Original Research Article

Toxicological, Biochemical and Haematological Studies of Ethyl acetate Extract of *Persea americana* Leaf in Albino Rats

ABSTRACT

The aim of this present study was to evaluate the effect of ethyl acetate extract of *P. americana* on liver and kidney function, lipid profile as well as haematological parameters in albino rats. A total of 20 albino rats were used for this experiment and they were divided into four groups of 5 (A-D) rats each. Group A served as normal control, group B-D served as experimental groups administered with different doses of ethyl acetate *Persea americana* leaf extract. This study was conducted in the Department of Biochemistry, Bayero University, Kano, in the month of May, 2018. The animals were administered with 100, 200 and 400mg/kg per day of the extract for 4 weeks. The activities of serum transaminases, alkaline phosphatase, total protein, albumin, globulin, electrolytes, urea and creatinine were determined. Biochemical parameters were evaluated by measuring the concentration of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and high density lipoprotein. Haemopoietic effect was evaluated by measuring the levels of packed cell volume, red blood cells, white blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelets. The levels of serum ALT and albumin were significantly ($P<0.05$) increased whereas total protein, globulin and potassium levels were significantly ($P<0.05$) decreased. Significant ($P<0.05$) decrease in total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein was observed. Haematological parameters were not significant affected. In conclusion, ethyl acetate extract of *P. americana* may be relatively safe and possesses hypolipidemic potentials but extremely high doses may not be advisable.

Keywords: *P. americana,* liver function test, kidney function test, lipid profile, haematological parameters

1. INTRODUCTION

Many medicinal plants used in ethnomedical practices in Nigeria are known or little known to scientific world [1]. Many important drugs used in medicine today are directly or indirectly derived from plants [2].

Plants and herbs have been a tremendous source of food and folk remedies for mankind and have served as starting material for the development of new synthetic drugs. They have the ability to synthesize a wide variety of chemical compounds which are used to perform biological functions and to defend against attack from predators such as insects, fungi, yeasts, bacteria, virus and other pathogens [3]. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs [4, 5]. *Persea americana* belongs to the family *Lauraceae*, unflatteringly known in the past as alligator pear, midshipman's butter and vegetable butter, it is one of those plants currently used by indigenous persons for it nutritional value and to manage health problems. It is well known in many parts of the tropical world including Nigeria [6]. The fruit tree can attain a height up to 20m, with large spreading and flat topped crown. The plant is reported to possess antidiabetic, antihyperlipidemic potentials [2, 7], antioxidant [8, 9], cancer risk reduction [10], wound healing [11], hepatoprotection [12], analgesic and anti-inflammatory [13], anticonvulsant [14], vasorelaxant and blood pressure reducing [15, 16]. The use of traditional medicines as substitutes to orthodox medicines has been on the increase [17]. The reasons, which have given rise to this trend, include the cheapness, availability and accessibility of these natural medicines. Besides, there has been the erroneous belief that these medicines are free from adverse effects [18, 19]. On the other hand they have been rejected because many of the acclaimed medicinal values have not been scientifically evaluated and their safety profiles uncertain [19]. It is, therefore, pertinent that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated. The World Health Organization has also recommended the evaluations of traditional medicine, because of the modern drugs are not safe [20]. It is, therefore, pertinent that safety assessments should be conducted on ethyl acetate extract of *P. americana* for which certain medicinal uses have been scientifically validated.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The leaf of *Persea americana* were collected from Jos, Plateau state of Nigeria. The plant part was authenticated by a Botanist at Plant Science Department, B.U.K. with accession number BUKHAN 0305 and was deposited in Bayero University, Kano herbarium for reference.

2.2 Extraction of Plant Material

Soxhlet extraction method was used for the extraction of the plant materials. The sample was chopped into small pieces and then shades dried and ground into powdered form. Ethyl acetate was used as the extraction solvent and later concentrated in vacuo using rotary evaporator at 40□.

2.3 Experimental animals

Experimental rats (80-100g) of either sex were obtained from the Physiology department, Faculty of Basic Medical Sciences, Bayero University, Kano. The animals were kept in cages and clean drinking water provided *ad libitum* while they were fed with standard commercial pelleted feed (Vital Feed® Nigeria). The temperatures varied between 27-30□ and relative humidity of about 55%-60% with 12-h light-dark cycle and adequate ventilation maintained in the animal house. Ethical conditions governing the conducts of experiments with life animals as stipulated were strictly observed. Also, the experimental protocol was approved by the College of Health Science ethical committee.

2.4 Experimental Design

A total of 20 rats were used for this experiment and they were divided into four groups of 5 rats each.

Group 1- normal rats

Group 2- treated with ethyl acetate extract of *P. americana* leaf.

Group 3- treated with ethyl acetate extract of *P. americana* leaf.

Group 4- treated with ethyl acetate extract of *P. americana* leaf.

The sub-chronic toxicity study lasted for a period of four weeks. The various doses (100, 200 and 400mg/kg b.w.) were administered to group 2, 3 and 4 respectively of normal rats. At the end of 4 weeks treatment the rats were sacrificed and blood samples collected for liver function (ALT, AST, ALP, ALB, TP

and GLO), kidney function indices [creatinine, urea, electrolytes (Na^+, K^+, Cl^- and HCO_3)], lipid profile (CHOL, TG, HDL, LDL and VLDL) and hematological parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC and PLT).

2.4 Assays

Liver function test were assayed using the methods of; serum alanine and aspartate aminotransferase activity [21], serum alkaline phosphatase activity [22], serum albumin concentration [23], serum total protein concentration and serum globulin concentration [24]. Kidney function test were assayed using the methods of; serum urea concentration [25], serum creatinine concentration [26], serum potassium concentration [27], serum sodium concentration [28], serum chloride concentration [29] and serum bicarbonate concentration [30].

Lipid profile was analysed using the methods of; serum total cholesterol concentration [31], triglycerides concentration and serum HDL-cholesterol concentration [32], serum LDL and VLDL-cholesterol concentration [33]. Haematological parameters were analyzed using SYSMEX XE-200 (QBC Autoread Plus, UK).

2.5 Statistical Analysis

The results were expressed as mean ± standard error of the mean and statistically analysed by one-way analysis of variance. The level of significant was set at $P< 0.05$.

3. RESULTS

3.1 Liver Function Test

Result of sub-chronic toxicity study showed no significant difference ($P>0.05$) in AST and ALP activities in all the treated groups when compared with normal control. However, significant increase ($P<0.05$) was observed in serum level of ALT of the entire treated group when compared with normal control (Figure 1). There was a significant decrease ($P<0.05$) in serum total protein and globulin in all the treated groups compared with the normal control (Figure 2). The level of serum albumin was found to increase significantly ($P<0.05$) in all the treated groups compared with normal control.

3.2 Kidney Function Test

Kidney function tests carried out include serum urea, creatinine, sodium, potassium, chloride and bicarbonate (Figures 3 and 4). No significant difference ($P>0.05$) was observed in serum urea, creatinine, sodium, chloride and bicarbonate in all the groups when compared with normal control group. Serum Potassium level was found to decrease significantly in group 2 (100mg/kg b.w.) When compared with normal and groups 3 and 4 (200 and 400mg/kg b.w.).

3.3 Lipid Profile

The levels of total serum cholesterol, triglyceride and VLDL in the treated groups were found to decrease significantly ($P<0.05$) when compared with normal control. No significant difference was observed ($P>0.05$) in serum high density lipoprotein level. Serum low density lipoprotein level was reduced significantly ($P<0.05$) in a dose dependent pattern within the treated groups when compared with normal control (Figure 5).

3.4 Haematological Parameters

There was no significant difference ($P>0.05$) between the normal control and the tested groups in all the haematological parameters assessed. These include, white blood cell, red blood cell, haemoglobin,

packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular heamoglobin concentration and platelets (Table 1).

4. DISCUSSION

Toxicity studies are essential tools in assessing the bioaccumulative effects of xenobiotics in biological systems. Assessment of liver and kidney function is very important in evaluating toxicity of modern and traditional medicines since these organs play major roles in metabolism of xenobiotics in the body. Elevated activities of liver enzymes are often diagnostic of underlying cellular injuries [34, 35]. From the biochemical result of this study, AST and ALP activities were not affected in all the groups administered with the extract. Serum concentration of ALT was found to increase in the treated groups, and this can be as a result of increase in hepatocytes proliferation or regeneration [36]. Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ [37]. Studies conducted by [38] showed that a large intake of tannins may cause liver damage. Reduction in total protein and globulin are indications of diminished synthetic function of the liver or might be due to impaired hepatocellular function [39, 40, 41, 42]. The increased level of albumin observed in this study depends on a number of factors such as the nutritional status, catabolism, hormonal factors, and urinary and gastrointestinal losses [42]. Ethyl acetate extract of *P. americana* was found to contain appreciable amount of saponins and tannins [8, 43] which can be related to the observed increase in ALT activity. Saponins may act by damaging cell membranes causing leakage of cellular materials, ultimately leading to cell death [44, 45].

Kidney function was evaluated by means of serum urea, creatinine and blood electrolyte concentrations. Urea is formed in the liver and is mainly excreted by the kidney, it is useful in evaluating kidney function in conjunction with creatinine which originates from the muscle and is filtered by the kidney. The serum urea and creatinine levels of treated groups in this study were not significantly changed. Increased blood urea and creatinine is a good indicator of compromised kidney function. These results suggest that the extract may not have altered the kidney function. In a similar study by [40] serum creatinine, urea and electrolytes such as sodium and calcium were not affected after *P. americana* extract treatment.

In this study, the administration of graded doses of EPAL significantly reduced serum levels of TC, TG, LDL and VLDL in treated rats. Saponins are known anti-nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion [46, 47]. Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol; hence saponins have been reported to have hypocholesterolaemic effect [48]. Thus, the observed hypolipidemic effect of EPAL may be linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract.

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals as it investigate the extent of damage to the blood [37]. It provides vital information regarding the status of bone marrow activity and intravascular effects such as haemolysis and anaemia [49]. Assessment of the haematological indices showed that the extract did not cause any significant effect on WBC, RBC, PCV, Hb, PLT, MCV, MCH and MCHC. The results on the haematological parameters indicate normal haemopoiesis and absence of anaemia confirming the non-toxic nature of the extract.

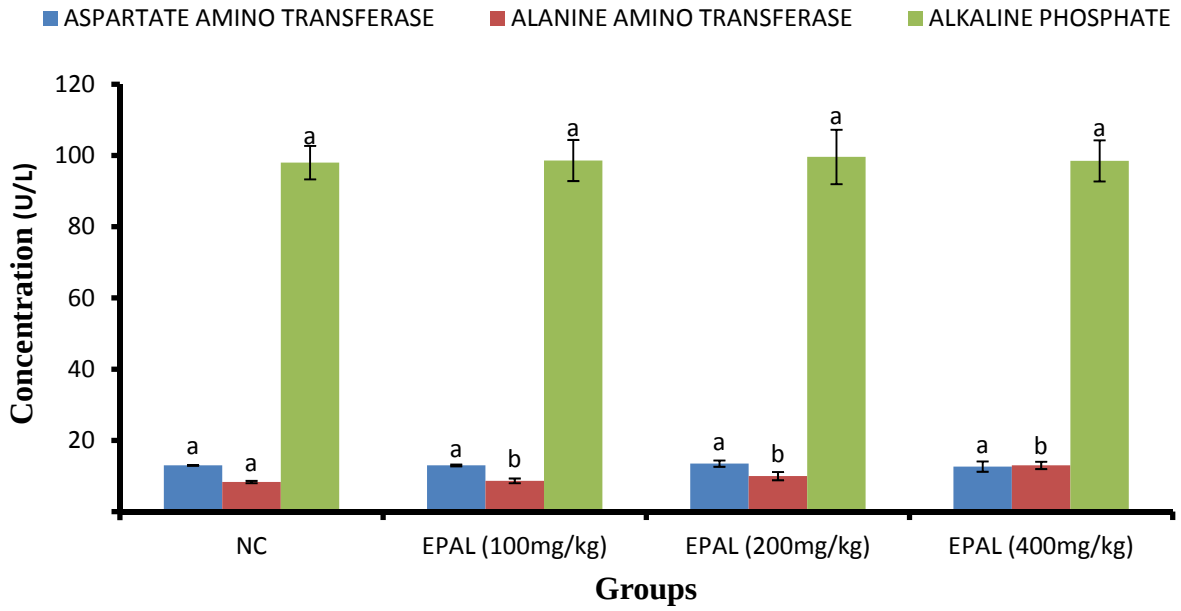

Figure 1: Serum Levels of Aspartate Amino Transferase, Alanine Amino Transferase and Alkaline Phosphatase of Rats Administered with Ethyl acetate Extract of *P. americana* leaf (100, 200 and 400mg/kg b.w.) for 4 Weeks

Results are presented as Mean ± SD, n=5. Values with different superscripts are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of *P. americana* leaf

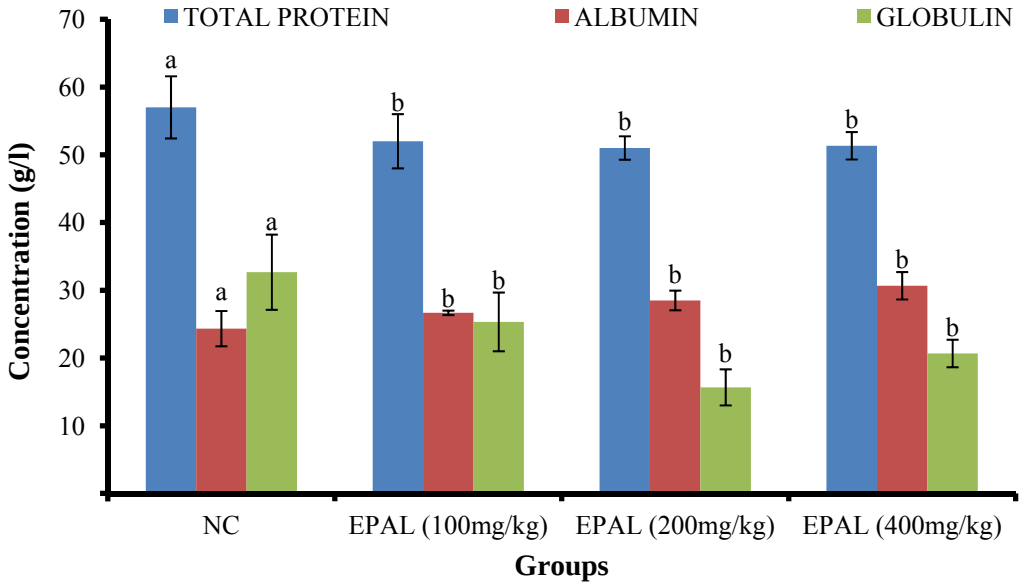

Figure 2: Serum levels of Total Protein, Albumin and Globulin of Rats Administered with Ethyl acetate Extract of *P. americana* Leaf (100, 200 and 400mg/kg b.w.) for 4 Weeks

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P<0.05) with respect to normal control, NC=Normal control, EPAL=Ethyl acetate extract of *P. americana* leaf

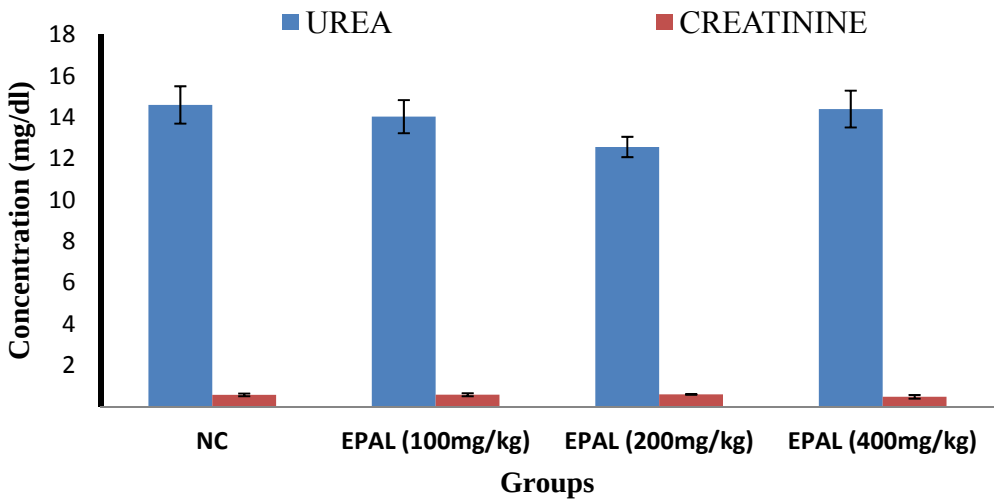

Figure 3: Serum Levels of Urea and Creatinine Levels of Rats Administered with Ethyl acetate Extract of *P. americana* Leaf (100, 200 and 400mg/kg b.w.) for a Period of 4 Weeks

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P< 0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of *P. americana*

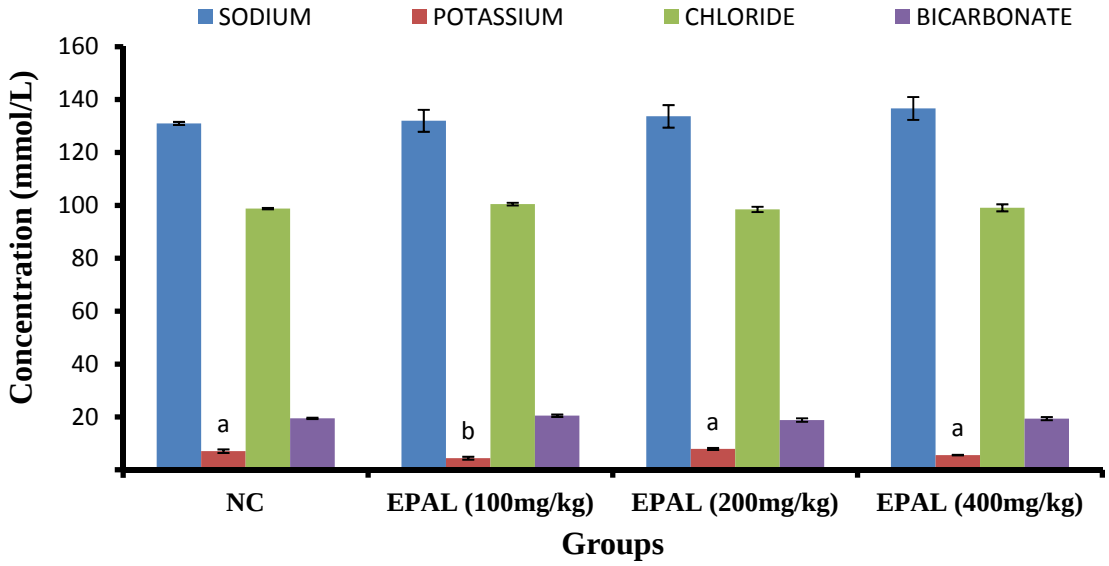

Figure 4: Serum Levels of Sodium, Potassium, Chloride and Bicarbonate of Rats Administered with Ethyl acetate Extract of *P. americana* Leaf (100, 200 and 400mg/kg b.w.) For 4 Weeks

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of *P. americana*

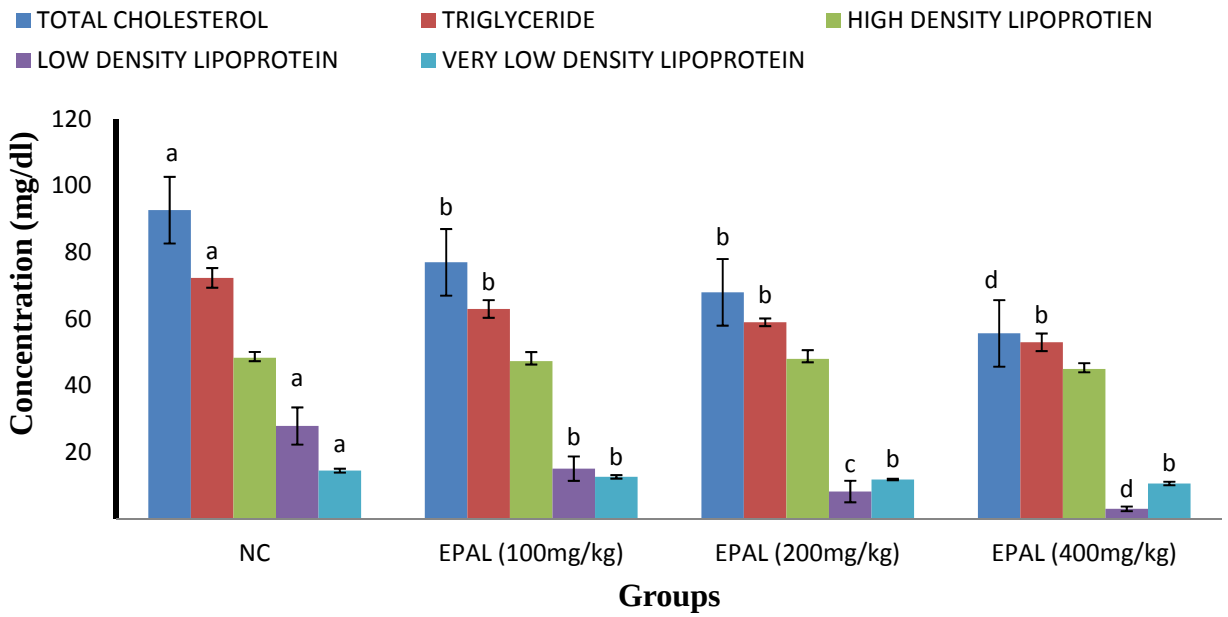

Figure 5: Levels of Serum Total Cholesterol, Triglyceride, High Density Lipoprotein, Low Density Lipoprotein and Very Low Density Lipoprotein of Rats Administered with Ethyl acetate Extract of *P. americana* Leaf (100, 200 and 400mg/kg b.w.) for 4 Week

Results are presented as Mean ± SD, n=5. Values with the different superscripts are significantly different (P<0.05) with respect to normal control, NC= Normal control, EPAL= Ethyl acetate extract of *P. americana* leaf

Table 1: Haematological Parameters of Rats administered with Ethyl acetate Extract of *P. americana* leaf (100, 200 and 400mg/kg b.w.) for 4 weeks

GROUPS	RBC(10^3/µl)	WBC(10^6/µl)	Hb(g/dl)	PCV(%)	MCV(FL)	MCHC(g/dl)	MCH(pg)	PLT(10^3/µl)
Normal Control	6.45 ± .10[a]	8.23 ± .20[b]	7.60 ± .36[c]	36.67 ± 1.86[d]	55.30 ± .06[e]	21.80 ± .23[f]	11.23 ± .35[g]	346.67 ± 3.22[h]
EE(100mg/kg)	6.47 ± .14[a]	8.73 ± .23[b]	6.97 ± .27[c]	37.67 ± 1.86[d]	58.07 ± .54[e]	22.93 ± .17[f]	11.83 ± .19[g]	336.67 ± 3.93[h]
EE (200mg/kg)	6.73 ± .04[a]	8.53 ± .35[b]	8.03 ± .35[c]	36.67 ± .88[d]	55.20 ± .47[e]	23.33 ± .98[f]	11.40 ± .42[g]	349.00 ± 8.50[h]
EE (400mg/kg)	6.63 ± .22[a]	8.77 ± .09[b]	7.47 ± .35[c]	37.67 ± 2.40[d]	53.00 ± .78[e]	22.23 ± .73[f]	11.60 ± .40[g]	357.33 ± 4.10[h]

Results are presented as Mean ± SD, n=5. Values with the different superscripts in the same column are significantly different ($p< 0.05$) with respect to normal control, EE= Ethyl acetate Extract of *P. americana* leaf

CONCLUSION

While the medicinal importance of the *P. americana* is increasingly becoming of scientific interest, the consumption of high dosage should be discourage, as oral administration of ethyl acetate extract of *P. americana* leaf results in an increase in the level of ALT. However, kidney function and haematological parameters were not affect with a significant decrease in the levels of total cholesterol, triglyceride, LDL and VLDL.

ETHICAL APPROVAL

All authors hereby declare that; principle of laboratory animals care (NHI publication number 829 revised 1985) were followed, as well as all experiment have been examined and approved by the appropriate ethic committee.

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