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Effect of Aqueous Extract of *Psychotria microphylla* Leaves on Some Biomarkers of Oxidative Stress in African Catfish (*Clarias gariepinus*)

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ABSTRACT

Psychotria microphylla Elmer is one of the *Psychotria species* found in the Eastern part of Nigeria. Infusion of the whole plant is used in Afikpo south Area of Ebonyi State, Nigeria for fishing and repelling of insects from crop vegetables. The work was planned to evaluate the possible toxicological effects of the aqueous extract of *Psychotria micophylla* leaves on some oxidative biochemical parameters of the widely consumed Afican catfish, *Clarias gariepinus*. *C. gariepinus* numbering 100 with mean body weight of 180 ±15 g and body length of 30±3cm were procured from a farm and acclimatized for two weeks prior to exposure. The fish were exposed for 1, 3, 7, 10 and 15 days to three sub-lethal concentrations: 0.016, 0.03 and 0.065mg/l of the aqueous extract of *Psychotria microphylla* leaf. Results showed a significant (P<0.05) increase in malondialdehyde (MDA) formation in the plasma and liver of the fish from day 3-15 following exposure to varied concentrations of the extract. Reduced glutathione (GSH) levels and superoxide activities significantly (P<0.05) decreased in plasma and liver. Catalase activities increased significantly (P< 0.05) in the plasma and liver from day 3. In conclusion, the altered antioxidant molecules and enzymes indicate that aqueous extract of *P. microphylla* leaves elicited oxidative stress. This may lead to other changes at molecular level in the fish that can eventually affect their survival.

Keywords: *Psychotria microphylla, Clarias gariepinus,* oxidative stress, antioxidant.

INTRODUCTION

Plants are the largest chemical industry in the whole universe as they possess the capacity to manufacture endless varieties of active principles. Generally, plants that produce chemicals having piscicidal activity are termed piscicidal plants [1, 2]. Research interest in piscicidal plants is predicated by dearth of information on their ecology, toxicological effects, insecticidal properties and an urgent need for effective and eco-friendly piscicides for aquaculture management [3, 4]. Botanical biocides are believed to be more environmentally friendly because they are easily biodegradable, cheap and leave no residues in the environment and are easily reversed in fish subjected to chronic concentrations [5, 6]. Synthetic piscicides are not

degradable, and hence, pose the problems of environmental resistance, pest resurgence and could have detrimental effects on non-target organisms [7].

Psychotria microphylla is one of the plants commonly used to catch fish in the South-eastern Nigeria, especially, Ebonyi State. It is also used to ward off insects from crop plants and has been reported very toxic to *C. gariepinus* [8]. Aquatic environments are habitat to a huge variety of organisms ranging from prokaryotes to higher vertebrates. They also serve as sinks for a great variety of anthropogenic contaminants, many of which are harmful. It is imperative to have an insight of the impact and effects of these harmful substances on aquatic life forms for instance fish. This knowledge will enable us to pinpoint sources of contamination, can provide governing bodies with useful information in management of aquatic ecosystems, information on which to base regulations concerning usage and handling of chemical compounds, and essentially help to protect aquatic ecosystems from the negative effects of anthropogenic activities. Toxic effects include the formation of reactive oxygen species with the ability to damage cellular molecules.

Fish are gifted with antioxidant defense system such as catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) to offset the impact of reactive oxygen species (ROS) ensuing from metabolism of diverse chemicals. CAT is a common enzyme found in nearly all the organisms where it functions to catalyze the breakdown of H_2O_2 to O_2 and H_2O. GSH is an antioxidant that aids to defend the cells from the ROS. However, these protective systems may be insufficient to entirely prevent the stress and accompanying damage induced by oxidative agents [9].

Oxidative stress is frequently used as a biomarker of the effects of exposure to environmental pollution in aquatic environments [10, 11]. The main reason to study oxidative stress in aquatic organisms is to understand not only whether animals are detrimentally affected by exposure, but also to understand the mode of action of the toxicants. Oxidative stress as a result of toxic substances has been documented in numerous fish species over the past decade [12, 13, 14, 15]. Many studies have shown that antioxidants that are affected by reactive oxygen species show adaptive responses to xenobiotics that produce oxyradicals [16] and are potential biomarkers for oxidative stress in fish [17].

Biomarkers of oxidative stress, such as changes in antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure to redox-active xenobiotics. These oxidative stress parameters have been associated with various disease

pathologies and organism longevity in a number of species, thereby establishing ecological relevance in these cases. A large number of biomarkers of oxidative stress have been used in fish studies and these include both the antioxidant defense mechanisms possessed by the cell, enzymatic and molecular, as well as oxidative damage products.

Studies have been conducted on several oxidative stress related parameters, addressing species differences, nutritional status, annual variations, and life cycle variations [18, 19, 20 21]. In fish, oxidative stress has been documented in both field and laboratory exposure studies. This work was designed to evaluate the effects of aqueous extract of *Psychotria microphylla* leaves on some oxidative biochemical parameters of *Clarias gariepinus* juveniles. *Clarias gariepinus* (African catfish) is a tasty, bottom and omnivorous feeder that sometimes also feeds at the surface. It is a choice experimental fish because it is resilient, stubborn and can withstand harsh aquatic conditions that other cultured fish species cannot survive.

MATERIALS AND METHODS

Sampling and Authentication of the plant

Psychotria microphylla Elmer leaves sample was collected from the wild at Afikpo South L.G.A of Ebonyi State, Southeastern Nigeria from August 2012- April 2013 and was identified by Mr. Ozioko of the International Bioresources and Research Centre, Nsukka, Nigeria.

Plate 1: The piscicide plant: *Psychotria microphylla* [20].

Preparation of *Psychotria microphylla* Leaves Sample

Samples of *P. microphylla* Elmer leaves were washed and shade-dried. It was then pulverized using mechanical blender and sifted using 0.25 mm sieve. Three hundred grams (300 g) of the leaf powder thus obtained was infused in one (1) liter of water (distilled) for two days. The set-up was sieved through a clean white cloth and the filtrate was obtained by hand pressure. The filtered extract was dried in an oven set at 45°C and the powder then used for evaluating its effect on the oxidative indices of *C. gariepinus.*

Procurement of the fish

Fresh water fish, *C. gariepinus* juveniles weighing 180±15 g and body length of 30±3 cm were procured from Chiboy's Farm, Abakaliki, Ebonyi State. They were safely brought to laboratory and stocked in 200 liter capacity rubber tank. The fish were acclimatized to laboratory conditions (25^oC) for 14 days before the exposure period using plastic aquaria. During the acclimation period the fish were feed twice daily using standard commercial fish feed.

Experimental design

The fish were exposed for 1, 3, 7, 10 and 15 days to three sub-lethal concentrations of 0.016, 0.03, and 0.065 mg/l of the aqueous extract of *Psychotria microphylla.* Two 60 liters capacity plastic containers were used for each concentration and in each of the containers ten 10 fish were placed and forty (40) liters of borehole water put into it. Similar set up was also simultaneously maintained in borehole water (0.00 mgl^{-l}) as the control.

At the end of day 1, 3, 7, 10 and 15, five fish were randomly taken from each group including the control. The fish were anaesthetized slightly in chloroform before the samples were collected. Holding the fish firmly, the operculum was lifted and blood collected by puncturing the cardiac into heparin containers for determining oxidative stress parameters. After blood collection, fish were sacrificed by sectioning the spinal cord, and the liver was carefully dissected out, washed in ice-cold 1.15% KCl solution, blotted and weighed. A sample, 0.5g of each of the liver tissue was homogenized in five ml homogenizing buffer (50 mM Tris-HCl mixed with 1.15KCl and pH adjusted to 7.4) using a motor-driven Teflon potter-Elvej-hem homogenizer. The resulting homogenate was centrifuged at 10,000 g for 20 minutes in a refrigerated centrifuge at 4^oC. The clear supernatants collected were used for assaying some oxidative biochemical parameters

Determination of MDA

The method of Buege and Aust (1978) [2] was employed to measure the quantity of MDA in the sample. 1.0 ml of the plasma sample was added to 2 ml of (1 :1 : 1) TCA-TBA-HCl reagent (thiobarbituric acid 0.37 %, 0.24N HCl and 15 % TCA) trichloro acetic acid-thiobabituric acid – hydrochloric acid reagent boiled at 100°C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifugation at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against blank with spectrophotometer. MDA was calculated using the molar extinction coefficient for MDA-TBA-complex of 1.56×10^5 $M^{-1}CM^{-1}$.

Estimation of superoxide dismutase (SOD) activity

The method described by Sun and Zigma (1978) [24] was used to determine the activity of Superoxide Dismutase. The plasma sample, 0.02 ml was mixed with 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, and 0.03 ml of epinephrine in 0.005 N HCl was employed to start the reaction. Similarly, the blank has 2.95 ml buffer, 0.03 ml of epinephrine and 0.02 ml of distilled water. SOD activity was determined by measuring the change in absorbance at 480 nm for 5 minutes with a spectrophotometer. $\Sigma = 4020M^{-1}$ cm^{-1}.

Assay of catalase (CAT)

The activity of catalase was measured using the method descrbed by Oyedemi *et al*. (2010) [21]. The plasma, 0.1 ml was mixed with 2.9ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. The phosphate buffers were used as blank and the absorbance was read against blank at 240nm at 30s interval for 1 minute. The enzyme activity was calculated using the molar extinction coefficient of 40.0 $M^{-1}cm^{-1}$ expressed as unit/mg protein.

Reduced glutathione determination (GSH)

This was determined using the described by Oyedemi *et al*. (2010) [21]. The plasma sample (0.2 ml) was mixed with 1.8 ml of EDTA solution and 3 ml of precipitating reagent was added, thoroughly mixed and left for five minutes before centrifugation. Two (2) ml of the supernatant, 4 ml of sodium hydrogen phosphate and I ml of DTNB (5, 5-dithio-bis-2-nitrobenzoic acid) were placed into it and the absorbance read at 412 nm. $\Sigma = 1.34 \times 10^4\ M^{-1}cm^{-1}$.

Statistical Analysis

Results were reported as mean ± standard deviation (SD). 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

The effects of 0.016, 0.03 and 0.065 mg/l of the distilled water extract of *P. microphylla* leaves on MDA formation in the blood plasma and liver tissues of *Clarias garienpinus* are presented on Figure 1 and 2 respectively. The exposure of 0.016, 0.03 and 0.065 mg/l of the water extract of *P. microphylla* leaves produced a marked increase ($P<0.05$) after day 1. The impact was both dose and duration of exposure dependent. The least malondialdehyde level was recorded on day 1 of exposure to the extract and then increased with the duration of exposure. At the dose of 0.065 mg/l of the water extract of *P. microphylla* leaves, the plasma MDA level increased from 51 to 76 %. The levels of GSH decreased significantly ($P<0.05$) in dose and duration dependent manner from 3rd day of exposure as displayed in Figure 3 and 4. The exposure of 0.016, 0.03 and 0.065 mg/l of the distilled water extract of *P. microphylla* leaves produced marked reduction ($P<0.05$) in the activity superoxide dismutase (SOD) in *C. gariepinus* as shown in Figures 5 and 6. Conversely, a significant increase ($P<0.05$) in the activity of catalase (CAT) was observed in the exposed fish as shown in Figure 7 and 8.

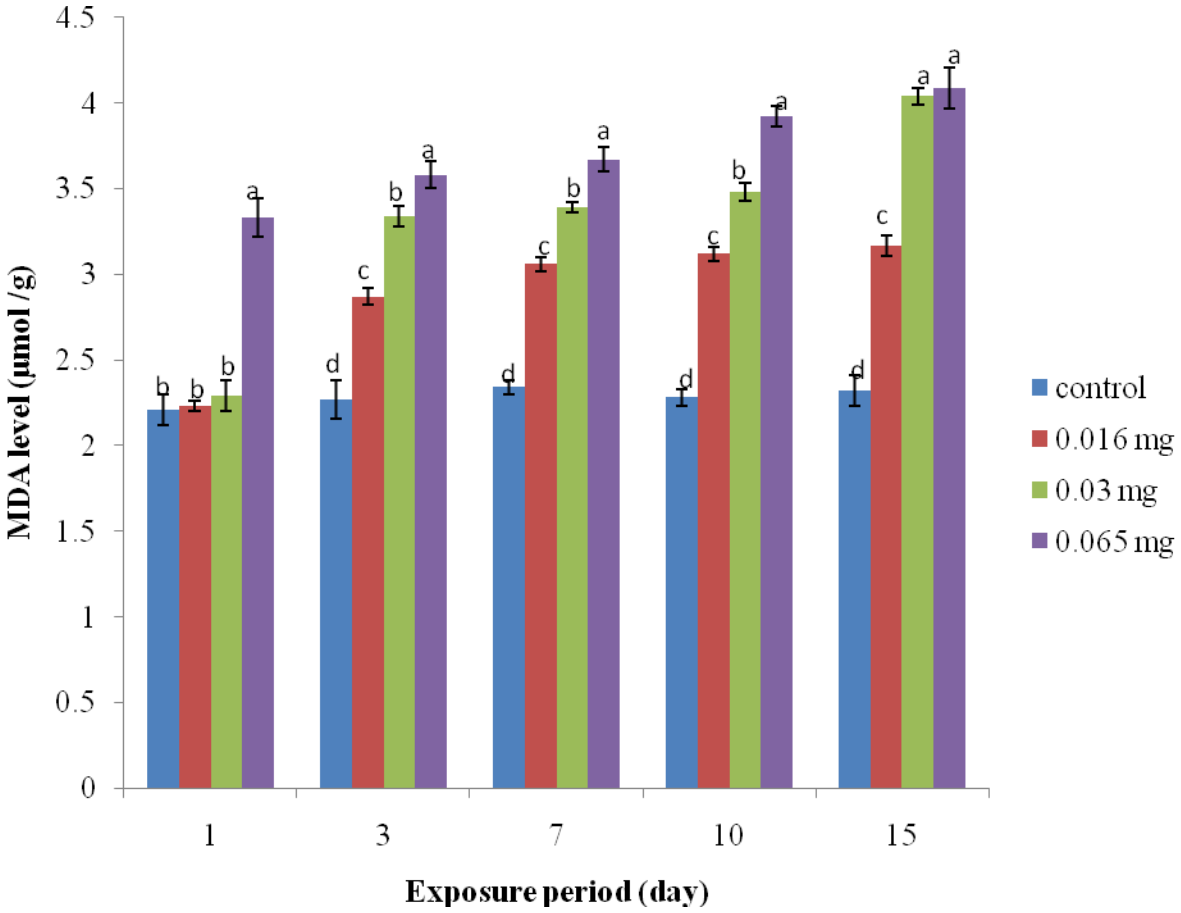

Figure 1: Plasma quantities of MDA in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish in each group. Ingots on the same day with different letters varied significantly (P< 0.05).

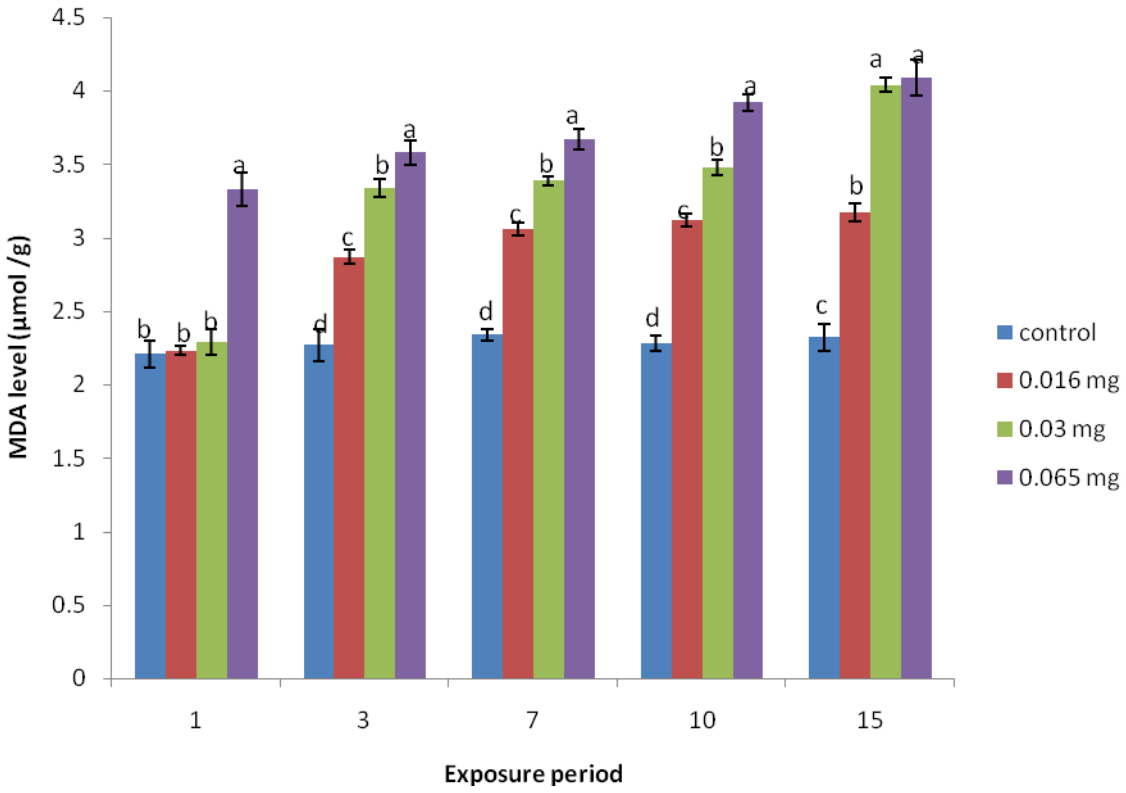

Figure 2 Liver quantities of MDA in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish in each group. Ingots on the same day with different letters varied significantly (P< 0.05).

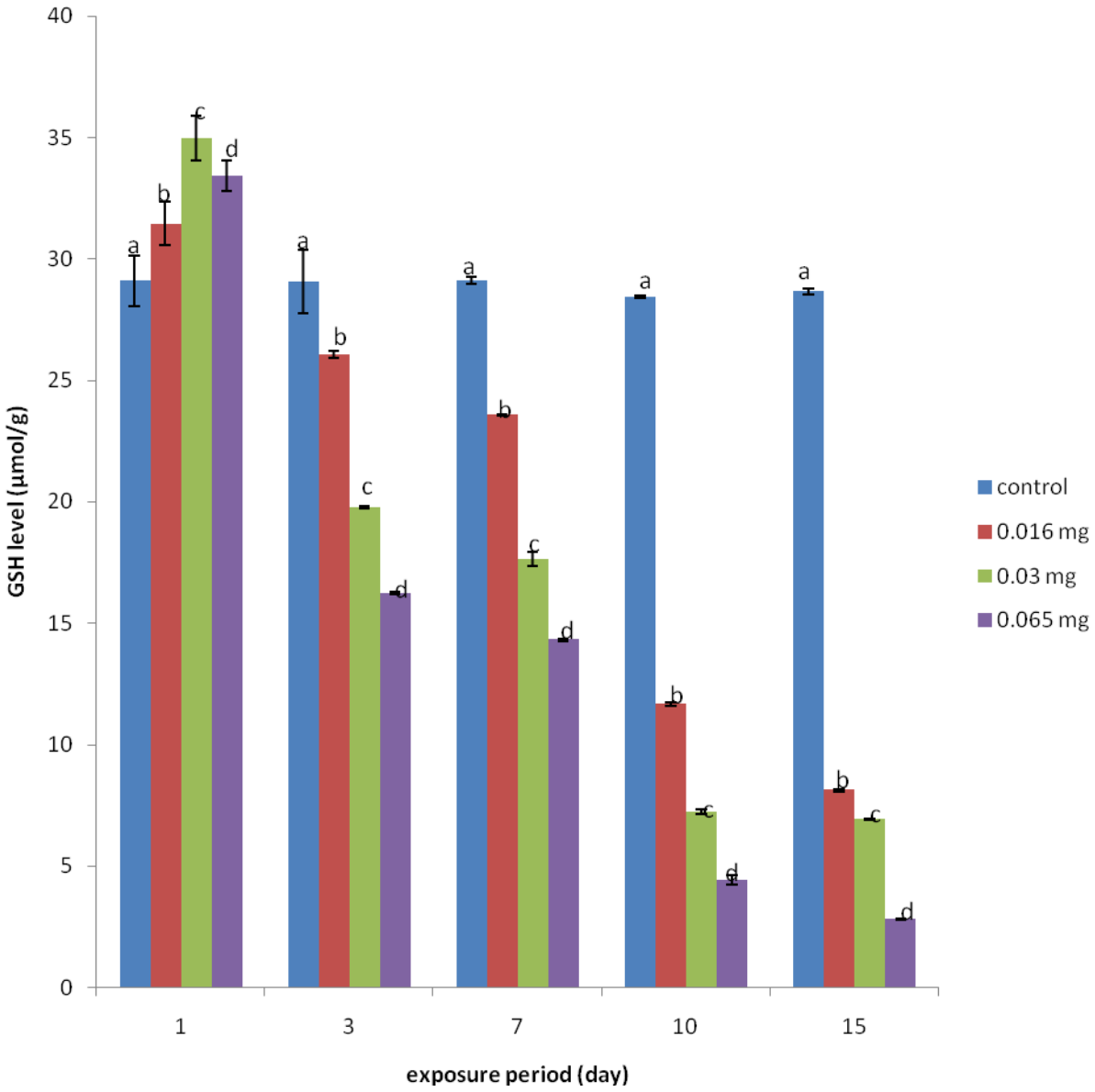

Figure 3: Plasma concentrations of GSH in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are presented as mean ±SD of 5 fish in each group. Ingots on the same day with distinguishing letters varied significantly (P< 0.05).

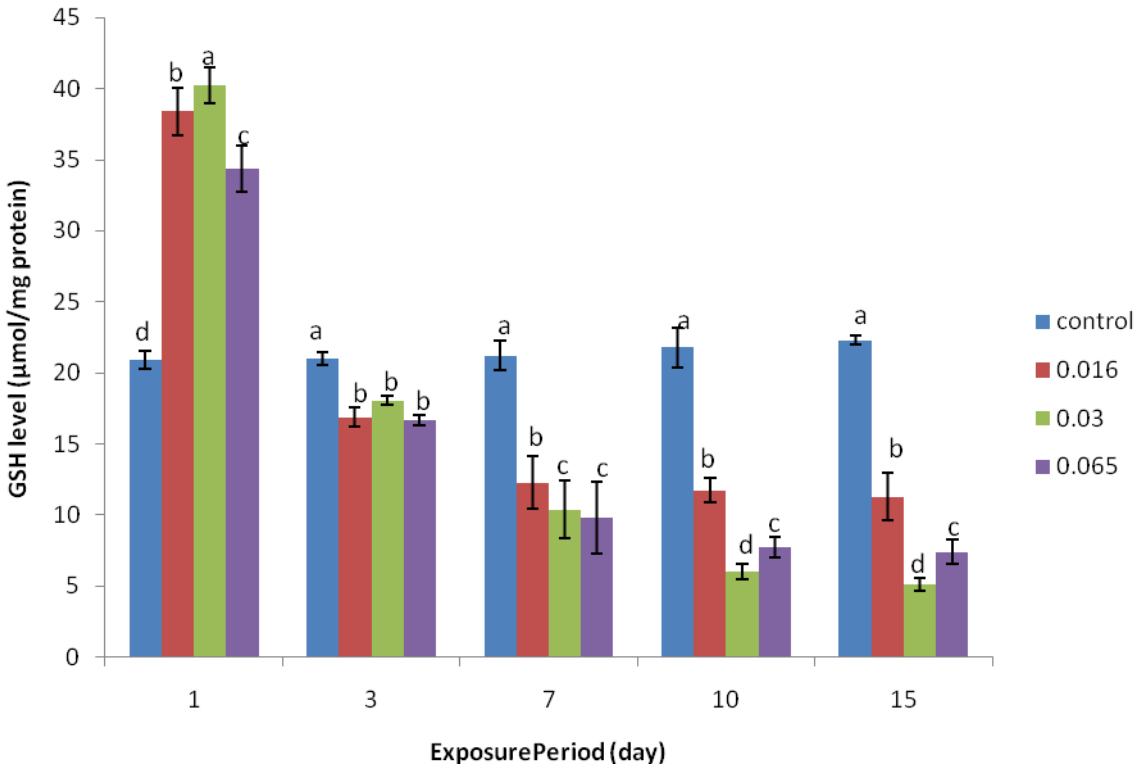

Figure 4 Liver levels of GSH in *C. gariepinus* exposed with the aqueous extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish in each group. Bars on the same day with distinguishing alphabets varied significantly (P< 0.05).

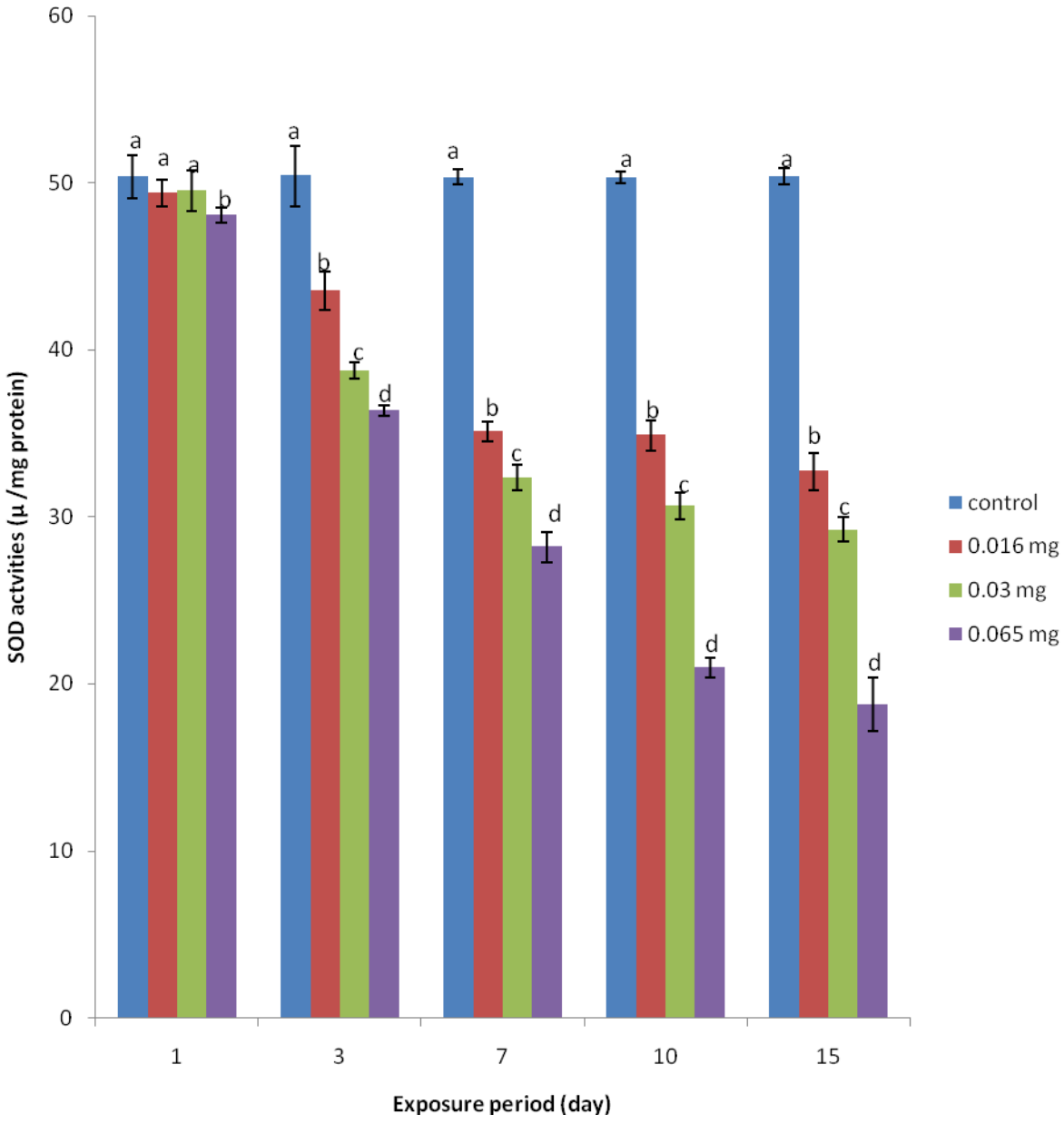

Figure 5: Plasma SOD activities in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are presented as mean ±SD of 5 fish. Ingots on the same day with distinguishing letters varied significantly (P< 0.05).

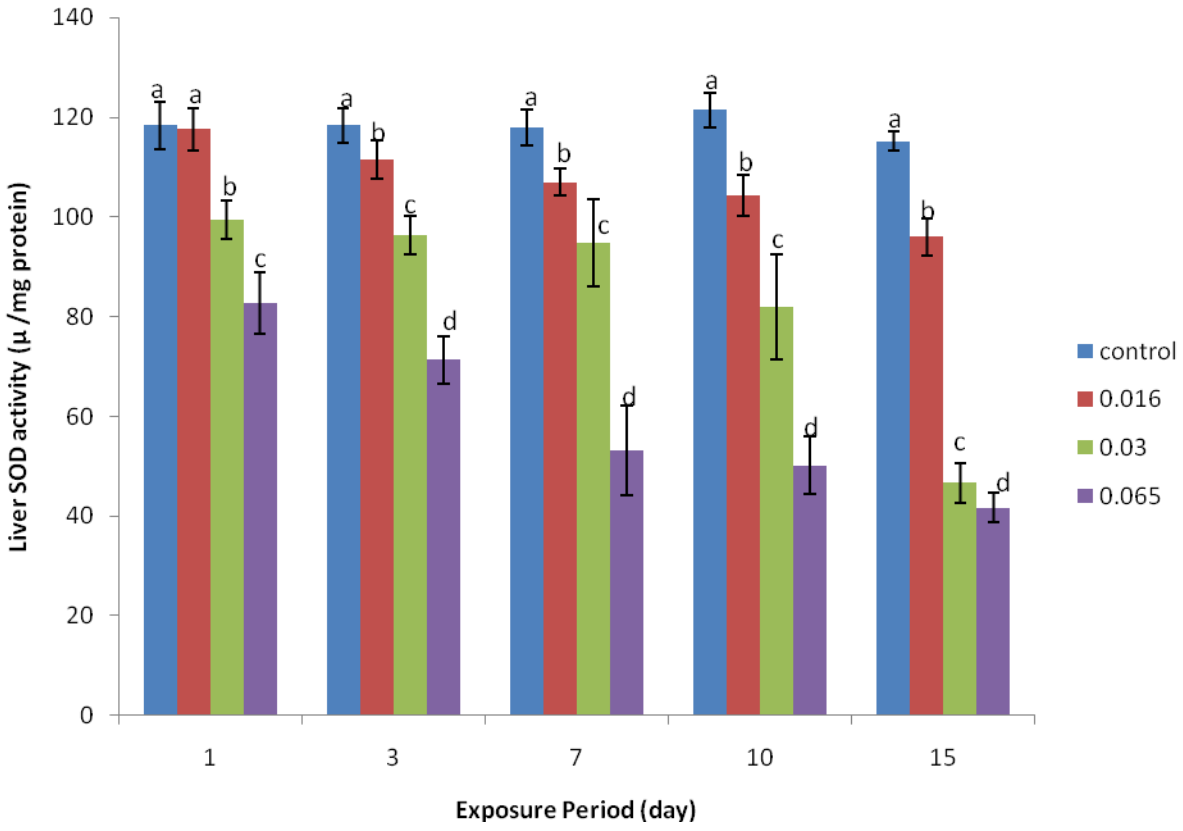

Figure 6 Liver SOD activities in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

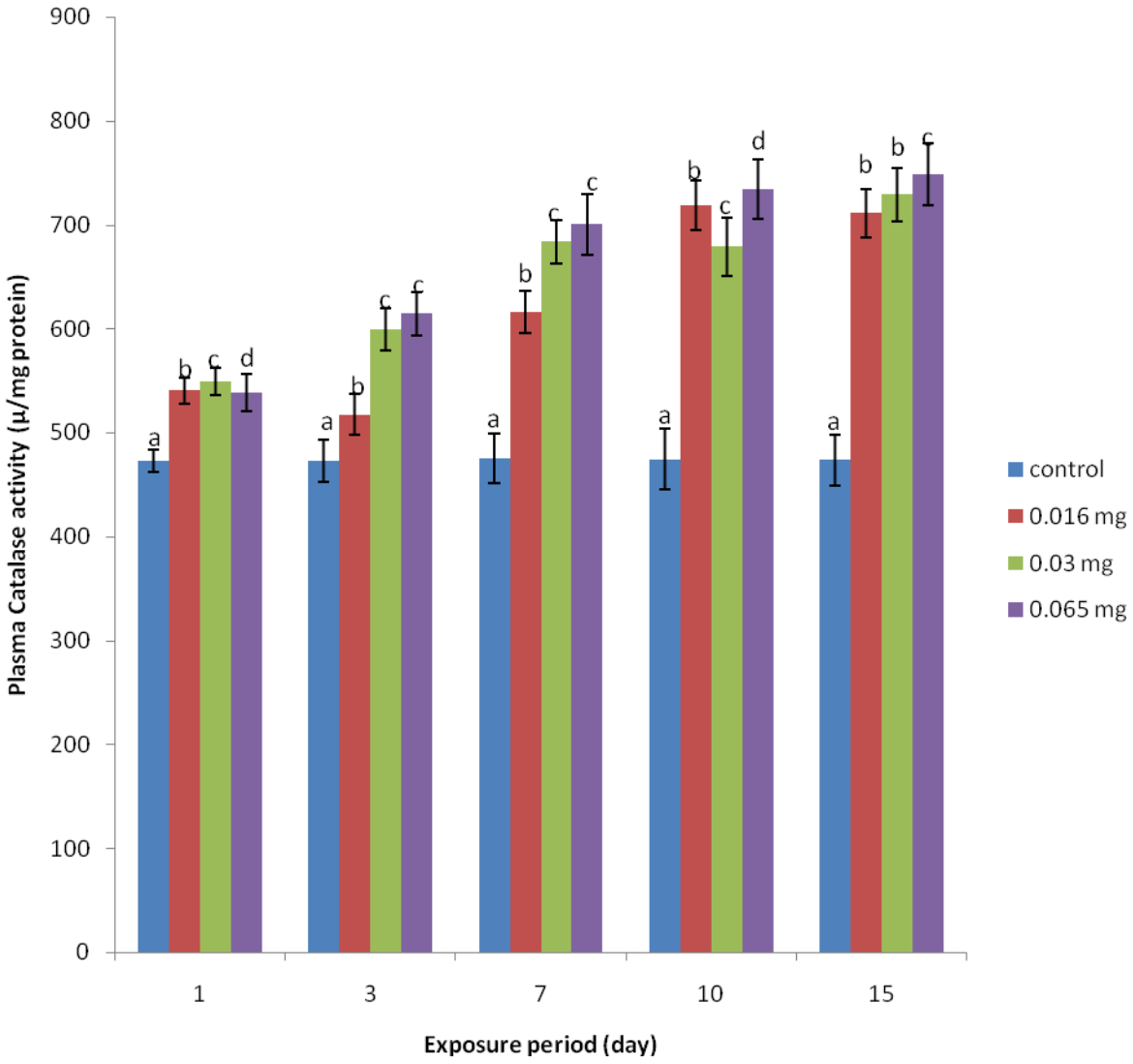

Figure 7: Plasma catalase activities in *C. gariepinus* exposed with the water extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

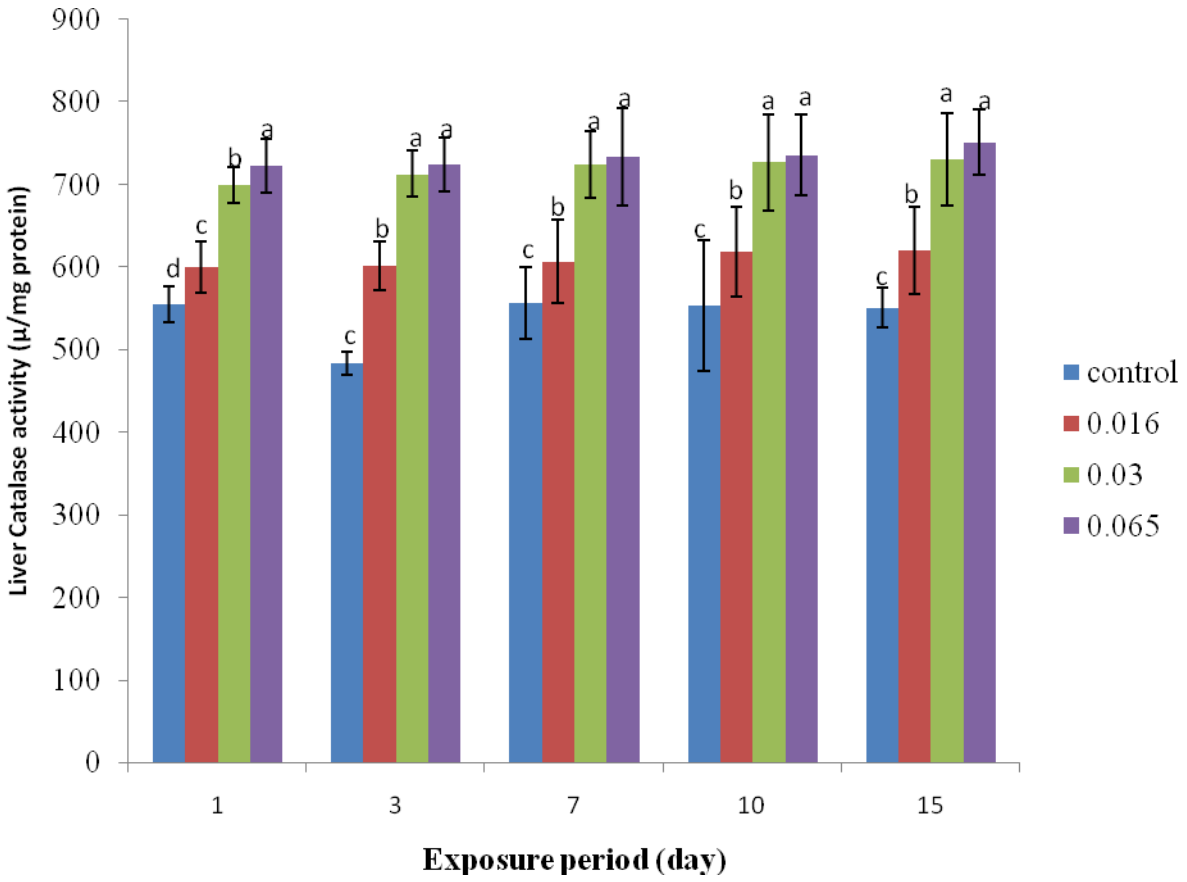

Figure 8 Liver catalase activities in *C. gariepinus* exposed with the aqueous extract of *P. microphylla* leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

DISCUSSION

Data from the present research clearly showed that catalase activity and MDA concentrations increased significantly (P<0.05) and a significant reduction of superoxide dismutase activity and GSH concentrations in the plasma of *Clarias gariepinus* exposed to the water extract of *P. microphylla* leaves. The levels of catalase and MDA increased by 71.67 and 76.29 % respectively, in the other hand, SOD and GSH reduced by 58.05 and 90.23 % in the experimental group exposed to 0.065 mg/l of the extract on day 15.

The observed increase in the action of catalase and significant decrease of SOD activity was not expected as these enzymes are usually induced simultaneously in the face of oxidative pressure [22]. [23] reported an increase in the activities SOD and catalase and MDA levels in the hepatic and kidney of *C. gariepinus* subjected to Butachlor. Nevertheless, similar observation have been made by some researchers [24, 25, 26]. The elevation of catalase may perhaps be because of higher presence of hydrogen peroxide levels. This rise in the action of catalase may be a demonstration of a fundamental role it plays to defend cells from the destructive effects of H_2O_2. It also implied that the phytoconstituents in the plant induced catalase to resist its toxicity. Conversely, the depressed activity of SOD suggests a compromised capacity to guard cells against superoxide radicals. This depression must have caused cellular injury as evidenced by the elevated liver marker enzymes and histological alterations. Disproportionate production of reactive oxygen species and oxidation of the cysteine residue in superoxide dismutase can cause the destruction of the enzyme thereby reducing its action [27, 28, 29, 30]. It is also probable that components of the extract may have inhibited this enzyme leading to a declined activity.

Our results caused a significant reduction of glutathione (GSH) concentration. [26, 27, 28] have also reported similar reduction of GSH in different fishes exposed to various toxicants. Glutathione is a powerful molecular antioxidant with a single cysteine residue that effectively scanvenge ROS and protect cells from their detrimental consequence [29]. But if oxidative stress is severe, GSH concentrations are repressed because of impairment of adaptive mechanisms and conversion of GSH to GSSH form [7]. The decreased GSH level in *C. gariepinus* subjected to aqueous extract of *P. microphyll* leaves may be indication of reduced cell protection abilities, more usage of GSH, impairment of adaptive mechanisms and insufficient regeneration because of severe oxidative stress [11, 15].

Malondialdehyde (MDA) is an end product of lipid peroxidation caused by free radicals that is usually employed as indicator of oxidative stress [13]. The determination of malondialdehyde level offers a relative appraisal of the capacity for toxic agents to induce oxidative damage [16]. Our results disclosed a significant elevation of MDA concentration in *Clarias gariepinus* exposed to the water extract of the plant. Previous studies [17, 19] have reported elevation of MDA in fishes exposed to toxicants. This rise in MDA concentration may be as a result of excessive production of ROS from the bioactive constituents of the aqueous extract of *P. microphylla* leaves. This increase in MDA can cause apoptosis [20]. In conclusion, the aqueous extract of *Psychotria micrphylla* leaves induced generation of reactive oxygen species and thus oxidative stress in the exposed fish.

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