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

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Abstract: The effect of aqueous stem bark extract of *Bridelia ferruginea* and butachlor on some kidney markers of *Clarias gariepinus* was done to determine the effect of the two toxicants on the kidney of *Clarias gariepinus.* There was a significant increase ($p<0.05$) in serum concentrations of creatinine, urea blood nitrogen, sodium and potassium in the catfish exposed to both test toxicants. But the effect was more pronounced in butachlor-exposed fish than the plant extract.

Key words: *Bridelia ferruginea* · Butachlor · *Clarias gariepinus* And Kidney Markers

INTRODUCTION

Fish plays a vital contribution to the world survival and wellbeing of an important section of the world's population. Giving its fairly low cost and abundance, fish has become the major sources of nutrient for the Africans [1]. The use of known natural biocides with pesticides properties obtained from plants by local fishermen has been widely reported in Nigeria [2].

The application of poisonous plant to harvest fish is an ancient way of harvesting fish but it is still used in many parts of the world today [3]. These plant poisons are not made for man's use but to protect plants from external attack and also for productivity [4].

Fish could also serve as bio-marker for evaluation of pollution in aquatic environment and plays an important function in detection 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 [5]. Hence, the aim of this research is to determine the effect of aqueous extract of the stem bark of *Bridelia ferruginea* and butachlor on some kidney markers of *Clarias gariepinus*.

MATERIALS AND METHODS

Collection of Biological Materials: The stem bark of *Bridelia ferruginea* plants was collected from 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 Plant Extract: The *Bridelia ferruginea* stem bark was cut into pieces and dried at room temperature for two weeks and pulverised into fine powder using grinding machine. Fifty grams 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 240g and average length 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 12hours of darkness (LD: 12:).

Determination of Creatinine Concentration: This was determined using the method as outlined below. The tubes were labelled according to the fractions of 96h LC_{50} ($^1/_{10}$, $^1/_8$ and ¼), control and standard. Then, 3.0 ml

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working reagent solution was dispensed to all test tubes in addition, 0.3 ml serum sample were added to all test tubes labelled with fractions of 96h LC_{50} and control. Next, 0.3 ml of the standard solution was added to the tubes labelled standard. These were mixed and the absorbance A1 of the samples read 30 seconds later. Within 2 mins later, absorbance A2 of standard and sample were read. The concentration of creatinine in serum was calculated using the formula below;

$$A_2\text{-}A_1 = \Delta A \text{ sample}$$

$$\text{Concentration (mg/dl)} = \left|\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}\right| \times \text{Standard conc. (mg/dl)}$$

Determination of Blood Urea Nitrogen Concentration: The blood urea nitrogen (BUN) enzyme reagent was reconstituted according to the manual instructions. 1.5 ml of BUN enzyme reagent was dispensed into labelled test tubes and allowed to equilibrate to room temperature followed by addition of 0.010 ml (10 µl) serum sample to its respective tubes and mixed gently while deionized water was used as blank. All the tubes were incubated for five mins at 37 °C. Next, 2 mls of BUN colour developer were put to the tubes except the reagent blank and mixed gently. The tubes were incubated again for five (5) minutes at 37 °C. The spectrophotometer was turned on to allow the light source to warm it up and zero it with the reagent blank at 630 nm. Thereafter, the absorbance of sample tubes were read and recorded while the concentration of Blood Urea Nitrogen was calculated using the formula below:

$$\text{Urea Nitrogen (mg/dl)} = \left|\frac{\text{Abs. of unknown}}{\text{Abs. of Standard}}\right| \times \text{Concentration of Standard}$$

Determination of Sodium Concentration: This was determined spectrophotometrically. The containers were labelled as blank, standard and sample. 1.0ml filtrate reagents were dispensed into all tubes and 50 µl of sample was added to all tubes and distilled water to the blank. The tubes were shook vigorously and mixed continuously for three minutes. Later, the tubes were centrifuged at high speed (1,500 G) for 10 minutes. The test tubes were labelled again for the filtrate tubes. Next, 1.0 ml Acids Reagents was dispensed to all tubes and 50 µl of the supernatant to the respective tubes and mixed gently. 50 µl of colour reagent was also added to all tubes and mixed. Thereafter, spectrophotometer was turned on in order to allow light source to warm it up and zeroed with distilled water at 550 nm. The absorbance of all tubes were read and recorded.

$$\text{Sodium calculation} = \left(\frac{\text{Abs. of blank - Abs. of S}}{\text{Abs. of blank - Abs. of Std.}}\right) \times \text{Conc. of Std (mEq/L)}$$

Determination of Potassium Concentration: The potassium concentration was determined by spectrophotometric method. The test tubes were labelled: standard, control and samples and 1.0 ml potassium solution dispensed to the test tubes. Then, 0.01 ml (10 µl) of samples was also added to the respective tubes, shook and placed at 25°C for three (3) minutes. After three (3) minutes, the wavelength of spectrophotometer was set to 500 nm and zero with reagent blank. Thereafter, the absorbance of all tubes was read and recorded [5].

$$\text{Potassium conc. (mEq/l)} = \frac{\text{Absorbance of Unknown}}{\text{Abs. of Standard}} \text{ X Conc. of STD. (mEq/l)}$$

RESULTS

The following parameters: creatinine, urea, sodium and potassium were tested for kidney functions. There was a significant ($p<0.05$) increase in serum concentrations of creatinine, urea blood nitrogen, sodium and potassium in the catfish exposed to both test toxicants. The effect was more pronounced in butachlor-exposed fish than the plant extract. The maximum threshold effect noted on the 16th day of sample collection while the least effect was observed on 4th day of sample collection at different concentration of $^1/_{10}$, $^1/_8$ and ¼ LC_{50} in both extract of the stem bark of *Bridelia ferruginea* and butachlor relative to control. Linear increase in the parameters tested was noted in a concentration and time dependent pattern.

DISCUSSION

The kidney performs a very vital role in the control of electrolytes and intracellular fluid volume and the excretion of metabolic waste from the body. The total homeostasis in the body depends on the function of the kidneys integrity [6, 7]. Substance, which is lethal to the kidney, will badly influence the total body metabolism. In the present study, the effect of the extract of stem bark of *Bridelia ferruginea* and butachlor on the kidney biomarkers of *C. gariepinus* was investigated.

The result showed that creatinine, blood urea nitrogen, sodium and potassium significantly increased in concentration ($p<0.05$) when compared to the control.

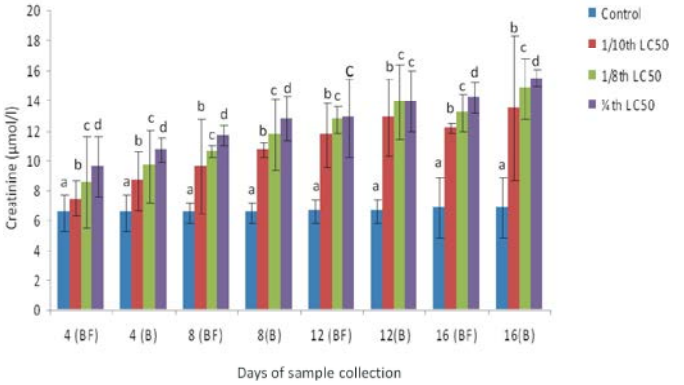

Fig. 1: Creatinine concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days.
All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are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

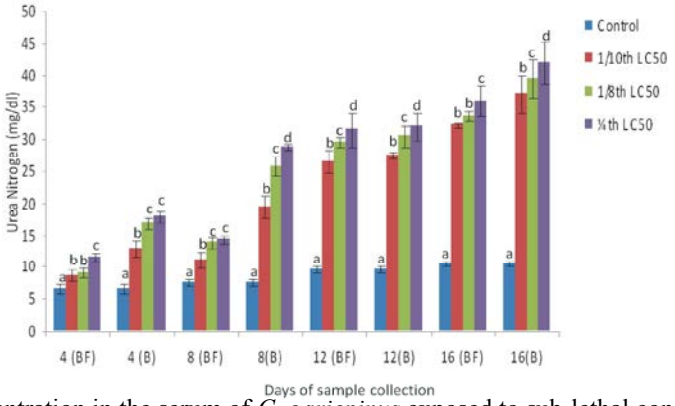

Fig. 2: Urea Nitrogen concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days.
All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters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

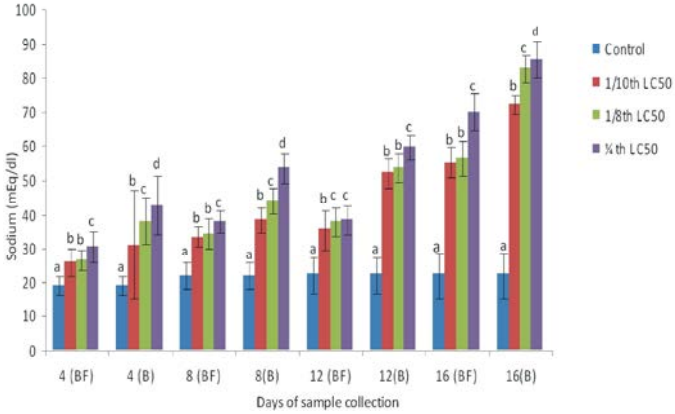

Fig. 3: Sodium concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days.
Bars in the same groups, control or test that have different letters are significantly different ($p<0.05$). BF = *Bridelia ferruginea,* B = Butachlor. The numbers 4,8,12 and 16 represents days of sample collection.

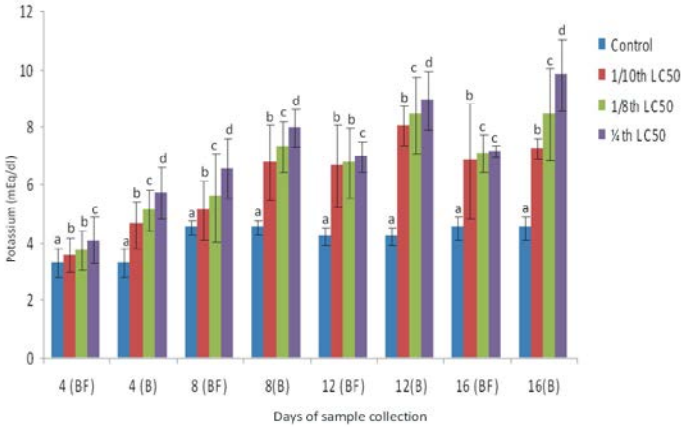

Fig. 4: Potassium concentration in the serum of *C. gariepinus* exposed to sub-lethal concentration of *B. ferruginea* and butachlor for 4 to 16 days.
All bars are mean + SD (standard deviation) of three (3) fishes in each group. Bars in the same groups, control or test that have different letters are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

Creatinine is a protein synthesized by muscle and moved into the blood stream. Creatinine concentration in the serum is proportional to the rate at which it is excreted and consequently a biomarker of kidney function [8].

The significant increase in the creatinine content of the serum following the exposure of the test toxicants may be attributed to an impairment of renal functional capacity. The toxicant could have been affected by creatinine metabolism causing increased production of tubular excretion [9, 10].

Blood urea nitrogen (BUN) is one of the usual blood indices of glomerula filtration rate. Most of the urea produced in the body is excreted in the urine by filtration across the glomerulus [9, 10].

Therefore, a decline in the glomerular filtration rate (GFR) results in the increase in the blood urea nitrogen concentration. Creatinine is excreted by renal excretion through glomerular filtration [9]. Furthermore, a pathologic process typically increases creatinine that cause decreased GFR, so raising BUN and creatinine activities reflect adverse effects of excess concentration of test toxicants on kidney function. In this research, the serum level of sodium and potassium in fish exposed to the toxicants increased significantly ($p<0.05$) relative to control with concentration and time dependent trend throughout the exposure period. This could also account to the kidney impairment. Thus, an impairment of the kidney interferes with excretion of Na^+ and K^+ leading to its accumulation in the serum [10].

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