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International Digital Organization for Scientific Research ISSN: 2550-794X
IDOSR JOURNAL OF SCIENTIFIC RESEARCH 3(1) 23-34, 2018.

Evaluation of Biochemical Indices of *Clarias Gariepinus* (African Catfish) Collected From Abakaliki Fresh Water Close to Mining Sites.

Nwali, B. U. And Okaka, A.N.C.

Department of Biochemistry, Ebonyi State University Abakaliki.
Email: bunwali2k2@yahoo.co.uk.

ABSTRACT

The assessment of oxidative stress and liver function indices of *Clarias gariepinus* from four different Ebonyi rivers were determined. Three of the rivers namely Enyigba, Ekwe-Agbaja and Ikwo-ihie are close to mining sites while Onu-Ebonyi river which is not close to mining site was used as control. A total of twenty adult fish of various sizes were collected from the fresh water bodies and used for the analysis. The oxidative stress indices; superoxide dismutase (SOD), catalase (CAT) (μ/mg protein), and glutathione peroxidase (GSH-PX μl) activities, reduced glutathione (GSH), and malondialdehyde (MDA) levels (μmol/g protein) as well as liver function indices viz; alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (μ/l) as well as albumin (ALB) concentrations (g/dl) of the fishes were evaluated using standard methods. The results of the oxidative stress indices showed that SOD, CAT and GSH-PX activities with GSH and MDA levels increased significantly at $P<0.05$ (3.14±0.22, 34.10±1.63, 25.28±4.84, 58.20±1.43, 1.24±0.72) when compared with the control (2.02±0.65, 16.20±2.64, 15.03±0.35, 12.07±1.34, 1.02±0.04 respectively). Similarly, the activities of ALT, AST and ALP (46.61±2.50, 58.02±4.65 and 37.74±1.74) in the serum increased significantly at $P<0.05$ when compared with the control (25.64±5.74, 21.15±2.75 and 15.88±1.84) respectively while the concentration of ALB decreased significantly at $P<0.05$ (3.5±0.47) when compared with the control (1.45±0.20). The findings of this study suggest a presence of environmental toxicants which could be responsible for the elevations of analysed parameters.

Keywords :*Clarias gariepinus,* Oxidative stress. Liver function indices, Mining and Toxicants.

INTRODUCTION

In recent years many research articles have appeared discussing the impact of man-made toxic compounds on the aquatic lives. Pollution of aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organism including fishes [1,2,3]. The agricultural drainage water containing pesticides, fertilizers and insecticides in addition to effluents have been seriously implicated in contamination of the ecosystem [4,5]. It can enter into the aquatic environment following agricultural use through runoff of dissolved chemicals or soil bound particles into water bodies [6,7].

Contamination of fresh water bodies with a wide range of pollutants has become a matter of concern over last few decades [8]. Heavy metals released from domestic, industrial and other man made activities may contaminate the natural aquatic ecosystem extensively [9]. Trace elements have harmful effects on ecological balance of the recipient environment and a diversity of marine population [10,11,12]. Heavy metals and chemicals are toxic to animals and many cause death or sub-lethal pathology of liver, kidneys, reproductive system, respiratory system or nervous system in both invertebrate and vertebrate aquatic animals

[13]. Accumulated trace elements may lead to morphological changes in the tissues of fish [14,15].

Cells naturally contain enzymes for their functions such that damages to cellular membrane lead to their escape into the blood where their presence or activities can easily be measured as an index of cell integrity [16,17]. Serum chemistry could be used to identify tissue damage [18,19]. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ACP) are normally found within the cells of the liver, heart, gills, kidneys and muscles [20, 21]. But their increase in the plasma indicates tissue injury or organ dysfunction (Wells *et al.*, 1986). However, changes in plasma glucose, total proteins and cholesterol concentrations can be indicative of a classical general adaptive response to stress in fishes exposed to pollutants [22,23]. This is because fish blood is very sensitive to pollution-induced stress (Patti and Kulkarni, 1993). Biochemical changes in fishes exposed to various pollutants have been documented [24].

Under normal physiological condition, there exists a balance between production and destruction of reactive oxygen species (ROS) in cells *of clarias gariepinus* provided by their antioxidant defence systems as toxicity of reactive oxygen species (ROS) is eliminated by the non enzymatic components of this system. As a result of exposure to environmental stressors, reactive oxygen species (ROS) can overcome antioxidant defence system leading to their excessive production thereby, damaging cell components and tissues of this fish, a condition known as oxidative stress and this can lead to adverse health effects and diseases [25]. Therefore, assays of antioxidant defence and oxidative damage parameters are used as biomarkers of oxidative stress for evaluation of oxidative stress indices in *clarias gariepinus.* In addition to the antioxidant defence system parameters, one of the most frequently used hallmarks of oxidative stress in damaging of membrane phospholipids in *clarias gariepinus* which lead to formation of secondary lipid peroxidation product is malondialdehyde (MDA) [26].

In order to evaluate the impacts on some biochemical parameters, some generally acceptable biomarkers are applied in monitoring aquatic organisms [27]. Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when in contact with the organs of fishes [28,29,30].

Ebonyi fresh water bodies are a water body located in Izzi and Abakaliki local government areas in Ebonyi State, Nigeria. The river basin is a booming fish farming area during dry and rainy seasons. This region is prominent because of occurrence of trace elements especially Lead-Zinc mineralization in the area. The major industrial activities that occur in this area include: stone crushing, metal mining (Pb-Zn) and smelting. These industries are located less than 100 meters near the river and they discharge their wastes directly into the river. When fish bio-accumulates these pollutants, it becomes a threat to fish survival as well as human health since consumers depend heavily in the fish for their dietary needs [31,32,33].

Clarias gariepinus is one of the most widespread catfish genera in the world

[34,35,36,37] which are found throughout Africa and the Middle East. *Clarias gariepinus* is one of the major fish species that inhabit Ebonyi fresh water bodies and has provided protein source for the people living around the River. Therefore, this work, aims to evaluate the biochemical indices of *Clariasgariepinus* harvested from Ebonyi fresh water bodies.

MATERIALS AND METHODS

Equipment/instrument

Spectrophotometer (spectro 2ID PEC MEDICALS USA), Oven (Gallenkamp), Analytical balance, Centrifuge, Refrigerator, Blender, Plastic aquaria, Micropipettes and general laboratory glasswares.

Procurement of the fish

Clarias gariepinus (African catfish) were used for the study. A total of twenty *Clarias gariepinus* (African catfish- mean weight 127.31 ± 21.41g and length 30.10 ± 3.44cm) were collected by net catch from the selected rivers under review viz: Enyigba, Ekwe-Agbaja and Ikwo-Ihie rivers and were transported to the laboratory for analysis. Onu-Ebonyi used as control also had five fish collected from it. Five (5) fish from each river with varied sizes were used for the biochemical studies. They were collected during rainy (between April-May).

Chemicals/Reagents

All reagents used were of analytical grades. Reagent kits were supplied by Randox Laboratories Limited, BT29 4QY, United Kingdom.

Sampling Area

The rivers in Enyigba and Ekwe-Agbaja were chosen as the study sites because of the mining activities (skilled and unskilled) going on in these areas, while Onu-Ebonyi river was used as control because of its non-proximity to mining sites. Enyigba, a town in Izzi Abakaliki L.G.A of Ebonyi State, Nigeria lies in the North eastern part of Ebonyi. It is positioned at latitude 6.19^{0}N and longitude 8.12^{0}E, and covers an area of 19km.

Ekwe in Agbaja - Ebonyi local government area of Ebonyi State, Nigeria, lies between Latitude 5.15^{o}N and longitude 8.09^{o}E of the Northeast part of Ebonyi State. It covers an area of 70km.

Onu-Ebonyi in Abakaliki, Ebonyi State, Nigeria, lie approximately 7º 3´N latitude and longitudes 5º 4´E and 6º 45´W. It is located in the eastern part of Nigeria .

These two water bodies, Enyigba river in Abakaliki, and Ekwe-Agbaja river in Izzi are highly contaminated by pollutants. Some of these pollutants are directly discharged by industrial and anthropological activities going on in these areas. As a result of this bioaccumulation, fish and other freshwater mammals may accumulate hazardous concentration of toxic chemicals

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Figure 1: The study map of Ebonyi State.

Experimental Design

This study was designed to assess some biochemical indices of *Clarias gariepinus* from Enyigba and Ekwe-Agbaja rivers close to mining sites in Ebonyi State, Nigeria. Onu-Ebonyi river was used as control because of its non-proximity to mining sites. Fifteen *Clarias gariepinus* (African catfish) were used for the study; five *Clarias gariepinus* each were collected from the three rivers.

Methods

Collection of Blood Samples

Blood samples were collected from *Clarias gariepinus* by cardiac puncture with the aid of a syringe, and then transferred into an anticoagulant bottle (EDTA container). The samples were spun in a laboratory centrifuge at 2000g for ten minutes and plasma and serum were separated and stored at -4 °C until analysis.

Determination of liver enzyme activities

The methods of Reitman and Frankel (1957) [28] were used to determine the activities of (AST), (ALT) and (ALP) while albumin level was determined by the method of Grant *et al.* (1987) [14]. The methods of [18], was used in determining SOD and CAT activities. GSH was determined according to the method described by Sedlak and Lindsay (1968) [29], MDA was determined using the method of [20] while the method described by Lawrence and Burk (1976) [17] was used in determining GSH-PX.

Statistical Analysis

Results were reported as mean ± standard deviation (SD) where appropriate. The averages were compared with one-way analysis of variance (ANOVA) and considerable variations amongst sets were determined by Ducan multiple range test using SPSS for windows version 20. The degree of significant was set at $P<0.05$.

RESULTS

Table 1: Measurements of liver function indices Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Albumin (ALB) activities in *Clariasgariepinus*from Abakaliki fresh water.

Sites	ALT	ALP	AST	ALB
Enyigba	**66.61±3.509**	**8.02±8.65**	**47.74±3.74**	**3.45±0.30**
Ekwe-Agbaja	**52.09±5.1211**	**1.17±9.60**	**53.15±7.87**	**3.59±0.09**
Ikwo-ihie	**58.12±2.16**	**75.81±19.28**	**36.60±2.00**	**3.51±0.25**
Onu-Ebonyi (Control)	**35.64±5.47**	**34.15±2.75**	**18.88±3.84**	**2.74±0.47**

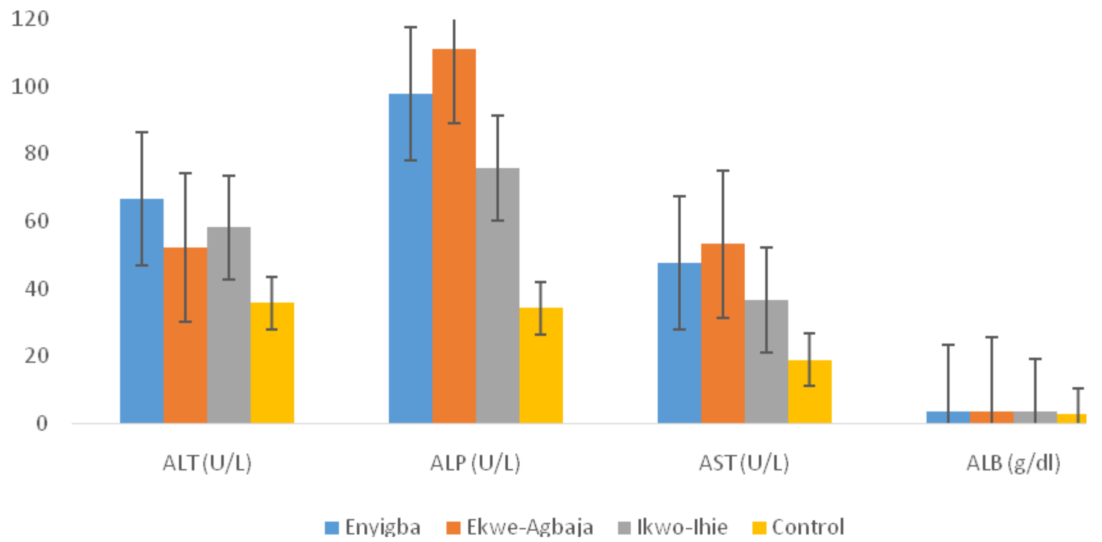
Results are mean ±SD of five fish samples from Ekwe-Agbaja, Enyigba, Ikwo-Ihie and Onu-Ebonyi.

Table 2. Measured oxidative stress indices Superoxide dismutase (SOD) (u/mg), Catalase (u/mg protein), Reduced glutathione (GSH) (μmol/g), Glutathione peroxidase (Gpx) (UI) and Malondialdehyde (MDA) (nmol/g protein) in *Clariasgariepinus*from Abakaliki fresh water

Sites	GSH	SOD	MDA	CAT	GPx
Enyigba	**68.40±1.93**	**7,16±0.20**	**3.34±0.82**	**42.10±2.83**	**24.76±3.14**
Ekwe-Agbaja	**5.58±1.75**	**5.58±1.41**	**4.79±0.93**	**39.03±5.36**	**24.56±3.32**
Ikwo-ihie	**11.78±3.18**	**6.27±1.21**	**2.77±0.91**	**37.09±3.45**	**25.28±4.48**
Onu-Ebonyi(Control)	**20.09±2.37**	**1.06±0.07**	**1.06±0.07**	**24.50±3.52**	**18.02±0.74**

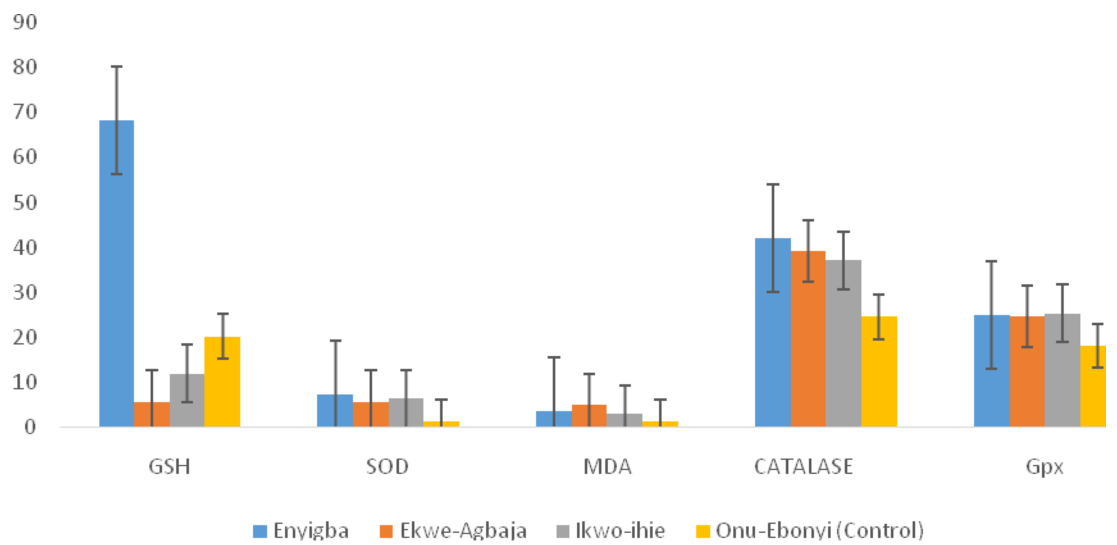
Results are mean ±SD of five fish samples from Ekwe-Agbaja, Enyigba, Ikwo-Ihie and Onu-Ebonyi.

Figure 2: Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatise activities and albumin levels in liver of *Clariasgariepinus*from Abakaliki fresh water.



Data are shown as mean ±SD (n=20). Bars with different letters differed significantly (P<0.05)

Figure 3: Superoxide dismutase (u/mg), Catalase (u/mg protein), Reduced glutathione (µmol/g), Glutathione peroxidase (UI) and Malondialdehyde (nmol/g protein) concentrations of *Clariasgariepinus*from Abakaliki fresh water.



Data are shown as mean ±SD (n=20). Bars with different letters differed significantly (P<0.05)

DISCUSSION

Many environmental pollutants such as heavy metals and other chemicals from industrial wastes dumped indiscriminately into the rivers are capable of inducing liver damage and oxidative stress in aquatic animals including fishes. The determination of biochemical indices in aquatic lives is very essential in assessing the health status of the liver.

In this study, the activities of AST and ALT increased significantly ($p < 0.05$) when compared with the control. This clearly suggests that the fish liver might be inflamed or injured as a result of diseases such as viral hepatitis. This was in accordance with the work of De Ritis, (1965) [8], who reported elevations of the serum AST and ALT in viral hepatitis as well as other hepatic diseases. The increase in serum ALT and AST level in the test samples can be attributed to heavy metal contamination of Abakaliki fresh water as a result of the mining activities in the region [23]. The levels of these enzymes in serum or plasma are reliable indicators of liver metabolism and wellness of organisms under chemical or physical challenge [26]. The observed general increase in the plasma enzymes in this study is an indication of underlying liver injury in the fish. The increase in levels of ALT and AST may indicate liver damage, while the increase in the ALP level may be indicator for renal and liver damage [5]. Also, it has been reported that alterations in enzymes activities in the plasma directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture, a signal of underlying pathological process [26].

Also, the result shows that serum ALP level significantly increased above the control. Alkaline Phosphates is an important liver enzyme in which high levels of this enzyme in the blood above the referenced standard values indicates liver injury. Serum alkaline phosphatase levels can be elevated by cholestatic or infiltrative diseases of the liver and by disease's causing obstruction to the biliary system, as well as tumors of the liver [11]. ALP in the cellular external membrane plays the major role in phosphate metabolism and it prevents the external membrane from being damaged [15]. Increase in plasma level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. Significant elevation of serum ALP is an indication of cholestasis. It has also been reported that increase in the serum levels of ALP indicate the extent of cellular damage on the liver [34]; its increase in activity is associated with necrosis of the liver and kidney [35,36,37]. This observation can be attributed to heavy metal contamination of Abakaliki fresh water which has the capacity to induce hepatic injury.

In the same way, the serum albumin concentration of the test sample is 1.45±0.20 g/dl. These values fall below the normal serum level of albumin which is 3.5 to 5.0g/dl. This observation indicates that the fish liver might have chronic liver disease which might have reduced the protein synthesis abilities of the liver. The extent of decrease in serum albumin level is directly proportional to the extent of liver damage. The half-life of serum albumin is as long as 20 days, hence serum albumin levels is not reliable indicator of hepatic protein synthesis in acute liver diseases whereas in all chronic diseases of liver, the albumin level is decreased [16]. The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice.

The result showed elevated MDA concentrations in *Clarias gariepinus* collected from the selected rivers close to mines. [5] reported similar findings of MDA elevation of *Clarias gariepinus* exposed to toxicants. Malondialdehyde (MDA) is the end product of lipid peroxidation caused by free radicals, and it is usually employed as indicator of oxidative stress [9] The determination of MDA level gives a clue about the capacity of pollutants to induce oxidative damage [36].

The levels of SOD, GSH, GST-PX and CAT also increased significantly ($p<0.05$) when compared with the control group. GSH as an antioxidant, plays a crucial role in protecting the cells from oxidative damage and change in the concentrations of GSH was observed during increases in oxidative stress [6]. Superoxide radicals or their transformation product, hydrogen peroxide (H_2O_2), are capable of causing the oxidation of cysteine which will lead to decreased SOD activity. Activities of SOD were markedly decreased by the tested samples which resulted in an increase in CAT activity, since the degradation of H_2O_2, a potent oxidant at high cellular concentration, is affected by CAT due to its induction against increased oxidative stress [2].

The heavy metals and organic compounds present in the well water samples and e-waste leachate are known to generate ROS that caused DNA, protein and lipid damage in eukaryotic of which these chemicals mostly heavy metals can bind to phosphate and base residues of DNA, to alter its primary and secondary structures [2]. The high concentration of the chemicals can cause severe degradation in the groundwater quality and palatability to human consumption. This has been implicated with human gastrointestinal irritation and laxative effects, abnormal sperm quality [2], chromosome aberration and DNA damage [18] and reduced fecundity and adverse birth effects [2].

This increase may be as a result of present of heavy metal like lead which was presumed to be present in that area. The contaminant was presumed to induce oxidative stress in the fish which the body tries to balance by producing more superoxide dismutase.

CONCLUSION/RECOMMENDATION

In conclusion, there is a significant increase in concentration of AST, ALT and ALP as well as decrease in albumin level when compared to the normal standard and also the oxidative stress indices such as SOD, CAT, GST, GSH and MDA levels increased significantly when comparing the test group to the normal standard. This indicates damage to the fish liver due to the high concentration of heavy metals and this can be very detrimental to the survival offish and other marine organism also the study revealed that the Abakaliki fresh river undergoes environmental degradation as this led to the observed increase in oxidative stress indices in *Claria garipinus*. Proper water management in areas of water quality and feed quality can help to reduce the incidence offish liver diseases. Also other tests such as haematological studies assessment should be carried out on the fishes to ascertain the health status.

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