Oxidative Stress in Tilapia guineensis Exposed to Paraquat Dichloride in the Laboratory

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ABSTRACT : *Changes in antioxidants (Catalase, Superoxide dismutase and Glutathione-S-Transferase) in the plasma of Tilapia giuneensis exposed to paraquat dichloride at concentrations of 0.00(control), 0.01, 0.02 and 0.03 mg/L, were determined spectrophotometrically to evaluate the oxidative stress induced by this pesticide. Blood samples were collected from these fish and were analyzed with Randox diagnostic test kits. Results from antioxidants analyses showed that catalase reduced significantly (P<0.05) when compared to the control values. Moreover, values of Superoxide dismutase and Glutathione transferase significantly increased (P<0.05) in all the concentrations of exposure. These variations were more pronounced in the fish exposed to 0.02 and 0.03 mg/L concentrations of the pesticide.*
Keywords: Pesticide; Antioxidants; Tilapia; Toxicity And Aquatic environment.

I.INTRODUCTION

Alterations in the balance between prooxidants and antioxidants in the cell of an organism, which results in biochemical and physiological effects, are known as oxidative stress [1]. This is a harmful condition in which increase in free radical production and decrease in antioxidant levels can lead to potential damage [2]. Indicators of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products. Oxidative stress is known to play a large role in the pathology of several human diseases, including atherosclerosis, pulmonary fibrosis, neurodegenerative diseases (i.e. Parkinson's, Alzheimer's) and cancer, as well as aging pathology [3]. Aerobic organisms have developed a comprehensive antioxidant defense system in a living organism, comprising both molecular and enzymatic defenses, against the dangers of oxygen radicals, thereby preventing excess oxidation and damage [4].

Oxidative stress can equally be described as veritable disparity between the production of reactive oxygen species and the cells' ability to reduce ROS, detoxify reactive intermediates, and/or repair damage that may occur in cellular molecules [5]. These differences may occur as a result of increased ROS production, a decrease in defense mechanisms, or both. ROS are endogenously produced from a wide number of sources within the cell. However, and very importantly, many environmental parameters are known to cause oxidative stress. Some of the most well documented in humans include irradiation, i.e. x-rays, γ-rays and UV light, air pollution, PAHs, drugs and their metabolites, and exposure to some heavy metals[6]. Xenobiotics have been reported by some authors to cause oxidative stress via the action of enzymes i.e. CYP1A, NAD(P)H oxidases, and flavoprotein oxidases, via metal-catalyzed oxidation systems, or via redox cycling [7,8,9].

A large number of contaminants that are present in the aquatic environment are as a result of human activities in these areas [10]. This can cause an increase in ROS production in the cells of the exposed organism especially fish. As the amounts and diversity of chemicals entering into the aquatic environment increase, biological loads in fishes have also increased with deleterious effects. Many pollutants mediate their toxicity through oxidative stress, resulting in changes in antioxidant defenses as well as damage to proteins, membrane lipids and DNA molecules. The result of such exposure leading to oxidative stress can impair cellular or biological function which can lead to disease [11]. One of such chemicals is paraquat dichloride, an herbicide commonly used in Nigeria.

Paraquat is an herbicide used to control weeds and grasses in the field. It is poisonous to humans, having toxic

effect on the liver, lungs and kidneys if ingested. Paraquat is easily broken down and can persist in the environment absorbed by soil particles [12] . Paraquat is a trade name for N,N-dimethyl 4, 4-bipyridinium dichloride, one of the most widely used herbicides in the world. Paraquat is quick acting and non-selective, killing green plant tissues on contact. It is also toxic to human beings when swallowed. In acute toxicity studies using laboratory animal's paraquat has been shown to be highly toxic by the inhibition route and has been placed in toxicity category. The acute toxicity of paraquat dichloride to fresh water fish species showed moderate sensitivity [13].

Biomarkers of oxidative stress, such as alterations in antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure of to contaminants. These oxidative stress parameters have been associated with various disease pathologies and organism longevity in a number of species, thereby establishing ecological relevance in these cases [14]. A large number of biomarkers of oxidative stress have been used in fish studies and these include both the antioxidant defense mechanisms possessed by the cell, both enzymatic and molecular, as well as oxidative damage products [15,16]. However, the relationship between exposure, either in laboratory or field situations, and the antioxidant response, is unclear with regards to many antioxidant parameters [17]. Knowledge is needed concerning alterations of various antioxidant mechanisms and damage effects in tropical fish species. The present study therefore investigates the oxidative stress status and associated antioxidant imbalance in the blood plasma of *Tilapia guineensis* fish exposed to paraquat dichloride in the laboratory.

II. EXPERIMENTAL WORK

Experimental Location and Fish

The experiment was performed at African Regional Aquaculture Centre, (ARAC), Buguma, Rivers State, Nigeria. The experiment was carried out in the hatchery and at the disease laboratory unit of the centre. One hundred and twenty (120) adult of *T.guineensis (*mean total length 14.63cm ± 2.61SD and mean weight 105.22 ± 3.14SD) were harvested from African regional Aquaculture Center, Buguma, Rivers State, Nigeria. They were transferred immediately in open, 50L tanks, half filled with water to the laboratory where they were acclimated to laboratory conditions for a period of seven day.

Preparation of test solutions and exposure of fish

The pesticide, paraquat dichloride (dragon), used were purchased from a commercial outlet, in Port Harcourt, Nigeria. Acute renewal bioassay was conducted in the laboratory following OECD guidelines No 203 to determine the toxicity of the chemicals to fish. Four concentrations 0.00 mg/l (control) , 0.01 mg/L, 0.02 mg/L, and 0.03 mg/L, were prepared following the methods of Gabriel et al. (12). They were dispensed into 30L tanks containing dechlorinated water. Ten fishes were randomly distributed into each test tank. The fish were exposed to the chemical for a period of 15 days

Collection of blood samples

Blood samples were collected at the end of the experimental period. Each blood collection was completed within 5 minutes of fish removal from the culture system. 5ml samples were drawn once and poured into Eppendorf tubes containing 500U of sodium heparin used as an anticoagulant. The blood samples were put in ice chest box and transported within 6 hours of collection to biochemistry laboratory for analysis.

Analytical procedure

Blood samples were centrifuged immediately for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20°C until assayed. The results were read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405).

Determination of Catalase

The activity of Catalase in centrifuged plasma was determined spectrophotometrically using the method of Bebianno, [18] . Into a centrifuge tube was pipetted 0.5ml of blood sample treated with 0.5ml of 10% TCA. This solution was immediately centrifuged for 15 minutes at 3,000rpm. Supernatant (0.4ml) was pipetted into two cuvettes. 0.8ml of 0.4M Tris buffer at pH 8.9 and 20µl was added to freshly prepared DTNB. Into each cuvette was pipetted 0.76ml of distilled water and 0.4ml of 0.059MH_20_2. The mixture was incubated at room temperature to achieve equilibrium. The absorbance was read within 5 minutes in addition to DTNB at 412 nm against a blank containing 0.5 ml of water.

The activity of Catalase was calculated thus:

$$\text{Units /mg protein} = \frac{\text{Absorbance at 240nm/min x 1000 x df}}{43.6}$$

Where 4.36 is the absorption coefficient of 1M H_2O_2

1000 = Catalase activity to hydrolyze 1mmol of H_20_2 in 1 minute

df = dilution factor

Determination of Superoxide Dismutase (SOD)

The activity of SOD was determined in the plasma using spectrophotometer (Dautremepuits, 2004). Two test tubes labelled test (T) and blank (B) were placed in a rack. Into the (T) test tube was pipetted 0.10ml of buffer, 0.83 ml distilled water, and 0.05 ml of centrifuged plasma. Into the test tube marked (B) was pipetted 0.15 ml of buffer, 0.83 ml of distilled water. The solution was incubated at 25^0C for 10 minutes. Later, Pyrogallol (reagent) was dissolved in 2ml HCL and 0.02 ml of the pyrogallol was pipetted into test tubes. The solution was mixed immediately by inversion and the record in the increase in absorbance at 340 nm for approximately 3 minutes was recorded. The reading of sample absorbance was obtained at 420 nm/minute using the maximum linear rate for both the Test and Blank.

The number of units of SOD in the assay was calculated thus:

$$\text{Units /mg protein} = \frac{(\text{Asorbance at 420nm/min of blank} - \text{Absorbance 420nm/min of sample})X100}{\text{Absorbance at 420nm/min of blank}}$$

Determination of Glutathione-S-Transferase

Glutatransferase concentration was estimated spectrophotometrically according to Dautremepuits 2004). Into one test tube labelled (T) was pipetted 1.00ml buffer, 0.05 G-SH, 0.05ml CDNB and 0.05ml of plasma sample. The solution was mixed immediately by inversion and the increase at absorbance 340 nm was recorded for approximately 5 minutes. The sample absorbance was read at 340 nm/minute using the maximum linear rate for both the Test and the Blank.

The activity of Glutathione-S-Transferasewas calculated as follows:

$$\text{Units /mg protein} = \frac{\text{Absorbance 340/min Test} - \text{Absorbence 340nm/min Blank (1.5) (df)}}{(9.6)(0.05)}$$

Where 1.15 = Total volume (in milliliters) of assay

df = Dilution factor

9.6 = Millimolar extinction coefficient of Glutathione-1-Chloro-2, 4- Dinitrobenzene conjugate at 340 nm

0.05 = Volume (in milliliter) of enzyme used.

Evaluation of Water Quality Parameters

Water quality parameters in the experimental tanks during the study were evaluated: Water temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013, Jenway, China). The values of dissolved oxygen and ammonia were evaluated using the method described by APHA [19].

Statistical analysis

Indices of oxidative stress were analyzed using one-way analysis of variance, (ANOVA) at 5% level of significance. Post-hoc comparison of significance of variance results gotten from ANOVA was done using DMRT (Duncan Multiple Range Test) tests. These analyses were carried out based on a computer programme SPSS 22.0.

III.RESULTS AND DISCUSSION

The result of the water quality in experimental tanks of fish exposed to paraquat is shown in Table 1. The result indicated that the temperature and pH of the water were within the same range with no significant difference. However, ammonia and dissolved oxygen values significantly reduced with increasing concentrations of the chemical. Changes in antioxidants in Tilapia fish exposed to paraquat are presented in Table 2. The results revealed that the values of catalase in all the concentrations decreased significantly ($P < 0.05$) when compared to the control. However, the values of Superoxide dismutase and Glutathione transferase were elevated significantly ($P < 0.05$) when compared to the control concentrations.

The antioxidant defense systems which include catalase (CAT), superoxide dismutase (SOD), and Glutathione-S-transferase(GST) can be altered by a slight oxidative stress as a compensatory response in the cell of an organism. Livingstone [6], reported that the oxidative stress caused by pesticides in aquatic organisms may lead to ROS production and alterations in antioxidants enzymes. The production of ROS may attack nearby molecules resulting in damage of the molecular structure and function or dysfunction of in the systems of the fish [19]. The excessive production of ROS production and their damaging effects can be minimized by the cellular antioxidant systems [20]. Generally, organisms under stress conditions utilize the antioxidant enzymes to adjust their systems to environmental stress and these alterations depend majorly on the dose, species, method and route of exposure of the toxicants [20]. The enzymes such as SOD, gluthatione and catalase play a major role in eliminating the ROS produced during bioactivation of contaminants in the cell of an organism [21]

Table 1: Water Quality Parameters in Experimental Tanks of *Tilapia guineensis* Exposed to Paraquat Dichloride (Mean ± SD)

Concentrations of Paraquat (mg/L)	Water Quality Parameters (mg/L)			
	Temperature	Dissolved Oxygen	Ammonia	pH
0.00 (control)	29.82±0.16[a]	6.53±1.31[c]	0.03±0.01[a]	6.73±1.31[a]
0.01	29.75±0.32[a]	5.97±0.67[b]	0.04±0.01[a]	6.53±1.11[a]
0.02	29.79±0.02[a]	4.98±2.00[b]	0.09±1.31[b]	6.13±1.21[a]
0.03	29.60±0.32[a]	3.25±0.22[a]	0.10±1.31[b]	6.03±1.01[a]

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

Table 2: Antioxidants in Plasma of *Tilapia guineensis* Exposed to Paraquat Dichloride (Mean ± SD)

Concentrations of Paraquat (mg/L)	Antioxidants (Umg/protein)		
	Catalase	Superoxide-dismutase	Glutathione-S-transferase
0.00 (control)	0.60±0.01[a]	1.98±0.12[b]	1.40±0.025[a]
0.01	0.77±0.02[a]	1.72±0.03[b]	1.22±0.55[a]
0.02	0.99±0.08[b]	0.91±0.07[a]	0.92±0.14[a]
0.03	1.90±0.01[c]	0.65±0.02[a]	0.74±0.16[b]

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

In this study, Antioxidant biomarkers in the blood of *Tilapia guineensis* exposed to different concentrations of paraquat were altered significantly. Glutathione (GST) is considered one of the most important antioxidant agents involved in protection of cell membranes from lipid peroxidation by scavenging oxygen radicals [20]. The high concentrations of the antioxidant biomarker (SOD and GSH) observed in the blood of Tilapia *guineensis* exposed to herbicide paraquat. Moreover, SOD and GST are highly sensitive and respond more quickly thereby protecting organisms from oxidative stress [22]. SOD is the first enzyme that responds to oxidative stress during any stress condition in animals [23]. The observed increase in SOD and GST levels in the plasma of the exposed fish indicates a detoxifying mechanism against the toxicity. A similar observation was also noted in tissues of *Brycon cephalus* exposed to toxicants in the laboratory [23].

However, catalase levels decreased in plasma of the exposed tilapia fish. Achilike *et al.* [24]) observed similar results in plasma of African catfish (*Clarias gariepinus*) reared in different culture facilities and suggested that the oxygen radicals or their transformation to peroxide may cause oxidation of cysteine in the enzyme which results in decrease of catalase activity. According to Bacanskas *et al.* [25] as observed in killifish exposed to different contaminants, excess production of ROS may also inhibit catalase activity. The inhibition of catalase activity may be due to binding of toxicants with –SH groups of enzymes, which increased peroxide and superoxide radical [26]. The significant decrease in catalase activity in the plasma might have resulted from its inactivation by the superoxide radical activated by paraquat di - chloride exposure in the plasma of the fish [27].

CONCLUSION

In conclusion, the alterations of antioxidant defenses suggest that these parameters could be used as biomarkers for toxicity. Considering the literature and our findings in this study, it appears conceivable to speculate that the pollution induced stress has caused alteration in the redox status and total antioxidant capacity to stabilize the damage to the biomolecules such as lipids and proteins in the plasma of Tilapia fish.

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