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The Effect of Aqueous Extract of the Stem Bark of *Bridelia ferruginea* and Butachlor on Some Liver Markers of *Clarias gariepinus*

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Abstract: The effect of aqueous stem bark extract of *Bridelia ferruginea* and butachlor on some liver markers of *Clarias gariepinus* was done to determine the effect of the two toxicants on the liver of *Clarias gariepinus.* There were significant increases ($p<0.05$) in aspartate amino transferase, alanine amino transferase and alkaline phosphatase activities of the aqueous stem bark extract of *Bridelia ferruginea* and butachlor when compared with the control group. Bilirubin increased significantly ($p<0.05$) in both extract of the stem bark of *Bridelia ferruginea* and butachlor when compared with the control. The alteration was more pronounced in butachlor-exposed fish than the plant extract. The maximum threshold effect was observed on 16th day of exposure at different concentrations of $^1/_{10}$, $^1/_8$ and ¼ 96 h LC_{50} in both aqueous extract of *Bridelia ferruginea* stem bark and butachlor while the least effect was observed on 4th day of exposure in both extract of *Bridelia ferruginea* stem bark and butachlor relative to control.

Key words: *Bridelia ferruginea* · *Clarias gariepinus* · Butachlor and liver markers

INTRODUCTION

Fish plays a vital contribution to the world survival and wellbeing of an important section of the world. Giving its fairly low cost and abundance, fish has become the major source of nutrition for the people of Nigeria. In Nigeria they hound fish for commercial and subsistence purposes in wild fisheries or farm them in ponds and cages in the oceans. The use of known natural biocides with pesticides properties obtained from plants by local fishermen has been widely reported in Nigeria [1, 2].

Tribal people in olden time use a variety of plants for the treatment of ailments and food utilization purposes, though application of fish poison plant to harvest fish is an ancient way of harvesting fish but it is still used in many places of the world today. The use of local plant to harvest fish was reported in many ways involved in harvesting fish from salt water free and seawater [3]. These plants poisons are not made for man's use but to protect plants from external attack and also for productivity [4].

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. 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. Hence, the aim of this research was to determine the effect of stem bark aqueous extract of *Bridelia ferruginea* and butachlor on some liver markers of *Clarias gariepinus.*

MATERIALS AND METHODS

Biological Materials

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.

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Fig. 1: Bridelia ferruginea Stem bark

Preparation of Plant Extract: The *Bridelia ferruginea* stem bark was cut into pieces and dried at room temperature of 25°C for two weeks and pulverised into fine powder using grinding machine. Fifty grammes of ground 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 (*Clarias gariepinus*) of mean weight 240 g + 25.5 – 30.8 cm were purchased from Chiboy's Fish Farm Abakaliki Ebonyi State. The fish were 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 Alanine Amino Transferase Activity: The test tubes were labelled according to fractions of 96 h LC_{50} ($^1/_{10}$, $^1/_8$ and ¼), control and reagent blank. Then, 0.1ml of fish exposed to the test toxicant was put to the tubes labelled with fractions of 96 h LC_{50} and 0.1ml serum of fish without test toxicant was dispensed to the tubes labelled as control. Later, 0.5 ml of phosphate buffer was also put to the tubes and 0.1 ml of de-ionized water was pipetted into the reagent blank. The tubes were subjected to incubation for 30 minutes at 25 °C. Then, 0.5 ml of 2, 4-dinitrophenolhydrazine solutions was put to test tubes, mixed and stood for 20 mins at 25°C. Next 5.0mls of Nacl solution was also put to all test tubes and homogenized. Thereafter, the absorbances of the sample against the reagent blank were read after 5 minutes. The enzyme activity was calculated using the formula below [5].

$$\text{Concentration (IU/L)} = \frac{\text{Sample absorbance}}{\text{Slope}}$$

Determination of Aspartate Amino Trasferase (AST) Activity: Test tubes were labelled according to fractions of 96 h LC_{50} ($^1/_{10,}$ $^1/_8$ and ¼), control and reagent blank. 0.1ml of serum sample with test toxicants were put to the test tubes labelled with fractions of 96 h LC_{50} and another 0.ml of serum without the test toxicant were added to the control tube. Then, 0.5 ml phosphate buffer solutions were added to all tubes while 0.1ml distilled water was dispensed to the reagent blank. The tubes were incubated for 30 mins at 37°C. Later, 0.5 ml 2,4-dinitrophenylhydrazine solution was poured to test tubes and stood for 20 mins. at 25 °C. Therafter, 5.0 ml of Na0H solution dispensed to all test tubes, homogenized and stood for 5 minutes. Absorbances of the sample were taken spectrophotometrically against the reagent blank. The enzyme activity was calculated using the below formula [5].

$$\text{Concentration (IU/L)} = \frac{\text{Sample absorbance}}{\text{Slope}}$$

Determination of Alkaline Phosphatase (ALP) Activity: This was determined by spectrophotometric method. The test tubes were labelled according to the fractions of 96 h LC_{50} ($^1/_{10}$, $^1/_8$ and ¼), control and reagent blank. To each sample, 0.5 mls of alkaline phosphatase substrate were added into labelled tubes and equilibrated at 37 °C for 3 mins. At time interval, 0.05 ml (50il) of each standard, control and sample were put to its individual test tubes and mixed very well. Deionized water was used as sample for reagent blank. Later, the test tubes were calculated for exactly ten (minutes) at 37 °C. Thereafter, 2.5 ml alkaline phosphatase colour developer were added to all the containers at time interval and homogenized. The absorbance of the sample was read and recorded while the enzyme activity was determined with the formula below [6]: Enzyme activity= (IU/L) Abs of unknown X values of standard.

Abs of Standard

Determination of Direct Bilirubin Concentration: The tubes were labelled according to the fractions of 96 h LC_{50} ($^1/_{10}$, $^1/_8$ and ¼), control and blank. 200ìl of sulphanilic acid solution was added to all the test tubes while 1 drop of (50ìl) sodium nitrite was put to the tubes with test toxicants. Then, 2000 ìl of 0.9 % NaCl solution was put to all tubes, 200 ìl serum sample was also added to all the tubes, mixed and incubated for 10 mins at 25°C. The absorbances of the sample was read spectrophotometrically against the sample blank. Thereafter, the concentration of direct bilirubin was determined with the formula below [7].

Direct bilirubin (mg/dl) = 14.4 X Absorbance of the sample

RESULTS

The following indices; aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein as well as bilirubin were tested for liver function. In the experimental groups there were significant increases ($p<0.05$) in aspartate amino transferase, alanine amino transferase and alkaline phosphatase activities of the aqueous stem bark extract of *Bridelia ferruginea* and butachlor when compared with the control group.

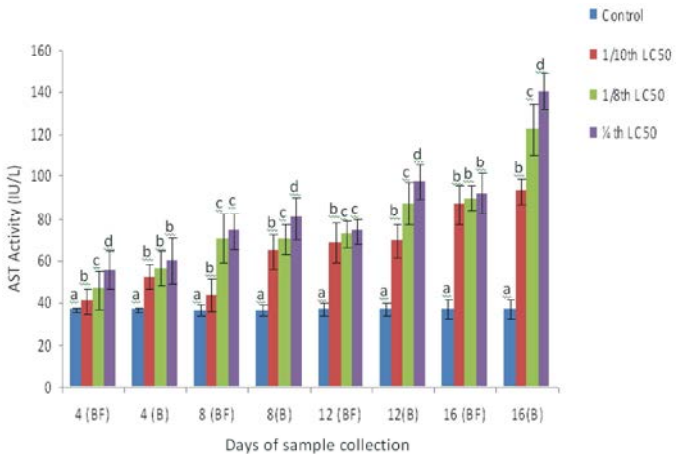

Fig. 2: Activity of aspartate amino transferase (AST) in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have 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

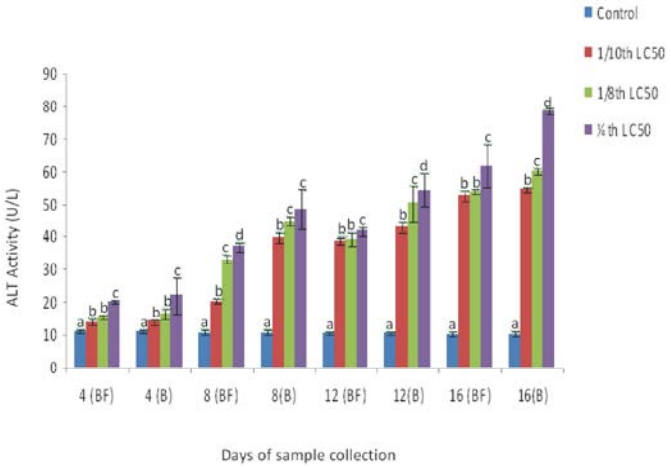

Fig. 3: Activity of alanine amino transferase in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different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

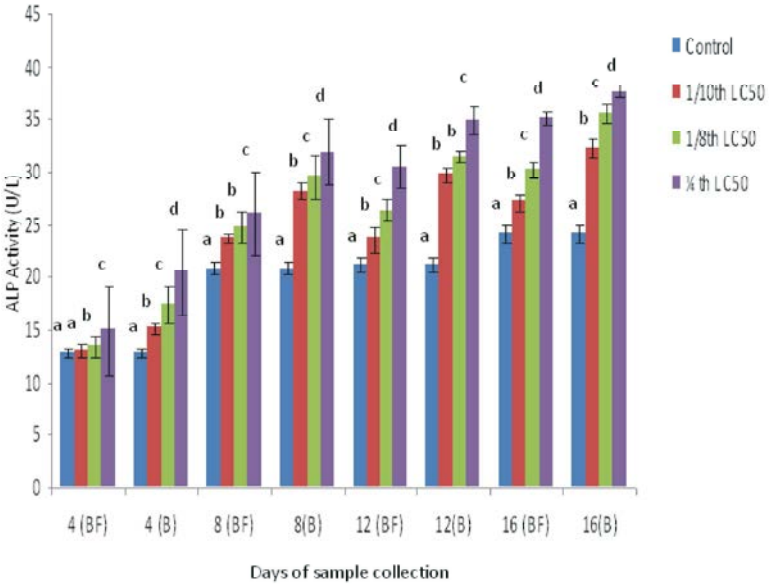

Fig. 4: Activity of alkaline phosphatase in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters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

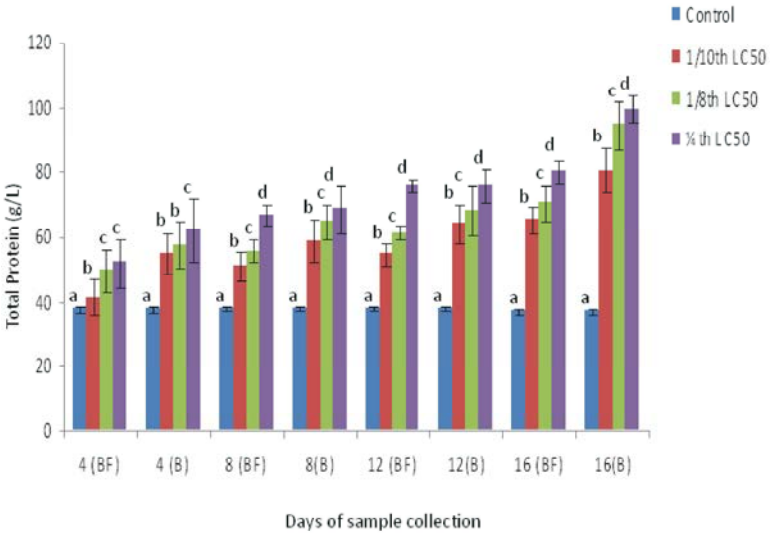

Fig. 5: Total protein concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters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

The total protein and bilirubin increased significantly ($p<0.05$) in both extract of the stem bark of *Bridelia ferruginea* and butachlor when compared with the control. The alteration was more pronounced in butachlor-exposed fish than the plant extract. The maximum threshold effect was observed on 16th day of exposure at different concentrations of $^{1}/_{10}$, $^{1}/_{8}$ and ¼ 96 h LC_{50} in both aqueous extract of *Bridelia ferruginea* stem bark and butachlor while the least effect was observed on 4th day of exposure in both extract of *Bridelia ferruginea* stem bark and butachlor relative to control. This shows that the effect followed concentration and time dependent pattern.

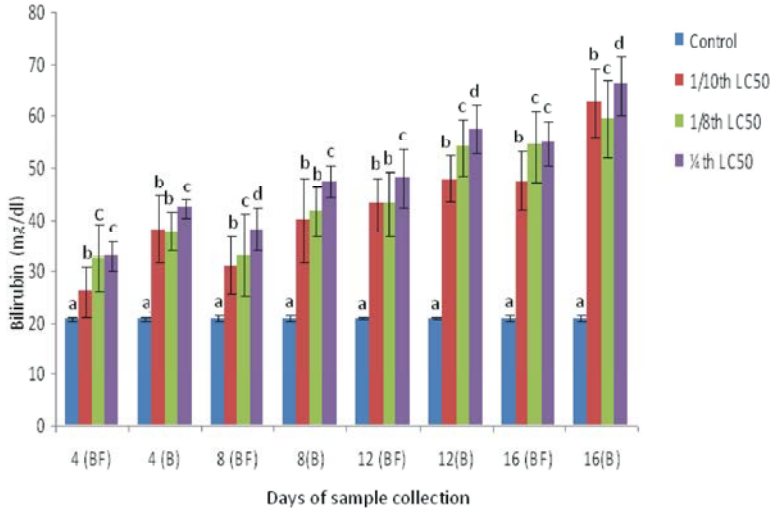

Fig. 6: Direct bilirubin concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days. All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are significantly different ($p<0.05$). BF = *Bridelia ferruginea,* B = Butachlor. The numbers 4,8,12 and 16 represents days of sample collection.

DISCUSSION

In a clinical serum examination, AST, ALT, ALP, total protein and direct bilirubin in serum represent biomarkers for liver function. The majority of the enzymes used in diagnosis or as biomarkers for cell damage rely on the very high level of such enzymes contained by the cell compared to that in plasma or serum. As a result, cell impairment arises from drug or other substance and disease regularly result in the damage cell. Their existence in serum could give information on tissue damage or organ impairment [8]. Significant increased observed within the ALT and AST activities in the serum of *Clarias gariepinus* exposed to the test toxicants relative to control group could be as a result of the movement of the enzymes from the cytosol to the blood which is a manifestation of the liver impairment. It may also be that both the plant extract and the butachlor leaked the enzyme from cytosol to the serum through changes in permeability of the membrane [9]. Cellular damage arising from the test toxicants exposure could result in movement of the biomarker enzymes to the extracellular fluid. Thus, alanine amino transferase activity increased significantly ($p<0.05$) in the serum of *Clarias gariepinus* exposed to plant extract and butachlor relative to control which could substantiate that injury has been imposed on the plasma membrane of the liver which could caused impairment of membrane integrity.

In this study, the serum activities of AST, ALT and ALP, of *C. gariepinus* exposed to *B. ferruginea* stem bark and butachlor increased significantly ($p<0.05$) relative to control. Data obtained in this investigation is in line with the findings of Mousa and Khattab (2003) [10] where it was found that there was an increase in AST and ALT activities in the serum of *C. gariepinus* following consumption of food with ochratoxin. This observation is also parallel with the work of Karmen *et al.* [8] that has a similar trend of result. Alterations of AST and ALT activities are specific to liver and used as a biomarker to analyze change in cell function and permeability in cellular membrane [8]. Our study also revealed significant ($p<0.05$) increase in total protein as well as direct bilirubin in the serum of *C. gariepinus* exposed to the test toxicants relative to control. The result followed concentration and time dependent pattern. The observed elevation of these liver function indices could be accounted for movement of these parameters from the cytosol to the blood that showed liver impairment. Changes observed were more pronounced in the butachlor-exposed fish than extract of the stem bark of *Bridelia ferruginea*. Total protein level could be affected by modification in hepatic synthesis, protein distribution, dehydration or overhydration and protein breakdown or excretion. High level of total protein is commonly the cause of tissue impairment [9].

The liver function indices indicated the synthesis and secretion function of the liver could be used to study liver dysfunction [6]. Bilirubin is the main catabolic product that occurs as a result of the damage of old red blood cells and transported from the blood by the liver; thus, it is a clear manifestation of the liver functioning. Bilirubin level is high in the blood by increased synthesis of bilirubin or reduction in liver uptake. Any rise in the level of serum

bilirubin indicates otherwise suggest liver damage since the liver functions as an excretory unit than in distribution unit for bilirubin. In this study, the bilirubin result increased significantly ($p<0.05$) in *C. gariepinus* exposed to aqueous stem bark extract of *B. ferruginea* and butachlor relative to control. These observations could suggest that the test toxicants have toxic effect on the liver.

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