

Research Article

Effect of the Aqueous Root Extract of *Urena lobata* (Linn) on the Liver of Albino Rat

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Abstract: The effects of the aqueous root extract of *urena lobata* on the rat liver was investigated using a total of twenty five (25) adult Wister rats of both sexes that were randomly divided into five groups of five rats each. Group I served as the control, while rats in groups II-IV were administered 100mg, 200mg and 300mg per kg body weight of the extract respectively for 28 days. Rats in group V were administered 300mg per kg of the extract for 28 days and allowed to stay for 14 days post treatment to observe for reversibility, persistence or delayed occurrence of toxic effects. At the end of the experimental period the animals were sacrificed and liver weight taken and fixed for routine histological examinations. Administration of the extract to rats had no effects on liver and body weights but the extract caused a decrease in albumin level and increases in the levels of Aspartate transaminases (AST), Alanine transaminases (ALT) and Alkaline phosphatase (ALP). Histopathological assessment of the liver revealed mild to severe interstitial hemorrhage, mononuclear cell infiltration, necrosis, congestion, and edema in the liver of the treated rats while withdrawal of the extract for 14 days showed a slight degree of recovery in the rats. This findings suggest that the biochemical and morphological organization of the liver can significantly be altered with continues and increase use of the extract, but further studies on the long term effect of the extract and a prolonged recovery period is recommended in further studies.

Keywords: Congestion, edema, interstitial hemorrhage, mononuclear cell infiltration, necrosis, urena lobata,

INTRODUCTION

For thousands of years people have looked to natural means of healing to treat various forms of illness and diseases, thus people are now turning to herbal medicine due to the fact that orthodox medicine are either too expensive or completely absent in rural and poor areas of the world. *Urena lobata* is a vascular plant and a member of the Malvaceae (mallow family), it grows to 2 m in height and is found mostly as a weed of waste land, roadsides and pastures in many places like New Guinea, Hawaii, Fiji, Tonga and New Caledonia (Henty and Pritchard, 1975; Wagner *et al.*, 1999; Smith, 1981; Yuncker, 1959; Mackee, 1994).

Medicinally *Urena lobata* extract have been used as an antibacterial agent (Mazumder *et al.*, 2001), as an expectorant, antitussive, depurative, diuretic, emmenagogue, emollient and have been used to treat unspecified male problems (Lans, 2007). Its use in the treatment of for angina pectoris, blenorhagia, boils, bronchitis, burns, scalds, colic, coughs, diarrhea, gingivitis, hangover, headache inflammation, toothache, tuberculosis, sore throat, sores, urogenital problems and

nephritis have also been reported (Parziale, 2005). Phytochemical analysis of *Urena lobata* has shown it to contain alkaloids, falconoid, tannin, saponin, coumarin, steroid/triterperoid, furocoumarin, mangiferin and quercetin, Imperatorin and triglycerides/polyunsaturated fatty acids (Keshab, 2004; Morelli *et al.*, 2006).

Urena lobata called Caesar weed in English is mostly found in the northern part of Nigeria and is commonly referred to as “uwar maganii” in Hausa (Hutchinson and Dalziel, 1957). This work was therefore designed to determine the effect of *Urena lobata* on the biochemical and histomorphological profile of the rat’s liver.

MATERIALS AND METHODS

Collection and identification of plant materials: The dry plant part (root) of *Urena lobata* was purchased from local herbalist located within the Monday market in Maiduguri Metropolis. The plant was identified and authenticated by Dr. S.S. Sanusi (plant taxonomist) of the Department of Biological Sciences, University of

Maiduguri, Borno state. The root was pulverized using a pestle and mortar and stored in cellophane bags at room temperature.

Preparation of extract: The World Health Organization procedure of extraction was adopted for this study (WHO, 1992). One hundred grams of the root powder of *urena lobata* were subjected to exhaustive soxhlet extraction in 500 mL of water for 72 h. The extract obtained was concentrated in a water bath until a constant dark sticky residue was obtained. This was further oven dried and maintained in a desiccator until a constant weight was obtained. The dried root extract obtained was stored in a tightly stoppered container in a refrigerator at -4°C until required. Stock solution of the extract was prepared by dissolving 5 g weight of the powdered root bark in 75 mL of normal saline and the concentration used was 0.1 g/mL.

Animals and husbandry: This study was carried out in the Departments of Human Anatomy and Human Physiology, University of Maiduguri, Nigeria between January and October, 2008. A total of 30 adult albino rats of the Wister strain weighing 70 and 180 g and 3-4 months of both sexes were used for the acute and repeated dose toxicity studies. They were purchased from the animal house of the Department of pharmacology and pharmaceutical sciences, University of Jos, Plateau State, Nigeria. Following an acclimation period of 2 weeks, the rats were individually identified by colour tattoo and weighed. The rats were kept in plastic cages at room temperature with a 12 h light/dark cycle. They had access to standard laboratory diet (Pelletised growers Feed by Grand Cereals and oil Mills Limited, Jos) and drinking water *ad libitum*.

Experimental design:

Acute toxicity study: This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) revised up and down procedure for acute toxicity testing (OECD, 2001). A limit dose of 2000mg/kg of the extract of *Urena lobata* was used on 5 healthy adult nulliparous female rats of the Wister strain, aged 4 months and weighing 150 to 200 g for this study. The rats were dosed sequentially at 48 h interval and observed for mortality and a clinical sign of toxicity and the LD₅₀ was predicted to be above 2000 mg/kg at the end of the experiment.

Repeated dose toxicity study: This study was conducted according to the U.S Environmental Protection Agency Health effects test guidelines for repeated dose 28-Day oral toxicity in rodents (U.S.EPA, 2000) by daily oral administration. A total of 25 rats were weighed and randomly divided into five

groups of 5 rats per dosage group (I-V). Group I served as control and were administered normal saline equivalent to the volume administered to the highest dosed experimental rats. Groups II, III and IV were administered 100, 200 and 300 mg/kg of the extract respectively for 28 days while rats in group V served as the recovery group and were administered the highest dose (300 mg/kg) of the extract for 28 days to observe for reversibility, persistence or delayed occurrence of toxic effects, for at least 14 days post treatment. At the end of the experimental period, body weights of all rats were taken and recorded. The rats were then sacrificed and the blood obtained was subjected to biochemical investigation. The liver obtained was trimmed of any adherent tissue, the wet weight taken and preserved in Bouins fluid for subsequent histopathological examination.

Biochemical analysis: Blood collected from the animals by transection of the jugular vein were put into sterile bottles and centrifuged at a rate of 12,000 revolutions per minute (rpm) for 10 min. The clear serum obtained was analyzed for Aspartatetransaminase (AST), Albumin, Alaninetransaminase (ALT), Alkaline phosphatase (ALP) and Bilirubin using Randox Laboratory kits at the department of chemical pathology, university of Maiduguri Teaching Hospital Maiduguri.

Histological analysis: The liver tissue obtained was carefully dissected out, weighed, fixed in Bouins fluid, embedded in paraffin and sectioned at 5 µm. Sections were stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was then carried out.

Statistical analysis: Numerical data obtained from the study were expressed as the mean value ± standard error of mean. Differences among means of control and treated groups were determined using statistical package (GraphPad InStat). A probability level of less than 5% (p<0.05) was considered significant.

RESULTS

Acute toxicity: None of the 5 rats died nor showed any sign of toxicity at the limit dose of 3000 mg/kg/oral in the first 48 hours and no evidence of toxicity was noted during 14 days of observation. LD₅₀ in rats was therefore taken as above 2000 mg/kg/oral.

Effect of the aqueous extract on mean organ and body weights: Statistical analysis of mean liver and body weights obtained from the control rats against the rats treated with the aqueous extract of the root of *urena lobata* showed no significant effect (Table 1).

Table 1: Effect of the aqueous extract of the root of *Urena lobata* on mean liver and body weights

Groups	Doses of extract (mg/kg)	Weight of liver (g)	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	Weight change (%)
I	0	4.80±0.51	118.54±26.47	155.18±20.24	36.76±7.24	27.94
II	100	5.40±0.65	143.82±10.24	190.02±5.79	46.20±6.86	24.56
III	200	4.71±0.63	139.56±13.47	180.52±12.23	41.08±5.93	23.16
IV	300	4.67±0.40	145.46±11.85	183.52±5.97	38.08±7.97	21.14
V	300 +14 days Post recovery	6.60±0.47	126.96±6.24	181.58±10.09	54.62±10.82	29.36

Results are presented as Means ± SEM, N = 5

Table 2: Effect of the aqueous extract of the root of *Urena lobata* on biochemical parameters of the liver

Groups	Doses of extract (mg/kg)	AST (IUL ⁻¹)	ALT (IUL ⁻¹)	ALP (IUL ⁻¹)	T/P (g/L)	ALB (g/L)
I	0	65.4±1.72	44.0±2.17	142.68±3.21	53.5±1.31	45.52±1.27
II	100	96.2±2.08*	63.8±1.32	156.34±12.10	55.1±3.66	57.08±3.31
III	200	98.7±1.07*	66.4±2.22*	194.6±6.01**	66.66±1.14	46.9±2.54
IV	300	101.0±4.16**	78.6±1.24**	199.12±6.54**	52.94±2.73	45.14±1.61
V	300 + 14 days Post recovery	99.0±4.03*	63.78±2.30*	193.84±16.47**	61.84±4.07	48.52±2.53

Significance relative to control (Group I) *: p<0.05, **: p<0.01, ***: p<0.001

Significance relative to group IV (300 mg/kg) a = p< 0.05, N = 5 Results are presented as

Means±SEM

AST = Aspartate Aminotransaminases, ALT = Alanine Aminotransaminases, ALP = Alkaline Phosphatase, T/P = Total Protein and ALB = Albumin

Effect of the aqueous extract on biochemical parameters: Biochemical analysis of the serum obtained revealed a significant increase (p<0.05-0.001) in the levels of AST, ALT and ALP in rats administered 200 and 300 mg/kg of the extract respectively when compared to control rats. The ALT and ALP levels were also significantly increased in the rats that were left for 14 days post treatment when compared to the rats administered the highest dose of the extract. There was also an elevation in the serum levels of total proteins and albumin though not significant (Table 2).

Histopathologic findings: No histological or macroscopic alterations were observed in the livers of the control rats, there was normal arrangement of the central vein, the sinusoids appeared normal and the hepatocytes were arranged in normal plates (Fig. 1). The histological changes observed in the group administered 100 mg/kg of the aqueous extract of the root of *Urena lobata* were mainly mild interstitial hemorrhage within the sinusoid and mononuclear cell infiltration (Fig. 2).

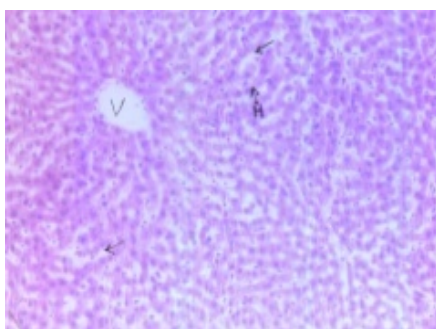


Fig. 1: Micrograph of the liver of a control rat showing normal arrangement of the Central vein (V), Sinusoids (arrows) and the Hepatocytes (H). H and E stain. Mag. x 100

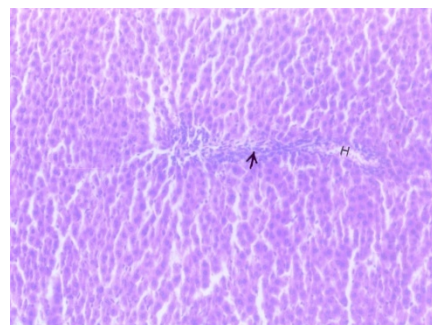


Fig. 2: Micrograph of the liver of a rat administered 100 mgkg⁻¹ of the aqueous extract of the root of *Urena lobata* showing mild interstitial hemorrhage of the sinusoid and mononuclear cell infiltration (arrow) H and E stain. Mag. x 200

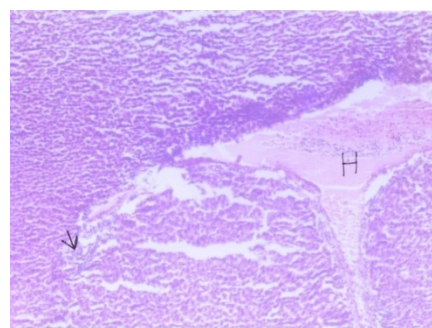


Fig. 3: Micrograph of the liver of a rat administered 200 mgkg⁻¹ of the aqueous extract of the root of *Urena lobata* showing severe hemorrhage (H) and mononuclear cell infiltration (arrow) H and E stain. Mag. x 100

The rats in group II that were administered 200 mg/kg of the aqueous extract of the root of *Urena lobata* presented with severe hemorrhage and mononuclear cell infiltration (Fig. 3). Interstitial

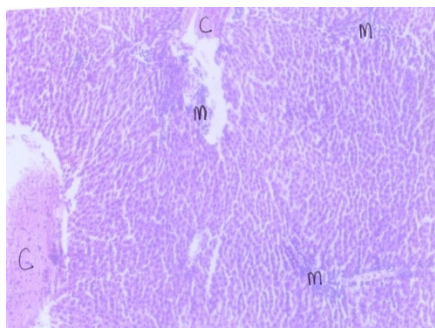


Fig. 4: Micrograph of the liver of a rat administered 300 mgkg⁻¹ of the aqueous extract of the root of *Urena lobata* showing congestion (C) and multifocal interstitial mononuclear cell infiltration (M) H and E stain. Mag. x 100

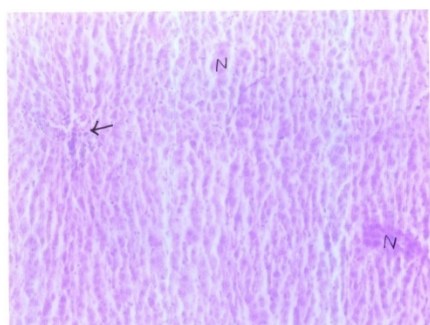


Fig. 5: Micrograph of the liver of a rat administered 300 mgkg⁻¹ of the aqueous extract of the root of *Urena lobata* and allowed a post treatment recovery period of 14 days showing necrosis (N), and interstitial mononuclear cell infiltration (arrow) H and E stain. Mag. x 200.

mononuclear cell infiltration were the histopathological findings observed in group III rats that were administered 300 mg/kg of the aqueous extract of the root of *Urena lobata* (Fig. 4). Group IV rats that were administered with 300 mg/kg of the aqueous extract of the root of *Urena lobata* and allowed to stay for 14 days post treatment to observed for the reversibility, persistence or delayed occurrence of toxic effects, presented liver tissues that had necrosis and interstitial mononuclear cell infiltration (Fig. 5).

DISCUSSION

The acute toxicity value of greater than 2000 mg/kg obtain after the administration of the aqueous root extract of *Urena lobata* in rats is an indication of the plants none or low toxicity. The US environmental protection agency had classified any substance with an LD₅₀ of > 2000 mg/kg to be practically non toxic (U.S.EPA, 2006). The administration of the extract showed no significant effect on both the body and liver weights, these findings agree with a similar work

carried out by Moundipa *et al.* (1999) who administered *Hibiscus macranthus* also a member of the Malvaceae family to rats and observed no effect on body weights. Administration of the extract produced a significant increase in the serum levels of ALT, AST, ALP and decrease in the level of albumin. It is known that increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture (Wittwer and Bohmwal, 1986; Benjamin, 1978) while the increase in ALP level, an enzymes produce in the liver, bone and placenta indicates liver injury (hepatocellular disease) or bile duct obstruction (Ganong, 2003). Low serum albumin (ALB) concentration observe in this study following the extract administration indicates poor liver function because albumin is synthesized by the liver and secreted into the blood because serum albumin concentration is usually normal in chronic liver disease until cirrhosis and significant liver damage is present (Schiff and Schiff, 1982). The experimental group shows mild to severe interstitial edema, necrosis, congestion, interstitial hemorrhage and mononuclear cell infiltration. Liver injuries are mostly caused by interference with the metabolic pathways essential for parenchymal cell integrity. They lead to diversion, competitive inhibition or structural distortion of molecules essential for metabolism or selective blockade of key metabolic pathways required to maintain the intact hepatocyte. The biochemical and physiological lesions induce by these agents lead to degenerative changes such as necrosis, such agents termed cytotoxic or indirect hepatotoxins induce hepatic injuries by mechanism that presumably relates to their selective interference with cell metabolism. Alkaloid and tannin are known for their cytotoxic effects on the liver (Zimmerman, 1978). Though the mechanism of necrosis is still unclear (Schiff and Schiff, 1982), it usually results from several disturbed extracellular environmental conditions and is associated with cell swelling and rupture, while saponins have been shown to have cytotoxic properties (Robbins and Cotran, 2004). Edema can be caused by inflammation, salt retention and lymphatic obstruction (Oakenfull and Sidhu, 1989), vascular obstruction (Bergh *et al.*, 1988) and also increases in the levels of hormones (Schrier and Abraham, 1999). Plant extract obtained from other members of the Malvaceae family have been shown to inhibit the formation of carrageenan, histamine and serotonin-induced paw edema (Vasudevan *et al.*, 2007).

Congestion of blood vessels usually results from vascular abnormality such as venous obstruction; it has been observed that edema and congestion occur together. This suggest that the extract administered to the rats probably caused impaired venous outflow in the rats liver and thus lead to congestion and interstitial edema and effects could be dose dependent.

Hemorrhage generally indicated extravasation of blood due to blood rupture and can occur under the condition of chronic congestion (Robbins and Cotran, 2004). There are infiltrations with mononuclear cell due to chronic inflammation and this is due to the administration of the extract which agrees with a similar work carried out by Matsuda *et al.* (2005) who demonstrated mononuclear cell infiltration in chronic inflammation after administration of a Japanese herbal extract. Plants of the same family as *Urena lobata* have also produce inflammation and haematoprotection, the protective nature of plants from the Malvaceae family might be due to the presence of flavonoids which have anti-inflammatory, anti-allergic, anti-thrombotic, vasoprotective, hepatoprotective properties and inhibits the tumors promotion (Trease and Evans, 1989).

CONCLUSION

In conclusion, the extract of *Urena lobata* was found to have no effects on body and liver weights but the histological and biochemical profile of the liver was significantly altered which was characterized by mild to severe hemorrhage and mononuclear cell infiltration with concomitant increase in the levels of AST, ALT and ALP. But further studies on the long term effect of the extract and a prolonged recovery period is recommended in further studies.

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