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***Phyllanthus amarus*: A hepatoprotective Agent in Acetaminophen induced Liver Toxicity in adult Wistar Rats**

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Abstract

The hepatoprotective activity of aqueous leaf extracts of *Phyllanthus amarus* (a small herb well known for its medicinal properties and widely used worldwide) was studied on acetaminophen induced hepatotoxicity in wistar rats. Animals in Group 1 served as control animals. Acetaminophen at 800 mg/kg body weight (bw) was administered daily to induce liver toxicity in Group 2 animals. Animals in group 3 were pre-treated with Silymarin (reference drug) at 50 mg/kg bw before acetaminophen (800mg/kg) administration. Animals in Groups 4 and 5 were pre-treated with aqueous leaf extracts of *Phyllanthus amarus* (100mg/kg and 200mg/kg) before acetaminophen (800mg/kg) administration. The experiment lasted for 14 days. Histological

findings suggests that pre-treatment with silymarin and aqueous leaf extracts of *phyllanthus amarus* significantly protected the liver against acetaminophen induced damage when compared to those administered with acetaminophen only. Biochemical analysis also showed significant ($p < 0.05$) increase in the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), with a reduction in total protein (TP) levels of rats administered acetaminophen only. The *phyllanthus amarus* extract's activity at 100mg/kg bw was comparable with 50 mg/kg bw silymarin. The results of the study therefore indicates that *Phyllanthus amarus* could be an effective hepatoprotective agent in acetaminophen mediated liver toxicity in adult wistar rats.

KEYWORDS: Hepatoprotective activity, *Phyllanthus amarus*, Acetaminophen, Histology, Biochemical analysis

Introduction

The liver is one of the most important internal organs in the body. Maintenance of life largely depends on the efficiency of this organ since it is directly related to several metabolic processes. In view of the importance of this organ to the body, special attention must be devoted towards ensuring that substances capable of compromising the integrity and functions of this organs do not get into the body or are made less toxic. Numerous numbers of drugs have been implicated in causing liver injury ¹ and it is the most common reason for a drug to be withdrawn from the market. Injury to the liver caused by chemicals often manifest as abnormal liver enzyme tests. Although drugs usually metabolize without injury to the liver, many fatal and near-fatal drug reactions occur. A few compounds produce metabolites that cause liver injury in a uniform, dose dependent fashion ^{2, 3}. Acetaminophen is a widely used over-the-counter analgesic-antipyretic drug. The toxicity of this substance stems from the intermediate produced during its metabolism by the cytochrome P450 enzyme system known as N-acetyl-p-benzoquinoneimine (NAPQI)^{4,5}. A great deal of work has gone into investigating the mechanisms by which acetaminophen is toxic^{6,7}. An overdose of acetaminophen is known to be the cause of acute hepatic necrosis in experimental animals ⁸ and humans ⁹. A number of medicinal plants are used in traditional system

of medicinal for the treatment and management of disorders involving the liver. A large number of medicinal plants exist in nature, many of which are yet to be explored and validated for their potency and medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases. Many natural and artificial agents possessing anti-oxidative properties have been proposed to prevent and treat hepatopathies^{10, 11}. *Phyllanthus amarus* is a world renowned plant which has been used since ages because of its rich medicinal values and ethnomedical importance. It is a small, erect, annual herb which is a rich source of phytochemicals that are attributed to biologically active lignans, glycosides, flavonoids, alkaloids, ellagitannins and phenyl propanoids that are present in the leaf, stem and root of the plant. Studies have shown antidiarrhoeal, antihyperlipidemic, antimicrobial, antidiabetic, anti-inflammatory, and antioxidant activities of *Phyllanthus amarus*¹², however little is known of its hepatoprotective activities. In the present study therefore, the herb *Phyllanthus amarus* possessing anti oxidative properties is selected to assess its hepatoprotective activity in adult wistar rats.

Materials and method

Experimental animals and their management

Twenty – Five adult wistar rats of both sexes weighing between 245 – 275g were used for this experiment. The rats were obtained and bred in the animal holding of the Department of Anatomy, University of Benin, Benin City. They were fed with livestock broiler finishers manufactured by top-feed limited and were given water *ad libitum*. The animals were exposed to natural room temperature and lighting conditions, and handled according to standard protocols for the use of laboratory animals (National Institute of Health Guide fro the Care and Use of Laboratory Animals -NIH, 1978). The animals (Five rats each) were randomly assigned into five groups 1, 2, 3, 4 and 5 as shown in the table below.

Plant material

Fresh leaves of *Phyllanthus amarus* were collected from the University of Benin, in Benin City, Nigeria. The plant was identified and authenticated at the Botany Department of University of

Benin, Benin City. The harvested fresh leaves were sun dried and ground into a fine powder. The dried material (1500g) was macerated in 10 liters of distilled water for 48hrs at 4°C in arefrigerator. The extract was sieved and the juice was filtered using Whatman No 1 filter paper. The filtrate was placed in a stainless-steel tray, and concentrated in an air-circulating oven at 42°C until totally dry.

Table 1: Showing Treatment Regimen in experimental groups

Groups	Dosage
Group 1	Distilled Water
Group 2	Acetaminophen control (800mg/kg of body weight)
Group 3	Silymarin (50mg/kg of body weight) + Acetaminophen (800mg/kg of body weight)
Group 4	<i>Phyllanthus amarus</i> leaf extracts (100mg/kg of body weight) + Acetaminophen (800mg/kg of body weight)
Group 5	<i>Phyllanthus amarus</i> leaf extracts (200mg/kg of body weight) + Acetaminophen (800mg/kg of body weight)

Assay of marker enzymes of liver damage

Blood samples were collected from the rats, transferred to sterilized tubes and allowed to clot at room temperature. The blood samples were centrifuged in a Denley BS400 centrifuge (England) for 5 mins at 5000 rpm to obtain serum. Serum obtained was used for estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total protein (TP) using Random diagnostic kits ¹³.

Histological study

The rats were sacrificed by cervical dislocation and the abdominal cavity was opened up using a pair of forceps to expose the liver which were quickly dissected out and fixed

in 10% formal saline for routine histological techniques. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 microns thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with hematoxylin and eosin (H&E) method ¹⁴. Photomicrographs of the desired sections were made for further observations.

Determination of the body weight and relative organ weight

The body weights of the animals were monitored every 72 hours with a weighing balance. At sacrifice, the weight of the liver was determined using a top loader sensitive balance (Mettler Toledo, Germany). To reduce the individual body weight differences, the relative organ weight (%) was calculated from the body weight at sacrifice and the absolute weights of each organ ¹⁵ as follows:

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

Statistical analysis

The data were analysed using descriptive and inferential statistics. All values were presented as mean \pm standard error of mean (SEM) for six rats each of seven groups. The significance of difference in the means of all parameters was determined using one way analysis of variance (ANOVA; 95% confidence interval). Least Square difference, *post hoc* tests was carried out for all groups with control and comparison of all pairs of groups respectively. All statistical analysis were carried out using Statistical package for Social Sciences (SPSS) (version 17).

Results

Body weight results

Figure 1 shows the bar chart of the body weight of animals in the experimental groups. There was no significant difference in ($p > 0.05$) weight. The bar chart shows a steady increase in weight in the control group 1, though this increase is not significant ($P > 0.05$). The animals in group 2 clearly show a steady decrease in body weight, this group was given only Acetaminophen. Analysis

however shows that there was no significant reduction in body weight ($p>0.05$). Animals in group 3, 4 and 5 show both decrease and increase in body weight at varying periods of the experiment. Again the weight changes shows no significant difference after analysis ($p>0.05$) as shown in figure 1. At the end of the experiment on the 15th day all groups experienced no significant ($p>0.05$) increase/decrease in body weight (figure 1).

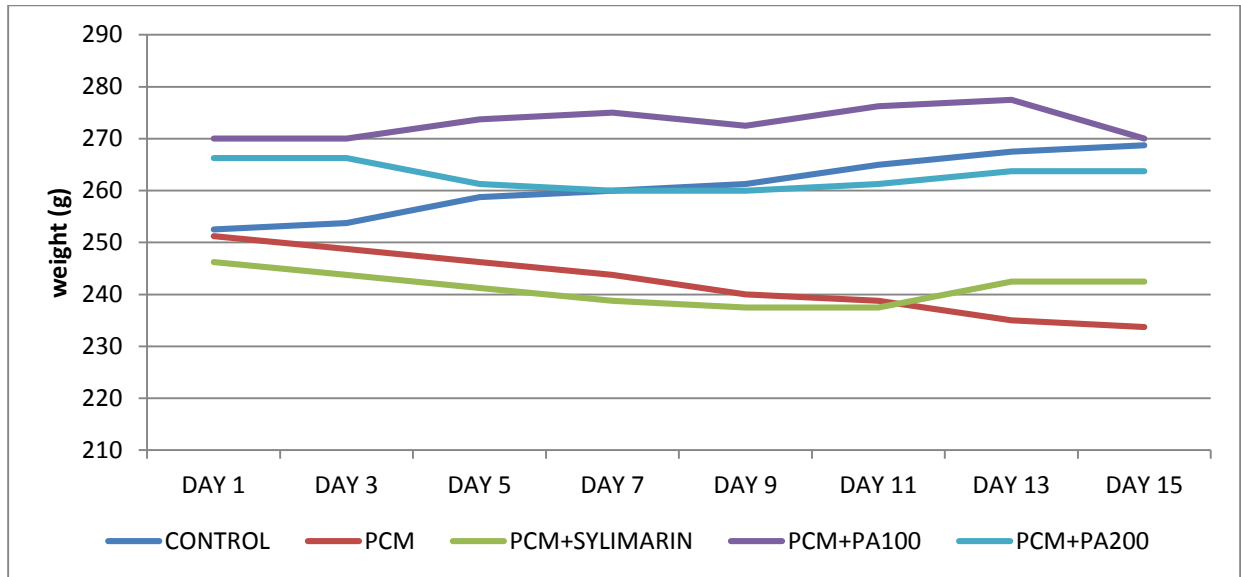


Figure 1: Line Graph Showing The Body Weights Of Animals In Experimental Groups

Relative liver weight results

Figure 2 shows the relative liver weight results of experimental groups 1-5. The relative liver weight of the Acetaminophen treated groups shows a decrease (3.30 ± 0.27) in relative liver weight when compared to the control group (3.47 ± 0.25) though not significant ($P > 0.05$). A further decrease in relative liver weight is shown in the Silymarin + Acetaminophen treated groups (3.23 ± 0.41) though also not significant ($p>0.05$) when compared to the control group. The *Phyllanthus amarus* treated group clearly shows increase in relative liver weight (3.51 ± 0.26 at 100mg/kg) and (3.44 ± 0.77 at 200mg/kg) respectively. Comparison of all experimental groups to control groups show no significant difference ($p>0.005$) as shown in figure 2.

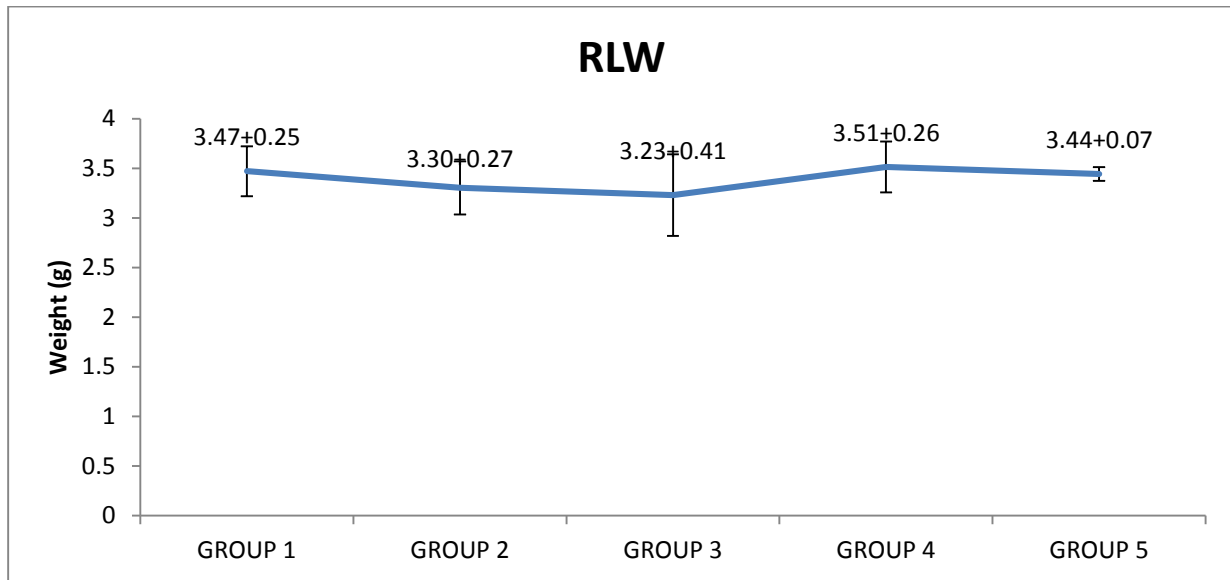


Figure 2: Line Graph Showing The Mean Relative Liver Weights Of Animals In Experimental Groups

Liver function enzymes and serum total protein results

Figure 3-6 illustrates the effects of Acetaminophen, Silymarin and aqueous leaf extracts of *Phyllanthus amarus* on the activity of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Total Protein respectively in the serum of experimental groups 1-5. The activity of AST, ALT and ALP significantly increased ($p < 0.05$) in Acetaminophen treated rats (Group 2) when compared to the control. The activity of these liver function enzymes is significantly lowered in the Silymarin and *Phyllanthus amarus* treated groups (3-5). Furthermore, the activity of total protein is significantly ($p < 0.05$) reduced in acetaminophen treated rats (Group 2) when compared to the control, Silymarin (group 3) and *Phyllanthus amarus* treated experimental groups 4-5.

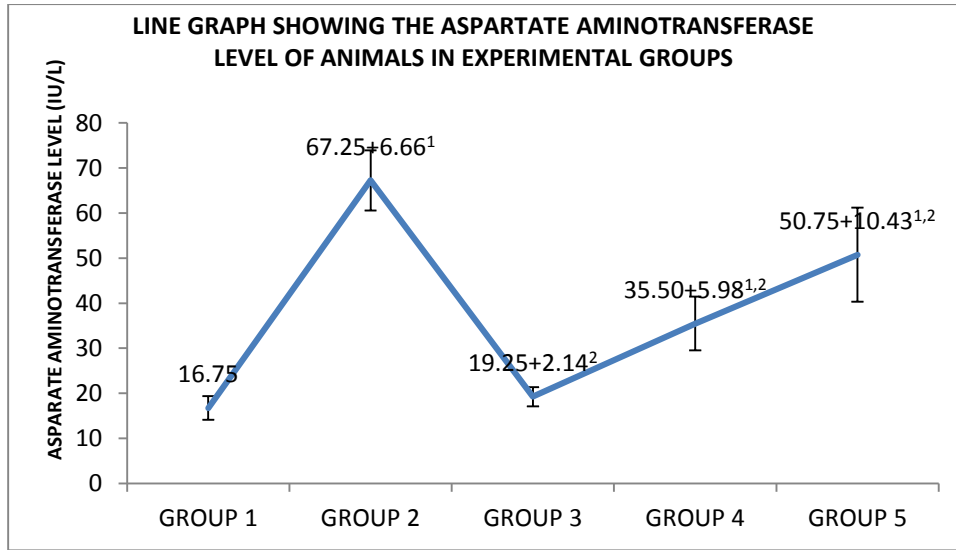


Figure 3: Line Graph Showing the Aspartate Aminotransferase Level Of Animals In Experimental Groups

^{1,2,3}Significant difference at (p < 0.05). ¹significant difference when compared to control group 1, ² significant difference when compared to acetaminophen group 2, ³significant difference when compared to silymarin + acetaminophen group 3

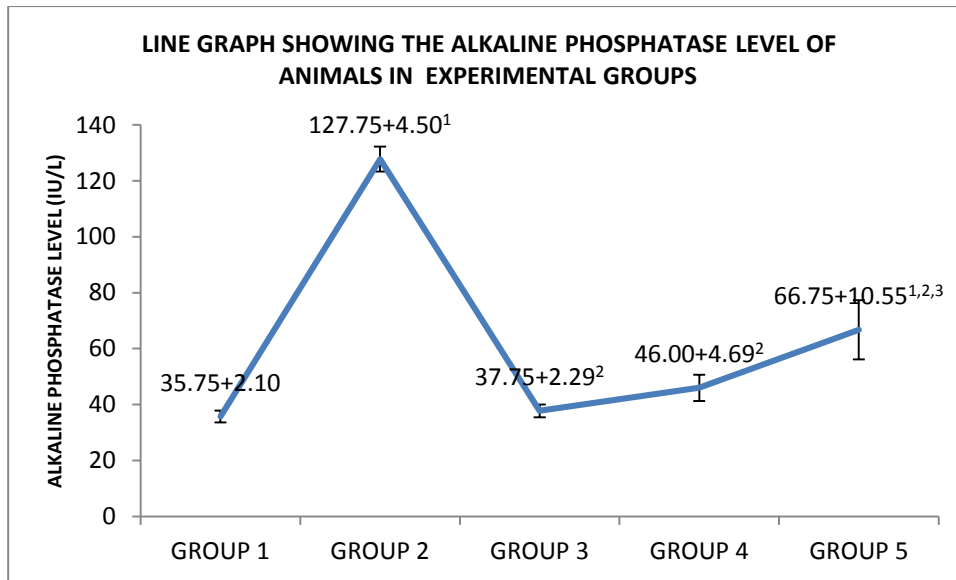


Figure 4: Line Graph Showing The Alkaline Phosphatase Level Of Animals In Experimental Groups

^{1,2,3}Significant difference at ($p < 0.05$).¹significant difference when compared to control group 1, ² significant difference when compared to acetaminophen group 2, ³significant difference when compared to silymarin + acetaminophen group 3

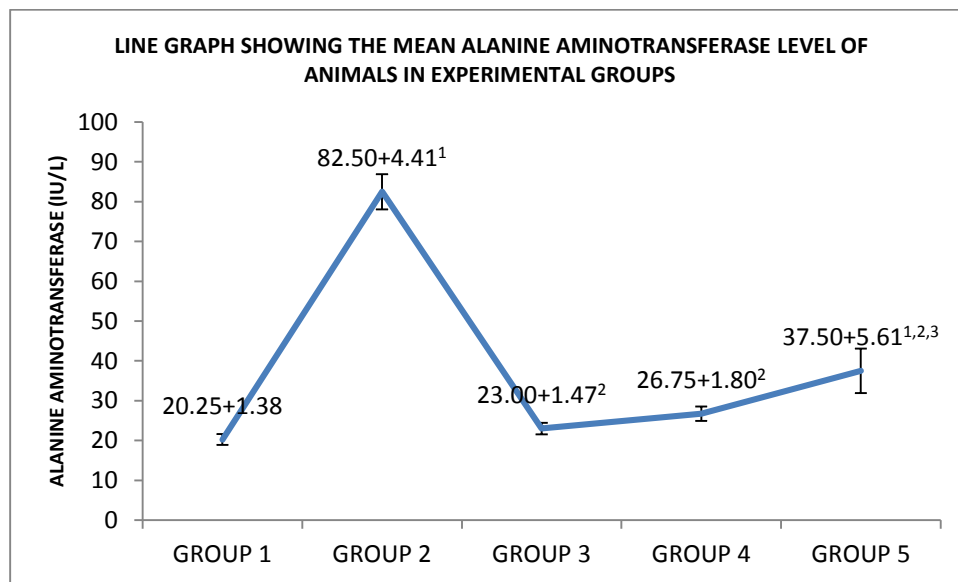


Figure 5: Line Graph Showing The Mean Alanine Aminotransferase Level Of Animals In Experimental Groups

^{1,2,3}Significant difference at ($p < 0.05$).¹significant difference when compared to control group 1, ² significant difference when compared to acetaminophen group 2, ³significant difference when compared to silymarin + acetaminophen group 3

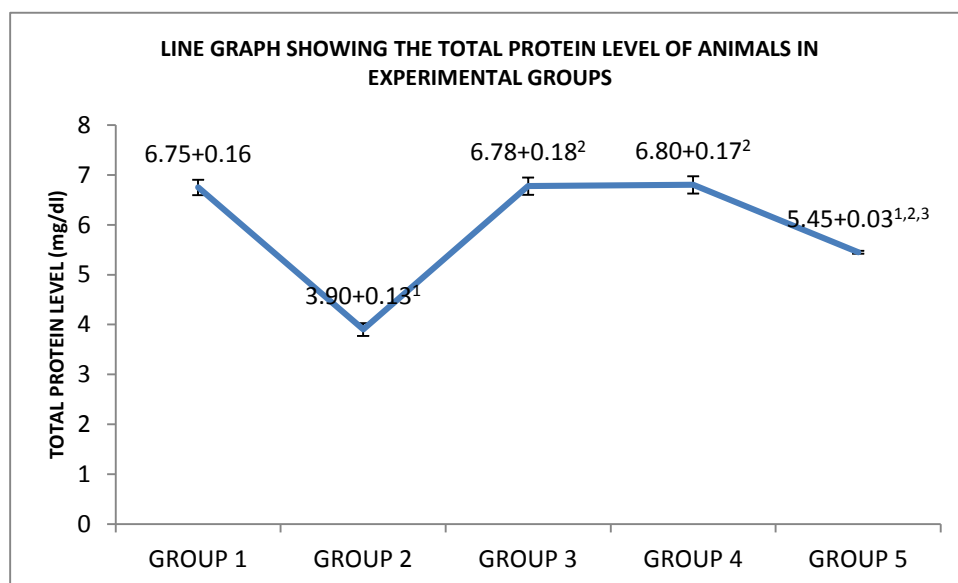


Figure 6: Line Graph Showing The Total Protein Level Of Animals In Experimental Groups

^{1,2,3}Significant difference at ($p < 0.05$). ¹significant difference when compared to control group 1, ² significant difference when compared to acetaminophen group 2, ³significant difference when compared to silymarin + acetaminophen group 3

Histology result

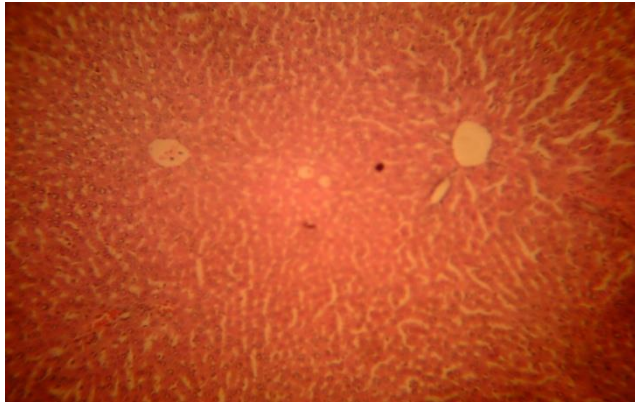


Plate 1: Liver tissue (Group 1 administered distilled water) showing normal Portal tract, central vein and Normal Hepatocytes (H and E X 100)

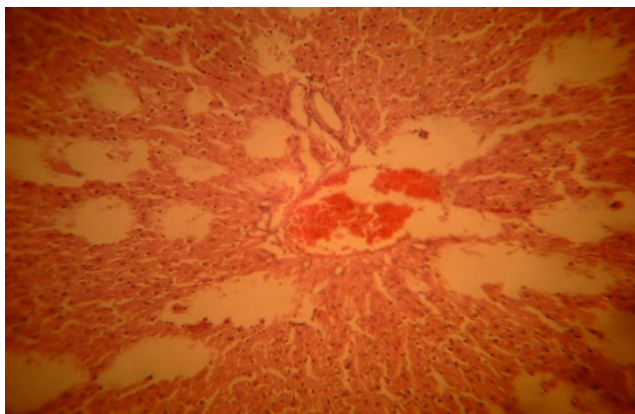


Plate 2: Liver tissue (Group 2 treated with Acetaminophen 800mg/kg) showing mild congested Portal Vein, severe distortion of the liver parenchymal (H and E X 100)

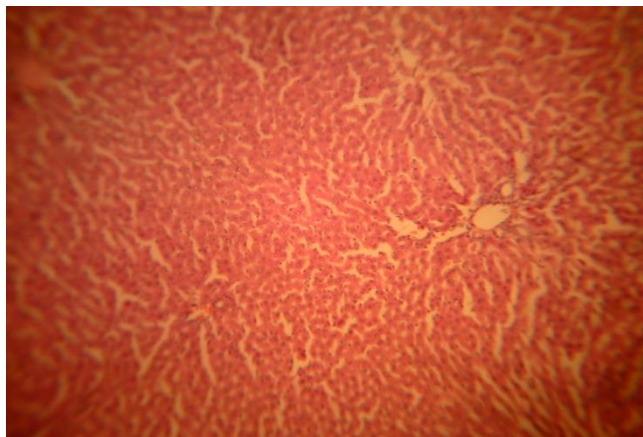


Plate 3: Liver tissue (Group 3 treated with 50mg/kg Silymarin and 800mg/kg Acetaminophen) showing mild portal area, mild tissue separation (H&E x 100)

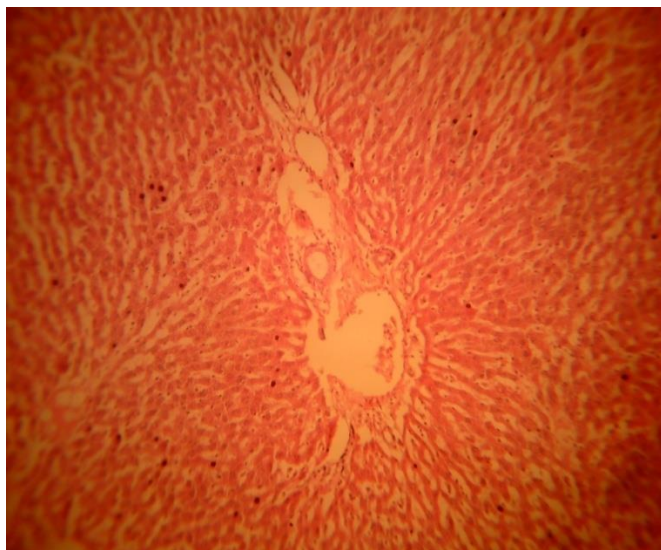


Plate 4: Liver tissue (Group 4 treated with 100mg/kg *Phyllanthus amarus* and Acetaminophen) showing mild portal area and moderate tissue separation (H&E x 100)



Plate 5: Liver tissue (Group 5 treated with 200mg/kg *Phyllanthus amarus* and Acetaminophen) showing mild portal congestion and mild tissue separation (H&E x 100)

Discussion

The purpose of this study was to investigate the hepatoprotective effect of *Phyllanthus amarus* leaf extracts in liver damage caused by acetaminophen. Administration of acetaminophen to normal rats increased serum levels of ALT, AST and ALP. These liver enzymes ALT, AST, and ALP are conventionally low in normal control, this is in agreement with the report by previous authors^{16, 17}. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells. Injury to the hepatocytes changes their membrane permeability and transport function thereby causing leakage of enzymes from the cells¹⁸. The release of these enzymes and their increased activity in animals treated with acetaminophen may therefore be as a result of liver cell destruction and alteration in the membrane permeability¹⁹. The reversal of increased serum enzymes in acetaminophen-induced liver damage could be attributed to the membrane stabilizing properties of *Phyllanthus amarus* extracts which consequently prevents the leakage of intracellular enzymes. The protective activity of *Phyllanthus amarus* extract confirms previous report²⁰. It is therefore worthy to state that the effect of any hepato-protective drug is

dependent on its ability of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a known hepatotoxin²¹. Silymarin, a standard hepatoprotective drug used in the treatment of liver injury at 50 mg/kg showed no significant difference in activity when compared to group treated with 100 mg/kg of *Phyllanthus amarus*. Both silymarin and the extracts of *Phyllanthus amarus* decreased acetaminophen induced elevated enzyme levels in tested groups, indicating its ability to either regenerate damaged liver cells or protect the structural integrity of the hepatocytes/cell membrane. However, from our results, *Phyllanthus amarus* extracts administered at 100mg/kg appears to be more effective than that administered at 200mg/kg in protecting the liver against acetaminophen induced liver damage. Furthermore, a primary consideration in the evaluation of the efficacy of a potential therapeutic agent for hepatic injury/damage is its effect on liver histology. Liver sections from acetaminophen treated rats demonstrated the destruction of architectural pattern, moderate inflammation of portal area and severe distortion of the liver parenchymal (Plate 2). *Phyllanthus amarus* extract in the dosage range of 100mg/kg and 200mg/kg administered alongside the liver damaging agent (acetaminophen) to rats apparently protected the liver against acetaminophen damage (Plate 4 and 5). Liver section of control rat shows normal hepatocytes and normal architecture (normal Portal tract, central vein and Normal Hepatocytes) (Plate 1). Liver sections from silymarin treated rats shows almost normal lobular architecture (Plate 3). These histological findings demonstrate a hepatoprotective effect of the extract against acetaminophen-mediated liver damage. Further in the present investigation, phytochemical analysis of the leaf extract revealed the presence of flavonoids, alkaloids, saponins, tannins, anthraquinone and terpenoids. Saponins²² and flavanoids²³ are well known for their hepatoprotective and antioxidant activities. The aqueous extract of *Phyllanthus amarus* may have exhibited significant hepatoprotective activity due to its possible antioxidant property which can be attributed to flavonoids.

Conclusion

The results of the study therefore indicates that *Phyllanthus amarus* could be an effective

hepatoprotective agent in acetaminophen mediated liver toxicity in adult wistar rats. It is also recommended that further studies aimed at corroborating these findings be carried out.

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