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Phytochemical and Toxicological Studies of Aqueous Leaves Extracts of *Erythrophleum africanum*

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Abstract: The leaves of *Erythrophleum africanum* is known in the arid land of tropical Africa to posses toxicological properties. Phytochemical, acute and sub-acute evaluation of the possible toxicity risk of *E. africanum* aqueous leaves extracts were investigated in this study. Phytochemical constituents detected in the leaves extracts were saponins (1.16% w/v), cardiac glycosides, tannins (0.17 true tannins and 0.23% w/v pseudotannins), flavonoid glycosides, free flavonoids and alkaloids (4.34% w/v). The Lethal Dose (LD_{50}) of the aqueous leaves extracts was greater than 3000 mg kg^{-1} *per os* (orally) in albino rats. Sub-acute administration of the extract for 28 days resulted in significant ($p<0.05$) changes in some renal and liver indices at 3000 and 2000-3000 mg kg^{-1} body weight, respectively. Histopathological lesions of the kidney and liver in form of moderate and marked infiltration with necrosis and perivascular lymphocytic cuff were observed. The observed lesions could be due to roles played by liver and kidneys in metabolism of xenobiotics and their elimination from the body. These investigations thus seem to indicate the toxic effects of the aqueous leaves extracts of *E. africanum* at 2000-3000 mg kg^{-1}. These could be attributed to the combined toxicity of the phytochemical constituents such as tannins, saponins, glycosides and alkaloids.

Key words: Phytochemical screening, toxicity studies, *Erythrophleum africanum*

INTRODUCTION

Erythrophleum sp. are extremely toxic to livestock all over the world especially to goats, sheep and cows. Species of *Erythrophleum* which include *E. lasicanthum*, *E. guineense*, *E. chlorostachys*, *E. invorense* and *E. africanum* are known to be poisonous (Koita, 1998; Tuulikki, 2003; Adeoye and Oyedapo, 2004; Agaie *et al*., 2007). The genus *Erythrophleum* is an endemic species in tropical Africa commonly used as a poison, or an ordeal brew for persons suspected of witchcraft or serious crime (Dongmo *et al*., 2001). Accidental human poisoning by the stem-bark and leaves extracts of *Erythrophleum* species from tradomedical uses have been reported (Adeoye and Oyedapo, 2004). Cases of consumption of phytochemicals (saponins, tannins and alkaloids) eliciting diverse biochemical effects on biological systems have been reported. These include drowsiness, haemolysis, loss of consciousness, laboured breathing, coma and death due to paralysis (Ahmad *et al*., 1994; Roberts and Wink, 1998; Hassan *et al*., 2006; Aceme, 2007).

Erythrophleum africanum (family Leguminoseae) is a tropical plant commonly found in the arid land in Nigeria. It is a fairly large tree about 30 cm and 15 m tall. The leaves are bi-pinnate; stem bark is dark brown, rough and cracked or slash reddish and gritty. The young fruits are green, about 5-10 cm long (Mabberley, 1997).

The local population in Nigeria uses the leaves extracts for poisoning wild animals (rats, dogs and other predators) around their farm lands. In northern Nigeria, several herbal claims of animal death after feeding on *E. africanum* were reported. The leaves of the plant were also among those used in national parks in east Africa to protect big animals (Caro, 2003). However, its toxicity has not yet been extensively studied. We therefore considered it worthwhile in this study to evaluate the toxicity of the aqueous leaves extract of *E. africanum* to substantiate the folklore claims and to detect the active principle(s).

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MATERIALS AND METHODS

Chemicals: All the chemicals used were of analytical grade.

Plant material: The leaves of *E. africanum* were obtained in August (2006), from Dogondaji farmlands, Sokoto, Nigeria. The plant was identified at the Herbarium, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria and the voucher specimen was retained in the Herbarium. The leaves collected were open-air-dried under the shade, pulverized in to a coarse powder (with a wooden pestle and mortar) and stored in plastic container until required for use.

Preparation of plant extract: The dried powdered leaves (50 g) were extracted with 500 mL of distilled water at room temperature for 24 h and filtered through Whatman No. 1 filter paper. The filtrate was concentrated to dryness in an oven at 45°C and the yield was 8.5% (w/w). The extract was stored in sealed plastic container until required. The dried powdered residue of the extract was further reconstituted in distilled water at different concentrations for oral administration to albino rats.

Animals: Twenty five Albino rats (Wister strain) of both sexes weighing 100-260 g were obtained from animal house, Faculty of Science, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were kept in wire mesh cages at room temperature for one week to acclimatize. They were fed with animal feeds (Bendel feeds and flour mills, Edo state, Nigeria), seasonal vegetables and tap water *ad libitum*, before and after daily administration of the extract between 09.00 to 10.00 h. The study was conducted from September to November, 2006.

Phytochemical analysis: This was done using standard procedures of Persinos and Quimby (1967), Harborne (1973), Trease and Evans (1978) and El-Olemyl *et al.* (1994).

Acute toxicity studies (Determination of LD_{50}): Water extract of *E. africanum* (3000 mg kg^{-1} body weight) was administered to 5 groups (one rat per group) of rats (one after the other at a grace observation period of 48 h) in a single oral dose by using a feeding needle. The control group received distilled water. Observations of toxic symptoms were made and recorded systematically at one, two and six hours after administration. The number of survivors was noted after 48 h for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated by using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD) (2001).

Sub-acute toxicity: A total of twenty rats, divided in to the following groups: Group 1 (n = 5) was control group and received distilled water. Groups II, III and IV (5 animals each) were orally administered (1 mL of 1000, 2000 and 3000 mg kg^{-1} body weight) aqueous leaves extract of *E. africanum* once daily for 28 days, respectively.

Parameters

Blood samples and clinical chemistry: Animals were sacrificed and blood samples were collected, allowed to clot and centrifuged to obtain sera. Serum Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined using Randox assay kit, based on standard methods of Reitman and Frankel (1957). Alkaline phosphatase activity was estimated by the Randox kit (colorimetric) of Rec GSCC (1972). Total bilirubin was assayed by the method of Malloy and Evelyn as reported by Varley *et al.* (1991). Albumin (Bromocresol green) was by the method of Cheesbrough (1991). Total protein was determined by the biuret method as described by Gornall *et al.* (1949). Urea (Diacetyl monoxime) was by the method of Wybenga *et al.* (1971) and uric acid by Collins and Diehl (1959) and Morin and Prox (1973). Electrolytes and bicarbonate were estimated by the methods of Uriyo and Singh (1974) and creatinine by the modified method of Jaffe (1986).

Histopathological assessment: Histopathological examinations were carried out on the liver and kidney of the rats. They were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin. Sections (4-6 μM thick) were prepared and then stained with Hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light microscope at high power magnifications (x 600 objective) (Wasfi *et al.*, 1994; Guntupalli *et al.*, 2006).

Statistical analysis: Results are expressed as mean±standard error. The data collected were subjected to one way Analysis of Variance (ANOVA), using Graph pad Instat Version 3.02, Benferoni compare all columns (San Diego, USA). Statistical significance was considered at $p<0.05$.

RESULTS

Phytochemical screening: Glycosides, tannins, saponins, cardiac glycosides, flavonoid glycosides, free flavonoids, saponin glycosides and alkaloids were detected in the aqueous leaves extracts, while cyanogenic glycosides and

Table 1: Liver function indices in rats administered aqueous leaves extracts of *Erythrophleum africanum*

Dose (mg kg^{-1} body weight)	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	ALB (gd L^{-1})	TP (g L^{-1})	TB (mg%)	CB (mg%)
1000	19.4±2.19	41.6±4.69*	115.8±23.06	48.2±4.71	94.0±6.51*	0.25±0.04	0.23±0.07
2000	23.8±3.32*	41.0±5.67*	253.6±23.42*	52.8±0.83	108.0±3.08*	0.72±0.34*	0.23±0.12
3000	26.2±4.36*	43.7±4.56*	281.4±81.50*	51.6±2.07	110.8±1.64*	0.85±0.05*	0.32±0.12
Control	17.3±1.44	22.4±4.87	77.2±23.06	36.4±2.88	61.6±2.51	0.26±0.05	0.17±0.06

Values are mean±standard error. *: Significantly ($p<0.05$) different from the control by using the analysis of variance (ANOVA) ($n = 5$), ALT = Alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase, ALB = Albumin, TP = Total Protein, TB = Total Bilirubin, CB = Conjugated Bilirubin

Table 2: Renal function indices in rats administered aqueous leaves extracts of *Erythrophleum africanum*

Dose (mg kg^{-1} body weight)	Urea (mmol L^{-1})	Creatinine (mg dL^{-1})	Uric acid (mmol L^{-1})	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})	HCO_3^- (mmol L^{-1})
1000	7.22±0.61	1.6±1.81	1.17±0.18	146.6±3.13	5.68±0.30	23.2±0.83
2000	6.68±0.22	3.0±2.82	1.60±0.66	142.6±3.50	4.64±0.08	21.6±2.19
3000	6.72±0.17	13.4±9.23*	2.10±0.71*	140.6±3.50	5.68±0.50	21.2±1.09
Control	6.22±0.95	1.2±0.84	1.18±0.20	141.0±4.58	5.00±0.14	22.8±2.58

Values are mean±standard error. *= Significantly ($p<0.05$) different from the control by using Analysis of Variance (ANOVA) ($n = 5$). Na^+ = Serum Sodium ion concentration, K^+ = Serum potassium ion concentration, HCO^-_3 = Serum bicarbonate ion concentration

Table 3: Histopathological features of liver and kidney of rats administered aqueous leaves extracts of *Erythrophleum africanum*

Dose (mg kg^{-1} body weight)	Kidney	Liver
Control	No visible Lesion (Normal)	No visible Lesion (Normal)
1000	No visible Lesion	No visible Lesion
2000	moderate infiltration and necrosis	Perivascular lymphocytic cuff.
3000	Marked infiltration and necrosis	Moderate infiltration and necrosis

anthraquinones were absent. The total amount of alkaloids and saponins detected in 50 g powdered extract was 4.34 and 1.16% *w/v*, respectively. True tannins and pseudotannins were 0.17 and 0.23%, respectively.

Acute toxicity study and behavioral effects: Acute toxicity test at 3000 mg kg^{-1} of *E. africanum* produced no mortality after 48 h of observation. The median Lethal Dosage (LD_{50}) of the aqueous leaves extract was greater than 3000 mg kg^{-1} body weight. Oral administration of low dose (1000 mg kg^{-1}) produced no obvious toxicity. However, higher doses (2000 to 3000 mg kg^{-1}) caused slow movement of the animals.

Sub-acute toxicity (Hepatorenal functions): Significant ($p<0.05$) differences in some kidney and liver function indices were observed. They were more pronounced in the experimental rats administered doses of 2000 to 3000 mg kg^{-1} compared with control (Table 1 and 2).

Histopathological studies: Aqueous leaves extracts produced histopathological lesions of the liver and kidney (Table 3), in form of marked and moderate infiltration with necrosis and perivascular lymphocytic cuffs (Fig. 1-8).

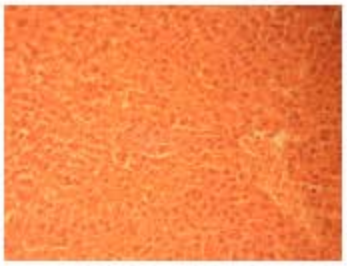

Fig. 1: Kidney photomicrograph section of normal rat (control). Hematoxylin and Eosin (H and E) X 600

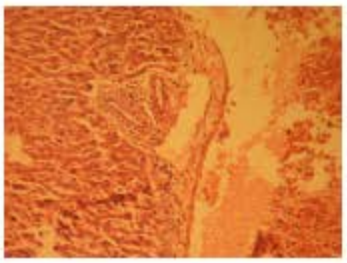

Fig. 2: Kidney photomicrograph section of rat administered aqueous leaves extract of *Erythrophleum africanum* (1000 mg kg^{-1}) showing no visible lesion. Hematoxylin and Eosin (H and E) X 600

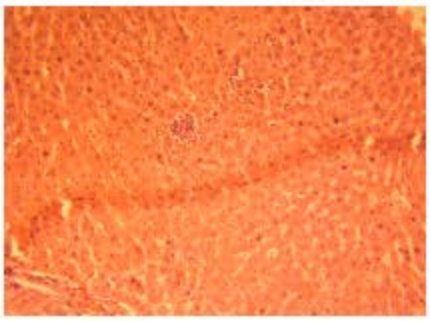

Fig. 3: Kidney photomicrograph section of rat administered aqueous leaves extract of *Erythrophleum africanum* (2000 mg kg^{-1}) showing moderate infiltration and necrosis. Hematoxylin and Eosin (H and E) X 600

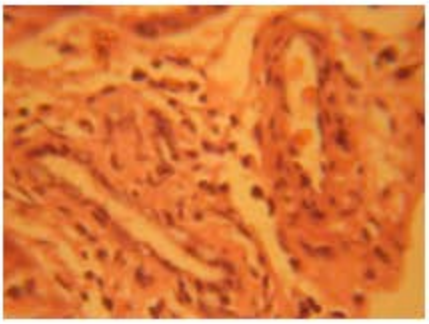

Fig. 4: Kidney photomicrograph section of rat administered aqueous leaves extract of *Erythrophleum africanum* (3000 mg kg^{-1}) showing marked infiltration and necrosis. Hematoxylin and Eosin (H and E) X 600

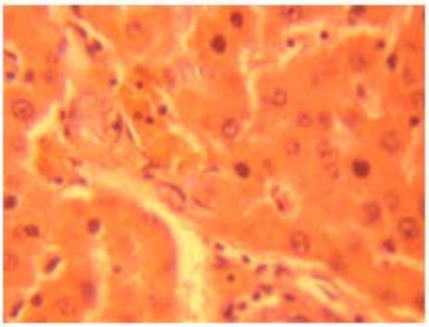

Fig. 5: Liver photomicrograph section of normal rat (control). Hematoxylin and Eosin (H and E) X 600

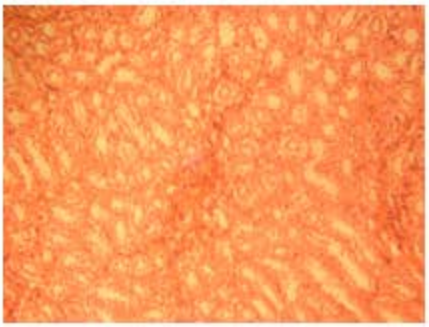

Fig. 6: Liver photomicrograph section of rat dministered aqueous leaves extract of *Erythrophleum africanum* (1000 mg kg^{-1}) showing no visible lesion. Hematoxylin and Eosin (H and E) X 600

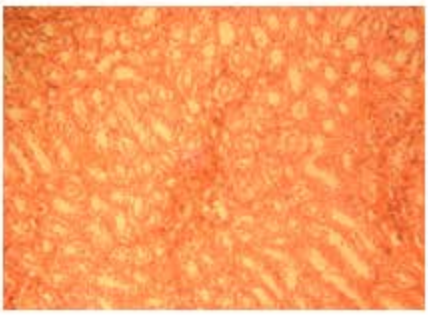

Fig. 7: Liver photomicrograph section of rat administered aqueous leaves extract of *Erythrophleum africanum* (2000 mg kg^{-1}) showing perivascular lymphocytic cuff. Hematoxylin and Eosin (H and E) X 600

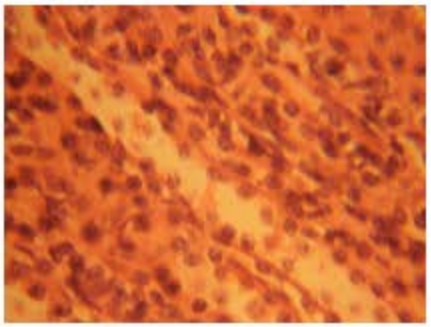

Fig. 8: Liver photomicrograph section of rat administered aqueous leaves extract of *Erythrophleum africanum* (3000 mg kg^{-1}) showing moderate infiltration and necrosis. Hematoxylin and Eosin (H and E) X 600

DISCUSSION

The assay of diagnostic enzymes in tissues plays a significant role in diagnosis, disease investigation and assessment of drug or plant extract for safety/ toxicity risk. The toxicological activity of one of the *Erythropleum* sp. (*E. guineense*) has been demonstrated in previous studies (Adeoye and Oyedapo, 2004). This study shows pronounced toxicity at higher doses (2000 to 3000 mg kg^{-1}) of orally administered aqueous leaves extracts of *E. africanum* to albino rats. Acute toxicity studies did not reveal grossly negative behavioral changes such as excitement, respiratory distress, convulsions or coma in the rats administered the various doses of *E. africanum*. The LD_{50} obtained was greater than 3000 mg kg^{-1}. However, no animal died but slow movements of animals were observed.

In the sub-acute toxicity studies, some of the indices of kidney and liver functions significantly varied compared to those in the control animals. Total bilirubin and proteins levels and the liver enzyme activities (aminotransferases and alkaline phosphatase) were significantly ($p<0.05$) raised consequent to administration of the extract at higher doses. Certain drugs and other substances are known to affect and influence circulation of bilirubin levels and elevation in bilirubin levels suggests increase in haemolysis (Orish *et al*., 2003). The rats administered the extract in doses of 1000 to 3000 mg kg^{-1} exhibited increases in serum total protein concentration. These data suggest liver injury (Donatien *et al*., 2005). Emerson *et al*. (1993) have reported that enhancement in the level of serum proteins is an indication of tissue injury and reflection of hepatic toxicity.

The liver enzymes, aspartate and alanine aminotransferases (AST and ALT) are involved in amino acid metabolism. Large amounts of AST are present in the liver, kidneys, cardiac muscle and skeletal muscle. However, ALT is known to be found principally in the liver (Cheesbrough, 1991). Serum ALT and AST levels were always found to increase in liver cell damage and the greater the degree of liver damage the higher the activities of both enzymes (Cheesbrough, 1991). In this study, increases ($p<0.05$) in ALT and AST levels were observed in the animals administered *E. africanum* extract at 2000 to 3000 mg kg^{-1}. The increased serum enzyme levels indicate that this extract at higher doses may cause cytotoxic effect on the liver. The aqueous leaves extract of *E. africanum* thus, may affect the permeability of the cell membrane making it leaky. These will cause their leakage in to the circulation in event of necrosis or hepatocellular injury leading to their subsequent elevation in the serum. Alkaline Phosphatase (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum. It is often used to assess the integrity of plasma membrane (Akanji *et al*., 1993). It is also related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme in presence of increasing biliary pressure. Significant elevation of serum alkaline phosphatase is an indication of cholestasis (Van Hoof and De Broe, 1994) with no effective control of ALP activity towards improvement in the secretory function of the hepatic cell.

Significant ($p<0.05$) increases in serum creatinine and uric acid caused by higher doses of the extract indicate compromised renal function (Chessbrough, 1991). Sodium, potassium and bicarbonate levels were not significantly altered. The present findings are in conformity with that of Adeoye and Oyedapo (2004) who established the toxicity of *Erythrophleum* species. The histopathological changes observed in the liver and kidneys of rats administered higher doses of aqueous extract of *E. africanum* have justified the significant alterations of the hepatorenal indices. These could be attributable to the effect of the extract. This may not be a surprise because liver and kidney are sites for xenobiotic metabolism and excretion, respectively. It is known that various antinutritional substances and xenobiotic chemicals like saponins and tannins cause haemolysis, nutrient malabsorption and abnormal haemopoesis which could arise from kidney and liver damage (Chubb, 1982; Connig, 1993). Some alkaloids have cytotoxic effect on organs; they damage the cells of the liver, lungs heart and kidney (Harborne, 1972). The toxicity of the leaves extract of *E. africanum* may be consequent upon the combined toxicity of constituents detected in the extract such as tannins, glycosides, alkaloids and saponins.

It is clear from the present findings that this plant has no clinical safety at higher doses especially at sub-acute administration of the extract to rats. It appears that *E. africanum* extract at higher doses have caused injury to liver and kidney but the mechanism(s) of toxicity of this plant is still being investigated.

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