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Oxidative Stress and Antioxidant Response in the Giant African Catfish (*Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809) under Chronic Paraquat Exposure

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ABSTRACT
The study assessed oxidative stress and antioxidant response parameters in the liver, kidney and gills of the Giant African catfish; *Heterobranchus bidorsalis*. Fish were exposed to different Paraquat concentrations of 0.0mg L^{-1}, 0.69mg L^{-1}, 1.37mg L^{-1} and 2.75mg L^{-1} for 28 days. Oxidative stress (Lipid peroxidation) was measured as malondialdehyde (MDA). Antioxidant response was measured as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GH-Px). The herbicide stress caused significant ($P<0.05$) increase in the MDA, SOD and CAT activity in all the organs examined except in the kidney in which the CAT was significantly decreased. The GH-Px activity, decreased in these organs and the changes in all these parameters were concentration dependent. These parameters significantly differ from the control (0.0mg L^{-1}) with no paraquat concentration. The study suggests that Paraquat induces oxidative stress in exposed fish. This antioxidant response has the potential to serve as a biomarker for the monitoring of bipyridyl herbicides in aquatic environments.

Key words: Paraquat, Oxidative stress, Antioxidant response, *Heterobranchus bidorsalis*

INTRODUCTION

The need to increase crop production to meet the ever increasing human population, most especially in the developing countries has necessitated the increasing use of Agrochemicals such as herbicides which saves the farmer's time by replacing the laborious manual weed control. There has, been great concern with the injudicious and indiscriminate use of these Agrochemicals since a large proportion of the chemical moves into the ecosystem (Pimentel and Levitan, 1986) in which water bodies such as ponds, lakes and low lying water filled areas are continuously polluted by these chemicals (Kumar and Saradhamani, 2004). Paraquat (1, 1, Dimethyl -4-4'-bipyridinium dichloride) is a bipyridyl herbicide widely used for general weed control (by contact) in all crops, a defoliant and also as a desiccant for some harvested agricultural produce. It is sold in Nigeria under different trade names such as Ravage, Grammazone, Lesher, Weed crusher (Dugje *et al*., 2008). Paraquat from decayed plants when in contact with soil is rapidly absorbed and binds strongly to the soil constituent which reduces the mobility of the herbicides due to leaching (Eisler, 1990; Moyer and Lindwall, 1985). The herbicide is repeatedly used for weed control and large quantities, therefore passes into the aquatic environment through rain and get rapidly accumulated by aquatic organism, especially fish (Ikpesu, 2013; Gabryelae and Klekot, 1985). Aquatic invertebrates are the most sensitive group of organism to this herbicide and adverse effects had been reported in crab larvae at a concentration between 0.5- 0.9µg/L (summers, 1980). Amphibians and fishes are usually unaffected at concentrations below 3,000µg/L although sensitive species such as frog tadpoles and common carp (*Cyprinus carpio*) were affected at 500µg/L (Anonymous, 1988). Bipyridyl herbicides are known for their potential to make redox cycles and cause oxidative stress in living organisms (Kappus and Sies, 1981). They interfere with intracellular electron transfer systems with reactive oxygen species (ROS) in plants which leads to cell death (Summers, 1980). Chronic exposure of *Clarias gariepinus* to Paraquat has been reported to cause marked changes in the electrolyte levels of the fish (Edori *et al*., 2013). A recent study (Nwani, *et al*., 2015) reported

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that Paraquat can impair physiological activities in *C. gariepinus*. Sub lethal concentrations of Paraquat have also been reported to cause significant changes in the plasma protein, plasma glucose and triglycerides of *C. gariepinus* (Kori- Siakpere *et al*., 2007). Oxidative stress is a condition associated with increased rate of cellular damage induced by oxygen free radicals and other reactive oxygen species (Valvanidis *et al*., 2006). It results from an imbalance between pro-oxidant and antioxidant in favour of pro-oxidant. Oxidative stress due to the action of free radicals plays a vital role in the regulation of many processes in living organisms. This includes the activation of certain transcriptional factors or in cell signalling pathways (Durackova, 2014; Halliwell, 2011). Antioxidant defence activities have proved to be of great diagnostic value as molecular markers of environmental pollution before oxidative damage in aquatic organisms (Vlahogiana and Valavandis, 2007) and the enzymes; superoxide dismutase, catalase and glutathione dependent enzymes (glutathione peroxidase, glutathione reductase and glutathione-s-transferase) constitute the antioxidant defence system in the organism (Martinez- Avarez *et al*., 2005).

Even though the toxic effect of Paraquat has been evaluated in some fishes (Ada *et al*., 2012: Ogamba, *et al*., 2011; Tortorelli, *et al*., 1990), there has not been any documented information on the effect of this herbicide exposure on oxidative stress and antioxidant response in any freshwater fish in Nigeria. The main thrust of this present study was to evaluate the effect of chronic exposure to Paraquat on *H. bidorsalis* by examining the oxidative stress and antioxidant response in the liver, kidney and gills at different Paraquat concentrations. *H. bidorsalis* was selected for the study because it is one of the commonly cultured *Clariid* catfish in ponds in Nigeria with high commercial value.

MATERIALS AND METHODS

Experimental fish

Two hundred, sixteen (16) weeks old *Heterobranchus bidorsalis* juveniles (62.5 ± 2.3g) were obtained from the hatchery complex of Federal College of Freshwater Fisheries Technology, New Bissau, and Niger State, Nigeria. The fish were transported in an open plastic container to the, Department of Aquaculture and Fisheries laboratory, University of Ilorin, Nigeria. The fishes were acclimated for seven (7) days in continuously aerated plastic tanks (3,500 litre capacities) filled to two-third of its volume with de-chlorinated water. During the conditioning period, they were fed with 3mm 40% crude protein commercial catfish diet (Coppens) at 3% of their biomass. Sealed Paraquat (276g/l), trade name - Grammazone (CAS number-CN33000217), manufactured by Anhui Zhongshan Chemical Industry Co. limited, Xianyu China was purchased from Aromokeye agro chemical company in Ilorin, Kwara State Nigeria and used as a stock solution.

Experimental design

The experiment was a semi static laboratory method conducted over 28 days in plastic aquaria (60 x 30 x 30 cm size). Twelve fishes were exposed each to Paraquat at the following concentrations: 0.0mgL^{-1} (control), 0.69mgL^{-1}, 1.37mgL^{-1} and 2.75mgL^{-1} which correspond to 0, 2.5, 5 and 10% of the 96h LC50 for *Clarias gariepinus* (a closely related catfish) previously examined in acute toxicity test on Paraquat (Nwani *et al*., 2014). The control group (0mgL^{-1}) did not contain Paraquat. The experimental conditions were in triplicates for the four treatments (n=144) and the fish were fed 3mm imported Coppens diet (40% Crude protein) in equal rations twice daily at 3% of their biomass. Seventy percent (70%) of the exposed solution was renewed every 48hours after feeding in order to maintain the appropriate concentration of the test solutions. The behaviour of the fish and survival were monitored during the experiment.

Collection and preparation of tissue homogenates

At the end of the experiment, the fish were euthanized by decapitation, dissected and the liver, kidney and heart were removed. The weighed organs were washed and homogenised in 50nM potassium phosphate buffer (pH 7.4) containing 0.5mm EDTA using a Teflon Homogenizer. The homogenates were centrifuged at 10,000rpm for 20 minutes at 4^{o}C and the supernatants obtained were stored in a freezer overnight until required.

Determination of oxidative stress and anti-oxidative parameters

Lipid peroxidation estimation was carried out as described by Lushchaks *et al*. (2005) through the determination of thiobarbituric acid reactive substance (TBARS) which are indices of membrane lipid peroxidation. Thiobarbituric acid reactive substance, values are reported as malondialdehyde (MDA) and expressed as nmol/mg protein. Superoxide dismutase activity (SOD) was determined by the method of Misra and Fridovich, (1972) in which the assay depends on the auto oxidation of adrenalin due to the presence of superoxide anion, measured spectrophotometrically at 420nm and expressed as a unit/mg protein. The catalase activity (CAT) assay based on the breakdown of H202 was performed as described by Clairborne, (1995) and the absorbance was measured at 240nm (pH7.0, 28ºC) and expressed as unit/mg protein. Gluthionine peroxidase activity (GH-Px) was determined by the method of Paglia and Valentine (1967) and expressed in nmol/mg protein.

RESULTS

Chronic exposure to Paraquat was significantly related to lipid peroxidation in the liver, kidney and gills of *H. bidorsalis* after 28 days (Table 1). The MDA concentration was higher in the liver. The MDA concentrations showed an appreciable increase with increasing concentrations of the herbicide and were significantly different ($P<0.05$) from the control group. The MDA content increased to 132% (2.16 ± 0.18), 128% (1.92 ± 0.21) and 111% (1.33± 0.14) in the liver, kidney and gills respectively after exposure to 10% 96H LC50 (1.37mgL^{-1}).

Table 1.0: Lipid peroxidation (nmol/mg protein of MDA) in three organs of *H. bidorsalis* after 28 days of exposure to various concentrations of Paraquat.

Concentrations (mg/L)	**Organs**		
	Liver	**Kidney**	**Gills**
0.0	1.63±0.23^{c}	1.49±0.31^{c}	1.19±0.17^{d}
0.69	1.74±0.5^{bc}	1.68±0.5^{bc}	1.24±0.09^{c}
1.37	1.86±0.4^{b}	1.77±0.14^{b}	1.20±0.32^{b}
2.75	2.16±0.17^{a}	1.92±0.21^{a}	1.33±0.14 ^{a}

Means with dissimilar superscripts in the same column are significantly different ($P<0.05$)

The highest SOD activity was also recorded in the liver of the fish exposed to 2.75mg/L of Paraquat concentration as shown in Table 2.0. The activity of SOD increased significantly ($P<0.05$) in the liver and kidney of the exposed fish while it decreased in the gills with an increase in concentrations of Paraquat. A significant difference ($P<0.05$) from the control group was obtained in the SOD activity of the organs examined. The SOD activity increased to 168% (24.1 ± 2.21) and 159.7 (21.4 ± 2.71) in the liver and the kidney while it decreased to 68.9% (9.75 ± 2.07) in the gills. The changes in the enzyme activities in these organs were dependent on the concentration of the herbicide.

Table 2.0: Superoxide dismutase (SOD) activity (unit/mg protein) in three organs of *H. bidorsalis* after 28days of exposure to various concentrations of Paraquat

Concentrations (mg/L)	**Organs**		
	Liver	**Kidney**	**Gills**
0.0	14.63±3.06 ^{c}	13.4±2.64^{c}	14.15±2.68 ^{a}
0.69	16.61±2.71^{c}	15.21±3.44^{bc}	12.87±1.89^{b}
1.37	19.25±1.78^{b}	17.52±2.08^{b}	11.85±2.35^{bc}
2.75	24.1±2.21 ^{a}	21.4±2.71^{a}	9.75±2.07^{c}

Means with dissimilar superscripts in the same column are significantly different ($P<0.05$)

Catalase activity in the liver, kidney and gills of *H. bidorsalis* in response to the various concentrations of paraquat for 28 days of exposure is shown in Table 3.0. The highest CAT activity was obtained in the gills of the exposed fishes to 2.75mg/L of the paraquat solution.

Table 3.0: Catalase (CAT) activity (unit/mg protein) in three organs of *H. bidorsalis* after 28 days of exposure to various concentrations of Paraquat.

Concentrations (mg/L)	Organs		
	Liver	Kidney	Gills
0.0	95.2 ±0.23 [d]	90.4±7.24 [a]	97.6±8.21[d]
0.69	115.7±6.13 [c]	81.62±9.13[b]	110.06±5.62[c]
1.37	121.18±0.41[b]	74.10±6.43[bc]	124.99±5.16[b]
2.75	132.16±0.17[d]	60.02±8.11[c]	145.7 ±6.08[a]

Means with dissimilar superscripts in the same column are significantly different (P<0.05)

Catalase activity was significantly increased in all the liver and the gills while it decreased in the kidney as Paraquat concentration increases. After 28 days of exposure to 10% 96H LC50 of Paraquat, the activity was found to be 138.8% (132.16 ±5. 32), 57% (60.02± 8.11) and 149.3% (145.7± 6.08) in the liver, kidney and gills. This represents an increase of 43.5% and 48.1% in the liver and gills respectively, and a corresponding decrease of 30.38% in the gills. There was also no significant difference in CAT activity at the different concentrations of exposure in the kidney of the fishes.

The GH-Px activity in the liver, kidney and gills of the exposed fish is shown in Table 4.0. The highest GH-Px activity was obtained in the liver exposed to 0.0mg/L of the solution which is the control treatment which did not contain Paraquat. The result shows significant (P<0.05) concentration dependent reduction in GH-Px activity in all three organs. The 10% 96H LC50 (2.75mg) dose of Paraquat caused levels of GH-Px to decreased by 66% (61.3 ± 7.13), 73.3% (58.24%) and 46% (27.26 ±4.21) in the liver, kidney and gills respectively compared to the control after 21 days of exposure.

Table 4.0: Glutathione peroxidase (GH-Px) activity (units/mg protein) in three organs of *H. bidorsalis* after 28 days of exposure to various concentrations of paraquat

Concentrations (mg/L)	Organs		
	Liver	Kidney	Gills
0.0	92.4 ±6.11[a]	79.42±3.27[a]	59.32±5.16 [a]
0.69	84.3±3.75[ab]	74.3±2.01[b]	44.10±3.41[b]
1.37	78.21±5.74[b]	71.1±4.22[c]	38.41±6.92[c]
2.75	61.3±7.13[c]	58.24±7.11[d]	27.26 ±4.21[d]

Means with dissimilar superscripts in the same column are significantly different (P<0.05)

DISCUSSION

In this study, there was a significant increase in oxidative stress and antioxidant activity of the liver, kidney and gills of *H. bidorsalis* exposed to different concentrations of Paraquat. This increase corroborates with findings on the increased antioxidant activities in the several fishes exposed to some herbicides (Elias *et al*., 2002; Farombi *et al*., 2008; Kadry *et al*., 2012).

Oxidative stress in aquatic organisms is induced by many chemical pollutants which may stimulate the production of reactive oxygen species and other oxygen free radical that can lead to alteration in antioxidant systems (Kadry *et al*., 2012; Valvanidis, *et al*., 2016). Lipid peroxidation results from oxidative breakdown of the lipids in the cell and organelle membranes and the concentrations of malondialehyde which is a by-product of this oxidative process, is a useful indicator of an increase in ROS concentrations and cellular injuries (Govindassamy *et al*., 2013). Studies have shown that Paraquat may prompt oxidative stress in fishes (Di Giulio and Meyer, 2008; Figueiredo-Fernandes, 2006; Stephensen *et al*., 2000). In this study, exposure of *H. bidorsalis* to Paraquat at the various concentrations induced an increase in MDA levels in the liver, kidney and the gills. These increases show the enhancement of LPO and suggest that ROS may be involved in the metabolism of Paraquat leading to peroxidation membrane lipids of these organs which can lead to loss of cellular function and mutations (Munkittrick *et al*., 2000; Bailey *et al*., 1996). Increase lipid peroxidation recorded in this

study has also been reported in several fish species exposed to other herbicides by Oropesa, *et al*. (2009), Elia *et al*. (2002) and Farombi *et al*. (2008).

The changes in enzyme activity during this study were dependent on the herbicide concentration. Such antioxidant systems can serve as biomarkers of exposure to pollutants and good indicators of aquatic toxicity whose activity could involve scavenging or hindering the formation of ROS, breakdown of hydrogen peroxide or blocks the formation of hydroxide in the tissues of affected organisms (Niwa *et al*., 2011). Superoxide dismutase is a class of all-enzymes whose antioxidant activity protects the organism from the toxic effects of superoxide radical.

The increased SOD activity observed in the liver and kidney of *H. bidorsalis* may be a response to the production of superoxide anions in which SOD converts the radical to H_2O_2. A similar increase has also been reported in the liver of the Nile tilapia exposed to the same herbicide by DI Giulio and Meyer, (2008) in *Clarias gariepinus* exposed to butachlor, Farombio *et al*. (2008) and in hybrid *Heteroclarias* exposed to sub–lethal concentrations of Monocrotophos (Abdulkareem and Owolabi, 2014). Unlike in the liver and kidney, SOD levels in gills were found to decline with increased paraquat exposure. Several studies have reported decreased SOD concentrations in tissues of fish and the decrease in exposed gills could be due to its poor tolerance to the toxicant (Abdelkhalek *et al*., 2015; Hamed, 2015; Govindassamy *et al*., 2013).

Catalase activity was found to increase in the gills and the liver of *H. bidorsalis* following Paraquat exposure to the various concentrations in this study while it decreased in the kidney. CAT detoxifies hydrogen peroxide to water and these elevated levels in the liver and the gills may be associated with the hydrogen peroxide produced by SOD activity in these organs. The SOD-CAT system is the primary defence mechanism against oxidative stress. Achuba *et al*. (2014) also reported increased CAT activity in the liver of *Heteroclarias* exposed to environmental pollutant while decreasing levels were, however obtained in the different tissues of some other fish species (Sayeed, *et al*., 2003; Alzbeta *et al*., 2014; Heba, 2015). Glutathione peroxidase catalyses the gluthathione dependent reduction of hydrogen peroxides which protects the oxidative damage of the erythrocytes due to lipid peroxidation.

The decreased GH-Px activity in the organs examined in this study suggests the defect in the protective role of the enzyme against lipid peroxidation. Similar decreased GH-Px activity were also recorded in the organs of *Cyprinus carpio* Govindassamy *et al*. (2013) and *Oreochromis niloticus* Abdelhalek *et al*. (2015) exposed to some pesticides. Antioxidant activity may be increased or decreased due to chemical stress depending on the intensity and duration of the stress as well as the tolerance of the exposed fish which is a function of the organism adaptation (Doyotte *et al*., 1997). There are variations in the antioxidant response to oxidative stress in the various tissues of different species (Ahmad, *et al*., 2000) and such fluctuations may be caused by a different concentration of xenobiotic in these systems due to the blood volume differences in the tissues of fish (Isik and Celik, 2008).

Conclusion

The present study shows that Paraquat induces oxidative stress and influences antioxidant response differently in the different tissues of *H. bidorsalis* and prolonged expose to this herbicide may lead to death. In order to prevent economic loss that can be associated with pollution of the fish ponds with paraquat, there is the need to put in place appropriate measures to prevent the discharge of this herbicide through runoff or erosion into culture facilities from crops that has been sprayed with the herbicides.

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