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**Effects of Anthraquinone Glycosides and Aqueous Ethanol
Extracts of *Ficus sycomorus* L. (Moraceae) on
Rat Liver and Kidney Functions**

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Abstract: The anthraquinone glycosides composition and toxicity studies of isolated anthraquinone glycosides and aqueous ethanol stem bark extracts of *Ficus sycomorus* on rat liver and kidney functions were conducted. The yield of anthraquinone glycosides in 40 g powdered stem bark extract was 16.0% (w/v). Liver and renal indices were significantly ($p < 0.05$) changed at higher doses of 617.86, 988.57 (anthraquinone glycosides), 767.80 and 1228.60 mg kg⁻¹ body weight (aqueous ethanol extract). Dose dependant decrease in weight ($p < 0.05$) was observed in the rats administered higher doses of the aqueous ethanol and anthraquinone glycosides extracts. The results suggest that extracts of *Ficus sycomorus* cause adverse effects to liver and kidney at higher doses.

Key words: *Ficus sycomorus*, anthraquinone glycosides composition, liver and kidney functions

Introduction

Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and are one of the main causes of morbidity and mortality world wide (WHO, 1998). The investigation of the efficacy of plant-based drugs used in traditional medicine have been paid great attention because they are cheap and have little side effects (Dharmasiri *et al.*, 2003). Recently, we have reported antifungal activity of stem bark extracts of *Ficus sycomorus* on some fungal isolates causing deep and superficial infections (Hassan *et al.*, 2006). Many medicinal plants used traditionally including *F. sycomorus* (for antifungal activity) have been in use without the real assessments of their toxic potential (s).

In Sokoto and other parts of northern Nigeria, the stem bark of *F. sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery. The extract was reported to contain tannins, saponins, reducing sugars, flavon aglycones, anthraquinone glycosides, flavonoid glycosides and condensed tannins (Hassan, 2005; Sandabe *et al.*, 2006). The plant was reported to have anthraquinone glycosides as the most potent antifungal compound in the stem bark extract (Hassan, 2005). The leaves of *F. sycomorus* contain calcium, phosphorus, iron, magnesium and zinc (Keay, 1989; Umar and Azare, 2006). The stem bark extract partially inhibits bacterial growth (Sandabe, 2002).

F. sycomorus is a rough leaved fig tree with trunk of up to 20 ft in diameter. The plant inhabits semi-arid parts of Nigeria (Williams *et al.*, 1980). The popular use of *F. sycomorus* in herbal therapy (for treating fungal diseases) demands scientific information on its toxicity risk assessment. This study

was aimed at providing information on the safety/toxicity risk potential of the isolated anthraquinone glycosides and aqueous ethanol stem bark extracts of *F. sycomorus* on the rat liver and kidney functions.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade.

Plant Material

The stem bark of *F. sycomorus* was purchased from Kara market, Sokoto, Nigeria. The plant was identified at the Herbarium, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The voucher specimen of the plant was retained in the departmental herbarium. The parts collected were open-air-dried under the shade, pulverized in to a moderately coarse powder (using a wooden pestle and mortar) and stored until required for use.

Preparation of Plant Extract

The dried powdered (40 g) stem bark was extracted with 50% ethanol at room temperature overnight and filtered through Whatman No.1 filter paper. The filtrate was concentrated to dryness in an oven at 45°C and the yield was 7.5% (w/w). The extract was stored in a sealed plastic container until required. The dried powdered extract was further reconstituted in distilled water at different concentrations for administration to albino rats.

Extraction of Anthraquinone Glycosides

The method employed was as gentle as possible so that the glycosides are maintained as entire molecules (Brain and Turner, 1975). Forty grams of the sample were extracted with 200 mL of chloroform for 8 h. The clear liquid was decanted and heated at 80°C for 3 min to denature proteins and allowed to stand for 24 h. It was filtered and the filtrate was evaporated under reduced pressure at a temperature of 45°C to obtain the residue. The final anthraquinone glycoside was weighed and the percentage was calculated with reference to the initial weight of the powder using the following formula:

$$\% \text{Anthraquinone glycosides} = \frac{\text{Weight of anthraquinone glycosiders residue} \times 100}{\text{Volume taken}}$$

Animals

Male albino rats (Wister strain) weighing 222-260 g were obtained from animal house, Faculty of Science, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were kept for one week to acclimatize at the animal house of the same Department in wire mesh cages. The animals were fed with pellet diet, seasonal vegetables and tap water *ad libitum*, before and after their daily administration of the extract between 09.00 to 10.00 h.

Administration of Extracts

The crude anthraquinone glycosides and aqueous ethanol extracts of *F. sycomorus* were used for sub-acute toxicity test. A total of thirty two albino rats were divided into two sets of 4 groups of 4 each. The first set (groups II, III and IV) was orally administered 1 mL of anthraquinone glycosides extract (247.14, 617.86 and 988.57 mg kg⁻¹ body weight, respectively) and the second set with

aqueous ethanol residue of stem bark extracts (307.14, 767.86 and 1228.57 mg kg⁻¹ body weight, respectively) daily for a period of 28 days. Group 1 in each set served as controls and received only distilled water by the same route.

Parameters of the Study

The following parameters were analyzed in all the animals.

Body Weight

The body weights of all the animals before and after 28 days of treatment were recorded.

Blood Samples and Clinical Chemistry

Animals were sacrificed and blood samples were collected, allowed to clot and centrifuged to obtain sera. The biochemical parameters; serum alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined using Randox kit by standard methods of Reitman and Frankel (1957). Alkaline Phosphatase (ALP) activity was estimated by the Randox kit (colorimetric) of Rec (1972). The 5'-nucleotidase (5'-NLT) was done by the methods of Campbell (1962) and Harold *et al.* (1980). Total bilirubin (Randox assay kit) was determined by the methods of Jendrassik and Grof (1938) and Sherlock (1951). Albumin (Bromocresol green) and urea (Diacetyl monoxime) were determined by the methods of Cheesbrough (1991) and Wybenga *et al.* (1971), respectively. Electrolytes and creatinine (Colorimetric with deproteinization) were estimated by the methods of Uriyo and Singh (1974) and Henry (1974), respectively. Uric acid was analysed by the method of Collins and Diehl (1959) and Morin and Prox (1973).

Statistics

Results are expressed as mean \pm standard error. The data were subjected to one-way analysis of variance (ANOVA), Benferoni compare all columns using Graph Pad InStat Soft ware, San Diego, USA. Statistical significance was considered at $p < 0.05$. The changes in rat body weight were subjected to student t-test.

Results and Discussion

The total amount of Anthraquinone glycosides detected in 40 g powdered extract was 16% (w/v).

There was significant ($p < 0.05$) changes in body weights of the rats administered higher doses of the isolated anthraquinone glycosides and aqueous ethanol extracts of *F. sycomorus* when compared with control groups (Table 1).

Some results observed for hepatorenal indices (Table 2 and 3) are significantly different at concentrations of 617.86 and 988.57 (anthraquinone glycosides) and 767.80 and 1228.60 mg kg⁻¹ body weight (aqueous ethanol extract).

Table 1: Mean body weight changes in rats administered anthraquinone glycosides and aqueous ethanol stem bark extracts of *Ficus sycomorus*

Concentration (mg kg ⁻¹)	WBT (g)	WAT (g)
Control (ATG)	222.50 \pm 1.29*	230.00 \pm 2.06
247.14 (ATG)	243.75 \pm 1.70	242.50 \pm 2.08
617.86 (ATG)	252.25 \pm 2.22*	244.50 \pm 1.29
988.57 (ATG)	260.00 \pm 2.75*	250.75 \pm 0.95
Control (AQE)	222.50 \pm 1.29*	230.00 \pm 2.06
307.14 (AQE)	236.50 \pm 2.08*	235.25 \pm 2.22
767.86 (AQE)	257.75 \pm 2.50*	246.25 \pm 0.96
1228.57 (AQE)	257.00 \pm 1.41*	237.50 \pm 1.29

WBT = Weight of rats before administration of extract, WAT = Weight of rats after administration of extract, ATG = Anthraquinone glycosides, AQE = Aqueous ethanol extract. Values are mean \pm standard deviation, * = Significantly different ($p < 0.05$) compared with weight of rats after (28 days) administration of extract by using the student t-test (n = 4)

Table 2: Serum liver function indices in rats administered anthraquinone glycosides and aqueous ethanol stem bark extracts of *Ficus Sycomorus*

Conc. (mg kg ⁻¹)	ALT (U L ⁻¹)	AST (U L ⁻¹)	ALP (U L ⁻¹)	5'-NLT (U L ⁻¹)
Control	5.60±0.45	10.00±1.23	62.10±6.90	17.50±2.50
247.14 (ATG)	6.40±0.66	11.50±0.87	75.90±6.90	20.00±4.08
617.86 (ATG)	16.15±0.49*	29.00±1.66*	475.10±6.90*	70.00±4.08*
988.57 (ATG)	25.85±0.85*	33.50±2.51*	648.60±13.83*	100.00±10.03*
307.14 (AQE)	6.00±0.75	10.75±1.43	69.00±7.97	18.00±2.50
767.86 (AQE)	14.50±1.03*	28.00±1.00*	338.10±20.70*	62.50±6.29*
1228.57 (AQE)	22.59±0.74*	31.50±4.50*	538.20±13.48*	75.00±5.04*

Table 2: Continued

Conc. (mg kg ⁻¹)	ALB (g L ⁻¹)	TB (μmol L ⁻¹)	CB (μmol L ⁻¹)	UCB (μmol L ⁻¹)
Control	1.86±0.06	6.48±0.54	5.54±0.62	0.48±0.15
247.14 (ATG)	1.53±0.03	6.94±0.89	4.92±1.00	2.02±0.53
617.86 (ATG)	0.97±0.03*	11.10±0.76	3.69±0.71	7.41±1.36
988.57 (ATG)	0.50±0.06*	13.88±0.93*	3.69±1.23	10.19±2.16*
307.14 (AQE)	1.81±0.06	6.93±0.47	4.31±0.60	2.63±0.16
767.86 (AQE)	1.34±0.04*	9.25±0.76	4.92±1.20	4.79±0.68
1228.57 (AQE)	0.84±0.05*	12.03±0.92*	2.92±1.24	9.11±0.70*

Values are mean±standard error of the mean. * = Significantly different from the control (p<0.05) by using the analysis of variance (ANOVA) (n = 4). ALT = Alanine transaminase, AST = Aspartate transaminase ALP = Alkaline phosphatase, 5'-NLT= 5'-nucleotidase, ALB = Albumin, TB = Total bilirubin, CB = Conjugated bilirubin, UCB = Unconjugated bilirubin, ATG = Anthraquinone glycosides and AQE = Aqueous ethanol extracts

Table 3: Serum kidney function indices in rats administered anthraquinone glycosides and aqueous ethanol stem bark extracts of *Ficus sycomorus*

Concentration (mg kg ⁻¹)	Urea (mmol L ⁻¹)	Creatinine (μmol L ⁻¹)	Sodium (ppm)	Potassium (ppm)	Uric acid (μ mol L ⁻¹)
Control	1.53±0.16	110.60±22.15	31.50±0.65	1.45±0.07	198.31±20.24
247.14 (ATG)	2.22±0.08	154.88±22.13	30.00±0.71	1.63±0.05	210.70±12.40
617.86 (ATG)	5.72±0.08*	1902.75±25.55*	19.75±0.25*	4.38±0.05*	842.90±20.25*
988.57 (ATG)	7.84±0.65*	2079.50±44.63*	16.50±0.49*	5.30±0.10*	1264.40±24.87*
307.14 (AQE)	1.75±0.18	132.80±25.55	30.75±0.75	1.53±0.05	185.91±12.39
767.86 (AQE)	4.52±0.12*	1393.80±42.37*	22.00±0.58*	3.95±0.03*	664.58±20.24*
1228.57 (AQE)	5.44±0.09*	1548.75±44.38*	18.50±1.50*	5.05±0.10*	1016.46±24.86*

Values are mean ± standard error of the mean. * = Significantly different from the control (p<0.05) by using the analysis of variance (ANOVA) (n = 4). ATG = Anthraquinone glycosides and AQE = Aqueous ethanol extracts

Animals administered higher doses of *F. sycomorus* extract experience reduced feed and water intake attributed to the presence of toxic components (saponins, tannins and anthraquinone glycosides). The toxic components depress appetite when present in sufficient concentration (Muyibi *et al.*, 2000). Anti-nutrients cause poor feed utilization expressed as decrease weight gain (Muyibi *et al.*, 2000).

Measurement of the activities of various enzymes and non-enzymic indices in tissues and body fluids play a significant and well-known aid in disease investigation and diagnosis (Malomo, 2000). Moreover, tissue enzyme assay can also indicate tissue cellular damage long before structural damage is revealed by some other conventional techniques (Akanji, 1986). Tissue damage is usually associated with the release of enzymes to the affected organ or tissue in to circulation. Risk assessments of so many medicinal plants used in herbal therapy for fungal infections have not been done. Sub-acute toxicity studies of the anthraquinone glycosides and aqueous ethanol root extracts of *F. sycomorus* were elucidated in albino rats. The doses of the extracts were calculated based on the body weight of the animals and it shows appreciable results in the albino rats studied in a dose dependant manner. The method used for quantification of anthraquinone glycosides presents an easy way for analyzing the composition of anthraquinone glycosides in plants. Presence of anthraquinone glycosides in the extract of *F. sycomorus* could probably contribute to antifungal activity of the extract (Hassan, 2005). Wuthi-Udomlert *et al.* (2003) reported extract of *Senna alata*, although a different family, to contain anthraquinone glycosides, as the major compound demonstrating antifungal activity.

In this study, anthraquinone glycosides and aqueous ethanol stem bark extracts of *F. sycomorus* had resulted in significant changes in hepatorenal indices at concentrations of 617 to 1229 mg kg⁻¹ body weight (Table 2 and 3). Hence, this result does not indicate clinical safety of *F. sycomorus* stem bark extract if used at such (higher) concentrations. Significant increases (p<0.05) of ALT and AST (at higher doses) as shown in the results suggest possible necrotic injury of the liver or cholestasis (Speech and Liehr, 1983; Lott and Wolf, 1986). From this, the extract has exerted adverse effect with possible hepatocellular necrosis. Increase of (p<0.05) of ALP and 5¹-NLT are indications of obstructive jaundice and intrahepatic cholestasis (Birkett *et al.*, 1986; Van Hoof and De Broe, 1994). ALP is also a marker enzyme of the kidney (Wright *et al.*, 1992). This suggests that there is damage to liver and kidney. The significant decrease (p<0.05) of albumin with increase (p<0.05) of UCBL and TBL are indication of compromised liver excretory function and possible impairment of the liver synthetic function (Harold *et al.*, 1980; Weiss *et al.*, 1983).

Chessbrough (1991) Albumin levels are usually reduced in chronic liver disease (Sclavo, 1987). Increase in serum Bilirubin may arise from excessive haemolysis, cytotoxicity to the liver or from obstruction of the bile ducts.

From the results, significant increase (p<0.05) of serum urea, creatinine, uric acid, potassium and decrease of sodium seen in rats administered higher doses of stem bark extracts suggest renal malfunction (Harold *et al.*, 1980; Chessbrough, 1991). Creatinine levels are indicators of renal functions, with increased levels appearing in the event of significant impairment (Tietz, 1982) as seen in this study. This shows that with significant increase in the levels of kidney markers, about 75% of the nephrons might have been damaged (Boyd, 1983). The altered biochemical indices of the liver and renal function may be strongly due to the phytochemical contents of the stem extract including anthraquinone glycosides.

Conclusions

In the light of this research, it is evident that aqueous ethanol and anthraquinone glycosides extracts of *F. sycomorus* at higher doses (617 to 1229 mg kg⁻¹) may be potentially toxic to the liver and kidney. It is therefore suggested that lower doses of the stem bark extract of *F. sycomorus* should be used cautiously for antifungal therapy to avoid toxicity.

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