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Original Research

# Effect of environmental pollution on oxidative stress biomarkers in african cat fish (*Clarias gariepinus*) from asejire river in oyo state, Nigeria

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## ABSTRACT

**Background:** Fishes are widely used as model organisms for the assessment of the quality of aquatic environment and can therefore serve as bioindicators of environmental pollution. In this study, the activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione S-transferase (GST), Reduced glutathione (GSH) concentration and Malondialdehyde (MDA) formation were determined in the organs of African Catfish, *Clarias gariepinus* from Asejire River in Ibadan, Oyo State, Nigeria. The River receives effluents discharged from various industries and it is suspected to be polluted. **Materials and Methods:** *Clarias gariepinus* weighing between 400g-600g were collected from Asejire River and *Clarias gariepinus* from a clean fish farm (Durantee fisheries) were used as the control. **Results:** Compared with control, a significant increase in malondialdehyde formation was observed in the liver, kidney and gills of *Clarias gariepinus* from Asejire River, while a significant decrease in superoxide dismutase, catalase and GST activities was observed in these organs. There was also a decrease in reduced GSH concentration in the liver and kidney but there was an increase in the gills of *Clarias gariepinus* from Asejire River. **Conclusion:** The results of this study show that there was induction of oxidative stress in the organs of *Clarias gariepinus* from Asejire River reflecting the pollution status of the River.

**KEY WORDS:** Asejire River; Oxidative stress; Environmental pollution; *Clarias gariepinus*; Effluent.

## INTRODUCTION

The importance of water for human sustenance is immeasurable [1]. However, sustainable water quality balance is dwindling each passing day [2]. Most water bodies are contaminated with a wide range of pollutants. Water is considered polluted when unwanted materials with potentials to threaten human and other natural systems find their ways into water sources (Rivers, lakes, well, boreholes) or reserved fresh water in homes or industries [3]. Human, industrial, domestic and agricultural activities result in the discharge of various pollutants into the aquatic environment, threatening the health of the population and damaging the quality of the environment by rendering water bodies unsuitable [4]. Water pollution affects plants and organisms living in these bodies of water [5].

Contamination of water bodies is one of the important environmental problems worldwide. Water pollution has become a very serious environmental challenge in Nigeria, particularly in the cities where there are lots of industries. Therefore, Nigeria is not left out in this global problem [6]. Most industries in Nigeria discharge their waste products directly into Rivers, rendering the Rivers polluted and unfit for domestic use and toxic to aquatic fauna [7]. It has been reported that most Rivers in southwestern Nigeria are polluted as a result of waste water discharge, industrial activities, agricultural and other anthropogenic activities taking place within the vicinities of these Rivers [8-10]. Waste water streams containing heavy metals are produced by many manufacturing processes and find their way into the aquatic environments [11-13]. Fishes in the River and natural habitats including human beings faces total collapse or extinction due to exposure to this polluted water.

Fishes are at the apex of the marine food chain and can bioaccumulate some toxic substances in their tissues [14,15]. Organic pollutants accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental stress. Oxidative stress occurs in fish usually as a consequence of environmental pollution and antioxidant defence system in fish is very sensitive to environmental conditions. These systems include various antioxidant defence enzymes such as superoxide dismutase (SOD), catalase, Glutathione-S-Transferase (GST) and Glutathione peroxidase.

Asejire River is located in Egbeda local government area at the outskirt of Ibadan, a major city in Oyo State of South-West Nigeria. It is one of the series of West African Rivers that discharge into coastal lagoons and creeks bordering the Atlantic Ocean [16]. Asejire River supports aquatic lives and is one of the major sources of water supply to the people of Ibadan and its rural environs. The River also provides raw water to the Asejire water treatment plant in Ibadan. But unfortunately, the River is perceived to be undergoing gradual pollution from human related activities at a rate that may constitute health and socio-economic problem to

the people. Lameed and Obadara [17] reported that Asejire River contains high level of contaminants and attributed this to discharge of effluents from various industries into the water body as well as breakdown of ecological balance caused by widespread destruction of flora and fauna diversities. An example of industry that discharges its effluent into Asejire River is Nigerian Bottling Company, Plc (NBC) which is located close to the River [6].

In this study, levels of certain biomarkers of oxidative stress (malondialdehyde and antioxidants) were investigated in the organs of *Clarias gariepinus* from Asejire River to further assess the extent of pollution of the River and the effect on the fresh water fish, *Clarias gariepinus*. *Clarias gariepinus* is the principal clarid catfish and probably the most widely distributed fish in Africa [18, 19]. It is also the most widely consumed fresh water fish in Nigeria [15], hence the choice of this specie for this study.

## MATERIALS AND METHODS

### Fish Samples

Ten *Clarias gariepinus* weighing between 400g- 600g with length of 27.8-31.5 cm were caught from Asejire River and transported with water from collection site in plastic containers to Biochemistry laboratory, Lead City University, Ibadan on the same day. Ten *Clarias gariepinus* purchased from Durantee fisheries (a clean fish farm which is devoid of any industrial discharge or any other facilities that could cause pollution) were used as control and were transported in the same manner like the test fish. The fishes were killed and dissected. The liver, kidney and gills were immediately removed, washed in ice cold 1.15% KCl. They were then homogenized in 4 volumes of homogenizing buffer (50Mm Tris- HCl mixed with 1.15% KCl, PH adjusted to 7.4). The resulting homogenate was centrifuged at 12,500g for 10minutes to obtain the post mitochondrial fraction which was used for biochemical analyses.

### Biochemical assays

Reduced glutathione (GSH) was determined in the post mitochondrial fraction of the liver, kidney, and gills of *Clarias gariepinus* according to the method described by Jollow et al., [20] 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., [21] 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 $9.6mM^{-1}cm^{-1}$.

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 as described by Magwere et al., [22]. 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, [23]. 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 570nm.

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale, [24]. 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., [25]. 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

Data were analysed using the Statistical Program for Social Sciences (SPSS) window versions 16.0 and Prism graph pad 6.0. Each of the test group was compared with the control group using unpaired t-test, and p- values less than 0.05 were considered statistically significant.

## RESULTS

The protein concentration in the kidney of *Clarias gariepinus* from Asejire River (26.0 ±2.2) was significantly ($p<0.05$) higher than that of the control (20.3 ±1.2), meanwhile, the gills of the test animals had a significantly ($p<0.05$) lower protein concentration (8.7 ± 1.9) compared with control (18.1 ± 1.9). Further information regarding the protein concentration in the liver, kidney and gills can be found in table 1 below.

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

| Groups | Liver | Kidney | Gills |
|---|---|---|---|
| Control | 41.7±1.2 | 20.3±1.2 | 18.1±1.9 |
| Test (Asejire samples) | 41.3±1.6 | 26.0±2.2 [a] | 8.7±1.9 [a] |

The results are expressed as Mean ± SEM. Significantly different from control, [a] $p<0.05$

There was a significant ($p<0.05$) increase in malondialdehyde formation, an index of lipid peroxidation in the liver (6.9 ± 0.3), kidney (14.2 ± 1.3) and gills (26.9 ± 2.4) of *Clarias gariepinus* from Asejire River compared with control (Figure 1).

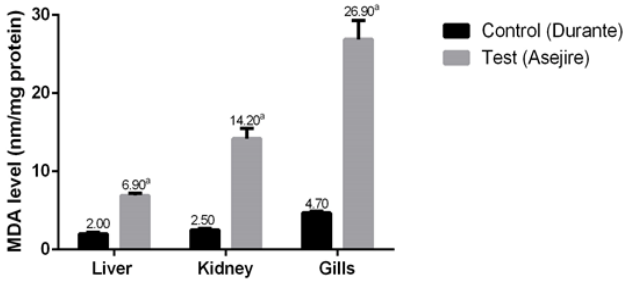


**Figure 1.** Levels of Malondialdehyde (nmol/ mg protein) in the organs of *Clarias gariepinus* from Asejire River compared with control

There was a significant ($p<0.05$) decrease in reduced glutathione concentration in the kidney (269.3±4.5) of *Clarias gariepinus* from Asejire River when compared with control (325.1±15.3) but there was a significant ($p<0.05$) increase in the concentration of this antioxidant in the gills (238.0±14.0) of the test animal compared with control (203.8±6.3). There was no significant difference in reduced glutathione concentration in the liver of the test and control group (Table 2).

**Table 2.** The concentration of Reduced Glutathione (nmol/ mg protein) in the organs of *Clarias gariepinus* from Asejire River

| Groups | Liver | Kidney | Gills |
|---|---|---|---|
| Control | 250.4±19.3 | 325.1±15.3 | 203.8±6.3 |
| Test (Asejire river) | 248.8±3.1 | 269.3±4.5 [a] | 238.0±14.0 [a] |

The results are expressed as Mean ± SEM. Significantly different from control, [a] $p<0.05$

There was a significant ($p<0.05$) decrease in the activity of superoxide dismutase in the liver (2.5 ± 0.2), kidney (2.2 ± 0.3) and gills (2.4 ± 0.3) of *Clarias gariepinus* from Asejire River when compared with control (Figure 2).

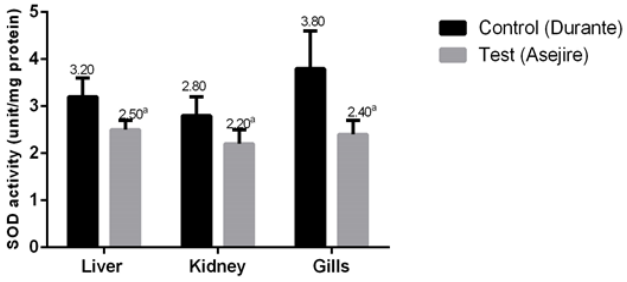


**Figure 2.** The activity of SOD (unit/ mg protein) in the organs of *Clarias gariepinus* from Asejire River

Table 3 shows the activity of Glutathione-S-trasferase, the activity of the enzyme decreased significantly ($p<0.05$) in the liver (1.6±0.5) and gills (4.0±1.1) of *Clarias gariepinus* from Asejire River compared with control but there was no significant difference in the activity of the enzyme in the liver of the test group compared with control.

**Table 3.** Activity of Glutathione-S-transferase (GST) (nmoles/min/mg protein) in the organs of *Clarias gariepinus* from Asejire River

| Groups | Liver | Kidney | Gills |
|---|---|---|---|
| Control | 2.5±0.3 | 3.5±1.3 | 9.6±2.4 |
| Test (Asejire samples) | 1.6±0.5 [a] | 3.4±0.3 | 4.0±1.1 [a] |

The results are expressed as Mean ± SEM. Significantly different from control, [a] $p<0.05$

Catalase activity was significantly ($p<0.05$) reduced in the liver (11.7±0.4), kidney (20.8±1.7), and gills (26.7±2.5) of *Clarias gariepinus* from Asejire River compared with control (Figure 3).

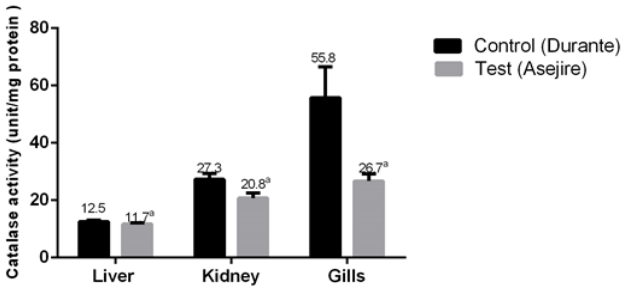


**Figure 3.** The activity of Catalase (Unit/mg protein) in the organs of *Clarias gariepinus* from Asejire River.

## DISCUSSION

Aquatic environment is a sink for many environmental contaminants which can be absorbed by aquatic organisms which may eventually disturb the antioxidant/pro-oxidant balance in fish [26, 27]. Fishes are used as a model for the bio-monitoring of the aquatic environment and as sentinel agents for pollutants [28]. The disturbance in antioxidant/ pro-oxidant balance in fish may cause oxidative stress which has been described as a state when antioxidant defenses are overcome by pro-oxidant forces [26].

Induction of lipid peroxidation as seen in the elevated levels of malondialdehyde (MDA) in the liver, kidney and gills of *Clarias gariepinus* from Asejire River compared with control point to the fact that *Clarias gariepinus* from Asejire River were under oxidative stress. Malondialdehyde is used as marker of oxidation of membrane phospholipids through lipid peroxidation [29]. An increase in MDA levels in organisms can be related to degradation of an environmental site due to decrease in water quality [30]. The significant increase in lipid peroxidation marker, MDA may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. The highest MDA levels were found in the gills compared with other tissues (Figure 1). According to Batista et al., [31] gills are particularly affected by pollutants amongst other organs in fishes because they are exposed directly to the contaminants in the environment. Gills are also known to exhibit a low-threshold response to

oxidative stress, as they are the first tissues to come into contact with water-borne contaminants [32]. These may explain the marked increase in MDA levels observed in the gills. Adedeji and Okocha, [33] reported the presence of some heavy metals in water samples and prawns from Asejire River; Lameed and Obadara, [17] detected heavy metals such as mercury, Cadmium, lead and iron in water samples from Asejire River, and Aladesanmi et al., [6] also detected Nickel, Iron, Magnesium, Zinc, Manganese, Calcium and Copper in *Clarias gariepinus* from Asejire River. The findings of these researchers confirm the presence of pollutants in Asejire River. The observed increase in lipid peroxidation in the organs of *Clarias gariepinus* from Asejire River can be attributed to high level of heavy metals as well as other pollutants in the River. The physicochemical parameters of the water medium usually affect the toxicity and bioavailability of xenobiotics in fishes. Our result corroborate the findings of other researchers who also reported increase in MDA levels in fishes from other polluted Rivers around the world [34-36], and also in fishes from Nigerian contaminated Rivers [37, 38].

Reduced glutathione (GSH) is the most copious non-protein thiol [39] found at millimolar concentrations in most cells. Glutathione is responsible for protection against reactive oxygen and nitrogen species and detoxification of endogenous and exogenous toxins of electrophilic nature. It also functions in maintaining the essential thiol status of proteins and other molecules; storage of cysteine reserves both in the cell and for inter organ transfer and signal transduction from the environment to cellular transcription machinery [27]. Reduced glutathione was depleted in the liver and kidney of *Clarias gariepinus* from Asejire River. A decrease in GSH levels is known to be closely associated with certain pathologies in both humans and animals [27]. Heavy metals and other toxicants present in the River might have overwhelmed the glutathione antioxidant system in the liver and kidney. Oxidative stress as reflected by induction of lipid peroxidation could have increased the utilization of this antioxidant in these organs to combat oxidative stress. However, it has been reported that severe oxidative stress may suppress GSH levels due to the impairment of adaptive mechanisms [40]. In contrast, there was an increase in GSH level in the gills. The levels of antioxidants in living organisms can increase in order to restore the imbalance caused by oxidative damage. Oxidative damage may lead to activation of expression of genes encoding antioxidants. This may explain the reason for the increase in GSH concentration observed in the gills.

Antioxidant enzymes such as Superoxide dismutase (SOD), Catalase and Glutathione S-transferase (GST) help to neutralize toxic effects of ROS on fish, just like in mammals. Superoxide dismutase is one of the key enzymes that provide the first line of defence against pro-oxidants and catalyses the transformation of superoxide radicals to $H_2O_2$ and $O_2$ [41]. Catalase is a heme-containing enzyme that facilitates the removal of $H_2O_2$, which is metabolized to oxygen and water. Glutathione-S-transferase (GST) in conjugation with reduced glutathione (GSH) act as defence against reactive oxygen species (ROS) and protect cells against oxidative injuries. Glutathione-S-transferase catalyses the conjugation reaction between reduced glutathione and xenobiotic metabolite to accelerate their excretion. The activity of SOD, Catalase and GST decreased in the liver, kidney and gills of *Clarias gariepinus* from Asejire River compared with control. When antioxidant defences are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents. In an aquatic environment, increased levels of oxidative damage occur in organisms exposed to contaminants that stimulate the production of ROS that can overwhelm antioxidant enzymes resulting in oxidative stress. The observed decrease in the activity of these enzymes may be as a result of high level of toxicants in Asejire River as confirmed by the findings of Lameed and Obadara, [6] and other researchers who reported that Asejire River contains high level of contaminants due to discharge of effluents from various industries into the water body, these toxicants might have caused generation of reactive free radicals that overwhelmed the function of these antioxidant enzymes, which also accounted for the high level of lipid peroxidation observed in the organs of the fish. Toxic stress is known to alter the activity of antioxidant enzymes in the vital tissues of fish. Maintenance of high constitutive levels of antioxidant enzymes is essential to prevent oxyradical-mediated lipid peroxidation. It has been reported in some cases that the superoxide radical by itself or after its transformation to $H_2O_2$ caused a strong oxidation of the cysteine in the enzyme superoxide dismutase and therefore decrease the enzyme's activity [42], this might be responsible for the observed decrease in SOD activity in the organs of the fish. Also decrease in CAT activity is known to be attributed to high production of superoxide anion radical [43], this might also explain the observed decrease in catalase activity. Similar to our findings, Ganesan et al., [44] also reported significant decrease in the activity of catalase and SOD in fish from a polluted lake in India, and Awoyemi et al., [45] also observed significant decrease in the activity of catalase and SOD in *Clarias gariepinus* exposed to lead. Also the decrease in GST activity observed in the organs of the fish corroborates the findings of Wilhelm Filho et al., 2001 who also reported a decrease in GST activity in the liver of *Geophagus brasiliensis* from a polluted River (Benedito River) in Southern brazil and Farombi et al., [38] who observed a decrease in GST activity in the gills of *Clarias gariepinus* from Ogun River in Nigeria. Dilek et al., [46] also observed a decrease in GST activity in Oreochromis niloticus exposed to cadmium and copper.

In conclusion, the results of this study show that there were alterations in biomarkers of oxidative stress in the organs of *Clarias gariepinus* from Asejire River, this shows that the fish were under oxidative stress probably as a result of high level of contaminants and industrial discharge from various industries into Asejire water body. This is an

indication that Asejire River is polluted. This is of serious health concern since Asejire River is one of the major sources of water supply to the people of Ibadan and people consume aquatic organisms, especially fishes from this River. Furthermore, the results also provide evidence that biomarkers of oxidative stress in fish can be used in aquatic ecosystem pollution biomonitoring and this should serve as an early warning signal of adverse effects of environmental pollution.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest regarding this study.

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