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Evaluation of the Antioxidant Properties of Aqueous Stem Bark Extract of the *Bridelia ferruginea* and Butachlor

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Abstract: The effect of various concentration of aqueous stem bark extract of *B. ferruginea* and butachlor on selected antioxidant markers in *C. gariepinus* was investigated. The effect on both concentration and days of sample collection were noted in the fish exposed to both test groups and toxicants.The effect of malondialdehyde (MDA) on both butachlor and *Bridelia ferruginea* followed concentration and time dependent pattern. But the effect was more pronounced in butachlor than aqueous extract *Bridelia ferruginea* stem bark. The antioxidant enzyme activities of glutathione transferase, glutathione reductase, superoxide dismutase and catalase significantly (p<0.05) decreased in a concentration and time dependent pattern following the exposure to toxicants.

Key words: *Bridelia ferruginea* · *C. gariepinus* · Butachlor And Antioxidants

INTRODUCTION

The application of herbicides in weed managements has been recognized as a form of practice in agriculture all over the world. Unfortunately, unsystematic application of herbicides to boost agricultural production and output could affect non-target organism, particularly aquatic forms and their environment [1]. Fish could also serve as bio-marker to evaluate pollution in aquatic environment and plays an important function in evaluation of impending hazard linked with pollution in aquatic environment since they have direct contact to chemicals arising from agricultural effluents or obliquely via food sequence of ecology [2].

Antioxidant is a molecule that stops the oxidation of other molecules. It is a chemical reaction that donates electron or hydrogen from a substance to an oxidizing agent. Damage to cell or cell death occurs when there is chain reaction initiated by free radical. Free radical intermediates are removed by antioxidant by stopping chain reaction as well as other oxidation reactions. Antioxidant oxidizes free radical and behaves as reducing agents such as thiols, ascorbic acids or polyphenols [3]. They are commonly used in nutritional argumentation and have been evaluated in preventing coronary heart disease and cancer etc. Previous investigation suggested that antioxidant intake might enhance health, while a lot of clinical trials with a limited number of antioxidants evaluated is not beneficial and affirms that high supplementation with some putative antioxidants could be detrimental to health [4]. Some of the antioxidant has several industrial applications in preservation of foods [5].

Aim and Objectives: The aim of this research is to determine the effect of stem bark aqueous extract of the *Bridelia ferruginea* and butachlor on some selected antioxidants.

MATERIALS AND METHODS

Collection of Biological Materials

Plant Material: The stem bark of *Bridelia ferruginea* plant was collected from the wild in Ntsuruakpa Ezzamgbo, Ohaukwu Local Government Area Ebonyi State, Nigeria. The stem bark was identified and authenticated by Prof. S.C. Onyekwelu of the Department of Applied Biology, Ebonyi State University Abakaliki.

Preparation of the Plant Extract: The *Bridelia ferruginea* stem bark wascut into pieces and dried at room temperature for two weeks and pulverised into fine powder using grinding machine. Fifty grams of ground

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stem bark of *Bridelia ferruginea* was weighed into a conical flask and 100mls of de-ionised water, mixed and shaken prior to filtration by means of a dried Whatman filter paper into a graduated 1litre measuring cylinder to obtain cold water extract. Thereafter, the extract was stored at -5°C.

Experimental Fish and Treatment: A total of two hundred and thirty four (234) juvenile African catfish (*Clariasgariepinus*) of mean weight 240g ± 25.5 – 30.8 cm were purchased from Chiboy's Fish Farm Abakaliki Ebonyi State. The fish was acclimatized for two weeks in a plastic container of 80 litre capacity in a laboratory condition where temperature was kept constant at 25 ± 1°C and lightening plan at 12 hours of daylight alternating with 12 hours of darkness (LD: 12:). The water in plastic container was changed daily with bore-hole water that was free from chlorine.

Determination of Glutathione Transferase (GST) Activity: The GST activity was determined using spectrophotometric method. Then, 0.15 ml of 1-chloro-2, 4- dinitrobenzene reagents was put to 3.00 mls of the buffer. The mixture was incubated at 37°C for 15 minutes. After incubation, the reaction begins by addition of 0.03 ml serum. Absorbance was read at 30 seconds, I minutes, 2 minutes and 3minutes interval. The incubated tubes without serum sample were used as the blank in each case. Readings were taken immediately after incubation. GST activity was determined with an extinction coefficient of 9.6 Mm 1cm for 1-chloro-2, 4-dinitrobenzene (CDNB) and was expressed in M/min.

Calculation

$$\text{GST Activity (M/Min)} = \frac{\text{Absorbance /min}}{9.6 \text{ X } 100}$$

Determination of Superoxide Dismutase Activity: This was determined using spectrometric method. 0.2 mls of serum were added to 2.5 mls of 0.05 M buffer (phosphate) with p^H 7.8. The tubes were equilibrated with spectrophotometer before adding adrenaline solution. The reaction begins when 0.3 mls of newly prepared adrenaline solution were added to the tubes followed by quick mixing by inversion in the cuvette. The standard cuvette therefore contained 3 mls buffer, 0.5 mls of adrenaline and 0.4 mls of the sample. The increase in absorbance was taken at 480 nm for 150 seconds at 30 seconds interval.

Calculation

$$\text{Increase in absorbance per minute} = \frac{A_a - A_o}{2.5}$$

where,

Ao = absorbance after 30 seconds

Aa = absorbance after 150 seconds.

$$\text{SOD (U/L)} = \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance for blank}}$$

A blank was prepared with 0.3ml of adrenaline in 2.5ml of buffer.

Determination of Catalase Activity: The cuvettes were labelled sample, standard and blank. And 20 µl of the sample was added to a 96-well microliter plate. Then, 100 µl of the hydrogen peroxide working solution (24 mM) were dispensed to the wells, shaken very well and incubated exactly 1 min. at 37°C. The reaction was inhibited via addition of 40 ml of the catalase quencher into wells and homogenized.

Next, 5 µl reaction well were transfer to fresh well. Thereafter, 250 µl of the chromogenic working solution were dispensed into the wells and incubated the plate for 60 minutes with vigorous shaking and the sample absorbance against blank was read spectrophotometrically at 520 nm.

Determination of Malondialdehyde: One (1) ml of serum sample was put into 2 mls each of trichloroacetic acid (TCA), thiobarbituric acid (TBA) and hydrochloric acid (HCl) solution and mixed very well. The tubes were heated for five (5) minutes using water bath and cooled. The precipitate formed was removed by centrifuging at 3000 r p m for 10 minutes. The absorbance was read spectrophotometrically at 535 nm against reagent blank. Thereafter, the concentration was obtained as follow;

$$\text{MDA (mg/ml)} = \Delta A/1.56 \text{ X } 10.$$

RESULTS

The results obtained were statistically studied by statistical package of SPSS version 16. The results were subjected to Duncan's multiple range tests and oneway analysis of variance (ANOVA) to evaluate the significance difference at 5% probability level.

Lipid Peroxidation Parameter: In this study, malondialdehyde (MDA) level was evaluated for lipid peroxidation, a marker of oxidative stress. The effect of various concentration of aqueous stem bark extract of

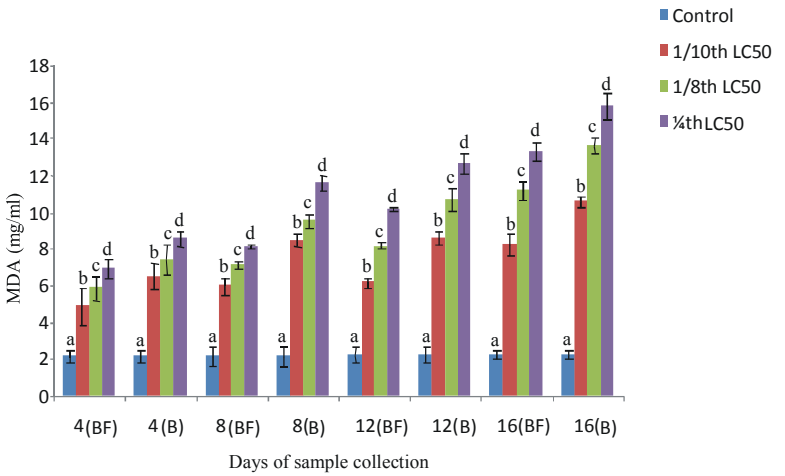

Fig. 1: Formation of malondialdehyde (MDA) in the Serum of *C. gariepinus*exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean ± SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different (p<0.05). BF = *Brideliaferruginea,* B = Butachlor. The numbers 4, 8, 12 and 16 represents days of sample collection

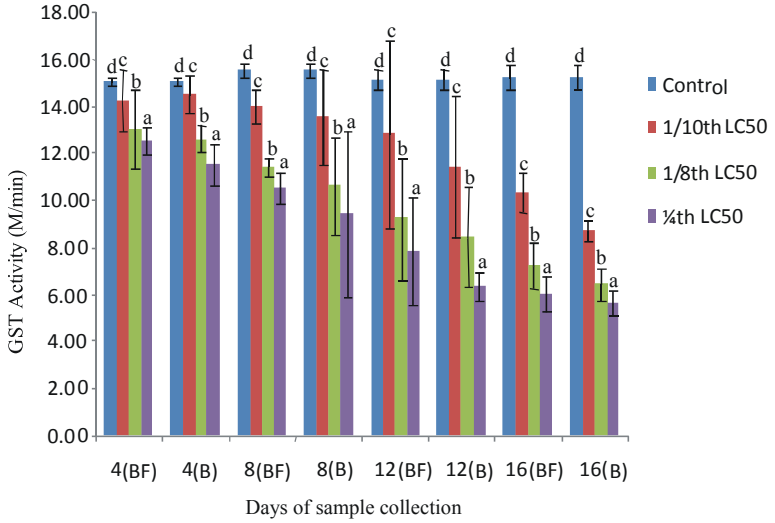

Fig. 2: Activity of Glutathione –S -Transferase (GST) in the Serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean ± SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different (p<0.05). BF = *Brideliaferruginea,* B = Butachlor. The numbers 4, 8, 12 and 16 represents days of sample collection.

B. Ferruginea and butachlor on MDA level in the serum of *C. gariepinus* are presented in Figure 1. The effect on significant (p<0.05) increase of both concentration and days of sample collection were noted in the fish exposed to both test groups and toxicants. Maximum threshold effect was noted on 16th day of sample collection while the least effect occurred on the 4th day of sample collection at different concentrations of $^{1}/_{10}$, $^{1}/_{8}$ and ¼ LC_{50} in both aqueous extract of the stem bark of *Bridelia ferruginea* and butachlor relative to control. The effect was more pronounced in butachlor than aqueous extract *Brideliaferruginea*stem bark. This shows that the effect is concentration and time dependent. No significant change was observed in the control.

Antioxidant Enzyme Status: The following enzyme activities; glutathione transferase, glutathione reductase, superoxide dismutase and catalase were evaluated for

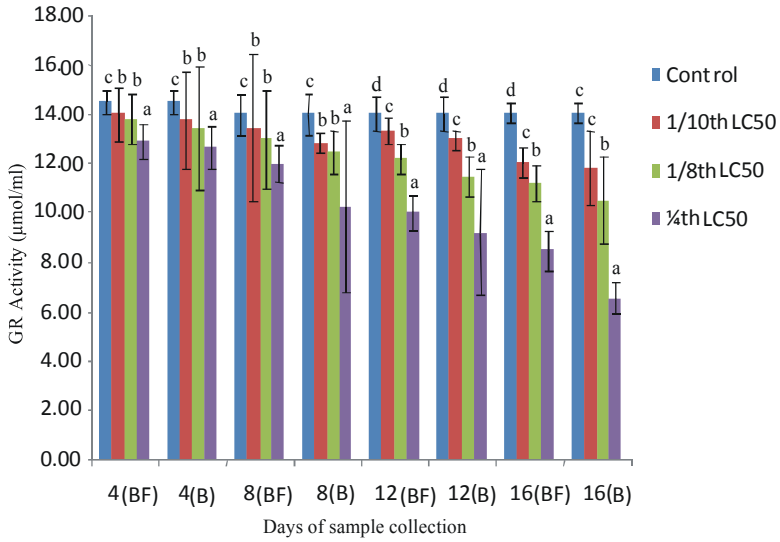

Fig. 3: Activity of Glutathione Reductase (GR) in the Serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean ± SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different ($p<0.05$). BF = *Brideliaferruginea,* B = Butachlor. The numbers 4, 8, 12 and 16 represents days of sample collection

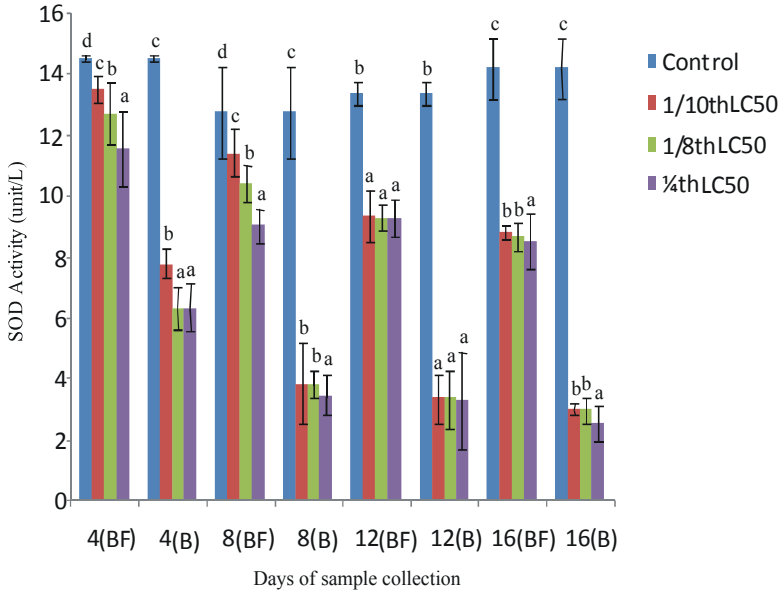

Fig. 4: Activity of Superoxide Dismutase (SOD) in the Serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different ($p<0.05$). BF = *Bridelia ferruginea,* B = Butachlor. The numbers 4, 8, 12 and 16 represents days of sample collection.

antioxidant enzyme status in fish exposed to aqueous extract of the stem bark of *Brideliaferruginea*and butachlor (herbicide) are presented in Figures 2, 3, 4 and 5. The antioxidant enzymes activities of glutathione transferase, glutathione reductase, superoxide dismutase and catalase significantly ($p<0.05$) decreased in a concentration and time dependent pattern following the exposure to test toxicants. The highest effect was observed on 16th day of exposure while the least effect was observed on 4th day of exposureat different concentration of $^{1}/_{10,}$ $^{1}/_{8}$ and $^{1}/_{4\ LC50}$ in both extract of the stem bark of *Bridelia ferruginea* and butachlor with

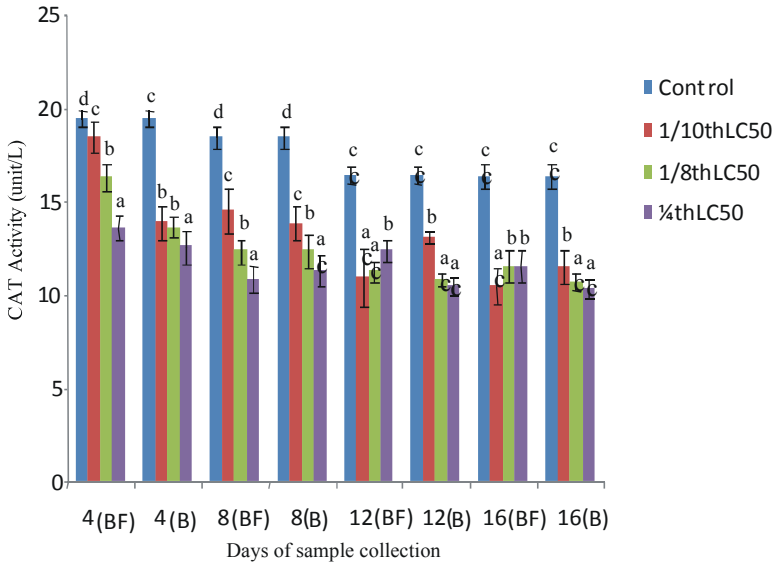

Fig. 5: Activity of Catalase (CAT) in the Serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different ($p<0.05$). BF = *Bridelia ferruginea,* B = Butachlor. The numbers 4, 8, 12 and 16 represents days of sample collection.

relative to control. The effect was more pronounced in butachlor-exposed fish than extract of the stem bark of *Bridelia ferruginea.* Thus, linear decrease in a concentration and time dependent pattern was also observed.

DISCUSSION

In the present study, the serum MDA level of *C. Gariepinus* exposed to the test toxicants increased significantly ($p<0.05$) relative to control. Alterations significantly ($p<0.05$) higher in butachlor-exposed fish than aqueous extract of *Brideliaferruginea*stem bark-exposed fish. These findings showed possible facts of free radical production and damage in *C. gariepinus* exposed to the test toxicants. The present data also show that ROS might be linked with the metabolism of the test toxicants leading to peroxidation of membrane lipids. Data obtained in this study follows the same fashion with the findings of Rekha and Joseph [6] in cyhalothrin intoxicated fish. The observed lipid peroxidation may be due to ROS generated by the compound leading to cell apoptosis because reactive oxygen species and oxidative stress have been shown to be cause of apoptosis [7]. Following the usual physiological condition, the antioxidant protection systems including superoxide dismutases, catalases, glutathione reductase as well as glutathione transferase could be influenced by a minor oxidative stress to compensate for the response and thus the reactive oxygen species could be removed to offer protection to the organisms from oxidative stress damage [8, 9 and 10]. Data from the present investigation showed significant decrease ($p<0.05$) in glutathione transferase, glutathione reductase, superoxide dismutase as well as catalase relative to control. Alterations in these biochemical parameters were more pronounced in the butachlor exposed fish than aqueous extract of *B. Ferruginea* stem bark. Thus, all alteration observed followed concentration-time dependent trend throughout the days of sample collection. SOD is the first line of defense against reactive oxygen species that catalyses the dismutation of the superoxide anion. Our findings revealed significant decrease ($p<0.05$) of SOD in *C. gariepinus* exposed to the test toxicants relative to the control. This decrease in the activity of SOD in serum of *C. Gariepinus* assumed to indicate a degradation process in which SOD is degraded by free radical during the de-toxifying process [11-14]. The decrease in catalase (CAT) activity might be as a result of superoxide radicals reported to inactivate CAT activity [15, 17]. This indicated a lowered activity to protect the cell against hydrogen peroxide radical. The same decreased activity of CAT was observed in the tissue of *Channapuntatus* Bloch exposure to deltametrin. Glutathione transferase activities and glutathione reductase significantly decreased ($p<0.05$) relative to control in concentration and time dependent pattern throughout the exposure period. This could be as the result of excessive generation of lipid peroxidation observed in this research.

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