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Induction of oxidative stress in *Clarias gariepinus* from Eleyele River in Nigeria

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Abstract. This study evaluated some markers of oxidative stress in the organs of African Catfish, *Clarias gariepinus* from Eleyele River in Oyo State, Nigeria. *Clarias gariepinus* (250 g-400 g) were collected from Eleyele River (a suspected polluted River) and *Clarias gariepinus* from a clean fish farm (Durantee fisheries) were used as the control. Levels of Malondialdehyde (index of lipid peroxidation), Glutathione (GSH) and activities of antioxidant enzymes- Superoxide dismutase (SOD), Catalase and Glutathione-S-Transferase (GST) were evaluated in the liver, kidney and gills of the fish. From the results, there were significant ($p<0.001$) increases in malondialdehyde and GSH levels in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control. The activity of GST increased significantly ($p<0.05$; $p<0.001$) in the liver and kidney of fish from Eleyele River compared with control. There was a significant decrease ($p<0.05$; $p<0.001$) in SOD activity in all the organs of *Clarias gariepinus* from Eleyele River compared with conrol and also a significant ($p<0.001$) decrease in catalase activity in the gills and kidney of the fish but catalase activity increased in the liver. Increase in lipid peroxidation and alterations in antioxidant status in *Clarias gariepinus* from Eleyele River show that the fish were under oxidative stress. These suggest that the River is polluted probably as a result of various wastes frequently discharged into the River. This could pose serious health risks to consumers of water and aquatic organisms from the River.

Keywords: environmental pollution; oxidative stress; *Clarias gariepinus*; Eleyele River; antioxidant

1. Introduction

Pollution of the aquatic environment is one of the major environmental problems all over the world as it affects aquatic organisms and even the health of human beings. Most Rivers are polluted as a result of industrial and agricultural wastes frequently discharged into them and this could cause various histological, pathological as well as biochemical alterations in fish (Reddy 2012) as well as other aquatic organisms.

Human beings depend on fish as a good source of protein and the harmful effects of pollution

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fishes could be transferred to man through the food chain. Fishes and other aquatic organisms can be used to monitor water pollution by using biomarker response (Reddy and Rawat 2013). Amongst aquatic organisms, fishes are frequently employed as models in understanding oxidative stress in aquatic environments. They can be used as biomarkers of aquatic pollution because they have the ability to bioaccumulate pollutants from water (Dautremepuits *et al.* 2004, Lopes *et al.* 2001).

Water pollution contributes greatly to oxidative stress in fish (Yildirim *et al*. 2011). Oxidative stress is induced by substances such as industrial wastes, agricultural wastes, fertilizers, landfill leachates, heavy metals, oil pollutants, pesticides and other substances that can generate reactive oxygen species (ROS) (Sevcikova *et al*. 2011, Slaninova *et al*. 2009, Lushchak 2011). Like human beings, fishes have antioxidant enzymes such as glutathione reductase, glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase that they use to nullify the harmful effects of ROS (Almeida *et al.* 2002, Pandey *et al.* 2003). They also have various non-enzymic antioxidants like vitamin A, vitamin C, vitamin E and glutathione that are also involved in the elimination of oxygen radicals (Van Der Oost *et al.* 2003, Yildirim and Asma 2010). But oxidative stress occurs in fish when there is an imbalance between the generation of ROS and production of antioxidants.

Many water bodies in Nigeria are polluted because many industries discharge their untreated wastes into Rivers, lakes and seas not minding their harmful effects on water quality and aquatic organisms. Eleyele River is one of the suspected polluted Rivers in Nigeria; it is located along Eleyele-Oluguneru road in Ibadan, Oyo State, Nigeria. It has been observed that Fan Milk Plc which is located close to the River discharge their effluent into the River. The River also receives effluents from a cassava processing site located close to it and there is evidence of regular discharge of waste water from domestic activities from neighboring homes (Akinyemi *et al*. 2014). The River serves as a major source of fish and drinking water for the people of Ibadan and its environs, therefore this study was carried out to evaluate the levels of some oxidative stress indices in *Clarias gariepinus* from Eleyele River to investigate whether the pollutants discharged into the water body induced oxidative stress and damage to the fresh water fish and also to further assess the pollution status of the River.

2. Materials and methods

Fish Samples

Nine *Clarias gariepinus* weighing between 250 g-400 g were caught from Eleyele River and transported to the laboratory on the same day. Nine *Clarias gariepinus* purchased from Durantee fisheries (a clean fish farm) were used as the control. The liver, kidney and gills of the fish were removed, washed in ice cold 1.15% KCl and homogenized in 4 volumes of homogenizing buffer (50 Mm Tris- HCl mixed with 1.15% KCl, PH adjusted to 7.4). The homogenate was then centrifuged at 12,500 g for 10 minutes to obtain the post mitochondrial fraction which was used for biochemical analysis.

Biochemical assays

Glutathione (GSH) was determined in the post mitochondrial fraction of the liver, kidney, gills and heart of *Clarias gariepinus* according to the method described by Jollow *et al*. (1974) at 412 nm using 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB).

Glutathione *S*-transferase (GST) activity was determined by the method of Habig *et al*. (1974) using 1 chloro 2, 4 dinitrobenzene as substrate. The specific activity of glutathione S-transferase is expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 $mM^{-1}cm^{-1}$.

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 as described by Magwere *et al*. (1997). One unit of SOD activity is the amount of SOD necessary to cause 50% inhibition of adrenaline auto oxidation.

Activity of catalase (CAT) was determined according to the method of Sinha (1972). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate produced was measured spectrophotometrically at 570 nm.

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990). Under acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid to yield a pink coloured complex with maximum absorbance at 532 nm.

Protein concentration was determined by the Biuret method as described by Gornall *et al*. (1949). The Biuret reaction involves a reagent containing copper (cupric) ions in alkaline solution. Molecules containing 2 or more peptide bonds associate with the cupric ions to form a coordination complex that imparts a purple colour to the solution with maximum absorbance at 540 nm.

Statistical analysis

Results are expressed as mean±standard deviation. Student's t test was used to determine differences between groups. Levels of statistical significance were analyzed by analysis of variance (ANOVA), using microcal origin 6.0 software and p-values<0.05 were considered significant.

3. Results

Protein concentration

The protein concentration in the liver of *Clarias gariepinus* from Eleyele River (25.7±1.83) was lower than that of the control (41.7±0.40), however there was increase in protein concentration in the kidney and gills (27.58±2.37; 27.62±2.17) of the test animals when compared with the respective controls (20.28±0.41; 8.87±0.67). Further information on the protein concentration in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 1 below.

Table 1 Protein concentration (mg/ml) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	41.71±0.40	20.28±0.41	8.87±0.67
TEST	25.70±1.83	27.58±2.37	27.62±2.17

Lipid peroxidation

There was a significant ($p<0.001$) increase in the level of malondialdehyde (an index of lipid peroxidation) in the liver (12.1±1.4), kidney (13.7±3.4) and gills (19.1±1.4) of *Clarias gariepinus* from Eleyele River when compared with control (2.1±0.1; 2.6±0.4; 5.0±1.0). Further information on lipid peroxidation in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 2 below.

Catalase Activity

There was a significant ($p<0.001$) decrease in the activity of catalase in the kidney (23.9±4.9) and gills (26.3±2.1) of *Clarias gariepinus* from Eleyele River when compared with respective controls (37.5±2.3; 96.3±9.0) but there was an increase in the activity of the enzyme in the liver (25.8±3.6) compared with control (17.9±0.6). Further information on catalase activity in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 3 below.

Super oxide Dismutase Activity

There was a significant ($p<0.05$, $p<0.001$) decrease in superoxide dismutase activity in the liver (1.6±0.6), kidney (1.7±0.6) and gills (2.1±0.5) of *Clarias Gariepinus* from Eleyele River when compared with respective controls (3.2±0.4; 3.0±0.7; 3.0±0.7). Further information on super oxide dismutase activity in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 4 below.

Table 2 Levels of Malondialdehyde (nmol/ mg protein) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	2.1±0.1	2.6±0.4	5.0±1.0
TEST	12.1±1.4**	13.7±3.4**	19.1±1.4**

Significantly different from control, * $p<0.05$ ** $p<0.001$

Table 3 The activity of Catalase (Unit/mg protein) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	17.9±0.6	37.5±2.3	96.3±9.0
TEST	25.8±3.6**	23.9±4.9**	26.3±2.1 **

Significantly different from control, * $p<0.05$ ** $p<0.001$

Table 4 The activity of SOD (unit/ mg protein) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	3.2±0.4	3.0±0.7	3.0±0.7
TEST	1.6±0.6 *	1.7±0.6 *	2.1±0.5 **

Significantly different from control, * $p<0.05$ ** $p<0.001$

Glutathione concentration

There was a significant ($p<0.001$) increase in glutathione concentration in the kidney (96.5±3.2) and gills (85.7±1.3) of *Clarias gariepinus* from Eleyele River when compared with respective controls (78.8±4.9; 53.8±5.1), there was also a non significant increase in the liver (65.4±3.1) compared with control (62.9±5.1). Further information on Glutathione concentration in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 5 below.

Glutathione-S- transferase Activity

There was a significant ($p<0.05$) increase in the activity of Glutathione-S-Transferase (GST) in the liver (6.2±2.3) and kidney (8.17±2.5) of *Clarias gariepinus* from Eleyele River compared with respective controls (2.3±0.5; 2.72±0.3), but there was a decrease in the activity of the enzye in the gills (5.33±0.5) compared with control (11.6±0.5). Further information on GST activity in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River compared with control can be found in Table 6 below.

4. Discussion

Levels of some oxidative stress indices were evaluated in the organs of *Clarias gariepinus* from Eleyele River. The results show that there were alterations in the antioxidant enzyme activities in the liver, kidney and gills of *Clarias gariepinus* from Eleyele River and also there was induction of lipid peroxidation in the organs of the fish as shown by elevated levels of malondialdehyde in these organs.

The significant increase in the level of MDA (an index of lipid peroxidation) in the organs of the fish (Table 2) shows that there was induction of oxidative stress in the fish which could be through generation of ROS by pollutants in the River. Oxidative stress arises in a situation where there is an imbalance between the generation of reactive oxygen species (ROS) and production of antioxidants (Nishida 2011). Pollutants can induce oxidative stress through alterations of the antioxidant enzyme activities or by ROS generation. Huang *et al*. (2007), Ferreira *et al*. (2005),

Table 5 The concentration of Glutathione (nmol/ mg protein) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	62.9±5.1	78.8±4.9	53.8±5.1
TEST	65.4±3.1	96.5±3.2 **	85.7±1.3**

Significantly different from control, * $p<0.05$ ** $p<0.001$

Table 6 Activity of Glutathione-S-transferase (nmoles/min/mg protein) in the organs of *Clarias gariepinus* from Eleyele River compared with control

GROUPS	LIVER	KIDNEY	GILLS
CONTROL	2.3±0.5	2.72±0.3	11.6±0.5
TEST	6.2±2.3*	8.17±2.5*	5.33±0.5 **

Significantly different from control, * $p<0.05$ ** $p<0.001$

Farombi *et al*. (2007), Sanchez *et al*. (2007), Bacanskas *et al*. (2004), Falfushynska *et al*. (2010) also reported increase in lipid peroxidation in the organs of fishes from polluted Rivers.

Catalase is one of the body's most powerful antioxidant enzymes; it is a heme enzyme that is present in the peroxisome of cells of almost all living organisms exposed to oxygen where it mitigates the toxic effects of hydrogen peroxide by converting it to water and molecular oxygen. It also helps to detoxify other toxic substances like phenols and alcohols (Matés 2000). The activity of catalase decreased in the kidney and gills of *Clarias gariepinus* from Eleyele River, however an induction response was triggered in the activity of the enzyme in the liver (Table 3).The observed decrease in catalase activity in the liver and kidney of the fish further strengthens the fact that the fish were under oxidative stress as a result of increased production of ROS by pollutants in the River, as increase in ROS production is correlated with decreased catalase activity as well as other antioxidants in the cells. This result is similar to our findings in *Clarias gariepinus* from Ogun River, a polluted River in Ogun State (Farombi *et al*. 2007). However, the increase in catalase activity in the liver of the fish could be an adaptive response to the increased production of reactive oxygen species in the liver cells.

Superoxide dismutase (SOD) is one of the primary internal antioxidant enzymes in the body that combats oxidative stress. It is considered to be possibly the most powerful antioxidant in the body present both inside and outside cell membranes. It is responsible for scavenging the highly reactive superoxide radicals. The observed decrease in SOD activity in the organs of the fish (Table 4) shows that the enzyme could no longer protect cells against superoxide radicals, which are the most dangerous of all the free radicals. The decrease may be due to damage to the SOD protein as a result of overproduction of superoxide radicals. This result is similar to the observations of Bacanskas *et al*. (2004), Falfushynska and Stolyar (2009), Falfushynska *et al*. (2010a) who also observed decrease in SOD activity in fishes from polluted Rivers. Impairment of antioxidant defense system may cause DNA damage, lipid and protein oxidation as a result of oxidative stress.

Glutathione which is a tripeptide of glycine, cysteine and glutamic acid is very important in all cells as it protects the cell against oxidative stress through detoxification of ROS; it also functions in the transport of amino acids as well as in the conversion of some antioxidants to their active forms (Nordberg and Arner 2001, Valko *et al.* 2006, Drake *et al.* 2003, Masella *et al.* 2005). There was increase in the level of GSH in the liver, kidney and gills of *Clarias Gariepinus* from Eleyele River when compared with control (Table 5). Usually antioxidants are depleted in cells during exposure to environmental pollutants, but sometimes antioxidants level may increase to compensate the imbalance caused by oxidative stress, this may explain the reason for the observed increase in GSH level in these organs.

Glutathione-S-Transferase (GST) activity was induced in the liver and kidney of the fish but was depleted in the gills (Table 6). Antioxidant enzymes are bioindicators of oxidative stress in aquatic organisms and they could be induced in response to pollutants (Borković *et al*. 2005). This could explain the induction mechanism of GST activity triggered in the liver and kidney of the fish. However, the activity of the enzyme was overwhelmed in the gills.

5. Conclusions

Alterations in antioxidant status as well as increase in lipid peroxidation in *Clarias gariepinus* from Eleyele River show that the fish were under oxidative stress. These suggest that the River is

polluted probably as a result of industrial, domestic and agricultural wastes frequently discharged into the River. This is of serious health concern as Eleyele River is a major source of drinking water to the people of Ibadan and its environs; people also consume aquatic organisms, especially fishes from the River.

Recommendation

Government should stop indiscriminate dumping of wastes into water bodies and people should be enlightened on the risk of consuming fishes and other aquatic organisms from polluted Rivers

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