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Histomorphological Effect of the Aqueous Root-Bark Extract of *Ficus sycomorus* (Linn) on the Liver and Kidney of Albino Rats

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Abstract: The aqueous root-bark extract of *Ficus sycomorus* (Linn) was screened for its chemical constituents, median lethal dose and its histomorphological effect on the liver and kidney of albino rats. A total of 114 adult albino rats of both sexes weighing between 150-320 g were used in this study. The animals were weighed and randomly divided into two batches for the acute toxicity and histomorphological studies. In the acute toxicity study the aqueous extract of the root-bark of *Ficus sycomorus* was administered intraperitoneally (ip) in a dose range of 0.2-12 g kg^{-1} and the rats were observed for the physical signs of toxicity for 24 h. For the histomorphological effect of the extract on the liver and kidney 320, 640 and 1,280 mg kg^{-1} were administered to the rats for 2, 4 and 6 weeks, respectively. At the end of each treatment period, the animals were weighed before been sacrificed and the liver and kidneys were extracted, weighed and processed for histological assessment. Phytochemical screening of the extract showed the presence of saponins, flavonoids, alkaloids, tannins and reducing sugar while the median lethal dose (LD_{50})was calculated as 3.20±0.60 g kg^{-1}. A significant decrease ($p<0.001$) in body weight was observed but weights of kidney and liver treated with the extract were not affected significantly. Microscopic examination of the liver tissues of rats treated with the extract showed degenerative changes ranging from cytoplasmic vacuolation of hepatocytes, necrosis, dilatation of the central vein and proliferation of bile ducts. There was no observable effect on the kidney. The results of the study suggest that the extract possess hepatotoxic potentials and should be used with caution but a further research to assess the pharmacokinetics of the extract on cell membrane stability, lipid peroxidation, parenchymal cell regeneration and ultra structural study will be useful and is recommended.

Key words: *Ficus sycomorus,* acute toxicity, histomorphological, phytochemical screening, hepatotoxic

INTRODUCTION

Ficus sycomorus (L) belongs to the family Moracea and is a plant that was venerated as a holy tree of outstanding importance in ancient Egypt. The Moracea are mainly shrubs and trees with milky latex and juices. It consists of about 70 genera and over 1400 species (Hutchinson and Dalziel, 1957; George and Lawrence, 1961). A total of nine (9) species have been identified in Borno State (Akinniyi and Sultanbowa, 1983) and is found scattered around Maiduguri, in the Sahel, Sudan and Guinea Savannas and usually grows on sites with a high ground water table. Usually it prefers fresh nutrient rich soils, particularly riverbanks and water holes. Its indigenous names include Tarmu (kanuri), Baure (Hausa) and Kamda (Babur/Bura).

Extracts obtained from the fruits, leaves, stem bark and root bark usually administered in the form of infusions, decoctions, tinctures, syrups and lotions have been used in the treatment of a wide range of diseases and disorders in various African countries (NAPRALERT, WHO, 2003). Oral administration of the dried fruit extract has been used in the treatment of tuberculosis (Arnold and Gulumian, 1984). The stem bark of the extract has also been used to induce lactation, its use as an ecbolic in pregnant females has also been reported (Samuelson *et al*., 1992). Abdurrahaman (1992) has also reported the use of the plant extract in the treatment of mental illness. Its sedative and anticonvulsive properties have also been documented in rats (Sandabe *et al*., 2003).

Due to its widespread use in most African countries without purification or standardization and the scanty information regarding the plants toxic effect this study was undertaken to investigate its effect on the liver and kidney.

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MATERIALS AND METHODS

Collection and identification of plant materials: The plant part (root bark) was collected from 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 .A specimen voucher (FS.01) of the plant was prepared and deposited at the herbarium of the Department of Veterinary Physiology and Pharmacology, University of Maiduguri. The root bark was sun-dried for 3 days and pulverized using a pestle and mortar. The pulverized parts were stored in cellophane bags at room temperature.

Preparation of extract: The world health organization (WHO, 1992) procedure of extraction was adopted for this study. One hundred grams (100 g) of the root bark powder of *Ficus sycomorus* (L) were subjected to exhaustive soxhlet extraction in 500 mL of distilled 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. This gave a mean extract yield of 8.5 g w/w. The dried root bark extract obtained was stored in a tightly stoperred container in a refrigerator at -4°C until required. Stock solution of the extract was prepared by dissolving 2 g weight of the powdered root bark in 20 mL of normal saline and the concentration used was 0.1 g mL^{-1}.

Phytochemical screening: Phytochemical screening of the extract was performed according to the methods of Clarke (1975), Odebiyi and Sofowora (1978) and Trease and Evans (1989). Tests for carbohydrates, tannins, saponins, flavonoids and alkaloids were carried out.

Experimental animals: This study was carried out in the Departments of Human Anatomy and Human Physiology, University of Maiduguri, Nigeria between March and October 2004.

A total of 114 adult albino rats of the Wister strain weighing 150 and 320 g and 3-4 months of both sexes were used. 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 of 32±4°C and <30% relative humidity with a 12 h light/dark cycle. They had access to standard laboratory diet (Sanders Nigeria Limited, Kaduna) and drinking water *ad libitum*.

Acute toxicity study: For the determination of the median lethal dose (LD_{50}), a total of 42 rats were randomly divided into seven groups of 6 rats each. The extract was administered intraperitoneally (ip) in a dose range of 0.2-12 g kg^{-1}. A maximum of 1.0 mL was administered. All the rats were observed for the physical signs of toxicity for 24 h. The LD_{50} was calculated by the method of Miller and Tainter (1944).

Histomorphological study: A total of 72 rats were used for this study. The rats were randomly divided into three broad groups (A, B and C) of 24 rats with each broad group consisting of subgroups 1, 2, 3 and 4. Each subgroup consisted of 6 rats, each which were administered in divided doses by orogastric intubation normal saline, 320, 640 and 1,280 mg kg^{-1} of the aqueous extract, respectively. At the end of every two weeks, rats from one broad group were anaesthetized and sacrificed by cervical exsanguinations. The liver and kidney of each rat were carefully dissected out macroscopically examined, cleaned, weighed and processed for light microscopic studies.

Histological analysis: The liver and kidneys obtained from rats in groups A to C were carefully dissected out, cleaned, weighed and fixed in 10% formalin solution for 48 h and then processed for paraffin sectioning.

Statistical analysis: All the data obtained were expressed as the mean value±SEM. Differences among means of various groups were determined by student's t-test. A probability level of less than 5% was considered significant ($p<0.05$). Computer software statistical package (SPSS10.0) was used.

RESULTS

Phytochemical screening: The phytochemical screening of the aqueous extract of the root-bark of *Ficus sycomorus* (L) showed that it contained reducing sugars, tannins, alkaloids, saponins and flavonoids.

Acute toxicity study: The physical signs of toxicity observed ranged from decreased motor activity, loss of appetite increased respiratory rate, which was followed by restlessness and gasping for air to death The median lethal dose of the extract was calculated to be 3.20±0.60 g kg^{-1} body weight. .

Morphologic findings: Physical examination of the livers and kidneys obtained from the rats revealed no macroscopic or morphologic changes.

Table 1: Effect of the aqueous root bark extract of *Ficus sycomorus* (L.) on the rat mean body weights

Dose of extract (mg kg^{-1})	Duration: 2 weeks Initial weight (g)	2 weeks Final weight (g)	2 weeks Difference (%)	4 weeks Initial weight (g)	4 weeks Final weight (g)	4 weeks Difference (%)	6 weeks Initial weight (g)	6 weeks Final weight (g)	6 weeks Difference (%)
0	241.97±15.19	264.49±18.13	8.5	214.85±9.43	240.45±12.18	10.65	227.20±16.70	240.15±17.58	5.39
320	259.57±16.94	228.13±19.34^{a*}	12.11	256.43±19.81	222.36±14.88^{a*}	13.29	269.38±17.57	252.68±18.44^{a*}	6.20
640	274.92±15.21	232.23±13.68^{a*}	15.53	241.55±20.39	209.42±16.53^{a*}	13.30	242.57±14.60	220.52±14.59^{a*}	9.09
1,280	248.45±19.54	211.05±12.61^{a*}	15.05	237.74±18.46	197.18±18.01^{a*}	17.06	243.98±19.66	228.01±21.39^{a*}	6.5

Significance relative to control a = p<0.001, n = 6, * = weight loss, Results are presented as mean±SEM

Table 2: Effect of the aqueous root bark extract of *Ficus sycomorus* on the rat mean liver and kidney weights

Dose of extract (mg kg^{-1})	Duration: 2 weeks weights (g) Liver	2 weeks weights (g) Rt. kidney	2 weeks weights (g) Lt. kidney	4 weeks weights (g) Liver	4 weeks weights (g) Rt. kidney	4 weeks weights (g) Lt. kidney	6 weeks weights (g) Liver	6 weeks weights (g) Rt. kidney	6 weeks weights (g) Lt. kidney
0	5.80±0.15	0.63±0.03	0.63±0.02	6.36±0.18	0.63±0.05	0.57±0.05	6.43±0.18	0.63±0.04	0.58±0.04
320	7.19±0.30^{b*}	0.73±0.04^{d*}	0.71±0.04	6.55±0.13	0.70±0.02	0.73±0.02^{d*}	6.46±0.21	0.58±0.04	0.58±0.02
640	5.90±0.24	0.59±0.02	0.66±0.03	6.64±0.35	0.60±0.01	0.66±0.02	6.27±0.21	0.58±0.02	0.58±0.02
1,280	5.79±0.25	0.68±0.05	0.72±0.05	6.97±0.17^{d*}	0.52±0.02	0.55±0.05	6.84±0.29	0.59±0.01	0.60±0.02

Significance relative to control b = p<0.001, d = p<0.05, n = 6, * Weight loss, Lt. = Left, Rt. = Right, Results are presented as mean±SEM

Fig. 1: Light micrograph of a paraffin section of the liver of a control rat showing normal hepatic parenchyma. Central vein (CV), Sinusoid (S) and Hepatocytes (H) H and E stain x 400

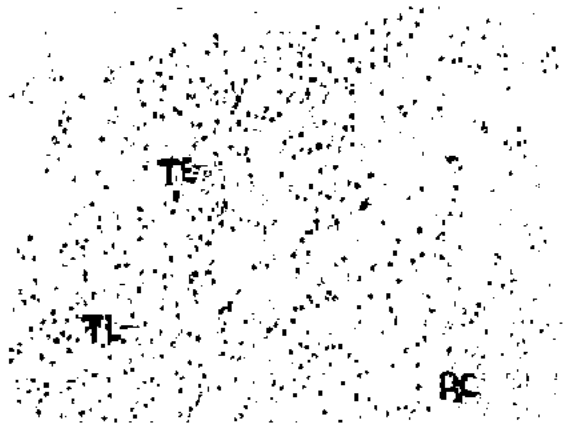

Fig. 2: Light micrograph of the paraffin section of the kidney of a control rat showing normal Tubular lumen (TL), Tubular epithelium (TE) and Renal corpuscle (RC). H and E stain x 200

Effect of the extract on mean body weight: The aqueous extract of *Ficus sycomorus* caused a significant (p<0.001) decrease in the body weight of all rats treated with all doses of the extract for the whole duration of the experiment (Table 1).

Effect of the extract on mean liver and kidney weights: There was no significant change in the liver and kidney weights of rats treated with the extract except the significant increase that was noticed in the liver weights of rats treated with 320 and 1,280 mg kg^{-1} of the extract for 2 and 4 weeks, respectively and the kidney weights of rats treated with 320 mg kg^{-1} of extract for 2 and 4 weeks, respectively (Table 2).

Histopathologic findings: No histological or macroscopic alterations were found in the liver or kidney tissues of the control rats (Fig. 1 and 2). The extract produced various effects on the liver tissues of the rats ranging from vacuolation in the cytoplasm of the hepatocytes, degenerative changes and dilatation of the central vein (Fig. 3). There was also marked bile duct proliferation (Fig. 4), cloudy swellings and degenerative changes (Fig. 5). No histopathologic changes were observed in the kidneys of rats treated with all doses of the extracts.

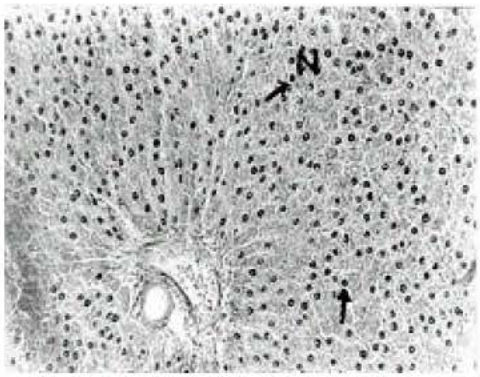

Fig. 3: Light micrograph of the paraffin section of the liver of a rat treated with 320 mg kg^{-1} of extract for 6 weeks showing dilatation of Central vein (X), vacuoles (arrow) in the hepatocytes and degenerative changes (N). H and E stain x 200

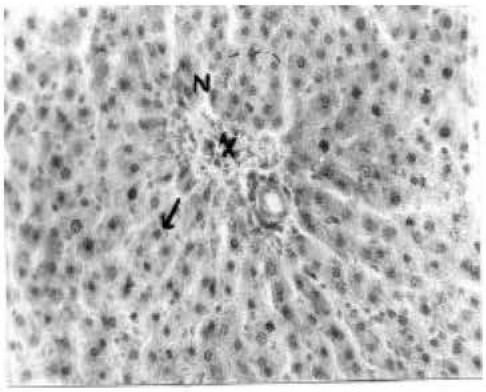

Fig. 4: Light micrograph of the paraffin section of the liver of a rat treated with 640 mg kg^{-1} of extract for 2 weeks showing vacuoles (arrow), degenerative changes (N) and marked bile duct proliferation (X). H and E stain x 400

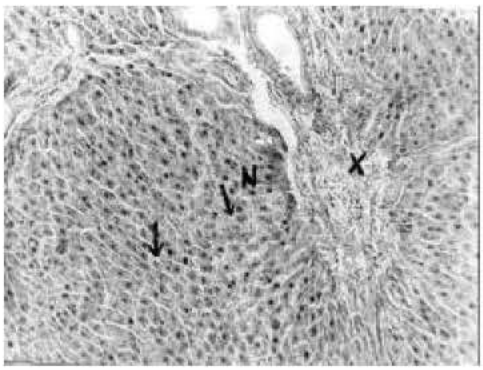

Fig. 5: Light micrograph of the paraffin section of the liver of a rat treated with 1,280 mg kg^{-1} of extract for 4 weeks showing proliferation of bile duct (X), cloudy swelling (arrow) and degenerative changes (N). H and E stain x 200

DISCUSSION

Some of the effects produced by the extracts in this study are reflections of its chemical constituents because alkaloids and tannic acid are known for their cytotoxic effect on the liver (Zimmerman, 1978). Saponins have also been shown to possess cytotoxic properties (Oakenful and Sidhu, 1989).

The physical signs of toxicity observed in this study (decreased motor activity; loss of appetite, increase in respiratory rate and gasping to death was dose dependent) thus suggesting that the effect of the extract are both central and peripheral. These correlates with the depressant effects of the extract on the central nervous system as described earlier for the aqueous extract of the stem-bark of *Ficus sycomorus* (Sandabe *et al*., 2003).

The administration of the extract also provoked a significant loss in body weights of all the treated rats and this might be attributed to the loss of appetite observed during the experimental period that lead to decrease in food intake or lesion in the intestine which might have affected the digestion and/or absorption of nutrients (Rabo, 1998). It may also have been the central effect of the plant extract on the satiety centre (Guyton and Hall, 1996). The significant increase that was noticed in the mean liver and kidney weights of some rats treated with the extract (Table 2) may be as a result of the saponin content of the extract because it has been established that most Saponins have similar structure to steroid hormones (Marletta, 1983; Evans, 1991).

The histopathological features observed in most of the liver slides of the rats treated with most doses of the extract for the 6 weeks duration of the experiments were severe degenerative changes and bile duct proliferation. 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 to selective blockade of key metabolic pathways required to maintain the intact hepatocyte. The biochemical and physiological lesions induced by these agents lead to degenerative changes such as steatosis, necrosis or both (Zimmerman, 1978). Such agents termed cytotoxic indirect hepatotoxins induce hepatic injury by mechanisms that presumably relate to their selective interference with cell metabolism. Alkaloids, tannic acid and other agents of plant origin are examples of these cytotoxic indirect hepatotoxins. Pyrrolidizine alkaloids and tannic acid interfere with protein synthesis by introducing selective biochemical lesions into the cell and necrosis follows. The consequent deficient or defective synthesis of the apoprotein moiety of the Low Density Lipoprotein

(LDL) or defective assembly of the triglyceride with apoprotein to form the LDL by which lipid is transported from the liver to the depots, can lead to steatosis and may contribute to the pathogenesis of steatosis. The mechanisms for necrosis are unclear (Schiff and Schiff, 1982). But necrosis usually results from severely disturbed extracellular environmental conditions and it is associated with cell swelling and rapture.

Proliferation of bile ductules occurs when the epithelium of small bile ducts are damaged and a cellular reaction which include lymphocyte, plasma cells, eosinophils and histiocytes occurs (Schiff and Schiff, 1982). As the bile ducts become destroyed, their sites are marked by aggregates of lymphoid cells and bile ductules begin to proliferate. The non-toxic effect observed in the kidney tissues might be attributed to either the doses, duration of the administration of the extract was minimal too induce any effect at light microscopic level or the kidney was able to clear the extract that has been detoxified by the liver

The result of this study suggest that the plant extract produced a significant decrease in body weight, caused degenerative changes and proliferation of bile ductules in the liver of most rats. But a further study to assess the effect of the extract on cell membrane stability, lipid peroxidation, parenchymal cell regeneration and ultra structural study is recommended and the use of the extract should be used with caution.

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