Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 6(3): 920-930, June 2012

International Journal of Biological and Chemical Sciences

ISSN 1991-8631

Original Paper

http://indexmedicus.afro.who.int

Evaluation of efficacious activities of aqueous extract of *Phyllanthus niruri* against acetaminophen-induced hepatitis in rats

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ABSTRACT

The efficacy of aqueous extract of *Phyllanthus niruri* against acetaminophen-induced hepatitis in rats was evaluated. The hepatic injury was induced with 200 mg/kg, p.o. of acetaminophen, which led to rise in serum levels of the biochemical parameters observed. These are the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which were elevated by 22% compared to respective negative control. Treatment with the plant extract (500, 1000 and 2000 mg/kg, p.o.) lowered the biochemical parameters of the respective serum AST 31 to 38%, ALT 20 to 31%, Bilirubin -2 to 4%, protein 5 to 15%, cholesterol 0.2 to 0.5%, and albumin 5 to 12%. The histopathological results indicated that, the effect of the extracts on the condition of the liver as compared to the normal control ranges from mild to moderate. The biochemical variations were as a result of the different treatment involved in the study. The result therefore, shows lowering of the elevated parameters in the serum and possible reversal of hepatic cell damage with aqueous extract of *Phyllanthus niruri*. The trend of the study shows that, the longer the period of treatment at lower doses, the better the efficacy of the plant extract.

Keywords: *Phyllanthus niruri*, hepatitis, acetaminophen, biochemical, histopathology, rats.

INTRODUCTION

Hepatitis disease is a major global public health problem with large number of people being infected worldwide; among which millions are chronic carriers of Hepatitis B Virus (HBV) resulting in high incidence of death per year (WHO, 2000). Many of the infected patients tend to develop cirrhosis, liver failure, or hepatocellular carcinoma (Lee, 1997; Mahoney, 1999), and liver cancer (hepatoma); a very fatal disease (Kathryn et al., 2010). The disease can be induced experimentally with carbon tetrachloride and acetaminophen (Moresco et al., 2007; Wang, 1985).

Plants have been used as a source of medicines from ancient times (Lev, 2003; Yesilada,, 2005; Alves and Rosa, 2006) and a significant portion of the currently available non-synthetic and/or semi-synthetic

DOI : http://dx.doi.org/10.4314/ijbcs.v6i3.1

pharmaceuticals in clinical use is comprised of drugs derived from plants. A great number of these natural products came from the scientific study of remedies traditionally employed by various cultures, most of them being plant-derived (Farnsworth and Morris, 1976; Farnsworth, 1988; 1990). Studies have revealed the hepato-protective activities of different plants such as *Silybum marianum*, *Cichorium intybus* and *Ficus carica* Linn. (Moraceae) (Madani et al., 2008).

The plant *Phyllanthus niruri* (Euphorbiacea) L.—Sp. Pl. 2: 1753 [1 May 1753] (IK) (*The International Plant Names Index www.ipni.org*) is a small, erect, annual herb which grows 30–40 cm in height. It is indigenous to the rainforests of the Amazon and other tropical areas including Nigeria and is a popular herbal remedy for different ailments (Naik and Juvekar, 2003) including liver disease (Sabir and Rocha, 2008). The plant is employed for numerous conditions by the indigenous peoples as natural remedy, usually by a standard infusion or weak decoction of the whole plant or its aerial parts in water against hepatitis, and other disorders including anemia, jaundice and liver cancer.

The present study was to ascertain the anti-hepatoxic efficacy of aqueous extract of *Phyllanthus niruri* and also its comparison with standard amino acids, by using acetaminophen for the induction of hepatic injury in rats. The extract's phytochemical constituents, physicochemical parameters as well as safety profile (LD_{50}) were also evaluated.

MATERIALS AND METHODS

Sample collection and preparation

The plant sample was collected fresh from Idu, Abuja Municipal Council, FCT-Abuja, Nigeria from the months of July-August, 2009. It was identified by plant taxonomist at the Department of Medicinal Plant Research and Traditional Medicine, NIPRD and assigned a voucher number NIPRD No. 3649. The plant material (whole plant) was air dried and powdered; from which about 400 g of the powder was macerated in cold water, then filtered and evaporated over a water bath in order to obtain the extract for subsequent use.

Phytochemical screening

Prior to testing, two grams (2.0 g) of the plant material were rapidly extracted with 20 ml of the solvent by shaking for 3-30 minutes or heating on water bath depending on the test in question. The solution was filtered through a whatman filter paper No.125 mm using funnel and the filtrate was used for the phytochemical test using Evans (2002) methods.

Physicochemical properties

Moisture: One grams (1.0 g) of the sample powdered were weighed on aluminium foil on the automated moisture analyser pan (Model MB 200, OHAUS Florham PK.USA) and set at 105 °C for 3 hours where % moisture content of the sample was obtained (WHO, 1998).

Total ash: Two grams (2.0 g) of the powdered sample were ignited in a previously ignited and tarred crucible at 500 °C for about 3 hours until the sample was white, indicating the absence of carbon. It was then cooled in desiccators, weighed and the moisture content calculated as %w/w (WHO, 1998).

Determination of the extractable matter (EM)

Four grams (4.0 g) of the powdered sample were macerated with 100 ml of distilled water by frequent shaking for 6 hours, allowed to stand for 18 hours and were then filtered; followed by evaporating 25 ml of the filtrate in a flat bottom platinum dish on a water-bath. The extract was dried at 105 °C for about 6 hours, cooled in desiccators for 30 minutes, weighed and calculated as mg per gram of the powdered sample (WHO, 1998).

Determination of bitterness value

The bitterness value was determined by finding the threshold bitter concentration

through tongue-tasting the dilutions of the quinine solutions and the plants material subsequently by different individual, beginning with the lowest concentration of the dilutions. The threshold bitter concentration at which a material continues to provoke a bitter sensation after 30 seconds was the concentration at which the bitterness was determined and calculated in units per g according to WHO (1998) method using the formula: 2000 x c / a x b. Where a = the concentration of the stock solution (S_T) (mg/ml)

b =volume of the ST (in ml) in the tube with the threshold bitter concentration

c =the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

Experimental animals

Adult Wistar rats (Six weeks old) were sourced from the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD. They were housed in plastic cages with saw-dust as beddings and maintained at normal environmental condition. They were given food and water *ad libitum.* Their usage was in accordance with *NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 83-27, 1985).*

Acute toxicity test

The oral acute toxicity test was carried out in healthy adult Wistar rats (140-200 g) according to Lorke's method (1983). Briefly, the rats were divided into four groups and treated orally with the extracts of the plant at doses of 1000 – 8000 mg/kg, p.o. They were observed for sign of toxicity in the first six hours and mortality within 24 h was monitored. The LD_{50} was estimated from the square root of the lowest lethal dose and the highest non-lethal dose (Vongtau et al., 2004).

Treatment protocol

Thirty-five adult rats of both sexes of weights ranging from 140-200 g were used for the study. The animals were housed in iron cages in well ventilated standard animal house and fed with standard animal feed and water throughout the period of the study. The animals were divided equally into eight groups (n = 5) as follows:

Group 1: Served as control group

Group 2: 200 mg/kg of acetaminophen (Dennis, 2011)

Group 3: 200 mg acetaminophen /500 mg plant extract

Group 4: 200 mg acetaminophen /1000 mg plant extract

Group 5: 200 mg acetaminophen /2000 mg plant extract

Group 6: 200 mg acetaminophen /950 mg cysteine (Wang et al., 1985).

Group 7: 200 mg acetaminophen /300 mg phenyl alanine

After the seven days exposure to treatment, the rats were sacrificed by decapitation and the blood was collected using syringe, allowed do clot, centrifuged at room temperature and the serum was stored in tubes for subsequent biochemical parameter analysis (Schmdt, 1993).

Biochemical parameter assessment

The following parameters were assessed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, total protein, albumin, cholesterol, using standard kits for activity as indicators of liver function and generalized tissue damage respectively by using appropriate methods (Nagini and Selvam, 1997; Jin et al., 2007).

Histopathological assessment

After blood was collected, the liver was fixed in 10 % neutral formalin for at least 24 hrs, dehydrated in graded (50 – 100 %) alcohols, embedded in paraffin, cut into 4 – 5 µm thick sections and stained with haematoxylin-eosin. It was then examined under photomicroscope (Ezeonwumelu et al., 2011) for characterization of histopathological changes in the tissues by a histopathologist in blind fashion (Alan et al., 2002; Jin et al., 2007).

Statistical analysis

Data collected were processed by using Microsoft excel, summarized as mean± SD, and interpreted in form of histogram.

RESULTS

The phytochemical screening of the extract shows the presence of alkaloids, balsam, carbohydrate, flavonoids, saponins, resins, tannins, phlobatannins, steroids, glycosides and traces of terpenoids. The extract's physicochemical parameters are presented in Table 1. The LD_{50} of the extract was above 5000 mg/kg, p.o., indicating that the experimental doses used were within the safe margin.

Biochemical parameters, AST, ALT, total bilirubin, total protein; triglyceride, creatinine, cholesterol, and albumin are presented in Figures 1 – 6; while the histopathological conditions of the liver for the different treatment groups are presented in Table 2.

The conditions of the representative samples of the liver of the rats are presented in form of slides A, B, C and D. Slide A shows the liver condition of normal control group showing normal hepatic cells, B shows the acetaminophen-induced group which indicated extensive injury with extensive portal fibrosis, while C indicated that 500 mg of the extract administered led to portal moderate condition showing mild portal fibrosis, and D shows liver section of showing mild condition.

Table 1: Physicochemical parameters.

Parameters	Loss on drying (%w/w)	Total ash (%w/w)	Bitterness value (Units/g)	Water extractable (%w/v)
Value	12.4057±0.45	6.9950±0.46	1636.88±0.74	1.3353±0.10

Table 2: Histopathological conditions of the liver.

Treatment	Normal control	200 mg Aceta.	500 mg extract	1000 mg extract	2000 mg extract	950 mg cysteine	300 mg Phenyl-alanine
Effect	normal cells	extensive portal inflam-matory	mild cell injury	moderate inter-tubular, interphase tract inflam-mation	moderate inter-tubular, interphase tract inflam-mation	moderate inter-tubular, interphase tract inflammation	extensive portal inflammation

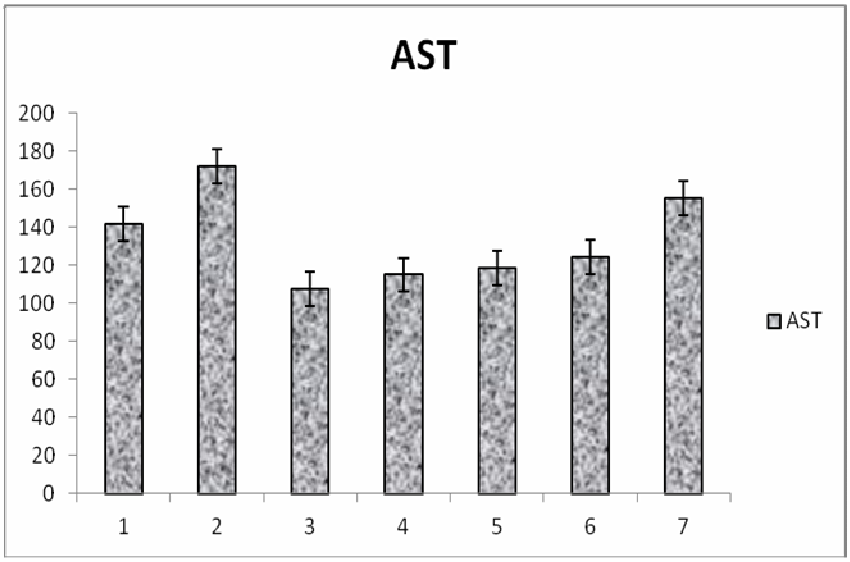

Figure 1: Average level of AST among the treatment groups.

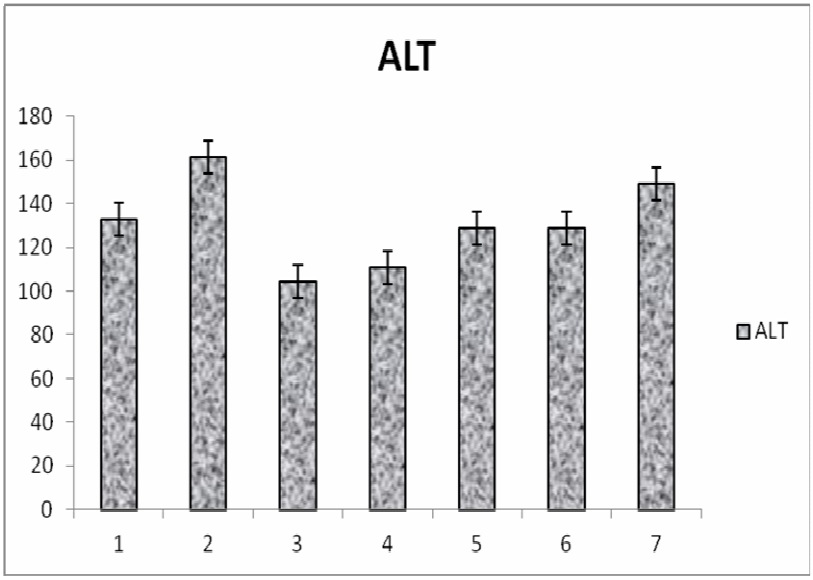

Figure 2: Average level of ALT among the groups.

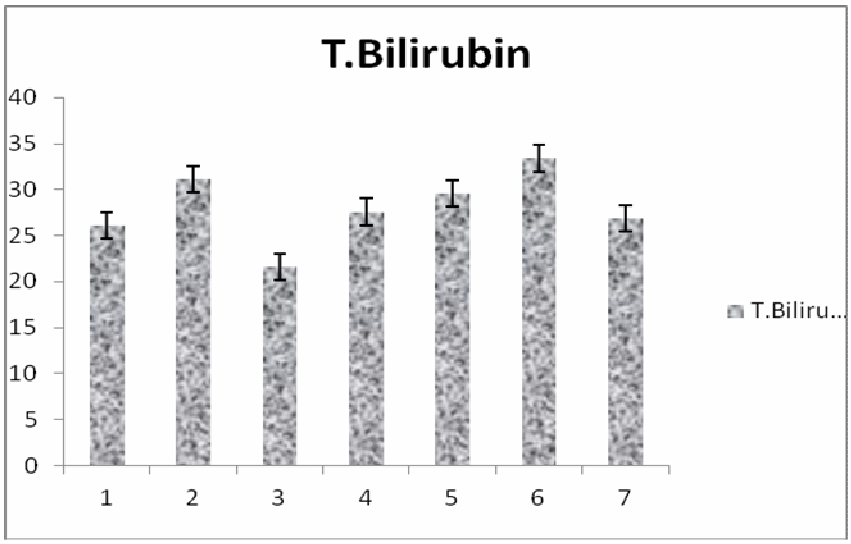

Figure 3: Average level of total bilirubin among the groups.

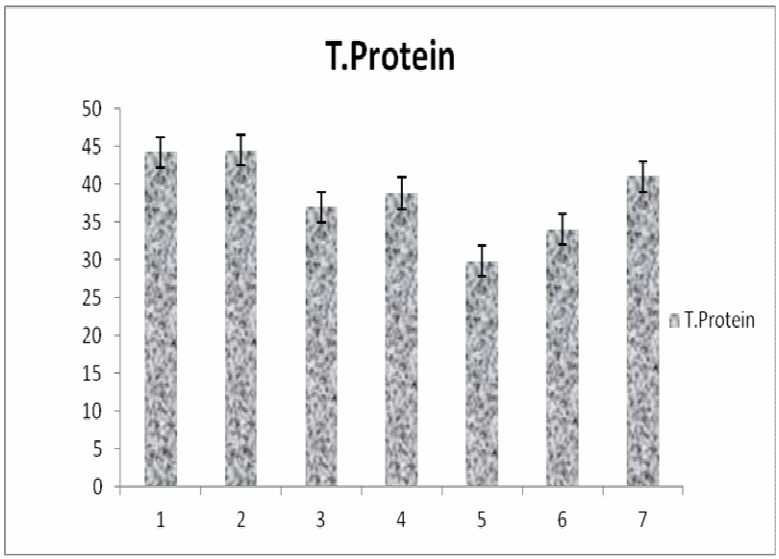

Figure 4: Average level of total protein among the groups.

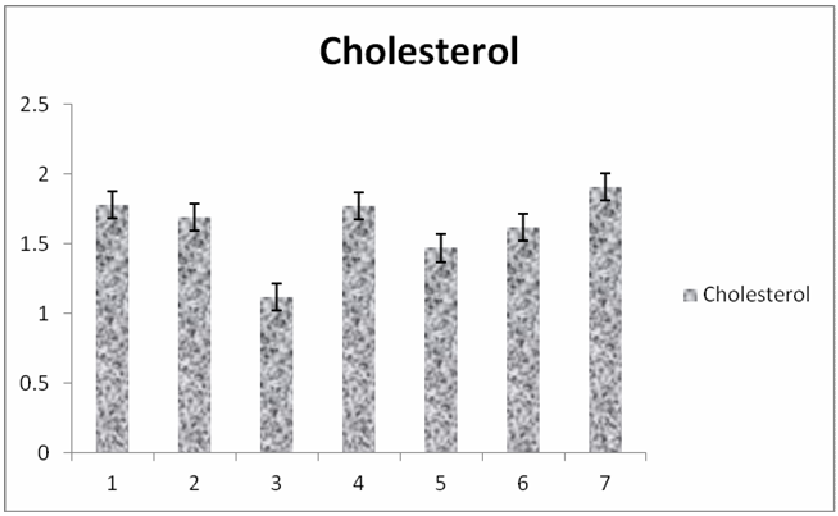

Figure 5: Average level of cholesterol among the groups.

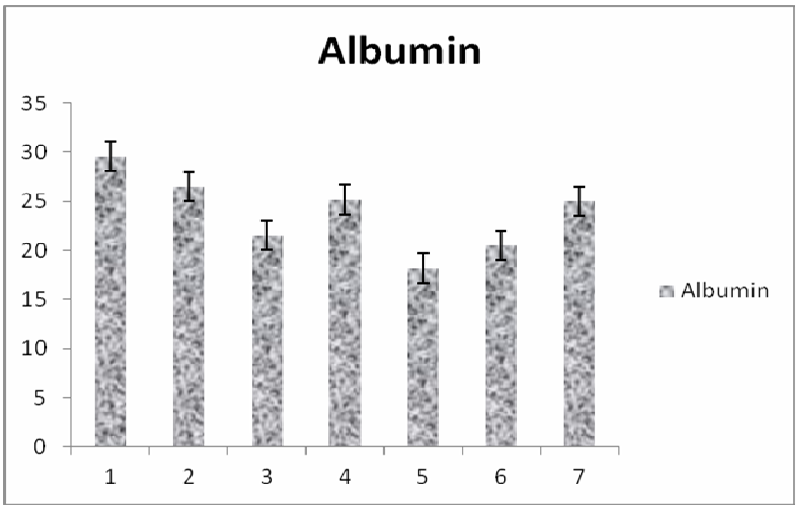

Figure 6: Average level of albumin among the groups.

Figures 1 - 6 are the histograms of the biochemical parameters (AST, ALT, total bilirubin, total protein, creatinine, triglyceride, cholesterol and albumin) respectively. These were chosen as the indicators to any changes in the condition of the liver which may result due to the inducement with the acetaminophen overdosed and the various treatments involved.

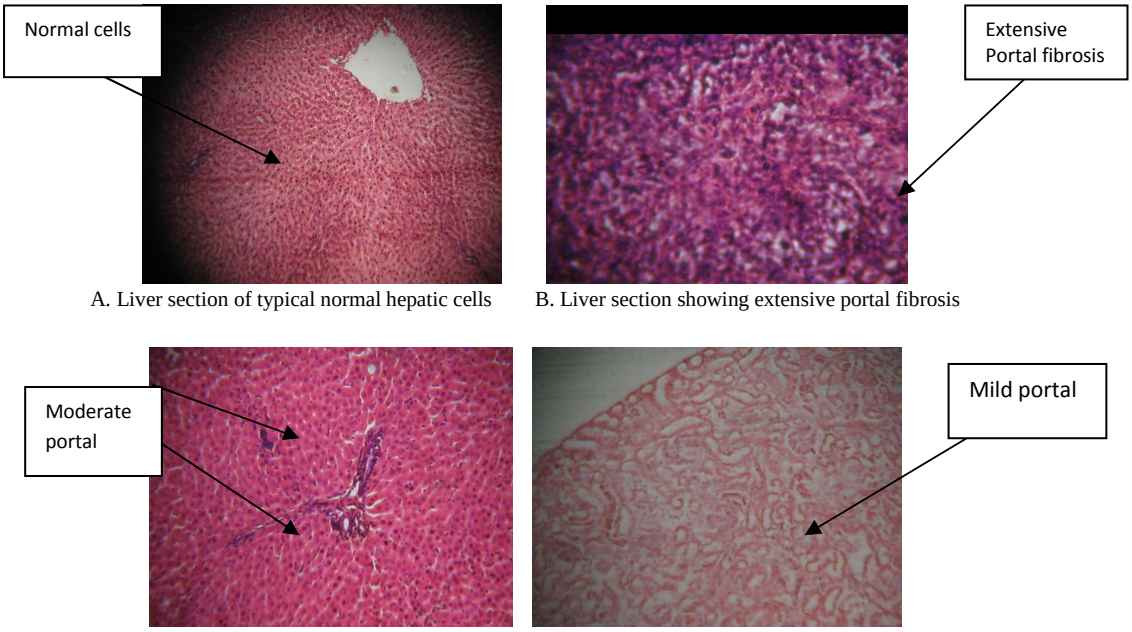

Figure 7: Hepatic cells (haematoxylin-eosin stain) sections of liver of various conditions observed. Rats were killed after seventh day administration of the different treatments. A, normal control group; B, acetaminophen (200 mg p.o); C, typical for extract groups (1000 mg, 2000 mg) and positive control group (950 mg cysteine/ 300 mg phenyl alanine); and D for 500 mg of the extract .

DISCUSSION

The phytochemical result indicated that aqueous extract of *Phyllanthus niruri* is rich in phytochemicals that are known to have effect on different form of diseases. Physicochemical parameters play a vital role in relating the properties of the plant and its activities; total ash is means of gauging elemental content. Water extractable matter is use as the quantitative variable by which physiological projection can be tested. The result obtained indicated that 1g of the extract is equivalent to 13 mg of the water extractable matter. The bitterness value serves as a marker for bioactive ingredient (pharmacological parameters) of plants (Balick, 1990; Ameh et al., 2009). The LD_{50} of the extract was above 5000 mg/kg, p.o., indicating that the experimental doses used were within the safe margin.

Biochemical enzymes monitored during the study indicated elevated AST, ALT, total bilirubin and total protein, which remained high throughout the period of the study in the acetaminophen induced group that wasn't treated, while those of the treated groups are low as shown in Figures 1, 2 and 3. The percentage activities of the extracts on these elevated enzymes as compared to the normal group were as follows; AST 31 to 38%, ALT 20 to 31%, Bilirubin -2 to 4%. From literature, leading factors to rise in bilirubin level are due to cirrhosis, hepatitis or Gilbert's diseases (Berk and Korenblat, 2007; Prat, 2010). In this study, 500 mg of the plant extracts (Figure 3) gave a better activity in protecting the liver.

The total protein test measures the total amount of two classes of proteins found in the fluid portion of blood; albumin and globulin. Albumin helps preventing fluid from leaking out of blood vessels, while globulins are an important part of your immune system. Higher-than-normal level protein in the blood

are due to chronic inflammation or infection, including HIV and hepatitis B or C; while lower-than-normal levels are due to bleeding (hemorrhage), liver disease or other related diseases (Tricot, 2005). In this study, the total protein lowered by the extracts treatment groups' ranged from 5 to 15% as shows in Figure 4, indicating that the higher dose (2000mg/kg, p.o.) has better activity than the positive control (cysteine).

High cholesterol level lead to clogging of arteries and thus contributing to heart diseases (Berk and Korenblat, 2007, Prat, 2010) In this study, the extracts lowered the elevated cholesterol level than the negative control group by 0.2 to 0.5% (Figure 5). Low than normal level of albumin as a blood protein; elevate bilirubin levels, clotting problems or other abnormalities which can point towards liver disease for example, hepatitis, cirrhosis, orascite (Berk and Korenblat, 2007, Prat, 2010). The results from the study indicated that 500 mg and 2000 mg/kg, p.o. of the extract exhibited good activities compared to the normal control group by 5 to 12% (Figure 6).

The findings from this study indicated that chemical potentials of the aqueous extract of *Phyllanthus niruri* had played vital roles which led to significant changes on the biochemical parameters observed as indicators to changes in condition of organs such as liver by inducement with acetaminophen.

Representative slides of the histopathological condition of the liver, presented as slide A, B, C and D shows condition ranges from mild, moderate to extensive portal tract inflammation with sign of focal necrosis. The cells show moderate, inter-tubular, inter-phase tract inflammation to extensive necrosis. Treatment with the extracts (groups 3, 4 and 5) showed inhibition of these conditions by the extract within the period of the treatment this corresponding to with the biochemical enzymes of the liver. The positive control groups which involved treatment with standard amino acids indicated the condition of the liver cells as, moderate to extensive portal inflammation with focal areas of necrosis as summarized in Table 2, while the hepatotoxicity induced by acetaminophen showed significant developments of inflammated lumps on the liver of some of the rats of the different treatment groups.

These results thus indicates that, *Phyllanthus niruri* extract has hepatoprotective activities against hepatitis diseases and other liver related problems (Naik and Juvekar., 2003) at the dose of 500 mg; the activities of the extract inhibited properties similar to the standard enzymes used (Wang et al., 1985). In conclusion, the present study proved the antihepatotoxicity of the aqueous extract of *Phyllanthus niruri* with significant changes both within and between the treatment groups by seven (7) days, due to different treatments and doses administered to the rats. There is therefore, the need to further investigate the plant in order to isolate the active phytochemical constituents responsible for the observed effect with the view to develop it for the treatments of liver and related diseases.

ACKNOWLEDGEMENTS

The research was funded by the authors. The authors thank Mr. Sunday Dzarma of Animal Facility Center, NIPRD for handling the animals during the study, Mr. Markus Kato and Mustapha Bakare of Teaching Hospital Gwagwalada for carrying-out the histopathological and the biochemical parameter assessment, respectively. Collectively we appreciate NIPRD for giving us atmosphere for carrying-out this work.

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