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ASSESSMENT OF PRECLINICAL IN VIVO SUB-ACUTE TOXICITY OF FICUS THONNINGII BLUME (MORACEAE) STEM BARK EXTRACT IN WISTAR RATS

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ABSTRACT

In recent decades, spectrum of disease has shifted and complex chronic diseases have become the main part. The complementary and alternative treatment, especially the herbal medicine, has gained more attention and has also become popular due to the adverse side-effects, and also the development of resistance against synthetic drugs. The use of medicinal plants for various ailments, ranging from minor to chronic, is strongly driven by the increased costs of western medicines. So far, the World Health Organization (WHO) estimates that about 80 % of the global population depends on herbal products as the first line of primary health care intervention. These remedies, with a considerable extent of effectiveness, are socially accepted and economically viable. The objective of this study was to evaluate *in vivo* the subacute toxicity profiles of the hydro-ethanolic extract of the stem bark of *Ficus thonningii* Blume (Moraceae) on albino Wistar rat models. This study was conducted in the Animal house affiliated with the laboratory of pharmaco-toxicology and pharmacokinetics of the Faculty of Medicine and Biomedical Sciences in the university of Yaoundé 1 from November 2021 to May 2022. The subacute toxicity study was performed in 6-week-old rats. Animals were orally treated with a daily dose of 125 mg/kg, 250 mg/kg, 500 mg/kg extract for 28 days. Hematological and blood biochemical parameters, as well as kidney and liver histology, were recorded at the end of each experiment. Following subacute dosing, biochemical analysis revealed a slight elevation of liver parameters at all dose levels, while no significant increase was observed for kidney parameters. The results of the study show that administration of the ethanol extract of *F. thonningii* to adult rats by gavage provoked reversible alteration of liver biochemistry parameters. Kidney microarchitecture and biochemistry remained unaffected by the plant extract. These modifications are in agreement with the slight modifications observed in liver histology. These findings call for caution in the use of *F. thonningii* especially at very high doses.

KEYWORDS: *Ficus thonningii*, Hydro-ethanolic extract, Stem bark, Toxicity, Wistar rat.

INTRODUCTION

About 100 years ago, natural herbs were the main remedy for treating human diseases.[1] However, in the late 19th and early 20th centuries, interest in the use of medicinal plants for therapeutic purposes declined as there were many shortcomings in the processing methods used and a paucity of information on their side effects.[2] Traditional medicine eventually ended up being overshadowed by modern medicine as the means of treatment for human diseases.[3]

In recent decades, spectrum of disease has shifted and complex chronic diseases have become the main part.[1] The complementary and alternative treatment, especially the herbal medicine, has gained more attention and has also become popular due to the adverse side-effects, and also the development of resistance against synthetic drugs.[4] Also, the use of medicinal plants for various ailments, ranging from minor to chronic, is strongly driven by the increased costs of western medicines.[5] So far, the World Health Organization (WHO) estimates that about 80 % of the global population depends on herbal products as the first line of primary health care intervention.[6] These remedies, with a considerable extent of effectiveness, are socially accepted and economically viable.[4] However, it is conventionally said

that all effective drugs may produce adverse drug reactions; herbal medicines are no exception.[1] This said it clearly appears that medicinal plants should be used with caution and toxicology studies should be conducted to increase the knowledge on the plant or plants preparation given to populations.[7]

Ficus thonningii Blume (Moraceae) also known as the strangler or common wild fig is a native species of Central Africa.[8] Ethnobotanic and ethno-pharmacologic data show that it has been widely used for the management of many several diseases.[9–11] This plant is widely distributed in the upland forests of tropical and sub-tropical Africa dense humid forest.[12] Many bioactive compounds have been identified such as alkaloids, terpenoids, flavonoids, tannins and active proteins, all of which contribute to its therapeutic potential of the plant. *In vitro* and *in vivo* pharmacological studies revealed that *F. thonningii* extracts have anti-inflammatory, analgesic, antimicrobial, anthelmintic, antioxidant, cardioprotective, hypotensive, anticonvulsant and hypoglycemic effects.[13,14] The remarkable therapeutic effects exhibited by *F. thonningii* are as a result of the presence of an array of phytochemicals which include flavonoids, alkaloids, tannins, stilbenes, terpenoids and other active proteins.[15,16]

A number of studies[17,18] have recently shown that *Ficus thonningii* has promising biologic activities, for the management of peptic ulcers. This has motivated the thought that it has the potential to be developed into an improved traditional medicine. In spite of the wide folk remedy related uses of this plant, there is a scarcity of information regarding its toxicity profile.[16] This prompted researchers like Tembe et al (2018)[17] to conduct an acute toxicity study on the hydro-ethanolic stem bark extract of the plant in order to contribute information in regards to its toxicity profile and preclinical pharmacology. Therefore, in the present investigation, we aimed to investigate the subacute toxicity of F. *thonningii* in order to increase the confidence in the safety to humans.

MATERIALS AND METHODS

Ethical considerations

The study was conducted after having approval from the institutional review board of the Faculty of Medicine and Biomedical Sciences. An authorization was obtained from the head of animal house affiliated with the laboratory of pharmaco-toxicology and pharmacokinetics.

Plant material

The powdered stem bark of *F. thonningii* which was previously obtained from the preserved plant material of the work conducted by Pougoue (2017)[5] was used in the present study. Fresh stem barks were collected from the plant growing at Bafoussam on the 03 of January 2017. The barks were then identified taxonomically and authenticated at the National Herbarium of Cameroon by comparison with a sample having the number 44042/HNC by Tadjouteu F.

Experimental animals

The animals used were male and female white albino rats of the *Wistar* strain less than nine weeks old. They were raised in the Laboratory for Preclinical Animal Studies and Pharmaco-Toxicology Research of FMBS under favorable conditions for their growth and development. The animal house has natural air-conditioned rooms with optimal air changes per hour, relative humidity, temperature and illumination cycles set to 12h light and 12 hours dark. The animals were grouped and housed in cages with stainless steel grill tops, together with facilities for food, water bottle and bedding of wood shavings. The animals chosen for the study were identified using a cage card and corresponding bold marker body markings. They were equally subjected to a gross observation to ensure that the selected rats were in a good health condition. Rats were randomly selected with respect to body weight for final allotment to the study. They were fed with a standard laboratory diet and tap water ad libitum.

Methods

Preparation of the hydro-ethanolic plant extract

Preparation of medicinal plants for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality and quantity of bioactive constituents before proceeding with the intended biological testing. According to the work done by Pougoue et al. (2017)[14,17] the hydro-ethanolic maceration of the stem bark of the plant was most active. Hence, a hydro-ethanolic extract was used during this study.

200 g of the powder was weighed and mixed with several fractions of a 50:50 hydro-ethanolic solution in order to obtain a final solution of 2000 mL in a flat-bottomed flask. This mixture was agitated several times within 48 h of maceration, after which the mixture was filtered using Whatman paper number 3. The macerate was then dried in an oven at 50°C for two days. The dark brownish residue obtained (15.16% yield)) was stored for subsequent experiments.

Subacute toxicity test

The OECD 407 Guideline for the testing of chemicals (Repeated Dose 28-Day Oral Toxicity study) was adopted in 2008[20] and applied in this study, with slight modifications.

A total of 60 animals were used in this study. At least twelve animals (six females and six males) were used at each dose level and control group. An additional satellite group of twelve animals (six per sex) in the top dose group were used for the observation of reversibility, persistence, or delayed occurrence of toxic effects, for at

least 14 days post treatment. The control group received distilled water at 10 mL/kg. The animals were administered escalating doses of 125 mg/kg, 250 mg/kg and 500 mg/kg while a further 500 mg/kg, was administered to a satellite group of animals. The animals were dosed with test substance daily 7 days each week for a period of 28 days. In the first 14 days, the satellite groups (500 mg/kg) were the only ones receiving treatment while the treatment groups (125, 250, 500 mg/kg) received nothing. From the 15th day, the treatment groups (125, 250, 500 mg/kg) started receiving the plant extract till the 42nd day. On the 29th day, the satellite animals (500 mg/kg) stopped receiving the extract and were just being observed for the remaining 14 days. This enabled us to observe all the study groups for a total of 42 days and a common control group was used for both satellite and treatment groups. When the test substance was administered by gavage, this was done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The volume did not exceed 1 mL/100g body weight as demanded by the OECD guidelines.[20,21] At least twice daily, all animals were observed for morbidity and mortality. Signs noted include, but not limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior were recorded. All animals were weighed at least once daily. Measurements of food consumption were made daily. On the 43rd day, all the animals were sacrificed by cervical dislocation. Blood sampling via the carotid artery was performed for the determination of biochemical markers of toxicity. The organs were isolated immediately and weighed.

Hematological examination

Blood samples were taken from the carotid artery just prior to or as part of the procedure for euthanasia of the animals, and introduced in EDTA tubes. The samples were then sent to the hematology laboratory at University Teaching Hospital (CHU) of the University of Yaoundé 1, for analysis. The following hematological examinations were carried out at the end of the test period: red blood cell count (RBC), white blood cell count (WBC), differential leukocyte count (lymphocyte, monocyte, granulocyte), platelets, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV).

Quantification of biochemical parameters

At the end of experimentation (43rd day), blood of each animal was collected from the carotid artery and submitted to clinical biochemical tests. For the hepatic function, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined, while for the renal function, blood urea nitrogen (BUN), uric acid and serum creatinine (CRE) were evaluated. Total protein (TP), albumin (ALB), globulin (GLO), total bilirubin (TBIL), LDL, HDL and total cholesterol were equally measured. Dosages were made using standard analytical Fortress Diagnostic kits.

Pathological examination

On the 28th day after blood collection for biological analysis, all the animals were euthanized, detailed gross necropsy carefully examination. Extracted brain, heart, liver, spleen, lungs and ovaries, testes, kidneys, adrenal glands were trimmed of any adherent tissue, and their wet weight was taken as soon as possible after dissection to avoid drying to cipher relative organ weight. The principal vital organs (liver and kidneys) were preserved in fixation medium of 10% solution of buffered formalin (pH 7.4). Five-micrometer sections were obtained and colored with hematoxylin–eosin for evaluation under an optical microscope and microphotographs of the sections were recorded.

Statistical analysis

The results were expressed in terms of mean ± standard deviation. The comparison between the groups were analyzed using one-way analysis of variance, the ANOVA test followed by *Dunnett's* post-hoc multiple comparison test using the GraphPad Instat version 5.0 software. A p-value of less than 0.05 was considered statistically significant.

RESULT

28-Day subacute oral toxicity

General signs

No relevant sign of toxicity was observed. Many animals died in different study groups following the administration of the hydro-ethanolic extract of *F. thonningii* (Blume). We observed that the mortality rate of male rats was higher than that of female rats. The mortality rate was not dose-dependent as illustrated in table 1.

Table 1: Summary of the number of deaths recorded during the subacute toxicity study of the plant.

Study groups	Number of animals / groups	Number of deaths			Mortality (%)
		Male	Female	Total	
Control group	12	2	0	2	16
Test 125 mg/kg	12	3	1	4	33
Test 250 mg/kg	12	2	1	3	25
Test 500 mg/kg	12	3	0	3	25
Satellite group 500 mg/kg	12	3	3	6	50

Weight gain, Food and Water consumption

The administration of the extract leads to a non-significant increase in weight gain in both females and males. The daily administration of the plant extract to the different groups resulted in a non-significant change in food and water intake by both males and females (see table 2).

Table 2: Comparative table of the zootechnical parameters of different study groups.

Groups	Control	125mg/kg	250mg/kg	500mg/kg	Satellite(500mg/kg)
Male					
Food intake (g)	123,1 ± 55,1	103,1 ± 44,9	150,3 ± 59,9	117,8 ± 61,3	107,0 ± 70,5
Water intake (mL)	123,1 ± 42,1	98,9 ± 23,4	205,1 ± 78,1	121,9 ± 40,1	135,3 ± 54,9
Weight gain (%)	48,2 ± 34,5	80,1 ± 35,4	90,4 ± 63,1	107,9 ± 20,3	153,0 ± 74,0
Female					
Food intake (g)	174,6 ± 94,9	142,2 ± 56,1	131,7 ± 57,7	158,3 ± 82,9	124,8 ± 47,7
Water intake (mL)	173,2 ± 52,3	161,5 ± 51,5	140,7 ± 32,6	195,8 ± 44,9	127,3 ± 26,7
Weight gain (%)	62,7 ± 19,5	54,3 ± 31,6	75,7 ± 29,2	62,9 ± 9,0	62,4 ± 48,8

The results are expressed as mean ± SEM with n = 5; Data analysis was performed using the ANOVA test, followed by Dunnet's post hoc multiple comparison test. The differences were considered significant from the *p-value* $*p<0.05$ compared to the rats of the healthy control groups.

Relative organ weight

There were no significant changes in the relative weights of the brain, heart, lungs, adrenal glands, spleen, brains and kidneys of both male and female treated rats in relation to control.

Histological analysis

Liver histology analysis

There no observed alteration in hepatic tissue apart from a slight infiltration of leukocytes in animals receiving the extract at the highest dose (500 mg/kg) in males. In the females, compared to the control, showed some alterations in the dilation of the sinusoidal capillaries, infiltration of leukocytes specifically in animals which received the highest dose of the extract (See figure 1)

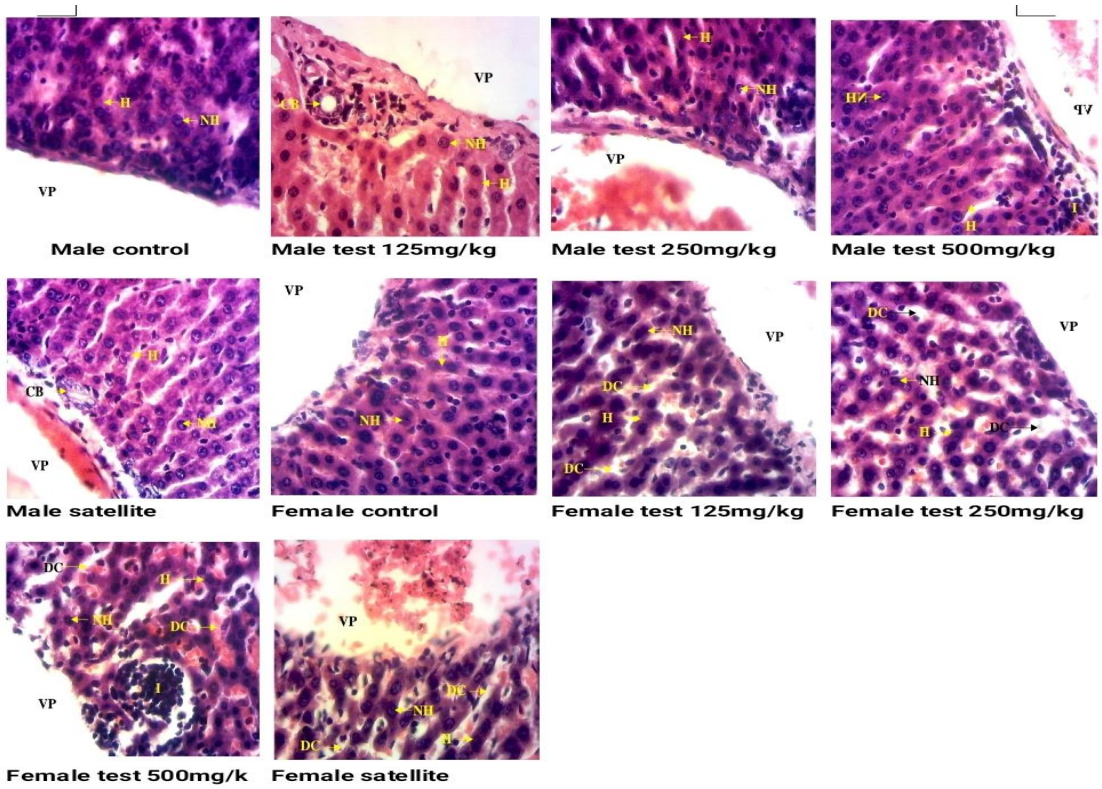

Figure 1: Effect of *F. thonningii* stem bark extract on liver histology of treated groups compared with the control.

Hematoxylin and eosin (250x). Satellite = Satellite (Extrait 500 mg/kg); **Control** = Control (distilled water 10 mL/kg); Extract doses: **125; 250; 500 mg/kg**
CB: Biliary canaliculi; **DC**: Hepatic sinusoid capillaries dilation; **H:** Hepatocyte lamina; **NH**: Hepatocyte nucleus; **VP**: Hepatic portal vein.

Kidney histology analysis
Figure 2 shows leukocyte infiltration and tubular clarification mainly in animals that received the extract at doses of 250 and 500 mg/kg as well as the satellite animals. These alterations were more or less reversible. Only the animals of the control group and those that received the extract at a dose of 125 mg/kg showed a normal renal microarchitecture.

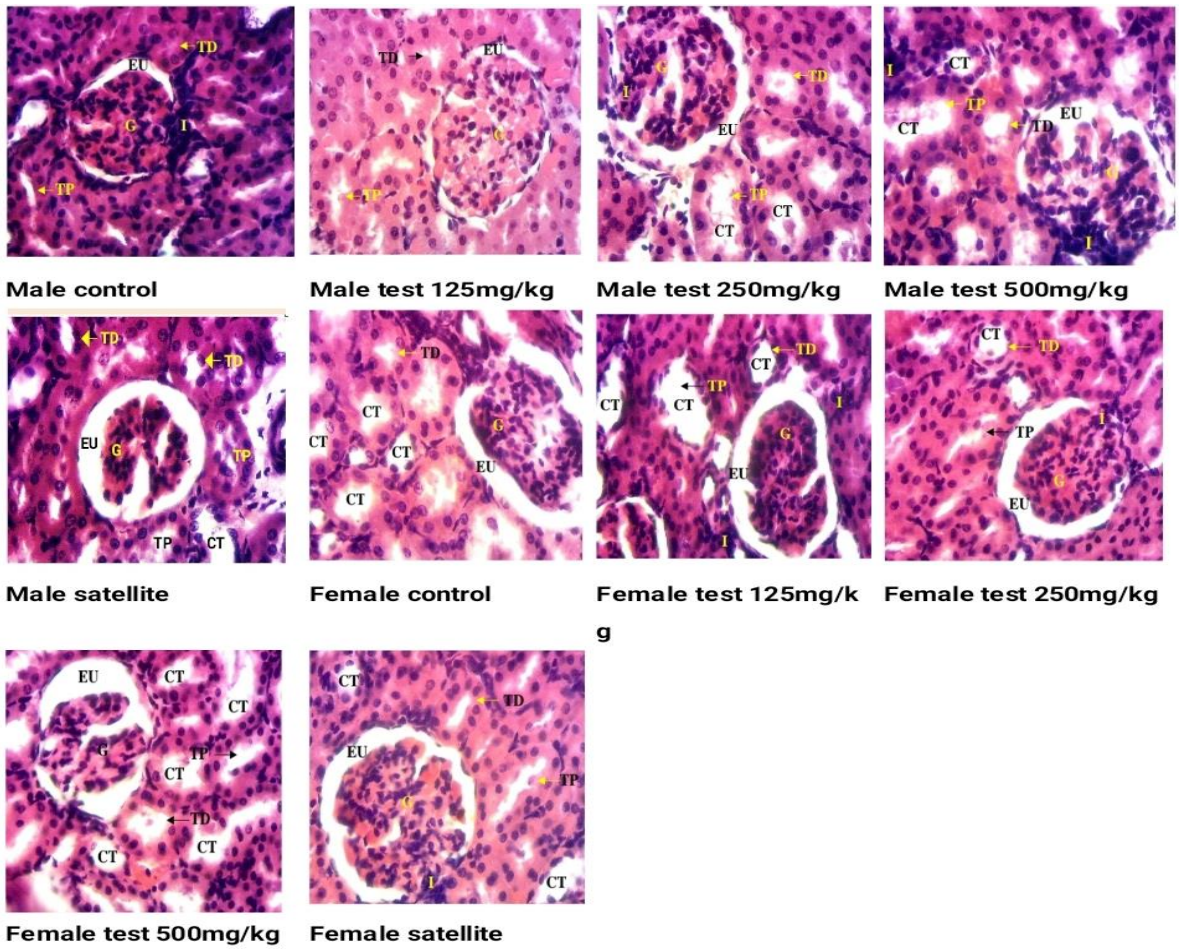

Figure 2: Effect of *F. thonningii* stem bark extract on liver histology of treated groups compared with the control.

Hematoxylin and eosin (250x). Satellite = Satellite (Extrait 500 mg/kg); **Control** = Control (distilled water 10 mL/kg); Extract doses: **125; 250; 500 mg/kg**
CT: Tubular clarification; **EU**: Urinary tract; **G**: Glomerulus; **I**: Inflammation; **TD**: Distal convoluted tubule; **TP**: Proximal convoluted tubule.

Hematology analysis
The administration of the plant for a period of 28 days did not significantly modify the hematological parameters, such as WBC, RBC, PLT, HGB, HCT, MCV, MCH, and MCHC, of the treated and satellite groups were within the reference range for rats. the hematological parameters in both control and experimental rats as observed in table 3.

Table 3: Hematological data of the different study groups.

Gender	Parameters			Dose (mg/kg)			
		Control	125	250	500	Satellite	Control
Male							
	RBC	8,90 ± 1,10	9,82 ± 0,29	8,73 ± 0,63	9,15 ± 0,75	9,68 ± 0,57	9,78 ± 0,10
	HGB	13,98 ± 1,99	16,63 ± 0,68	14,10 ± 1,41	14,53 ± 0,65	15,84 ± 0,83	16,02 ± 0,27

	HCT	47,38 ± 5,47	59,55 ± 2,47	49,70 ± 3,68	52,58 ± 2,89	53,24 ± 2,45	57,58 ± 1,91
	MCV	53,28 ± 1,41	60,97 ± 0,12	56,95 ± 0,07	57,63 ± 3,64	55,06 ± 2,09	58,88 ± 1,67
	MCH	15,68 ± 0,52	16,97 ± 0,65	16,15 ± 0,49	15,95 ± 1,11	16,36 ± 0,61	16,38 ± 0,22
	MCHC	29,45 ± 1,14	27,83 ± 1,00	28,35 ± 0,78	27,63 ± 0,33	29,76 ± 0,86	27,84 ± 0,63
	PLT	609,25 ± 104,34	678,00 ± 304,30	742,00 ± 387,494	701,00 ± 181,21	820,40 ± 166,86	779,40 ± 139,46
	WBC	15,82 ± 5,09	21,14 ± 7,35	12,23 ± 3,06	15,06 ± 5,81	18,12 ± 5,27	14,94 ± 5,37
	NEUT	1,49 ± 1,26	2,59 ± 1,11	1,84 ± 0,74	3,93 ± 2,81	2,05 ± 1,92	2,88 ± 1,67
	LYMPH	11,9 ± 4,11	16,78 ± 7,09	9,64±4,17	9,18 ± 2,88	14,54 ± 4,02	11,08 ± 4,49
	MONO	2,28 ± 0,42	1,56 ± 0,33	0,72 ± 0,36	1,83 ± 0,68	1,34 ± 1,19	0,91 ± 1,08
	EO	0,12 ± 0,06	0,12 ± 0,11	0,01 ± 0,00	0,08 ± 0,04	0,11 ± 0,07	0,03 ± 0,02
	BASO	0,07 ± 0,04	0,09 ± 0,05	0,03 ± 0,01	0,05 ± 0,01	0,08 ± 0,04	0,05 ± 0,03
Female							
	RBC	8,33 ± 0,66	9,02 ± 0,39	8,51 ± 0,48	8,99 ± 1,21	8,84 ± 0,68	8,94 ± 0,16
	HGB	14,30 ± 1,17	15,13 ± 0,57	14,78 ± 1,45	15,25 ± 1,32	15,00 ± 0,87	14,73 ± 0,45
	HCT	46,80 ± 3,56	56,23 ± 2,77	51,26 ± 1,88	54,53 ± 5,88	49,92 ± 3,15	51,90 ± 1,18
	MCV	56,24 ± 3,59	62,38 ± 0,79	60,30 ± 1,46	60,80 ± 1,74	56,52 ± 1,47	58,07 ± 0,99
	MCH	17,22 ± 1,22	16,78 ± 0,26	17,36 ± 1,52	17,03 ± 0,9	16,98 ± 0,61	16,50 ± 0,44
	MCHC	30,56 ± 0,68	26,93 ± 0,39	28,82 ± 2,26	28,00 ± 0,94	30,06 ± 0,80	28,40 ± 0,26
	PLT	731,80 ± 217,70	744,25 ± 149,05	670,60 ± 142,41	694,50 ± 481,47	800,40 ± 290,01	600,67 ± 171,16
	WBC	13,75 ± 3,29	13,44 ± 2,67	10,03 ± 5,28	8,46 ± 2,38	12,22 ± 2,49	14,85 ± 6,25
	NEUT	2,38 ± 1,54	3,03 ± 1,02	1,32 ± 1,08	1,48 ± 1,56	1,03 ± 0,67	1,81 ± 2,51
	LYMPH	10,33 ± 2,76	9,61 ± 2,17	6,56 ± 4,86	4,78 ± 3,02	9,58 ± 1,79	11,67 ± 4,46
	MONO	0,93 ± 1,29	0,59 ± 0,53	2,00 ± 1,62	0,60 ± 0,49	1,41 ± 1,61	1,29 ± 0,91
	EO	0,05 ± 0,04	0,16 ± 0,08	0,12 ± 0,11	0,09 ± 0,03	0,15 ± 0,07	0,06 ± 0,01
	BASO	0,07 ± 0,04	0,05 ± 0,03	0,03 ± 0,02	0,03 ± 0,01	0,05 ± 0,01	0,06 ± 0,006

The results are expressed as mean ± SEM with n = 5; Data analysis was performed using the ANOVA test, followed by Turkey Kramer post hoc multiple comparison test. The differences were considered significant from the *p-value *p<0.05*.

RBC=red blood cell ($x10^6$ /mm^3). HGB=hemoglobin concentration (g/dl). HCT=hematocrit (%). MCV=mean corpuscular volume (fl). MCH=mean corpuscular hemoglobin (pg). MCHC=mean corpuscular hemoglobin concentration (g/dl). WBC=white blood cell (10^3 /mm3). PLT=platelets (10^3 /mm^3). NEUT= neutrophils (%). LYMPH=lymphocyte (%). MONO=monocyte (%). EO=eosinophilic leukocyte (%).). BASO= basophils (%)

Biochemical analysis

Table 4 shows the concentration of different biochemical parameters following the analysis of blood samples obtained from treatments following in the subacute toxicity. The administration of the hydro-ethanolic extract of the bark of *Ficus thonningii* (Blume) showed a non-dose-dependent increase in liver enzymes, specifically, AST and ALT in the treated animals, which was restored after stopping the administration of the extract in the satellite groups (500 mg/kg). A similar phenomenon was observed for alkaline phosphatase, total proteins, total bilirubin and direct bilirubin. There was a non-dose-dependent decline in albumin levels. The administration of the extract led to an increase in serum lipid parameters (total cholesterol, triglycerides, LDL-Cholesterol) in a dose-dependent manner. This was more prominent in females than in males. A dose-dependent decline in HDL-cholesterol was equally observed in both male and female groups. The lipid parameters returned to their values comparable to those of the control groups after stopping the administration in satellite animals. With regard to renal damage, a non-significant rise in creatinine, urea and uric acid was observed in all treated animals as well as satellite animals (500 mg/kg).

Table 4: Biochemistry of Liver and Kidney function in the different experimental groups.

	Dose (mg/kg)				
	Control	**125**	**250**	**500**	**Satellite**
Male ALT (µmol/min/mL)	3,89 ± 1,23	10,77 ± 2,89	9,31 ± 5,52	10,32 ± 5,00	4,66 ± 3,54
AST (µmol/min/mL)	8,04 ± 3,59	22,97 ± 4,84	31,44 ± 24,01	38,20 ± 25,78	9,51 ± 0,77
ALP (µmol/min/mL)	5,19 ± 0,39	6,13 ± 0,71	6,64 ± 2,67	8,16 ± 1,37	5,23 ± 0,94

Total proteins (mg/mL)	4,40 ± 3,44	5,48 ± 0,39	10,53 ± 6,45	10,76 ± 4,17	4,95 ± 3,89
Albumine (g/dl)	46,67 ± 10,93	56,17 ± 3,44	64,58 ± 16,59	38,61 ± 6,94	43,33 ± 9,82
Total bilirubin (mg/dL)	0,63 ± 0,49	0,99 ± 0,26	0,85 ± 0,39	0,79 ± 0,61	1,36 ± 0,53
Direct bilirubin (mg/dL)	0,35 ± 0,044	0,91 ± 1,03	0,93 ± 0,59	0,84 ± 0,65	0,68 ± 0,34
Creatinine (mg/dL)	4,46 ± 0,55	3,85 ± 0,62	4,19 ± 0,55	4,26 ± 1,34	3,79 ± 0,49
Urea (mg/dl)	73,44 ± 9,38	89,58 ± 3,61	93,75 ± 8,84*	83,33 ± 9,55	93,75 ± 0,00*
Uric acid (mg/dL)	18,38 ± 14,61	59,67 ± 39,83	38,63 ± 31,83	46,00 ± 15,39	20,83 ± 3,18
Total cholesterol (mg/dL)	313,04 ± 150,11	531,88 ± 66,56	531,52 ± 133,79	482,61 ± 56,86	365,22 ± 7,53
Triglycerides (mg/dL)	262,75 ± 38,45	390,00 ± 40,00	412,50 ± 88,08*	466,67 ± 35,12**	353,33 ± 25,17
LDL (mg/dL)	2676,04 ± 288,26	2992,25 ± 574,15	3180,95 ± 296,17	3698,22 ± 111,31	3001,75 ± 553,51
HDL (mg/dL)	53,24 ± 9,12	45,45 ± 10,80	31,53 ± 5,74	30,73 ± 1,53	35,15 ± 10,37
Female					
ASAT (µmol/min/mL)	3,24 ± 1,31	10,48 ± 0,58	14,96 ± 6,19**	12,22 ± 3,92*	6,60 ± 1,21
ALAT (µmol/min/mL)	6,06 ± 2,75	7,77 ± 2,84	14,55 ± 9,85	17,83 ± 17,12	7,95 ± 2,043
ALP (µmol/min/mL)	4,61 ± 1,54	7,17 ± 3,97	7,50 ± 3,90	8,09 ± 3,28	4,60 ± 3,31
Total proteins (mg/mL)	5,12 ± 1,61	9,17 ± 4,41	10,37 ± 4,46	9,30 ± 8,30	3,79 ± 1,70
Albumine (g/dl)	43,61 ± 18,62	38,67 ± 23,80	68,83 ± 8,78	31,81 ± 20,83	37,92 ± 3,99
Total bilirubin (mg/dL)	0,81 ± 0,58	1,64 ± 0,67	1,45 ± 0,89	2,02 ± 0,45*	1,86 ± 0,38
Direct bilirubin (mg/dL)	0,65 ± 0,07	0,63 ± 0,19	1,12 ± 0,16	1,59 ± 0,18	1,42 ± 0,42
Creatinine (mg/dL)	4,13 ± 0,47	4,40 ± 0,46	4,22 ± 0,40	4,31 ± 0,62	4,72 ± 0,18
Urea (mg/dl)	84,38 ± 6,56	100,00 ± 22,10	82,50 ± 16,77	80,21 ± 29,95	89,06 ± 28,58
Uric acid (mg/dL)	34,33 ± 3,22	64,00 ± 17,47	68,20 ± 6,27	101,25 ± 68,70*	37,67 ± 4,75
Total cholesterol (mg/dL)	273,19 ± 98,24	333,04 ± 157,41	511,30 ± 166,18	813,04 ± 375,70**	330,43 ± 45,18
Triglycerides (mg/dL)	273,33 ± 80,21	382,50 ± 89,21	414,00 ± 100,40	433,33 ± 48,03	305,00 ± 73,26
LDL (mg/dL)	2707,80 ± 412,97	3494,71 ± 161,05	3825,05 ± 121,2	3984,78 ± 167,3	2845,60 ± 77,41
HDL (mg/dL)	52,15 ± 11,72	42,08 ± 5,51	40,89 ± 34,94	39,29 ± 10,32	43,99 ± 9,88

The results are expressed as mean ± SEM with n = 5; Data analysis was performed using the ANOVA test, followed by Turkey Kramer post hoc multiple comparison test. The differences were considered significant from the *p-value* *$p<0.05$.

DISCUSSION

The scientific validation of potential toxic effects of plant medicines is crucial in light of their widespread use and the common misconception that green medicine is always safe.[12] This study was done to evaluate the toxic effects of *F. thonningii* stem bark considering the fact several researchers[15,22,23] have shown that it has promising biological activity in the management of peptic ulcers, a globally prevalent disease, with little attention accorded to the study of its toxicity.

The administration of daily doses (125 mg/kg, 250 mg/kg, 500 mg/kg) of the stem bark extract of *F. thonningii* for a period of 28 days resulted in no abnormal changes in the behavior and physical appearance. A higher mortality rate was observed in males as compared to females in both control and test groups. This may not only be related to the administration of the plant but could also be due to the aggressive nature of males among themselves until an alpha male has been established according to Blanchard et al. (1984).[29]

The daily administration of *F. thonningii* stem bark extract to both male and female treatment groups caused a non-significant decrease in food consumption except for the dose of 250 mg/kg in males which showed a non-significant increase in food intake. A significant increase in water intake was observed in the male test group treated with a dose of 250 mg/kg and a significant decrease in the female satellite group. The administration of the extract led to a non-significant increase in weight gain in both females and male treatment groups compared with the control. This showed that the plant extract may not interfere with carbohydrate, protein or fat metabolism in these experimental animals. This point is also buttressed by the fact that there were no significant changes in the relative weights of all the essential organs investigated. These results concur with the work done by Stanley and collaborators (2008).[20]

Concerning the hematological parameters, the overall analysis of the results showed that the administration of the plant extract did not significantly affect to the blood cells of animals.

Histological examination of the liver and kidneys showed that at the dose of 250 mg/kg and 500 mg/kg, there were leukocyte infiltrates which persisted after stopping the treatment in hepatic and renal tissue. This indicates possible response to tissue injury, cellular stress or inflammation.[30] These results show that the plant might have caused injury to the liver and kidneys. The administration of the hydro-ethanolic extract of the bark of *Ficus thonningii* (Blume) leads to a non-dose-dependent increase in liver enzymes, specifically, AST and ALT, which suggests a slight hepatic cytolysis in the treated animals, which is restored after stopping the administration of the extract in the satellite groups (500 mg/kg). A similar phenomenon is observed for alkaline phosphatase and total protein.

Total plasma bilirubin reflected both hepatic and renal function. The liver converted non-conjugated bilirubin to conjugated bilirubin. Then, the kidney excreted conjugated bilirubin. The change in plasma bilirubin showed a hepatic or renal dysfunction.[28] There was a non-significant increase in total bilirubin and direct bilirubin in treatment groups which was reestablished after the treatment was interrupted. There was also a slight decline in albumin levels. With regard to renal damage, non-significant rise in creatinine, urea and uric acid was observed in treated animals, which was restored after stopping treatment in satellite animals. In general, this plant caused a non-significant effect on kidneys.

The administration of the extract leads to an increase in serum lipid parameters (total cholesterol, triglycerides, LDL-Cholesterol) in a dose-dependent manner. This was more prominent in females than in males. A dose-dependent decline in HDL-cholesterol was equally observed in both male and female groups, indicating possible fatty liver disease. The lipid parameters returned to their values comparable to those of the control groups after stopping the administration in satellite animals (500 mg/kg), thus indicating a transient effect.

These biochemical data are confirmed by histological analysis of liver and kidney which showed a few signs of slight liver and kidney injury of rats exposed to the extract of *F. thonningii* for 28 days. Based on these results, the administration of the hydro-ethanolic extract of the bark of *F. thonningii* would result in alteration of liver and kidney functions. However, the fact that the animals spent a lot of time fighting each other exposed them to a state of stress. A study conducted by Joung et al. (2019)[31] and Marchon et al. (2018)[32] illustrated the impact of stress on liver and kidneys respectively in animal models. Their studies showed that social, environmental and psychological stress had been related to liver and kidney impairment, confirmed by abnormal histology of these organs as well as an elevation of hepatic and renal biochemical parameters via several mechanisms, many of which are yet to be discovered. Therefore, the administration of the plant extract to already stressed animals may have simply worsened the condition of already impaired liver and kidneys.[33,34] This assumption is buttressed by the fact that most of the animals that died during the study did so only a few days following the administration of the plant.

CONCLUSION

Taken together, our data suggested that the subacute oral administration of the hydroethanolic extract of *F. thonningii* produced no significant toxic effects in male and female Wistar rats, which could stand as an assurance for the medicinal use of this plant in folk medicine. However, since the extract had some effect on liver biochemical parameters, caution should be exercised in its use at very high doses for prolonged periods. Further investigations (chronic, reproductive, developmental and genetic toxicity studies) need to be done for the complete elucidation of the safety profile of *F. thonningii* (Blume).

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Authors contribution

NBMO, ETF, FCN conceived and designed the study and drafted manuscript. AP, NNBL and NBMO coordinated laboratory analysis and data assembly. NBMO, EBB, NB did the data mining. All authors read and reviewed the final draft of manuscript for publication.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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