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Biochemical and toxicological implications of ethylacetate fraction of the methanolic extract of *Plumbago zeylanica* (Linn) root

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ABSTRACT

Plumbago zeylanica, Linn. is an important plant with numerous medicinal values. This study was aimed to evaluate the biochemical and toxicological effects of the administration of the ethylacetate fraction of the methanolic extract of *Plumbago zeylnica* root (EAME). In sub-chronic study, extract doses of 100, 200 and 400 mg/kg body weight were administered orally in rats for 28 days. Biochemical and histological evaluations were carried out on the rats. At the highest dose of EAME, organ-body weight ratio increased by 170% in the liver, spleen by 85%, but reduced in kidney by 62%, lung 17% and heart 7%. In the plasma, administration of EAME at the highest dose increased the concentrations of protein by 11%, albumin 32%, glucose 153%, direct bilirubin 151%, total bilirubin 656%, creatinine 35% and uric acid 29%. Activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltansferase were also increased by 6%, 39%, 76% and 46% respectively. The concentrations of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol were not significantly different ($p>0.05$) compared with the control. EAME induced histopathological alterations in the cellular architecture of the liver and lungs. Mild congestion in sinusoids and bile duct proliferation were observed in the liver, while the lung showed necrosis, oedema and haemorrhage. The alterations were more prominent in the lung of rats treated with 200 and 400 mg/kg b.wt. doses. No histopathological alteration was observed in the kidney. These findings indicate that EAME of *P. zeylanica* root contains bioactive compounds which are toxic to the organism but the action was organ specific.

Keywords: *Plumbago zeylanica*, Biochemical, Toxicological, Histopathological parameters.

INTRODUCTION

The tropical forest of Africa is endowed with various medicinal plants. Many of these medicinal plants are utilized in African Traditional Medicine (ATM) to solve the health issues of the local communities. The medicinal values of these plants are dependent on the presence of inherent substances which produce definite physiological actions on human body [1]. A World Health Organization (WHO) report indicated that about 80% of the world population especially in developing countries, including Nigeria depends on herbal traditional medicine for treatment of a variety of diseases [2]. The last two decades have witnessed a huge attention from both the developed and developing countries on medicinal plants. Moreover, the economic importance of medicinal plants has also attracted the attention of various world bodies, in particular, the World Health Organization [3]. In 2003, WHO highlighted the importance of quality control, safety and proper validation of medicinal plants to prevent wrong plants species use, contamination by chemicals or unwanted foreign substances, adulteration, factors that may induce over dose, wrong use by health providers and consumers, and undesirable interaction by various medicinal plants [4].

Plumbago zeylanica (Linn) is a perennial multipurpose medicinal herb which belongs to the family of Plumbaginaceae. The plant is distributed throughout most of the tropics and subtropics, growing in deciduous woodland, savannas and shrub lands from sea level up to 2000 metres altitude [5]. This species is known by several names in different part of the world viz: white leadwort or ceylon leadwort (in English), bleiwurz or zahnkraut (in Germany), Chitrak or chitramol (in India), ensain or enkin (in Arabia), sanza (in Swahili), and inabiri (in South-West Nigeria) [6]. In Nigeria, the roots pounded with vegetable oil are used as a treatment for rheumatic swellings [5]. Powdered bark, root or leaves are used as a conventional method to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, swellings and wound treatment system in Ethiopia. Other parts of Africa use the paste of the root in vinegar, milk and water to treat influenza and black water fever; and the root decoction is used in the treatment of shortness of breath; inflammation in the mouth, throat and chest; diarrhea and dyspepsia [5, 7-8].

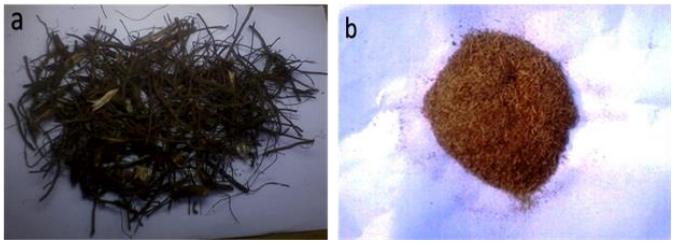

Figure 1: *Plumbago zeylanica* (a) Dried root (b) Crushed root

The roots of *P. zeylanica* and its constituents are credited with lots of therapeutic properties. Various solvents extracts have been investigated and reported to possess pharmacological properties which include alcoholic or ethanolic extracts that show anti-inflammatory [9-12], antibacterial [13-14] and hyperglycaemic [15]. Methanolic extracts also have activity against skin diseases [7], and hepatoprotective activity [16] while hydroalcoholic extract served as nephroprotective [17], cytotoxic and antioxidant agents [18].

However, there has been limited scientific evidence as regards the safety and efficacy to support the continued therapeutic use of many of these herbal medicines. The only evidence available is often linked to the ancestral experience. The upsurge in the demand for herbal medicines therefore necessitates a thorough scientific evaluation of biochemical and toxicological properties of these herbs. In case of *Plumbago zeylanica*, there are scanty reports on its toxicological properties especially, the ethylacetate fraction from methanolic extract of the plant. It is therefore pertinent to have a thorough study on the biochemical and toxicological properties of the *Plumbago zeylanica* found in Nigeria.

MATERIALS AND METHODS

Chemicals and equipment

All the reagents used were of analytical grade purchased mainly from renowned U.S.A., Sigma-Aldrich Laborchemkalien GmbH, Seelze, Germany; and British Drug House (BDH) Ltd., Poole, England. The reagent diagnostic kits used for various biochemical assays were products of ERBA Diagnostics Mannheim GmbH, 68219 Mannheim, Germany. Equipment used include Oven which was supplied by Genlab Ltd., England; balance (Mettler Toledo, Switzerland); rotary evaporator (Buchi Rotavapor-R) coupled with Speedmac Edwards High Vacuum Pump, a product of Edward vacuum component, a division of British Oxygen Company Ltd., England and Churchill Chiller Thermo Circulator, product of Churchill Instrument Co. Ltd., Privale, Middx, England. Centrifuge was a product of Hettich Zentrifugen – Universal 320, D-78532 Tuttlingen, Germany and ERBA Mannheim XL-600 Automated Random-Access Clinical Chemistry Analyzer, manufactured by ERBA Diagnostics Mannheim GmbH, Mallaustrasse 69-73, 68219 Mannheim, Germany.

Collection and identification of plant materials

The roots of *Plumbago zeylanica* were collected at Babajakan village, Ayedande Local Government Area, Osun State, Nigeria in the months of June-August, 2010 and 2011. The plant identification and authentication has been done as reported by Olagunju *et al.* (2006) and Mr. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The voucher specimen has been deposited at the IFE Herbarium with code QC 488.

Preparation of methanolic extract of *P. zeylanica*

Roots were removed from the stalks and gently but thoroughly washed with tap water to remove sands. Cleansed roots were spread on black polythene on the laboratory table for 7 days to air-dry. It was further oven-dried completely at 35ºC overnight. This was then crushed to powder with a local grinder. The powdered *P. zeylanica* root (700 g) was soaked in 2.6 L 70% (v/v) methanol for 48 hours and then filtered with a piece of white nylon cloth. The residue was re-extracted five more times with the same solvent and time duration till the extract became colourless. The filtrates were combined and allowed to settle and decanted to obtain a clear solution of extract. The filtrates were pooled together and evaporated to complete dryness at 30ºC with rotary evaporator to obtain the methanolic extract of *P. zeylanica* (ME), a dark-brown residue.

Preparation of ethylacetate extract by solvent partitioning

Fractionation of crude ME by solvent partitioning was carried out according to a procedure based on the methods of Aruajo *et al.* 2011 [19] and Lin *et al.* 2003 [20]. 30 g of *P. zeylanica* extract (ME) was dissolved in 300 mL water: methanol (4:1) and poured into 1.0 L separating funnel. This was partitioned with 150 mL ethylacetate, shaken vigorously to allow the solvent systems to separate into two layers as ethylacetate is immiscible with water. Compounds soluble in the upper ethylacetate layer (ethylacetate being lighter than water) were collected and the lower aqueous layer was extracted three more times. All fractions of ethylacetate were pooled together and evaporated to complete dryness at 30 ºC with rotary evaporator to obtain ethylacetate fraction (EAME).

Evaluation of sub-chronic toxicity of ethylacetate fraction of *P. zeylanica* root

Animals

A total of thirty-two (32) healthy male and female albino rats (Wistar strain) weighing between 130 and 180 g used for this study, were purchased from the Animal House Unit, College of Medicine, University of Lagos, Idi-Araba, Surulere, lagos, Nigeria. The rats were housed in standard aluminium cages with saw-dust on its floor to absorb urine and feaces of animals, under clean environmental conditions (23 ± 1°C, with 80 ± 5% humidity and 12 h / 12 h light / dark cycles) and allowed to acclimatize for 14 days. They were fed with pelletized feed from Grand Cereals Nigeria Limited, a subsidiary of UAC Vital Feed, Km 17, Zawan Round about, Jos, Plateau State, Nigeria and tap water *ad libitum.*

Grouping and treatment of experimental animals

The thirty-two (32) rats were randomly distributed into four groups of eight rats per group. Group I served as Control and was administered orally using cannula with 1 mL 2% (v/v) Tween-20 (the vehicle). Groups II, III and IV were administered orally with 100, 200 and 400 mg/kg body weight EAME of *P. zeylanica* respectively. The extract was administered daily for 28 days. The animals were weighed before the commencement of administration and every 7 day of the experiment till the completion of extract administration. The amount of feed consumed was weighed daily while the level of water consumed was also measured daily till the end of administration. At the end of extract administration, the animals were fasted overnight.

Animal sacrifice and collection of samples

On the 29th day, rats were anaesthesized with chloroform. The rats were opened and blood samples were collected by cardiac puncture into a set of sample bottles containing heparin. The Blood samples were centrifuged at 5000 x g for 10 minutes using Hettich Zentrifugen – Universal 320, D-78532 Tuttlingen, Germany, to prepare the plasma meant for biochemical analyses. The animals were then dissected and organs (liver, lung and kidney) harvested for histopathological studies.

Determination of relative organ body weight ratio

Organs such as the heart, liver, lungs, spleen and kidneys were weighed and volumes were measured by simple water displacement. The relative

organ body weight ratio (ROW) was calculated using the formula below:

$$ROW = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}}$$

Estimation of biochemical parameters

Biochemical parameters were estimated in the blood plasma samples collected. These parameters are Total protein, Albumin, Glucose, Total bilirubin, Direct bilirubin, Creatinine, Urea, Uric acid, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma-glutamyltranferase (GGT), Triglycerides, Total cholesterol, High density lipoprotein-cholesterol (HDL-c), Low density lipoprotein-cholesterol (LDL-c) and Very low density lipoprotein-cholesterol (VLDL-c). The assay was conducted using the ERBA Mannheim kit (ERBA Diagnostics Mannheim GmbH, Mannheim, Germany) and ERBA Mannheim XL-600 Automated Random Access Clinical Chemistry Analyzer, manufactured by ERBA Diagnostics Mannheim GmbH, Mallaustrasse 69-73, 68219 Mannheim, Germany according to the instructions of the manufacturer.

Histopathological study of the organs

The histopathological study of organs (liver, kidney and lung) excised from the experimental rats were carried out. These organs harvested were first rinsed with water and fixed in 10% (v/v) formaline. They were then processed routinely by having a cut-up from the organs and the cut-up tissues was allowed to go through different alcohol concentration in ascending order (70%, 90%, 95%, and 100%) and then, xylene to remove alcohol and prepare the tissues for waxing. The tissues were embedded in paraffin wax and tissue sections were sliced at 5 microns using a microtome. The sliced sections were mounted on slides and stained with haematoxylin and eosin (HE).

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). The statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 20. The levels of homogeneity among the groups were assessed using Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Tukey HSD. Results were considered statistically significant when the value of $p<0.05$.

RESULTS

Body weight changes, feed and water consumption patterns

The effect of ethylacetate extract of *P. zeylanica* on weekly body weight change, feeding and water consumption patterns of rats in sub-chronic toxicity studies are summarized in Tables 1-3. There was no significant ($p>0.05$) weekly body weight gain in all treated rats compared to the control. The highest weight gain of 14.1% was observed in rats administered dose 200 mg/kg b.wt (week 3), 12.3% with dose 400 mg/kg b.wt (week 3) and 7.2% with 100 mg/kg b.wt dose (week 1) (Table 1). However, a statistically non-significant decrease in weight (28.7%) of the control animals was observed in week 4 whereas, the weight of treated animals increased marginally in the same week (Table 1). Compared to the control, the animals administered 100 and 200 mg/kg b.wt ethylacetate extract of *P. zeylanica* experienced decrease in weekly food consumption at weeks 2, 3 and 4 whereas, animals administered 400 mg/kg b.wt experienced an increase at week 2, 3 and 4 (Table 2). Generally, the weekly water consumption of all groups of animals (control and treated) was reduced. Highest reduction of 18.4% in control animals (week 4), 55.5% and 20.6% in animals given 100 mg/kg b.wt (week 2) and 200 mg/kg b.wt (week 4) doses respectively was observed (Table 3).

Organs weight

Table 4 is the summary of the relative organ weight of rats administered ethylacetate extract of *P. zeylanica* root in sub-chronic toxicity studies. Increase and decrease were observed in the relative organ weight of animals depending on the dose of ethylacetate extract and organ of the animals. The relative organ weight of liver and spleen increased by 170.3% and 84.8% respectively at 400 mg/kg b.wt whereas, the kidney and heart were reduced by 62% and 6.7% respectively at 400 mg/kg b.wt dose. In the lung, the relative organ weight increased by 56.9% at 100 mg/kg b.wt and reduced by 16.7% at 400 mg/kg b.wt compared with the control.

Biochemical parameters in sub-chronic toxicity studies

The effects of ethylacetate extract from methanolic extract of *P. zeylanica* root on plasma biochemical parameters in sub-chronic toxicity studies are presented in Figures 2-5. A significant increase ($p<0.05$) was observed compared with the control in the plasma concentrations of protein by 15.5% and 11.4% with doses 200 and 400 mg/kg b.wt (Figure 2a), albumin by 25.8% and 32.2% (Figure 2b). The plasma total bilirubin and direct bilirubin increased significantly by 6.5 and 1.5 folds respectively compared with the control in the highest dose of 400 mg/kg b.wt ethylacetate extract administered rats (Figures 2c and 2d). There was a steady but insignificant ($p>0.05$) increase observed in creatinine whereas, urea was increased at 400 mg/kgbwt dose by 4% and uric acid increased significantly at 100 mg/kg b.wt dose by 39% compared with the control (Figures 3a, b, c).

Enzymes activities in sub-chronic toxicity studies

The effects of the ethylacetate extract of *Plumbago zeylanica* on plasma enzymes activities in sub-chronic toxicity studies are depicted in Figures 4. A steady increase was observed in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) as the dose increased. At the highest dose of 400 mg/kg b.wt, the activities of AST increased significantly ($p<0.05$) by 6.3% (Figure 4a), ALT by 39.4% (Figure 4b), ALP by 75.5% (Figure 4c) whereas, GGT was increased by 46% (Figure 4d) when compared with the control.

Lipid profile in sub-chronic toxicity studies

The results of the effect of ethylacetate extract of *Pumbago zeylanica* on the lipids concentrations in sub-chronic toxicity studies are depicted in Figures 5. Plasma concentrations of total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c) were not significantly different ($p>0.05$) compared with the control. At dose 400 mg/kg b.wt, the increase in total cholesterol was 1.8% (Figure 5a), triglyceride was increased by 0.9% (Figure 5b), HDL-c by 11.2% (Figure 5c), LDL-c by 4.0% (Figure 5d) whereas, VLDL-c was decreased by 0.9% (Figure 5e) compared with the control.

Histopathological study

The histopathological effects of ethylacetate extract of *Plumbago zeylanica* in some organs of treated rats and control in sub-chronic toxicity studies are presented in Figures 6-8. The extract induced histopathological alterations in the cellular architecture of the liver and lung of treated rats compared with the control. Mild congestion in the sinusoid, presence of hepatic plate and bile duct proliferation were observed in the liver sections of treated rats. These alterations were pronounced in the liver of rats treated with extract doses of 200 and 400 mg/kg b.wt compared with the control (Figure 6). Likewise, necrosis of the alveoli wall, haemorrhage and oedema were observed in the lung sections of the treated animals compared with the control. The alterations were more prominent in the lung of rats treated with 200 and 400 mg/kg b.wt doses (Figure 7). Whereas, no histopathological alteration was observed in the kidney of treated rats as well as the control (Figure 8).

Table 1: Effect of ethylacetate extract of *P. zeylanica* on weekly body weight (g) of rats during sub-chronic toxicity studies

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Control	136.00±6.32[a]	139.98±5.81[a]	138.02±6.94[a]	95.86±3.47[a]
100mg/kgbwt	143.25±7.59[a]	138.33±7.60[a]	141.88±7.08[a]	138.16±5.42[b]
200mg/kgbwt	156.88±7.15[a]	160.34±7.56[a]	163.46±7.17[a]	152.86±6.93[b]
400mg/kgbwt	130.62±7.60[a]	137.31±7.91[a]	144.30±7.42[a]	137.17±1.78[b]

Values were expressed as Mean±SEM of 8 rats. Groups with different alphabets are significantly different at $p<0.05$.

Table 2: Effect of ethylacetate extract of *P. zeylanica* on weekly feeding (g) pattern of rats during sub-chronic toxicity studies

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Control	170.86±27.73[a]	337.97±39.65[a]	182.39±24.57[a]	157.17±21.66[a]
100mg/kgbwt	216.43±24.18[a]	143.06±39.02[b]	179.96±20.72[a]	163.27±17.80[a]
200mg/kgbwt	164.86±14.26[a]	139.46±26.55[b]	154.30±26.45[a]	129.04±16.45[a]
400mg/kgbwt	148.47±20.09[a]	228.67±33.49[a]	225.21±23.12[a]	205.66±28.33[a]

Values were expressed as Mean±SEM of 8 rats. Groups with different alphabets are significantly different at $p<0.05$.

Table 3: Effect of ethylacetate extract of *P. zeylanica* on weekly water (mL) consumption pattern of rats during sub-chronic toxicity studies

GROUP	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Control	99.66±17.74[a]	118.51±29.34[a]	95.57±17.86[a]	81.33±11.92[a]
100mg/kgbwt	226.29±16.64[b]	101.57±15.61[a]	144.79±43.79[a]	199.57±24.98[b]
200mg/kgbwt	197.57±11.31a	177.57±6.14[b]	181.00±9.99[a]	156.93±14.51[b]
400mg/kgbwt	128.89±12.58[b]	128.29±12.68[a]	122.14±17.84[a]	148.50±12.34[b]

Values were expressed as Mean±SEM of 8 rats. Groups with different alphabets are significantly different at $p<0.05$.

Table 4: Relative organ weight of rats administered ethylacetate extract of *P. zeylanica* in sub-chronic toxicity studies

GROUP	LIVER ($x10^{-3}$)	LUNG ($x10^{-3}$)	KIDNEY ($x10^{-3}$)	HEART ($x10^{-3}$)	SPLEEN ($x10^{-3}$)
Control	26.87±1.86[a]	9.00±0.68[a]	20.50±11.38[a]	3.88±0.23[a]	2.50±0.27[a]
100mg/kgbwt	38.50±1.79[a]	14.12±2.74[b]	8.25±0.41[a]	4.00±0.19[a]	4.75±0.53[b]
200mg/kgbwt	33.75±3.16[a]	8.62±1.02[a]	7.62±0.46[a]	3.88±0.23[a]	4.88±0.58[b]
400mg/kgbwt	72.63±38.20[a]	7.50±o.27[a]	7.75±0.31[a]	3.62±0.18[a]	4.62±0.32[b]

Values were expressed as Mean±SEM of 8 rats. Groups with different alphabets are significantly different at $p<0.05$.

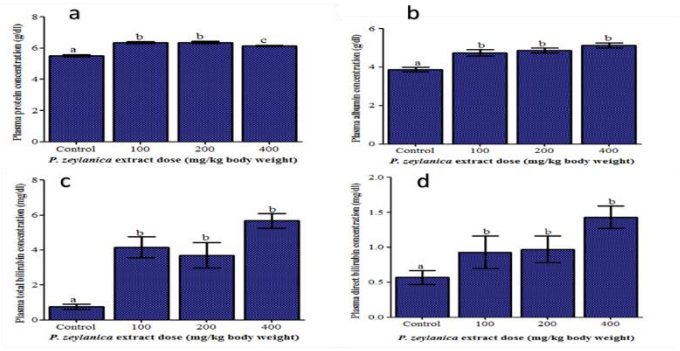

Figure 2: Effect of EAF on plasma concentrations of **(a)** Total protein **(b)** Albumin **(c)** Total bilirubin **(d)** Direct bilirubin in rats

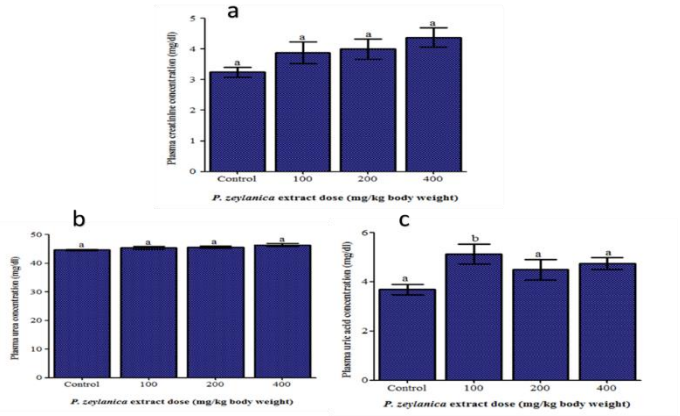

Figure 3: Effect of EAF on plasma concentrations of **(a)** Creatinine **(b**) Urea **(c)** Uric acids in rats

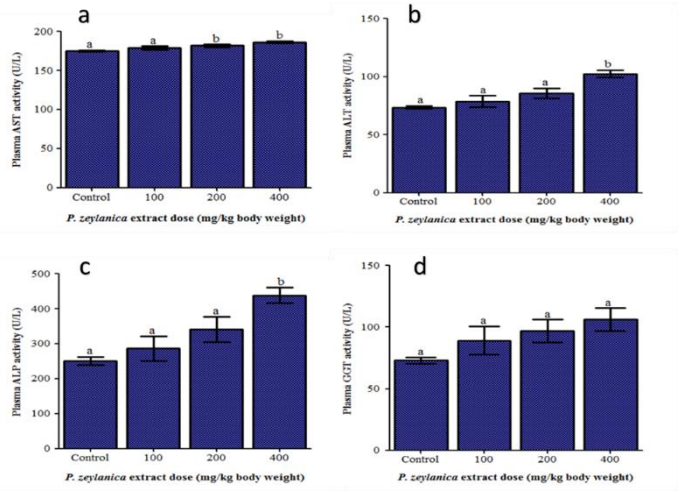

Figure 4: Effect of EAF on plasma enzyme activities of **(a)** AST **(b)** ALT **(c)** ALP **(d)** GGT in rats

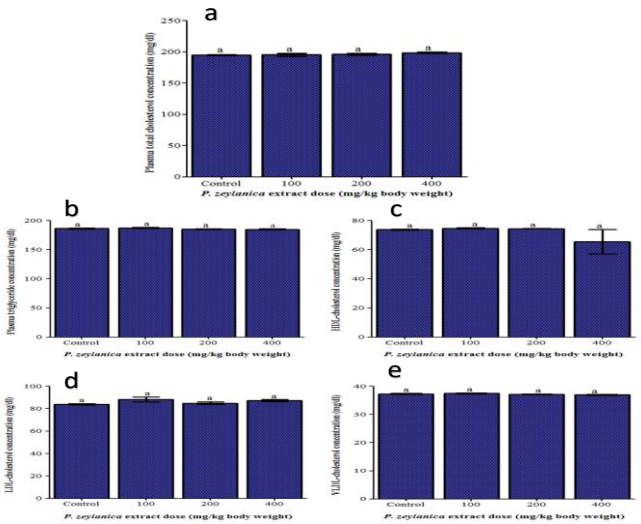

Figure 5: Effect of EAF on plasma lipid profile **(a)** Total cholesterol **(b)** Triglycerides **(c)** HDL-c **(d)** LDL-c **(e)** VLDL-c

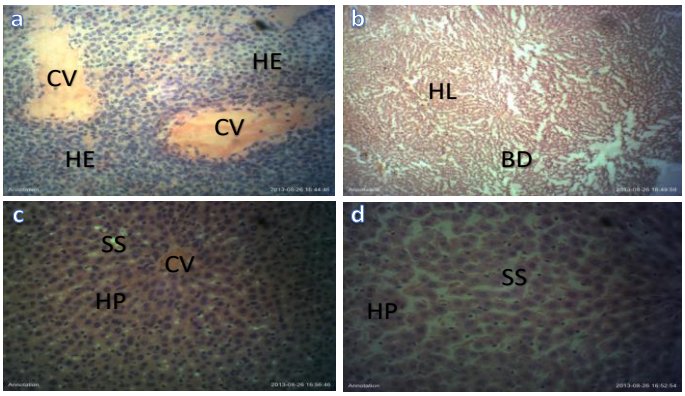

Figure 6: Effect of EAF on liver of rats **(a)** Control **(b)** 100 mg/kg b.wt **(c)** 200 mg/kg b.wt **(d)** 400 kg b.wt. CV (Cental veins), HE (Hepatocytes), HL (Hepatic lobules), BD (Bile ducts), SS (Sinusoids), HP (Hepatic plates) x 400.

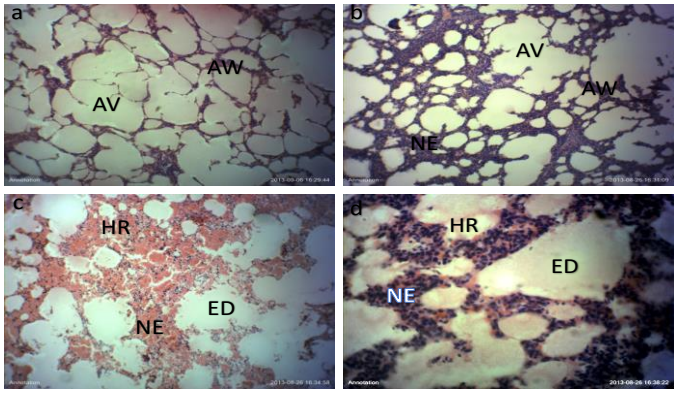

Figure 7: Effect of EAF on lung of rats **(a)** Control **(b)** 100 mg/kg b.wt. **(c)** 200 mg/kg b.wt. **(d)** 400 kg b.wt. AV (Aveoli), AW (Alveoli wall or membrane), NE (Necrotic membrane), HR (Haemorrhage), ED (Oedema) x 400.

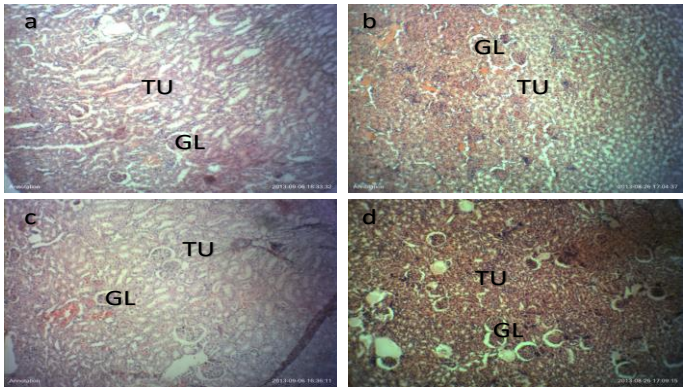

Figure 8: Effect of EAF on kidney of rats **(a)** Control **(b)** 100 mg/kg b.wt. **(c)** 200 mg/kg b.wt. **(d)** 400 mg/kg b.wt. GL (Glomerulus), TU (Tubule) x 400

DISCUSSION

A number of studies have reported the toxic effects of herbal medicines [21] and a slight body weight change serves as a sensitive indication of the general health status of animal [22]. In sub-chronic toxicity study, the decline in water and feed consumptions may contribute to the observed decrease in body weight. The organ-body weight ratio can be used to indicate organ swelling, atrophy or hypertrophy [23]. No effect was observed on heart-body weight ratio, the increase observed on liver, spleen and lung (100 mg/kg dose) suggests that the extract did caused inflammation at the cellular levels of these organs while the decrease observed in kidney-body weight ratio suggests constriction of the kidney at the cellular level.

Evaluation of biochemical parameters such as liver and kidney functions, as well as lipid profile of animals following the administration of chemical compounds, and/or plant extracts, plays important role in the evaluation of toxicity risk or safety of such compounds [24]. It could be inferred that increase in protein synthesis due to ingestion of a mixture of plant components especially saponin might be the means through which the treated rats compensated for the production of enzyme or protein lost as a result of necrosis or means to meet the increased demand of detoxification [25]. Elevation of protein concentration observed in this study contradicts another report where a significantly high reduction of 73.6% in the serum total protein level was recorded in rats treated with ethanolic extract of *P. zeylanica* [26]. However, the extract administered in this case was extracted with ethanol and administered intraperitoneally, while the present study utilized ethylacetate fraction of methanolic extract. It is not unlikely that the composition of the active principles will be different and one would expect to have different responses triggered in the animal.

Animal plasma normally contains 25-35 gm/L of albumin which constitutes 40 -60% of the total protein concentration. It is responsible for transportation of fatty acids and bilirubin through the blood stream to the liver. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM [27]. However, fluid may accumulate with higher albumin concentrations if hypertension, and loss of vessel integrity, are present. The significant elevation in the plasma albumin of treated rats may have contributed immensely to the increased level of protein concentration observed as well as affected the transportation of materials within the blood stream.

There was significant elevation of plasma total sugar level in treated rats as the concentration of ethylacetate extract increased compared with the control in this study. Oyedapo and Amos had earlier reported an increase in serum blood sugar when crude extract of *P. zeylanica* was administered orally to rats [28] whereas, Zarmouth *et al*. 2010 observed a plasma glucose lowering effect in diabetic-induced rats [29]. The increase in the blood glucose could be due to enhanced gluconeogenesis and reduction in the activity of key enzymes of glycolysis [30].

Liver plays a major role in metabolism and has a number of functions in the body which includes glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and excretion of waste materials [25]. Damage to the liver could be confirmed by changes in the activities of hepatic enzymes in serum or plasma by their increased or decreased synthesis, released from damaged cells and extrahepatic tissues [31]. Any significant change in the activities of plasma and liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and bilirubin levels are good indices for detecting liver damage [24, 32]. In this study, the activities of plasma aspartate aminotransferase (AST), Alanine aminotransferase (ALT) were elevated in treated rats compared with the control. The activities of alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were also increased. An increased serum alanine aminotransferase (ALT) activity is suggestive of hepatocellular damage and is specific in liver diseases [27]. In addition to these marker enzymes of hepatocellular injury, measurement of serum levels of protein, albumin and bilirubin are considered as adjunct markers of acute or chronic hepatic damage. Significant increase in plasma total bilirubin and direct bilirubin concentrations were observed in treated rats compared with the control. The observed increase in protein, albumin and bilirubin may be the result of selective damage caused to the liver tissue.

Renal function indices such as creatinine, urea and uric acid can be used to evaluate the functional capacity of the kidney nephrons of animals [33]. The increase in creatinine was not significant whereas increase in urea and uric acid were significant at 400 mg/kgbwt and 100 mg/kgbwt doses respectively in treated rats. Creatinine, urea and uric acid are major catabolic products of muscle, protein and purine metabolism respectively. The increase in these parameters might probably be due to impairment in the renal functional capacity.

The important lipids whose elevations are implicated in cardiovascular diseases (CVD) are cholesterol and glycerides. Elevation of low-density cholesterol, triglycerides and total cholesterol with reduced high-density lipoprotein-cholesterol (HDL-c) enhances the development of antherosclerosis and cerebrovascular disorders [34]. No change was observed in the levels of total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c) in ethylacetate extract treated rats compared to the control. Therefore, the observed lipid profile in this study shows that oral administration of the ethylacetate extract of *P. zeylanica* may not be seriously associated with the risk of antherosclerosis or cardiovascular diseases (CVD).

Based on the histopathological study of the liver, lung and kidney of control and treated rats, there was no cellular architectural changes observed in control rats and kidney of treated rats. However, the liver of treated rats showed mild hepatic degeneration due to bile duct proliferation and sinusoidal congestion. Likewise, the lung of treated rats showed gross alterations in the cellular architecture as a result of observed necrosis of alveoli walls, haemorrhage and oedema which is more noticeable at 200 and 400 mg/kg bwt doses. Functional studies in toxicology coupled with the appropriate histological studies were said to be useful, especially during anatomical localization of the action of a toxin [35]. These histopathological damages could be responsible for the alterations in the biochemical and haematological parameters observed.

CONCLUSION

Administration of ethylacetate extract of *P. zeylanica* resulted in elevation of plasma glucose level while it has no significant effect on lipid metabolism. Ethylacetate extract was slightly toxic with 400 mg/kgbwt or less dose when administrered orally. The mild cellular alterations observed in liver and lung corroborated the biochemical and haematological changes found in the investigated experimental animals.

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