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Phytochemical analysis, assessment of acute and subacute toxicological profile of the ethanolic extract of *Ficus elastica* Roxb. Ex Hornem (Moraceae) lianas in experimental Wistar rats

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Abstract
Ficus elastica Roxb. Ex Hornem, also called Rubber fig tree is a plant of the Moraceae family widely used in traditional medicine for the treatment of various ailments including allergies and skin infections, as well as a diuretic agent. This study points out the acute and sub-acute toxicological profile of the ethanolic extract of *Ficus elastica* lianas. The plant material was collected, dried, ground and soaked in ethanol. After filtering, the filtrated were dried and the dried material was stored in the shade prior to analysis. The phytochemical screening was done according to Harbone methods. Acute and sub-acute toxicity tests were performed according to an amended Organisation for Economic Co-operation and Development (OECD) Guidelines 423 and 407 respectively on Wistar rats. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, polyphenols and tannins. The single oral administration of the extract did not induce an abnormal variation of the physiological and behavioural parameters in rats, at the doses of 2000 and 5000 mg/kg body weight after 14 days of observation. Also, the extract showed a good safety profile after 28 days of repeated dosing of 200, 400, and 800 mg/kg body weight. No deaths were recorded and biochemical, hematological, and physiological parameters analyses revealed a non-significant statistical difference as compared to control. In addition, histological studies indicated that all tested doses induced no damage to liver, kidney, heart or lung whether in male or female animals. Therefore, the use of the ethanolic extract of *Ficus elastica* lianas as herbal remedy at tested doses is not associated with any toxic effects thus, justify its traditional use.

Keywords: *Ficus elastica*, lianas, phytochemical analysis, acute toxicity, subacute toxicity

1. Introduction
The use of plants is as old as mankind itself and has always been part of human culture. In Africa, plants are used in traditional medicine to treat various infectious and non-infectious diseases [1]. The use of herbal medicines for various ailments, ranging from minor to chronic, is strongly driven by limited access to modern health services, the rising cost of western medicines, as well as the side effects often associated with prolonged use of these drugs [2]. According to the World Health Organization (WHO), about 40 to 90% of people living in developing countries frequently use traditional medicine [3]. These medicinal plants can therefore constitute important resources for new substances with therapeutic potential and at low cost [4]. However, it should be emphasized that the traditional use of any plant for therapeutic purposes does not guarantee its safety [5]. While the pharmacological effects of many plants have been proven, their toxicity is often ignored. Therefore, evaluating the toxicity of herbal preparations is important to determine their safety [24]. In order to improve knowledge about medicinal plants, we chose to conduct our work on *Ficus elastica* Roxb. Ex Hornem (Moraceae). The selection of this plant was motivated on one hand by its use in traditional medicine for the management of allergies and on the other hand, a number of reports standing for their pharmacological efficacy. However, to the best of our knowledge, only one paper is reporting the toxicity effect of the hexanic fraction, a less-used preparation of this plant [7]. This paper therefore aimed at evaluating the innocuity of the ethanolic crude extract of *Ficus elastica* lianas.

2. Material and methods

2.1. Plant materials collection and extraction

Ficus elastica lianas were harvested in Bonaberi in the Douala 4th district, Littoral region of Cameroon with the help of Botanist Dr Tankeu Sévérin. The plant was authenticated at the National Herbarium of Cameroon by comparison with specimen number 65646 HNC previously deposited.

Ethanolic extract was performed according to the method described by Adesokan *et al*. [8]. The harvested lianas were washed several times with tap water to eliminate impurities. The plant material was then dried at room temperature out of direct sunlight to avoid decomposition of natural product contents. Thereafter, it was pulverized and yielded a mass of 2912.8 g of powder. The resulted powder was mixed with 15 L of ethanol 90% and the resulting mixture was stirred for 72 hours at room temperature (25 °C) with multiple-daily shaking. The mixture was filtered three times through cotton wool and on 3 mm Wattman filter paper. Finally the filtrate was evaporated at 60 °C using a rotary evaporator (Heidolph LABOROTA 4000). The obtained crude extract was weighed (44.4 g) and was used to carry out the various tests, being store at 4 °C.

Experimental Animals

Healthy female and male Wistar rats, nulliparous, non-pregnant, and aged from eight to ten weeks, which have not been subjected to previous experimental activities, were used. Their weights were determined prior to feeding. The rats were acclimatized for two weeks. The experimental animals were housed in standard plastic cages and provided access to standard animal food and water *ad libitum.*

2.2. Qualitative phytochemical analysis of plant extract

Detailed phytochemical screening was performed on the ethanolic extract of *Ficus elastica* lianas using standard methods, as reported in the literature [9, 10]. Other specific phytochemical tests were also realized, all based on a precipitation reaction via the generation of insoluble complexes called precipitates, and on colorimetry reaction through the formation of colored soluble chemical species. The colored reactions were carried out in test tubes in the presence of the positive controls. The following tests were carried out: Drangendorff test (alkaloids), Tannins test (Gallic tannins), Libermann Burchard test (steroids and triterpenoids), Shinoda test (flavonoids), Foam Index test (saponins), $FeCl_3$ test (polyphenols) and Reducing Sugars test. All observations were recorded.

2.3. Acute Toxicity

A total number of 12 female Wistar rats were randomly selected and divided into four (4) batches of three animals each. Batch 1, the neutral control group, treated with 1 ml/100 g of distilled water body weight. Batch 2, the negative control group received virgin olive oil used as solvent to dissolve the extract at 1 ml/100 g body weight. Batch 3 was treated with ethanolic extract of *Ficus elastica* lianas at unique dose of 2000 mg/kg of body weight and batch 4 was treated with ethanolic extract of *Ficus elastica* lianas at 5000 mg/kg of body weight. Acute toxicity experiment was conducted according to guideline 423 of the OECD protocol [11] at the Pharmacology and Toxicology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. Rats were fasted over the night prior the experiment from 8 p.m. to 8 a.m. Once treated, the animals were observed for 2 hours (H) after the administration of the extract. They were then fed and observed after 4, 8 H and then 14 days during which the symptoms of intoxication (stool appearance, noise sensitivity, groupement, locomotion, motility, coat modification, reaction to noise, grooming, trembling, as well as deaths) were noted. The dead rats in each batch were counted for the determination of the median Lethal Dose (LD_{50}). The extract was administered to animals orally.

2.4. Subacute Toxicity

A total number of 30 Wistar rats were randomly selected and divided into five batches of 6 animals each (three males and three females). Batch 1, the neutral control group was treated with 1 ml/100 g of distilled water body weight. Batch 2, the negative control group, received virgin olive oil at 100 ml/100 g of body weight. Batch 3, 4 and 5 were treated with extract at the doses of 200, 400, and 800 mg/kg of body weight respectively. Subacute toxicity was performed as per OECD Guideline 407 with slight modifications [12] at the Pharmacology and Toxicology Laboratory of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. The rats were fasted the night before the experiment from 8 p.m. to 8 a.m. All substances were daily administrated per os as a single dose for 28 days. On the 29th days, animals were anesthetized using ketamine and diazepam then sacrificed. Blood was collected into EDTA-containing tube and empty tube for hematological and biochemical analysis respectively. Some organs including liver, kidneys, heart, lung, and spleen were rapidly identified, dissected out, rinsed with normal saline solution, weighed, and fixed in a freshly prepared 10% formalin buffer for histological analysis.

The blood collected into EDTA-containing tube was immediately subjected to full haematological parameters determination including red blood cell count (RBC), haemoglobin (Hb), platelet count (PLT), total and differential white blood cell (WBC) count, neutrophil, % neutrophil, mean cell haemoglobin concentration (MCHC), mean red cell volume (MCV), mean cell haemoglobin (MCH), and packedell volume (PCV) using commercial test kits and "Mindray BC-2800" hematology counter. This machine operates by drawing blood from an EDTA tube and performs red blood cell and platelet counts, as well as hemoglobin levels, hematocrit and erythrocyte constants. Differential lysis of the red blood cells is then performed and the different leukocyte populations are counted using an enzymatic activity specific to them. The different parameters measured and calculated are transcribed on a graph and printed [13].

For biochemical parameters determination namely urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), triglyceride (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C), the blood collected into empty tube was centrifuged at 1500 rpm for 10 minutes and the supernatant (serum) was used, being stored at -4 °C. These assays were carried out using standard protocol provided with commercial kits.

The histological investigation was carried out following the method described by Biswas *et al*. with slight modifications [25]. The procedure comprises multiple stages including fixing in formalin, trimming and dehydration in alcohol, inclusion in paraffin, cutting using a microtome, staining with haematoxylin-eosin, assembly and observation under light microscope. The experiment was carried out at the Animal Physiology Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1.

2.5. Statistical Analysis
Results are expressed as a mean ± Standard Error on the Mean (SEM). The statistical analysis was performed using PRISMA software (GraphPadInstat software, Inc, San Diego, CA, version 8.0.1.). The difference between treated groups and control groups was compared using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc-test. p values less than 0.05 were considered significant.

3. Results
3.1. Extraction efficiency
The extraction yield of *Ficus elastica* lianas using ethanol as solvent was found to be 1.52% (w/v) after maceration. The crude extract was deep green in colour with good flavour and odour, indicting its valid contribution for pharmaceutical product.

3.2. Phytochemical screening results
Table 1 summarize the secondary metabolites content of *Ficus elastica* lianas ethanolic extract. The qualitative phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, and triterpenoids amongst other. Steroids and reducing sugar were not detected.

Table 1: Phytochemical constituents of the ethanolic extract of *Ficus elastica* lianas

Performed tests	Phytochemicals	Results
Dragendorff	Alkaloids	+
Foam index	Saponin	+
Shinoda	Flavonoids	+
Libermann-burchard	Triterpenoids	+
Libermann-burchard	Steroids	-
Ferric Chloride ($FeCl_3$)	Polyphenols	+
Stiasny	Tannins	+
Fehling	Reducing sugars	-

(+) = present; (-) = absent

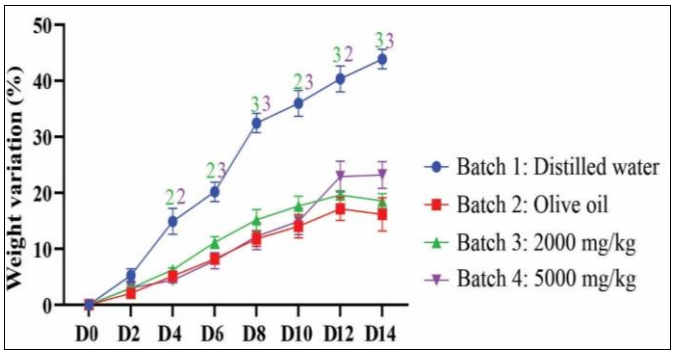
3.3. Acute toxicity results
3.3.1. Effect of the ethanolic extract of *Ficus elastica* lianas on physiological and behavioural parameters
The oral administration of a single dose of the ethanolic extract of *Ficus elastica* lianas induced no abnormal variation of physiological behavioural parameters throughout the experimental period as compared to control (Table II). Also, no death was recorded and the LD_{50} was estimated greater than 5000 mg/kg.

Table 2: Physiological and behavioural parameters observed after single administration of the ethanolic extract of *Ficus elastica* lianas

Observed Parameters	Batch 1	Batch 2	Batch 3	Batch 4
Number of rats	3	3	3	3
Number of death	0	0	0	0
Coat modification	A	A	A	A
Impaired gait	A	A	A	A
posture and reaction to manipulation	N	N	N	N
Excessivity	A	A	A	A
Trembling	A	A	A	A
Convulsions	A	A	A	A
Stool appearance	N	P	P	P
Reaction to sound	N	N	N	N
Intense thirst	A	A	A	A
Vomiting	A	A	A	A
Salivation	A	A	A	A

A = Absent; N = Normal; P = Pasty; Batch 1: batch that received distilled water; Batch 2: batch that received the virgin olive oil; Batch 3 and Batch 4: groups receiving the extract at the doses of 2000 and 5000 mg/kg, respectively.

3.3.2. Effect of the ethanolic extract of *Ficus elastica* lianas on rat's body weight
Figure 1 show the effect of a single dose of ethanolic extract of *Ficus elastica* lianas on rat body weight recorded during fourteen days. No significant change was recorded in treated animals as compared to negative control group. However, the treatment induced a significant ($p< 0.01$ and $p< 0.001$) decrease of body weight gained when compared with neutral control. No further change was noted between the batches.



Each point represents the mean ± Standard Error on the Mean (SEM); n = 3;[2] $p < 0.01$, [3]$p < 0.001$: significant differences compared to Batch 1. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3 and Batch 4: groups receiving the extract at the doses of 2000 and 5000 mg/kg, respectively.

Fig 1: Rat body weight evolution in acute toxicity

3.4. Subacute toxicity results
3.4.1. Effect of repeated administration of the ethanolic extract of *Ficus elastica* lianas on physiological and behavioural parameters
Table III shows the effect of the ethanolic extract of *Ficus elastica* lianas on animal's physiological and behavioural parameters following 28 days oral post-treatment. Prolonged administration of the extract induced no change in treated (males and females) as compared with control batches. Furthermore, no death was recorded throughout the experimental period.

Table 3: Behavioral and physiological patterns of rats following 28 days repeated administration of *Ficus elastica* lianas ethanolic extract

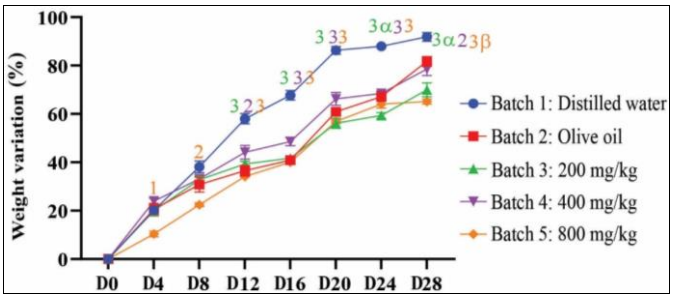
Observed Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Number of rats	6	6	6	6	6
Number of death	0	0	0	0	0
Coat modification	A	A	A	A	A
Impaired gait	A	A	A	A	A
Posture and reaction to manipulation	N	N	N	N	N
Excessivity	A	A	A	A	A
Trembling	A	A	A	A	A
Convulsions	A	A	A	A	A
Stool appearance	N	P	P	P	P
Reaction to sound	N	N	N	N	N
Intense thirst	A	A	A	A	A
Vomiting	A	A	A	A	A
Salivation	A	A	A	A	A

A = Absent; N = Normal; P = Pasty; Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 1: batch that received distilled water; Batch 2: batch that received

virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

3.4.2. Effect of repeated administration of the ethanolic extract of *Ficus elastica* lianas on body weight in female rats

Although all animals exhibited increasing body weight throughout the experimental period. The repeated dosing of the ethanolic extract of *Ficus elastica* lianas did not induced any significant change in animal's body weight as compared with negative control, except from the 24th day in Batch 3 ($p <$ 0.05, $p<$ 0.01) and on day 28 for batch 5 ($p<$ 0.01). Furthermore, the body weight gain of neutral control batch was significantly ($p<$ 0.01, $p<$ 0.001) higher than all tested doses both in female (Figure 2).

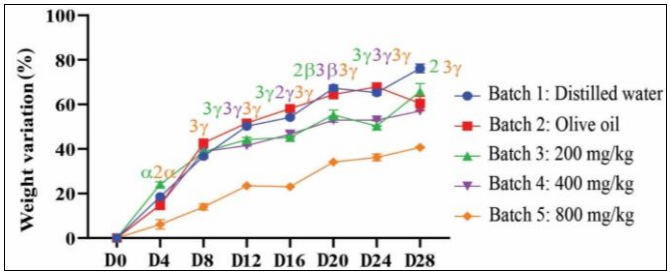


Legend Each point represents the mean ± Standard Error on the Mean (SEM); n = 3; $^{1}p < 0,05$, $^{2}p < 0,01$, $^{3}p < 0,001$: significant differences compared to Batch 1;$^{\alpha}p < 0,05$, $^{\beta}p < 0,01$: significant differences compared to Batch 2; Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 2: Body weight spectral evolution of female rats during subacute toxicity test

3.4.3. Effect of repeated administration of the ethanolic extract of *Ficus elastica* lianas on body weight in male rats

It was noted that all animals exhibited increasing body weight throughout the experimental period. The body weight gain of neutral control and negative control batches were significantly ($p<$ 0.05, $p<$ 0.01, $p<$ 0.01) higher than all tested doses in male, except from the 4th day in Batch 3 ($p<$ 0.05) (Figure 3).

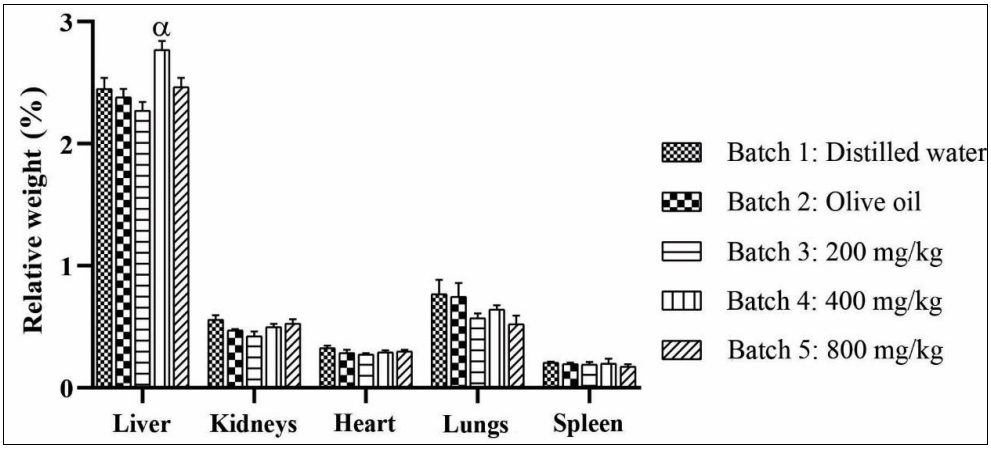


Each point represents the mean ± Standard Error on the Mean (SEM); n = 3; $^{1}p < 0,05$, $^{2}p < 0,01$, $^{3}p < 0,001$: significant differences compared to Batch 1;$^{\alpha}p < 0,05$, $^{\beta}p < 0,01$, $^{\gamma}p< 0,001$:significant differences compared to Batch 2; Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 3: Body weight spectral evolution of male rats during subacute toxicity test

3.4.4. Effects of subacute administration of the ethanolic extract of *Ficus elastica* lianas on absolute relative organs weight in male rats

As depicted in Figure 4, the oral administration of the ethanolic extract of *Ficus elastica* lianas resulted in no significant difference on the variation on absolute relative organ weight in male rats at all tested doses as compared with negative control.However, the liver relative weight of animal treated with significantly increased ($p<$ 0.05) as compared with neutral control.

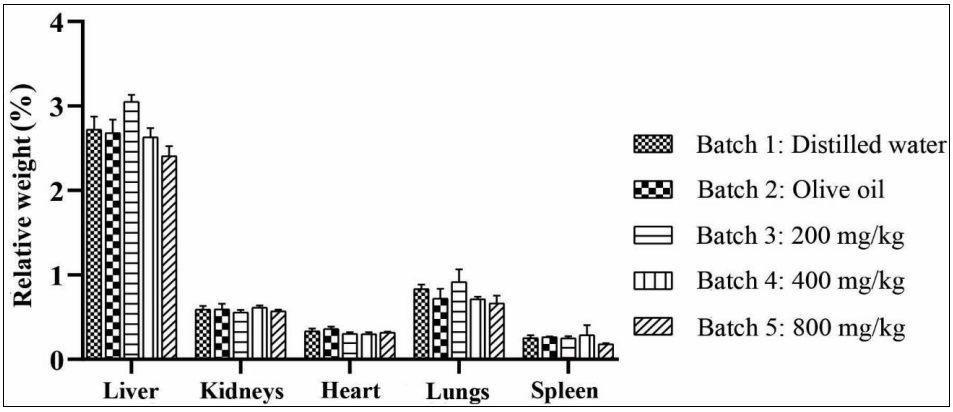


$^{\alpha}p < 0.05$ significant differences compared to batch 1. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 4: Organs relative weight of male rats during subacute toxicity test

3.4.5. Effects of subacute administration of the ethanolic extract of *Ficus elastica* lianas on absolute relative organs weight in female rats

The results show no significant difference in batches 3, 4 and 5 in comparison with the reference batches (Batch 1 and 2) for the various organs.

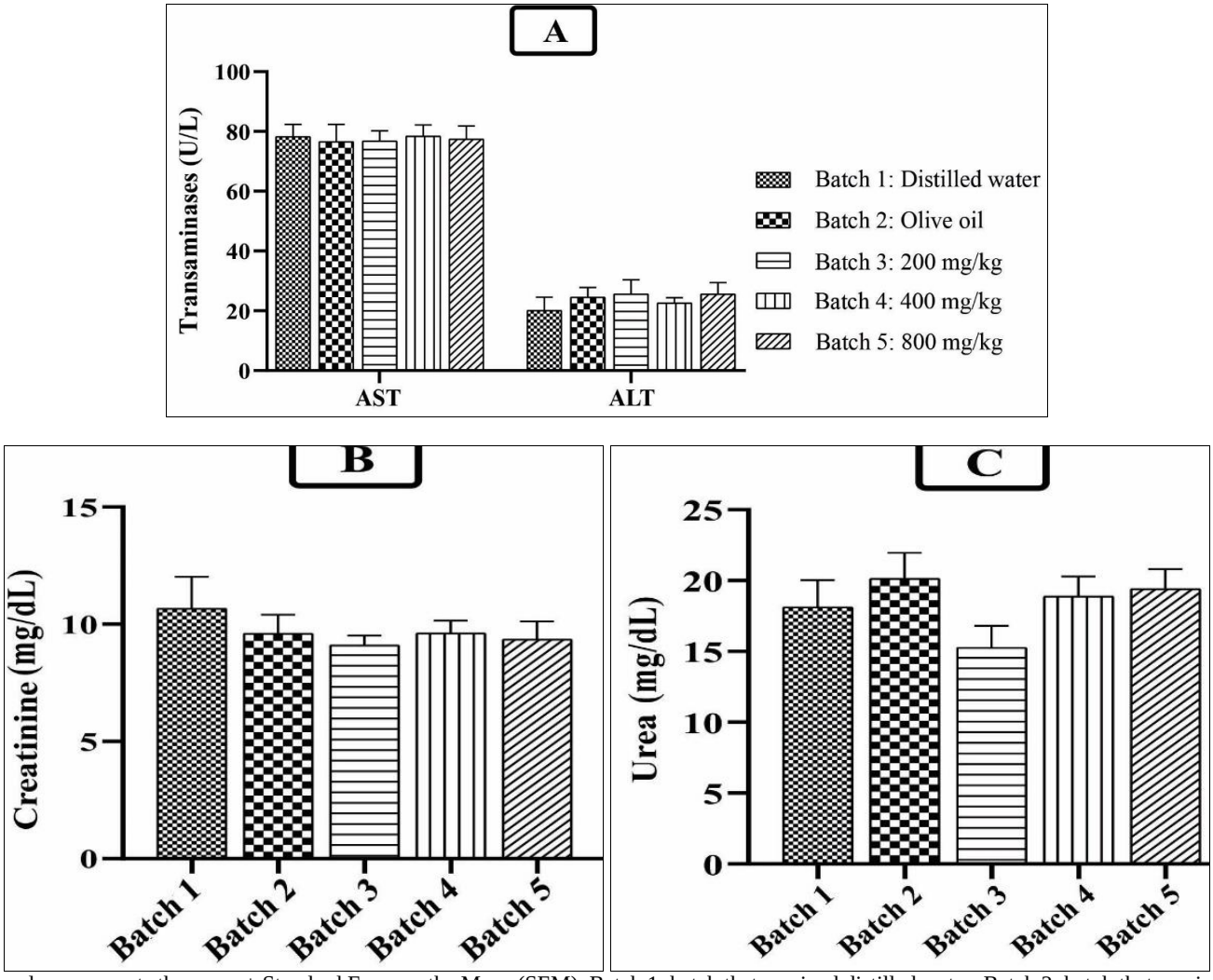


Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 5: Organs relative weight of female rats during subacute toxicity test

3.4.6. Effects of the ethanolic extract of *F. elastica* lianas on some parameters of hepatic and renal function in male rats

The effects of the ethanolic extract of *F. elastica* lianas on the activity of transaminases (AST, ALT), on the levels of creatinine and urea in male rats are summarized in the Fig. 6. The results show that after 28 days of treatment no significant difference was observed either in the control batches or in the test batches (Figure 6A, B and C).

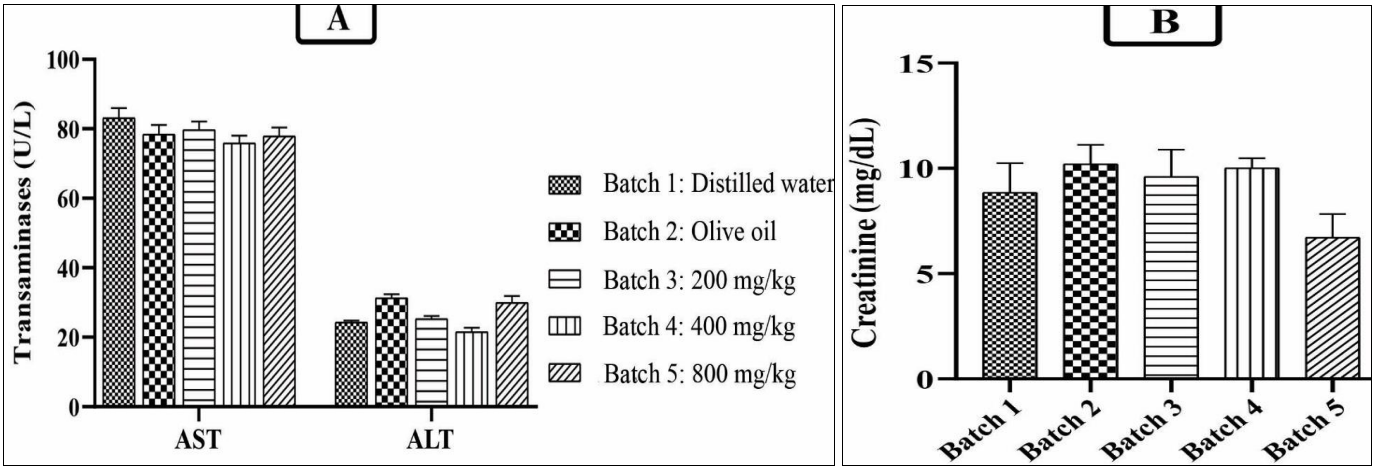


Each value represents the mean ± Standard Error on the Mean (SEM). Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 6: Effects of the ethanolic extract of *F. elastica* lianas on some parameters of hepatic and renal function in male rats

3.4.7. Effects of the ethanolic extract of *F. elastica* lianas on some parameters of hepatic and renal function in female rats

As in the males, the results show that after 28 days of treatment no significant difference was observed either in the control batches or in the test batches (Figure 7A, B and C).



Each value represents the mean ± Standard Error on the Mean (SEM). Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Fig 7: Effects of the ethanolic extract of *F. elastica* lianas on some parameters of hepatic and renal function in female rats

3.4.8. Effects of the ethanolic extract of *F. elastica* lianas on the lipid profile in male rats

The table below presents the effects of the ethanolic extract of *F. elastica* lianas on the lipid profile in male rats. Compared to Batch 1, the results show a significant increase ($p< 0.05$) in the total cholesterol level in the Batch 5. Compared to the Batch 2, a significant decrease ($p< 0.01$) in the cholesterol level was observed total in Batch 2 and Batch 3. A significant increase in HDL-cholesterol was noted in Batch 2 ($p< 0.001$) and Batch 5 ($p< 0.05$) compared to Batch 1; Compared to the Batch 2, a significant decrease is noted in the Batch 3 ($p< 0.001$) and Batch 5 ($p< 0.05$). Regarding the level of triglycerides, the results show a significant decrease in Batch 4 ($p< 0.001$, $p< 0.001$) and an increase in Batch 5 ($p< 0.01$; $p< 0.001$) compared to Batch 1 and at Batch 2 respectively. The LDL-cholesterol level was low in Batch 4 ($p< 0.05$) and high in Batch 5 ($p< 0.01$) compared to Batch 1 and high in Batch 5 ($p< 0.001$) compared to Batch 2.

Table 5: Effects of the ethanolic extract of *F. elastica* lianas on the lipid profile in male rats

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
TC (mg/kg)	59.52 ±1.87	71.42±1.60	56.63±0.62$^{\beta}$	55.69±2.72 $^{\beta}$	70.05±2.58^{1}
HDL-C (mg/kg)	35.44±1.90	51.64±0.47	38.56±1.65$^{\gamma}$	49.95±0.60^{3}	43.77±1.88$^{1\alpha}$
TG (mg/kg)	30.88±0.50	26.76±1.87	31.20±0.47	13.05±1.03$^{3\gamma}$	39.79±1.71$^{2\gamma}$
LDL-C	12.27±0.60	9.47±1.94	10.65±0.45	5.99±0.25^{1}	20.79±1.24$^{2\gamma}$

TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Each value represents the mean ± Standard Error on the Mean (SEM); n=3;$^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$: significant differences compared to Batch 1, $^{\alpha}p< 0.05$, $^{\beta}p< 0.01$, $^{\gamma}p< 0.001$: significant differences compared to Batch 2;

3.4.9. Effects of the ethanolic extract of *F. elastica* lianas on the lipid profile in female rats

The results show a significant decrease in the total cholesterol level in Batch 3 ($p < 0.001$), Batch 4 ($p< 0.01$) and Batch 5 ($p< 0.01$) compared to Batch 1. Compared to Batch 2, a significant decrease ($p< 0.01$) in the total cholesterol level was observed in the Batch 3 ($p< 0.001$). A decrease ($p< 0.001$) in the HDL-cholesterol level was noted in the different test batches compared to the Batch 1; Compared to Batch 2, there is a significant decrease in Batch 3 ($p< 0.001$) and a significant increase in Batch 4 ($p< 0.01$) and Batch 5 ($p< 0.001$). Regarding the triglyceride level, the results show an increase in the Batch 3 ($p< 0.001$) compared to the Batch 1; Compared to Batch 2, an increase in triglycerides was observed in Batch 3 ($p< 0.001$) and Batch 5 ($p< 0.05$). LDL-Cholesterol level was increased in Batch 2 ($p< 0.001$) and high in Batch 5 ($p< 0.001$) compared to Batch 1 and low in Batch 3 ($p< 0.001$), Batch 4 ($p< 0.001$) and Batch 5 ($p< 0.001$) compared to Batch 2.

Table 6: Effects of the ethanolic extract of *F. elastica* lianas on the lipid profile in female rats

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
TC (mg/kg)	74.25±2.06	63.37±1.50	45.87±2.28$^{3\gamma}$	60.12±2.57^{2}	59.17±1.58^{2}
HDL-C (mg/kg)	58.82±0.56	33.31±0.30	25.32±0.36$^{3\gamma}$	39.67±1.76$^{3\beta}$	41.77±0.85$^{3\gamma}$
TG (mg/kg)	26.17±2.05	22.19±1.24	38.81±1.23$^{2\gamma}$	29.60±2.45	31.56±1.44$^{\alpha}$
LDL-C	11.25±1.44	36.11±0.82	10.72±0.63$^{\gamma}$	15.53±1.06$^{\gamma}$	24.88±1.18$^{3\gamma}$

TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: batches that received the extract at the doses of 200, 400 and 800 mg/kg respectively

Each value represents the mean ± Standard Error on the Mean (SEM); n=3; $^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$: Each value represents the mean ± Standard Error on the Mean (SEM); n=3;$^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$: significant differences

compared to Batch 1, $^{\alpha}p< 0.05$, $^{\beta}p< 0.01$, $^{\gamma}p< 0.001$ significant differences compared to Batch 2

3.4.10. Effect of repeated doses of ethanolic extract of *F. elastica* lianas on some hematological parameters in male rats

In comparison to Batch 1 and Batch 2, no significant difference was noted in the levels of RBCs, Hb, WBC, LYM, GRA, MCHD and MCHC in the different batches treated with the ethanolic extract of *Ficus elastica* lianas. With regard to hematocrit, a significant increase in its level was observed in Batch 2 ($p< 0.05$), Batch 3 ($p< 0.001$) and Batch 4 ($p< 0.001$) compared to Batch 1, an increase significant in the Batch 4 ($p< 0.05$) compared to the Batch 2. MGV decreased in Batch 3 ($p< 0.001$), Batch 4 ($p< 0.05$) and Batch 5 ($p< 0.01$) compared to Batch 1, also compared to Batch 2 in Batch 3 ($p< 0.01$) and Batch 5 ($p< 0.05$). The number of blood platelets increased ($p< 0.05$) in Batch 2 and in Batch 5 compared to Batch 1; compared to Batch 1 and Batch 2, the number of PLTs decreased in the Batch 3 ($p< 0.01$; $p< 0.001$) and increased in the Batch 4 ($p< 0.001$; $p< 0.01$) respectively.

Table 7: Mean ± ESM of hematological parameters of different of male rats for the different samples compared to the control batch

	TED	THO	FE 200	FE 400	FE 800
RBCs (10^6/µl)	3.71±0.29	4.72±0.23	5.19±0.12	4.15±0.06	4.48±0.83
Hb (g/dl)	12.67±0.88	10.93±0.75	11.17±0.43	11.07±0.05	11.67±0.25
HCT (%)	44.57±0.72	50.17±0.44^{1}	54.20±1.33^{3}	56.33±1.76$^{3\alpha}$	49.50±0.87
WBC (10^3/µl)	5.84±1.31	8.15±1.51	6.67±0.67	5.91±0.95	5.63±1.18
MGV (µm^3)	89.67±2.73	83.00±3.46	58.00±2.65$^{3\beta}$	73.67±3.53^{1}	67.00±4.04$^{2\alpha}$
LYM (10^3/µl)	1.23±0.62	1.25±0.52	1.67±0.67	1.56±0.31	1.53±0.43
GRA (10^3/µl)	2.67±0.18	2.12±0.49	2.30±0.12	2.57±0.26	2.65±0.32
MCHD (g/dl)	33.33±1.20	34.67±1.45	31.04±0.44	32.00±1.99	32.60±0.95
MCHC (g/dl)	30.33±1.20	26.01±1.30	32.67±0.33	27.00±1.53	29.00±2.31
PLTs (10^3/µl)	142.30±0.67	146.30±1.20^{1}	137.50±0.29$^{2\gamma}$	152.00±0.58$^{3\beta}$	146.50±0.29^{1}

RBCs = Red blood cells (106/mm^3); WBC = White blood cells (mm^3); Hb = Hemoglobin (g/l); HCT = Hematocrit (%); MGV = mean globular volume in ftl; MCHD = mean corpuscular hemoglobin content in Pg; MCHC = mean corpuscular hemoglobin concentration in (g/dl); PLTs = Platelets (103 mm^3); LYM = lymphocyte (%); pg = pictogram; ftl = fentoliter. THE: batch that received distilled water; THO: batch that received virgin olive oil; FE 200, 400 and 800: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Each value represents the mean ± Standard Error on the Mean (SEM); n=3;$^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$: significant differences compared to Batch 1, $^{\alpha}p < 0.05$, $^{\beta}p< 0.01$, $^{\gamma}p< 0.001$ significant differences compared to Batch 2

3.4.11. Effect of repeated doses of ethanolic extract of *F. elastica* lianas on some hematological parameters in female rats

In comparison to Batch 1 and Batch 2, no significant difference was noted in the levels of WBC, LYM, GRA and MCHC in the different batches treated with the extract. The number of RBCs was increased in Batch3 ($p< 0.01$) compared to Batch1 and low ($p< 0.05$) in Batch4and Batch 5; the Hb level was high in Batch5 ($p< 0.05$; $p< 0.05$) compared to Batch 1 and Batch 2 respectively; HCT concentration was high in Batch4 ($p< 0.05$; $p< 0.05$) compared to Batch1 and Batch2 respectively; the number of blood platelets was low in Batch3 ($p< 0.001$; $p< 0.001$), Batch4 ($p< 0.001$; $p< 0.01$) and Batch5 ($p < 0.001$; $p < 0.001$) compared to Batch1 and Batch2 respectively; the MCHC was high in the Batch3 ($p< 0.05$) compared to the Batch 2; the MGV was high in the Batch 4 ($p< 0.05$) compared to the Batch 1.

Table 8: Mean ± ESM of hematological parameters of different of female rats for the different samples compared to the control batch.

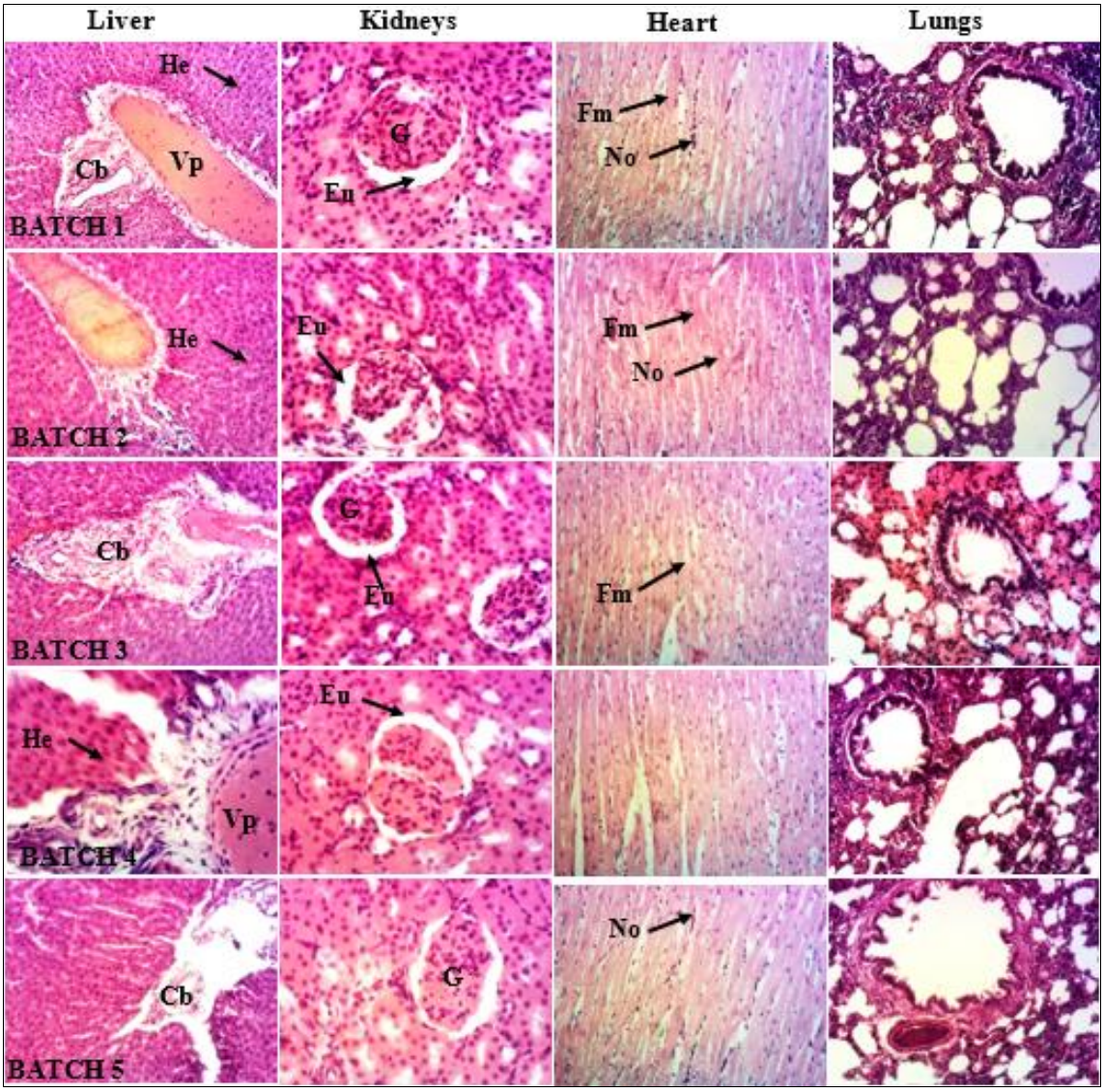
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
RBCs (10^6/µl)	3.73±0.37	5.55±0.32^{2}	4.53±0.26	4.17±0.12$^{\alpha}$	4.21±0.30$^{\alpha}$
Hb (g/dl)	10.16±0.22	10.36±0.27	11.39±0.86	11.73±0.35	13.05±0.55$^{1\alpha}$
HCT (%)	40.50±0.29	39.87±1.36	46.10±1.10	58.67±6.39$^{1\alpha}$	44.67±3.76
WBC (10^3/µl)	4.90±0.86	5.97±0.52	6.17±1.23	5.27±1.04	7.16±1.52
MGV (µm^3)	80.00±1.53	84.00±3.64	80.00±1.00	90.00±0.58^{1}	87.67±0.88
LYM (10^3/µl)	1.00±0.46	0.71±0.60	1.67±0.70	1.56±0.13	1.53±0.62
GRA (10^3/µl)	2.70±0.15	1.91±0.15	2.00±0.58	3.75±0.75	2.28±0.13
MCHD (g/dl)	30.31±1.97	26.86±1.36	37.33±2.19$^{\alpha}$	34.00±2.52	33.33±1.20
MCHC (g/dl)	29.67±0.67	25.50±1.61	29.17±0.44	28.83±1.09	27.00±1.00
PLTs (10^3/µl)	185.00±4.51	182.00±3.22	147.30±4.18$^{3\gamma}$	153.00±4.16$^{3\beta}$	147.00±0.58$^{3\gamma}$

RBCs = Red blood cells (106/mm^3); WBC = White blood cells/ (mm^3); Hb = Hemoglobin (g/l); HCT = Hematocrit (%); MCV = mean globular volume in ftl; MCHD = mean corpuscular hemoglobin content in Pg; MCHC = mean corpuscular hemoglobin concentration in (g/dl); PLTs = Platelets (103 mm^3); LYM = lymphocyte (%); pg = pictogram; ftl = fentoliter. FE 200, 400 and 800: tested batches receiving the extract at the doses of 200, 400 and 800 mg/kg respectively

Each value represents the mean ± Standard Error on the Mean (SEM); n=3;$^{1}p< 0.05$, $^{2}p< 0.01$, $^{3}p< 0.001$: significant differences compared to Batch 1, $^{\alpha}p< 0.05$, $^{\beta}p< 0.01$, $^{\gamma}p< 0.001$ significant differences compared to Batch 2

3.4.11. Effect of repeated doses of ethanolic extract of *F. elastica* lianas on histological section of livers, kidneys, hearts and lungs of male rats

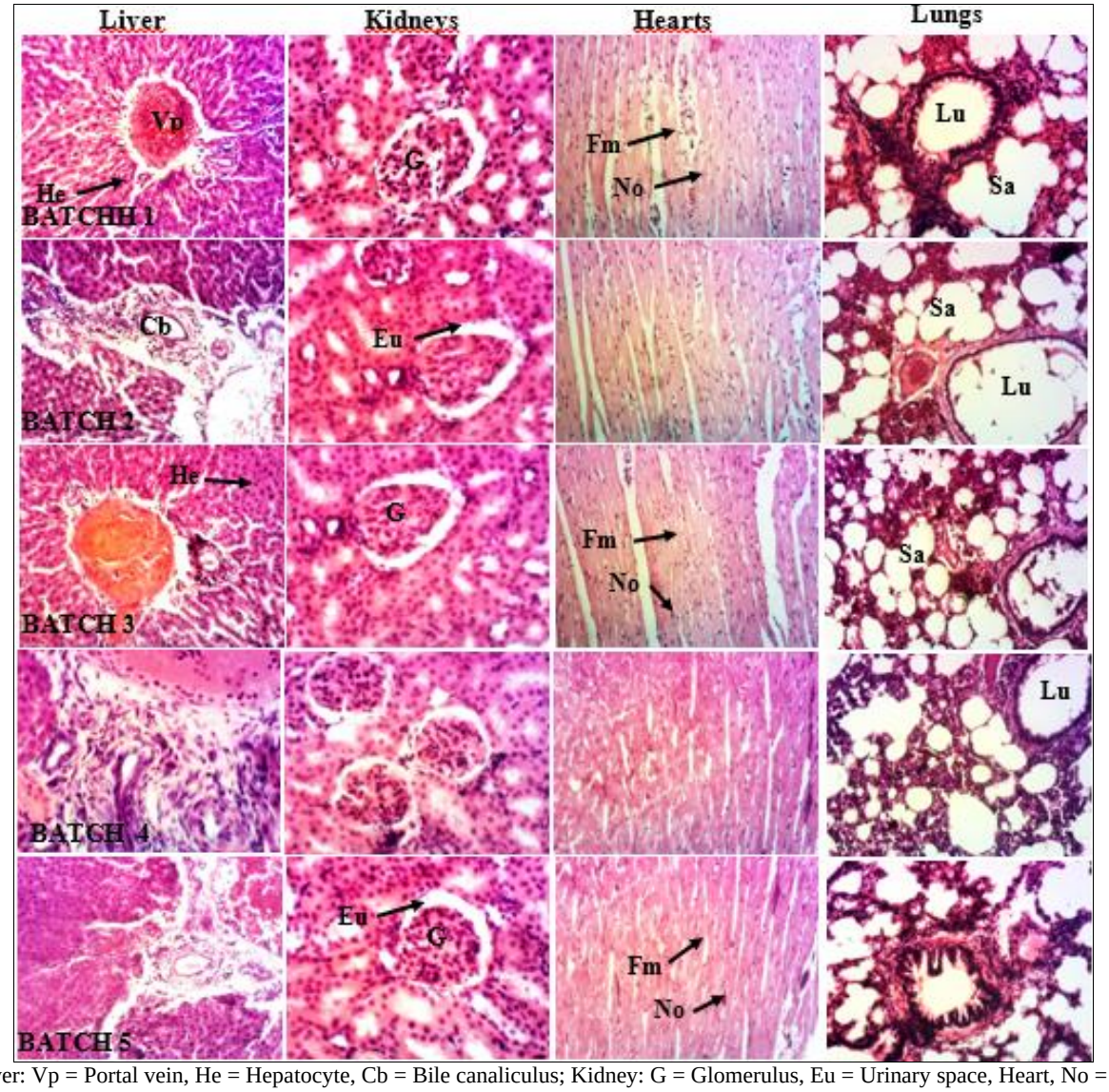
The figure bellow shows the effects of ethanolic extract of *F. elastica* lianas on the structure of the liver, kidney, heart and lung of the male rats of the control groups and those treated with the extract at the respective doses of 200, 400 and 800 mg/kg. The sections show a normal architecture of the liver (hepatic parenchyma with a normal portal vein and distinct hepatocytes), of the kidney (normal parenchyma with a distinct glomerulus and urinary space, of the heart (muscle fibers and distinct nuclei), lung (Presence of a pulmonary epithelium, a lumen and distinct alveolar sacs). No signs of alterations at the tissue level were observed.



Legend: Liver: Vp = Portal vein, He = Hepatocyte, Cb = Bile canaliculus; Kidney: G = Glomerulus, Eu = Urinary space, Heart, No = Nucleus, Fm = Muscle fibre; Lung: Lu = Light, Sa = Alveolar sac. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: batches that received the extract of *Ficus elastica* lianas mixed with virgin olive oil at the doses of 200, 400 and 800 mg/kg respectively.

Fig 8: Effects of ethanolic extract of *F. elastica* on the structure of liver, kidney, heart and lung (X200, H-E) in male rats

3.4.12. Effect of repeated doses of ethanolic extract of *F. elastica* lianas on histological section of livers, kidneys, hearts and lungs of female rats: Similar to males, normal structure of liver, kidneys, heart and lungs was observed. No signs of tissue toxicity were observed.



Legend: Liver: Vp = Portal vein, He = Hepatocyte, Cb = Bile canaliculus; Kidney: G = Glomerulus, Eu = Urinary space, Heart, No = Nucleus, Fm = Muscle fibre; Lung: Lu = Light, Sa = Alveolar sac. Batch 1: batch that received distilled water; Batch 2: batch that received virgin olive oil; Batch 3, Batch 4 and Batch 5: batches that received the extract of *Ficus elastica* lianas mixed with virgin olive oil at the doses of 200, 400 and 800 mg/kg respectively.

Fig 9: Effects of ethanolic extract of *F. elastica* on the structure of liver, kidney, heart and lung (X200, H-E) in female rats

4. Discussion

The widely use of *Ficus elastica* in traditional medicine for the quest of well-being aroused attention for its potential risk for humans and animals health. In this study, the ethanolic extract was prepared and investigated for its toxicological profile following a qualitative phytochemical screening. Different classes of secondary metabolites including alkaloids, flavonoids, saponins, triterpenes, polyphenols and tannins were found in the extract. These compounds are known to exhibit various biological and pharmacological activities, justifying its utilization in pharmacopeia. Similar observations were previously reported while using as solvent for extraction. However, flavonoids were not detected in the methanolic extract [7]. This difference may be related to the place of harvest (Yaoundé vs Bonabéri; approximately 250 km) or to the solvent used for extraction (methanol vs ethanol).These results corroborates previous work on *Ficus elastica* as flavonoids, triterpenes, quinones, and saponins have previously been isolated from leaves [15], aerial roots bark [16], and aerial roots wood [17].

To study the acute toxicity effect of the ethanolic extract of *Ficus elastica* lianas, male and female Wistar rats were orally given 2000 mg/kg and 5000 mg/kg b.w at a single dose and their behaviour was monitor during 14 days. Results showed that the extract induced no death in both male and female throughout the experiment. Therefore the lethal dose 50 (LD_{50}) of the tested extract was estimated greater than 5000 mg/kg, assuming that the substance is practically non-toxic. Further, no sign of toxicity including behavioural (irritability, fearfulness), neurologic (convulsion, gait), autonomous (defecation), physiological (vomiting, trembling) was observed. Additionally, no change in organs relative weight was recorded suggesting that the ethanolic extract of *F. elastica* is less toxic or non-toxic to these organs. Such has been observed with the hexane fraction of *F. elastica* lianas administered to female rats [7]. This high safety margin through oral route application supports its therapeutic use in traditional medicine.

During the study of subacute toxicity, the ethanolic extract of *F. elastica* lianas administered orally at repeated doses of 200, 400 and 800 mg/kg b.w. did not cause any deaths. The extract would have a toxicity index equivalent to 5, according to the Hodge and Sterner toxicity scale of a chemical substance depending on the LD_{50} by the route of administration [18]. However, no signs of toxicity were observed during the first

hours following the administration of the extract. The mean body weight of rats from all batches increased over the observation period with a greater gain in male rats. These results are consistent with those of Etame *et al*. in 2017 and Tankeu *et al*. in 2020 who in their work found that weight gain in both males and females generally increased regardless of the chosen batch and that weight gain was greater in male rats compared to female rats [19-20].

The ethanolic extract of *F. elastica* lianas on the relative weight of some organs in rats show no significant difference in batches 3, 4 and 5 in comparison with the reference batches (Batch 1 and 2) in general in both males and females, except in the relative weight of the liver which significantly increase ($p < 0.05$) in Batch 4 compared to Batch 2. This could be due to fat accumulation in the liver caused by high oil consumption [21].

Regarding the dosage of transaminases, it was observed that with compare to batch 1 and batch 2, there was no significant difference after 28 days of treatment in batch 3, batch 4 and batch 5 whether in males or in females. Similarly for the haematological analyses, there were no significant differences between the different batches. These results are consistent with those of Timothy *et al*. in 2017 on the subacute toxicity of *Ficus sycomorus* leaves where the administration of the ethanolic extract did not significantly affect hematological, renal and hepatic functions [22]. Regarding the lipid profile, the different variations observed could be explained by the fact that cholesterol and triglyceride levels can be influenced by hereditary factors, such as genetic mutations that affect the way the body metabolizes fats [23].

Histological analysis of some major organs involved in detoxification mechanisms revealed no damage to the livers, kidneys, hearts and lungs of the rats. Whether in males or females, a normal structure was observed in the liver (hepatic parenchyma with a normal portal vein and distinct hepatocytes), kidney (normal parenchyma with a distinct glomerulus and urinary space, of the heart (muscle fibers and distinct nuclei) and of the lung (presence of a pulmonary epithelium, a light and distinct alveolar sacs). All these results reinforce the hypothesis according to which the ethanolic extract of *F. elastica* lianas administered to the repeated doses for 28 days showed no signs of toxicity, which is in line with the results of Tankeu *et al* in 2020 on the subacute toxicity study of the combination of the aqueous extracts of stem bark of *Myosotis scorpioides R. Br.* (Cecropiaceae) and Fruits of *Picralima nitida* (Stapf) T. Durand and H. Durand (apocynaceae) [20].

5. Conclusion

Phytochemical screening of the ethanolic extract of *Ficus elastica* lianas revealed the presence of alkaloids, flavonoids, saponins, polyphenols, triterpenes, tannins and the absence of sterols and reducing sugars. Extract did not cause the death of any rat at the limit doses of 2000 mg/kg and 5000 mg/kg of body weight. Repeated dose administration for 28 days of the extract did not disturb the biochemical and haematological metabolism of rats and instead contributed to non-significant weight gain in rats at all doses regardless of gender.

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7. Author's contributions

Jean Emmanuel Mbosso Teinkela oversaw the entire project and critically reviewed the manuscript. Joseph Crépin Kouopmtchop Sado, Alain Njoya Mbouombouo and Judith Caroline Ngo Nyobe performed the experiments and analyzed the data. Philippe Belle Ebanda Kedi and Jean Baptiste Hzounda Fokou revised the manuscript. All authors have read and approved the manuscript.

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9. Conflicts of interest

There is no conflict of interest among the authors.

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