IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 4 Ver. V (Jul - Aug. 2015), PP 60-68
www.iosrjournals.org

Hepatotoxic effects of Aqueous Extract of Psychotria Microphylla leaves on Clarias giriepinus Juveniles

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Abstract: *Psychotria microphylla (Akwukwo iyi, Igbo) is one of the common herbs used to harvest fish from rivers and streams by many villages in South-East Nigeria and not much, if any, has been reported on the toxicity of this plant. The results showed that the mean values of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and total bilirubin increased significantly ($p< 0.05$), while total proteins and albumin siginificantly reduced ($P<0.05$).The histological analysis of the hepatic tissues of exposed fish showed congestion of central vein, vacoulation of hepatocyte, cellular infiltration and cellular necrosis. Thus aqueous extract of psychotra microphylla leaves is hepatotoxic to C. garienipinus and caused hepatic lesion after 15 days of exposure*

Key words: *Psychotria microphylla, Clarias gariepinus, toxicity, juveniles, lethal concentration*.

I. Introduction

Psychotria microphylla Elmer is one of the Psychotria species found in the Eastern part of Nigeria. It is a small evergreen shrub with a slender stem. Infusion of the whole plant is used in Afikpo south Area of Ebonyi State, Nigeria for fishing and prevention of insects from destroying crop vegetables (personal account). Its leaves are shiny, light to dark green, opposite, elliptic to ovate, often acuminate (taper to a long narrow point) with more lateral veins, the flowers are white and the fruit is ribbed. Its natural habitat ranges from sea level to 1 500 m in evergreen forests, forest margins, shrub and dune bush, edges of rivers and rocky outcrops in high rainfall grassland.

Stress response is characterized by biochemical and physiological changes which may be manifest in both acute and chronic toxicity tests (Singh and Singh, 2002; Tiwari and Singh, 2004). The metabolic pathways of fish can be severely altered by a variety of biological, chemical and physiological factors, which could be accessed through several biochemical procedures. Biochemicals are the assessable body fluid contents for checking the toxicity of any chemicals (Singh et al, 2010); they are of fundamental importance in the evaluation of physiological state of organisms and are often used when clinical diagnosis of fish physiology is needed to determine the effects of external stressors and toxic substances. Most often, the disruption of biochemical and physiological integrity is assessed by the changes in the enzyme activities in functional organs or body fluids (de la Torre et al., 2000, van der Oost et al., 2000). Maintenance of internal homeostasis through biochemical processes may be reflected by variation in the levels of AST, ALT, or ALP in the serum (or plasma) occasioned by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Heath, 1991). Both serum AST and ALT are raised when disease process affects cell integrity and ALT is a more liver specific enzyme (Gabriel and George, et al., 2005).

Although psychotra microphylla has been used over the years for harvesting fish from streams in many parts of Ebonyi State, as in other parts of Nigeria, there is yet no scientifically based study on its toxicity to fish or other aquatic organisms. This work was therefore designed to study the toxicity of psychotra microphylla to the Afican catfish, Clarias gariepinus using biochemical and histopathological indices.

The African catfish, Clarias gariepinus (Burchell, 1822), was selected as the test organism in this study for its great aquaculture and commercial value in Nigeria and elsewhere in the developing world. C. gariepinus is a benthopelagic (bottom feeder); omnivorous feeder that occasionally feeds at the surface. C. gariepinus, also referred to as mudfish, is very hardy and tasty. They are able to tolerate adverse aquatic conditions, where other cultivable fish species cannot survive. It is widely cultivated and used as experimental fish (Musa and Omoregie, 1999).

II. Materials And Methods

Materials

Equipment/instrument

Spectrophotometer (spectro 2ID PEC MEDICALS USA), oven (Gallenkamp), analytical balance, Gas chromatography-mass spectrophotometer, incubator, centrifuge, refrigerator, blender, plastic aquaria, micropipettes and general laboratory glassware

Biological material and chemical reagents

Psychotria microphylla leaves and Clarias gariepinus (African catfish) were used for the study. All reagents used were of analytical grades. Reagent kits were supplied by Randox Laboratories Limited, BT29 4QY, United Kingdom.

Collection and authentication of the plant sample

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.

Procurement of the fish

Fresh water fish, C. gariepinus juveniles weighing 180±15 g and body length of 30±3 cm were procured from Chiby's Farm, Abakaliki, Ebonyi State. They were safely brought to laboratory and stocked in 200 litre 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.

Methods

Preparation of Psychotria microphylla Leaves Sample

Samples of P. microphylla Elmer leaves were washed and shade-dried. It was then pulverised 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) litre 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 55°C and the powder then used for evaluating piscicidal activity of Psychotria microphylla.

Sub-Acute Toxicity test for biochemical assays and histopathological analysis

The fish were exposed for 15 days to three sub-lethal concentrations of 0.016, 0.03, and 0.065 mg/l of the aqueous extract of Psychotria microphylla. Three 60 litres capacity plastic containers were used for each concentration and in each of the containers ten 10 fish were placed and forty (40) litres of borehole water put into it. A set of 6 fish were also simultaneously maintained in borehole water (0.00 mgl^{-l}) as the control each time the test was repeated.

At the end of every 1, 3, 7, 10 and 15 days, six fish were sampled from each group. Blood samples were quickly collected from the caudal vein with heparinized syringes. Plasma was obtained by centrifugation at 5400g for 10 minutes. Activity of transaminases (AST and ALT) was determined by method of Reitman and Frankel (1957), alkaline phosphatase (ALP) activity was based on Tietz, (1990). Total bilirubin was assayed using the procedure detailed by Jendrassik and Grof (1993).

Estimation of albumin levels

The procedure of Grant et al. (1987) was used to measure plasma levels of albumin

Three (3) test tubes were set in a rack and were identified as reagent, standard and sample test. Each of reagent, standard and sample test tubes, 10 µl of distilled water, standard and sample respectively was pipetted into each of them. In all the tubes, 3000 µl of the albumin reagent was added, mixed and incubated for 5 minutes at 25°C. The absorbance of sample (Asample) and standard (Astandard) was measured against the reagent blank at 578 nm using spectrophotometer.

$$\text{Albumin concentration} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{Conc. of standard albumin (mg/dl)}$$

Total protein determination

Three test tubes were arranged in a rack and labelled as blank, standard and sample. To each of the test tube, 1000 µ of biuret reagent was pipetted into it. To the the blank, standard and sample tube was added 20 µ of distlled water, standard and sample respectively. Each of them was mixed and placed in an incubator set at 25°C for 30 minutes. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) was measured against the reagent blank at 546 nm with spectrophotometer (Tiez, 1995).

Total protein conc = A_{sample} x standard conc

After blood collection, fish were sacrificed by sectioning the spinal cord, and the liver was carefully dissected out prepared for histopathological observation. They will be fixed in bouin's fluid for 24 h, washed with 70% ethanol and dehydrated through a graded series of ethanol (Kelly, 1979; Schalm et al., 1995). They will be embedded in paraffin, sectioned at 4 – 5 µm thickness, stained with hematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989).

Statistical analysis

The probit method of Finney (1971) was applied to estimate the 96 hour $LC_{50.}$ Results were reported as mean ± standard deviation (SD) where appropriate. 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 $P<0.05$.

III. Results

The results of the influence of the aqueous extract on plasma total protein (TP) and albumin (ALB) are presented in Figures 1 and 2 respectively. The exposure of C. gariepinus to aqueous extract of Psychotria microphylla leaves resulted in significant decrease ($P<0.05$) in the plasma total protein and albumin levels. The effects of were both duration and dose dependent. The result of the impact of aqueous extract of P. microphylla leaves on plasma ALP, ALT and AST are presented in Figures 3-5. Exposure to the aqueous extract of Psychotria microphylla leaves caused marked rise ($P<0.05$) in the activities of plasma alkaline phosphatase and aspartate aminotransaminase (AST). The exposure of 0.03 and 0.065 mg/l of the aqueous extract of P. micrphylla leaves to C gariepinus caused a significant ($P<0.05$) non linear rise in the activities of alanine aminotransaminase (ALT) as shown on Figure 4. The increase was time dependent as the activities of enzyme rose with the days of exposure. The groups exposed to 0.016 mg/l revealed insignificant change ($P>0.05$) after 72 h of exposure, the activites of these enzymes however increased significantly ($P<0.05$) from 7th day of exposure. The exposure of 0.03 and 0.065 mg/l of the aqueous extract of P. microphylla leaves to C. gariepinus caused a marked elevation ($P<0.05$) of bilirubin quantity. The highest increase was recorded in the group exposed to 0.03 mg/l on day 15 of exposure. There was insignificant difference ($P>0.05$) in groups exposed to 0.016 mg/l after 24 h of exposure; bilirubin levels however increased significantly ($P<0.05$) from the 3rd day of exposure as displayed in Figure 6.

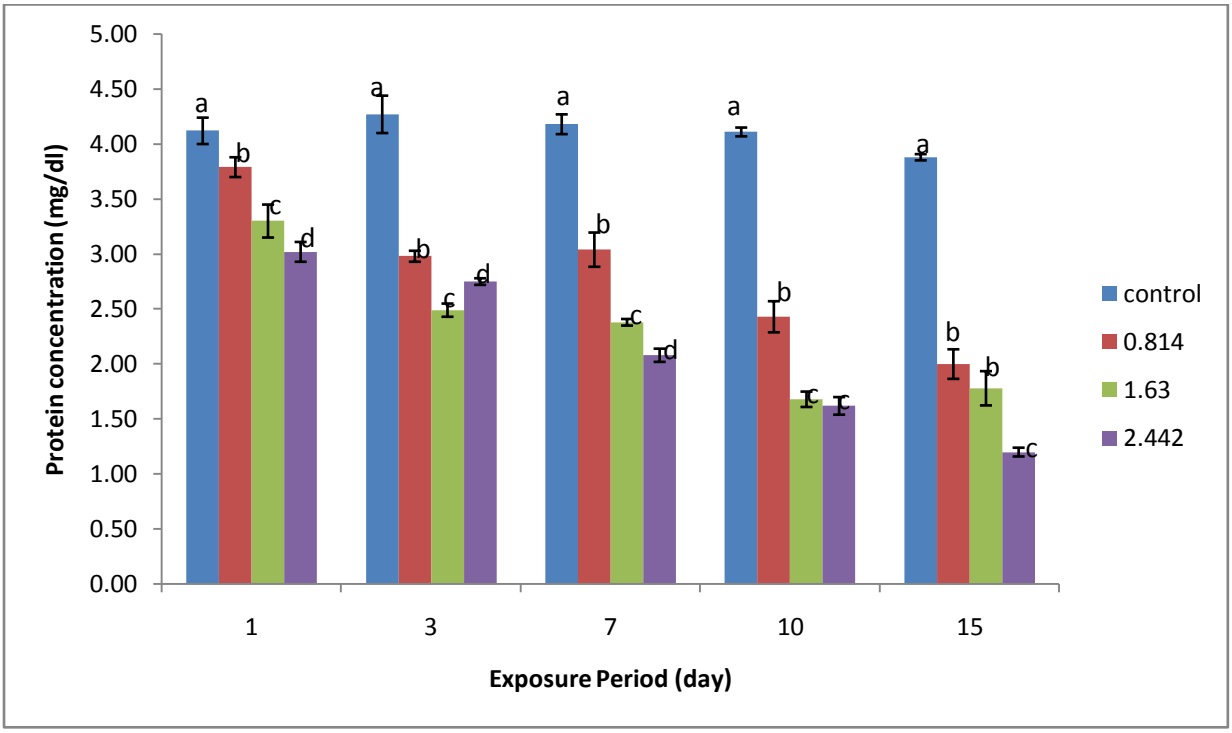


Figure 1: Protein levels in C. gariepinus exposed to sub-lethal doses (mg) of P. microphylla leaf powder. The result indicates a significant decrease ($P<0.05$) in protein concentration. The results are mean ±SD of 6 fish in each group

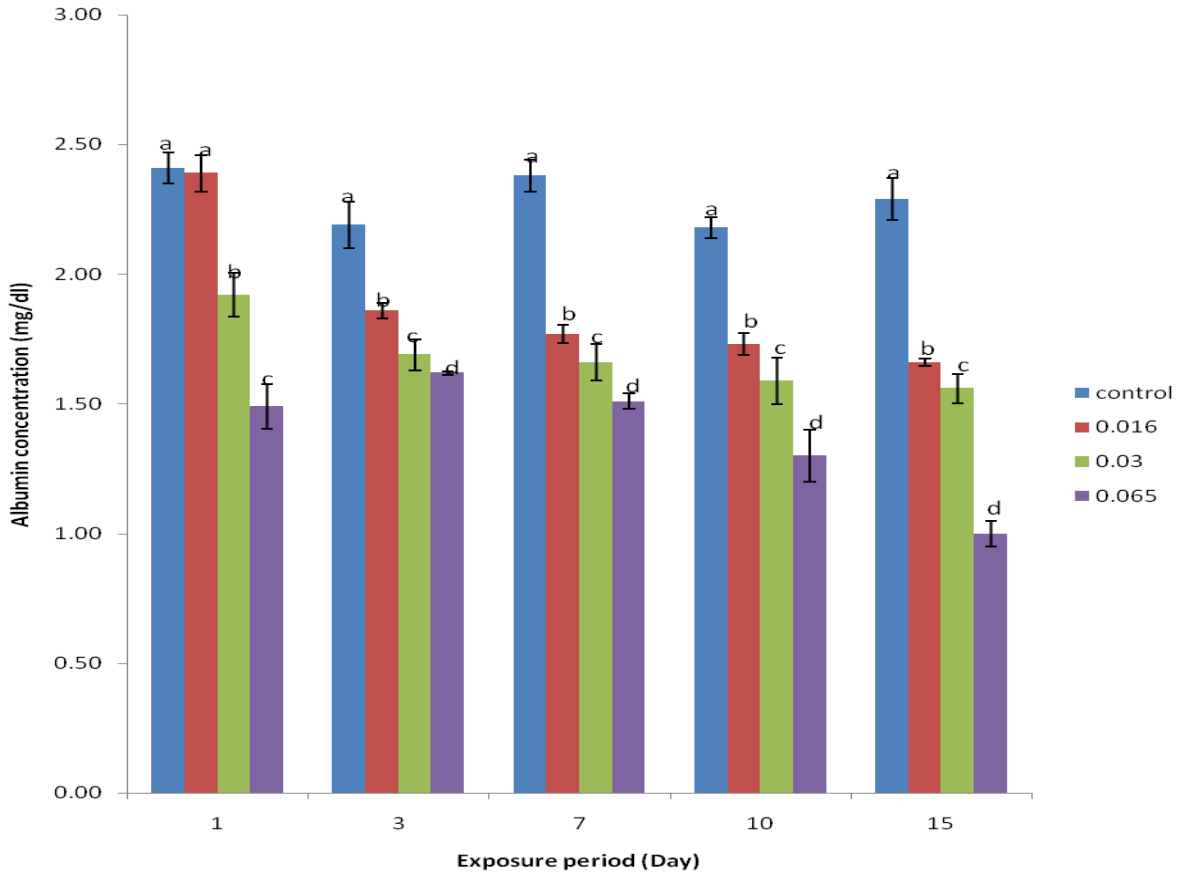


Figure 2: Plasma Albumin level in C. gariepinus exposed to leaves powder of P. microphylla. The results are mean ± SD of 5 fish. Ingots with distinguishing letters differed significantly (P< 0.05).

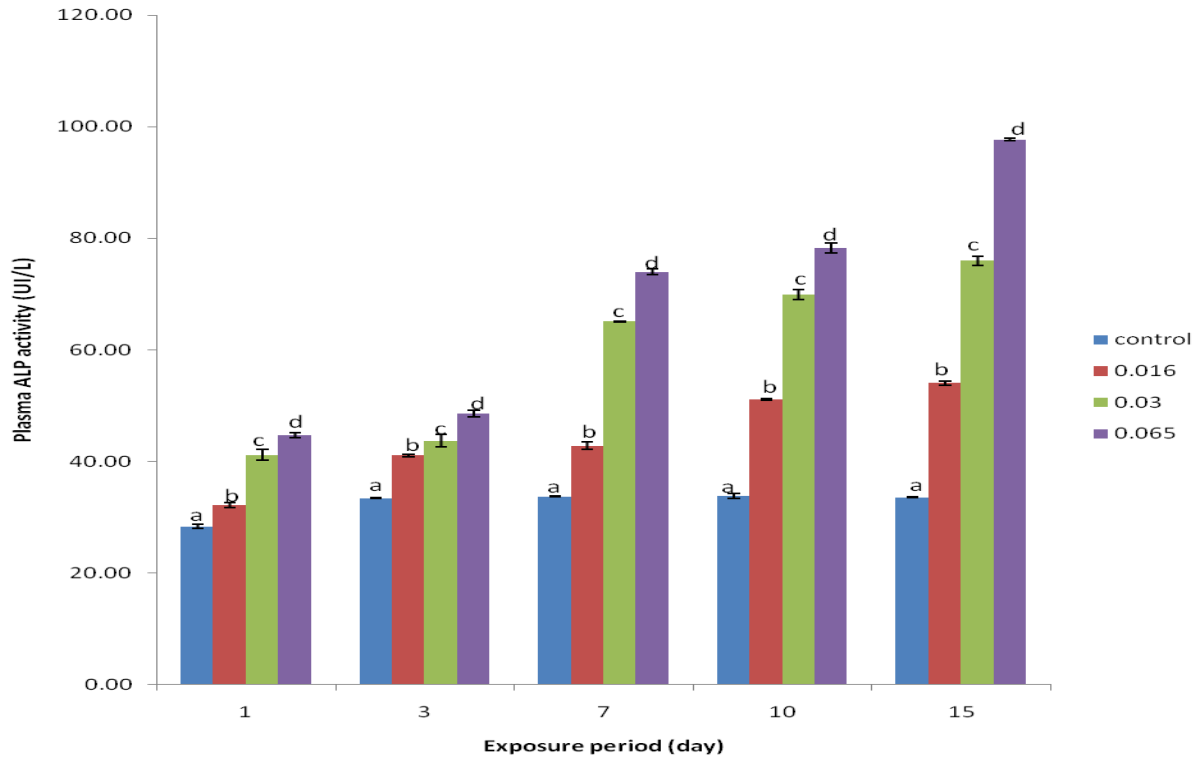


Figure 3: Plasma ALP activity in C. gariepinus exposed with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

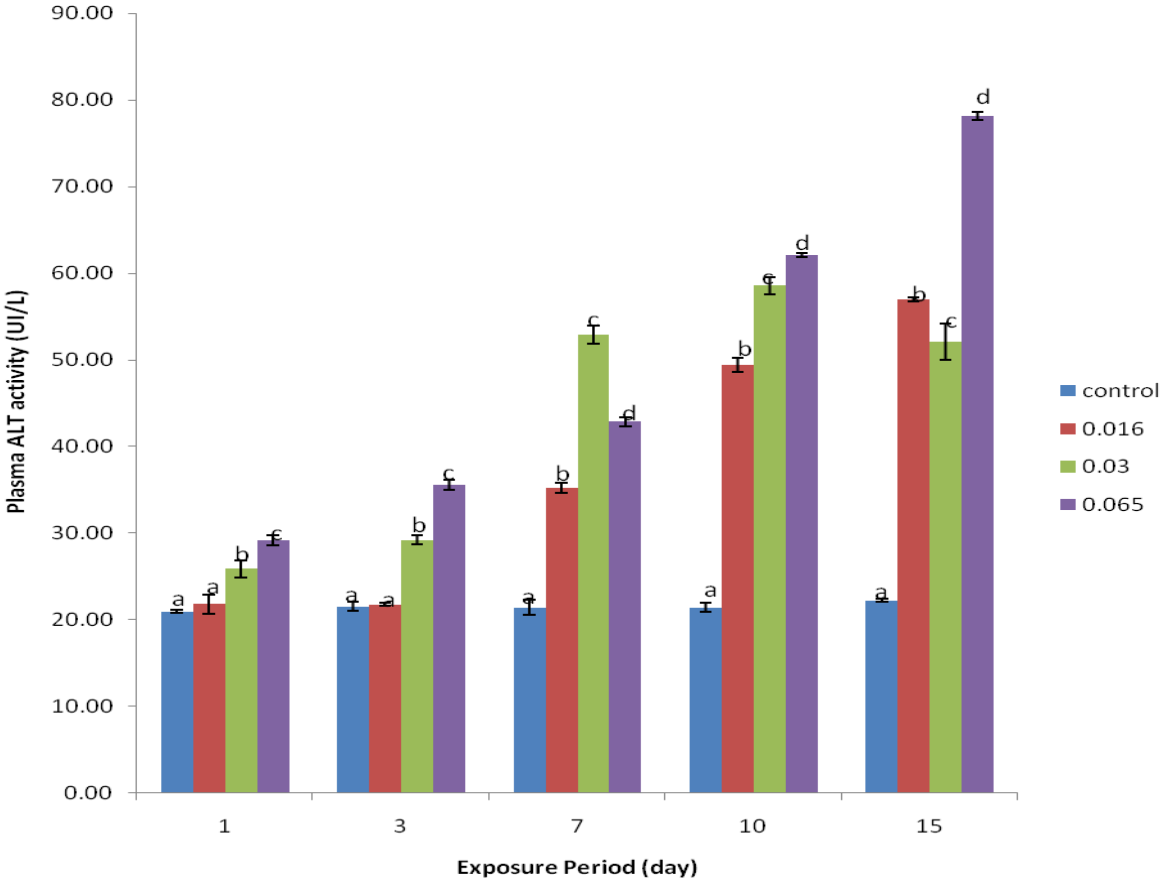


Figure 4: Plasma ALT activities in C. gariepinus exposed with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

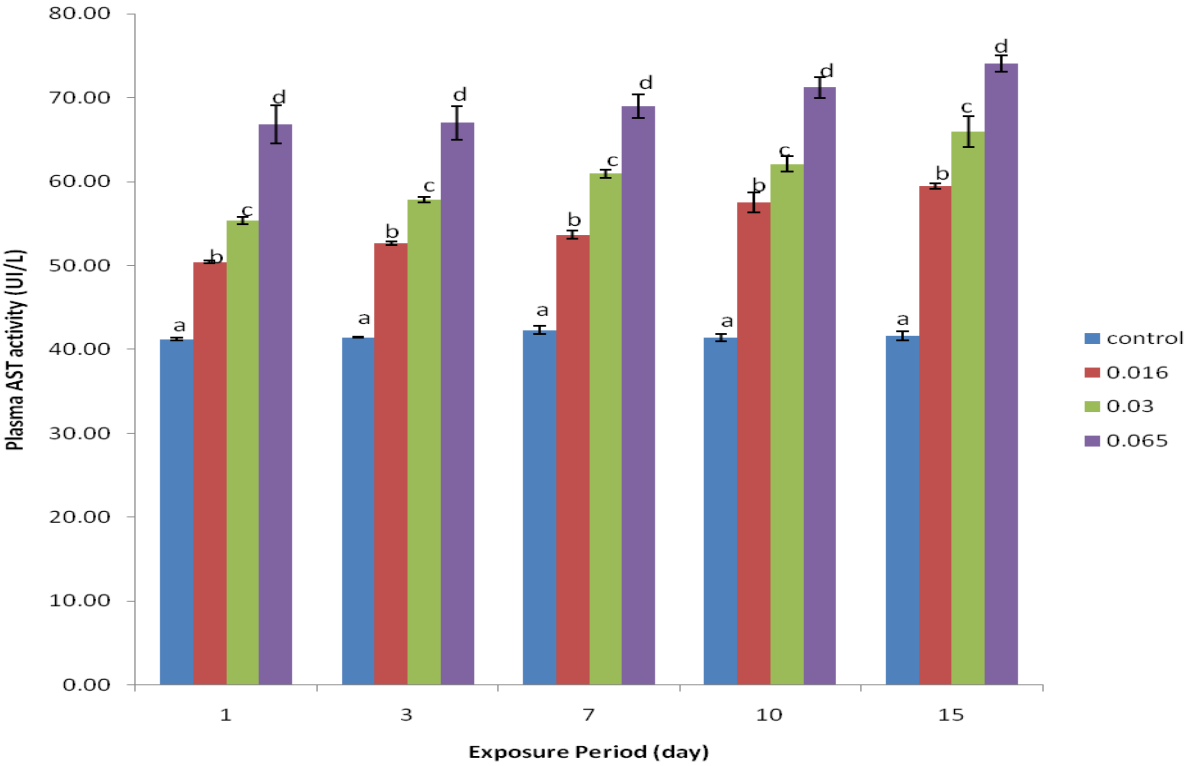


Figure 5: Plasma AST activities in C. gariepinus exposed with the water extract of P. microphylla leaves. The results are mean ± SD of 5 fish. Ingots with distinguishing letters varied significantly (P< 0.05).

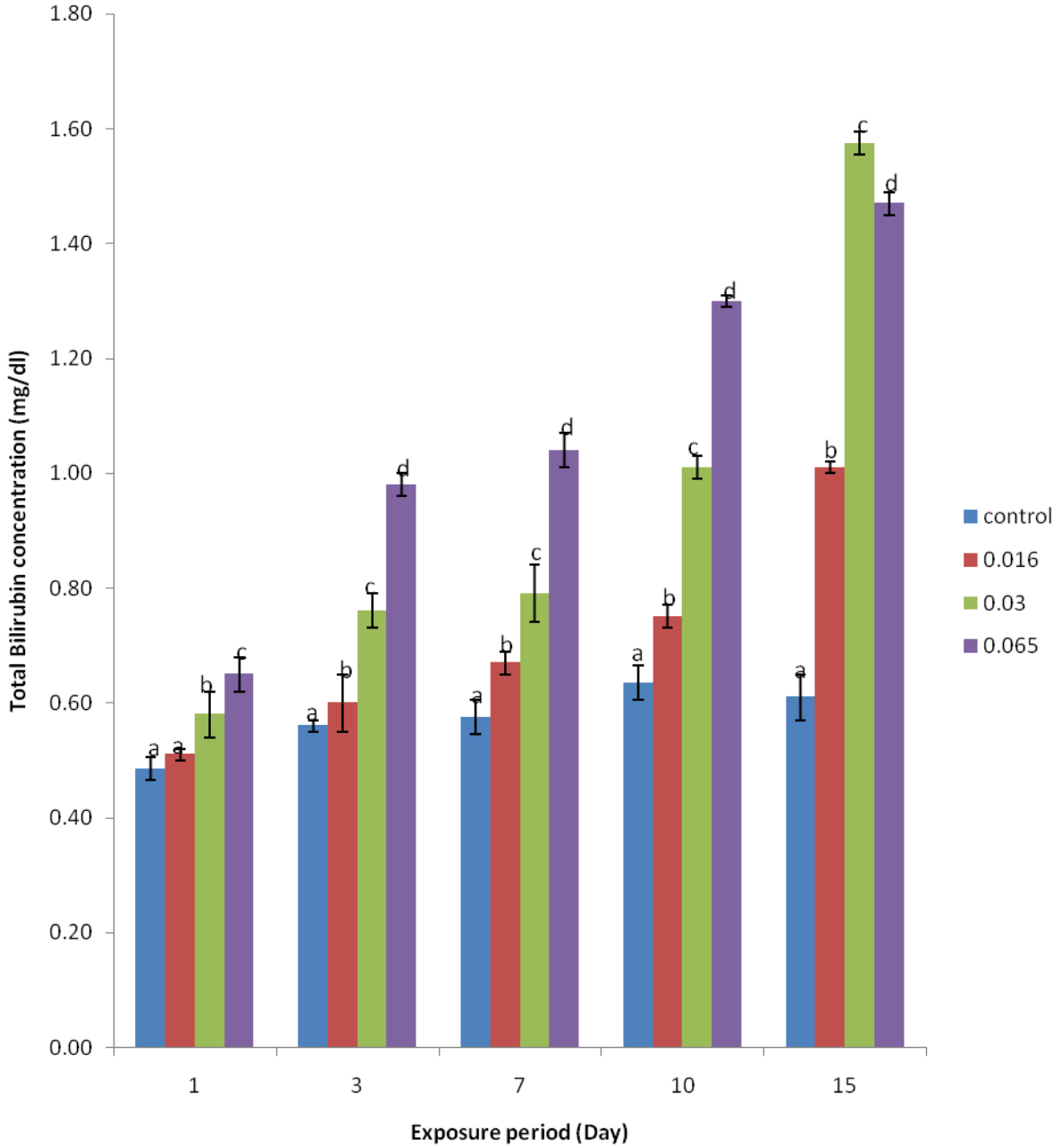


Figure 6: Plasma Total bilirubin level in C. gariepinus exposed to the aqueous extract of P. microphylla leaves. Ingots with distinguishing letters varied significantly (P< 0.05).

Photomicrograph of Hepatic tissues of C. gariepinus exposed to sub-lethal doses (mg) of P. microphylla leaf powder

The photomicrographs of the excised liver are presented in Figures 7-8. In the control experiment, the photomicrograph of the liver showed the typical network of parenchymatous appearance forming an irregular clump made up of hepatocytes (H) and the sinusoids were properly arranged (SD). The interspaces are the sinusoids (S) which are characteristics of a thin sparse connective tissue and are regularly converge into the large central vein (V). The sinusoids make continuous communication as they are seen converging into the central vein (7) x 40. The hepatic tissue of fish exposed to higher doses of the leaf powder revealed varied degree vacoulation, lymphocytic infiltration, congestion of the central vein, loss of the normal lattice of the hepatocytes.

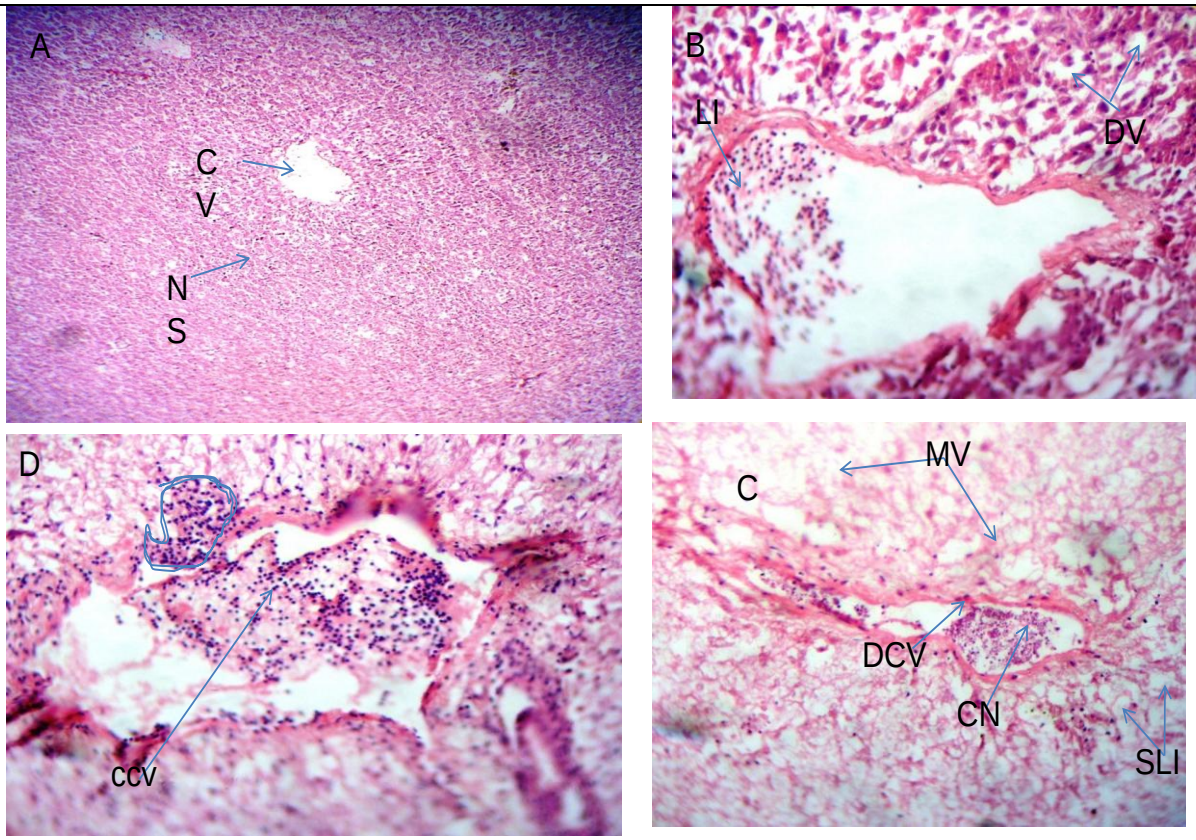
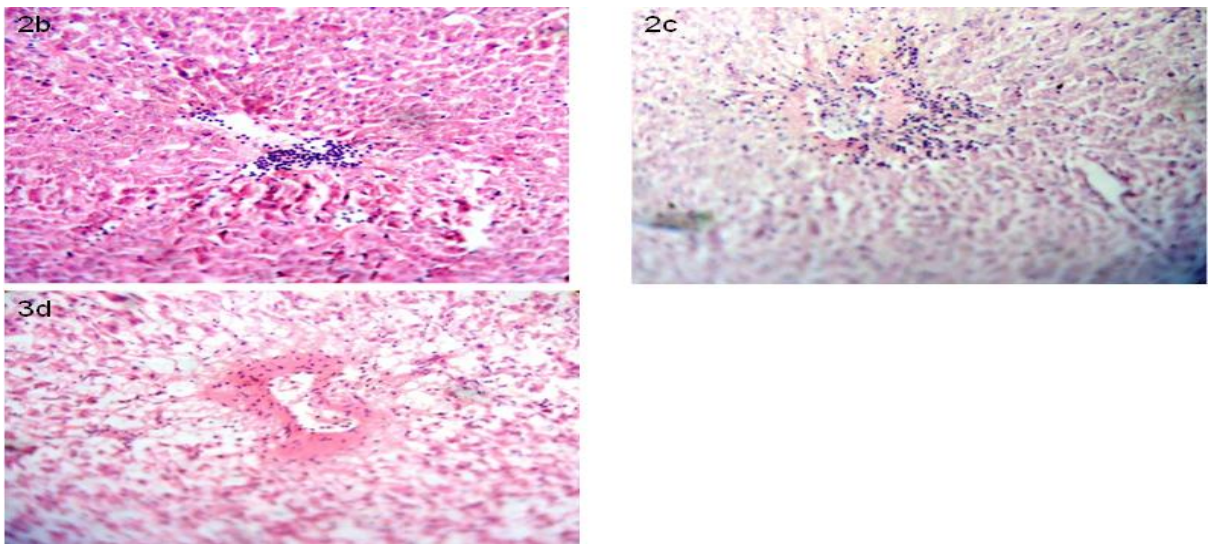


Figure 6 A is a photomicrograph of control experiment (0.000mgl-1).hepatic tissue showed normal lattice network of parenchymatous cells. Central vein (CV), prominently shown without a central nucleus, with normal sinusoid (NS). Showed unremarkable changes around a central vein, B is a photomicrograph fish liver exposed to 0.814 mg/l. showed diffused vacuolation of hepatocytes,distorted central vein and mild lymphotic infiltration. **C** is photomicrograph of fish liver treated with 1.63 mg/l showed scanty lymphocytic infiltration (SLI) of the portal area, marked vacuolation of hepatocytes (MV) and slight degeneration of central vein (DCV) became evident with mild congestion of nuclei (CN) and **D** is the photomicrograph fish liver exposed with 2.442 mg/l showing marked



2c is a photomicrograph of fish liver treated with 0.814 mg/l after 15 day showing moderate lymphocytic infiltration (LI) of the portal area, slight degeneration of central vein (DCV), and distortion of the sinusoids. **3c** is a photomicrograph of fish liver treated with 1.63 mg/l for 15 days showing marked lymphocytic infiltration of the portal area (H &E stain x 100). **3d** is a photomicrograph of fish liver treated with 2.442 mg/l showed loss of normal lattice network of parenchymatous cells, marked vacuolation hepatocytes around an arteriole, slight degeneration of central vein (DCV) (H &E stain x 100).

IV. Discussion

Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Yeragi et al., 2003; Remia et al., 2008; Pang-Hung et al., 2008). The result of the present study showed significant ($P<0.05$) decrease in plasma protein content (Figure 3). The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia et al., 2008).; Adedeji, (2010) reported that after 96 hr diazinon produced a significant decrease ($P < 0.05$) in protein concentration in the blood plasma of the test catfish, as compared with the control. A number of other workers have reported decline in protein level of various organs and tissues under toxic stress of various chemicals. For instance, Begum and Vijayaraghavan (1996) have reported that the protein content of muscle tissues of fish Clarius botrachus was significantly depleted during 192 h of dimethoate exposure; Kumar and Saradhamani (2004) observed significant decrease in protein content in all the tissue of the fish Cirrihinus mrigala upon exposure to the pesticide avaunt. Dalela et al., (1981) observed a decrease in protein content in Mystus vittatus under pesticide exposure and reported that the depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disorders. A similar mechanism may also be operative in the present study.

Our results also showed significant ($P<0.05$) increases in ALT, AST and ALP enzymes activities in C. gariepinus exposed to sub-lethal concentrations of psychothra microphylla leaf powder for fifteen days. The response was both time and concentration-dependent. The levels of these enzymes in serum or plasma are reliable indicators of liver metabolism and wellness of organisms under chemical or physical challenge (Sreekala and Zutshi, 2010; Iweala and Okeke, 2005; Uboh et al., 2011). ALT, AST and ALP are metabolic enzymes involved in amino acid metabolism (Bhattacharya et al., 2008). The observed general increase in the plasma enzymes in this study is an indication of underlying liver injury in the fish. The increase in levels of ALT and AST may indicate liver damage, while the increase in the ALP level may be indicator for renal and liver damage (Bhattacharya et al., 2008; Gill and Bruland, (1990) or indicates active transamination, so as to maintain energy cycle (Adams et al., 1996). Also, it has been reported that alterations in enzymes activities in the plasma directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture (Benjamin, 1978), a signal of underlying pathological process (Hayes et al., 2002).

ALP in the cellular external membrane plays the major role in phosphate metabolism and it prevents the external membrane from being damaged (Hayes et al., 2002). Increase in plasma level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. Significant elevation of serum ALP is an indication of cholestasis. It has also been reported that increase in the serum levels of ALP indicate the extent of cellular damage on the liver (Wannang, 2007); its increase in activity is associated to necrosis of the liver and kidney (Ochmanski and Barabasz, 2000)

The results of histopathological analysis of liver of exposed and none exposed rats are presented in Figures 6 and 7. For the control liver, the photomicrograph of the liver showed the typical network of parenchymatous appearance forming an irregular clump made up of hepatocytes (H) and the sinusoids were properly arranged (SD). The interspaces are the sinusoids (S) which are characteristics of a thin sparse connective tissue and are regularly converge into the large central vein (V). The sinusoids make continuous communication as they are seen converging into the central vein. But exposure to increasing concentrations of P. microphylla caused a distortion of the liver architecture. For example, the photomicrograph of hepatic tissue of fish exposed to high concentrations of the leaf powder, the result revealed varied degree vacoulation, lymphocytic infiltration, congestion of the central vein, loss of the normal lattice of the hepatocytes.

In conclusion, it is evident from the results presented here that aqueous extract of P. microphylla leaves is hepatotoxic to the freshwater fish, C. gariepinus. Exposure to sub-lethal concentrations of the extract resulted in significant biochemical and histological alterations in the fish.

Acknowledgement

We acknowledge Tertiary Education Trust Fund Nigeria (TETFUND), and the DERIC, Ebonyi State University, Abakaliki for providing the fund and the conducive environment, respectively, to carry out the research

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