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Biotransformation and Oxidative Stress Markers in *Clarias gariepinus* from Petroleum Exploration Area in Delta State, Nigeria.

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ABSTRACT: *In this study Clarias gariepinus were collected from three sites (site A: Eku axis of Ethiope River (control site), site B: the swampy environment of Kokori-Erhoike petroleum flow station and site C: an earthen fish pond located within Kokori-Erhioke area). Biochemical analyses in serum, gill, liver, brain and muscle tissues of Clarias gariepinus (n=8/site) were performed using standard methods. Results indicated that blood reduced glutathione was significantly ($p < 0.05$) lower in Clarias gariepinus from sites B and C as compared with site A but elevated ($p < 0.05$) in the liver of fish from site B and C as compared with that of site A. Activities of glutathione S-transferase, superoxide dismutase, catalase and lactate dehydrogenase and values of malondialdehyde indicate high measure of oxidative stress in serum, gill, liver, brain and muscle tissues of fish from sites B and C as compared with that of site A. The changes observed in biochemical parameters showed that the fish were relatively under stress in their natural habitat (Erhoike swamp) and fish pond as compared with Ethiope River (Eku axis) and could be employed as biomarkers of contamination in environmental monitoring of crude oil pollutants and their metabolic changes as early warning signs of adverse effects of environmental pollution.*

Keywords: *Reduced glutathione, Antioxidant enzymes, Lactate dehydrogenase, Malondialdehyde, Kokori-Erhoike,*

Introduction: Crude oil is discharged into the aquatic environment during exploration activities through operational, transportation or other means and it affects marine organism directly exposed to it. The Niger Delta area of Nigeria has the worst hit of environmental havoc emanating from oil production. The pollution of streams and rivers is one of the environmental problems affiliated with oil exploration and production in the Niger Delta, Nigeria [1]. Exposure to crude oil and its derivatives can induce a variety of toxic symptoms in experimental animals and capable of inducing oxidative stress in aquatic animals including fish [2, 3 and 4]. These toxic signs can be estimated through analysis of biochemical changes in organisms inhabiting that region [5]. The response of aquatic organism to pollution is given by changes through exposure of several key enzymes especially those of biotransformation (glutathione S- transferase) and oxidative stress markers (superoxide dismutase, catalase, lactate dehydrogenase and malondialdehyde) [6 and 7].

For over 35years of oil exploration activities in Kokori-Erhoike community in Delta State, Nigeria, there are no available biochemical information on fish exposed to crude oil in their natural habitat. Hence, this study investigates changes in biotransformation and oxidative stress markers in African catfish (*Clarias gariepinus*) from swamps around Kokori-Erhoike petroleum flow station in Delta State, Nigeria.

MATERIALS AND METHODS

Sampling Sites:

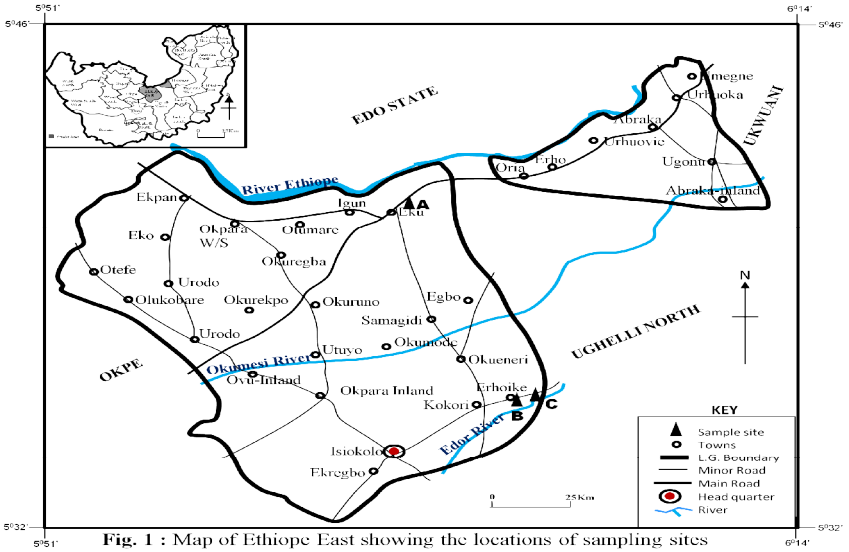

Fig. 1 : Map of Ethiope East showing the locations of sampling sites
Source: *Ministry of Lands, Surveys & Urban Development, Asaba, (2008)*

Three sites were used for this research and have been previously described by [8]. Site A (Eku axis of Ethiope River, control site), Site B (Erhoike swamp) and Site C (an earthen fish pond located in Erhoike environment) (Fig. 1).

Fish: African catfish (*Clarias gariepinus*, n=8/ site) with mean weight 162.37 ± 4.62 g were collected from each site in October, 2011. Fish were caught with the help of professional local fishermen at site A (Ethiope River, Eku axis) and site B (Erhoike swamp), and at site C (Erhoike Fish pond), fish net was used to catch the fish.

Collection of Blood from fish: About 1.2 ml of blood was collected by caudal arterial puncture from each fish into a sterilized plain tube. A portion (100 µl) of blood was collected in a 5ml heparinized tube and was used for the estimation of blood reduced glutathione. The preparation of serum and tissue homogenate has been previously outlined [9].

Biochemical Analysis: Blood reduced glutathione was estimated according to the method of [10]. Tissue reduced glutathione was determined according to the method of [11]. The activities of superoxide dismutatse, catalase and glutathione S- transferase were determined using the methods of [12, 13 and 14]. Lactate dehydroenase activity was determined using TECO commercial kits (TECO Diagnostics, USA) employing the method of [15]. The method of [16] was used for the determination of acetylcholinesterase while malondialdehyde was determined using the method of [17].

Statistical Analysis: Data obtained from the biochemical analysis were presented as mean ± SD and analyzed using analysis of variance (ANOVA). Group means were compared by the Duncanôs Multiple Range Test (DMRT). Values were considered statistically different at 5 % probability level. All statistical analyses were performed using SPSS version 16.

RESULTS:

TABLE 1: Reduced glutathione, activities of superoxide dismutase, catalase and malondialdehyde level in *Clarias gariepinus* from sampling sites

Tissue(s)		A	B	C
		Reduced glutathione		
Blood (mg% in blood)	(n=8)	32.68±2.13[a]	28.13±2.57[b]	28.93±2.79[b]
Liver (Unit/ml)	(n = 8)	14.31±1.69[a]	29.75±1.04[b]	28.56±0.62[b]
		Superoxide dismutase (unit/ml)		
Gill	(n = 8)	5.39±0.66[a]	7.24±1.18[b]	7.23±0.87[b]
Liver	(n = 8)	10.23±1.24[a]	11.31±0.19[b]	12.25±0.85[b]
Brain	(n = 8)	3.34±0.02[a]	3.38±0.04[a]	3.35±0.02[a]
		Catalase (unit/g of wet tissue)		
Gill	(n = 8)	22.70±1.46[a]	24.22±3.15[a]	24.41±2.68[a]
Liver	(n = 8)	20.72±1.04[a]	20.96±0.68[a]	21.04±0.75[a]
Brain	(n = 8)	1.92±0.05[a]	1.95±0.02[b]	1.96±0.01[b]
		Malondialdehyde (unit/ml)x10^{-6}		
Serum	(n = 8)	0.280±0.01[a]	0.290±0.03[a]	0.280±0.03[a]
Gill	(n = 8)	0.171±0.01[a]	0.190±0.01[b]	0.176±0.01[a]
Liver	(n = 8)	0.216±0.02[a]	0.233±0.02[a]	0.231±0.02[a]
Brain	(n = 8)	0.350±0.02[a]	0.420±0.02[b]	0.430±0.02[b]

Values are expressed as Mean ± SD. Means not sharing the same superscript alphabet in a given row differ significantly at p < 0.05.
A = Ethiope River (Eku axis), B = Erhoike Swamp; C = Erhoike Fish Pond.

Blood reduced glutathione content of *Clarias gariepinus* in Table 1 is significantly reduced ($p < 0.05$) in fish from sites B and C as compared with that of site A, while liver reduced glutathione is increased ($p < 0.05$) in fish from Sites B and C as compared with fish from Site A. Activities of superoxide dismutase (SOD) is elevated ($p < 0.05$) in the gill and liver of *Clarias gariepinus* from sites B and C as compared with fish from site A. Brain SOD activity was comparable ($p > 0.05$) in fish from the three sites (A, B and C). Catalase activity was comparable ($p > 0.05$) in the gill and liver of *Clarias gariepinus* from sites A, B and C but significantly higher ($p < 0.05$) in the brain of fish of site B as compared with that of site A. Table 1 also showed that malondialdehyde level in gill and brain of African Catfish of sites B and C was higher ($p < 0.05$) as compared with that of site A. Serum and liver malondialdehyde levels were comparable ($p > 0.05$) in fish from all sites (A, B and C).

TABLE 2: Activities of glutathione S-transferase, lactate dehydrogenase and acetylcholinesterase in selected tissues of *Clarias gariepinus* from sampling sites

Tissue(s)		A	B	C
		Glutathione S- transferase (nmol/mg protein/min)		
Gill	(n=8)	1.04±0.22[a]	1.84±0.49[b]	1.72±0.52[b]
Liver	(n=8)	0.13±0.03[a]	0.48±0.04[b]	0.44±0.02[c]
		Lactate dehydrogenase (IU/L)		
Gill	(n=8)	16.99±2.35[a]	20.51±2.01[b]	21.45±2.4[b]
Liver	(n=8)	14.63±1.74[a]	25.02±1.54[b]	25.57±2.18[b]
Muscles	(n=8)	25.60±1.97[a]	69.09±3.16[b]	75.44±2.80[b]
		Acetylcholinesterase (nmol/mg protein/min		
Brain	(n=8)	0.019±0.002[a]	0.020±0.002[a]	0.021±0.002[a]
Muscle	(n=8)	0.123±0.04[a]	0.129±0.004[a]	0.127±0.006[a]

Values are expressed as Mean ± SD. Means not sharing same superscript alphabet in a given row differ significantly at p < 0.05.
A = Ethiope River (Eku axis); B = Erhoike Swamp; C = Erhoike Fish Pond.

Table 2 showed that there is an increase ($p < 0.05$) in the activity of the gill and liver glutathione S- transferase of African catfish from site B and C as compared with site A. The gill, liver and muscle activity of lactate dehydrogenase is statistically elevated ($p < 0.05$) in fish from site B and C as compared with that of site A.

Table 2 also revealed that the activity of acetylcholinesterase was comparable ($p > 0.05$) in the brain and muscle tissues of *Clarias gariepinus* from all sites (A, B and C).

DISCUSSION: Biochemical markers of contamination are important indices in fish toxicity tests and for field monitoring of aquatic pollution. They confirm contact of the specimen with specific groups of chemical compounds and clarify their further metabolic fate. Biochemical investigations allow cause-effect relationship to be established at an early stage of pollution and these are sensitive and predictive diagnostic tools (biomarkers) for assessing animal exposure and toxic effects of chemical contaminants are needed as aquatic environmental contamination assessment indicators. Exposure to many environmental pollutants including petroleum contaminants are capable of inducing oxidative stress in aquatic animals including fish [2, 3 and 4]. Oxidative stress occur when the production of reactive oxygen species (ROS) overwhelms the endogenous protection afforded by antioxidant enzymes like catalase, superoxide dismutase, glutathione S-transferase and redox sensitive thiol compound, reduced glutathione.

Glutathione is the most copious non-protein thiol [18] found at millimolar concentrations in most cells. Reduced glutathione acts as the fundamental line of defense to cope with the destructive effects of reactive oxygen species [19] and protect cells against oxidative injury as it conjugates with compounds of exogenous and endogenous origin [20]. Blood glutathione level was significantly lower in *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond as compared with that from the control site (Ethiope River, Eku axis). Decreased level of blood GSH observed in this study corroborate with the observation of [21]. This reduction in blood GSH could represent an increased utilization due to oxidative stress which is evidence in the high level of lipid peroxidation marker (malondialdehyde) observed in the serum of *Clarias gariepinus* from Erhoike swamp (Table 1) that has been reported to contain higher amounts of total petroleum hydrocarbon as compared with the two other sites [8, 22].

The liver is the primary site for the synthesis of many substances including plasma proteins and short peptide like glutathione. It is also the principal organ of metabolism and has a role to play in many body processes most especially detoxification of chemical compounds. The increase in the liver GSH content could be due to increased synthesis prior to contamination. This result agrees with [23. 24 and 25] who reported elevated levels of liver GSH in *Clarias gariepinus* from Ogun River, Lagos Lagoon and fish treated with Successor 600.

The SOD-CAT system provides the first defense against oxygen toxicity. *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond have significant ($p < 0.05$) elevated gill-SOD activities as compared with that of Ethiope River Eku axis (control site). Similar increase in SOD activity was observed in liver tissue. The increase in the activity of SOD in the gill of *Clarias gariepinus* in this study could be a response to oxidative stress caused by the presence of contaminants in the water. The gill remains in close contact with the external environment and particularly sensitive change in quality of water are considered the primary target organ of the contaminants. Gills also remain the greatest surface area of the fish in contact with external environment. Increase in the activities of CAT and SOD is usually observed in the face of environmental pollutants [26] and the induction of antioxidants represents a cellular defense mechanism to counteract toxicity of ROS. The high lipid peroxidation observed in the gill and liver of *Clarias gariepinus* from Erhoike swamp together with the elevated activities of SOD provides information that the fish in Erhoike swamp were most stressed because changes in enzyme activity has been advocated to provide an early warning of potentially damaging changes in stressed fish [27].

Superoxide dismutase activity in the brain of *Clarias geriepinus* is comparable among the three sites. Brain catalase activity is significantly elevated in fish from Erhoike swamp and Erhoike fish pond as compared with that from Ethiope River (Eku axis). High catalase activity indicate high level of enzyme induction which could be as a result of the presence of contaminants and this is evidenced in the level of malondialdehyde (lipid peroxidation marker) which serves as a reliable marker of oxidative stress-mediated lipid peroxidation in tissues. Results of this study corroborate with the previous findings of [4, 23, 24 and 28].

Glutathione S-transferase (GST) activity plays a role in the detoxification of oxidative stress products as well as in the conjugation of glutathione to xenobiotic metabolite to accelerate their excretion [29]. GST has been identified in environmental pollution dealing with oil and petroleum hydrocarbons, heavy metals, pesticides and organochloride contaminants [30]. A marked elevation in GST activity was recorded in *Clarias gariepinus* gill sample from Erhoike swamp and Erhoike fish pond as compared with gill GST activity of *Clarias gariepinus* from the control site (Ethiope River, Eku axis). The hepatic GST activity was higher in fish from the swamp and pond than the control site. The induction in gill and

hepatic GST levels of *Clarias gariepinus* from Erhoike swamp and Erhioke fish pond may not be unconnected with the presence of crude oil and other forms of organic and inorganic pollutants present in the swamp and fish pond [8]. Elevated levels of GST indicate a defense response to chemical or oxidative stress in cells [6]. Increased activity of GST observed in this study is in agreement with the reports of [23, 25, 31, 32 and 33]. Lactate dehydrogenase (LDH) is an important glycolytic enzyme, which is present in all animal tissue [34]. The enzyme has been employed as an indicative criterion of exposure to chemical stress [35]. LDH forms the centre for a delicately balanced equilibrium between catabolism and anabolism of carbohydrates. According to [36], LDH is a potential marker for assessing the toxicity of a chemical and the alteration of normal LDH activity pattern are found to be oxygen (O_2) stress after exposure. The marked elevation of LDH activity in gill, liver and muscle tissues recorded in *Clarias gariepinus* from Erhoike swamp and Erhoike fish pond as compared with LDH activity in fish from Ethiope River (Eku axis) could be due to a shift from aerobic metabolism to anaerobic pathway. It may also be due to the release of the enzymes from damaged tissues as a result of environmental pollution. Erhoike swamp and Erhoike fish pond have significantly reduced level of dissolved oxygen as compared with Eku River [8] and this implies that oxygen concentration in these waters is low which could lead to anoxic condition thereby shifting metabolism from aerobic to anaerobic with a resultant effect of elevating LDH activity. The observed elevation in LDH activity in fish tissues in this study supports the findings of [27, 37 and 38] who reported higher LDH activity in fish exposed to different types of contaminants.

Acetylcholinesterase (AChE) is an enzyme responsible for hydrolysing the neurotransmitter acetylcholine into choline and acetic acid. AChE is a well confirmed specific biomarker of exposure and its inhibition is linked directly to the presence of organophosphorus and carbamate insecticides in the environment [39]. Results of the study show that the activity of acetylcholinesterase in the brain and muscle of *Clarias gariepinus* is comparable ($p > 0.05$) in the experimental sites. This indicates that *Clarias gariepinus* were not exposed to substances that could cause relevant anticholinesterase effect. It also suggests that the observed biochemical differences in tissues of *Clarias gariepinus* from the experimental areas could not be attributed to the presence of organophosphate or carbamate insecticides.

Conclusion: This research reveals the effect of oil exploration activities on *Clarias gariepinus* in the natural habitat of the fish in Kokori-Erhoike environment. The observed changes in markers of biotransformation and oxidative stress (GST, GSH, SOD, CAT, LDH and MDA) showed that the fish were relatively under stress in their natural habitat (Erhoike swamp) and Erhoike fish pond as compared with fish from Ethiope River (Eku axis) and these biochemical alterations could be employed as biomarkers of contamination in environmental monitoring of crude oil pollutant and their metabolic changes as early warning signs of adverse effects of environmental pollution. It is also of interest to note that all sites under investigation were not polluted with organophosphate or carbamate insecticides thus, observed biochemical changes could be attributed to the presence of petroleum hydrocarbon in the Kokori-Erhoike community as a result of the over 35years of oil exploration activities in that area.

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