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EVALUATION OF THE TOXICITY OF PHYLLANTHUS AMARUS IN WISTAR ALBINO RATS

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ABSTRACT

Aim: The study evaluated the acute and sub acute toxicity of the aqueous leaf extract of Phyllathus *amarus* Schum. and Thonn using Wistar albino rat.

Methods: Sixty-one adult Wistar rats were grouped four groups with ten rats per group for acute study. Rats in group 2, 3 and 4 received 2000mg/kg, 4000mg/kg, and 8000mg/kg of the extract respectively. For sub acute study, rats were divided into three groups with seven per group. Rats in groups 2 and 3 received 2000mg/kg and 4000mg/kg of the extract respectively. Blood was obtained by cardiac puncture. The heart, liver and kidney of each rat were also excised, weighed and used for biochemical and histopathological analysis.

Results: The acute toxicity study revealed that the oral administration of the extract was safe up to the dose level of 8,000mg/kg. Besides the significant ($P<0.05$) reduction in plasma ALT and significant ($P<0.05$) increase in kidney AST 48hrs after acute exposure and significant reduction in plasma ALP in sub-acute exposure, no abnormality in plasma and tissue biochemical markers was observed. There was non-effect on the renal function indices but increase in WBC after sub-acute exposure. The gross and histopathological examination of the kidney and liver after both acute and sub acute study showed minor abnormalities.

Conclusion: Therefore, it can be concluded that *P.amarus* aqueous extract is safe, as oral administration has no adverse effect on rat liver and kidney.

Keywords: Acute, subacute toxicity, *Phyllanthus amarus*, Medicinal plant

INTRODUCTION

Medicinal plants have been used all over the world for treatment and prevention of various sicknesses in both developed and developing world. Herbal medicines are believed to be safer than synthetic medicine because phytochemicals in the plant extract target its biochemical pathways (Zaiden et al., 2005;Sandigawqd, 2015) This believe in the safety of medicinal plants have resulted in the indiscriminate use of plants by the public however, the incidence of adverse effects and toxicity resulting from plant remedies have been reported (Chan, 2003). Therefore, it is important to determine the toxicity profile of medicinal plants. *Phyllanthus* has been used in folk remedies; therefore this genus is of great importance in traditional medicine (Foo, 1993). The genus *Phyllanthus* has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal, parasites. Species like *P. amarus* Schum. and Thonn., *P. niruri* L.and *P. urinaria* L. are used in the treatment of kidney/gallstones, other kidney related problems, appendix, inflammation and prostrate problems (Heyde, 1990). Extracts obtained from *Phyllanthus amarus* has been used as blood purifiers, boiled leaves is considered to be diuretic and therefore used in treating diabetes, dysentery, menstrual disorders and skin disorders (Heyde, 1990). The young shoots of plant are administered in the form of an infusion for the treatment of chronic dysentery. Fresh leaf paste has wound healing capacity and used to cure white spots on skin and jaundice. The stem juice is also used as wound healers. The whole plant extract is used

in urinary problems and swelling of liver. The root extract is used to cure stomach pain. The flower paste of plant is applied externally as antidote against snake bite (Adegoke et al., 2010,;Zingare,2013). Due to the many application of this plant in ethnomedicine,it is therefore important to investigate the toxicity profile of *P. amarus* it is in this respect that the acute and sub acute toxicity studies was carried out on *P. amarus* aqueous leaf extract.

MATERIALS

Ethical Consideration

Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) and all NIH Guide for Care and Use of Laboratory Animals (Pub. no, 85-23, revised 1985). The experimental protocol was approved by the institutions ethical committee for the use of laboratory animals.

Preparation of leaf extract

The leaves of *Phyllanthus amarus* Schum and Thonn were collected from their natural habitat in Abraka, Delta State. The plant was identified in the Department of Botany, Delta State University, Abraka. Plant leaves were washed with distilled water and then air dried. The aqueous extract of the plant was prepared by weighing 200g of dried powdered leaves, soaked in 2 litres of sterile distilled water and heated in a water bath at 80^0C for 1 hour. The crude extract was filtered with Whatman No. 1 Filter paper and evaporated in vacuo at 40^oC using rotary evaporator. The dried extract was obtained by further heating using oven at temperature 35^oC. The weight of extract was noted and labeled and then kept at 4^oC until required.

Experimental design

Sixty-one female albino rats (Wistar strain) weighing between 70-230g were divided into ten per group. Rats in group 2, 3 and 4 received 2000mg/kg, 4000mg/kg and 8000mg/kg of the extract respectively. The control rats in group 1 received an equal volume of distilled water/kg body weight. Prior to the administration of extract, the rats were starved of food but not water overnight. After administration of extracts food was withheld for another period of three hours. Following the treatments the rats were allowed free access to food and water. The initial and final body weights of the rats in each group were also determined. The extract was administered once orally thereafter, rats were sacrificed at the end of 12hours, 24hours and 48hours after the period of treatment. In sub-acute study, the rats were divided into seven rats per group, rats in group 2 and 3 received 2000mg/kg and 4000mg/kg of the extract respectively. The control group 1 did not receive any treatment. The modification of Taziebou et al. (2007) was adopted; extract was administered to the group once in three days until the twenty eight day. In both acute and sub acute, the extracts were administered with oral cannula and then the rats were sacrificed at the end of 28th day.

Collection of Tissue

The thoracic and abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture by means of a 5ml hypodermic syringe and needle into lithium heparin bottle. Plasma was obtained from the blood by centrifugation at 3000 rpm for 5 minutes. The heart, liver and kidneys of each rat were also excised, weighed and used for biochemical analysis.

Preparation of Tissue Homogenate

Ten percent (10%) homogenates of the organs were prepared by homogenizing appropriate weight of the organs in ice-cold normal saline. The homogenates were centrifuged at 2000 rpm for 15 minutes and the supernatant obtained were used for biochemical analysis.

Biochemical Assays

The plasma, and the homogenates of kidney, heart and liver of each rat was analyzed for activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase. The creatine, creatinine kinase, and urea concentrations were also estimated in the plasma. The L-Alanine aminotransferase (L-ALT) and The L-Aspartate Aminotransferase (L-AST) activities in the plasma and tissue homogenates was estimated using Randox laboratory kit based on the method of Reitman and Frankel (1957).Alkaline phosphatase activity was determined using Randox Laboratory kit based on the method of Annino and Giese (1976).The plasma creatine levels was determined by the method of Bonsnes and Taussky (1945). The urea was measured using the Urease-Berthelot colorimeter method which is based on the fact that urea in tissue is hydrolyzed to ammonia in the presence of urease. The creatinine kinase was measured by using the standard biuret method which is based on the reaction between creatine phosphate and adenine-5'-diphosphate (ADP). The

measurements were done using Randox commercial kit,

Haematological Parameters
Packed Cell Volume (PCV), Red Blood Cell (RBC), haemoglobin (HB), Total and Differential White Blood Cell (WBC)counts were determined according to standard methods.

Preparation of Tissue for Microscopic Examination
The liver and kidney tissues were dehydrated by being passed from low concentration (70%) to high concentration (100%) of alcohol, cleared in xylene for 1.30 min and embedded in paraffin wax. The sections of liver and kidney were cut in 5micronsand mounted on slides. The slides were stained with heamatoxylin and eosin, and viewed under light microscopy.

Statistical Analysis
The values are reported as mean±SEM. Statistical difference in the means where determined using analysis of variance (ANOVA). The significant level was 0.05.

RESULTS

Clinical Signs and Mortality Patterns

There was no overt signs or death observed in rats 48hours after post oral (p.o.) treatment with 2000mg/kg, 4000mg/kg and 8000mg/kg doses of *P. amarus* aqueous leaf extract. The oral median lethal dose (LD50) of the extract in rats was therefore greater than 8000mg/kg p.o During the period of treatment, all the animals were observed daily for clinical signs and mortality pattern once before dosing and immediately after dosing and up to 4hours after dosing. There was no record of death after dosing and 48hours after dosing. Restlessness, large food intake was observed, however, in animal treated with 8000mg/kg of extract that received the highest dose.

Organ/Body Weight Ratio and Body Weight Gain
The effect of *P.amarus* on organ/body weight ratio and body weight gain of rats is presented in Table 1.No significant difference was observed in body weight gain of rats after 48 hours post administration of *P. amarus* extract. Similarly, no significant difference was observed in the organ/body weight ratio for heart, liver and kidney for the specified periods after acute exposure to *P. amarus* extract.

Table 1: Body weight gain and Relative organ weight percent of rats after exposure to acute doses of *P. amarus*

Test Amount (groups	Weight gain (g)	Relative Heart weight (%	Relative Liver weight (%)	RelativeKidney weight (%).
Control	15.5±4.15 [a]	0.53 ±0.04[a]	4.85± 0.19 [a]	0.74± 0.606[a]
Group 1	13.3± 1.06 [a]	0.53 ±0.02 [a]	5,31 ±0.24 [a]	0.8±0 0.57 [a]
Group 2	11.24 ±1.72	0.45± 0.02 [a]	5.0±4 0.66 [a]	0.84 0.18 [a]
Group3	16.16 ± 2.083 [a]	0.39 0±.05	4.10 ± 0.53[a]	0.40± 0.02[b]

The values are reported as mean±SEM. Statistical difference was determined using analysis of variance (ANOVA). The significant level was 0.05.
Means of the same column followed by different letters differ significantly (P<0.05)

Table 2: Effects of aqueous extract of *P. amarus* on haematological indices of albinoWistar rats over time

	HAEMATOLOGICAL INDICES			
Cell type	**Control**	**Group 1***	**Group 2****	**Group 3*****
RBC (10^6mm^3)	12.3±3.21[a]	3.83±49.07[a]	6.23±4.42[a]	6.83±2.36[a]
WBC (10^2mm^3)	59.0±3.61[a]	53.7±6.35[a]	61.0±10.15[a]	69.33±6.03[a]
PCV (%)	4.33±1.53[a]	45.7±1.53[a]	40.7±3.05[a]	44.3±2.1[a]
HB (g/100ml)	14.9±0.75[a]	14.6±1.49[a]	13.7±1.65[a]	13.5±1.50[a]
Differential (Neutrophils)	59.3±0.58[a]	59.7±0.58[a]	58.0±1.00[b]	6.00±0.00[a]
Differential (Lymphocytes)	40.3±0.58[a]	40.0±0.00[a]	40.0±0.00[a]	39.3±0.58[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05). Concentration of extract applied (mg/ml) *2000mg/kg **4000mg/kg ***8000mg/kg

Table 3.1 : Effect of aqueous *P. amarus* on aspartate transaminase activity in plasma and organs of albino Wistar rats after acute exposure over time

	Aspartate Transaminase Activity (u/ml)			
Organs	**Control**	**Group1***	**Group2****	**Group 3*****
Plasma	49.3±2.51[a]	47.3±6.43[a]	32.7±13.58[b]	36.3±5.51[a]
Heart	2030.00±4.58[a]	2219.00±2.89[a]	2310.00±7.21[a]	2779.00±8.50[a]
Kidney	2520.00±5.29[a]	3428.00±10.69[b]	3572.00±18.48[b]	3708.00±6.35[b]
Liver	2344.00±1.53[a]	2584.00±20.03[a]	3896.00±38.94[a]	1624.00±11.85[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05). Concentration of extract applied (mg/ml) *2000mg/kg **4000mg/kg ***8000mg/kg

Table 3.2: Effect of aqueous extract of *P. amarus* on alanine transaminase activity in plasma and organs of albino Wistar rats after acute exposure

	Alanine Transaminase Activity (u/ml)			
Organs	**Control**	**Group1***	**Group2****	**Group 3*****
Plasma	25.3±2.52[a]	18.0±1.00[b]	16.3±1.15[b]	18.0±1.73[b]
Heart	2030.00±4.58[a]	2219.00±2.89[a]	2310.00±7.21[a]	2779.00±8.30[a]
Kidney	748.00±6.11[a]	732.00±6.66[a]	748.00±12.66[a]	1308.00±2.31[a]
Liver	2376.00±4.04[a]	2080.00±6.00[a]	1976.00±4.63[a]	2136.00±6.43[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05). Concentration of extract applied (mg/ml) *2000mg/kg **4000mg/kg ***8000mg/kg

Table 3.3: Effect of aqueous *P. amarus* on alkaline phosphatase activity in Plasma and organs of albino Wistar rats after acute exposure over time

	Alkaline Phosphatase Activity (u/ml)			
Organ	**Control**	**Group1***	**Group2****	**Group 3*****
Plasma	217.7±53.11[a]	204.3±49.41[a]	234.3±41.67[a]	180.0±22.11[a]
Heart	1330.00±3.61[a]	1281.00±4.73[a]	1281.00±11.72[a]	630.00±3.61[a]
Kidney	12512.00±8.41[a]	10448.00±55.49[a]	11800.00±24.93[a]	11992.00±63.80[a]
Liver	426.80±2.082[a]	280.00±2.000[a]	386.80±3.512[a]	226.80±2.887[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05). Concentration of extract applied (mg/ml) *2000mg/kg **4000mg/kg ***8000mg/kg

Table 3.4: Effect of aqueous extract of *P. amarus* on renal function indices

Parameter	**Control**	**Group1***	**Group2****	**Group 3*****
Plasma urea (mg/dL)	67.9±4.52[a]	40.9±9.09[b]	37.9±7.08[b]	37.5±7.82[b]
Plasma Creatinine(mg/dL)	0.77±0.058[a]	0.64±0.151[a]	1.11±0.693[a]	0.62±0.157[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05). Concentration of extract applied (mg/ml) *2000mg/kg **4000mg/kg ***8000mg/kg

Table 3.5: Effect of aqueous extract of *P.amarus* on plasma and heart creatine kinase activity

Parameter	**Control**	**Group1***	**Group2****	**Group 3*****
PlasmaCreatinine Kinase(u/ml)	21.7±7.02[a]	29.3±25.01[a]	87.3±2.08[b]	52.3±6.66[b]
Heart Creatinine Kinase(u/ml)	528.7±123.25[a]	750.7±469.03[a]	678.7±3.58.89[a]	2420.00±198.02[a]

* Results expressed as mean ± SEM. Means of the same row followed by different letters differ significantly (P<0.05). * 2000mg/kg ** 4000mg/kg *** 8000mg/kg

Histopathological Examination

The histopathological changes in the organs of rats treated with the extract (2000mg/kg, 4000mg/kg and 8000mg/kg) are presented in figure 1 to 4(liver) and figure 5 to 8 (kidney). There was no abnormality seen in the morphologies /features and consistencies in liver and kidney of the rats treated with the various extract concentrations

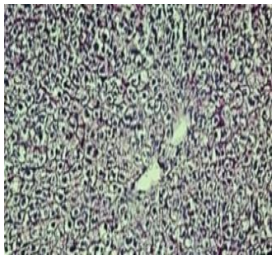


Figure 1 Control

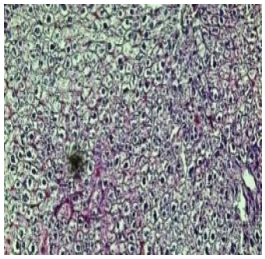


Figure 2Group. 2

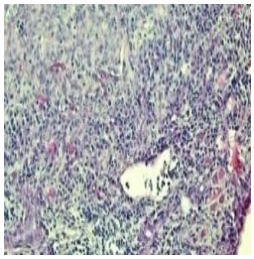


Figure 2 Group. 2

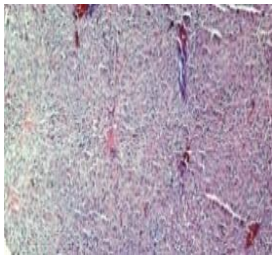


Figure 4Group 4

Figures 1 to 4 show photomicrographs of liver cells of rats treated with 2000mg/kg, 4000mg/kg and 8000mg/kg of aqueous extract of *P. amarus* sacrificed after 48 hours after treatment (H&E×100). Figure 1 is control treated with distilled water. No significant morphological changes.

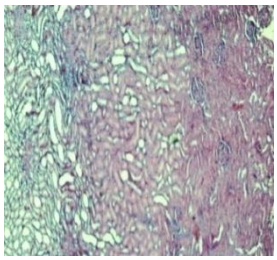
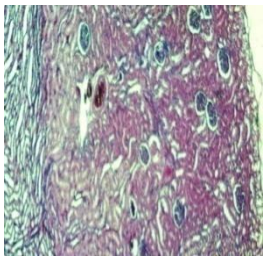


Figure 5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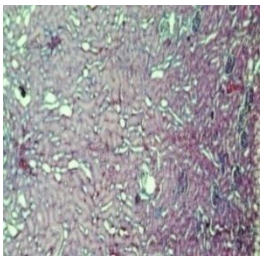
Figure 7

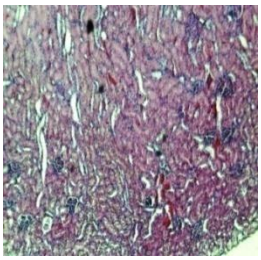
Figure 8

Acute toxicity test kidney tissues

Figures 5 to 8 show photomicrographs of kidney cells of rats treated with 2000mg/kg, 4000mg/kg and 8000mg/kg of water extract of *P. amarus* sacrificed after 48 hours after treatment (H&E×100). Figure 5 is control treated with distilled water. No significant morphological changes observed.

Sub-Acute Study

Clinical Signs and Mortality Patterns

No adverse clinical signs or toxicity sign was observed in all the rats during 28days treatment. No mortality was recorded in all the groups.

Table 4.2: Body weight gain and organ/bodyweight ratio of rats after sub acute exposure to *P.amarus*

		Ratio		
Test Amount (groups)	**Weight gain(g)**	**Heart: body weight**	**Kidney:body weight**	**Liver: body weight**
Control	1.67±1.32[a]	0.76±3.60[a]	7.17±0.27[a]	26.62± 5.82[a]
Group 1 2000mg/kg	-16.04±3.16[b]	1.28±3.28[a]	6.27±0.29[a]	34.62±1.66[a]
Group 2 4000mg/kg	2.56±2.42[a]	1.13±2.78[a]	6.21±0.69[a]	35.81±1.66[a]

* Results expressed as mean ± SEM Means of the same column followed by different letters differ significantly ($P<0.05$)

Table 4.3 : Effects of aqueous extract of *P. amarus* extract on the haematological indices of albino Wistar rats after sub acute exposure.

	Control	Group1*	Group2**
PCV (%)	35.71± ±2.63[a]	41.43±5.88[b]	39.14±1.95 [a]
WBC (102mm3)	2055.99±354.99[a]	12221.43±7305.35[b]	10392.86±4881.63.5[b]
RBC (mm3)	736.43±237.08 [a]	896.71±314.6 [a]	929.57±150.85 [a]
HB (g/100ml)	13.21±.59 [a]	14.41±2.65 [a]	12.67±1.74 [a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05) * 2000mg/kg ** 4000mg/kg

Table 4.4.1 : Effect of aqueous extract of *P. amarus* on plasma and tissue ALP activity of rats after sub acute exposure

	Control	Group1*	Group 2**
Liver ALP (u/ml)	58228±138.49[a]	55064.8±295.77[a]	55515.2±335.63[a]
Heart ALP	17131.8±68.62[a]	18187.4±153.03[a]	18657.8±100.24[a]
Kidney ALP	1829.6±29.46[a]	1562.4±24.31[a]	1984.423.01[a]
Plasma ALP	452.25±123.47[a]	263.78±39.128[b]	308.73±125.91[b]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05) * 2000mg/kg ** 4000mg/kg

Table 4.4.2:Effects of aqueous extract of *P. amarus* extract on plasma AST activity of rats after sub acute exposure.

	Control	Group 1*	Group 2**
Kidney AST (u/ml)	1862.8±7.91	1994.4±7.44	2040±9.78
Heart AST (u/ml)	2240±15.23[a]	3019.8±13.74[a]	2039.8±5.11[a]
Liver AST (u/ml)	5474.4±7.34[a]	5200.00±13.14[a]	1222.4±1.38[b]
Plasma AST (u/ml)	23.71±7.158[a]	32.14±12.46[a]	23.86±3.38[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05) * 2000mg/kg ** 4000mg/kg

Table 4.4.3: Effects of aqueous extract of *P. amarus* extract on plasma and tissue ALT activity of rats after sub acute exposure.

	Control	Group 1*	Group 2**
Kidney ALT (u/ml)	645.6±3.57[a]	588±2.69[b]	708±2.81[a]
Liver ALT (u/ml)	2731±4.84[a]	2914.43±4.72[a]	2714.4±5.11[a]
HEART ALT (u/ml)	1229.92±3.10[a]	1519.7±4.34[b]	1419.6±1.38[b]
Plasma ALT (u/ml)	24.43±1.98[a]	25.71±4.88[a]	23.86±3.39[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05) * 2000mg/kg ** 4000mg/kg

Table 4.4.4 : Effects of aqueous extract of *P. amarus* on levels of plasma urea, creatinine andcreatinine kinase and heart creatinine kinase of albino rats after subacute exposure. .

	Control	Group1*	Group2**
Plasma creatinine (mg/dL)	0.90±0.22 [a]	0.86±0.99 [a]	0.99±1.0[a]
Plasma urea (mg/dL)	60.16±11.53 [a]	56.56±15.216[a]	51.73±10..08[a]
Plasma creatine kinase (u/ml)	83.13±51.10 [a]	73.11±46.12[a]	51.29±21.95[a]
Heart creatine kinase (u/ml)	1054.74±195.72[a]	897.92±286.32[a]	976.95±265.19[a]

* Results expressed as mean ± SEM Means of the same row followed by different letters differ significantly (P<0.05) * 2000mg/kg ** 4000mg/kg

Histopathological Examination

The histopathological changes in the kidney and liver tissues are presented in figures 9-14. No gross abnormality was observed in the morphologies/features consistencies and appearance of the kidney and liver of rats treated with 2000mg/kg extract (Group 2) and 4000mg/kg extract (Group 3) for 28 days.

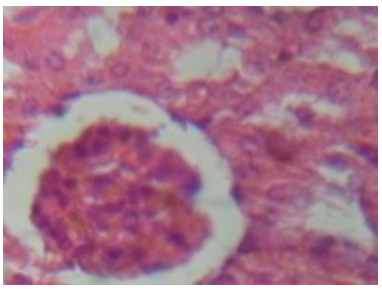

Figure 9 Control

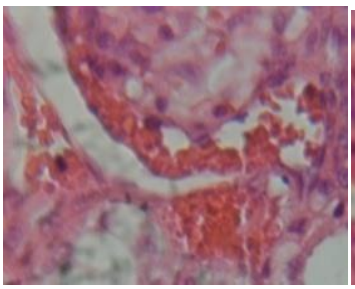

Figure 10 Group 2 (Low dose)

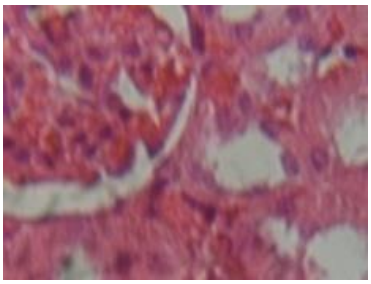

Figure 11 Group 3 (High dose)

Sub acute toxicity of kidney tissues

Figures 9 to 11 show photomicrographs of kidney cells of rats treated with 2000mg/kg (low dose), and 4000mg/kg (high dose) of water extract of *P. amarus* sacrificed after 28days of treatment (H&E×400) magnification). Figure 9 is control treated with distilled water. Cells are normal.

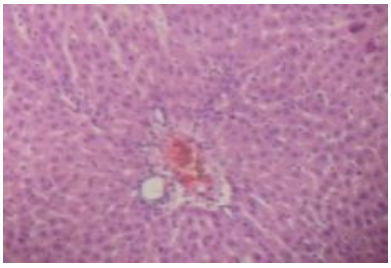

Figure 12 Control

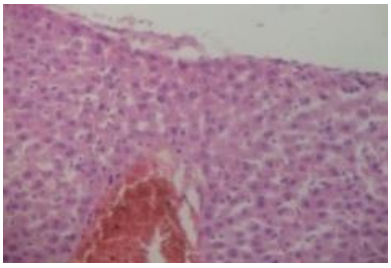

Figure 13 Group 2 (Low dose)

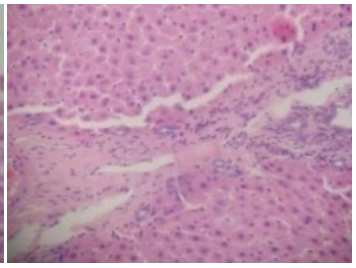

Figure 14 Group 3 (High dose)

Sub acute toxicity of liver tissues

Figures 12 to 14 show photomicrographs of kidney cells of rats treated with 2000mg/kg(low dose), and 4000mg/kg (high dose) of aqueous extract of *P. amarus* sacrificed after 28days of treatment (H&E×4 00) magnification). Figure 12 is control treated with distilled water. Cells are normal

DISCUSSION

Most herbs used in ethno medicine lack toxicological records. Though most herbs used are believed to be safe, compared to synthetic drugs, scientific evidence is lacking to this believe, hence the need to carry out the toxicological studies on *Phyllanthus amarus* leaves using albino Wistar rats. The oral LD50 of the extracts of *P. amarus* was estimated to be ≥ 8000mg/kg in rats, which is a likely indication that the extract is non-toxic. The absence of overt toxicity sign or death in rats after 48hours post oral treatment in this study support this claim. The organization of Economic cooperation and Development (OECD), Paris, recommended LD50 value of 50mg/kg as toxic;> 50≤500mg/kg as harmful and >500≤ 2000mg/kg as not harmful or toxic (Walum,1998). However it is noteworthy that LD50 has not been considered as a biological constant because many variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions and time of the day can all affect the LD50 value obtained. Hence, there are considered uncertainties in extrapolating the LD50 values obtained for a species to other species (Appidi *et al.*, 2009). The results obtained for bodyweight gain of rats indicate that no significant difference occurred in this parameter, in rats after acute exposure to various doses of the extracts(Table 1). Similarly, with exception of those administered at low dose, significant (P>0.05) difference was observed in the bodyweight gain of rats after subacute exposure to the extract (Table 4.2). Parallel analysis of the organ/body weight ratio for the liver, kidney and heart showed that this parameter was not significantly (P >0.05) altered after acute and sub acute exposure to various doses of the extracts. Changes in body weight and organ/body weight ratio have often been used as indices of toxicity of chemicals (Timbrell, 2002). Increase in organ/body weight ratio is an indication of inflammation, while reduction in the same parameter can be adduced to cellular

constriction(Moore and Dalley,1999). The non effect of *P. amarus* extract on bodyweight gain and organ/body weight ratio for the liver, kidney and heart in this study is also a likely indication that extract is non-toxic.The non-significant (P>0.05) effect of the extracts on haematological parameters at the end of acute exposure (Table 2) might be due to the fact that there was no destruction of matured red blood cells. Many plant extracts have been reported to cause anaemia by destruction of RBCs or by its decreased production in the bone marrow (Adedapo *et al*., 2007). The increase in WBC by the extract after subacute exposure (Table 4.3) may indicate boost in the immune system (Yakubu *et al*., 2007). It is therefore conceivable that *P. amarus* extract may have potential application as immunostimulants and could act against a broad spectrum of pathogenic microorganisms. Increased level of creatine kinase in the plasma of rats after acute exposure to the extract (Table 3.5) may be due to leakage from any of the internal organs (Panda, 1989). Creatine kinase is of clinical importance and its level are routinely used as an indicator of acute myocardial infarction (Nigam, 2007). However, parallel analysis of heart creatine kinase revealed no significant (P.>0.05) alteration in the level of the enzyme 48 hr after acute exposure to various doses of the extract (Table 3.5). This finding is a likely indication that the elevated plasma creatine kinase may not be due to myocardial infarction but increase activity occasioned by increased energy requirements by other organs other than the cardiac muscle(Williamann *et al*., 2011: Hornikova *et al*., 2009). As for rats sub acutely exposed to *P. amarus* extract, the plasma and heart creatine kinase activity were not significantly P>0.05) different from control (Table 4.4.4) . Thus the findings suggest that myocardial infarction may not have occurred in these rats. Certainly, histopathology of the heart would have lent credence to this finding.The liver is the primary place of biotransformation of xenobiotics which makes it vulnerable to xenobiotics (Lee, 1993: Sturgill and Lambert, 1999). Besides the significant (P<0.05) reduction in plasma ALT (Table 3.2) and significant (P<0.05) increase in kidney AST (Table 3.1), No abnormality in plasma and tissue biochemical markers for liver damage was observed 48hrs after acute exposure to the extract. Besides, the significant increase in heart ALT (Table 4.4.3) and significant reduction in plasma ALP (Table 4.4.1), no abnormality in plasma and tissue biochemical markers was observed after sub acute exposure to various doses of the extract.Thus liver damage did not occur after acute and sub acute exposure to the extract which further suggests that *P. amarus* extract may be safe for oral consumption. the non-effect of the extract of *P.amarus* on this renal function indices after acute (Table 3.4) and sub acute (Table 4.4.4) exposure may suggest that the normal functioning of the nephrons at the glomerulus level was not affected.The gross and histopathological examination of the kidney and liver after both acute and sub acute exposure to *P. amarus* extract showed minor abnormalities which further corroborate that hepatic and renal function indices were not impaired.

Summary and Conclusion

Acute and sub acute study on *P.amarus* extract on Wistar albino rats was carried out, no mortality or abnormality was observed in rats. Treatment ranged from 2000mg/kg to 8000mg/kg in acute study while 2000mg/kg and 4000mg/kg for sub acute study. Biochemical and histological results showed no toxic effect. Therefore, it can be concluded that *P.amarus* aqueous extract is safe, as oral administration has no adverse effect on rat liver and kidney.

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