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Acute and sub-chronic toxicity of the aqueous extract of *Ficus vogelii* (Miq.) Miq. stem bark in rats

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ABSTRACT

Ficus vogelii is a medicinal plant mainly found in tropical Africa and reported to treat inflammatory complaints. This study aims to evaluate the acute and sub-chronic toxicity of the aqueous extract of *Ficus vogelii* stem bark in wistar rats. For acute study, aqueous extract at a single dose of 5000 mg/kg body weight was administered to female rats and observed for 14 days. In the sub-chronic study, the extract was administered daily to both sex rats at the doses of 100, 200, 400, and 600 mg/kg body weight for 28 consecutive days. Body weight was measured weekly, while hematological, biochemical, and histopathological parameters were analyzed after euthanize. Aqueous extract of *Ficus vogelii* at all tested doses didn't produced any mortality or significant change on the body weight and relative weight of rats on acute and sub-chronic studies. The lethal dose 50 was estimated greater than 5000 mg/kg (DL_{50}>5000 mg/kg). Hematological parameters were recorded non-significant in all treated rats. Aqueous extract at 600 mg/kg significantly changed transaminases and alkaline phosphatase activities, these changes were reversible in satellites. The concentrations of bilirubin was increased at 200 and 600 mg/kg in male rats, at 100, 400 mg/kg in female rats. The levels of lipids markers didn't changed, except the significant decrease of LDL-cholesterol. Histological examination didn't showed any change in the architecture of the liver and kidney of rats treated compared to control. Thus aqueous extract of *Ficus vogelii* stem bark didn't produced adverse effects in rats after oral acute and sub-chronic treatment.

Keywords: Acute toxicity, Aqueous extract, *Ficus vogelii*, rats, Sub-chronic toxicity.

INTRODUCTION

Traditional medicines are used all over the world. It has been practiced for a long time in order to preserve health or prevent and treat diseases [1]. The World Health Organization (WHO) estimates that 80% of the African population use traditional medicine for primary health care [2]. Consumers must therefore be informed and have the means to obtain suitable, effective and safe treatment [3]. With the global explosion of herbal medicine, the safety of herbal medicines has become a public health problem [4]. However, research has shown that not all herbal medicines are safe, as they produce a toxic effect on evaluation, which can result from the inherent toxic effect of the active ingredient, overdose, chronic use, interactions, allergies, contaminations [5]. Extremely toxic substances like strychnine, digitoxines, cyanogenic glycosides, among others are extracted from plants. Therefore, we can only assure that, the use of particular specie is secure after a careful investigation [6].

Ficus vogelii (Miq.) Miq. (*F. vogelii*) is a plant belonging to the Moraceae family. It is found mainly in the vegetation belt of the Guinean savannah, by the river and on all types of soil in West and Central Africa [7]. Synonymous with *Ficus lutea* Vahl, *F. vogelii* is a terrestrial tree 10-12 meters high with a dense and regular crown with leaves gathered in tufts at the end of the branches. The leaves are alternate, arranged in spirals, leathery, elliptical oblate to oblate ovate shortly acuminate with rounded or subcorded base, glabrous, dark green glossy above and matte below. The bark is smooth on the bole, scaly on the branches, gray to light brown, with a pink edge, exuding an abundant white latex[7]. In Obudu culture (Nigeria), the specie is called *Kujung* and the leaves are used as vegetables in various local dishes. In Cameroun, the plant is called *Adjolo* in Baka culture (East-Cameroun), *Ekekam* in Ewondo culture (Centre- Cameroon) and *Ekekap* in Yemba culture (West-Cameroun) and used by herbalists to treat rheumatism. Empirically, stem bark is used as a calming agent in gastritis, gastralgia, colic and dysentery; latex is used as a healing agent and is used to make rubber [7].

Recent pharmacological studies reported that *Ficus vogelii* extract demonstrated efficiency in the prevention and therapy of ulcers [8]. Therefore, the ethanolic extract leaves of *Ficus vogelii* improving of dyslipidemia in diabetic rats [9] and anemia [10] and also antihepatotoxic [11]. The leaf is also reported to be highly effective in improving hemoglobinopathies, and used to boost immunity in HIV-infected patients [12]. The phytochemical composition of air-dried leaves of *Ficus vogelii* shown quantitatively

(mg/100g) and respectively the following metabolites: polyphenols, saponins, flavonoids, carotenoids, coumarins, steroids, triterpenoids, glycosides cardiaques [9]. The evidence describing the biological effects of *F. vogelii* is increasing. However, there are no reports regarding its safety or toxicity. The objective of this study is therefore to assess the acute and sub-chronic toxicity of the aqueous extract of *F. vogelii* stem bark by considering its use in folk medicine.

MATERIALS AND METHODS

Materials

Plant collection

The fresh bark of *Ficus vogelii* (Miq.) Miq. was harvested in June 2018 in Dschang (West-Cameroon). The botanical identification was made to the National Herbarium of Cameroon (HNC) in comparison with the sample *67461/HNC*. The bark was washed, dried at room temperature for two weeks and crushed.

Preparation of the aqueous extract

500 g of powder was decocted in 5 liters of distilled water for 30 minutes then filtered using wattman paper N°3 and the filtrate obtained was dried for 72 hours at 45°C using the laboratory oven. After drying, 50 g of brown powder were obtained, giving a yield of 10%, stored in the refrigerator and used for futures studies.

Chemicals and reagents

The kits (*in vitro* diagnostic medical device) used for the various assays were supplied by SGM Italia.

Animal material

Wistar albino rats of approximately 8-10 weeks of age weighing 120-160 g were used. They were obtained from the bred colony in the animal house of the Faculty of Sciences of the University of Douala under ambient temperature conditions, sufficient ventilation, under natural lighting (approximately 12 hours of light and 12 hours of darkness). All the experiments have been approved by the Institutional Ethical Committee of the University of Douala (N°2042 CEI-UDo/08/2019/T).

Methods

Acute toxicity

Acute toxic study was conducted in compliance with the Organization for Economic Cooperation and Development guideline for the testing of chemicals N° 423 [13]. The rats were fasted 12 hours before start. Six (6) nulliparous and non-pregnant female's rats weighing 120–130 g were randomly divided into two groups of three each. Group I serving as control and received distilled water10 mL/kg and group II received a single dose of 5000 mg/kg of the aqueous extract of *Ficus vogelii* stem bark (*p.o*). The animals were observed individually for the first 4 hours and then daily for 14 days. Observations were focused on parameters such as pilo-erection, locomotion, salivation, drowsiness, convulsions, aggressiveness and appearance of feces. The number of survivors was noted after 24 hours. Animals were weighed on day 0, and then on days 7 and 14. At the end of the study, all surviving animals were euthanized and the organs such as liver, kidneys, spleen, lungs and heart were removed and weighed. A gross pathological examination of these organs was also performed. The LD_{50} was estimated [13].

Sub-chronic toxicity

The study was conducted in compliance with the OECD guideline for the testing of chemicals N° 407: repeated dose 28-day oral toxicity study in rodents [14]. Study design both the male and female rats of 8–10 weeks of age weighing 140–160 g were used in this study. Sixty rats were randomly divided into 6 groups of 10 animals each (5 males and 5 females) and studied in parallel. The aqueous extract of *F. vogelii* stem bark (100, 200, 400, 600 mg/kg) and satellites (600 mg/kg) or distilled water (10 mL/kg), was administered (*p.o*) daily to animals for 4 weeks (28 days). The animals were weighed weekly and observed for behavioral changes. On the 29thday, the animals were anesthetized with the exception of those of satellite group. After sacrifice, the blood was collected for hematological and biochemical analysis. The serum obtained was aliquoted and stored at -20°C for biochemical assays. After dissection, liver, kidney, spleen, lungs, and heart of each animal were removed from the connective tissue and weighed to determine the relative mass of these organs. A section of the liver and kidney was fixed and stored in 10% formalin buffered for histological analysis. The animals in the satellite groups (600 mg/kg) were observed for two additional weeks and at the end they followed the same process as described on the 29thday above. The changes in general behavior, mortality, body weight and relative mass of organs were recorded.

Determination of hematological parameters

Blood samples taken in EDTA tubes were used for hematological analyze using an automated hematology analyzer.

Determination of biochemical parameters [15]

The assays was carried out using the SGM Italia brand kits for different parameters such as: total proteins, ALT, AST, alkaline phosphatase, bilirubin, urea, creatinine, cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol level was obtained by deduction.

Histological studies [16]

Liver and kidney were fixed in 10 % phosphate-buffered formalin, embedded in paraffin wax, cut into 5µm sections and stained with hematoxylin and eosin (H-E). Preparations were examined under a light microscope and any alteration compared to the normal structure was registered.

Statistical analysis

All the values are expressed as mean ± SEM. Statistical analysis was performed in Graph Pad Prism 5.03 using one-way and two ways analysis of variance (ANOVA) followed by Bonferonni's test. The value of $p < 0.05$ was considered to be statistically significant

RESULTS

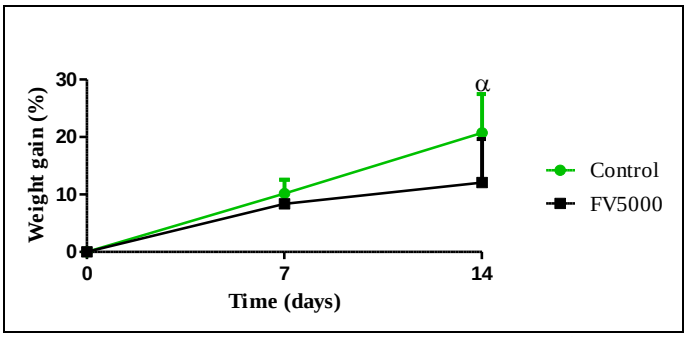
Acute toxicity study

Effect of a single dose of aqueous extract of *Ficus vogelii* stem bark on animal behavior and mortality

14 days observations post treatment showed that the aqueous extract of *F. vogelii* stem bark did not caused any change in animal behavior compared to the control. At a dose of 5000 mg/kg body weight, the extract did not induced any sedative effect in rats, in addition no mortality was recorded and the lethal dose 50 was estimated greater than 5000 mg/kg body weight (DL_{50}>5000 mg/kg).

Effect of a single dose of aqueous extract of *Ficus vogelii* on body weight

Figure 1 shows that the *F. vogelii* aqueous extract at a dose of 5000 mg/kg body weight resulted in a non-significant increase of weight gain in treated animals compared to control (Figure 1).

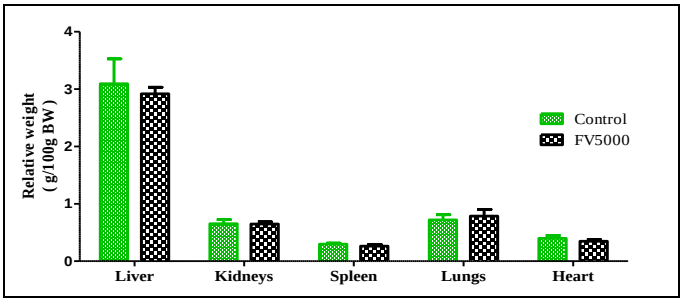


Each data represents the mean ± ESM; n = 3; FV 5000 = aqueous extract of *Ficus vogelii* at a dose of 5000 mg/kg body weight; Control = distilled water 10 mL/kg body weight.

Figure 1: Effect of the aqueous extract of *Ficus vogelii* on weight gain

Effect of a single dose of aqueous extract of *Ficus vogelii* on the relative mass

Administration of the aqueous extract of *F. vogelii* at a dose of 5000 mg/kg body weight did not caused any significant difference in the mass of the liver, kidneys, spleen, lungs and heart of treated animals compared to control animals (Figure 2).



Each bar represents the mean ± ESM; n=3; FV 5000 = aqueous extract of *Ficus vogelii* at a dose of 5000 mg/kg body weight; Control = distilled water 10 mL/ kg body weight; BW= body weight.

Figure 2: Effect of the aqueous extract of *Ficus vogelii* on the relative mass of organs

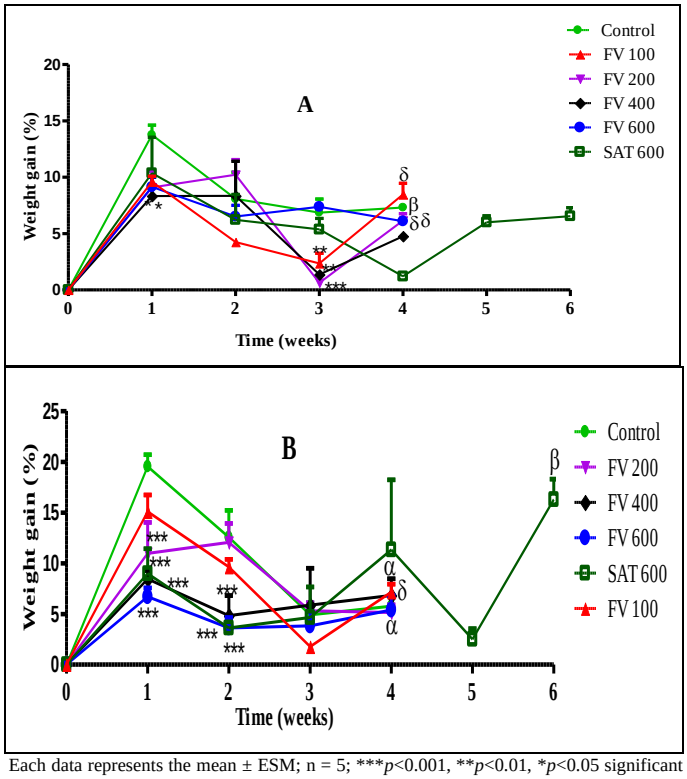
Sub-chronic toxicity study

Effects of the aqueous extract of *Ficus vogelii* on animal behavior and mortality

Oral administration of the aqueous extract of *Ficus vogelii* stem bark at daily repeated doses (100, 200, 400 and 600 mg/kg body weight) for 28 days did not caused any change in animal behavior compared to the control. No mortality was recorded. In addition, satellite animals (males only) showed aggression towards each other from the 16thday of treatment until the 28thday, then for the first week of additional observation. On the second week of additional sighting, this aggressiveness disappeared and the animal behavior returned to normal.

Effect of the aqueous extract of *Ficus vogelii* on weight gain

Figure 3 shows the variation of weight gain in animals during the experiment. On week 1, it was observed a significant ($p<0.05$) weight gain of animals treated with *Ficus vogelii* aqueous extract at 400 mg/kg body weight. Also, on week 3 a significant increase ($p<0.05$, $p<0.01$, $p<0.001$) of weight gain was observed in animals treated with *Ficus vogelii* respectively at the doses of 100, 200 and 400 mg/kg body weight. In addition, a significant gain ($p<0.01$) of weight was observed in animal treated with extract at 600 mg/kg body weight at the week 4 compared to control. But, this gain was lower than that observed in the control animal. However, a significant ($p<0.001$) increase of weight gain was noted at the end of treatment (week 4) at doses 100, 200 and 400 mg/kg body weight compared to the weight at the beginning of treatment in male rat. In female rat, a significant ($p<0.001$) increase weight gain was observed at all tested doses at week 1, in addition a significant ($p<0.001$) increase of weight gain was observed in animals treated at the doses of 400, 600 mg/kg body weight and satellite animal at day 14 compared to the control. Only, this gain was lower than that observed in the control. However, a significant ($p<0.001$, $p<0.05$) weight gain were noted respectively at the end of treatment (week 4) in rats treated with *Ficus vogelii* aqueous extract at 100, 200 and 600 mg/kg body weight compared to the start of treatment (Figure 3).



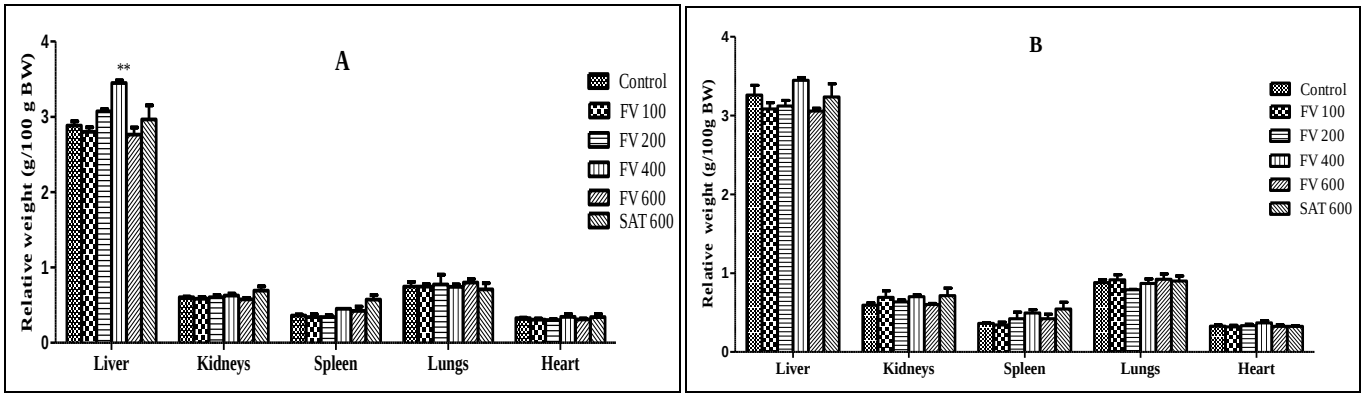
Each data represents the mean ± ESM; n = 5; ***$p<0.001$, **$p<0.01$, *$p<0.05$ significant difference compared to control; δ$p<0.001$, β$p<0.01$, α$p<0.05$ significant difference at the end (week 4) compared to the start (week 0), ANOVA followed by the Bonferoni post-test; Control = distilled water 10mL/kg body weight; FV100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellite at 600 mg/kg body weight.

Figure 3: Effect of the aqueous extract of *Ficus vogelii* stem bark on the weight gain male (A), female (B)

Effect of the aqueous extract of *Ficus vogelii* stem bark on the relative weight of the organs

Figure 4 shows the variation in the relative mass of the organs of male and female rats treated with aqueous extract of *Ficus vogelii* stem bark for 28 days at doses 100, 200, 400, 600 mg/kg body weight and satellite animals. In both sex, regardless of the dose of *F. vogelii* extract, there was no significant change in the relative weight of the

kidneys, the spleen, the lungs and the heart of treated animals compared to control animals. However, aqueous extract of *F. vogelii* at the dose of 400 mg/kg body weight caused a significant (p<0.001) increase of the relative mass in liver in males rats compared to the control (Figure 4).



Each bar represents the mean ± ESM; n = 5; **p<0.001 significant difference compared to control, ANOVA followed by the Bonferoni post-test; Control = distilled water 10 mL/kg body weight; FV100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight; BW= body weight.

Figure 4: Effect of the aqueous extract of *Ficus vogelii* stem bark on the relative weight male (A), female (B)

Effect of the aqueous extract of *Ficus vogelii* stem bark on hematological parameters

The table 1 below shows the effect of the aqueous extract of *F. vogelii* on hematological parameters analyzed in rats after 28 days at the doses of 100, 200, 400 and 600 mg/kg body weight and 42 days for the satellite. No significant variation was observed for the various hematological parameters observed in the rats treated with the plant extract compared with control rat.

Table 1: Effect of prolonged administration of the aqueous extract of *Ficus vogelii* on hematological parameters

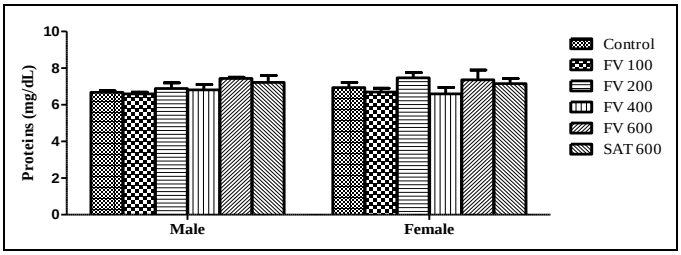
Hematological Parameters (Units) [Standard value]	Male						Female					
	Control	FV100	FV200	FV400	FV600	SAT600	Control	FV100	FV200	FV400	FV600	SAT600
WB (10^3µL) [4-10]	4±0.0	4±0.0	4±0.0	4±0.0	5±0.2	4±0.0	3.8±1.3	3.6±1.0	3.9±0.3	4.75±1.0	3.8±0.5	4±0.0
Lymphocytes (%) [76-98]	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.7±0.1	0.8±0.0	0.6±0.1	0.8±0.1	0.7±0.1	0.7±0.1	0.8±0.0	0.8±0.0
Monocytes (%) [0-6]	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.1	0.2±0.0	0.2±0.1	0.12±0.1	0.3±0.2	0.3±0.2	0.2±0.0	0.2±0.0
Granulocytes (%) [2-27]	2.5±0.3	2.5±0.3	2.5±0.3	2.5±0.6	2.2±0.3	2.1±0.3	2.7±0.3	2.5±0.2	2.2±0.24	3.3±0.3	2.5±0.2	2.7±0.3
RB (10^3µL) [4,5-6,5]	3.1±0.2	3.1±0.3	3.1±0.3	3.1±0.0	4.7±0.3	3.1±0.5	3.7±0.1	3.7±0.2	4.3±0.1	4.3±0.3	3.1±0.0	3.3±0.1
Hematocrit (%) [40-54]	40.4±0.2	39±0.3	39.7±0.3	40,95±0.0	38.8±0.3	47.1±0.5	40.15±0.4	39.5±0.3	40.2±0.4	41±0.4	39.4±0.3	44.5±0.5
Hemoglobin(g/dL) [13-17]	14±0.4	13±0.2	12.8±0.3	15±0.2	12.2±0.2	14±0.3	12.4±0.2	13±0.2	12±0.2	12.3±0.3	11±0.2	15.2±0.4
VGM (µL) [80-100]	83.8±0.4	77±0.7	80.6±0.2	83.5±0.3	71±0.8	87.5±0.5	65.8±0.8	77±0.8	75.2±0.7	76±0.5	79.4±0.7	82.6±1.0
TCMH (pg) [27 -33]	25.6±0.4	26±0.4	25.7±0.3	27.1±0.3	25±0.2	29±0.5	25±0.3	26±0.4	27±0.3	25±0.4	26±0.2	28±0.5
CCMH (g/dL) [30-35]	31.2±0.4	31.6±0.4	32±0.3	34±0.6	28.4±0.4	34.5±0.6	28.8±0.3	31.6±0.4	28.6±0.2	31±0.3	29.2±0.3	31.4±0.3
Platelets (10^3µL) [150-450]	139±1.0	147±0.7	143.5±1.0	147.5±0.4	197.2±2.1	180±1.1	139±0.9	147±0.7	144.6±0.6	240.63±2.3	146.2±0.5	179.4±1

Values represent means ± ESM; No significant difference compared to the control. Control = distilled water 10 mL/kg body weight; FV 100, 200, 400 and 600: Aqueous extract of *Ficus vogelii* at 100, 200, 400 and 600 mg/kg body weight; SAT 600: satellites; The parameters were: white blood cells (WB), Lymphocytes, Monocytes, Granulocytes, red blood cells (RB), hematocrit, Hemoglobin, the average globular volume (VGM), the average corpuscular concentration of hemoglobin (CCMH), the average corpuscular content of hemoglobin (MCT) and platelets.

Effect of the aqueous extract of *Ficus vogelii* on biochemical parameters

Effect of the aqueous extract of *Ficus vogelii* on the level of total proteins

After 28 days of treatment, the aqueous extract of *F. vogelii* stem bark at doses of 100, 200, 400 and 600 mg/kg body weight did not caused any significant change in the serum protein level compared to control. Two weeks after the last dose of aqueous extract of *F. vogelii* (SAT) was administered, no significant difference was observed (Figure 5).

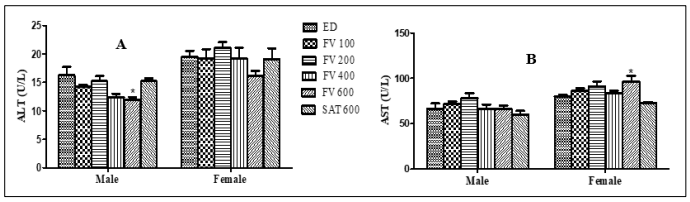


Each bar represents the mean ± ESM; n = 5; No significant difference were observed compared to control, ANOVA followed by Bonferoni's post-test; Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight.

Figure 5: Effect of the aqueous extract of *Ficus vogelii* on the level of total serum proteins

Effect of the aqueous extract of *Ficus vogelii* on the activity of transaminases

The activity of transaminases are represented by figure 6. After 28 days of treatment, a significant (p<0.05) decrease of the alanine aminotransferase (ALT) activity was observed in female rats treated with extract at the dose of 600 mg/kg body weight. At the same dose, the extract induced a significant (p<0.05) increase of the aspartate aminotransferase (AST) activity in male rats compared to control. Two weeks post treatment, no significant difference was observed in satellites rats (Figure 6).

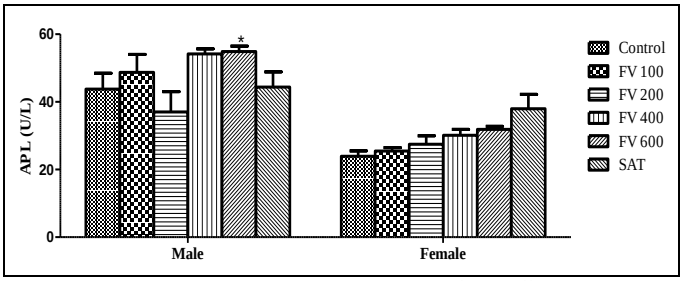


Each bar represents the mean ± ESM; n = 5; *p<0.05 significant difference compared to control, ANOVA followed by Bonferoni's post-test; Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight.

Figure 6: Effect of the aqueous extract of *Ficus vogelii* on the activity of alanine aminotransferase (A) and aspartate aminotransferase (B).

Effect of the aqueous extract of *Ficus vogelii* on the alkaline phosphatase level

Excepted the treatment at the dose of 600 mg/kg which induced a significant (p<0.05) increase of alkaline phosphatase activity in male rat, the administration of the aqueous extract of *F. vogelii* at other doses in male as well as female rat and thus satellites (SAT) did not caused any significant modification of the activity of alkaline phosphatase compared to control (Figure 7).

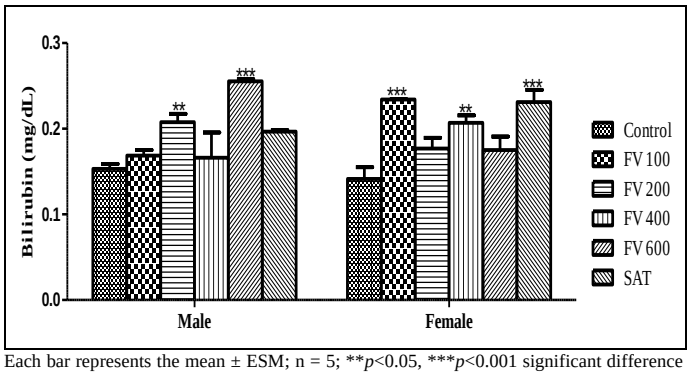


Each bar represents the mean ± ESM; n = 5; *p<0.05 significant difference compared to control, ANOVA followed by Bonferoni's post-test; Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight

Figure 7: Effect of the aqueous extract of *Ficus vogelii* on the level of alkaline phosphatase

Effect of the aqueous extract of *Ficus vogelii* stem bark on total bilirubin level

Administration of aqueous extract of *F. vogelii* resulted in a significant increase of total bilirubin at the dose of 200 mg/kg (p<0.05) and 600 mg/kg (p<0.001) after 28 days of treatment in male rat compared to control. In female rat, the treatment resulted in a significant increase of total bilirubin at doses of 100 mg/kg (p<0.001) and 400 mg/kg (p<0.05) respectively. Two weeks after the treatment, only female rat showed a significant (p<0.001) increase of total bilirubin level in satellite group compared to control (Figure 8).

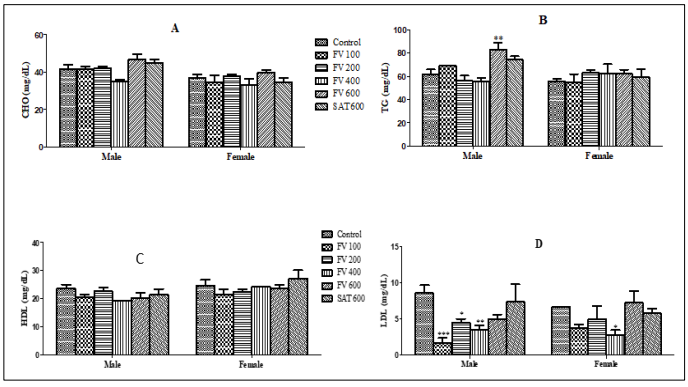


Each bar represents the mean ± ESM; n = 5; **p<0.05, ***p<0.001 significant difference compared to control, ANOVA followed by Bonferoni's post-test. Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight.

Figure 8: Effect of the aqueous extract of *Ficus vogelii* on the total bilirubin level

Effect of the aqueous extract of *Ficus vogelii* on the lipid profile

After treatment, the aqueous extract of *F. vogelii* caused a significant (p<0.01) increase in the triglycerides level at the dose of 600 mg/kg body weight in the male rat compared to control. Total cholesterol and HDL-cholesterol did not showed any modification at all tested doses compared to control. In addition, a significant decrease in the level of LDL-cholesterol was observed in both sex rat compared to control (Figure 9).

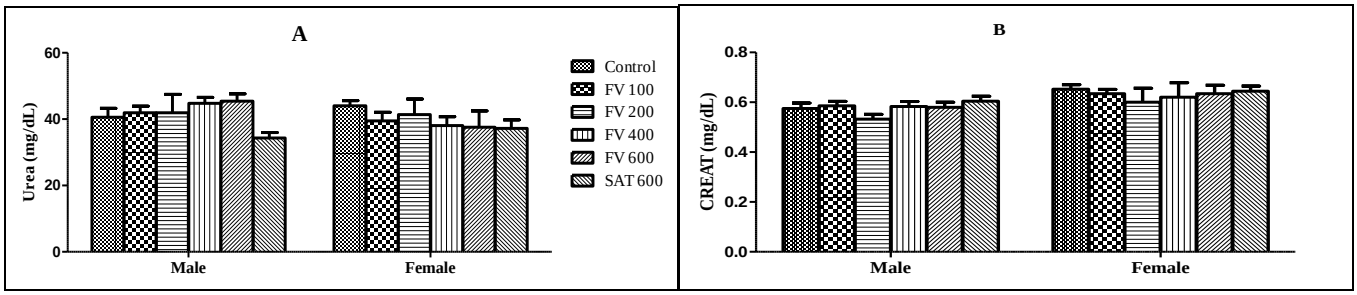


Each bar represents the mean ± ESM; n = 5; *p<0.001, **p<0.001, ***p<0.001 significant difference compared to control, ANOVA test followed by Bonferoni's post-test; CHO=Total cholesterol (A), TG=Triglycerides (B), HDL=HDL-cholesterol (C), LDL=LDL-cholesterol (D); Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight.

Figure 9: Effect of the aqueous extract of *Ficus vogelii* on the lipid profile

Effect of the aqueous extract of *Ficus vogelii* on the urea and creatinine level

The results show that *F. vogelii* aqueous extract administered at doses of 100, 200, 400, 600 mg/kg body weight to treated rats as well as satellites rats did not caused any modification in the level of urea and creatinine in male and female rats treated compared to control rats (Figure 10).



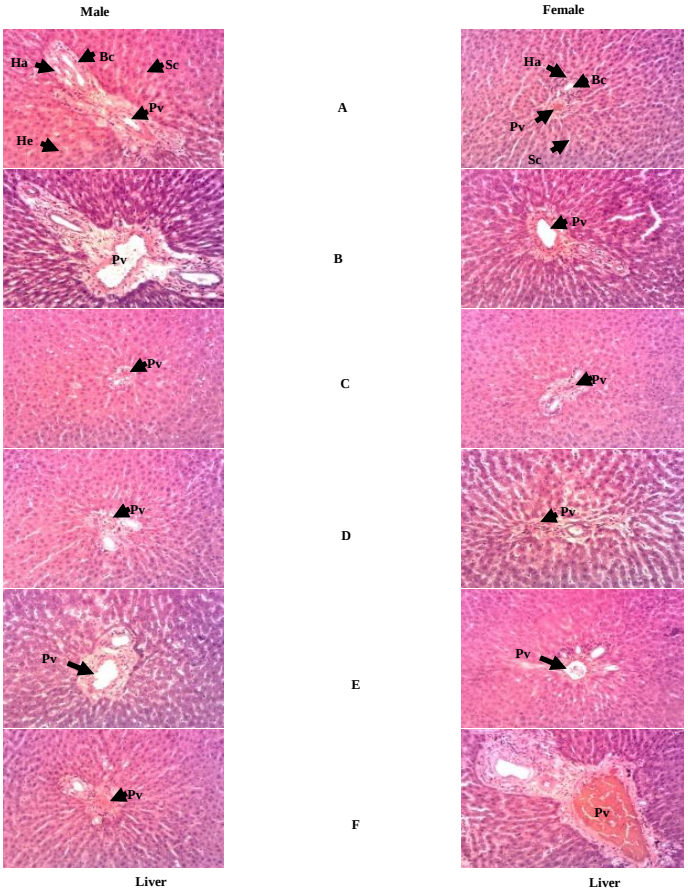
Each bar represents the mean ± ESM; n = 5; ANOVA followed by Bonferoni's post-test; Control = distilled water 10 mL/kg body weight; FV 100, 200, 400, 600 = aqueous extract of *Ficus vogelii* at doses of 100, 200, 400 and 600 mg/kg body weight; SAT = satellites at 600 mg/kg body weight.

Figure 10: Effect of the aqueous extract of *Ficus vogelii* on urea (A) and creatinine levels (B)

Effect of prolonged administration of the aqueous extract of *Ficus vogelii* on the histology of the liver and the kidneys

Effects on histology of the liver

Histopathological study of the hepatic sections of the control group [Figure 11 (A)] have shown in male as well as in female rat a normal appearance of the portal vein (Pv) and hepatic sinusoids (Hs) with normal radiating hepatocytes (He). There was also a normal appearance of the bile duct (Bd) and the branches of the hepatic artery (Ha). The rats treated with the *F. vogelii* aqueous extract at the doses of 100, 200, 400, 600 mg/kg body weight and satellites [Figure 11 (C, D, E, F and B)] presented the similar architecture like the control rat (A) without any alteration signs (Figure 11).

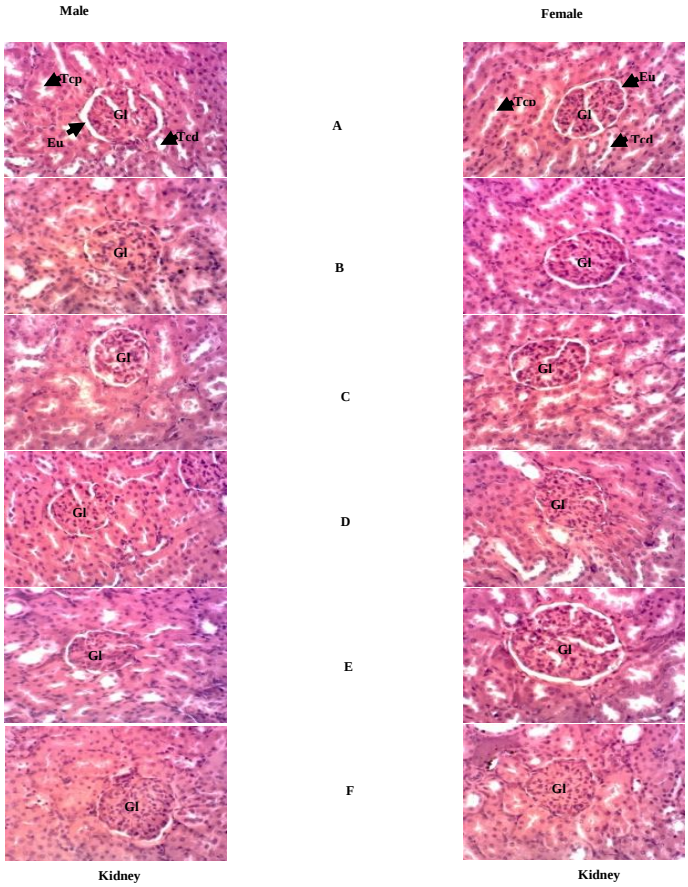


A = control, B = Satellite at 600 mg/kg, C = FV 100 mg/kg, D = FV 200 mg/kg, E = FV 400 mg/kg, F = FV 600 mg/kg; Vp = Hepatic portal vein; He = Hepatocyte; Sc = Sinusoid capillary; Ha = Hepatic artery; Bc = Biliary canaliculus; X100= magnification calibration; H&E = hematoxylin and eosin.

Figure 11: Microphotography of the liver rat after prolonged administration of aqueous extract of *Ficus vogelii* (X100, H&E)

Effects on histology of the kidneys

Histopathological study of renal sections of rats treated with *F. vogelii* aqueous extract stem bark at doses of 100, 200, 400, 600 mg/kg body weight and satellites [Figure 12 (C, D, E, F and B)] did not showed any significant microscopic alteration in either sex compared to control. In the control rats, the renal sections revealed a normal glomerulus (G), urinary space (Us), proximal alveolar tubules (Tp) lined with simple cuboid epithelium with brush border, distal alveolar tubules (Td) lined with epithelium simple cubic (Figure 12).



A = control, B = Satellite at 600 mg/kg, C = FV 100 mg/kg, D = FV 200 mg/kg, E = FV 400 mg/kg, F = FV 600 mg/kg; G = Glomerulus; Us = Urinary space; Td= Distal convoluted tubule; Tp = Proximal convoluted tubule; X200 = magnification calibration; H&E = hematoxylin and eosin.

Figure 12: Microphotography of the kidney rats after prolonged administration of the aqueous extract of *Ficus vogelii* (X200, H&E)

DISCUSSION

Considered safe because they are natural, all natural products used in therapy must first be subjected to safety tests. The evaluation of the toxic characteristics of an extract, a compound or a fraction of natural products and general observations of behavior generally constitute a first step of a toxicity study [17-18]. A part from the beneficial pharmacological effects of the aqueous extract of *Ficus vogelii*, details and in-depth knowledge of the toxicologycal study of this plant remain to be investigated. Therefore, the present study was undertaken to assess the acute and sub-chronic toxicity of the aqueous extract of *Ficus vogelii* stem bark in rats. When assessing the toxic characteristics of medicinal plants, determining the LD_{50} is usually the first step. Data from the acute toxicity study can help determine the LD_{50} values that provide many clues to potential types of drug activity [19]. The results of the acute toxicity study showed that the aqueous extract of *F. vogelii* administered at a single dose of 5000 mg/kg follow by 14 days observation had no effect in the rats' behavior and no mortality. Similar results was obtained by Ping *et al* [17]. Who reported that *Euphorbia hirta* showed no signs of toxicity or mortality up to 5000 mg/kg in rats in an acute toxicity study. This result indicated that *F. vogelii* aqueous extract do not caused deleterious effects in a single dose administrated up to 5 000 mg/kg. The LD_{50} was estimated greater than 5000 mg/kg. In principle, the limit test method is not intended to determine a precise LD_{50} value, but it serves to classify the crude extract according to the expected dose at which the animals should survive [20]. According to the chemical labeling and classification of acute systemic toxicity recommended by the [13] OECD (2001), the aqueous extract of *F. vogelii* has received class 5 status (LD_{50}> 5000 mg/kg), which was the lowest toxicity class. According to Kennedy *et al*. [21] (1986), substances with a LD_{50} greater than 5000 mg/kg orally are considered safe or practically non-toxic.

Since no toxic effects were found during the acute toxicity study, a more in-depth evaluation was conducted to assess the sub-chronic toxicity of the aqueous extract of *F. vogelii* for 28 days in rats in order to estimate the effect of prolonged administration of the aqueous extract of *F. vogelii* on animal behavior and physiology. Therefore, in the sub-chronic toxicity, the aqueous extract of *F. vogelii* stem bark was evaluated in rats at doses of 100, 200, 400 and 600 mg/kg/day in both sexes for 28 days, with an additional satellite group treated with the higher tested dose in order to observe reversibility, persistence or delay of systemic toxic effects during 14 days post treatment.

After 4 weeks of treatment and 2 additional week's observation for satellite group, no significant trouble of animal behavior and any mortality was recorded. The relative mass of organs has been observed in toxicity study as a relatively sensitive indicator for some organs which define toxicity as significant changes observed in these particular organs [22]. The results revealed that the essential organs were not affected and did not showed clinical signs of toxicity throughout the treatment in males and females compared to control. In addition, the treated rats showed a progressive increase in their body weight.

The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological condition which generally gives vital information on the body's response to injury or stress [23-24]. The hematological profile was carried out for all the animals treated with *F. vogelii* and control. The results showed no significant difference of hematological profile of treated animal compared to control. The non-significant effect of the extract on red blood cells, mean corpuscular volume, Hemoglobin, mean corpuscular content and platelets indicates that *F. vogelii* stem

bark does not affect erythropoiesis, morphology or osmotic fragility of red blood cells [25]. Leukocytes are the first line of cell defense that responds to infectious agents, tissue damage or inflammatory processes. In addition, no significant changes were observed in white blood cells, lymphocytes, monocytes and granulocytes, which confirmed the above results. A normal hematological profile would also justify the non-toxic nature of the *F. vogelii* aqueous extract. These results corroborate those obtained by Million *et al.* [26] on the methanoic extracts of *Syzygium guineense*.

Analysis of liver and kidney function is very important in assessing the toxicity of drugs and plant extracts, as they are both necessary for the survival of an organism [27]. The liver is the primary organ involved in drug metabolism, and the kidneys are the site of drug reabsorption and excretion [28]. Increased activities of AST and ALT and alkaline phosphatase in serum are associated with liver toxicity. Transaminases are used as biomarkers to predict possible toxicity[29]. In the present study, only rats which received the aqueous extract of *F. vogelii* at the 600 mg/kg showed a significant variation in the activities of ALT/AST and ALP. However theses variations was reversible 2 weeks post treatment. These results shows that the aqueous extract of *F. vogelii* would not deteriorate hepatic function and would allow the hepatocytes to maintain their normal structure. Similar results have been observed by Mabozou *et al.* [30] on *Combretum micranthum* study. An increase in the bilirubin plasma level is due to the breakdown of hemoglobin, it is an indicator of liver diseases such as jaundice, ineffective erythropoiesis and hepatic cholestasis [31]. The results obtained show a significant increase in the both sex at some tested doses indicating a possible toxic effects of the extract of *F. vogelii*. However, this increase was not accompanied by a decrease in the number of red blood cells, or in the hemoglobin level. It would probably be due to a drop in renal filtration of conjugated bilirubin or to obstruction of the bile ducts.

The liver is the organ responsible for the synthesis, elimination or breakdown of cholesterol. The alteration of fatty acid metabolism is characterized by an increase in high cholesterol and triglycerides levels [32]. In this study, the prolonged administration of the *F. vogelii* aqueous extract caused an increase in the triglycerides level in male animals at 600 mg/kg body weight. The levels of total cholesterol, HDL did not changed. The significant decrease in the level of LDL-cholesterol showed that *F. vogelii* is protective against metabolic diseases. Urea, uric acid and creatinine are considered important markers of kidney function [33, 24]. Kidney function can be assessed by simultaneous measurements of urea and creatinine, and their normal levels reflect a reduced likelihood of kidney problems [34]. Urea and creatinine level did not changed in this study indicating normal kidney function.

Macroscopic examinations of the organs of rats treated with *F. vogelii* did not showed any change in appearance compared to control. An enlarged organ is a direct indication of the toxicity of the chemical or biological substance. However, no organ hypertrophy was observed in this study in all the groups studied. In addition, microscopic examination revealed no changes in the cellular structure of the liver and kidney under the light microscope using multiple amplification power. No alteration was recorded in the histological sections of the vital organs (liver, kidney). In general, any damage to the parenchymal liver cells leads to an elevation of the two transaminases in the blood [35]. Thus, in harmony with the results, the sub-chronic administration of the *F. vogelii* did not affected the hepatocytes and consequently, the liver tissue has kept a normal architecture. Any increase in urea and creatinine levels is observed only if there is marked damage to the functional nephrons [36]. There was no change in urea and creatinine levels during the sub-chronic administration of the *F. vogelii* aqueous extract compared to control animals. These results was confirmed by normal histopathological observations of renal tissue in this study. Consequently, the results recorded in this study demonstrate that *F. vogelii* did not impaired liver or renal function and further supports the non-toxic nature of the aqueous extract of *F. vogelii*.

CONCLUSION

The results obtained indicate that the LD_{50} of aqueous extract of *F. vogelii* stem bark is greater than 5000 mg/kg and therefore classified relatively as non-toxic substances. From the sub-chronic toxicity study, the animals treated at 600 mg/kg exhibited a significant modification of the activity of ALT, AST, ALP and increase level of TG. These changes were reversible during the 2 weeks of observation in satellite animals. No alteration of the liver and the kidney was observed, thus demonstrating the non-toxic effect of oral administration of aqueous extract of *F. vogelii* stem bark at the doses tested and consider safety for its use in traditional medicine.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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