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Hepatoprotective Effect of Ethanol Leaf Extract of *Ficus thonningii* (Blume) against Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Drug-induced liver injury is a potential complication of virtually all medications because the liver has a central role in the metabolism of drugs and toxic substances The study aimed to evaluate the hepatoprotective activity of the ethanol leaf extract of *F. thonningii* (Blume) against carbon tetrachloride (CCl_4)-induced liver damage in Wistar rats. The hepatoprotective effect was assessed by evaluating the biochemical parameters and histopathological examination of the liver of treated and control animals. The Phytochemical constituents of the extract was further studied using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The results showed that the hepatoprotection of the extract was confirmed by the significant ($P < 0.05$) decrease in the serum levels of the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), and Total bilirubin (TB) especially at higher doses. Histopathological examination revealed cytolysis in the liver of untreated animals, while treated animals showed typical standard liver architecture. The GC-MS analysis revealed the presence of palmitic acid, phytol, oleic acid, iconic acid, 9,12-Octadecadienoic acid, and 9-Octadecenal. Findings of this study substantiate the folkloric use of *F. thonningii* in the management of hepatic ailments.

Keywords: *Ficus thonningii* (Blume), Carbon tetrachloride (CCl_4), Wistar rats, Hepatotoxicity.

Introduction

The liver is one of the largest organs in the human body and the main site of metabolism and excretion. It therefore has a big role during the routine maintenance, and homeostasis of the body, fight against infections, nutrient supply, and reproduction.[1] The primary functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile, and storage of vitamins. Furthermore, it's continuous and varied exposure to environmental toxins, abuse by poor drug habits, chronic alcoholism contribute to various liver ailments like cirrhosis, hepatitis and other related liver diseases.[2,3] The hepatoprotective effects of herbs are usually studied either *in vitro* or *in vivo* against hepatotoxicity induced by drugs like carbon tetrachloride (CCl_4), *β*-galactosamine, thioacetamide, paracetamol, nimesulide, isoniazid and Rifampicin at a different doses and varying duration.[4] These hepatoxic agents are considered as oxidative stress inducers due to elevated levels of reactive oxygen species (ROS); hydrogen peroxide (H_2O_2), hydroxyl radical (HO) and peroxy radicals in biomolecules,[5] that binds to proteins and lipids to initiate lipid peroxidation and contribute to liver injury.[6] Hence, detoxification by antioxidants is essential in order to restore co-factors and repair altered biomolecules.[5] In the absence of reliable liver protective drugs in medical practices, herbs with antioxidant properties such as silymarin, a flavonol lignan mixture extracted from the milk thistle (*Silybum marianum*) have been used in the management of various liver diseases like hepatitis and cirrhosis.[7] Liver diseases have increased rapidly in recent years.[8] Also, the development of liver diseases has become a global issue; their treatment and management mostly result in more economic problems, especially in Africa.

Synthetic drugs used in the treatment of hepatic disorders mostly come with side effects. Therefore there is a need to search for other natural products of plant origin for their hepatoprotective effect and further evaluate their modulatory effect on endogenous liver antioxidant enzymes.

For this reason, the hepatoprotective effect of a commercial drug (Silymarin) and ethanol leaf extract of *Ficus thonningii* was compared. *Ficus thonningii* (Common Name: Strangler Fig. Hausa Name: Cediya) belongs to the family Moraceae. All parts of the plant are medicinally useful; the latex-rich leaves are preferred because latex has been traditionally associated with potency.[9] Macerations of this leaves, that is usually administered orally, have been applied by traditional healers for treating diarrhea, gonorrhea, and diabetes mellitus.[10] Decoctions of *F. thonningii* leaves are used for healing wounds. Leaf extracts are also widely utilized during the treatment of bronchitis and urinary tract infections.[11] Furthermore, decoction of this extract is used in Mali for treating urinary schistosomiasis.[12] In Nigeria, maceration of the leaves is used for treating stomach pains, gastritis, gastric ulcers, and other stomach conditions in animals.[13] However, this leaves were reported to be effective for the treatment of bone movement disorders, ringworm, thrush, scabies, and athlete's foot rot.[14, 15]

An ethnobotanical study revealed that *Ficus thonningii* (Blume) was among the most cited species used locally in the management of hepatic ailments in Katsina, Northern part of Nigeria.[16] Despite the excessive use of these taxa, there are little or scanty data proving the efficacy of this specie. This study is therefore designed to validate the folkloric use of *F. thonningii* in search for effective and safe medicine for the management of liver diseases.

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Materials and Methods

Chemicals, Drugs, and Reagents

The Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Commercial kits were obtained from Randox laboratories, United Kingdom; Carbon Tetrachloride (Cardinal Scientific Supplies, Zaria, Kaduna State, Nigeria), Silymarin (Micro Pharmaceuticals, India). Ethanol, chloroform, and the rest of the chemicals utilized in this research were of analytical grade.

Plant material

Fresh leaves *of Ficus thonningii* were collected from Umaru Musa Yar'adua University (UMYU), Katsina, Nigeria in August-September 2018. Its botanical identities were determined and authenticated by Dr. Abubakar Bello of Biology Department UMYU Katsina, with a voucher specimen number; SSK150. The leaves were washed with water, cut into pieces, dried in the shade for five days, and then dried in an oven below 60ºC. The dried plant materials were then pulverized into coarse powder using mortar and pestle. Five hundred grammes of the plant sample was extracted with 1000 mL ethanol using soxhlet apparatus. Solvent from the sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract.

Experimental animals

Wistar rats weighing between 120-150 g of both sexes were explored in this assessment. These rats aged between 2 and 2.5 months were procured from the animal house of the Pharmaceutical Sciences Department at Ahmadu Bello University, Zaria, Nigeria. They were housed in well-ventilated stainless-steel cages at room temperature (24 ± 2ºC) in the appropriate environmental condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *ad libitum*. Animals handling and experiments conducted in this study approved by Ahmadu Bello University ethical committee on animal use and care, and the research was carried out in compliance with the National institute of health guidelines for care and use of Laboratory Animals (Pub No 85-23, revised 1985).

Acute toxicity study: median lethal dose (LD_{50})

The median lethal dose (LD_{50}) of the extract was determined using Lorke's method.[17] The analysis was conducted in two phases. In phase 1, three groups of three animals each were used. The extract was administered orally in widely differing doses (10 mg/kg, 100 mg/kg, and 1000 mg/kg). The treated animals were observed frequently for up to twenty-four hours post-administration for signs of toxicity. The second phase of the experiment was based on the outcome of the first phase (presence or absence of death). After 24 hours, in the absence of mortality, stage 2 was carried out. In stage 2, four groups of one animal each were orally treated with extract with doses of (1200 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The square root of the smallest dose that caused death and the highest dose that did not was taken as the median lethal dose (LD_{50}) of the extract.

Experimental design for hepatoprotective activity

The CCl_4 model described by Obi *et al*.[18] was used for scheduling the dose regimen. Carbon tetrachloride (0.5 mL/kg *i.p*.) diluted in vegetable oil (1:1) was employed for inducing liver damage. Thirty-six (36) Wistar rats of both sexes used in this study were divided randomly into Six (6) groups (I, II, IIIa, IIIb, IIIc, and IV) of six rats each. The hepatoprotective activity of this extract was tested using the CCl_4 model. Group I (Normal Control) received neither the plant extract nor CCl_4 for 72 hours, that is, they received food and water only; Group II (Induction Control) were given a single intraperitoneal dose of CCl_4. Groups IIIa, IIIb, and IIIc were given a single intraperitoneal dose of CCl_4 followed by ethanol-leaf extract of *Ficus thonningii* at 500 mg/kg, 250 mg/kg and 125 mg/kg orally, respectively). Finally, Group IV received a single intraperitoneal dose of CCl_4 followed by Silymarin (Standard Drug) at a dose of 100 mg/kg. The suspension of the test samples was then administered to rats at 1 hour, 2 hours, and 48 hours after CCl_4 injection.

Assessment of the hepatoprotective activity

After three days of drug treatment, the animals were dissected under chloroform anesthesia. Blood from each rat was withdrawn from the carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature.[19] Separation of serum was done by centrifugation at 3000 rpm for 15 minutes. The separated serum was used for the estimation of some biochemical parameters using commercial reagent kits (Randox Laboratories, United Kingdom) by the following methods; Alanine aminotransferase (ALT/SGPT) and Aspartate aminotransferase (AST/SGOT),[20] Alkaline phosphatase (ALP) and Total bilirubin (TB).[21]

Histopathological studies

For the histopathological examination, the liver from each animal was removed after dissection and preserved in 10% formalin solution for a period of 24 hours. Then representative blocks of liver tissues from each lobe were taken and embedded with paraffin wax using the standard micro-technique.[22] Segments (5 µm) of livers stained with hematoxylin and eosin, was observed microscopically for histopathological studies.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the plant using ethanol extract of *Ficus thonningii* leaves was done at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. The prepared leaves extracts were analyzed using GCMS-QP2010 plus Shimadzu Japan, equipped with a VF-5 ms fused silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 mm film thickness. For GC-MS recognition, it was achieved by an electron ionization system with an ionization energy of 70 eV. Helium gas was used as a carrier gas at a constant flow rate of 1.58 mL/min. Injector and mass transfer line temperature was set at 230 and 250°C, respectively. The oven temperature was programmed from 80 to 200°C at 10°C/min, held isothermal for 1 min, and finally raised to 280°C. Identification of the constituent was achieved by comparison of the mass spectra and those in the library, National Institute of Standard Technology (NIST).

Statistical analysis

The results were analyzed by one way ANOVA followed by Tukey's test using SPSS 16.0. The results were expressed as means ± Standard deviation. A probability level of < 0.05 was considered statistically significant.

Results and Discussion

Acute toxicity study for oral median lethal dose (LD_{50})

The acute lethal study of ethanol leaf extract of *Ficus thonningi* on rats shows no observable signs of toxicity following post-treatment observation in both the first and second phase experiment, and no animal died within the first and second day after treatment with the extracts (Table 1). The oral median lethal dose (LD_{50}) for the plant extract is therefore greater than or equal to 5000 mg/kg b.wt which is a safe dose suggested by lorke.

Table 1: Acute lethal effect of ethanol leaf extract of *Ficus thonningii* administered to wistar rats

Experiment	Dose (mg/kg b.wt)	No. of Dead Rats after 24 hours of Treatment
Phase 1	10	0/3
	100	0/3
	1000	0/3
Phase 2	1200	0/1
	1600	0/1
	2900	0/1
	5000	0/1

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Hepatoprotective activity

The effect of ethanol leaves extract of this plant *Ficus thonningii* on liver function in carbon tetrachloride (CCl_4)-induced Wistar rats at different doses of 500 mg/kg b.wt, 250 mg/kg b.wt, and 125 mg/kg b.wt is illustrated in Table 2.

The carbon tetrachloride group significantly increased the serum level of the biochemical markers of hepatotoxicity viz; ALT (115 ± 8.39 U/L), AST (178.81 ± 5.67 U/L), ALP (75.56 ± 1.52 U/L) and TB (2.25 ± 0.33 mg/dL) after 72 hours of CCl_4 administration.

The ethanol leaf extract of the sample at a dose of 125 mg/kg showed insignificant changes, while at 500 mg/kg and 250 mg/kg b.wt exhibited significant protection against CCl_4-induced liver injury as shown by the reduction in toxin-mediated rise in ALT, AST, ALP activities and TB concentration in rats, with 500 mg/kg dosage showing the highest recovery of the biochemical markers which is near to that of the standard drug, i.e., Silymarin.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are the most common biochemical markers used to evaluate liver injury.[23]

Elevation of these liver enzymes is associated with cell necrosis of many tissues, especially the liver.[24] This was confirmed in this study, as the ALT and ALP activities were elevated in untreated carbon tetrachloride (CCl_4)-induced hepatotoxic rats. The increase in ALP and ALT concentrations following CCl_4 administration is in line with existing literature that synthesis of these enzymes is increased by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure.[24] The decrease in activities of these enzymes in rats pre-treated with the ethanol leaf extract indicate that the leaf extract may offer some protection and maintain the functional integrity of hepatic cells. This protective effect has been attributed to its ability to stabilize the plasma membrane, thereby preserving the structural integrity of cells as well as the repair of hepatic tissue damage caused by CCl_4.[25]

Following the administration of the ethanol leaf extract of *Ficus thonningii*, the result showed that the extract at 500 mg/kg significantly ($p < 0.05$) reduced ALT (89.00 ± 1.00 U/L) and ALP (35.22 ± 0.76 IU/L) activities when compared to rats induced with CCl_4 only (115.00 ± 8.39 U/L and 75.56 ± 1.52 IU/L for ALT and ALP, respectively).

The inconsistencies in the values of AST, when compared with negative control rats, maybe because the enzyme is not specific to the liver. It is found in other organs like heart, muscle, brain, and kidney. Although, AST is useful in detecting liver injury it is considered a less specific biomarker for hepatocellular injury as it can also signify abnormalities in the heart, muscle, brain or kidney.[26]

Histopathological studies

Histopathological examination of the liver tissues of rats administered with ethanol leaf extract of *Ficus thonningii* (FT) is presented in Figure 1.

Liver sections of Normal/Control rats (Plate a) showed remarkable liver tissues, healthy hepatic cells with intact cytoplasm and nucleus. In contrast, that of the untreated, i.e., CCl_4 only (Plate b) showed severe hepatocellular necrosis with Kupfer hyperplasia. Rats treated with 250 mg/kg FT (Plate d) showed slight hepatocellular necrosis. In comparison, those given 500 mg/kg FT (Plate c) and Standard Drug silymarin (Plate f) showed typical architecture and well-defined nucleus when compared with the control.

Rats treated with extract 125 mg/kg FT (Plate e) showed severe to moderate hepatocellular necrosis and vascular congestion, as shown in Figure 1.

The hepatoprotective nature of the ethanol leaf extract of the sample was also seen during cross-examination of the histopathological features of the liver sections among the different groups of the rats. Regular standard control group showed remarkable liver tissues with normal hepatic cells, cytoplasm, and nucleus. The negative control rats showed severe cytolysis with degrees of fatty degeneration and the loss of cellular boundaries as the nucleus were seen in blue stains exposed to the cytoplasm following disruption of the nuclear membrane. Rats treated with 250 mg/kg of *Ficus thonningii* ethanol extract showed proliferative hepatocytes with mild degenerative changes and increased intercellular space. But as the dose increases (500 mg/kg), the liver showed standard architecture and well-defined nuclei compared with the normal control group and that of the standard drug, silymarin, which showed normal architecture with well-defined nucleus as well.

Gas chromatography-mass spectrometry (GC-MS) analysis of the leaves extract of Ficus thonningii

Results from the GC-MS analysis of the ethanol extract of the sample from the library of National Institute of Standards (NIST14.L) showed about 26 compounds from *Ficus thonningii* (Table 3). Out of which six compounds have been found to possess hepatoprotective potentials. n-Hexadecanoic acid (Palmitic Acid)[27] have shown to possess both antioxidant and anti-inflammatory properties. Others with anti-inflammatory properties include 9,12-Octadecadienoic acid,[28] Phytol[29] and Eicosenic acid.[30] Oleic acid was also reported to have both anti-inflammatory and antioxidant properties[31] as well as antimicrobial properties,[32] and 9-Octadecenal was also said to have a control effect on human pathogens.[33]

Table 2: Hepatoprotective effect of ethanol leaf extract of *Ficus thonningii* on some liver function test parameters in carbon tetrachloride-induced Wistar rats

Biochemical Markers	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (mg/dL)
Control	75.00 ± 1.53	133.0 ± 2.00	35.50 ± 1.76	0.63 ± 0.02
CCl_4 (0.5 mL/kg)	115 ± 8.39[a]	178.81 ± 5.67[a]	75.56 ± 1.52[a]	2.25 ± 0.33[a]
F. thonningii (500 mg/kg)	89.00 ± 1.00[b]	140.4 ± 0.74[b]	35.22 ± 0.76[b]	0.71 ± 0.02[b]
F. thonningii (250 mg/kg)	95.00 ± 1.00[b]	151.80 ± 1.61[b]	45.60 ± 0.79[b]	0.90 ± 0.01[b]
F. thonningii (125 mg/kg)	100.30 ± 1.53[b]	160.10 ± 1.05[b]	60.40 ± 0.72[b]	1.07 ± 0.07[b]
Silymarin (100 mg/kg)	84.80 ± 2.27[b]	132.90 ± 4.04[b]	36.89 ± 1.48[b]	0.64 ± 0.07[b]

Mean ± Standard deviation. [a] $P < 0.05$ Compared with control, [b] $P < 0.05$ Compared with standard

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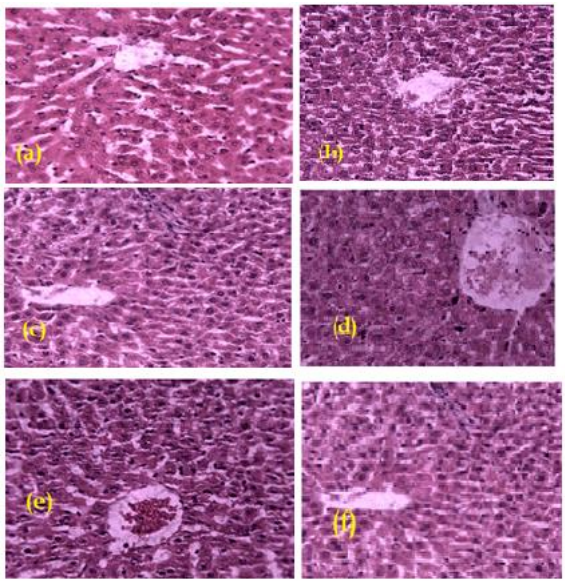

Figure 1: Photomicrophotograph (200X) of H & E stained liver tissues (a) (Normal Control); (b) (CCl_4 Induction); (c) A500 (CCl_4 + 500 mg/kg *Ficus thonningii* ethanol leaf extract); (d) A250 (CCl_4 + 250 mg/kg *Ficus thonningii* ethanol leaf extract); (e) A125 (CCl_4 + 125 mg/kg *Ficus thonningii* ethanol leaf extract); (f) (CCl_4 + Standard Drug, Silymarin).

Table 3: Phytoconstituents of *Ficus thonningii* ethanol leaf extract Identified by GC-MS

S/NO	Retention Time	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Area Percentage (%)
1	4.711	2-Propanol,1,1-Oxybis	$C_6H_{14}O_3$	134	2.91
2	5.006	1-Propanol, 2-(2-hydroxyPropoxy)	$C_6H_{14}O_3$	134	3.65
3	5.239	7-Octen-2-ol,2,6-dimethyl	$C_{10}H_{20}O$	156	0.84
4	7.688	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	$C_{17}H_{23}NO_2$	273	0.37
5	10.437	Aromadendrene	$C_{15}H_{24}$	204	0.29
6	12.029	Trans-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	220	0.70
7	12.327	7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)	$C_{10}H_{16}O_2$	168	0.82
8	12.897	Isocitronellol	$C_{10}H_{20}O$	156	0.61
9	13.141	Eudesm-4(14)-en-11-ol	$C_{15}H_{26}O$	222	0.79
10	15.322	Octadecyne	$C_{18}H_{34}$	250	1.28
11	15.429	Octadecane, 1-(ethenyloxy)	$C_{20}H_{40}O$	296	0.73
12	16.102	11-Tetradecen-1-ol, acetate, (Z)	$C_{16}H_{30}O_2$	254	0.63
13	16.526	13-Docosenoic acid, methyl ester	$C_{23}H_{44}O_2$	352	0.44
14	16.981	Pentadecanoic acid, 14-methyl ester	$C_{17}H_{34}O_2$	270	8.34
15	18.215	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256	11.48
16	19.996	9,12-Octadecadienoic Acid, methyl ester (E,E)	$C_{19}H_{34}O_2$	294	8.89
17	20.089	11-Octadecenoic acid, methyl ester	C19H36O2	296	8.54
18	20.326	Phytol	$C_{20}H_{40}O$	296	4.67
19	20.426	Octadecanoic Acid, methyl ester	$C_{19}H_{38}O_2$	298	5.59
20	21.000	Oleic Acid	$C_{18}H_{34}O_2$	282	16.00
21	21.241	Octadecanoic Acid	$C_{18}H_{36}O_2$	284	5.69
22	22.548	Decane, 1-Fluoro	$C_{10}H_{21}F$	160	5.84
23	22.857	Eicosanoic Acid, methyl ester	$C_{21}H_{42}O_2$	326	1.65
24	24.359	9-Octadecenal	$C_{18}H_{34}O$	266	5.31
25	24.580	Octane, 4-ethyl	$C_{10}H_{22}$	142	1.81
26	27.916	Squalene	$C_{30}H_{50}$	410	2.12

Conclusion

This study validated the traditional use of *Ficus thonningii* (Blume) in the management of hepatic ailments and confirmed that this plant contains biochemical agents that have hepatoprotective potential against carbon tetrachloride (CCl_4) overdose.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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