

**PHYTOCHEMICAL SCREENING AND SUB-ACUTE TOXICITY STUDIES OF
AQUEOUS LEAF EXTRACT OF *Ficus aurea* FROM SOUTH-EAST NIGERIA**

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ABSTRACT

In this study, the toxicity of aqueous leaf extract of *Ficus aurea* on male albino rats was determined after 28 days of administration using standard analytical procedures. The experimental animals (same age) were divided randomly into four (4) groups of five (5) animals each. The group 1 animals were orally given 500mg/kg body weight of the aqueous leaf extract while group 2 had 1000mg/kg and group 3, 1500mg/kg body weight of the aqueous leaf extract respectively. The control (4) was given water in place of the extract. Qualitative phytochemical screening of aqueous *Ficus aurea* leaf extract indicated the presence of alkaloid, tannin, flavonoid, saponin, phenol, cardiac glycoside and steroid. Alkaloid present had the highest value of 6.00 ± 0.00 mg/g while cardiac glycoside had the lowest value of 0.02 ± 0.00 mg/g in the aqueous leaf extract of *Ficus aurea*. The acute toxicity study indicated that the aqueous leaf extract of *Ficus aurea* was safe at 5000mg/kg body weight (b.w.) dose to the experimental animals. All the animals given the aqueous leaf extract of *Ficus aurea* had increased ($p < 0.05$) serum globulin concentration and alkaline phosphatase activity compared to the control group. Similarly, serum urea increased significantly ($p < 0.05$) in groups 2 and 3 animals administered with the leaf extract of the test plant compared to the control (group 4). However, there was a significant reduction ($p < 0.05$) in unconjugated bilirubin of animals administered with 1000mg/kg body weight of the plant extract when compared to the control (group 4). Significant reductions ($p < 0.05$) in serum cholesterol concentration and very low density lipoprotein (VLDL) concentration were observed in group 3 animals when compared to the control. These results suggest low toxicity of aqueous leaf extract of this plant at a dose of 1500mg/kg body weight after prolonged administration in these experimental animals.

KEYWORDS: *Ficus aurea*, toxicity, aqueous leaf extracts.**INTRODUCTION**

Plant-derived products are now gaining recognition worldwide as a source of medicine, but only a few plants have received rigorous scientific investigation. Phytomedicine also known as botanical medicine or herbal medicine is a branch of science in which plant based formulations are used to alleviate diseases. Lately, phototherapy was introduced as a more accurate synonym of herbal or botanical medicine. In the early twentieth century, herbal medicine was a prime health care system as antibiotics and analgesics were not as yet discovered. In the rural communities, people depend mostly on traditional medicine which also recognizes their socio-cultural and religious background which orthodox medicine neglects.^[2] The use of plants in traditional medicinal systems forms indispensable sources of medicinal preparations. Hundreds of species are recognized as having medicinal values. Many of today's synthetic drugs originated from the plant kingdom but with the advent of allopathic system of medicine, herbal medicine gradually lost its popularity

among people, which was based on the fast-therapeutic actions of synthetic drugs.^[2]

Ficus, a large genus in the family Moraceae, is a large pan-tropical genus of trees, shrubs, and vines with more than 1,000 members and is distributed in tropical and subtropical regions. Collectively known as fig trees or figs, they are native throughout the tropics with a few species extending into the semi-warm temperate zone.

The fig specie of greatest commercial importance, *Ficus carica* L. (the common fig), consists of numerous varieties with significant genetic diversity.^[27] Other notable species of *Ficus* are *Ficus religiosa* L. (the Bo tree), *Ficus elastic* Roxb. exHornem (the rubber tree), *Ficus benghalensis* L. (the banyan tree) and *Ficus racemosa* L. (syn.glomerata, the giant cluster tree).^[27]

In the rural settings of South East Nigeria, inhabitants administer aqueous leaf extracts of *Ficus aurea* as a treatment to alleviate malaria attacks. Presently, not

much is known of the toxicity of the aqueous leaf extract to such consumers. This work is therefore aimed at investigating the toxicity of the aqueous leaf extract to male albino rat models using standard biochemical tests.



Figure 1: *Ficus aurea* leaves.

A toxic agent is anything, be it in chemical, physical or biological form which is capable of producing adverse biological effects. Toxic plants are plants which contain active agents, which through contact, inhalation or ingestion are capable of causing injury, disease and even death in animals and humans. These compounds may be alkaloids, tannins, oxalates, saponins, glycosides. Plants toxicity depends on several factors among which is the chemical content of such plants. Toxins, be it neurotoxins or cytotoxins affect the nervous system as well as the kidneys, liver, muscles, reproduction, heart and respiration.

MATERIALS AND METHODS

PLANT COLLECTION

The leaves of *Ficus aurea* were harvested from a bush located in Umuahia, Abia State, Nigeria and identified at the Department of Botany, Micheal Okpara University of Agriculture, Umudike. The fresh plant material was washed with clean water and milled using an electric blender.

EXTRACTION

Fresh leaves of *Ficus aurea* were milled and subjected to hot water extraction. Exactly 100ml of distilled water was added to 20g of the milled plant leaves and set to boil for 20 minutes in an aluminum pot using an electrical heater. This represents the method of extraction practiced by local people before use as an antimalaria drug. It was then filtered using Whatman no. 1 filter paper. The filtrate was administered to experimental animals.

For the phytochemical analysis, fresh plant materials were milled to fine powder using an electric blender. Exactly 1g of the fine powdered plant sample was

weighed and soaked in 10ml of distilled water and left to stand for 24hrs. The solution was filtered using a Whatman no. 1 filter paper to obtain the extract.

DETERMINATION OF THE CONCENTRATION OF THE EXTRACT

A 250ml beaker was weighed, 50 ml of the extract added to the beaker and heated in a hot water bath to evaporate to dryness. After evaporation, the dried sample and beaker was reweighed.

Weight of beaker= W1 (g-mg)

Weight of beaker + 50ml of extract = W2

Weight of beaker after evaporation to dryness = W3 (g-mg)

Therefore weight of solid after evaporation to dryness is
 $W3-W1=X1$

50ml of extract after evaporation =X1

The volume of the extract to be administered will be calculated as thus;

$$\text{Required volume (ml)} = \frac{\text{Animal weight (g)} \times \text{Dose (mg/kg)}}{\text{Concentration of extract (mg/kg)}} \times 1000$$

PHYTOCHEMICAL STUDIES

Qualitative test

The freshly prepared crude extract was qualitatively tested for the presence of alkaloids, tannins, flavonoids and saponins as described by (14). Similarly, cardiac glucoside was measured by the method of (10).

Quantitative test for phytochemical

Quantitative test for alkaloids in the aqueous leaf extract was as described by (14) and tannin by method of (35). Similarly, the method of (4) was used for quantitative determination of flavonoids while procedures of (21) was employed for measuring saponins in the aqueous plant leaf extract.

EXPERIMENTAL DESIGN

Randomized complete block experimental design was used in the study.

The experimental animals were divided randomly into four (4) groups of five (5) animals each:

- **Group I:** Normal control.
- **Group II:** Treated orally with 500mg/kg body weight of aqueous leaf extract of *Ficus aurea*.
- **Group III:** Treated orally with 1000mg/kg body weight of aqueous leaf extract of *Ficus aurea*.
- **Group IV:** Treated orally with 1500mg/kg body weight of aqueous leaf extract of *Ficus aurea*.

EXPERIMENTAL ANIMALS

A total of 47 healthy male albino rats (100 - 130g) were allowed to acclimatize to laboratory conditions for two weeks prior to commencement of the experiment. The rats were kept under normal standard environmental condition of temperature (25-28°C), humidity (35-60 %)

and 12 h/12 h light/darkness cycles. They were also fed *ad libitum* with standard rat feed and allowed free access to water. Ethical principles (World Health Organization) of good laboratory practice regulations of 1998 and United States guidelines for experimental animals was strictly adhered throughout the study.

ETHICAL CLEARANCE

Ethical Committee of Animal Care Use of Abia State University, Uturu gave clearance for the use of these animals.

TREATMENT OF EXPERIMENTAL ANIMALS

The experimental animals were administered with the extract by oral intubation daily for 28 days. On the 28th day, the animals were starved, anaesthetized with chloroform and blood samples collected through cardiac puncture.

The experimental animals' body weights were measured on days 0, 7, 14, 21 and 28 during the administration of the aqueous plant leaf extract. The body weights were expressed as mean \pm SD body weight (g). The collected blood samples were discharged into EDTA containers (plasma for hematological analysis) and plain bottles for other biochemical assays. Acute toxicity test (LD₅₀) was performed on the test animals using (16) method.

Biochemical Analysis

Haematological Parameters

Haematological indices were determined by standard procedures as described by (22).

Lipid Profiles

Serum total cholesterol of the experimental animals administered aqueous leaf extract of *Ficus aurea* was determined using the method of (3) while serum triacylglycerol was monitored as described by (13). Similarly, the method of (34) was used to determine serum HDL- Cholesterol. The barbiturate reactions (TBARS) methods as described by (24) was used to measure lipid peroxidation.

Liver enzymes

Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined by the method of (25) while Alkaline phosphatase was measured using commercial diagnostic kits (Randox, United Kingdom).

Renal Functions

Serum creatinine and urea were determined using diagnostic kits from Randox laboratories, United Kingdom. However, serum sodium was measured as described by (15), Serum potassium by (7), serum chloride by method of (32) and serum bicarbonate by method of (5).

Antioxidant estimation

Superoxide dismutase (SOD) activity was measured by method of Sun and Sigma as described by (23) and catalase activity according to method of (31). However reduced glutathione (GSH) was determined in the experimental animals using the method described by (12).

RESULTS

The preliminary phytochemical screenings of aqueous leaf extract of *Ficus aurea* are as presented in tables 1 and 2 below.

Table 1: Result of the qualitative phytochemical screening of aqueous leaf extract of *Ficus aurea*.

Phytocomponent	Inference
Alkaloid	+++
Tannin	+++
Flavonoid	+
Saponin	+
Phenol	+++
Cardiac glycoside	+
Steroids	+++

Key:

+++	Highly present
++	Moderately present
+	Slightly present
-	Absent

In Table 1 above, the qualitative screening of aqueous leaf extract of *Ficus aurea* is presented. This indicates that alkaloid, tannin, steroids and phenol were highly present while flavonoid, saponin and steroids were slightly present.

Table 2: Result of quantitative phytochemical screening of aqueous leaf extract of *Ficus aurea*.

Phytocomponent	Amount (mg/g)
Alkaloid	6.00 \pm 0.00
Tannin	0.18 \pm 0.01
Flavonoid	4.86 \pm 0.12
Saponin	1.13 \pm 0.14
Phenol	3.45 \pm 0.00
Cardiac glycoside	0.02 \pm 0.00
Steroids	5.89 \pm 0.24

In table 2 above, the results of quantitative phytochemical screening of aqueous leaf extract of *Ficus aurea* is presented. Results expressed in Mean \pm SD of triplicate analysis indicate that alkaloid has the highest value (6.00 \pm 0.00 mg/g) while cardiac glycoside has the least value of 0.02 \pm 0.00mg/g.

Table 3: Acute (Oral) toxicity study of male albino rats after 24 hours administration of aqueous leaf extract of *Ficus aurea*.**PHASE I**

Group	Dose (mg/kg)	Mortality
Control	0	0/3
Treatment 1	10	0/3
Treatment 2	100	0/3
Treatment 3	1000	0/3

PHASE II

Group	Dose (mg/kg)	Mortality
Control	0	0/3
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Acute toxicity study of albino rats after 24h administration of aqueous leaf extract of *Ficus aurea* is presented in table 3 above.

plant leaf extracts according to Lorke's method were administered to groups of test animals and observed for 24 hours to monitor behaviour and mortality rates.

The experimental animals did not show any sign of aqueous leaf extract toxicity. Different doses of aqueous

Table 4: Mean body weights of male albino rats post 28 days administration of aqueous leaf extract (grams) of *Ficus aurea*.

Days	Normal control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
0	107.81±3.05 ^a	107.99±1.52 ^a	106.67±4.99 ^a	106.21±6.21 ^a
7	109.26±2.71 ^a	118.35±3.39 ^b	122.98±0.43 ^b	129.11±3.96 ^b
14	117.00±2.21 ^a	127.33±3.37 ^b	132.44±1.17 ^b	141.07±6.22 ^b
21	128.74±14.95 ^a	142.97±7.96 ^a	142.39±11.66 ^a	149.64±9.17 ^b
28	145.39±3.38 ^a	168.55±5.10 ^b	181.41±5.25 ^b	192.87±2.17 ^b

Table 4 represents the effects of aqueous leaf extract of *Ficus aurea* on the body weight of male albino rats. Values are Mean ± SD for N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at $p>0.05$. The result showed a consistent increase in the body weight of the male albino rats in a dose dependent manner. There was no significant increase ($p>0.05$) in the body weight of the male albino rats administered 500mg/kg and 1000mg/kg post 21 days administration of aqueous leaf extract of *Ficus aurea* respectively, a

significant increase ($p<0.05$) however was observed in male albino rats administered with 1500mg/kg of *Ficus aurea* aqueous leaf extract. However, significant increases ($p<0.05$) in body weights of the animals administered aqueous leaf extract of *Ficus aurea* were obtained on the twenty-eight (28) day. These changes in body weights were dose dependent with animals in group 3 gaining most weight after being administered 1500mg/kg body weight of the aqueous *Ficus aurea* leaf extract.

Table 5: Effect of aqueous leaf extract of *Ficus aurea* on haematological indices of male albino rats post 28 days administration.

Indexes	Normal Control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
RBC($\times 10^6$ /mm)	7.94±0.21 ^a	8.33±0.14 ^a	8.43±0.29 ^a	8.50±0.04 ^a
PCV (%)	48.43±1.35 ^a	49.87±1.08 ^a	50.67±1.52 ^a	51.13±0.91 ^a
Hb(g/dl)	15.80±0.18 ^a	15.70±0.27 ^a	16.50±0.45 ^a	16.40±0.47 ^a
WBC($\times 10^3$ /mm ³)	11.86±0.87 ^a	13.43±0.23 ^a	15.15±0.40 ^b	15.93±0.77 ^c
MCV(fl)	60.97±0.79 ^a	59.83±0.46 ^a	60.13±0.27 ^a	59.94±0.55 ^a
MCH(pg)	19.90±0.35 ^a	18.84±0.56 ^a	19.61±1.21 ^a	19.29±0.48 ^a
MCHC(g/dl)	32.64±0.77 ^a	31.50±1.02 ^a	32.61±1.86 ^a	32.07±0.35 ^a
Neutrophils(%)	59.67±1.37 ^a	62.00±2.37 ^a	61.00±3.90 ^a	60.33±2.73 ^a
Lymphocytes (%)	30.67±1.86 ^a	29.00±3.10 ^a	29.67±3.61 ^a	31.00±1.79 ^a

Monocytes(%)	5.33±0.52 ^a	5.33±0.52 ^a	5.00±0.89 ^a	4.67±0.52 ^a
Eosinophils(%)	3.33±0.52 ^a	3.00±0.00 ^a	3.33±1.03 ^a	3.33±0.52 ^a
Basophils(%)	1.00±0.00 ^a	0.67±0.52 ^a	1.00±0.00 ^a	0.67±0.52 ^a

Legend

PCV: Packed Cell Volume, Hb: Haemoglobin, RBC: Red Blood Cells, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, WBC: White Blood Cell.

Table 5 represents the effects of aqueous leaf extract of *Ficus aurea* on the haematological indices of male albino rats. Values are Mean ± SD for N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at (P>0.05).

A significant increase (p<0.05) of the WBC concentration was observed in group 2 (1000mg/kg) and

group 3 (1500mg/kg) of the animals administered with aqueous leaf extract of *Ficus aurea* when compared to the control. However, there were no significant (p>0.05) changes observed in the RBC, PCV, Hb, MCV, MCH, MCHC, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils between the test groups and control.

Table 6: Effects of aqueous leaf extract of *Ficus aurea* on antioxidant biomarkers of male albino rats post 28 days administration.

Parameter	Normal control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
GSH(μ/l)	39.97±1.32 ^a	39.63±0.21 ^a	41.20±0.75 ^a	41.23±0.60 ^a
SOD(μ/l)	10.27±0.77 ^a	10.83±0.42 ^a	11.57±0.76 ^a	12.07±0.67 ^b
CAT(μ/l)	14.82±1.55 ^a	15.29±0.52 ^a	15.71±0.60 ^a	14.65±0.28 ^a
MDA(μ/l)	0.39±0.02 ^a	0.41±0.02 ^a	0.37±0.03 ^a	0.42±0.04 ^a

Legend

GSH: Reduced Glutathione, SOD: Superoxide Dismutase, CAT: Catalase, MDA: Malondialdehyde.

Table 6 represents the effects of aqueous leaf extract of *Ficus aurea* on antioxidant biomarkers of male albino rats. Values are Mean ± SD for N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at (P>0.05).

A significant (p<0.05) increase of the SOD was observed in group 3 (1500mg/kg) when compared to the control. The alteration in Glutathione, CAT and MDA were not significant (p>0.05) when compared to the control.

Table 7: Effects of aqueous leaf extract of *Ficus aurea* on serum proteins and hepatocellular indices of male albino rats post 28 days administration.

Parameters	Normal control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
Total Protein(g/dl)	6.79±0.15 ^a	7.16±0.11 ^a	7.41±0.04 ^b	7.40±0.26 ^c
Albumin g/dl)	3.77±0.46 ^a	3.24±0.19 ^a	3.66±0.37 ^a	3.88±0.39 ^a
Globulin(g/dl)	3.25±0.27 ^a	4.01±0.08 ^b	3.93±0.14 ^c	3.71±0.17 ^d
AST(μ/L)	39.70±2.60 ^a	38.37±1.46 ^a	38.90±2.86 ^a	41.53±2.30 ^a
ALT(μ/L)	30.47±2.46 ^a	30.30±1.51 ^a	30.93±1.59 ^a	32.37±1.59 ^a
ALP(μ/L)	110.33±6.51 ^a	121.00±3.61 ^b	124.67±2.52 ^c	130.00±2.00 ^d
Total bilirubin(mg/dl)	0.61±0.08 ^a	0.53±0.06 ^a	0.54±0.02 ^a	0.61±0.02 ^a
Conjugated bilirubin (mg/dl)	0.38±0.06 ^a	0.37±0.04 ^a	0.39±0.02 ^a	0.39±0.02 ^a
Unconjugated bilirubin(mg/dl)	0.23±0.03 ^a	0.16±0.03 ^a	0.16±0.01 ^{ab}	0.21±0.03 ^a

Legend

AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase.

Table 7 represents the effects of aqueous leaf extract of *Ficus aurea* on the hepatocellular indices of male albino rats. Values are Mean ± SD for N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at (P>0.05).

The oral administration of *Ficus aurea* leaf extract increased the concentration of total protein in the groups administered with 1000mg/kg (group2) and 1500mg/kg (group 3) of aqueous leaf extract of *Ficus aurea* significantly (p<0.05). The concentration of globulin increased in group 1 (500mg/kg), group 2 (1000mg/kg)

and group 3 (1500mg/kg) significantly ($p < 0.05$) when compared to the control. The ALP concentration increased significantly ($p < 0.05$) in a dose dependent manner. Unconjugated bilirubin reduced significantly

($p < 0.05$) in group 2 (1000mg/kg) when compared to the control. The concentration of albumin, AST, ALP, total bilirubin and conjugated bilirubin were not significantly ($p > 0.05$) altered.

Table 8: Effects of aqueous leaf extract of *Ficus aurea* on renal function indices of male albino rats post 28 days administration.

Parameters	Normal control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
Urea(mg/dl)	31.00±1.39 ^a	32.80±1.57 ^a	39.77±1.36 ^b	50.77±0.32 ^c
Creatinine(mg/dl)	0.80±0.02 ^a	0.73±0.10 ^a	0.69±0.09 ^a	0.80±0.03 ^a
Na ⁺ (mEq/L)	142.67±3.06 ^a	141.67±3.06 ^a	144.00±2.00 ^a	144.00±1.00 ^a
K ⁺ (mEq/L)	5.56±0.17 ^a	5.04±0.26 ^a	5.25±0.06 ^a	7.17±0.29 ^b
Cl ⁻ (mEq/L)	103.37±4.11 ^a	103.43±3.13 ^a	103.47±4.16 ^a	106.17±1.12 ^a
HCO ₃ ⁻ (mEq/L)	22.60±0.56 ^a	22.20±1.45 ^a	22.87±1.36 ^a	25.07±1.90 ^a

Legend

Na⁺- Sodium, K⁺- Potassium, Cl⁻- Chloride ion, HCO₃⁻

Table 8 represents the effects of aqueous leaf extract of *Ficus aurea* on the renal function indices of male albino rats. Values are Mean ± SD for n=3 (Triplicates) and N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at ($P > 0.05$).

A significant ($p < 0.05$) increase in serum urea concentration was observed in group 2(1000mg/kg) and

group 3 (1500mg/kg) when compared to the control. A significant ($p < 0.05$) increase in potassium concentration was observed in group 3 (1500mg/kg) when compared to the control. No significant changes ($p > 0.05$) were observed in serum creatinine, Na⁺, Cl⁻ and HCO₃⁻ when compared with the control.

Table 9: Effects of aqueous leaf extract of *Ficus aurea* on the Lipid profile biomarkers of male albino rats post 28 days administration.

Parameters	Normal Control	Group 1 (500mg/kg)	Group 2 (1000mg/kg)	Group 3 (1500mg/kg)
CHOL (mg/dl)	77.70±1.25 ^a	74.70±2.55 ^a	70.70±2.55 ^a	70.70±1.06 ^b
HDL (mg/dl)	39.10±3.60 ^a	43.03±2.27 ^a	43.50±2.69 ^a	44.27±0.47 ^a
LDL (mg/dl)	23.63±0.50 ^a	24.03±0.64 ^a	23.43±0.21 ^a	23.80±0.79 ^a
VLDL (mg/dl)	14.97±4.35 ^a	7.63±0.51 ^b	9.17±2.12 ^a	2.63±1.37 ^c
TAG (mg/dl)	118.20±2.69 ^a	120.10±3.22 ^a	116.93±1.34 ^a	118.97±3.78 ^a

Legend

TAG- Triacylglycerol, CHOL- Cholesterol, HDL- High density lipoprotein, LDL- Low density protein
VLDL- Very low-density lipoprotein

Table 9 represents the effects of aqueous leaf extract of *Ficus aurea* on the lipid profile biomarkers of male albino rats. Values are Mean ± SD for N=5 (Sample size; number of animals per group). Values across the row bearing the same letter of alphabets are not significantly different at ($P > 0.05$).

A dose dependent significant ($p < 0.05$) reduction was observed in total serum cholesterol concentration. A significant reduction ($p < 0.05$) in VLDL concentration was observed in the groups administered with 500mg/kg and 1500mg/kg. The reduction noticed in the group administered with 1000mg/kg was not significant ($p > 0.05$). No significant changes ($p > 0.05$) were observed in HDL, LDL and TAG concentration when compared with the control.

DISCUSSION

It is a well-known fact that herbs play an indispensable role in medicine. The pharmacological treatment of disease began long ago with the use of herbs.^[20] Medicinal plants are the “backbone” of traditional medicine, and it has been reported that 3.3 billion people in less developed countries utilize medicinal plants on a regular basis for treatment and management of various ailment.^[20]

The different *Ficus* species have been known to contain several secondary compounds, some of which are flavonoids, tannins, saponins, steroids, terpenoids, glycosides, alkaloids, phenolic acids and coumarins.^[29] The qualitative assessment of *Ficus aurea* revealed the presence of alkaloids, tannins, cardiac glycosides, saponins, flavonoids, steroids and phenols at varying

concentrations. The results of quantitative phytochemical screening of aqueous *Ficus aurea* leaf extract indicate that alkaloid has the highest value of 6.00 ± 0.00 while cardiac glycoside has the least value of 0.02 ± 0.00 . Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity, anticancer actions and analgesic properties.^[19] In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals.^[11] Flavonoids exhibit antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions.^[33] Saponins can be utilized for their insecticidal, antibiotic, fungicidal, and other pharmacological properties while cardiac glycosides have long served as the main medical treatment to congestive heart failure and cardiac arrhythmia, due to their effects of increasing the force of muscle contraction while reducing heart rate. Phenolics derived from various natural sources are linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, anti-arthritis and antimicrobial activities.^[8]

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours). The value of LD_{50} for a substance is the dose required to kill half the members of a tested population after specified test duration. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity.^[6] Different doses of *Ficus aurea* plant leaf extracts according to Lorke's method were administered to groups of test animals and observed for 24 hours to monitor behaviour and mortality. Acute toxicity study of albino rats after the administration of aqueous *Ficus aurea* leaf extract showed no mortality even at the highest dose of 5000 mg/kg as presented in Table 3. No secondary sign of toxicity was noticed at all doses.

Sub-acute toxicity test is a repeated dose study performed to expose any deleterious change in organ, haematological and biochemical indices that may arise in the course of the repeated administration of a test substance, usually ranging from weeks to few months.^[30] The result showed a consistent increase in the body weight of the male albino rats in a dose dependent manner. There was no significant increase ($p > 0.05$) in the body weight of the male albino rats administered 500 mg/kg and 1000 mg/kg post 21 days administration of aqueous leaf extract of *Ficus aurea*. However, significant increase ($p < 0.05$) in body weights of the animals administered aqueous leaf extract of *Ficus aurea* were obtained on the twenty-eight (28) day. These changes in body weights were dose dependent with animals in group

3 gaining most weight after being administered 1500 mg/kg body weight of the aqueous *Ficus aurea* leaf extract. No mortalities were recorded in rats over the period of 28 days of treatment with aqueous leaf extract of *Ficus aurea* at the doses of 500, 1000 and 1500 mg/kg body weight through oral gavage. None of the male albino rats after administration of aqueous leaf extract of *Ficus aurea* at the doses of 500, 1000 and 1500 mg/kg body weight showed any obvious morbidity or clinical symptoms of toxicity such as changes in the skin and fur, eyes, respiratory rate, autonomic (salivation, perspiration and piloerection) and stereotype activities throughout the experimental period of 28 days. There were no clinical signs of toxicity observed for the normal control group. Any minor changes or activities in rats found in the study period can be considered common findings for albino rats. The body weight of the albino rats were recorded at an interval of 7 days over the treatment period of 28 days and statistically significant increase in body weight was compared with the control group. Increases in body weight of the experimental animals were dose dependent for the study period. Monitoring body weight can serve as a good and sensitive indicator to assess the overall health of experimental animals as a decrease in body weight may be a sign of adverse effects.

The investigation of haematological parameters and their concentrations such as packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell total and differential, erythrocytes indices such as mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) are very essential consequent upon exposure to xenobiotics. These indices more often than not, compromise during oxidative attack by free radicals and foreign compounds.^[17] In the group treated with 1000 mg/kg and 1500 mg/kg body weight of *Ficus aurea* aqueous leaf extract, there was an increased count of the white blood cells when compared to the control and the group treated with 500 mg/kg of the plant extract. The proliferation of white blood cells is a responsive signal to oxidative stress, toxins and free radicals. This WBC increase is also attributed to the cell mediated first line of defence consequent upon the administration of the plant extract.^[17] The administration of the plant extract did not significantly ($p > 0.05$) alter the concentrations of the PCV, RBC, Hb, MCHC, MCH, MCV, neutrophils, eosinophils, basophils, monocytes and lymphocytes as shown in the table. The non-alteration of RBC and factors relating to it might be an indication that the balance between the rate of production and destruction of blood corpuscles (erythropoiesis) was not adversely affected. It also indicates that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cell was affected.^[11] This pattern of result is similar to the findings of (26) following the administration of aqueous leaf extract of *Ficus exasperata* to albino rats.

Failures of the endogenous antioxidant defense mechanisms promote formation of excessive free radicals and consequent tissue damage.^[18] Parameters such as GSH (reduced Glutathione), SOD (Superoxide Dismutase), CAT (Catalase) and MDA (Malondialdehyde) can be indicative of oxidative stress status. As observed in this research, there was a significant ($P < 0.05$) increase in the superoxide dismutase activity. Superoxide Dismutase is an important endogenous antioxidant enzyme that prevents the production of free radicals. The increased concentration of SOD in the treatment group when compared to those in the control group may indicate intra and extra hepatic lipid peroxidation injury.^[9] The alteration in Glutathione, CAT and MDA were not significant ($p > 0.05$) when compared to the control.

The liver plays a central role in biochemical homeostasis and in xenobiotic metabolism, detoxification and biotransformation. The analysis of liver function parameters may provide useful information on the safety or toxicity effects of therapeutic agents. The liver enzymes; alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are closely associated with hepatic injury. Similarly changes in serum concentration of total protein, bilirubin and albumin are closely associated with hepatic injury.^[28] This study revealed that the biochemical parameters of the liver showed very little variation (non-significant) when compared to the control. However, there was a significant ($P < 0.05$) increase in the concentrations of total protein in the group treated with 1000mg/kg and 1500mg/kg body weight of *Ficus aurea* aqueous leaf extract respectively. Total protein biomarker is used to assess a number of health conditions, including: liver diseases, renal diseases and malnutrition. The increase in serum total protein following the administration of the aqueous leaf extract of *F. aurea* could be as a result of an increase in immunoglobulin content due to rise in WBCs of the experimental animals.^[26] The group treated with 500mg/kg, 1000mg/kg and 1500mg/kg body weight of the plant extract showed a significant ($P < 0.05$) increase in globulin concentration when compared to the control. High levels may indicate infection, inflammatory disease or immune disorders. A dose dependent increase in the concentrations of ALP was observed in Table 7. Increase in the concentration of serum ALP indicates an increased hepatocytic activity in hepatobiliary disease. Higher ALP levels in serum are observed when bile ducts are blocked as in the case of obstructive jaundice.

Serum urea, creatinine and electrolytes (Cl^- , Na^+ , K^+ , HCO_3^-) concentrations have been utilized in the diagnosis of the insults of the renal system. Urea, the waste product of protein catabolism undergoes reabsorption at the Loop of Henle and excretion through the kidney confirms its relevance in the examination of kidney's functionality. Consequently, urea clearance may be used as a measure of glomerular filtration rate (GFR). Nephrotoxicity which results in the impairment of these

homeostatic functions of the kidney are often initiated by the metabolism of toxicants. The blood electrolytes (sodium, potassium, chloride, and bicarbonate) help in the regulation of nerve-muscle functions, maintenance of acid-base and water balances. The kidney helps in striking electrolyte concentration balance by filtering them alongside with water in the blood. Some of the electrolytes are returned to the blood, while excess are excreted through urine. In this study, experimental animals administered with 1000mg/kg and 1500mg/kg of the plant leaf aqueous extract showed a significant ($P < 0.05$) increase in the concentration of Urea when compared with the control. Potassium is a necessary element playing physiological roles in multiple processes such as the electrical impulse conduction and the contraction of smooth and skeletal muscles, including the heart. It is the increased efflux of K^+ ions from cardiomyocytes that determines their return to the resting state. It also facilitates cell membrane function and proper enzyme activity. Its role is especially significant in the excitable cells, such as neurons. The resting potential of these cells depends mainly on potassium, since their membrane is the most permeable to this ion. Increased potassium is known as hyperkalemia. A significant ($P < 0.05$) increase in the concentration of Potassium was observed in the group treated with 1500mg/kg body weight of the plant leaf extract. Thus, *Ficus aurea* aqueous leaf extracts do not have renal protective effect from this study. Lipid profile is a panel of clinical chemical assays that serve as an initial preliminary broad medical screening for abnormalities associated with lipid metabolism. Imbalances in the concentration of key lipids in the system such as total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triacylglycerols (TAG) are held as major culprits to lipid-related metabolic diseases and the risk of cardiac diseases. There was a significant ($P < 0.05$) reduction in the concentration of total cholesterol and VLDL when compared to the control group which suggests that the aqueous leaf extract of *Ficus aurea* possess a hypolipidemic agent.

In conclusion, these results suggest a low toxicity of aqueous leaf extract of *Ficus aurea* at a dose of 1500mg/kg body weight after a prolonged administration in experimental animals.

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