collected, crushed and treated with deionized water for electrolyte assessment, while blood samples from experimental fishes were centrifuged for 15 minutes at 3000 rpm for metabolite assay in the laboratory. Metabolites in the blood like total protein, albumin, bilirubin and creatinine values declined while urea value appreciated, compared to the control. Muscle electrolytes such as sodium (Na^+) appreciated, potassium (K^+) declined, whereas magnesium and chloride values in the muscle stabilized across various treatment groups, hence, not significantly different ($p>0.05$) compared to the control group. The result confirms the toxicity of glyphosate 360 g/L on metabolic and electrolyte parameters of exposed fishes and should be used cautiously.
Keywords: *Clarias gariepinus*, Glyphosate, Electrolytes, Metabolites, Pesticide.

INTRODUCTION

Pesticides are biocides which alters metabolic process of organisms in the ecosystem. They are a menace to farming activities, and non-target bio-life forms in the ecosystem. "Pesticides are of different formulations which act sometimes as microbial agents or disinfectant against target pests" (US-Environmental Protection Agency, 2008). Due to their toxic effect on both target and non-target organisms in the environments, they by extension present a serious risk to humans and other bio-life forms in the environment by bioaccumulating in food chains and tissues of organisms (Hamilton, et al., 2004). Feed and fodder used in feeding animals are also frequently infected with pesticides (Sandhu, 1980; Raikwar & Nag, 2003), which accumulates in the adipose tissues of affected animals (Prasad & Chhabra, 2001). In the last few years, research has revealed the trend and toxic effect of pesticide on human health and animals in general, especially as it regards human food poisoning in different countries of the world.

Herbicides like glyphosate containing ammonium salt is a selective pre-emergent and post-emergent systemic herbicide which reduces the population of broad-leaved weeds and additional plants on farms or agricultural lands. Its application on grasses, golf courses, woods, roadways and playgrounds also help

control the population of unwanted weeds and target organisms in such areas (Material Safety Data Sheet, 2018). The use of these anthropogenic chemicals in agriculture and food preservation as well as security have caused untold environmental problems, and the effects of human acute and chronic exposure to these xenobiotics remain basically understudied (Kurenbach, et al., 2015). Though, the usage of chemical (pesticides) substances in contemporary farming has significantly increased food security and yield but not without its accompanying harmful influence on people and animal wellbeing as reported by Richter, 2002 who revealed that yearly, there are plenty issues of poisoning through insect killer globally (Richter, 2002).

The use of fish models like African catfish in scientific or biomedical studies has been given a positive backing for the purpose of validating existing literature and procedures (Sanada, et al., 2003; Miranda, et al., 2005; Ferreira, et al., 2004). Such prototype models possess very close phylogenic characteristics that is relatively frequent with the item for the test and should also be easily controlled devoid of the restrictions of that item (Fagundes, et al., 2004). Biomarkers such as metabolite and electrolyte macromolecules direct metabolic procedures of organisms. Hence, a minor deviation in their concentration or measurement from homeostatic equilibriums in life forms would influence the metabolic procedures of the organisms in general (Roy, 2002). Therefore, significant alterations in the levels of electrolytes like sodium, potassium, magnesium as well as metabolites such as bilirubin, total protein and albumin in the serum would be key investigative signs of liver illnesses in animals, fishes and humans as reported by authors like Chatterjea & Shinde, 2005; Attia & Nasr, 2009; Saafi, et al., 2011; Khan, et al., 2013. The findings of this research will therefore help provide the needed information to guide against human exposure to chemicals through unintentional and unguarded feeding practice that are capable of contaminating the human food chain.

MATERIALS AND METHOD

A total eighteen (18) juvenile *Clarias gariepinus* (African catfish) were used in the present study. These fishes were obtained from Ikuligan fish farm at Agudama Epie, Yenagoa, Bayelsa State, Nigeria and was transported individually in plastic jerricans to the laboratory. The herbicide, Glyphosate 360 g/L with active ingredient Ammonium SL was purchased from Swali chemical market within Yenagoa metropolis of Bayelsa State, Nigeria. Following the method described by Inyang, 2008, sublethal doses were measured in ml (s) during a trial experiment also known as range finding test after a period of acclimation for seven (7) days. Three concentrations (2 ppm, 4 ppm, and 6 ppm) were prepared following the trial test and was used for the main experimental run. This was also followed by a renewal bioassay throughout the period of the trial test.

The main test lasted for three weeks (21days) following a randomized renewal bioassay. During this period, experimental fishes were randomly selected and exposed to three sub-lethal concentrations (2 ppm, 4 ppm and 6 ppm) of the toxicant obtained from the trial test. This was aimed at testing the toxic effect of chemical on exposed fishes. At the end of the exposure period, a clinical syringe (10 ml) and needle was used to collect blood samples from the lower abdominal cavity and centrifuged for 15 minutes at 3000 rpm. The supernatant (serum) was then poured into a plain sample bottle for analysis to determine the effect of toxicant on a number of metabolites in exposed fish following the method described by APHA, (1998). A small portion of muscle from experimental fishes were also collected by dissection, crushed in a ceramic mortar and homogenized with deionized water. The mixture was then centrifuged for 15 minutes at 3000 rpm and the supernatant was collected in a sample bottles for electrolytes assay following the method described by Malmstadt and Winefordner, (1959).

Statistical Analysis

All data obtained were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20 software and expressed as mean ± standard deviation. A one-way analysis of variance was also conducted at α=0.05 to determine the significance in the different experimental groups assessed in this study. Duncan multiple range test (DMRT) was also applied to distinguish discrete mean values.

RESULTS

Table 1. Effect of Glyphosate 360 g/L Containing Ammonium salt on Muscle Electrolytes IN *Clarias gariepinus*.

Conc. of Glyphosate (ppm)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Mg^{2+} (mmol/L)
0.00	1.95±1.63[d]	13.30±4.24[a]	26.62±1.63[a]	0.85±0.02[a]
2.00	2.70±1.41[c]	11.95±0.92[b]	26.20±0.42[a]	0.82±0.15[a]
4.00	4.25±1.06[b]	9.84±1.22[c]	26.60±1.41[a]	0.62±0.98[a]
6.00	5.05±2.47[a]	8.62±3.66[d]	26.20±1.98[a]	0.67±0.19[a]

All data are expressed as mean ± standard deviation using the software Statistical Package for Social Sciences version 20.

Table 2. Effect of Glyphosate 360 g/L Containing Ammonium salt on metabolites in the serum of *Clarias gariepinus*

Conc. of Glyphosate (ppm)	T.P (nmol/L)	ALB (g/L)	T.BIL (g/L)	UREA (nmol/L)	CREAT (mmol/L)
0.00	64.27±3.20[b]	29.76±0.48[a]	27.51±4.32[a]	5.46±1.52[b]	139.13±77.6[a]
2.00	60.48±2.90[b]	27.64±2.48[b]	19.40±3.88[b]	3.43±3.56[d]	89.45±4.33[b]
4.00	70.40±5.49[a]	24.06±2.20[c]	12.39±13.15[c]	6.30±0.17[a]	79.29±0.17[c]
6.00	47.19±10.35[c]	29.19±1.10[a]	9.95±6.57[d]	4.88±0.23[c]	69.31±30.83[d]

All data is expressed as mean ± standard deviation using the software Statistical Package for Social Sciences version 20.

DISCUSSION

Electrolytes in Muscle

Electrolytes are ionized molecules found throughout the blood tissues and cells of animals generally. These molecules, which are either positive (cations) or negative (anions), conduct electrical signals in organisms and assist to balance pH and acid-base levels in animals which are often compromised with stress (Ogamba, et al., 2011). Skeletal muscles also need sodium, potassium, chlorides and magnesium ions to contract. When these materials become lopsided, it can result in either weakness of muscles or extreme tightening. The heart, muscles and nerve cells use electrolytes to convey electrical impulse to other cells. From the result presented in Table 1 of this study, there were observed changes in the values of certain electrolytes measured in the muscle of exposed fishes.

The value of Sodium (Na^+) ions measured in the muscle of fish exposed to Glyphosate increased to 2.70±1.41 mmol/L at 2.00 ppm, 4.25±1.06 mmol/L at 4.00 ppm and 5.05±2.47 mmol/L at 6.00 ppm when compared to the control value of 1.95±1.63 mmol/L. The appreciation in the value of sodium ion in the muscle was observed to be dose dependent, thus, increase in toxicant concentration leads to increases in the value of sodium ion in the muscle. This observation is similar and in line with the report of previous authors such as Capcarova, et al., (2013). In their experiment, they recorded appreciation in the value of sodium in the blood of probe Fishes intoxicated with bendiocarbamate.

The observed increase in the value of sodium ion (Na^+) compared to the control in this study is also an indication of hypernatraemia in the blood which is usually associated with dehydration. This could be attributed to the toxic effect of xenobiotic on exposed Fishes. Similarly, Inyang *et al.* (2016) also reported related result after studying the effect of glyphosate on some electrolytes in *Heterobranchus bidorsalis*. Their result also recorded increase in the value of some electrolytes. Inyang and Patani, (2015) also reported similar findings and confirmed an increase in the value of electrolytes in the muscle of exposed fishes after studying the haematological aberrations and electrolyte stabilization in *Heterobranchus bidorsalis* induced by Rhonasate 360 SL containing glyphosate.

The increased value of sodium in the muscle as recorded in this study could also be an indication of xenobiotic poisoning caused mainly by the effect of the toxicant on fishes and this could lead to an increased activity of diarrhea, sweating and vomiting which were common observations at the intoxication stage of probe Fishes in this study. Furthermore, the loss in weight occasioned by excessive dehydration as was observed among exposed animals in this study could be attributed to increased muscular activity and stress induced by xenobiotic which may actually lead to lethargy in exposed fishes. More so, the increase in sodium concentration in the muscles of experimental fishes as opposed to potassium reduction in muscle cells in this study is also suggestive of alteration in physiological activity, osmoregulation as well as disturbance of the functions of neuromuscular, endocrine and excretory system of animals. This report is in line with the finding of Cox, (1996) who also reported that cypermethrin alters nerve impulse which travels along nerves of vertebrates and other animals due to alteration in the normal level of electrolytes in animals. Similar results have also been documented by Logaswamy, et al., (2007) and Khan et al. (2013) on electrolyte variance in tissues and organs of animals exposed to similar xenobiotics.

Potassium is a vital mineral that has numerous functions in animals generally. It assists control muscle tightening; enhance healthy functioning of the nerve as well as fluid balance. Potassium ordinarily is concentrated inside the cells of muscles, and other tissues of animals. The variance in concentration of potassium from within the cell as juxtaposed to plasma is essential in producing electrical impulse in the body of animals for proper muscle and brain function. On the contrary, as presented in table 1 above, the measured value of potassium (K^+) in the muscles of fishes treated with toxicant declined significantly from 11.95±0.92 mmol/L at 2.00 ppm, to 9.84±1.22 mmol/L, and 8.62±3.66 mmol/L at 6.00 ppm, compared to the control (13.30±4.24 mmol/L). This could be as a result of the influence of poisons on renal function, altering the kidney's ability to regulate the level of potassium thereby causing its levels to decline as observed in this study. This is a direct pointer to the damaging influence of toxicant on the kidney. Related results were also accounted by Inyang et al. (2016) after exposing *Heterobranchus bidorsalis* to varying concentrations of glyphosate. The decreasing effect of potassium (K^+) ion in this study, if it continues could lead to conditions such as hypokalaemia, muscular weakness, cramps, spasm and fatigue, digestive disorder, heart palpitation and breathing difficulties in exposed fishes. These in most cases induce osmotic imbalance that can lead to abnormal haemo-concentration and circulatory collapse.

The variance in the value of potassium ion in probe animals as compared to the control in this study also possibly defines the toxic effect of toxicant on electrolyte activities in exposed fishes, thus interfering with potassium (K^+) ion homeostasis. Related to this study is the report by Mahjoubi-Samet, et al., (2005). After exposing rats to the effect of dimethoate, they also recorded variance in the levels of various electrolytes such as high calcium level (Hypercalcaemia) and low phosphorus level (hypophosphatemia) as compared to the control. According to the authors, exposure to dimethoate altered bone mineral composition such as calcium and phosphorus levels in probe animals. Also the recorded decline in the value of potassium could lead to ionic imbalance and this may trigger or activate muscle weakness or excessive contraction as well as poor heart, nerves and muscle electrical impulse coordination to other cells in exposed animals.

From the result obtained in this study as presented in Table 1 above, values of Chloride (Cl^-) and Magnesium (Mg^{2+}) ions were not significant ($p>0.05$), compared to the control. This possibly indicate that the xenobiotic (Glyphosate 360 g/l containing ammonium salt) may be less toxic to the activities of chloride (Cl^-) and magnesium (Mg^{2+}) ions in the muscle or it may be due to the low concentration of toxicant used in this study. Thus, the stabilization of some electrolytes values according to Ogamba *et al.*, (2011) could be a pressure stimulated reply caused by the effect of animals exposed to toxicant which could activate definite physiological and metabolic means that may result to a quick uptake of electrolytes from water and food substance or decrease of ion-efflux.

Metabolites in the Blood

Metabolites are small intermediate end products of metabolic activity that is aided by enzymes in animal cells (Fiehn, 2002). They serve as cellular fuels to generate cellular energy and also stimulate, and conduct cell signaling and play inhibitory roles on enzymes. They also have various functions including

structure, catalytic activities, usually as cofactors to enzymes, defense and interactions with other organisms such as pigments, odorants and pheromones (Semanta et al., 2014). Metabolite summary has been recognized as a multi-parallel plan for virtual calculation of a blending of compounds or compound groups utilizing different techniques (Weckwerth, et al., 2004).

Regardless of its beginning since late 1960s, it was barely in 1980s that its usage was recognized to analyze metabolic disarray in animals, particularly for quick testing of inborn errors such as diabetes in humans and cardiovascular risk factor evaluation (Krull & Swartz, 1999). Thus, profiling blood samples of metabolic parameters can be adopted to inspect definite biochemical reaction (Fiehn, 2002). Examples of metabolites measured in this experiment include total protein, albumin, bilirubin, urea and creatinine. The mean levels of metabolites (total protein, albumin, total bilirubin, urea and creatinine) in the blood of control fishes and those treated each day with Glyphosate toxicant for 21 days are presented in Table 2 of this study.

From the result obtained, the value of total protein in the blood of probe fishes conclusively declined from 63.46±2.87 nmol/L at 2.00 ppm to 57.17±11.32 nmol/L at 6.00 ppm compared to 65.26±3.30 nmol/L of the control. But there was slight increase in the value of total protein to 67.38±5.58 nmol/L at 4.00 ppm as compared to the control (65.26±3.30 nmol/L). This slight increase in the value of total protein at concentration 4.00 ppm could be due to pressure stimulated by poison and probe animal reaction to maintain physiological equilibrium. Proteins are energy molecules responsible for providing needed energy for animals to carry out their physiological functions. Full protein experiment is a straightforward, schedule blood check. It examines regular or irregular levels of protein in the body.

According to Chatterjea and Shinde, (2005), "the decrease in the levels of total protein and albumin in the serum, are the major diagnostic symptoms of liver diseases". Related results were accounted in other research such as Attia and Nasr, (2009) and Salih, (2010) as a result of oral administration of different doses of dimethoate. They also recorded decline in the levels of various metabolic parameters. The decrease in serum protein could be credited to modifications in protein and free amino acid metabolism and their mixture in the liver. Moreover, the protein stage repression might be as a result of loss of protein either by reduction in protein mixture or improved proteolytic actions or deprivation as reported by Heikal et al. (2012). It is therefore, imperative that the low level of total protein measured in the serum of animals treated with Glyphosate is a show of the toxic effect of the pesticide on nutritional activity, absorption, liver function, as well as renal function which may lead to nephritic syndrome or glomerulonephritis, and cardiac functions of treated animals.

Albumin is one of the most abundant blood proteins in the body of animals. It is produced in the liver and plays several roles in animals as well as responsible for maintaining arterial pressure, by keeping water in the blood vessels (Yeragi, et al., 2003). It transports several substances ranging from various hormones to drugs. Therefore, changes in albumin level in the blood, either decrease or increase can be used to diagnose various health problems that involve the kidney or liver and it may be caused as a result of malnutrition, inflammatory disorders, infections, as well as dehydration (Inyang et al., 2010). Increased level of albumin in the blood is seen in most cases when the blood is too concentrated, as in the case with dehydration leading to excess water loss occasioned by the effect of a drug, clinical condition or toxic chemical effect. Reduced level of albumin could also be due to reduced liver function or increased albumin losses and are often associated with inflammation of liver or malnutrition as well as kidney diseases. This variance in the value of serum albumin in probe animals as compared to the control perhaps is indicative of the toxic effect of Glyphosate which could result in associated risk of liver inflammation, nephrotoxicity or malnutrition.

The reduced levels of albumin in Glyphosate treated animals may also indicate or present the condition of abnormal control of arterial pressure and osmoregulation in the blood vessels, which further indicate reduced body mass and weight reduction in animals treated with toxicant in this study. This finding is related to that of Vasilenko and Grebenev, (1990) and Inyang and colleagues, (2016). They also recorded a decline in the serum albumin when male fishes and *Parpohiocephalus obscurus* where exposed to the activity of dimethoate and diazinon as well as Lamda cyhalothrin pesticides respectively and concluded that the decline may be possibly due to renal failure and inability for probe animals to osmoregulate, thereby causing albumin to be excreted with urine.

Urea level in the blood slightly varies with the control in an irregular pattern. At the initial exposure of probe animals to 2.00 ppm of toxicant concentration, its average value declined to 4.53±5.38 nmol/L as compared to the control of 6.56±1.32 nmol/L. This shows the response of probe fish to stress induced by their sudden exposure to xenobiotic in this study. The observed decrease in urea level could also be indicative of liver disease if the trend continues. But as the concentration of toxicant was increased to 4.00 ppm, urea value appreciated to 7.27±0.12 nmol/L as compared to the control (6.56±1.32 nmol/L) and later stabilized to 6.74±0.43 nmol/L at 6.00 ppm, but still slightly higher compared to the control of 6.56±1.32 nmol/L. The elevation of urea level is again a major sign of glomerular filtration damage as reported by Chatterjea and Shinde, (2005). The result of this research is related to that of Elias, (2010) who also exposed male fishes to the activities of Dimethoate and Diazinon respectively, and recorded similar increase in the level of serum urea in treated animals compared to the control. But this observation is slightly dissimilar to the findings of previous authors like Nyblom et al. (2004). In their publication, they reported a decline in the value of urea and concluded that a decreased urea level is indicative of liver disease.

Bilirubin is an orange-yellow material formed during the usual breakdown of red blood cells. Bilirubin passes via the liver and is finally excreted, thus controlling the level of bilirubin in the body. More than average stages of bilirubin might signify diverse forms of liver issues. Sporadically, higher bilirubin levels might signify an improved pace of obliteration of red blood cells (Inyang & Patani, 2015). In the present study, the administration of Glyphosate containing 2, 4-dimethylamine salt caused gradual and regular dose dependent decrease in bilirubin level throughout the experiment. Values of bilirubin declined to 14.30±3.79 g/L at 2.00 ppm, 13.59±10.04 g/L at 4.00 ppm and 11.63±2.47 g/L at 6.00 ppm as compared to 23.61±4.11 g/L of the control. Consequently, a lower than normal level of bilirubin in the blood is usually not much of a clinical concern as it only shows how far the liver is able to clear bilirubin to prevent jaundice. Rather, higher than normal level could mean that liver damage or diseases is possible. Sastry and colleagues, (1982) as well as Das & Mukharjee, (2000) also opined that contact of fish for a long period to most poison like insect killer obstruct protein metabolism, reduction of full protein in the plasma and serum of fish. Generally, changes in serum bilirubin which is acknowledged as sign of liver function might offer more proof on hepatotoxicity stimulated by toxicant as reported by Khan et al. (2013). Consequently, the variance of the value of bilirubin in Glyphosate treated fishes as compared to the control is an indication of hepatotoxicity on exposed animals.

Creatinine is a material the body generates in the course of regular metabolism and eradicates it nearly wholly via the kidneys process of filtration (Higgins, 2016). Thus, dimension of creatinine is a precise evaluation of how well the kidney processes of filtration are functioning. Something that changes the capacity of the kidneys to sieve creatinine well (such as lack of moisture) can lead to alterations in creatinine and urea levels in the blood (Baun, 1975). Creatinine is also a dispel product of muscle turnover and is indicative of muscle mass, which is calculated as a simple blood test (Felber, 1988).

The value of Creatinine in the blood regularly declined from 95.27±2.51 mmol/L at 2.00 ppm to 77.27±0.12 mmol/L at 4.00 ppm and 67.71±29.96 mmol/L at 6.00 ppm compared to the control (149.12±87.70 mmol/L). The observed decrease was dose reliant as the concentration of poison increases. This trend is a clear indication of the toxic effect of toxicant to metabolic parameters such as creatinine in probe animals. The significant decline in creatinine as seen in this study does not conform to the report by previous authors like Capcarova et al. (2013) in bendiocarbamate-intoxicated fishes. They reported increase in the values of creatinine measured in the blood of probe animals. Also, the observed decrease in the value of creatinine in this study might be an indication of increased kidney function as well as reduced muscle activity which could result in gastrointestinal bleeding, liver disease or malnutrition as well as muscle spasm and atrophication in exposed fishes. Similar to this finding is the report by Attia and Nasr, (2009) and Salih, (2010). They due to oral administration of diverse doses of dimethoate on rats confirmed a reducing trend of creatinine in the blood of rats exposed to dimethoate.

CONCLUSION

Conclusively, the changes in the values of serum metabolites in Glyphosate treated fishes in this study is an accepted sign of liver function which provides proof on hepatotoxicity stimulated by the toxicant. In addition, increase in serum metabolites such as urea in this study can also be credited in part to the harmful impact of xenobiotic on liver cells. Thus, results of the current study are indicative of hepatotoxicity and nephrotoxicity. Most importantly, in order to curb the rising trend of food poisoning in Nigeria due to bio-accumulation and magnification of xenobiotics in food chains, there is therefore the need to comprehensively pursue solution based research at this time to gather data about the use and toxicity of herbicides such as Glyphosate, containing 360 g/L of Ammonium salt. This should be followed with the analysis of bio-concentration of herbicides concentrations or residues in fish, especially those that serves as a direct source of nutrition to man.

Ethical Issues

The authors are all aware of ethical issues as stipulated by the laws of the land and thus completely complied with best practices while carrying out this research.

Competing Interest

Authors declare that there is no conflict of interest that would jeopardize the authenticity and originality of this scientific manuscript.

Authors Contribution

All authors of this research made equal imputes both for data collection, analysis and manuscript writing.

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