OXIDATIVE STRESS BIOMARKERS IN MALE CLARIAS GARIEPINUS (AFRICAN CATFISH) EXPOSED TO SUBLETHAL CONCENTRATIONS OF GLYPHOSATE

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Abstract - This study investigated the toxicity effect of sublethal concentrations of glyphosate in Clarias gariepinus with respect to the oxidative stress biomarkers, Catalase (CAT), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), and Malondialdehyde (MDA) in the liver, kidney and gills. Healthy mature (4 months) male Clarias gariepinus were used for the study. The fish were distributed into four groups (A–D) of five animals each. After 16 days of acclimatization the fish were exposed for 9 days to 300 mg/l, 60mg/l and 10mg/l of glyphosate. Results obtained showed that the MDA concentration of the liver, kidney and gill of exposed fish were significantly ($p < 0.05$) higher than the control group. GPx and SOD activities in the test groups were significantly ($p < 0.05$) lower than that of the control group while CAT activity was significantly ($p < 0.05$) higher in the test groups. This study showed that exposure to glyphosate may induce oxidative stress in the liver, kidney and gill of mature male Clarias gariepinus which could be evidence of toxicity. Hence such herbicides should be used with caution.

Keywords - Clarias Gariepinus, Glyphosate, Oxidative Stress

I. INTRODUCTION

Environmental preservation is important to the survival of the human race. Although some of man's activities to the environment have brought benefits to man, some have equally resulted in many environmental problems such as pollution, waste generation and management, land degradation, desertification, erosion, etc [1], [2]. Various types of agricultural pesticides such as herbicides, insecticides and fungicides are commonly in use globally because of their crop yield boost [3], [4]. Drainage and irrigation systems may become polluted through the application of these pesticides during agricultural and pest control activities. Farmland effluents frequently find their way into fresh water sources and other bodies of water and this has led to diverse contamination of aquatic ecosystem [3], [4]. High levels of pesticide contamination in aquatic ecosystems results in poisoning, oxygen depletion and resultant mass mortality of aqua-fauna such as fish [5].

Herbicides, used to control weeds, are the major pesticides used in farm lands [6] and glyphosate is one of the most widely used herbicides in many countries. [7], [8]. It is commonly applied to kill a wide range of unwanted plants in agriculture, forestry and in canals, lakes, fish ponds and low running water [7], [8]. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) activity in plants and impairs the synthesis of the aromatic amino acids in plants and some bacteria [9]. It was thought that since EPSP enzyme was not found in animals, that glyphosate will not be toxic to them. However, studies have shown that glyphosate was toxic to various species of fish [10], [11], [12], [13]. Contaminants in the environment such as herbicides have been implicated in modulating antioxidant defense systems thereby causing oxidative damage in aquatic life forms through the generation of Reactive Oxygen Species (ROS) [14], [15], [16].

Reactive Oxygen Species are produced as a result of an imbalance in the ratio of pro and antioxidants and consequently, oxidative stress develops. Fish like many organisms are equipped with a defensive system to counter the influence of ROS arising from the breakdown of chemicals and other xenobiotics. The essential enzymes involved in detoxification of ROS in an organism are Catalase (CAT), Glutathione peroxidase (GPx) and Superoxide dismutase (SOD). Under oxidative stress, these enzymes become easily inducible indicating an ability to adapt to stress conditions. Antioxidant mediated toxicities may occur if these systems are inhibited [17]. Oxidative stress can also cause lipid peroxidation in different tissues giving rise to oxidative damage products. One such product is Malondialdehyde, MDA which can also be used as a biomarker of oxidative stress [11], [18]. The use of biomarkers to evaluate the toxicity of chemical pollutants on animals is very relevant in verifying potential toxicants. This is because they can give information on biological effects of pollutants rather than a mere quantification of their environmental levels [19], [20].

The African catfish (Clarias gariepinus) is a major source of animal protein in Nigeria due to its relative abundance in natural fresh water bodies, affordability, ease of rearing them in local ponds, etc. It is therefore necessary to monitor the health of fish as whatever negatively affects it can also affect man who uses it as a source of protein. Hence the aim of this investigation is to evaluate the toxicity of sublethal

concentrations of glyphosate on C.gariepinus with respect to the oxidative stress biomarkers CAT, GPx, SOD and MDA in the kidney, liver and gill.

II. MATERIALS AND METHOD

Fish samples

Twenty (20) adult male Clarias gariepinus weighing 425–430g purchased from Fishery and Aquaculture Department of the Federal University of Technology Owerri (FUTO) Imo State, Nigeria were used for the investigation. The sex of the fish was identified by Mr Ezeafulukwe of the Fishery and Aquaculture Department of the Federal University of Technology Owerri, by observing the genital papilla of the fishes. The male Clarias gariepinus have a distinct sexual papilla located above the anus.

Animal grouping

The animals were weighed and separated into four groups of five fish, each were placed into 100 liter aquarium filled with water and allowed to acclimatize for 16 days before exposing it to glyphosate for 9 days. Commercial formulations of glyshosate were purchased from an outlet in Owerri, Imo State, Nigeria. The glyphosate concentrations were 300 mg/l, 60mg/l and 10 mg/l. The concentrations represent 150%, 30% and 5% of LC_{50} respectively measured in a previous study. Water was changed at two days interval to prevent the build of metabolic wastes and the glyphosate was replenished with same concentration. The animals were maintained on standard fish feed at 4% body weight twice daily. This study adhered to the guidelines on the care and well-being of laboratory animals and was approved by the ethical committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. Blood samples were collected from the fish after immersion in ice cold water. The blood samples were processed and stored.

Processing of tissues

Feeding of the fish was stopped 24hrs before the experiment and thereafter they were sacrificed to obtain the liver, kidney and gills. Each of the required organs was homogenized in potassium chloride buffer (1.15 %) with Ethylene Diamine Tetra Acetic acid (EDTA) at pH 7.4 using a Teflon homogenizer. Centrifugation of the homogenate obtained was at $4^{O}C$, 12,000g for 10 min. The supernatant was used to assay oxidative stress parameters.

Determination of oxidative stress parameters

Malondialdehyde (MDA) assayed in the form of thiobarbituric reactive substances (TBARS) concentration was carried out by the method as described by [21]. Glutathione peroxidase (GPx) activity was determined by the method of [22]. Superoxide dismutase (SOD) activity was determined using the method of [23]. Catalase (CAT) activity was determined according to the method of [24].

Statistical analyses

The results were expressed as mean ± standard deviation and the test for statistical significance was done using analysis of variance (ANOVA) with SPSS software (Standard Version 10). Means were separated using Duncan Multiple Range Test (DMRT) where applicable. Test of significance was at 5% level.

III. RESULTS

In all the results presented, bars with different letters are significantly different. Figs 1 and 2 show the effect of glyphosate on concentration of malondialdehyde (MDA) in the kidney and gill respectively of the African cat fish from control group and glyphosate polluted groups. In the kidney, there was a significant ($p < 0.05$) increase in the concentration of MDA only in the 300g/ml group compared to the control while in the gill all test concentrations showed significant ($p < 0.05$) increase in the concentration of MDA when compared to the control.

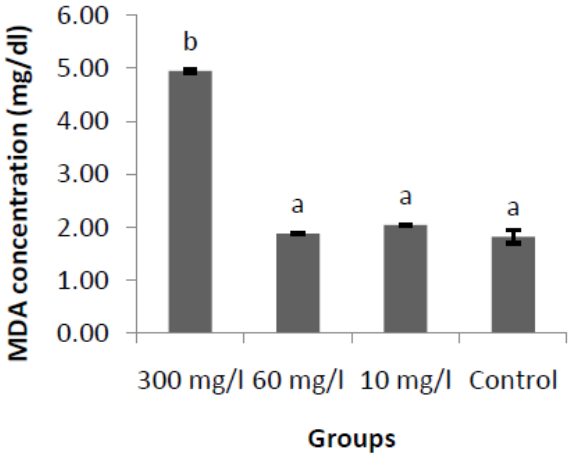


Fig 1: Malondialdehyde concentration in the kidney of African Cat fish

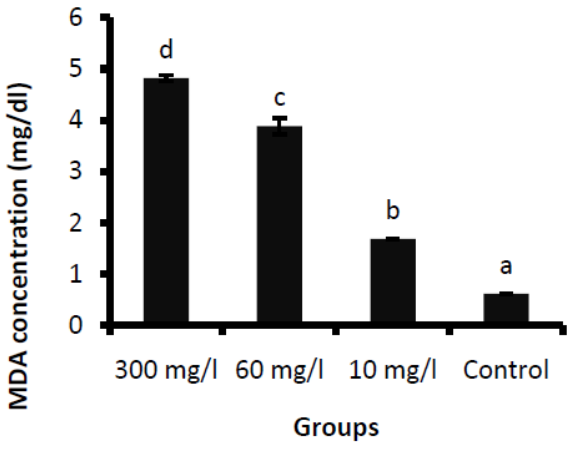


Fig 2: Malondialdehyde concentration in the gill of African Cat fish

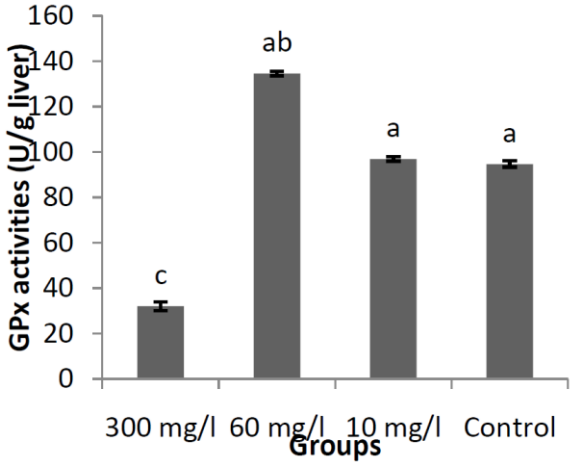


Fig 3: Glutathione peroxidase activity in the liver of African Cat fish

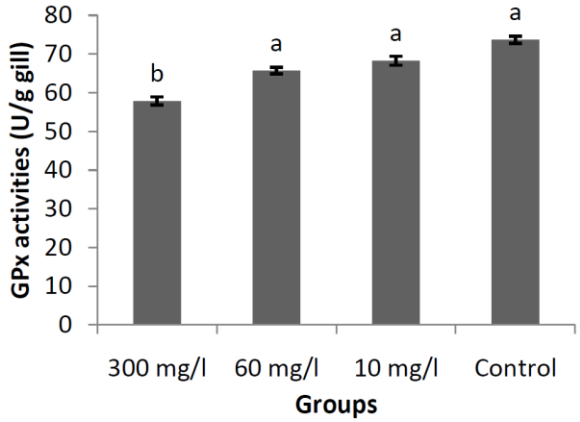


Fig 4: Glutathione peroxidase activity in the gill of African Cat fish

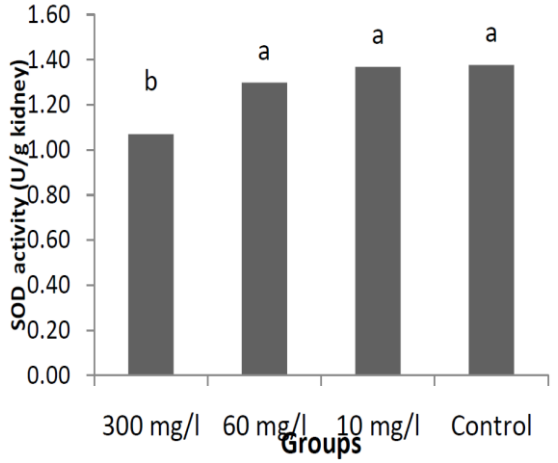


Fig 5: Superoxide dismutase activity in the kidney of African Cat fish

Figs 3 and 4 present the effect of glyphosate on Glutathione peroxidase (GP_x) activity in the liver and gill respectively of African cat fish from control group and glyphosate polluted groups. While significant ($P < 0.05$) decrease in the concentration of Glutathione peroxidase was observed only at the 300mg/l in the liver (fig. 3), the gill showed a significant decrease in GP_X in all the test concentrations (fig. 4).

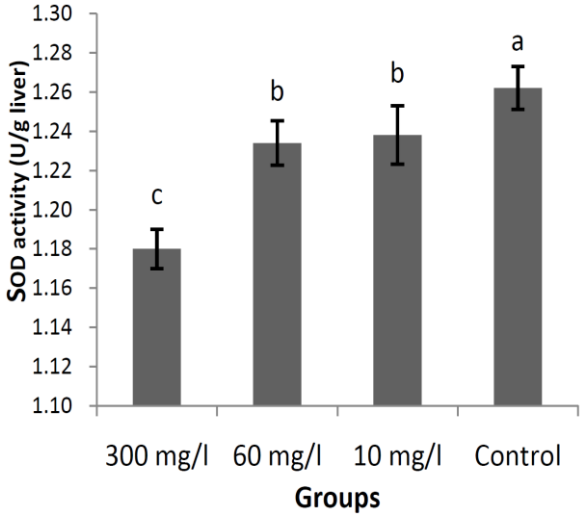


Fig 6: Superoxide dismutase activity in the liver of African Cat fish

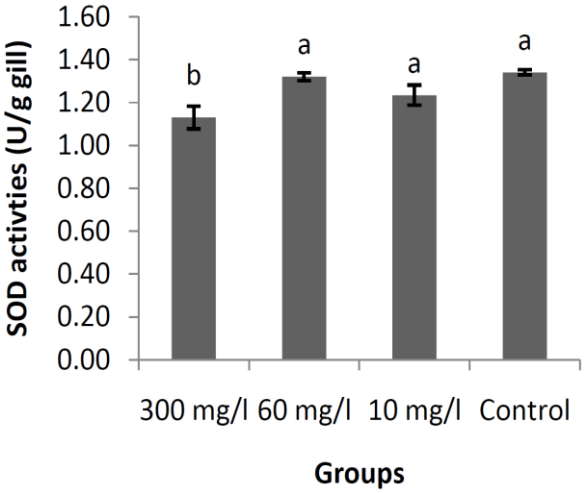


Fig 7: Superoxide dismutase activity in the gill of African Cat fish

In the kidney and gill, a significant decrease ($P < 0.05$) in the SOD was observed only in the 300g/ml test group (figs. 5 and 7 respectively) while in the liver (fig. 6) all the test groups showed significant ($P < 0.05$) decrease in SOD concentration.

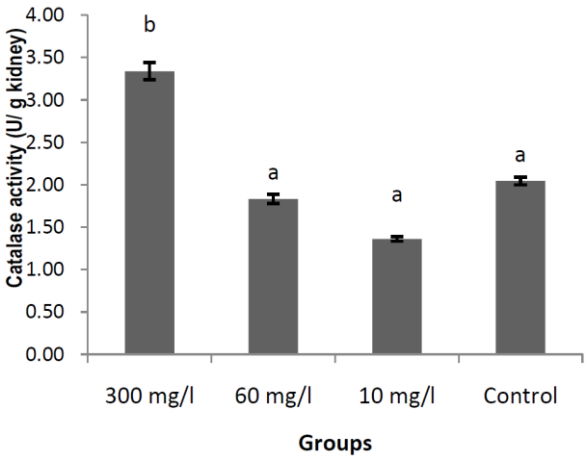


Fig 8: Catalase activity in the kidney of African Cat fish

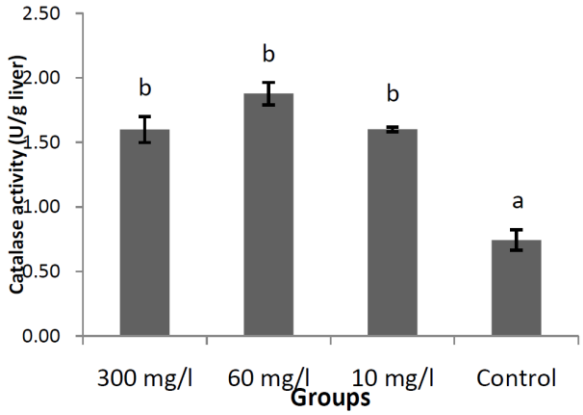


Fig 9: Catalase activity in the liver of African Cat fish

Figs 8, 9 and 10 present the effect of glyphosate on catalase activity in the kidney, liver and gill respectively of African cat fish from control group and glyphosate polluted groups. In the kidney and gill significant ($p < 0.05$) increase in CAT activity was observed only in the 300mg/l test group when compared to the control (fig. 8 and 10 respectively). In the liver all the test concentrations showed significant ($p < 0.05$) increase in CAT activity when compared to the control (fig. 9).

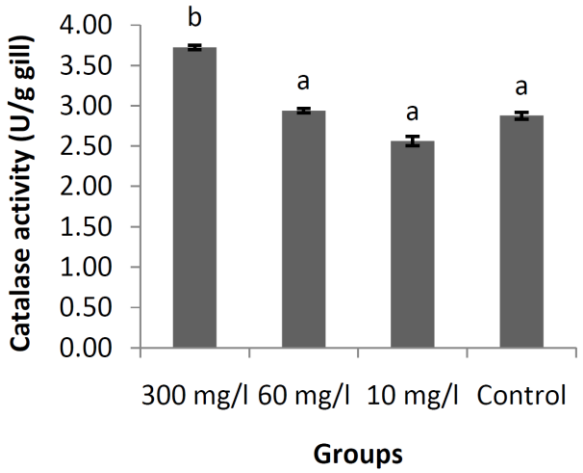


Fig 10: Catalase activity in the gill of African Cat fish

IV. DISCUSSION

Glyphosate is a herbicide that is widely used in agriculture. It is known to have long half life in aquatic environments [8] and its residues can remain stable in foods for a year or even more even if the foods are frozen, dried or processed [25]. The increased levels of MDA observed in this study (figs. 1 and 2) following exposure of Clarias gariepinus to glyphosate suggest that there were increased levels of lipid peroxidation due to increase in the production of Reactive Oxygen Species (ROS). ROS enhances the oxidation of polyunsaturated fatty acid which can end up in peroxidation of lipids [15] with production of oxidative damage products such as MDA [18]. Thus reactive oxygen species are generated in fish by glyphosate herbicide leading to peroxidation of lipids. Similar observations were reported by other researchers in fish exposed to glyphosate [17], [26].

There is a natural balance between the quantity of free radicals that the body generates and the antioxidants needed to scavenge them so as to protect the body cells against their damaging effects. Under physiological conditions, these protective antioxidants are present in quantities that are only enough to deal successfully with the physiological rate at which free radicals are generated [17]. Hence, whenever there is an external influence responsible for the generation of free radicals, there may be an imbalance in the free radical/antioxidant ratio, a situation where the antioxidants produced will not be enough to clear the free radicals generated.

In the present study, the antioxidant enzyme glutathione peroxidase (GP_X) was significantly decreased with exposure to glyphophate (figs. 3 and 4) when compared to the control. This suggests that exposure to glyphosate for 9 days caused production of ROS which could not be adequately contained by the Glutathione antioxidant system. Reference [27] reported a similar decline in the activities of GSH – dependent antioxidant enzyme, GPx and GST in the liver of tilapia (Oreochromis mossambicus) exposed to Iambda cyhalothrin.

The results of this study showed significant decrease in SOD activity upon exposure of Clarias gariepinus to glyphosate for 9 days (figs 5, 6, 7). SOD converts the superoxide anion radical to oxygen and hydrogen peroxide [28]. In this study, the production of ROS caused by exposure to glyphosate within the 9 days period may have overwhelmed the available SOD causing its reduction in activity when compared to the control. A similar decrease in SOD activity was obtained by [26] in the liver of Prochilodus lineatus exposed to glyphosate. It is also strongly believed that the production and accumulation of high levels of hydrogen peroxide by the activity of SOD on ROS inhibits enzymatic activity of SOD [18].

Catalase activity CAT was found to significantly increase in this study with the exposure of Clarias gariepinus to glyphosate (figs. 8 and 9). This finding is consistent with the results obtained by [18] in his studies on the effect of glyphosate exposure to Chlorella kessleri and [17] in the toxicity studies of glyphosate and paraquat herbicides on Clarias gariepinus.

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