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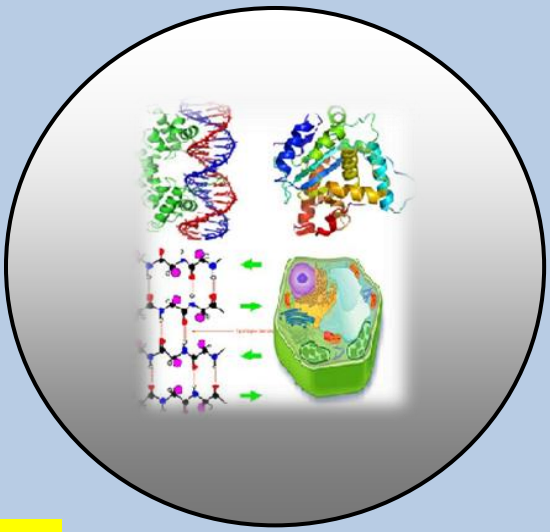

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Toxicological Implications of Aqueous Extract of *Phyllanthus muellerianus* Leaves in Albino Rats

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ABSTRACT

The possible safety/toxicological risk of the aqueous extract of Phyllanthus muellerianus leaves in albino rats were determined using physical and biochemical 'markers'.
There were significant (P<0.05) reduction in some key metabolites as portrayed by loss of Na^+, K^+, urea, bicarbonate, creatinine in the case of kidney and total protein and albumin in the case of liver . The significant reduction in the activities of liver alkaline phosphatase (ALP), gamma glutammyltransferase (GGT) and arginase further lend credence to the "possible injury" in the liver. The significant reduction in blood parameters (Hb, WBC, PCV, MCV and MCH) also attest to the possible negative effect of the consumption of the extract. Furthermore, the histoarchitecture of the injured liver as well as alterations in the biochemical parameters lends credence to the toxicity of the extract at 25.00 and 50.00 mg/kg body weight when administered twice daily, for seven days.
The kidney and the liver suffered "possible injury" from the consumption of the extract at 25.00 and 50.00 mg/kg body weight of the extract.
Keywords: Phyllanthus muellerianus leaves, Aqueous extract, Biochemical analysis, Haematological studies and Histopathological studies.

INTRODUCTION

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects occasionally caused by synthetic chemicals (Nafiu *et al*., 2011). For a variety of reasons, more individuals nowadays prefer to take personal control over their health with the use of herbal medicines, not only to prevent diseases but also treat them (Kincheloe, 1997). World Health Organization estimated that about three-quarters of the World's population currently use herbs and

other forms of traditional medicines to treat their diseases (Farnsworth *et al*., 1985; Nafiu *et al*., 2011). For example, Farnsworth *et al*., (1985) reported that 80% of the population in the developing World still relies on traditional medicine for their primary health care needs. However, with the upsurge in the use of herbal medicine, thorough scientific investigations of these plants are imperative in order to provide information on their safety and toxicity risk (Palambo, 2006; Nafiu *et al.,* 2011). One of such plants that is widely claimed in Traditional Medicine of Nigeria to be used in the management of several ailments such as diarrhoea, toothache, sexual dysfunction, chest pain, conjunctivitis, fever, paralysis, sore throat, urethritis, gonorrhea, wound (James, 2008); also used as an aphrodisiac, a purgative and as a snuff (David *et al*., 1990) and generally claimed to possess antimicrobial activities (Iwu, 1993; Onocha *et al*., 2003) without recourse to its safety or toxicity risk is *Phyllanthus muellerianus*. *P. muellerianus* otherwise known as Eegun eja Yoruba-Western Nigeria was formally classified as one of the flowering trees of spurge family Euphorbiaceae. However, a recent revision of classification has categorized *Phyllanthus* under the family Phyllanthaceae (Hoffman*, et al., 2006). P. muellerianus* is widely spread in tropical Africa (Burkill, 1994). Apart from Nigeria, it is also found in other countries like China, Cuba, Philippines, central and southern India (Adedapo *et al*., 2007). Although common in the tropics, the *Phyllanthus* specie of interest is generally often scattered in distribution (David *et al*., 1990). It has short, twig-like thorns, which are lined with rows of alternating leaves. It grows up to 23-26 metres high with many flowers and fruits of different colors. These acid-sour fruits are always present in clusters. It is bitter, astringent harsh or sharp in taste (David *et al*., 1990).

MATERIAL AND METHODS

The Plant

Phyllanthus muellerianus leaves were obtained near Government Day Secondary School, OkeAdinni, Ilorin, Kwara State, Nigeria. It was authenticated at Forestry Research Institute of Nigeria FRIN, Ibadan, where a voucher specimen FHI 108364 was deposited.

Experimental Animals

Adult, albino rats (*Rattus norvegicus*) of both sexes used in this study were obtained from the small Animal House of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Preparation of aqueous extract of *Phyllanthus muellerianus* leaves

The preparation was carried out following the method described by Adedapo *et al* (2007). The leaves of *P. muellerianus* were harvested as always freshly harvested by the traditional medicine practitioners after which 500 g was blended and 2.75 litres of distilled water added, allowed to stand for 48 hours but shaken intermittently with electric stirrer. The mixture was then filtered using Whatman No. 1 filter paper and the filterate was lyophilized with 1.5 litre ice capacity model of FS400-05 Freeze Dryer, Micromodulyo; USA . The lyophilized sample was reconstituted in distilled water to prepare different doses 12.50, 25 and 50 mg/kg body weight that were administered to different groups of animals.

Animal grouping and extract administration

Twenty (20) adult albino rats (*Rattus norvegicus*), of both sexes weighing 224.8 ± 15.8 g were randomly divided into four groups consisting of 5 rats each. Group I representing negative control was administered twice daily with 1.0 ml of distilled water.

Groups II, III and IV were administered with the same volume of extract containing 12.50, 25.00 and 50.00 mg/kg body weight twice daily. This administration lasted for seven days. On the 8^{th} day, the animals were anaesthesised with diethyether and the blood was collected; and organs kidney, liver and small intestine were aseptically removed for various toxicological studies.

Collection of blood and preparation of serum

The rats were anaesthesised with diethyether according to the method described by Akanji and Yakubu, (2002). Aliquots of the blood were collected and used for thedetermination of the haematological parameters as described by International Committee for Standardisation in Haematology, (ICSH 1980; Dacie and Lewis, 1991).

Blood sample was also collected into clean, dry sample bottles and centrifuged at 503 g X 15 minutes, then the sera were collected using Pasteur pipette Akanji and Ngaha, (1989). Serum sample was stored at 0^0 C and used within 12 hours of preparation for the biochemical analysis (Molomo, 2000).

Preparation of tissue homogenates

Tissue homogenates were prepared as described by Akanji and Yakubu, (2002).

Determination of protein concentration of homogenate

The method described by Plummer (1978) was used to determine the protein concentration of the homogenate.

Determination of bilirubin concentration

The method described by Nwanjo (2007) was adopted for the determination of bilirubin content in the animals.

Determination of albumin concentration

The method described by Doumas *et al* (1971) was used for the determination of albumin concentration in the serum of the animals.

Determination of electrolyte concentration

The method used for the determination of sodium and potassium is as described by Tietz (1995) using flame photometry.

Determination of urea concentration

The method described by Kaplan (1962) was used for the determination of serum urea concentration.

Determination of creatinine concentration

The method described by Blass *et al* (1974) was used for the determination of serum creatinine concentration.

Organ: body weight ratio

The rats were weighed before they were sacrificed. The liver, kidney and small intestine were excised, blotted with tissue paper and weighed. The organ: body weight ratios were calculated using the expression:

$$\% \text{ organ: body weight ratio} = \frac{\text{weight of the organ}}{\text{total weight of the animal}} \times 100$$

Assay of gamma-glutamyltransferase activity

The method of assay described by Tietz (1987) was used.

Assay of alkaline phosphatase activity

The method described by Wright *et al;* (1972) was used.

Assay of arginase activity

Arginase activity was determined using the method described by Davis and Mora (1968).

Histopathological study

The procedure described by Krause (2001) was used.

Statistical analysis

Data were expressed as means ± S.D of five determinations except otherwise stated. The statistical tools used were one-way analysis of variance (ANOVA) and Duncan Multiple Range Test. Differences were considered statistically significant at P < 0.05 (Mahajan, 1997).

RESULTS

At the end of the experimental period, there was a significant reduction (P<0.05) in the final body weights of rats treated with the extract when compared with distilled water treated rats. Compared with their initial body weight, the extract significantly reduced the final body weight of the animals. The organs liver, kidney and small intestine: body weight ratios of the extract treated animals were not significantly different from the control values (Table1).

Administration of aqueous extract of *P. muellerianus* leaves at 12.50, 25.00 and 50.00 mg/kg body weight significantly reduced the levels of Na^+, urea and creatinine in the serum of the animals. The administration of the extract did not significantly alter the levels of K^+ in the serum of the animals when compared to the distilled water treated animals (Table 2).

Aqueous extract of *P. muellerianus* leaves at 12.50, 25.00 and 50.00 mg/kg body weight significantly reduced the concentration of total protein, albumin and bilirubin in the serum of the animals, except the bilirubin of animals treated with 25.00 mg/kg body weight, which showed a significant increase when compared with the distilled water treated animals. The 12.50 mg/kg body weight of the extract reduced the total protein by 25.88% while 25.00 mg/kg body weight reduced it by 14.12%. The 50 mg/kg body weight of the extract reduced the total protein by 27.06%. Whereas the 12.50 and 25.00 mg/kg body weight of the extract reduced the albumin by 62.35 and 71.76% respectively, the 50.00 mg/kg body weight reduced the albumin by 68.24% (Table 3). The activities of ALP, GGT and arginase in the liver of rats treated with the aqueous extract of *P. muellerianus* leaves were significantly (P<0.05) reduced. The 12.50 and 25.00 mg/kg body weight of the extract reduced the ALP activity by 72.43 and 71.67% respectively while the 50 mg/kg body weight reduced it by 73.72%. Whereas the 12.50 and 25.00 mg/kg body weight of the extract reduced the GGT activity by 40.00 and 45.00% respectively, the 50.00 mg/kg body weight reduced the activity by 59.17% when compared with the distilled water treated animals. Furthermore, the 12.50, 25.00 and 50.00 mg/kg body weight of the extract reduced the activity of arginase by 69.09, 73.45 and 60.36% respectively compared with distilled water control (Table 3). Whereas the activities of ALP and arginase in the serum of extract treated animals were significantly increased, that of the GGT was significantly reduced compared with distilled control treated animals (Table 3). All the doses of the extract significantly reduced the levels of MCV, MCH and WBC when compared with the distilled water treated animals. The 12.50, 25.00, and 50.00 mg/kg body weight of the extract reduced the levels of MCV by 11.58, 4.50 and 26.04% while these doses also reduced the level of MCH by 8.65, 2.88 and 25.01%, respectively.

The 12.50 and 25.00 mg/kg body weight of the extract reduced the level of WBC by 32.12 and 18.65% respectively whereas 50.00 mg/kg body weight of the extract had 24.42% reduction level of WBC compared with the distilled water treated animals. The animals treated with 12.50 and 50.00 mg/kg body weight of the extract had the percentage reduction in the level of Hb by 10.49 and 23.26 respectively. Similarly, the 12.50 and 50.00 mg/kg body weight of the extract reduced the level of PCV by 9.49 and 25.01% respectively. The 50.00 mg/kg body weight of the extract slightly reduced the platelets level by 0.34%. However, when compared with distilled water control, the 12.50 mg/kg body weight of the extract increased the levels of RBC and platelets by 5.04 and 13.08% respectively. Similarly, the 25.00 mg/kg body weight of the extract increased the level of platelets by 6.69% compared with distilled water control. Furthermore, there were no significant difference in the levels of Hb and PCV of the animals treated with 25.00 mg/kg body weight of the extract when compared with the distilled water treated animal (Table 4).

Table 1: Organ-body weight ratios of rats administered with aqueous extract of *P. muellerianus* **leaves for seven days**

Treatment	Doses (mg/kg body weight)	Initial body Weight (g)	Final body Weight (g)	Change in weight (%)	Ratio		
					Liver	Kidney	Small Intestine
Water	Control	224.80±15.81^{a}	255.00±14.91^{a}	11.84‡	2.75±0.23^{a}	0.49±0.08^{a}	1.66±0.17^{a}
Extract	12.50	234.00±11.44^{d}	181.20±20.58^{c}	29.14†	2.64±0.65^{a}	0.49±0.10^{a}	1.65±0.50^{a}
	25.00	229.00±12.60^{c}	199.80±5.13^{b}	14.61†	2.67±0.22^{a}	0.45±0.04^{a}	1.65±0.31^{a}
	50.00	221.00±5.72^{b}	178.60±7.90^{d}	23.74†	2.66±0.45^{a}	0.49±0.07^{a}	1.66±0.18^{a}

Values are mean of five determinations ± SD

‡ Increase in the % of final body weight of rats in distilled water control group

† Decrease in the % of final body weight of rats treated with different concentrations of the extract

Values carrying different superscript from the control are significantly different (P<0.05).

Table2: Kidney function indices of rats administered with aqueous extract of *Phyllanthus muellerianus* **leaves**

Treatment	Doses (mg/kg body weight)	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	Urea	Creatinine
		mM/L				µmol/L	
Water	Control	40.20±3.18^{a}	1.20±0.09^{a}	18.20±0.30^{a}	27.60±0.24	3.00±0.00^{a}	44.60±1.47
Extract	12.50	31.60±0.67^{d}	1.30±0.07^{a}	15.00±0.40^{b}	20.40±0.40^{b}	2.50±0.00^{d}	17.40±1.47^{c}
	25.00	36.30±0.60^{c}	1.33±0.03^{a}	19.60±0.20^{c}	30.20±0.37^{c}	2.30±0.05^{c}	36.20±0.73^{b}
	50.00	39.20±1.39^{b}	1.20±0.06^{a}	19.40±0.20^{a}	30.40±0.24^{c}	2.10±0.05^{b}	30.80±0.73^{c}

Values are mean of five determinations ± SD

Values carrying different superscript from the control are significantly different (P<0.05).

Table 3: Liver function indices of rats administered with aqueous extract of *Phyllanthus muellerianus* leaves

Treatment	Doses (mg/kg body weight)	Total protein (g/L)	Albumin (g/L)	Bilirubin (g/L)	ALP Specific Activity (*nM/min/mg protein*)		GGT Specific Activity (*nM/min/mg protein*)		Arginase Specific Activity (U/l)	
					Liver	Serum	Liver	Serum	Liver	Serum
Water	Control	17.00±0.44^{a}	7.60±0.24^{a}	8.40±0.24^{b}	335.25±0.00^{a}	20.60±0.19^{a}	1.20±0.09^{a}	0.58±0.02^{a}	165.00±0.00^{a}	1.38±0.01^{a}
Extract	12.50	12.60±0.24^{c}	6.40±0.24^{b}	6.80±0.37^{a}	92.43±0.03^{c}	36.94±0.40^{b}	0.72±0.04^{b}	0.45±0.04^{b}	51.00±0.00^{c}	1.63±0.01^{b}
	25.00	14.60±0.24^{b}	4.80±0.37^{c}	11.40±0.24^{c}	94.98±0.00^{b}	36.11±0.00^{b}	0.66±0.04^{c}	0.59±0.0a	43.80±0.73^{d}	1.80±0.00^{b}
	50.00	12.40±0.60^{c}	5.40±0.24^{c}	8.60±0.24^{b}	88.09±0.28^{d}	35.09±0.98^{b}	0.49±0.04^{d}	4.33±0.40^{c}	65.40±1.47^{b}	1.36±0.04^{a}

Values are mean of five determinations ± SD
Values carrying different superscript from the control are significantly different P<0.05 .

Table 4: Heamatological parameters of rats administered with aqueous extract of *Phyllanthus muellerianus* leaves

Treatment	Doses (mg/Kg body weight)	Hb (g/dl)	PCV (%)	RBC (C/L)X 10^{12}	MCV (FL)	MCH (Pg)	MCHC (g/dl)	WBC (C/L) X 10^{9}	PLATELETS (C/L) X 10^{9}
Water	Control	12.77±0.37^{a}	38.67±0.67^{a}	3.77±0.09^{a}	103.67±3.67^{a}	34.67±1.67^{a}	33.67±0.33^{a}	5.20±0.60^{a}	970.00±10.00^{a}
Extract	12.50	11.43±0.47^{b}	35.00±0.0^{b}	3.97±0.04^{b}	91.67±2.67^{c}	31.67±0.67^{c}	34.00±0.00^{a}	3.53±0.33^{d}	1041.33±60.67^{b}
	25.00	13.10±0.10^{a}	38.67±0.33^{a}	3.90±.00^{ab}	99.00±1.00^{b}	33.67±0.33^{b}	34.00±0.00^{a}	4.23±0.03^{b}	1116.00±4.00^{c}
	50.00	9.80±0.00^{b}	29.00±0.00^{e}	3.80±0.06^{a}	76.67±0.67^{d}	26.00±0.00^{d}	34.00±0.00^{a}	3.93±0.13^{c}	966.67±3.33^{b}

Values are mean of five determinations ± SD
Values carrying different superscript from the control are significantly different (P<0.05).

Compared with the distilled water control, administration of the extract at all doses for seven days did not cause any histoarchitectural changes in the kidney as the glomeruli and tubules were intact. The arrowed spots show preserved medullary and cortical architecture as well as proximal and convoluted tubules with intervening loop of Henle (Fig. 1).

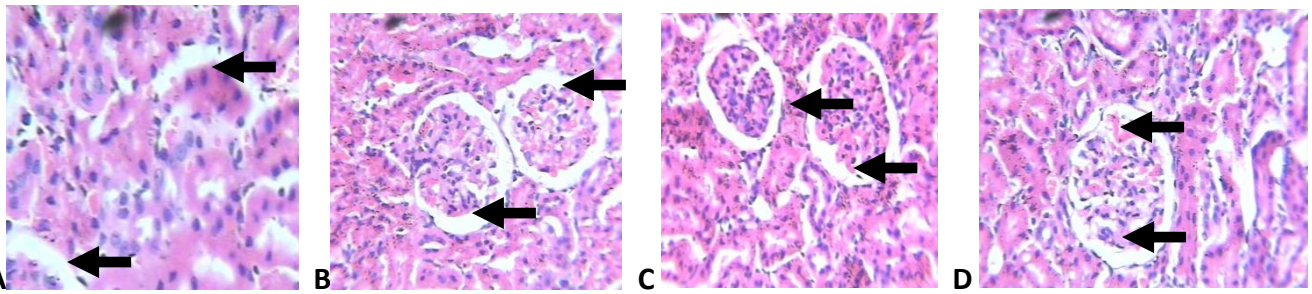

Fig. 1: Photomicrographs of the cross section of rat kidney orally administered with (A) distilled water control, (B) 12.50 mg/Kg, (C) 25 mg/Kg and (D) 50 mg/Kg body weight of aqueous extract of *P. muellerianus* leaves. (**A**) The arrows indicate intact glomeruli and tubules (x 400; H&E). (**B**) The arrows show normal architectural structure of the kidney (x 400; H&E). (**C**) The arrows show normal glomeruli and tubules (x 400; H & E). (**D**) The arrows show normal glomeruli and tubules (x 400; H & E).

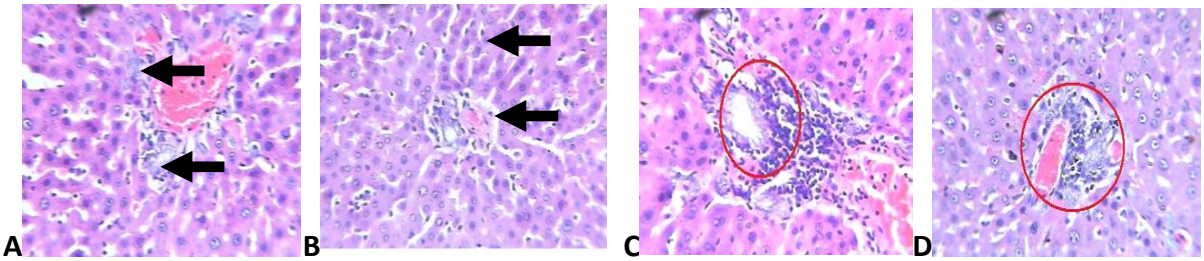

Fig. 2: Photomicrographs of the cross section of rat liver orally administered with (A) distilled water control, (B) 12.50 mg/Kg, (C) 25 mg/Kg and (D) 50 mg/Kg body weight of aqueous extract of *P. muellerianus* leaves. (**A**) The arrows show portal tract with normal hepatocytes (x 400; H & E). (**B**) The arrows show normal histology (x 400; H & E). (**C**) The circled spot shows the area of lymphocytic infiltration of the portal tract (x 400; H & E). (**D**) The circled spot shows area with mild lymphocytic infiltration of the portal tract (x 400; H & E).

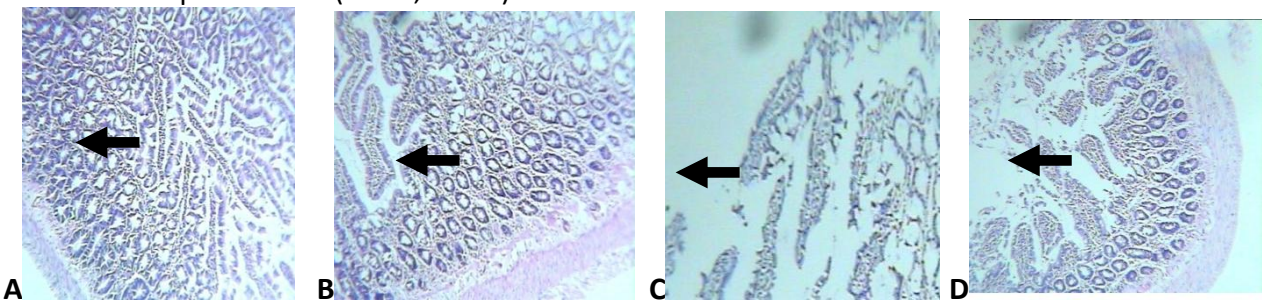

Fig. 3: Photomicrographs of the cross section of rat small intestine orally administered with (A) distilled water control, (B) 12.50 mg/Kg, (C) 25 mg/Kg and (D) 50 mg/Kg body weight of aqueous extract of *P. muellerianus* leaves. Plates A to D indicate normal histology as shown with the arrow (x 100; H & E).

DISCUSSION

Organ: body weight ratio can be used to indicate organ swelling, atrophy or hypertrophy (Schmidt *et al*., 2007; Amresh *et al.,* 2008). However, the extract neither caused atrophy (wither) nor hypertrophy in the animals treated with it for seven days. This result agreed with the findings of Amresh *et al* (2008) in which the administration of *Cissampelospareisa* did not produce any effect on the organ: body weight ratio investigated. Large quantities of inorganic electrolytes always occur in both extracellular and intracellular fluids. These electrolytes compromised the single most important factor in the transfer and movement of water and electrolytes between the three divisions of the extracellular and intracellular components (Zilva *et al*., 1991). Renal function indices such as serum electrolytes, urea and creatinine could be used to evaluate the functional capacity of the nephrons of animals (Isnard *et al*., 2004). The extract has selective effects on the electrolytes. The profile of the renal function parameters observed in this study following the administration of aqueous extract of *P. muellerianus* leaves for seven days corroborated the selective and dose-dependent effect of the extract. For example, the level of K^+ was within normal range whereas those of Na^+, urea and creatinine were reduced at all doses of the extract. Furthermore, the observed significant decrease in the serum Na^+ concentration suggested possible effect on the pump that maintains the constancy of its extracellular concentration even though the serum K^+ concentration was unaffected.

In addition, the increase in serum Cl^- might be an indication of glomerular and tubular dysfunction. The levels of Cl^- were only elevated at 25.00 and 50.00 mg/kg body weight of the extract. All these alterations might be attributed to physiological response of the animals to either counteract the effect of the extract or recover from the assault of the extract as these alterations were not sustained beyond specific doses (Nakanishi and Goto, 1975). Urea and creatinine are major catabolic products of muscles and proteins respectively. The significant decrease in urea and creatinine content of the serum following the administration of the aqueous extract of *P. muellerianus* leaves may imply that normal renal functional capacity was not impaired. Albumin, total bilirubin and globulins are biomolecules that are used to test the integrity of glomeruli and regulation of osmotic pressure respectively (Ganong, 2001). They are used to assess the synthetic ability of the liver as well as damage to the hepatocytes. Low albumin content in the serum suggests chronic damage to the liver. Similarly, the reduction in the levels of serum albumin by the extract might be an indication of diminished synthetic function of the liver, resulting probably from hepatocellular damage (Woodman, 1996) or an infection and/or when albumin is lost continuously from the body (Naganna, 1989). This agreed with the findings of Pendote *et al* (2010) who reported decrease in albumin content of the serum of male rats following the administration of 200 mg/kg body weight of aqueous extract of *Hippobromuspauciflorus* leaves. Bilirubin is an important catabolic product of blood with biological and diagnostic values (Chowdhury *et al*., 1989; Moudgil and Narang, 1989). Hepatocytes convert bilirubin to a polar form by adding glucuronic acid or sulphate molecules to it in a process referred to as conjugation. Conjugation increases solubility in water and this enhances the ease with which bilirubin becomes excreted in the bile. The non -specific pattern of effect on the bilirubin could possibly suggest physiological response as a result of exposure to the extract which is not part of the normal diet and is most likely not to be toxicologically relevant, more so when no definite pattern was produced (Yakubu *et al*., 2009). The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis, and tissue cellular damage (Malomo, 2000; Yakubu *et al*., 2003). Alkaline phosphatase (ALP), a marker enzyme of damage to the plasma membrane and endoplasmic reticulum (Shahjahan *et al*., 2004), is often used to assess the integrity of the plasma membrane (Akanji *et al*., 1993). The reduction in ALP activity of the liver homogenates at all the doses investigated might be the consequence of damage to the plasma membrane leading to loss of membrane components including ALP into the extracellular fluid or the serum (Malbica and Hart, 1971) or inhibition of the enzyme activity at the cellular/molecular level (Akanji *et al*., 1993). Damage to structural integrity of tissues is always reflected by an increase in the activities of these enzymes in the serum/plasma, probably through leakage from the affected organs/organelles. Therefore, the corresponding increase in serum ALP activity confirmed damages to the liver plasma membrane, leading to compromise of its integrity (Yakubu *et al*., 2003). The loss of ALP activity from the liver to the serum could possibly be attributed to disruption of the ordered lipid bilayer of the membrane structure. Loss of ALP activity from the tissue may hinder adequate transportation of required ions or molecules across the cell membrane (Akanji *et al*., 1993). It may also affect other metabolic processes such as the synthesis of nuclear proteins, nucleic acids, phospholipids, and cleavage of phosphate esters that require the enzyme (Ramalingam and Vimaladevi, 2002).

GGT is another membrane-localised enzyme that plays a major role in glutathione metabolism (Kaplan and Peace, 1996). The reduction in the liver GGT activity at all the doses investigated may be attributed to leakage from the organ to the extracellular fluid because there was also a corresponding increase in the activity of GGT in the serum. Liver arginase is primarily located in the cytoplasm of the liver. It functions in the urea cycle by converting L-arginine into L-orthinine and urea (Wu and Morris, 1998). The decrease in the activity of liver arginase in this study may imply the impairment of the liver structure. The significant reduction in the urea observed in this study further corroborates that the liver may have been impaired. Assessment of haematological parameters can be used to determine the extent of deleterious effect of extracts on the blood of an animal. It can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al*., 2007). Platelets when present in sufficient size, number and function are involved in the process of normal coagulation of the blood (Williams and Levine, 1989). Therefore, the reduction in the Platelets levels of animals treated with 50.00 mg/kg body weight may be attributed to the diminished effect on thrombopoietin (Li *et al*., 1999). The non-definite effect of the extract on the platelets can be attributed to adaptation by the animals to the effect of the extract. Lymphocytes are the main effector cells of the immune system (Mc Knight *et al.*, 1999). The reduction in the lymphocytes in this study may affect the effector cells of the immune system. Furthermore, the decrease in WBC may be due to impairment in the rate of its entrance into the blood from the bone marrow and an enhanced rate of removal from circulation. The altered levels of WBC accompanied with lack of RBC suggest selective and localized stimulatory effect on the bone marrow. This may be an indication of localized systemic toxicity of the extract which may adversely affect the normal functioning of the WBC and its related indices. This agrees with the findings of Adebayo *et al* (2005) and Yakubu *et al* (2007) on ethanolic extract of *Bougainvillea spectabilis* leaves and on aqueous extract of *Fadogiaagrestis* stem respectively. The significant effect of the extract on the RBC may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was altered. Hb, RBC and PCV are associated with the total population of red blood cells while MCV, MCH and MCHC relate to individual red blood cells. Therefore, the significant effect of the extract on Hb, RBC, PCV and MCH could mean that the incorporation of haemoglobin into red blood cells or the morphology of osmotic fragility of the red blood cells was altered (Adebayo *et al*., 2005). The decreased MCV and non-significant effect by the extract on the MCHC suggest selective toxicity of the extract and or its components. Histological examination of tissues could serve as complementary evidence to enzymes studies towards revealing any distortion/damage to the normal structure of the tissue cells. The incidence of infiltration of the portal tract displayed by the liver following administration of 25.00 and 50.00 mg/kg body weight of the extract may be due to damage to the hepatocyte by the extract. This may disrupt the normal flow of blood through the liver (Singh, 2002). It can therefore be deduced that some indices of liver function as well as the selective morphological changes in the liver of rats following the administration of the aqueous extract of *P. muellerianus* leaves for seven days is dose specific. The liver functional indices were affected probably because the liver is a vulnerable target to a number of toxicants since it metabolises foreign substances/compounds that may be hepatotoxic as well. The hepatotoxic effects of the extract may adversely affect the normal hepatic functioning of the animals. Therefore, the extract might not completely be safe in the rats when repeatedly consumed at 25 and 50 mg/kg body weight twice daily for seven days.

CONCLUSION

The effects of aqueous extract of *P. muellerianus* leaves in rats revealed that

i The extract did not affect the organ: body weight ratio;

ii The aqueous extract exhibited systemic toxicity as evidenced from heamatological changes in rats administered with 12.50, 25 and 50 mg/kg body weight of the extract for seven days;

iii The functional indices of the kidney and liver in the extract treated rats were adversely affected and thus indicating its possible toxicity on the kidney and liver at all the concentrations used.

iv The extract altered the histomorphology of the liver in rats, thus confirming its liver toxicity.

v The higherconcentrations 25 and 50 mg/kg body weight used exhibited adverse effects on the liver, as such care must be exercised when the plant is to be employed as an alternative drug.

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