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Evaluation of the acute and subacute toxicity of the aqueous extract of *Ficus vallis-choudae* leaves in Wistar rats

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ABSTRACT

In Cameroon, *Ficus vallis-choudae* Delile leaves are empirically used to treat diabetes mellitus. However, no studies have been carried out to ensure the plant's safety. This work aimed to assess the acute and subacute toxicity of the aqueous extract of *Ficus vallis-choudae* (FVE) leaves in rats. To assess acute toxicity, FVE at a single dose of 5000 mg/kg was administered to female rats (n = 6), while distilled water (1 mL/100 g) was force-fed to animals in the control group (n = 6). Behavioral changes and mortality were assessed at 30 min, 4h, 24h, 48h and regularly for 14 days. For the subacute toxicity study, animals were divided into 6 groups of 10 rats each (5 males and 5 females) and treated orally for 28 days. A control group received distilled water (10 mL/kg), three groups received FVE at doses of 220, 440 and 880 mg/kg respectively, and two satellite groups received distilled water (10 mL/kg) and FVE at 880 mg/kg respectively. Body mass, food and water consumption, biochemical, and hematological parameters, and liver and kidney histology were evaluated. For acute toxicity, no behavioral changes and no mortality were observed. The mean lethal dose (LD_{50}) of FVE was greater than 5000 mg/kg. With regard to subacute toxicity, the FVE tested appeared to be tolerated by rats, as no major clinical signs of toxicity, no mortality, no significant changes in body weight, internal organ weights, food and water consumption were noted in either female or male rats. Similarly, biochemical and hematological parameters, as well as histological aspects of the liver and kidneys of FVE-treated animals of both sexes, showed no significant changes. Short- and medium-term consumption of FVE is safe in rats. However, it will be useful to assess the sub-chronic and chronic toxicity of this plant extract.

Keywords: *Ficus vallis-choudae*, Acute toxicity, Sub-acute toxicity, Aqueous extract, Wistar rats.

INTRODUCTION

Nowadays, much research is focused on medicinal plants, considered a huge source of multiple phyto-therapeutic substances. These plants are endowed with pharmacological activities that can constitute a weapon to deal with oxidative stress and its damage to the organs of living beings. They have secondary metabolites which represent an important source of molecules by humans [1]. The WHO estimates that around 80% of populations living in developing countries use medicinal plants for their primary health care needs [2]. On the other hand, the majority of people believe that medicinal plants are natural and therefore harmless, unlike synthetic medicines. Previous studies have shown that herbal medicine is not without risks. Toxic damage affects most organs. We can cite in particular renal failure, cardiac damage by poisoning, pulmonary damage and liver damage which are also the most significant [3,4]. Thus, the safety assessment of a substance must be carried out to prevent certain possible damage to the organs involved. This evaluation can be done either by studying its acute toxicity after administration of a single dose or by studying its chronic toxicity after repeated administration of the substance. According to the Commission for Occupational Health and Safety (CSST), the harmful effect is linked to the dose, the route of absorption, the type and severity of the lesions as well as the time necessary for the appearance of a lesion [5].

Ficus vallis-choudae Delile is a plant belonging to the Moraceae family. It is a shrub or small tree reaching 8 m in height, with a well-developed and spreading crown and a short bole, gray-brown bark slightly cracked and a little scaly and pink edge exuding a scant white latex. Leaves simple, alternate, leathery; oval blades up to 20 x 22 cm, with smooth upper surface; petiole up to 8 cm long; stipules 1-3 cm long, deciduous. Solitary figs, axillary leaves, globose, slightly flattened at the top; yellowish with around ten reddish meridian stripes. The fruits are solitary figs in the leaf axils [6]. Decoctions of leaves and young leafy stems of *Ficus vallis-choudae* are used as a local medicine for diabetes, jaundice, nausea, bronchial and gastrointestinal disorders. The figs of this plant are eaten and appreciated by children [7].

Previous studies reported that *Ficus vallis-choudae* Delile exhibits antifungal, anticonvulsant [8,9], anti-inflammatory and antinociceptive activities [10]. The methanol extract of the bark of this plant exhibited inhibitory activities on urease and α-glucosidase enzymes and showed potent DPPH radical scavenging capacity. It also showed low chemiluminescent activity compared to ibuprofen [11, 12, 13]. Kolefer et al. [14] showed that the aqueous extract of *Ficus vallis-choudae* leaves has antidiabetic, antioxidant and antidyslipidemic properties in the type 2 diabetes model induced by high-fat diet and streptozotocin. Previous phytochemical studies have shown that *Ficus vallis-choudae* is rich in flavonoids, glycosides, alkaloids, tannins, and saponins [10,14]. However, no toxicity studies have been conducted on the aqueous extract of *Ficus vallis-choudae* leaves. The aim of this study was therefore to evaluate the acute and subacute toxicity of the aqueous extract of *Ficus vallis-choudae* leaves (FVE).

MATERIALS AND METHODS

Plant material

The plant material used consisted of the leaves of *Ficus vallis-choudae*, harvested in July 2019 in the locality of Koza (Cameroon). The identification of the plant was made at the National Herbarium of Yaoundé (Cameroon) by comparison with the specimen registered under number 5115 SRF/Cam. The harvested leaves were first washed in tap water, dried away from the sun and crushed until a fine powder was obtained.

Preparation of FVE

A mass of 250 g of powder obtained was dissolved in 2 L of distilled water for 24 hours. The mixture obtained was filtered using Wattman Nº1 paper. The filtrate thus obtained was evaporated in an oven at 45°C for 72 hours to obtain a raw dry matter (30.80 g) with a yield of 12.32%.

Animal material

The animal material consisted of male and female albino rats of Wistar strain, 8 to 10 weeks old and weighing between 180 and 220 g, with healthy, nulliparous and non-pregnant female rats as inclusion criteria. The rats were bred in the animal house of the Department of Biological Sciences at the University of Ngaoundere (Cameroon). The animals were housed in polypropylene cages under standard environmental conditions (temperature 22 ± 2°C in a 12 h/12h light/dark cycle). They received a standard diet and drinking water ad libitum. They were acclimatized for 14 days under laboratory conditions before the start of the test. All animal experiments were conducted in accordance with the Cameroon National Ethics Committee (ref. N° FWIRB 00001954) and all experiments were reviewed and approved.

Acute toxicity study

The toxicity test was carried out following the "dose adjustment" method of according to organization for economic cooperation and development line 425 and consisted of testing FVE at a dose of 5000 mg/kg [15]. After 4 hours of fasting, the rats were divided into 2 groups of 6 rats each and treated as follows:

• Group 1: rats treated with distilled water (1 mL/100 g);

• Group 2: rats treated with FVE (5000 mg/kg).

A behavioral observation was carried out 4 hours after administration of the substances. Then the animals received food and water daily for 14 days. During this period, signs of toxicity including coat appearance, motility, tremors, grooming, breathing, appearance of stools, mobility as well as death were noted.

Subacute toxicity study

The evaluation of the subacute toxicity of FVE was carried out according to OECD guideline 407 [15].

Distribution of rats and treatment

Albino rats (60), i.e. 30 males and 30 females, were divided into 6 groups of 10 rats each (5 males and 5 females) and treated daily for 28 days as follows:

• Group 1: rats treated with distilled water (10 mL/kg);

• Group 2: rats treated with FVE (220 mg/kg);

• Group 3: rats treated with FVE (440 mg/kg);

• Group 4: rats treated with FVE (880 mg/kg);

• Group 5: satellite rats treated with FVE (880 mg/kg);

• Group 6: satellite control treated with distilled water (10 mL/kg).

Body weight and food and water consumption were assessed each week of the experiment.

Blood and organ collection

On day 28, rats were fasted for 24 h. Blood was collected from the jugular vein after cervical dislocation then introduced into dry tubes and centrifuged at 3000 rpm for 20 min. The supernatant obtained was collected and stored at -18°C for the determination of biochemical parameters. After blood collection, the abdominal cavity was opened and organs such as the liver, kidneys and heart were removed, cleaned with NaCl 0.9% solution, drained and weighed. A fragment of kidney and liver was placed in formalin 10% for histological sections.

Determination of mineralogical parameters

Sodium determination was carried out according to the method of Helgeson et al. [16]. The determination of the serum concentration of chlorine, calcium and iron was done according to the method of Ono et al. [17]. Serum potassium and magnesium concentrations were measured using the enzymatic method of Berry et al. [18].

Assay of biochemical parameters

Total cholesterol (TC) and triglycerides (TG) were measured according to the method used by Kaplan [19]. The activities of aspartate aminotransferase (AST), and alanine aminotransferase (ALT), and the concentrations of creatinine, total bilirubin, urea, and uric acid were determined according to the method of Murray [20].

Evaluation of hematological parameters

The hemogram is carried out from whole blood contained in EDTA tubes using an automatic hematological analyzer (Myndray BC-3000) which directly gives the values of the following parameters: red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin content (MHCT), platelets (PLT), lymphocytes, neutrophils, monocytes, eosinophils, and basophils.

Histopathological analysis

The histological section of the liver and kidney was carried out following the classic technique of Houlot [21]. After removing the kidney and liver from alcoholic Bouin (26 mL Formalin, 7 mL acetic acid, 45 mL 1% picric acid in 95% ethanol and 22 mL distilled water), each organ was dehydrated and immersed in paraffin baths. Sections were made using a microtome with a thickness of 4 to 7 microns. Staining was done using the hematoxylin-eosin technique.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Data in different groups were compared using one-way analysis of variance (ANOVA) followed by Turkey's test and two-way ANOVA followed by Bonferroni's test. The Graph Pad Prism software version 8.0.1 was used to analyze results. Values were considered significant at $p < 0.05$.

RESULTS

Acute toxicity of FVE

During the 14 days of experiment, the animals showed no behavioral changes. Indeed, signs of toxicity such as tremor, convulsion, aggressiveness, sleep and digestion disorders were not observed. Likewise, all the animals survived after these 14 days of observation, the mean lethal dose (LD_{50}) was therefore greater than 5000 mg/kg of body weight.

Subacute toxicity of FVE

Effect of FVE on relative body weights

Relative body weights of rats during 28 days of treatment showed no significant differences in all treatment groups including satellite groups (Figures 1A and 1B). Nevertheless, normal rat growth was observed in all experimental groups.

Effect of EFV on the relative organ weights

Table 1 shows the effect of FVE on the relative weights of the heart, kidneys, liver, and lungs of male and female rats treated for 28 days. No significant differences in organ weights were observed after treatment with FVE at doses of 220, 440 and 880 mg/kg, compared with the control group. The same observations were made in satellite groups after a further 14 days of observation.

Effect of FVE on ionograms

Table 2 shows the effects of FVE on the serum ion composition of male and female rats treated for 28 days. In the present study, compared to the control group, there was no change in the levels of sodium, potassium, chloride, calcium, iron, and magnesium ions in the blood of male and female rats after administration of FVE at all dose levels. Similarly, the satellite groups showed no significant change in the ionogram compared to the satellite control group.

Effect of FVE on biochemical parameters

The effects of FVE on transaminase activities (ALT and AST) and levels of urea, creatinine, bilirubin, uric acid, total cholesterol, and triglycerides in male and female rats are shown in Table 3. The values of these different biochemical parameters did not vary significantly in male and female rats treated with 220, 440 and 880 mg/kg FVE, compared with the control group. However, in female rats, at the 880 mg/kg dose, there was a significant decrease ($p < 0.05$) in serum TC concentrations of around 17.95% and in TG of around 13.10%, compared to the control.

Effect of FVE on haematological parameters

The numbers of WBC, RBC and PLT, hematocrit levels, MCV, MCHC and MHCT of male and female rats did not vary significantly from those of the control group (Table 4). However, a significant increase in lymphocyte count of approximately 14.54% ($p < 0.05$), 18.42% ($p < 0.01$), and 6.87% ($p < 0.01$) was noted in male rats treated with VFE at doses of 220, 440 and 880 mg*/kg, respectively, compared to* the control group. In female rats, there was a significant increase in lymphocyte levels at doses of 220 (14.13%; $p < 0.05$), 440 (15.82%; $p < 0.05$), and 880 mg/kg (18.12%; $p < 0.01$) and in PLT levels at 440 mg/kg (22.56%; $p < 0.05$) compared to the control group. In satellite batches of male rats, FVE resulted in a significant reduction in hemoglobin (21.53%; $p < 0.001$) and monocyte (30.95%; $p < 0.01$) levels compared to the satellite control. However, in the satellite group of female rats, there was a significant reduction in MCV ($p < 0.01$) and monocytes ($p < 0.001$) compared to the satellite control.

Effect of FVE on the liver tissue of male and female rats. Analysis of histological sections of the liver of male (A) and female (B) FVE rats during subacute toxicity showed that animals in the control group, as well as those in the 220, 440, and 880 mg/kg dose groups, exhibited normal liver architecture with well-distinct hepatic parenchyma and hepatocytes (Figure 2).

Effect of EFV on the kidney tissue of rats. Histological sectioning of the kidneys of all experimental male (A) and female (B) rats treated with EFV at 220, 440, and 880 mg/kg for 28 days revealed no significant abnormalities. In fact, the kidneys of the animals had a normal architecture with normal glomeruli, distal and proximal convoluted tubules (Figure 3).

Table 1: Effect of FVE on the relative organ weights of rats in subacute toxicity

	Relative weights of organs (g/100g m.c)			
Groups	**Heart**	**Liver**	**Kidney**	**Lungs**
Male				
Normal control	0.42 ± 0.05	2.29 ± 0.13	0.88 ± 0.04	1.19 ± 0.39
FVE 220 mg/kg	0.44 ± 0.04	2.44 ± 0.10	0.91 ± 0.50	1.17 ± 0.28
FVE 440 mg/kg	0.45 ± 0.04	2.58 ± 0.21	0.91 ± 0.07	1.20 ± 0.19
FVE 880 mg/kg	0.45 ± 0.01	2.40 ± 0.18	0.93 ± 0.06	1.19 ± 0.22
Satellite control	0.42 ± 0.04	2.25 ± 0.06	0.86 ± 0.08	1.18 ± 0.17
Satellite extract	0.45 ± 0.01	2.23 ± 0.07	0.92 ± 0.04	1.16 ± 0.14
Female				
Normal control	0.41 ± 0.02	2.31 ± 0.21	0.85 ± 0.04	1.18 ± 0.34
FVE 220 mg/kg	0.42 ± 0.03	2.23 ± 0.24	0.88 ± 0.04	1.21 ± 0.12
FVE 440 mg/kg	0.42 ± 0.05	2.16 ± 0.22	0.89 ± 0.06	1.20 ± 0.19
FVE 880 mg/kg	0.43 ± 0.03	2.60 ± 0.41	0.90 ± 0.04	1.22 ± 0.11
Satellite contol	0.39 ± 0.03	2.46 ± 0.37	0.84 ± 0.05	1.23 ± 0.16
Satellite extract	0.42 ± 0.03	2.27 ± 0.22	0.91 ± 0.04	1.19 ± 0.21

Results were expressed as mean ± MSE (n=5)

Table 2: Effect of VFE on the ionogram of rats in subacute toxicity

Parameters	Control	FVE 220 mg/kg	FVE 440 mg/kg	FVE 880 mg/kg	Satellite control	Satellite Extract
Male						
Sodium (mmol/L)	136.69±1.83	137.66±1.24	144.00±3.20	139.42±0.76	137.82±1.86	140.22±5.43
Potassium (mmol/L)	2.33±0.22	2.22±0.20	2.28±0.13	2.59±0.10	2.39±0.28	2.34±0.17
Chloride (mmol/L)	92.98±1.79	95.39±2.03	99.39±2.69	94.76±2.42	91.58±2.43	97.64±2.66
Calcium (mmol/L)	30.77±0.90	31.09±0.67	31.08±1.35	30.97±0.79	28.43±1.91	28.41±1.96
Magnésium (mmol/L)	6.45±0.18	6.53±0.21	6.98±0.06	6.82±0.12	6.40±0.21	6.62±0.19
Iron (mmol/L)	5.34±0.13	5.64±0.15	5.34±0.28	5.89±0.33	5.22±0.34	5.46±0.17
Female						
Sodium (mmol/L)	140.98±1.56	140.33±1.71	138.19±2.24	146.13±2.41	139.35±1.02	140.56±2.84
Potassium (mmol/L)	2.26±0.17	2.53±0.09	2.59±0.15	2.51±0.13	2.32±0.15	2.78±0.10
Chloride (mmol/L)	95.96 ±2.19	96.36±2.27	99.78±1.62	96.27±3.14	95.96±2.06	96.29±2.04
Calcium (mmol/L)	30.34 ±1.06	30.57±0.99	32.45±2.14	30.68±0.99	30.98±0.60	31.63±0.64
Magnesium (mmol/L)	6.58±0.20	7.06±0.07	7.02±0.06	7.05±0.07	6.49±0.24	6.46±0.24
Iron (mmol/L)	5.80±0.15	5.81±0.18	5.36±0.33	5.75±0.17	5.56±0.18	5.63±0.18

Results were expressed as mean ± MSE (n=5)

Table 3: Effect of FVE on biochemical parameters of rats in subacute toxicity

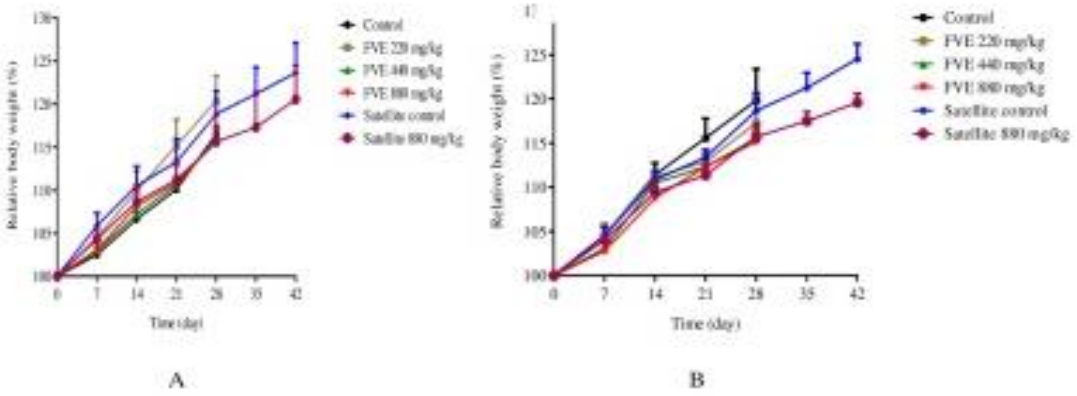
Parameters	Normal control	FVE 220 mg/kg	FVE 440 mg/kg	FVE 880 mg/kg	Satellite control	Satellite extract
Male						
Urea (mmol/L)	6.72±0.18	6.65±0.17	6.76±0.22	7.05±0.17	6.54±0.20	6.90±0.18
Créatinine (mg/dL)	0.65±0.04	0.67±0.05	0.61±0.02	0.81±0.05	0.63±0.03	0.79±0.05
Uric Acide (mol/L)	85.89±1.56	83.76±1.79	83.80±2.98	91.29±2.76	83.48±1.65	84.30±3.73
ALT (U/L)	54.93±1.82	51.97±1.11	53.13±1.98	54.76±2.36	54.63±2.00	57.81±1.74
AST (U/L)	88.73±1.76	87.08±1.21	88.54±1.92	90.10±1.48	88.97±2.14	90.34±1.99
Bilurbin (mg/L)	4.79±0.22	4.312±0.18	4.43±0.30	4.17±0.16	4.74±0.19	4.08±0.21
TC (mg/dL)	57.73±3.03	61.54±1.18	61.35±2.06	53.33±2.22	60.79±2.04	51.2±2.78
TG (mg/dL)	103.96±3.49	105.93±3.19	88.11±1.83	90.91±1.62	105.59±3.18	93.04±2.51
Female						
Urea (mmol/L)	5.34±0.16	5.64±0.15	5.70±0.21	6.15±0.20	5.33±0.22	6.22±0.23
Creatinine (mg/dL)	0.57±0.01	0.6±0.02	0.59±0.01	0.66±0.03	0.55±0.02	0.66±0.03
Uric acid (mol/L)	83.30±2.22	80.63±1.22	82.81±3.10	88.85±1.62	83.24±2.69	87.71±2.70
ALT (U/L)	52.85±0.70	53.44±1.13	50.28±1.34	52.33±1.41	52.05±1.42	55.00±2.11
AST (U/L)	89.23±1.26	88.90±0.83	87.81±1.01	90.17±1.89	89.28±1.28	94.69±1.85
Bilurbin (mg/L)	4.23±0.20	3.82±0.15	3.96± 0.23	3.87±0.14	3.64±0.33	4.81±0.43
TC (mg/dL)	60.59±1.94	59.61±1.03	58.85±1.77	49.71±1.2$^{\alpha}$	59.05±2.78	54.76±1.69
TG (mg/dL)	103.94±3.18	104.69±2.59	102.50±3.44	90.32±1.43$^{\alpha}$	103.33±3.56	94.31±2.84

Results were expressed as mean ± MSE (n = 5). TC: total cholesterol; TG: triglycerides. ALT: alanine aminotransferase; AST: aspartate aminotransferase.

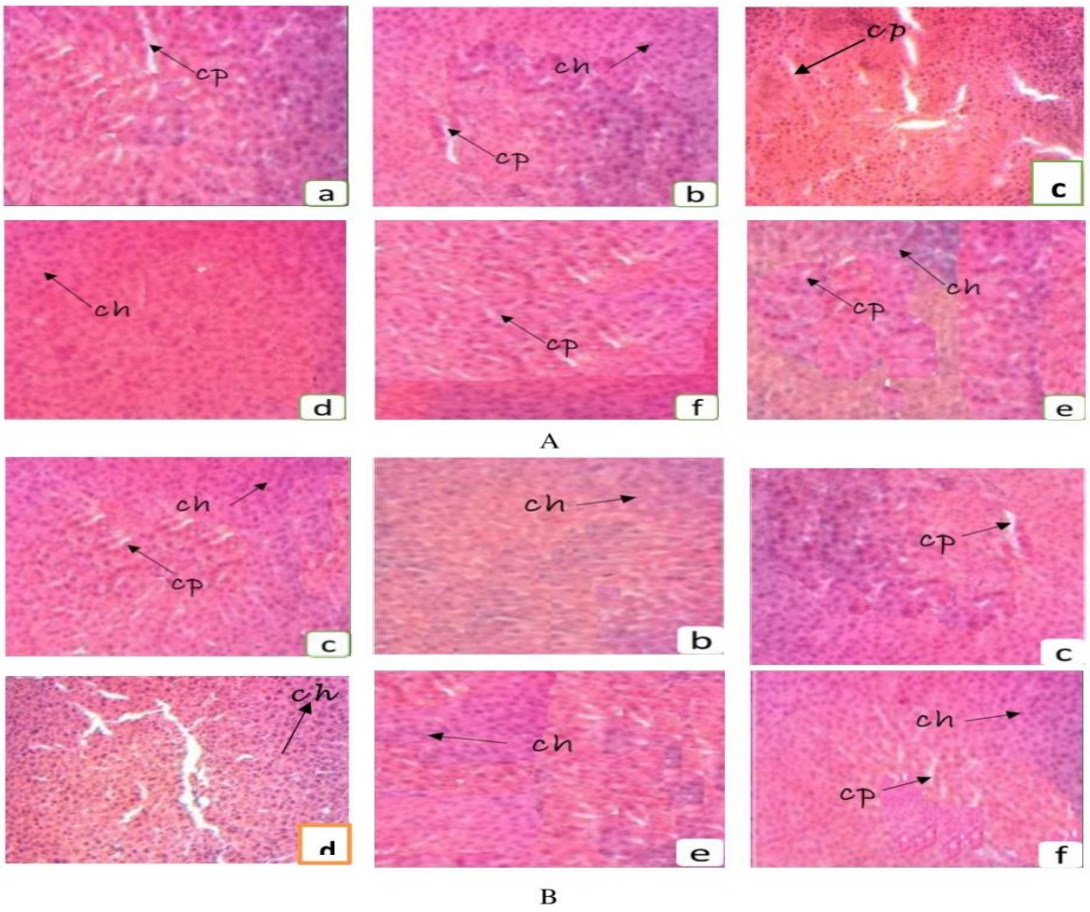
Table 4: Effect of FVE on hematological parameters of rats in subacute toxicity

	Control	EFV 220 mg/kg	EFV 440 mg/kg	EFV 880 mg/kg	Satellite control	Satellite extract
Male						
RBC ($10p^6/mm^3$)	6.10± 0.25	5.99± 0.16	6.80± 0.32	6.40± 0.22	6.11± 0.33	6.17±0.28
Heamoglobin (g/dL)	15.99± 0.69	14.75± 0.33	13.89± 0.41	17.09± 0.47 $^{\gamma}$	16.11± 0.39	12.64±0.65$^{\alpha}$
Heamatocrit (%)	38.61± 0.56	39.59± 0.64	39.61± 0.60	40.78± 0.59	39.08± 0.76	38.99±0.91
MCV (μ^3)	56.99 ± 0.78	53.19± 0.90	59.16± 0.75	50.73± 1.48	56.52± 2.57	48.73±2.38 $^{\gamma}$
MCHT (pg)	18.59±0.60	18.88± 0.35	19.58± 0.49	17.15± 0.58	17.81± 0.65	15.67±0.44
MCHC (%)	31.58±0.58	30.83± 1.37	32.82± 0.92	32.08± 1.03	30.34± 1.41	29.04±1.41
WBC ($10p^3/mm^3$)	10.04± 0.46	10.19± 0.43	10.61± 0.37	9.56± 0.72	10.04± 0.34	9.60±0.70
Neutrophil (%)	19.93± 0.89	20.26± 0.43	20.77± 0.76	19.00± 0.44	19.73± 0.77	17.72±0.42
Lymphocytes (%)	60.90± 1.06	69.76± 0.80 $^{\gamma}$	72.12± 2.06$^{\beta}$	70.06± 1.05$^{\beta}$	58.73± 2.18	67.14±2.30$^{\gamma}$
Monocytes (%)	11.68± 0,29	10.06± 1.01	13.37± 0.47	8.52± 0.30$^{\beta}$	11.37± 0.60	7.85±0.35$^{\beta}$
Eosinophils (%)	2.23± 0.09	2.10± 0.04	2.25± 0.09	2.25± 0.10	2.45± 0.17	2.25±0.19
PLT ($10p^3/mm^3$)	945.14± 76.33	1063.24± 68.72	1119.57± 81.18	763.42± 51.86	950.55± 72.88	744.55±64.75
Female						
RBC ($10p^6/mm^3$)	6.03±0.22	6.438±0.24	5.92±0.26	5.98±0.29	6.08±0.31	4.97±0.27
Heamoglobin (g/dL)	15.99± 0.38	15.45±0.42	15.53±0.61	15.22±0.24	16.61±0.32	12.46±0.82
Heamatocrit (%)	39.44±0.89	43.29±2.33	41.60±1.16	39.82±1.64	38.32±2.07	42.90±2.51
MCV (µ3)	59.06±1.16	52.03±1.57	57.43±2.63	48.58±1.84$^{\beta}$	58.64±1.00	48.20±1.14$^{\beta}$
MCHT (pg)	18.34±0.49	18.72±0.32	20.24±0.90	16.87±0.43	17.62±0.74	14.22±0.83$^{\gamma}$
MCHC(%)	31.16±0.936	33.05±1.10	30.70±1.42	30.42±1.06	31.65±1.00	30.54±2.76
WBC($10p^3/mm^3$)	10.10±0.52	8.826±0.37	10.57±0.63	9.03±0.71	10.39±0.50	9.97±0.59
Neutrophil (%)	20.22±0.68	22.44±1.88	22.63±1.78	18.48±0.55	20.74±0.83	16.63±0.59
Lymphocytes (%)	60.98±1.24	69.60±2.04$^{\gamma}$	70.63±3.17$^{\gamma}$	72.03±1.04$^{\beta}$	65.18±1.69	69.90±1.67
Monocytes (%)	12.05±0.18	10.38±1.08$^{\gamma}$	10.75±0.42$^{\gamma}$	7.89±0.24$^{\alpha}$	11.65±0.56	6.96±0.28$^{\alpha}$
Eosinophil (%)	2.336±0.17	2.02±0.06	2.11±0.06	2.13±0.08	2.34±0.15	2.00±0.06
PLT ($10p^3/mm^3$)	947.18±75.56	1103.83±56.26	1167.04±18.93$^{\gamma}$	768.21±55.83	943.04±76.46	673.30±37.14$^{\gamma}$

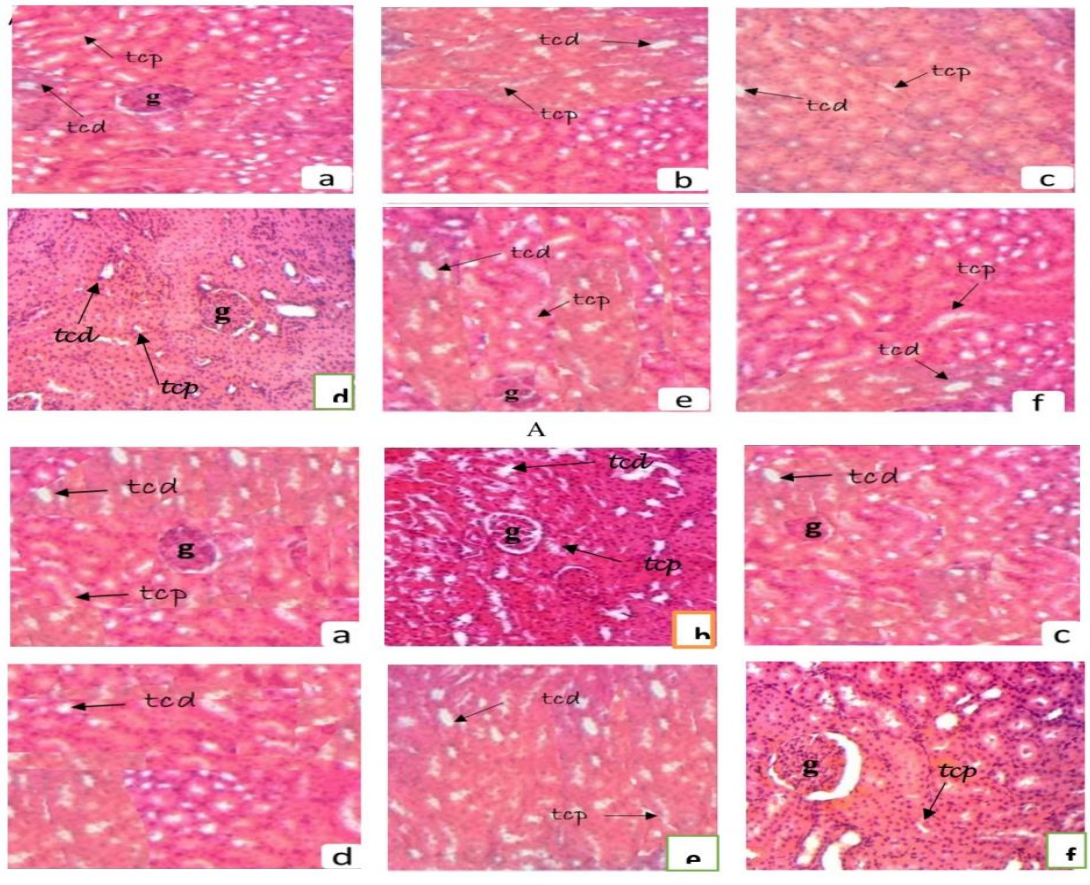
Each value represents the mean ± MSE (n=5). γp <0.05, βp <0.01, αp <0.001 statistically significant to normal control. MCV: mean corpuscular volume; MCHT: mean corpuscular haemoglobin content; MCHC: mean corpuscular haemoglobin concentration. WBC : white blood cell, RBC : red blood cell, PLT: blood platelets.



Fgure 1: Effects of FVE on relative body weight as a function of time in subacute toxicity in male (A) and female (B) rats. Results were expressed as mean ± MSE (n = 5).



Fgure 2: Histological section of liver tissue of male (A) and female (B) in subacute toxicity (haematoxylin-eosin x100). a = Control; b = FVE 220 mg/kg; c = FVE 440; d = FVE 880 mg/kg; e = satellite control; f = satellite extract; cp = blood capillary, ch = hepatocytes.



Fgure 3: Histological section of kidney tissue of male (A) and female (B) in subacute toxicity (Haematoxylin-eosin x100). a = normal control b = FVE 220 mg/kg; c = FVE 440 mg/kg; d = FVE 880 mg/kg; e = satellite control; f = satellite extract; tcd = distal convoluted tubule, tcp = proximal convoluted tubule, g = glomerulus.

DISCUSSION

The main aim of studying plant toxicity is to understand the nature and extent of the secondary effects of extracts or molecules at appropriate doses, in order to prevent any risk to humans. In addition, identifying the toxic agent will enable either its elimination to obtain non-toxic active extracts, or the modification of its dose, or its structure if it itself carries biological activity [22].

The acute toxicity study in rats showed that FVE (5000 mg/kg), administered orally, caused no clinical signs of toxicity and no mortality. The LD_{50} is therefore greater than 5000 mg/kg. This observation enables FVE to be classified in category 5 of the globally harmonised classification system for chemical substances. This category characterises substances with low toxicity [23].

Subacute oral administration of FVE did not cause any deaths or clinical signs of toxicity. During 28 days of treatment, FVE induced normal growth in female and male rats. The evaluation of organ weights and histological sections is used to predict the toxic effect of substances, and also to identify possible target organs [24]. In the present study, no significant differences were found in the relative weights of the liver and kidney. Similarly, analysis of histological sections of the liver and kidney of male and female rats showed normal aspects, suggesting that FVE did not have deleterious effects on these two organs.

Mineral elements play an important role in the construction of human tissues and in the regulation of vital reactions as cofactors of numerous metalloenzymes. In the present study, the quantitative determination of blood ionic composition such as Na+, K+, Cl^-, Ca^{2+}, Fe^{2+}, and Mg2+ was performed. However, increased levels of these ions are generally linked to renal failure or a reduction in glomerular filtration. As a result, the Na+ retained in the blood will cause an overload of Na+, which will lead to water retention, resulting in an increase in blood volume and hence in blood pressure [25]. Increased levels of Ca^{2+} and Mg^{2+} ions impair renal function. Hypercalcaemia also reduces the renal capacity to concentrate urine by inhibiting the creation of the corticomedullary osmotic gradient essential for the action of ADH on the collecting tube [25]. No significant variation in the levels of these ions was observed in male and female rats in this study. In addition, excess chlorine in the blood can cause digestive and respiratory disorders with mucous membranes, leading to thick secretions, fibrinous exudates and even dehydration [25]. The FVE showed no digestive problems, since the stool appearance was normal, and no respiratory problems.

Elevated ALT activity is an indicator of the degree of cell membrane degradation and elevated AST activity is an indicator of mitochondrial damage [26]. Renal nitrogen constituents including urea and creatinine have also been assessed in order to demonstrate renal function. Typically, these compounds are filtered by the kidneys with little or no tubular reabsorption and their elevated levels in the blood generally indicate renal failure [27]. FVE did not produce significant variations in transaminase activities and serum urea, creatinine and bilirubin concentrations in any of the groups studied. These results provide sufficient evidence of the low toxicity of our extract, which also justifies the results of the histological analyses, which showed that there were no variations in the size of the liver and kidney cells of male and female rats. On the other hand, in female rats treated with FVE at the dose of 880 mg/kg, our results showed a reduction in serum TC and triglyceride concentrations. The reduction in these two biochemical parameters are indicators of protection against risk factors for the development of atherosclerosis and hence cardiovascular disease [28]. These results suggest that our extract could reduce the risk of obesity and cardiovascular disease. Similar results were reported by Oyebanji et al. [29].

The haematopoietic system is one of the targets most exposed to toxic substances. It is also an important marker of physiological and pathological status in animals [30]. Analysis of haematological parameters is of vital importance for all toxicological studies. A variation in these parameters in a subject is synonymous with an abnormality in the function, morphology or metabolism of erythrocytes, leukocytes and thrombocytes [31]. In this study, haematological values showed non-significant variations in lymphocyte, erythrocyte and platelet lineage in animals treated with FVE. However, there was a significant increase in haemoglobin in male rats treated at 880 mg/kg and in lymphocyte counts in male and female rats treated at 220, 440, and 880 mg/kg. The decrease in haemoglobin, hematocrit and red blood cell levels are the permanent causes of anemia [29]. The increase in haemoglobin levels observed in male rats suggests a beneficial effect of FVE on haematopoiesis and the increase in lymphocytes and monocytes in male and female rats treated at 220, 440, and 880 mg/kg suggests that FVE may enhance the body's defence system.

CONCLUSION

FVE appears to be tolerated by rats, as no major clinical signs of toxicity on biochemical and haematological parameters, no mortality and no adverse effects on the liver, kidney, heart and lung were recorded at the doses used, suggesting that this extract is of low toxicity.

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Conflict of interest

The authors declared no conflict of interest.

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