

Preliminary toxicity and phytochemical studies of the aqueous extract of *Ficus platyphylla* in female albino rats

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Ficus platyphylla Del. Holl. known as gutta percha tree, grows widely in the Northern part of Nigeria. It has been used traditionally to promote fertility. The present study was aimed to investigate the safety of *Ficus platyphylla*. Phytochemical, acute and repeat dose toxicity studies were conducted on aqueous extract of the leaves, seeds and bark of *Ficus platyphylla*. A Limit dose of 3000mg/kg of the aqueous extract was administered orally to female albino rats in sequence to test for acute toxic effect. A dose of 700mg/kg was administered orally daily for 28days to another group of female albino rats to ascertain the repeat dose effect. The control group of female albino rats received 5ml/kg of distilled water (diluent of the aq. extract) for 28days. Phytochemical studies revealed that the extract contains saponins (1%), tannins (16.75%), flavonoids (24.3%), volatile oils, glycosides (2.47%) and steroids. The acute toxicity results showed that the extract has LD₅₀ above 3000mg/kg and repeat dose toxicity studies of the extract revealed possible damage to the glomeruli of rat kidney. Though the extract is relatively safe, its prolong use may carry risk of renal damage.

Keywords: Toxicity, *Ficus platyphylla*, Albino rats, Phytochemical studies

INTRODUCTION

Ficus platyphylla Del. Holl (Moraceae) is a deciduous plant locally known as “gamji” in Hausa and widely distributed throughout the savannah region of West African coast. The common name is Gutta percha tree. In Northern Nigeria, the plant is used by herbalists for treating several diseases such as insomnia, psychosis, depression, and as analgesic (Chindo *et al.*, 2003; Audu, 1989). In Sokoto state, the decoction of the stem bark, leaves and seeds of this plant are used in combination as a medicine to promote fertility. Previous studies revealed

that the stem bark of the plant possesses antinociceptive, anti-inflammatory, and gastrointestinal activities (Amos *et al.*, 2001, 2002). in rodents. Preliminary phytochemical analysis of the stem bark revealed the presence of flavonoids, tannins and saponins (Amos *et al.*, 2001). The central nervous system (CNS) activity of *F. platyphylla* has also been evaluated for the scientific basis for the use of this plant in traditional medicine for the treatment of CNS disorders (Chindo *et al.*, 2003).

The present study is designed to identify the phytochemical constituents of the stem bark, leaves and the seeds and to evaluate the toxicity of the combine plant parts in female albino rats. The three parts of the plant were used in combination as it is used traditionally to treat infertility.

MATERIALS AND METHODS

Plant materials

The bark, leaves and seeds of *Ficus platyphylla* (Gamji) were obtained from the surroundings of Usmanu Danfodiyo University teaching hospital Sokoto on the 6th of November 2008. They were taken to the Taxonomy unit of the Department of Botany, Usmanu Danfodiyo University Sokoto for identification. Voucher specimen was also deposited in the herbarium with voucher accession number 003.

Preparation of plant material and extraction

The bark, leaves, and seeds were washed with tap water, cut into pieces and air dried for about a week to constant weight. The dried materials were pulverised mechanically using a grinding machine into a dry powder and weighed. The powder was subjected to Soxhlet extraction using distilled water. The filtrates were evaporated in the oven at 50°C. The percentage yield was calculated. The extract was stored in a freezer from where it was used when required

Experimental animals

Female albino rats of 8-10 weeks old weighing between 150-180g were obtained from Veterinary institute Vom, Jos and kept in the animal house of the Department of Pharmacology Usmanu Danfodiyo University Sokoto.

The animals were kept in well constructed cages that allowed freedom of movement for two (2) weeks for acclimatisation to the laboratory conditions before commencement of study. Water and standard rat chow were provided *ad libitum* throughout the period of acclimatization and the study. The study was conducted in accordance with the Organization for Economic Development (OECD) guidelines on good laboratory practice (OECD, 2008).

Phytochemical analyses

The quantitative and the qualitative analyses of the plant's constituents were assessed by the methods described by (Trease and Evans, 1999; El-Olemmy *et al.*, 1994; Harbone 1993). The tests were done to find the presence of the active chemical constituents such as alkaloids, tannins, saponins, flavonoids, glycosides, cardiac glycosides and saponin glycosides, anthraquinones, volatile oil, and steroids. The quantitative analyses were estimated for saponins, glycosides, flavonoids, and tannins.

Acute toxicity studies

The acute oral toxicity study was done by 'Up-and- Down' method in healthy adult female albino rats according to 'OECD' guideline no. 425 (OECD, 2001). A limit dose of 3000mg/kg was used for the study. Five female rats were randomly selected through a computer generated random numbers and labelled with picric acid for identification. An animal was picked at a time, weighed and dosed with the equivalent volume of extract containing 3000mg/kg body weight dissolved in distilled water as a vehicle after overnight fasting. Oral administration of drug was done using gastric feeding tube.

Each animal was observed after dosing for the first 5 minutes for signs of regurgitation and kept in a metallic cage. Each was then observed every 15 minutes in the first 4 hours after dosing, every 30 minutes for 6 hours and daily for 48 hours for the short-term outcome according to the specifications of the OECD. The animals were monitored for a total of 14 days for the long-term possible lethal outcome. Other behavioural manifestations of acute oral toxicity were also noted.

Repeat dose oral toxicity study

The study was conducted on thirteen female rats according to the method used by Adeneye *et al.*, 2006. The animals were divided into two groups, the control (n=5) and the test groups (n=8).

At the beginning of the experiment, the animals were weighed and their weights recorded. 700mg/kg body weight of the crude extract of *Ficus platyphylla* dissolved in distilled water was administered to the test group by gastric feeding daily for 28 days. Administration of the drug was discontinued after the 28th day. The control group received 5ml/kg of distilled water for the same period of time. Both the control and the test group were treated under the same experimental conditions.

On the 29th day, rats were weighed and anaesthetised with chloroform. Blood samples were collected by cardiac puncture for biochemical and haematological analyses. The samples for haematology were put in bottles containing anticoagulant, ethylene diamine tetra-acetic acid (EDTA) while that for biochemical analysis were placed in plain bottles gently to avoid haemolysis of the blood cells. The heart, liver, lungs, kidney, ovary, spleen and brain were excised, weighed, trimmed and then fixed in Bouin's solution for histological analysis.

Methods for the bioassays

The hematocrit was determined by the micro-hematocrit method. The total and differential leucocytes count and platelets count were made from blood smear stained with

Table 1: Phytochemical constituents of *Ficus platyphylla*

S/N	Constituent	Qualitative analysis	Quantitative analysis
1	Alkaloids	—	
2	Tannins	+++	16.75%
3	Saponins	++	1.00%
4	Flavonoids	+++	24.30%
5	Glycosides	++	2.47%
6	Cardiac Glycosides	—	
7	Saponin Glycosides	—	
8	Anthraquinone	—	
9	Volatile Oil	+++	
10	Steroids	+++	

Trace (+); Moderate (++); Large (+++); Absent (-)

Giemsa (Schlam *et al.*, 1975). Beckman model spectrophotometer was used to determine the Haemoglobin concentration using cyanomethemoglobin method (Drabkin and Austin, 1932). The biochemical parameters- alkaline phosphatase was estimated by methods of (Sigma Diagnostic 1987) while those of aspartate aminotransferase (AST) and alanine amino transferase (ALT) were estimated by colorimetric method (Sigma Diagnostic 1985). Sodium and potassium were estimated by modified diacetylmonoamine method (Marsh 1965). Estimation of creatinine was done by the Jaffe's reaction method (Biod and Sirota, 1948). The total protein was estimated by Biuret method (Treitz, 1970) while the albumin was determined by bromocresol green (Lowry *et al.*, 1957).

For the histological analysis the fixed tissues were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin wax melting at 60°C. Serial sections (5-µm thick) were mounted on 3-aminopropyl triethsilane – coated slides and dried for 24 hours at 37°C (Baravalle *et al.*, 2006).

The sections on the slides were deparaffinised, hydrated, and stained with Mayer's hematoxylin and eosin dyes, dried and mounted.

Statistical analysis

Data were analyzed by students T-test. Statistical evaluations were performed using STATS programs and GraphPad prism, and a difference was considered statistically significant at $P < 0.05$.

RESULTS

The percentage yield and Phytochemical analyses

The percentage yields of extracts obtained from 2.2 kg powder of leaves, bark and seeds of *Ficus platyphylla* was found to be 4.74 % w/w.

The qualitative analyses of the phytochemical constituents of *Ficus platyphylla* showed the presence of tannins, saponins, flavonoids, volatile oils, steroids and glycosides with their varying quantitative values as shown in the (table 1 above).

Acute toxicity study

No death of rats was recorded on administration of 3000mg/kg body weight of the plant extract within the short- and long-term outcome of the limit dose test of Up and Down Procedure (Table 2 below).

Nevertheless, the observed behavioural signs were reduced motility, increase drowsiness, and ruffled fur.

The LD₅₀ was therefore assumed to be greater than 3000mg/kg body weight per oral route of administration.

Repeat dose oral toxicity study

Effect of *Ficus platyphylla* on the Weight of rats

(Table 3 below) shows the dose of the extract per kilogram body weight, the number of animals used for the

Table 2: Result of the Acute Toxicity Study

S/N	Dose (mg/kg)	Short-term (48 h)	Long-term (14 days)	Initial (g)	wt	Wt(g) wk 1	Wt(g) wk 2
1	3000	Survived	Survived	156.1		173.3	196.5
2	3000	Survived	Survived	168.9		171.9	194.6
3	3000	Survived	Survived	169.5		184.3	204.5
4	3000	Survived	Survived	160.5		160.9	164.4
5	3000	Survived	Survived	155.0		181.8	196.2

Table 3: Effect of *Ficus platyphylla* on the weight of rats

Dose (mg/kg)	No. of rats	M ₀ wt on day 0 (g)	M _f wt on day 28 (g)	M _c wt (g)	%M _c wt
Control	5	157.3 ± 2.3	161.6 ± 5.3	4.3 ± 3.2	2.6 ± 2.0
700	8	156.2 ± 1.3	149.3 ± 5.4	-6.9 ± 4.9	-4.5 ± 3.2

M₀=initial mean weight, M_f=final mean weight, M_c=changes in mean weight.
 Values are expressed as mean ± S.E.M of the rats. P < 0.05

Table 4: Effect of *Ficus platyphylla* on Haematological parameters (Full blood count parameters)

Dose (mg/kg)	700mg	control
RBC=10 ^{12/L}	7.13 ± 0.31	6.95 ± 0.56
MCV=LFL	46.55 ± 0.22	46.85 ± 1.65
HCT=L%	33.18 ± 1.34	32.60 ± 3.80
WBC 10 ^{9/L}	8.33 ± 2.04	13.70 ± 5.20
PLT 10 ^{9/L}	336.75 ± 62.63*	177.50 ± 4.50
HGB g/dl	11.58 ± 0.53	11.20 ± 0.80
MCH pg	16.30 ± 0.17	16.15 ± 0.15
MCHC g/dl	34.98 ± 0.31	34.60 ± 1.60
PCT %	0.20 ± 0.03*	0.11 ± 0.01

Values are expressed as mean ± S.E.M of the rats.

*Values significant at P < 0.05, n=6

WBC- total white blood cell count, RBC-total red blood cell count, HGB-haemoglobin concentration, HCT-haematocrit, MCV-mean cell volume of RBCs, MCH-mean cell haemoglobin, MCHC-mean cell haemoglobin concentration, PLT-total platelet count, PCT-platelet crit.

study, the initial mean body weight, the final mean body weight, the mean changes in the weight of the rats and its percentage.

F. platyphylla administered to rats for 28 days showed a slight decrease in the weight of the animals when compared to the control though it was not significant at the P value < 0.05.

Effect of the extract on Haematological responses

The extract showed no statistical significant difference (P>0.05) on the haematological parameters except for the Granulocytes, Absolute lymphocyte, and Total plate-

let count, when compared to the control (table 4 and 5 respectively).

However, the extract showed a remarkable decrease in the Total white blood cell count as compared to the control. Table 4 shows the effect of *F. platyphylla* on full blood count parameters while Table 5 shows the effect on differential count parameters.

Effect of *Ficus platyphylla* on Biochemical parameters

Table 6 shows the effect of *F. platyphylla* on the serum electrolytes, urea, creatinine, total proteins, albumin and

Table 5: Effect of *Ficus platyphylla* on Haematological parameters (Differential blood count parameters)

Dose (mg/kg)	700mg	control
LYM%=H%	80.58 ± 4.00	87.15 ± 2.75
GRA%=L%	12.38 ± 3.02*	5.70 ± 2.40
MID%=%	7.05 ± 0.98	7.15 ± 0.35
LYM=H 10 ⁹ /L	6.60 ± 1.36	11.75 ± 4.15
GRAN=L 10 ⁹ /L	1.08 ± 0.53	0.85 ± 0.65
MID=10 ⁹ /L	0.65 ± 0.22	1.10 ± 0.40

Values are expressed as mean ± S.E.M of the rats.

*Values significant at P< 0.05, n=6

LYM=H-absolute Lymphocyte, LYM%-Lymphocyte percentage, MID%-Mid cell population percentage, MID=H-absolute mid cell population, GRAN%-Granulocyte percentage, GRAN=L-absolute granulocyte.

Table 6: Effect of *Ficus platyphylla* on Biochemical parameters

Dose (mg/kg)	700mg	Control
Na ⁺ (mmol/L)	131.83 ± 3.27	134.6 ± 1.63
K ⁺ (mmol/L)	5.02 ± 0.32	4.86 ± 0.28
Cl ⁻ (mmol/L)	105.56 ± 4.15	108.57 ± 3.16
HCO ₃ ⁻ (mmol/L)	20.83 ± 1.47	22.60 ± 1.86
Urea (mmol/L)	5.57 ± 0.09	5.76 ± 0.25
Creatinine (mmol/l)	1.72 ± 0.39*	1.08 ± 0.16
T. Protein (g/dl)	6.47 ± 0.70	6.73 ± 1.53
Albumin (g/dl)	3.91 ± 0.25	4.02 ± 0.43
AST (IU/L)	111.35 ± 28.54	68.30 ± 9.51
ALT (IU/L)	64.85 ± 7.86	56.08 ± 8.27
ALP (IU/L)	394.50 ± 97.22	387.38 ± 52.51

AST-aspartate aminotransferase, ALT-alanine aminotransferase, ALP-alkaline phosphatase. T. Protein-total protein. Values are expressed as mean ± S.E.M of the rats. *Values significant at P< 0.05, n=6

liver enzymes. The extract elevated the serum creatinine significantly (P<0.05). All the other parameters showed no significant difference when compared to the control.

However the AST showed a remarkable increase when compared with the control.

Histopathological effects of *Ficus platyphylla*

The histological views of all the organs appeared normal except for the kidney. The capsule of the kidney treated with 700mg/kg *F. platyphylla* was intact, lined by simple squamous epithelium. The renal corpuscles were present. Some of the glomeruli appear to have been damaged (figure 1) when compared to control group of animals (figure 2). Some of the bowman's capsules were empty as shown in figure 1. Figure 2 showed the control with intact glomeruli.

DISCUSSION

In this study, the seeds, leaves and bark of *Ficus platyphylla* were used together. Currently, it is considered best practice in ethnopharmacology to evaluate ethnomedicines as it is; i.e in the form it is used in the traditional medical setting (Reyes-García, 2010). For this reason, this study was conducted on the extract of the leaves, plants and roots (combined) thus mimicking the way it is prepared for fertility purposes in the ethnomedicinal setting. In the previous study only the bark of *Ficus platyphylla* was used and it was shown to contain flavonoids, saponins, and tannins (Amos *et al.*, 2001) while in this study the phytochemical result showed the presence of flavonoids, saponins, tannins, steroids, glycosides and volatile oils. Other constituents identified may probably be as a result of the other parts used in combination.

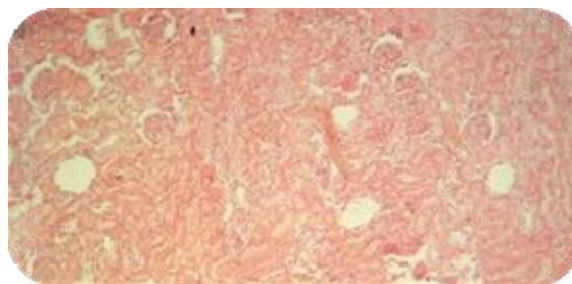


Figure 1: Section through the kidney of the treated group with empty bowman's capsule at magnification x400 stained with Heamatoxylen and Eosin



Figure 2: Section through the kidney of the control group with intact bowman's capsule at magnification x400 stained with Heamatoxylen and Eosin

The acute toxicity study of *Ficus platyphylla* aqueous bark leaves and seeds extract showed that no mortality of rats occurred at a limit dose of 3000mg/kg body weight given by oral route. Amos *et al.*, 2001 obtained LD₅₀ greater than 5000mg/kg with aqueous extract of bark of same plant indicating that aqueous extract of *Ficus platyphylla* is safe. According to (Clarke and Clarke, 1977). substances with LD₅₀ of 1000mg/kg body weight are regarded as safe or of low toxicity. In our studies, we observed changes in behavioral response (reduced movement, increase drowsiness and ruffled fur lasting.) thus confirming the earlier work of (Chindo, *et al.*, 2003). on the central nervous effect of *Ficus platyphylla* in rats. In the repeat dose toxicity study, the weights of the treated rats at the end of 28 days were lower than the weight of the control though this was not significant. This suggests that the extract may have some level of toxicity on the animals that interfered with the normal physiology of weight gain. The organs weights in the two groups were similar. Haematological analysis showed a significant increase in the number of platelets. Therefore exposure of animals for a long time to this extract may cause thrombosis. Estrogens have been found to increase the incidence of thromboembolic diseases (Tripathi, 2004) suggesting that the extract's steroids may

possess an estrogenic activity. It was also noticed that the extract caused a reduction in the quantity of the white blood cell and the lymphocytes when compared to the control. This could be due to the presence of steroids in the extract as discovered in this work (Giltay *et al.*, 2000). Sex steroids are known to cause decreased immunity (Giltay *et al.*, 2000).

All the biochemical parameters tested appeared the same with the control group except for creatinine which showed a significant increase with the control. The rise could be as a result of reduced body weight in the rats treated with the extract. Secondly from the histological pictures in figure 1 it showed a loss of glomeruli in the bowman's capsule. Reduced creatinine could result in the decrease rate of creatinine excretion in the body and hence a rise in blood creatinine.

The result also showed marked rise in one of the liver enzymes-AST but not ALT. The histological picture of the liver showed no damage to the liver cells. These findings suggest that the extract may have mild or no deleterious effect on the liver. AST is also found in other organs and tissues (USDVA, 2007) and it is probable that the increased AST may be as a result of the damaged glomeruli and the loss of body weight in the rats. The damaged glomeruli suggest renal toxicity.

CONCLUSION

The aqueous extract of *Ficus platyphylla* obtained from Northern part of Nigeria (Sokoto) gave a yield of 4.74%w/w. It has been shown to contain saponins (1%), tannins (16.75%), flavonoids (24.3%), volatile oils, glycosides (2.47%) and steroids.

Aqueous extract of the leaves, bark and leaves of *Ficus platyphylla* has been shown to have LD₅₀ above 3000mg/kg body weight. The extract appears to be toxic to the glomeruli of the kidney at the dose of 700mg/kg and has also been found to reduce immunity.

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