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Biochemical and Oxidative Stress Responses in *Clarias gariepinus* Exposed to Sublethal Concentrations of Benzo[a]pyrene

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Authors' contributions

All authors collaboratively carried out the laboratory experiments and statistical analysis of experimental data. All authors read and approved the final draft of the article.

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ABSTRACT

The effect of benzo[a]pyrene (BaP) on selected plasma biochemical parameters of the tropical African catfish *C. gariepinus* was investigated. Apparently healthy juvenile fish (n = 90; mass = 19.7 ± 1.8 g) were exposed to sublethal concentrations of BaP over a period of 35 days after which haematological and plasma biochemical analysis were carried out on whole blood and plasma respectively. While there were significant declines in red blood cell count (RBC), hemoglobin, haematocrit and platelet count, significant elevations were observed in mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and white blood cell (WBC) count. Significant increases were also observed in the activities of the liver enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). There were significant increases in

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the activities of the oxidative stress enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione s-transferase (GST). Findings from this study reveal that benzo[a]pyrene causes changes in haematological and plasma biochemical profiles in exposed aquatic organisms.

Keywords: Benzo[a]pyrene; haematological; biochemical; plasma; aquatic; pollution.

1. INTRODUCTION

"Aquatic pollution is a cause for concern because of its impact on aquatic organisms which serve as food for humans and also maintain the ecological balance. One of the causes of aquatic pollution are polycyclic aromatic hydrocarbons (PAHs). PAHs are a collection of carbon-based organic compounds which consist of two or more benzene rings" [1]. PAHs are found widespread in the environment. These compounds are formed from either natural or anthropogenic activities [2]. "The dominant sources of PAHs in the environment are from human activity. Such activities include but are not limited to wood-burning, combustion of fossil fuels, mining activities etc. One of the most studied PAHs in the terrestrial but least studied in the aquatic environment is Benzo[a]pyrene (BaP). BaP is a five-ringed PAH that is formed by the incomplete combustion of organic compounds just like other PAHs. In recent times, BaP has been confirmed as having properties capable of causing cancer" [3].

This study aimed to evaluate the effect of BaP on haematological parameters, some enzymes involved in amino acid metabolism and possible oxidative response of the tropical catfish to BaP. We intended to provide data that will help in the prediction of the effects of sublethal concentrations of BaP on aquatic organisms. The tropical catfish was chosen for this work because of its abundance in tropical climes together with its hardiness and relative ease of handling. This study will contribute immensely to the toxicological database and also enhance the ecological risk assessment of BaP in aquatic environments.

2. MATERIALS AND METHODS

2.1 Chemicals

All reagents used were of analytical grade. Benzo[a]pyrene (BaP) was obtained from Sigma Aldrich (Germany). Acetone was obtained from British Drug Houses (BDH) chemicals (UK). Kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were obtained from Randox laboratories ltd. (Antrim, UK). Adrenaline, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), and hydrogen peroxide (H_2O_2) were all purchased from Sigma Chemical Company (London, UK).

2.2 Animals

Juvenile catfish, *C. gariepinus* (n = 90) weighing 19.7±1.8 g were obtained from a commercial fish farm in Aba, South east Nigeria. The fish were acclimatized for 2 weeks in tap water prior to experimentation. The fish were fed twice daily *ad libitum* with commercial fish feed. Fish fecal matter and uneaten food was removed daily to prevent organic pollution with the attendant algal growth.

2.3 Sublethal Toxicity Tests

Stock solutions of BaP were prepared by dissolving BaP in distilled water taking acetone (0.01 ml/L) as solvent carrier. Test solutions were prepared by dilution of stock (64 μg/L) solutions in tap water. The acute LC_{50} value of BaP was determined in a pilot study using semi-static method [4]. During sublethal studies, fish were exposed to 1/2 and 1/4 of the LC_{50} value (corresponding to treatment levels 1 and 2). A solvent control was included in the experimental design. Fish were kept in groups of 10 in 30L plastic tanks containing the test solutions. Experiments were performed in triplicates. Period of exposure lasted 35 days

2.4 Assays

At the end of the exposure period, fish were anesthetized using non-chemical method by hypothermia. Blood was then collected from the immobilized fish by caudal vein puncture method as described by Argungu et al. [4] using a 5ml sterile disposable syringe with a 22 gauge needle. The blood was transferred to EDTA tubes. The blood samples were spun with a bucket centrifuge at 4000 rpm for 5 minutes to separate the plasma from the packed cells. The plasma obtained was kept in plain blood containers and used for biochemical analysis.

2.4.1 Haematology

Haematological parameters were determined using automated haematology analyzer machine (Mindray BC 2300, USA).

2.4.2 Liver enzymes

Randox diagnostic kits were used to assay the activities of plasma ALT, AST and ALP. Assay of AST and ALT activities was based on the principle as modified by Tietz et al. [5]. “AST activity was assayed by monitoring the concentration level of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm, while ALT activity was assayed by monitoring levels of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. ALP was assayed in accordance with the principles of Tietz [6]. The p-nitrophenol formed by the hydrolysis of p-nitrophenyl phosphate confers a yellowish colour on the reaction mixture. Its intensity is monitored at 405 nm to measure enzyme activity.

2.4.3 Oxidative stress enzymes

“The procedure of Misra and Fridovich [7] as described by Magwere et al. [8] was used to determine plasma superoxide dismutase (SOD) activity by measuring the inhibition of auto-oxidation of adrenaline at pH 10.2 and 30°C. SOD activity was expressed in U/mL”. “Plasma catalase activity was determined according to the method of Sinha [9] by measuring the reduction of dichromate in acetic acid to chromic acetate at 570 nm”. “Catalase activity was expressed in kU/L. Plasma glutathione S-transferase (GST) activity was determined by the method described by Habig et al. [10] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. GST activity was expressed in U/L”.

2.5 Statistical Analysis

Results were expressed as mean ± standard error. Data from the different treatment groups were compared by a one-way analysis of variance (ANOVA) followed by a Scheffes test to determine statistically different groups. All differences were considered significant at $p < 0.05$. Statistical analysis was performed using Microsoft Excel and the SPSS statistical package (ver. 24.0 SPSS Company, Chicago, IL, USA).

3. RESULTS

The results showed that exposure to sublethal concentrations of BaP affected some of the haematological parameters. There was a statistically significant ($p < 0.05$) reduction in the red blood cell count of exposed fish. The observed reduction appeared to be dose-dependent with a higher dose of the chemical (8 µg/L) causing a greater reduction in the reduction compared to the lower dose (4 µg/L). There was also an observed reduction in haemoglobin concentration and hematocrit. The impact of BaP on the erythrocyte indices showed mixed results. While there were increases in MCV and MCHC, there were no observed changes in MCH. There was a dose-dependent increase in WBC count. Though there was a decline in platelet count, the reduction was not statistically significant ($p < 0.05$).

Table 1. Haematological parameters of *C. gariepinus* exposed to sublethal concentrations of benzo[a]pyrene

Parameter	Control group (0 µg BaP/L)	Benzo[a]pyrene Concentration (µg/L)	
		4	**8**
RBC ($x10^9$cell/L)	$2.86±0.09^a$	$2.28±0.05^b$	$2.23±0.13^b$
Hb (g/L)	$100.25±3.9^a$	$88.75±0.63^b$	$91.5±0.67^b$
Hct (%)	$37.52±2.38^a$	$30.25±0.32^b$	$29.68±0.32^b$
MCV (fL)	$132.8±1.71^a$	$132.55±1.36^a$	$120.25±1.07^b$
MCH (pg)	38.75±0.19	36.9±1.15	38.98±0.49
MCHC (g/L)	$272±3.4^a$	$294.25±0.85^b$	$313.25±3.5^b$
WBC ($x10^9$ cell /L)	$90.87±4.2^a$	$125.83±3.32^b$	$132.2±2.5^b$
PLT ($x10^9$ cell /L)	20±1.2	18±2.2	16±1.6

Means not sharing the same letter (a or b) are statistically different at p < 0.05

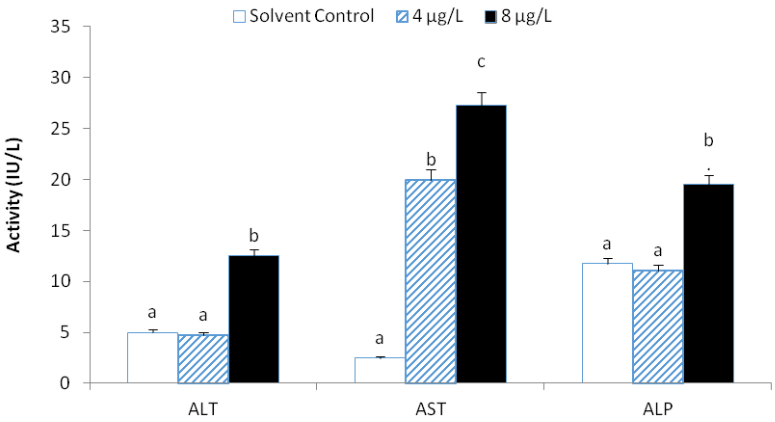

Fig. 1. Plasma ALT, AST and ALP activities of *C. gariepinus* exposed to sublethal concentrations of benzo[a]pyrene. Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$

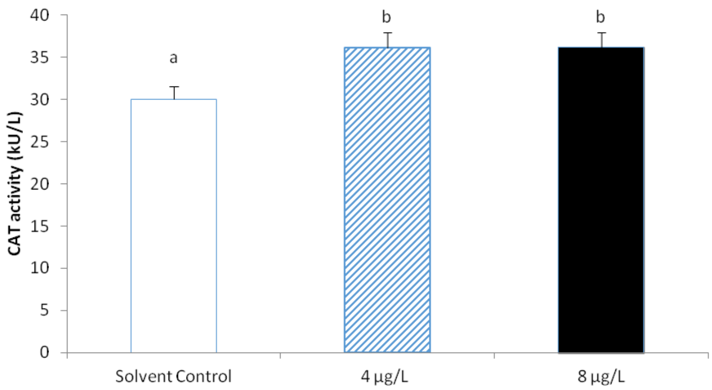

Fig. 2. Plasma CAT activity of *C. gariepinus* exposed to sublethal concentrations of benzo[a]pyrene. Means not sharing the same letter (a or b) are statistically different at $p < 0.05$

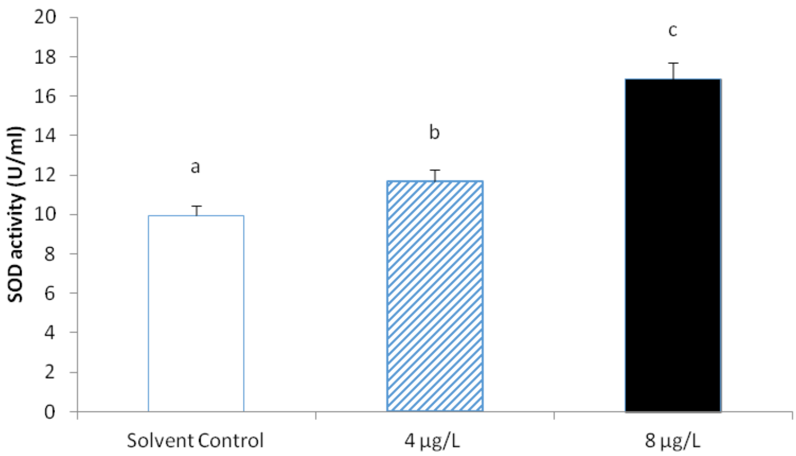

Fig. 3. Plasma SOD activity of *C. gariepinus* exposed to sublethal concentrations of benzo[a]pyrene. Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$

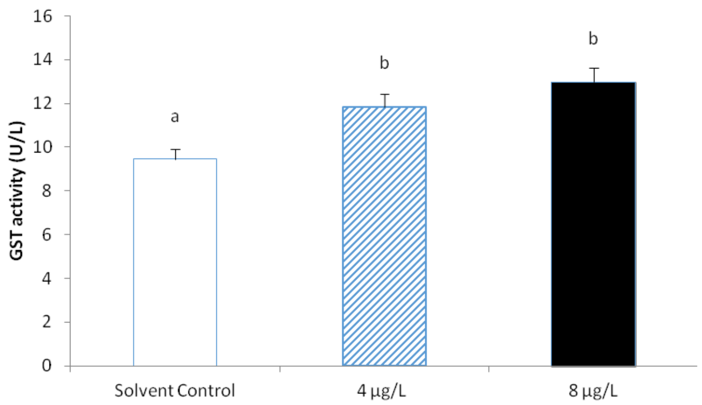

Fig. 4. Plasma GST activity of *C. gariepinus* exposed to sublethal concentrations of benzo[a]pyrene. Means not sharing the same letter (a or b) are statistically different at p < 0.05

Fish exposed to 8 µg/L BaP showed significant ($p < 0.05$) increases in the activities of the liver enzymes with AST showing the highest activity. BaP induced the expression of the oxidative stress enzymes. All three enzymes CAT, SOD and GST showed statistically significant ($p < 0.05$) increases in their activities compared to the control.

4. DISCUSSION

BaP had adverse effects on some of the studied haematological parameters. At the end of the experiment (35 days). Fish exposed to BaP showed significant reductions in the RBC count compared to the control. The potential of BaP to cause anemia is likely due to changes in the metabolism of iron. BaP may also inhibit the synthesis of red blood cells in the exposed fish. Many inorganic and organic contaminants have been shown to minimize the concentrations of red blood cells in circulation [11]. Findings by Kim et al. [12] show that subchronic dietary B[a]P exposure caused a significant reduction in erythrocytes. Similar results were also observed in common carp exposed to ethinylestradidol [13] and rainbow trout exposed to heavy metals [14]. Both studies demonstrated dose-dependent reductions in the red blood cell counts in fish exposed to the contaminants.

Haemoglobin (Hb) is a polypeptide containing heme and globin as prosthetic group and apoprotein respectively [15]. BaP exposure caused a significant reduction in the observed Hb concentration. As in red blood cell count, the reduction in Hb concentration can be attributed to the inhibition of erythropoiesis in the exposed fish. A similar finding was made by Kim et al. [12] where BaP exposure led to a significant decline in Hb concentration. Recently, Dey et al. [16] showed that naphthalene caused a significant reduction in Hb concentration. The authors attributed the observed reduction in Hb concentration to disruptions in oxygen supply to the tissues. This impairment could have caused a decline in metabolism and subsequently led to a reduction in energy generation.

Hematocrit (HCT) also known as packed cell volume (PCV) assays the volume of packed red blood cells compared to the whole blood [17]. Fish exposed to BaP showed significant reductions in the HCT value. The low HCT values in the present study could be attributed to the combined effects of destruction of erythrocytes, inhibition of erythropoiesis and destruction of erythrocytes [18]. Glusczak et al. [19] reported the reduced HCT value in L. obtusidens to glyophosate exposure. Earlier, Barcellos et al. [20] showed that lower HCT could indicate the anemic status of the fish in contact with contaminants.

While there were significant increases in MCV and MCHC, there were no observed changes in MCH on exposure to BaP. Red blood cell parameters are very useful in understanding the nature of anemias. Anemias can be classified according to the size of the erythrocyte, as being normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV) [21].

The present work showed that BaP was immunotoxic to *C. gariepinus.* There was an observed increase in the levels of circulating white blood cells in exposed fish. Leukocytes which are white blood cells are a crucial part of the immune system in most vertebrates and take part in immune responses. They circulate in the blood and mount inflammatory respond to toxicants or pathogens [22]. Ramesh and Saravanan [23] reported an elevation in WBCs in Indian carp *L. rohitato* exposed to deltamethrin at sublethal concentrations. More recently, Parma and Shar [24] demonstrated that reactive red (RR) azo dye 120 caused an increase in WBC count in Indian catfish exposed to the insecticide, malathion.

Platelets perform important roles in wound healing and inflammation. It is proposed that they perform these functions via interaction with cells of the immune system [25]. Platelets are however not cells, but rather fragments of cytoplasmic origin derived from megakaryocytes [25]. In the present study, though there was a decline in platelet count, though the observed change was not significant ($p > 0.05$). The results of Dey et al. [16] also disclosed a reduction in platelet count in A. testudineus under naphthalene exposure.

ALT, AST and ALP are enzymes involved in amino acid metabolism. Changes in the activities of these enzymes allow the identification of tissue damage in organs like the liver and kidney [26]. The present study showed that there were increases in the activity of the enzymes in fish exposed to BaP and these increases could be indicative of liver damage and possible disruptions in the permeability of the hepatic membrane. Such damages have potential of subsequently releasing enzymes to the blood [27]. Derakhshesh et al. [3] examined the effect of BaP on liver cell culture of *E. coioides*. The authors reported increases in activities of all three enzymes in varying concentrations of BaP. Increases in all three enzymes have also been reported in *S. schlegeil* [28] and in C. carpio on exposure to pyrene [29].

Oxidative stress is known to be caused as a result of an imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological system to mop up the oxidizing agents [30]. In the present study, BaP was found to induce the expression of the antioxidant enzyme systems. The scientific literature is rich in publications which show that toxicants lead to the production of free radicals and that these free radicals induced the expression of CAT, SOD and GST to ameliorate the effects of oxidative stress on the exposed biological system. Jiffa et al. [31] observed significant increases in SOD and CAT activities in a dose-effect related pattern in *L. japonix* exposed to benzo[a]pyrene. Palanikumar et al. [32] also reported increases in GST activity of BaP-exposed fish. The present result is in agreement with those earlier findings as just stated affirming deleterious effects of contaminants such as benzo[a]pyrene on exposure to fish.

5. CONCLUSION

In conclusion, BaP causes changes in haematological parameters in *C. gariepinus* and has a damaging effect on the liver tissue via oxidative stress. These effects could predispose aquatic organisms to disease and parasite infestation. There is need for further research to study the impact of BaP in aquatic organisms at the genetic level and also to study metabolism and biotransformation pathways of BaP in *C. gariepinus.* The information obtained from this study will help to assess the sublethal effects of PAHs in general and BaP in particular and to establish water quality criteria for control policies and conservation strategies in tropical region

ETHICAL APPROVAL

Animal ethic committee approval was obtained before the commencement of experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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