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Acute and Subchronic Toxicity Evaluation of *Ficus thonningii* Leaves Hexane Extract in Albino Rats

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ABSTRACT

Medicinal plant has a significant contribution in providing knowledge to the researchers in the field of ethnobotany and ethnopharmacology, However, toxicological studies contribute significantly to human health. The present study aimed to evaluate acute and subchronic toxicity of *Ficus thonningii* hexane extract. Phytochemicals screenings were assayed using standard laboratory procedures, while acute and subchronic oral toxicity of *Ficus thonningii* n-hexane leaves fraction extract was evaluated in albino rats as per OECD guide line. Phytochemical analysis of the extract revealed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides, steroids, balsams, saponin and glycoside. While anthraquinones and volatile oils are not detected, the LD50 of this plant fraction was calculated to be above 5000mg/kg. The result of liver function test revealed a non-significant ($P<0.05$) difference in all parameters ALT, AST, ALP, TP, ALB, DB and TP activities compared to normal control group. No significant ($P>0.05$) decreases in serum (Creatinine, Uric acid, K and Cl^-). While there are significant ($P<0.05$) decreases in urea and HCO_3 concentration in group administered with highest dose (750mg/kg body weight) compared to normal control. Na^- revealed significant ($P<0.05$) decreases only in groups treated 750 and 1000mg/kg compared to normal control. A non-significant decrease ($P>0.05$) in WBC, MCHC, MCV, RDW and lymphocytes in all extract treated groups compared to normal control. HGB and hematocrits significantly ($P<0.05$) decreased in groups treated with extract 500, 750 and 1000mg/kg respectively compared to normal control. MCH significantly ($P<0.05$) decreased only in group treated with 750mg/kg compared to normal control. While, PLT showed significant ($P<0.05$) increase only in group treated with extract 750mg/kg compared to normal control. In conclusion, the present findings revealed that *Ficus thonningii* n-hexane leaves fraction doesn't show any sign of effect at acute dose and it's long-term oral administration for 28days, hence the extract relatively non-toxic at both acute and subchronic administrations.

Keywords: Toxicity, Acute, Subchronic, *Ficus thonningii*, liver, kidney

1. INTRODUCTION

Medicinal plants play an essential role in providing knowledge to researchers in ethnobotany and ethnopharmacology (Ghorbani *et al*., 2006). Although the number of cases of diseases is decreasing globally, parasite resistance to current synthetic drugs and resistance to insecticides by vectors such as mosquitoes threaten the prospects of malaria elimination in endemic areas (Mendis *et al*., 2009). Interestingly, there is a scientific approach toward discovering new drug agents from natural sources. Because drugs such as quinine and artemisinin and combined therapy were discovered through thorough research of the indigenous knowledge of plants, bioprospecting Sub-Saharan Africa's enormous plant biodiversity may be a source of new and better drugs to treat malaria (Mendis *et al*., 2009).

However, toxicological studies are contributing to human health more than ever (Wu and Sun, 2012). Reports on the toxicological studies of plants, which are continuously growing, are documented in several literatures. Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care, and almost 7% of the herbal drugs used worldwide were derived from medicinal plants (Ukwuani *et al*., 2012). There are thousands of chemical substances synthesized from plants for drug development. However, many plants were reported to be toxic to humans and animals.

Therefore, evaluating the toxicity profile of plants used in pharmaceuticals is of paramount importance; toxicological studies will give insight regarding their safety. Otherwise, *Ficus thonningii* is a species of plant that takes the form of a tree or shrub that reaches 10–30 m high. The crown is wide open, with spreading branches (Nongonierm *et al*., 2005). Ethnopharmacological investigations show that *F. thonningii* is widely used in the treatment of many diseases such as hepatitis, jaundice, sickle-cell disease (Asase *et al*., 2005), malaria (Nongonierm *et al*., 2005), dysentery, cholera, hemorrhagic diarrhea, gingivitis (Kerharo & Adam, 1974), cough, throat inflammation, tuberculosis, and some urogenital (Adjanohoun *et al*., 1989). This plant is also used as a tonic, stimulant, and diuretic in cases of difficult childbirth (Adjanohoun *et al*., 1989). In the present study, both the acute and subchronic effects of *F. thonningii* hexane fraction were examined.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Sample

The plant sample was collected in January 2024 from Kontagora town, Kontagora Local Government Area of Niger State. It was authenticated by a Taxonomist from the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro, with a voucher specimen (25) deposited in the herbarium of the same Department.

2.2. Plant Preparation and Extraction

The leaves of *Ficus thonningii* was collected and is washed with tap water and air dried at room temperature under shade. It was pounded to a coarse powder using a mortar and pestle. Five hundred grams (500g) was diluted with 1500ml of methanol for 74 hours. It was filtered using a muslin cloth and the filtrate was evaporated in an electric oven at 45^0C. The methanol crude extract from *Ficus thonningii* was suspended in water (60 mL). Then it was extracted successively with different organic solvents which include hexane, chloroform, ethyl acetate and water to obtain hexane, ethyl acetate, chloroform and butanol (40 mL) and residual methanol fractions, respectively. All crude extracts were filtered separately through Whatman No. 1 filter paper to remove particles. The free crude extract was evaporated completely under reduced pressure to obtain dry crude extracts. The residue left in the separatory funnel was re-extracted twice following the same procedure and filtered. All extracts were concentrated and dried by using a rotary evaporator under reduced pressure. The percentage yield was calculated using the formula below.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

2.3. Experimental Animals

The albino rats used in this study are purchased from Animal House, Usman Danfodiyo University, Sokoto in February, 2024. They were transported in well-ventilated cages to Animal House, Faculty of Life Sciences, Kebbi State University of Science and Technology,

Aliero. The rats were housed in clean cages and were allowed to acclimatize for fourteen (14) days before the commencement of the experiment. The rats were fed with a standard rat feed and were allowed free access to water and libitum.

2.4. Phytochemical Screening

Phytochemical screenings were carried out according to the methods described by Trease and Evans, (1989).

2.5. Acute Oral Toxicity Studies (LD_{50}) of *Ficus thonningii* hexane Fraction

The acute oral toxicity study was conducted according to the method of Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001). This method has two phases which are phase 1 and 2 respectively.

Phase 1

This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals is administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behavior as well as mortality.

Phase 2

This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality). The animals were observed for signs of drowsiness, hair loos, and loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were then observed for a period of 14 days for any signs of delayed toxicity (Lorke, 1983).

2.6. Sub-chronic Oral Toxicity Study of *Ficus thonningii* Hexane Fractions

Twenty (20) albino rats weighing 100-250g were randomly divided into five (5) groups of four (4) animals each. The animals are acclimatized for 2 weeks on a regular feed with pelletized feed and water. Group one served as [control] and was administered with distilled water only, other groups (2-5) were administered 5%, 10%, 15%, and 20% of the calculated extract LD50, respectively. The animals were administered at different doses of the extract for 28 days based on varying individual body weights as follows:

Group I	5ml/kg body weight of distilled water only.
Group II	250mg/kg body weight of *Ficus thonningii* hexane fraction
Group III	500mg/kg body weight of *Ficus thonningii* hexane fraction
Group IV	750mg/kg body weight of *Ficus thonningii* hexane fraction
Group V	1000g/kg body weight of *Ficus thonningii* hexane fraction

The extract was orally administered to the animals daily for 28 days. The rats were sacrificed on the twenty-ninth day of the experiment. Blood samples were collected in heparinized bottles for biochemical analysis

2.6.1. Measurement of Liver Function Test

Alkaline phosphatase activity was estimated using the method of Sood, (2005). Aspartate aminotransferase catalytic activity and alanine aminotransferase activity were determined by the method of Reitman and Frankel, (1957). Albumin was determined using the bromocresol green method as modified by Doumas *et al*., (1971). Total protein was estimated using the Biuret reaction method by Lowry *et al*., (1951). Total and Direct Bilirubin were determined by the calorimetric method of Jendrassik and Grof, (1938).

2.6.2. Measurement of Renal Function Markers

Serum urea was determined according to Berthelot colorimetric method of Young, (1997). Serum creatinine was determined using Jaffe's method as described by Bartels and Bohmer, (1971). Serum uric acid concentration was determined using the method of Henry *et al*., (1957). Serum sodium and potassium ions were measured using flame photometry. Serum bicaronate and chloride ions are measured using titration/volumetric method described by Chapman, (1961).

2.6.3. Haematological Analysis

Hematological parameters including, white blood cells count (WBC), hemoglobin concentration, packed cell volume (PCV), lymphocytes, neutrophils, esosinophils, monocytes and basophiles, were analysed using an automated hematological analyzer Sysmex XS800i (Sysmex corporation, USA) (2004).

2.7. Data Analysis

The data generated from the study are presented as Mean ± Standard deviation and subjected to one-way analysis of variance (ANOVA) statistical difference between means were seperated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$.

3. RESULTS

3.1. Phytochemical Constituents of *Ficus thonningii* Hexane Fraction

Several phytochemicals in methanol leaf extract of *Ficus thonningii* were detected and are presented in Table 1. The result revealed the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides, steroids, balsams, saponin and glycoside. Anthraquinines and volatile oils are not detected.

Table 1: Qualitative Phytochemical Constituents of *Ficus thonningii* Methanol Leaves Extract

Phytochemicals	Inference
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Cardiac glycosides	+
Steroids	+
Glycosides	+
Quinines	+
Anthraquinone	-
Volatile oils	-

Key: + = Detected; - = Not detected

3.2. Acute Toxicity Effect of *Ficus thonningii* N-hexane Leaves Fraction in Albino Rats

The acute oral toxicity (LD_{50}) effect of *Ficus thonningii* hexane extract is calculated to be greater than 5000 mg/kg body weight since there was no sign of toxicity observed which include, convulsion, bulging of eyes, restlessness, weakness, hair loss, and paralysis or mortality after 14 days of monitoring.

3.3. Sub-chronic Toxicity Effect of *Ficus thonningii* Hexane Fraction

3.3.1. Effect of *Ficus thonningii* N-hexane Leaves Fraction on Bodyweight of Rats

The effect of *Ficus thonningii* n-hexane leaves fraction on bodyweight on bodyweight is presented in Figure 1. A progressive increase in animal bodyweight was observed in all the treatment groups throughout the experimental period (week 1, 2, 3 and 4) respectively.

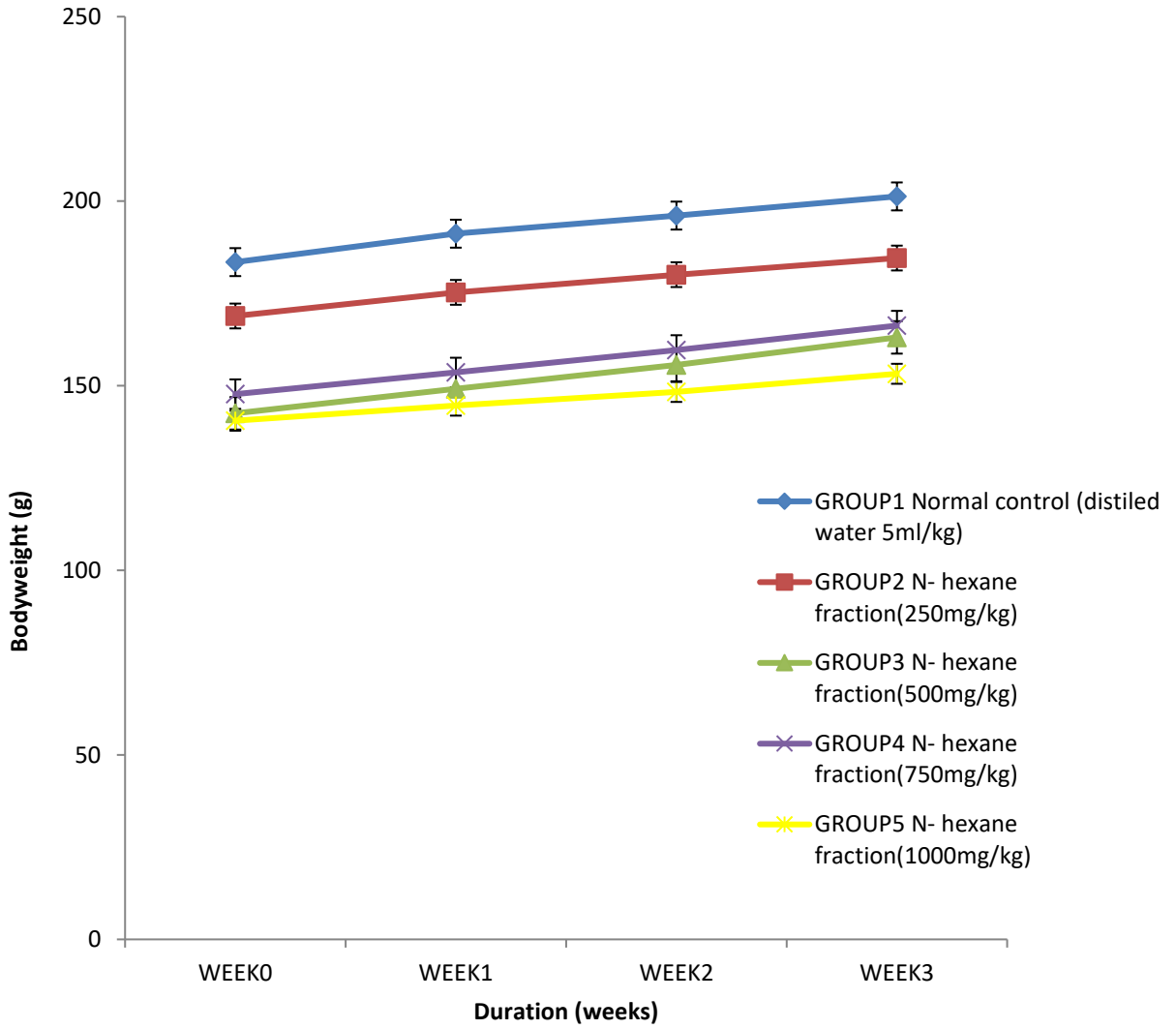

Figure 1: Effect of *Ficus thonningii* Hexane Leaf Fraction on Bodyweight

3.3.2. Effect of *Ficus thonningii* Hexane Leaves Fraction on Liver Function Parameters

The effect of *Ficus thonningii* solvent fractions extract on biomarkers of liver toxicity is presented in (Table 2). The Result revealed non-significant ($P<0.05$) differences in all the parameters ALT, AST, ALP, TP, ALB, DB AND TP activity compared to the normal control group.

3.3.3. Effect of *Ficus thonningii* Hexane Leaves Fraction on Kidney Function Parameters

The effect of *Ficus thonningii* leaf hexane fractions on kidney toxicity is presented in (Table 3). There was no significant ($P<0.05$) decrease in (Creatinine, Uric acid, K and Cl^-). There was a significant ($P<0.05$) decrease in urea and HCO_3 concentration in the group administered with the highest dose (750mg/kg body weight) compared to the normal control. While, Na^- revealed significant ($P<0.05$) decreases only in groups treated with 750 and 1000mg/kg compared to the normal control.

3.3.4. Effect of *Ficus thonningii* N-hexane Leaves Fraction on Hematological Parameters

The subchronic effect of *Ficus thonningii* hexane fractions on hematological indices is presented in (Table 4). A non-significant decrease ($P>0.05$) in WBC, MCHC, MCV, RDW and lymphocytes in all extract treated groups compared to normal control. HGB and hematocrits

significantly (P<0.05) decreased in groups treated with 500, 750, and 1000mg/kg respectively compared to normal control. MCH significantly (P<0.05) decreased only in group treated with 750mg/kg compared to normal control. However, PLT significantly (P<0.05) increased only in group treated with 750mg/kg compared to normal control.

Table 2: Effect of *Ficus thonningii* Hexane Leaf Fraction on Liver Function Parameters

PARAMETER	Control (2ml/kg Distilled water)	Hexane Fraction (250 mg/kg)	Hexane Fraction (500 mg/kg)	Hexane Fraction (750 mg/kg)	Hexane Fraction (1000 mg/kg)
ALT (U/L)	62.28±0.03 [a]	67.83±3.29 [a]	71.84±3.44 [a]	59.01±8.78 [a]	61.59±10.81 [a]
AST (U/L)	154.57±10.58 [a]	163.23±5.20 [a]	158.80±3.56 [a]	161.94±9.64 [a]	160.51±5.14 [a]
ALP (U/L)	63.48±1.39 [a]	70.00±0.57 [a]	67.24±1.43 [a]	63.56±7.29 [a]	63.47±7.29 [a]
TP (g/dl)	3.42±0.48 [a]	4.09±0.27 [a]	4.35±0.19 [a]	3.22±0.63[a]	4.20±0.23 [a]
ALB (g/dl)	2.01±0.36 [a]	2.05±0.44 [a]	1.78±0.35 [a]	2.14±0.23 [a]	1.89±0.31[a]
DB (mg/dl)	1.20±0.01 [a]	0.91±0.15 [a]	1.28±0.31 [a]	1.09±0.01 [a]	1.19±0.02 [a]
TB (mg/dl)	3.58±0.02 [a]	3.82±0.09 [a]	3.87±0.43 [a]	3.65±0.27 [a]	3.50±0.30 [a]

Values are expressed as mean ± standard error of the mean (n = 5). Mean values having common superscript letters in rows are significantly different (P>0.05) analyzed one-way ANOVA followed by Duncan's multiple range test). ALT- Alanine Amino Transferase, AST- Aspartate Amino Transferase, ALP- Alkaline Phosphatase, TP- Total Protein, ALB- Albumin, DB- Direct Bilirubin, TB- Total Bilirubin. MLEIT - Methanol Leaves Extract of *Ficus thonningii*

Table 3: Effect of *Ficus thonningii* Methanol Leaf Extract on Kidney Function Parameters

Parameters	Control (2ml/kg Distilled water)	Hexane Fraction (250 mg/kg)	Hexane Fraction (500 mg/kg)	Hexane Fraction (750 mg/kg)	Hexane Fraction (1000 mg/kg)
Urea (mmol/l)	25.53 ± 1.76 [a]	27.56±1.32 [ab]	27.72±0.44 [a]	28.24±2.08 [b]	24.94±0.89 [a]
Creatinine (µmol/l)	3.85±0.67 [a]	4.18±0.84 [a]	3.88±0.93 [a]	4.34±0.75 [a]	4.63±0.54 [a]
UA (mg/dl)	5.67±0.08 [a]	5.75±0.53 [a]	5.34±0.16 [a]	5.39±0.68 [a]	5.12±0.20 [a]
Na^+ (mg/l)	96.67±1.67 [b]	103.33±6.01 [b]	100.00±2.87 [ab]	100.33±4.84 [a]	103.33±4.41 [a]
Cl^- (mg/l)	74.40±2.27 [ab]	72.08±1.08 [a]	74.42±1.08 [ab]	74.33±2.30 [ab]	78.00±1.30 [b]
K^+ (mg/l)	12.17±0.60 [a]	11.17±0.73 [a]	11.67±0.67 [a]	12.00±1.15 [a]	11.00±1.00 [a]
HCO_3^-(mg/l)	20.33±1.21 [a]	22.28±1.10 [ab]	22.56±0.91 [ab]	24.15±1.13 [b]	21.89±0.60 [ab]

Values are expressed as mean ± standard error of the mean (n = 5). Mean values having common superscript letters in rows are significantly different (P>0.05) analyzed one-way ANOVA followed by Duncan's multiple range test). Uric Acid (UA), Potassium (K+), Sodium (Na+), Bicarbonate (HCO3) MLEIT - Methanol Leaves Extract of *Ficus thonningii*.

Table 4: Effect of *Ficus thonningii* N-hexane Leaves Fraction on Haematological Parameters

Parameter	Control (2ml/kg Distilled water)	Hexane Fraction (250 mg/kg)	Hexane Fraction (500 mg/kg)	Hexane Fraction (750 mg/kg)	Hexane Fraction (1000 mg/kg)
WBC (10^9/L)	34.25±7.93 ^{ab}	31.36±7.58 ^{ab}	18.06±4.36 ^{a}	17.11±3.23 ^{a}	42.43±6.50 ^{b}
RBC (10^{12}/L)	7.47±0.04 ^{c}	7.57±0.05 ^{c}	6.57±0.22 ^{ab}	7.05±0.10 ^{bc}	6.26±0.38 ^{a}
HGB (g/dl)	15.57±0.07 ^{b}	15.20±0.26 ^{b}	12.30±0.35 ^{a}	13.13±0.54 ^{a}	12.07±0.93 ^{a}
MCHC (g/dl)	118.07±88.47 ^{a}	28.20±0.60 ^{a}	28.80±0.56 ^{a}	29.77±0.99 ^{a}	29.20±0.00 ^{a}
MCH (pg)	20.83±0.23 ^{b}	19.97±0.39 ^{ab}	18.77±1.02 ^{ab}	18.67±0.52 ^{a}	19.23±0.49 ^{ab}
MCV (fL)	70.47±1.23 ^{a}	70.93±2.19 ^{a}	65.03±3.01 ^{a}	62.87±3.90 ^{a}	65.23±1.11 ^{a}
PLT (%)	311.00±48.27 ^{a}	396.67±78.88 ^{ab}	315.33±53.72 ^{a}	565.67±102.57 ^{b}	482.33±63.01 ^{ab}
Hematocrits (10^9/L)	52.63±0.67 ^{b}	53.70±1.68 ^{b}	42.70±0.55 ^{a}	44.40±3.31 ^{a}	41.33±3.27 ^{a}
RDW (%)	18.80±0.06^{a}	16.63±0.64 ^{a}	16.73±0.61 ^{a}	16.60±0.23 ^{a}	17.90±1.61 ^{a}
Lymphocytes (%)	81.37±1.20 ^{ab}	86.37±3.41 ^{b}	71.37±4.97 ^{a}	87.83±1.28 ^{b}	78.07±5.47 ^{ab}

Values are presented as mean ± SEM (n = 5) Mean values having common superscript letters in rows are significantly different (P>0.05) analyzed one-way ANOVA followed by Duncan's multiple range test). WBC-white blood cell, RBC-red blood cell, HGB-haemoglobin, MCHC-mean cell haemoglobin concentration, MCH-mean cell haemoglobin, MCV-mean corpuscular volume, PLT-platelets.

4. DISCUSSION

Many indigenous plants have been reported to contain numerous constituents of different chemical classes of secondary metabolites, such as alkaloids, terpenoids, essential oils, glycosides, steroids, phenolic constituents, aliphatic compounds, and polysaccharides. Leaves, stems, and roots of these plants are a rich source of proteins, flavonoids, alkaloids and glycosides (Hussein and El-Anssary, 2019). These active compounds are reported to have several biological activities, including anti-septic, anti-inflammatory, anti-cancer, anti-malarial and anti-diabetic activities (Abdelkhalek *et al*., 2024). Elshafie *et al*., (2023) reported that plant-derived secondary metabolites are small molecules or macromolecules biosynthesized in plants, including steroids, alkaloids, phenolic, lignans, carbohydrates, and glycosides, etc., that possess a diversity of biological properties beneficial to humans, such as their anti-allergic, anti-cancer, anti-microbial, anti-inflammatory, anti-diabetic and anti-oxidant activities. In the present study, the anti-malarial activity observed might be due to these phyto-constituents in *Ficus thonningii* leaf methanol extract.

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short time (usually less than 24 hours). Acute toxicity tests in animals (i.e. rats) use mortality as the primary observational endpoint in order to derive an LD_{50} (González-Martín *et al*., 2021). Substances with LD_{50} below 5 mg/ kg are classified to be highly toxic, 5-50mg/kg highly toxic, 50-500mg/kg moderately toxic, 500-5000mg/kg slightly toxic, 5000-15000mg/kg practically non-toxic, in comparison, substances with LD_{50} above 15,000 mg/kg are termed relatively harmless (Aminu *et al*., 2024). In the present study, the LD_{50} of *Ficus thonningii* leaf hexane extract is found to be above 5000mg/kg, suggesting that the extract is relatively non-toxic at acute doses.

The increase in body weight might indicate increased adiposity, which can decrease the glucose level in the blood, as supported by previous findings. The non-significant changes of toxicity biomarkers may be due to the fraction of non-toxicity effect. An increase in AST level occurs due to liver damage and AST is considered a less specific marker for liver injury than ALT, as it is also found in other tissues, such as the brain, myocardial cells, and skeletal muscle cells. A decrease in ALT and ALP levels indicates the plant extract has no toxic effect in the liver cells of the experimental animals (Thakur *et al*., 2024). In addition to this, damaged liver cells release ALP into the blood. ALP concentration in plasma also elevates with significant bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver. However, as the changes in the level of other liver enzymes are not significantly altered, it is clear that the plant extract has less or no toxic effect, and it can be a potential candidate for a new natural drug source (Aminu *et al*., 2024). The usual serum

test, which checks the functionality of the kidneys, measures the levels of urea, creatinine, and certain dissolved salts (serum electrolytes) (Ugwah-Oguejiofor *et al*., 2019).

High serum level of urea indicates that the kidneys may not be functioning properly, or that the animal is dehydrated, whereas low urea level is associated with acute liver failure or overhydration (Njinga *et al*., 2020). Creatinine, is an indicator of glomerular filtration rate and is used for assessing kidney function (Nelson *et al*., 2006). *Ficus thonningii* leaf hexane fraction did not cause any apparent signs of alteration in kidney function parameters, suggesting that the extract is not nephrotoxic.

Analyses of hematological parameters are used to study the extent of toxicity of drug substances, including plant extracts (Ibrahim *et al*., 2016). Hematopoiesis is the process of blood cell formation. Changes in the hematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies (Fornari *et al*., 2019). All blood cells are derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet) (George-Gay and Parker, 2003). Lymphocytes are dynamic cells which mediate immune response to foreign substances (Pearce *et al*., 2013). They also produce antibodies which enabled the destruction of intracellular microbes and cancer cells; an abnormally high platelet count may point to hemostatic disorders and eventually lead to thromboembolic diseases (Lisman and Porte, 2010). In the present study, there was no alteration in all hematological parameters, suggesting that *Ficus thonningii* leaf hexane fraction does not have adverse effect on hematological parameters.

5. CONCLUSION

In conclusion, the findings of the present studies do not show any sign of effect at acute dose, and its long-term oral administration for 28 days shows no toxicity; hence, the extract is relatively non-toxic at both acute and sub-chronic administrations.

Acknowledgement

We appreciate participants who contributed in the conduct of this research especially Abdulhamid Zubairu.

Ethical approval & declaration

In this article, the animal regulations followed as per the ethical committee guidelines of Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria; the authors observed the acute and subchronic toxicity evaluation of *Ficus thonningii* Leaves Hexane Extract in Albino Rats. The albino rats used in this study are purchased from Animal House, Usman Danfodiyo University, Sokoto. The Animal ethical guidelines are followed in the study for species observation, identification & experimentation.

Consequently, as per the plant regulations followed in the Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria; the authors observed the acute and subchronic toxicity evaluation of *Ficus thonningii* Leaves. The plant sample was collected from Kontagora town, Kontagora Local Government Area of Niger State. The ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation.

Informed Consent

Not applicable.

Conflict of Interest

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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