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Research Article

Evaluation of acute, subacute toxicity and in vivo impact of aqueous decoction of *Flemingia faginea* Guill. & Perr. (Barker) leafy stems on NMRI mice and normotensive Wistar rats

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Abstract

Introduction: *Flemingia faginea*, a Fabaceae family medicinal plant, has been used for a long time in Burkina Faso for the treatment of hypertension and excess salt. However, the safety of the preparations derived from this plant has not yet been scientifically documented. This study aimed to evaluate the acute and subacute oral toxicity of the leafy stems aqueous decoction of *F. faginea* (FAD) in healthy normotensive mice and rats and the impact on their normal blood pressure. **Material and Methods**: The acute oral toxicity study was conducted according to the toxicity class method of the Economic Cooperation and Development Organization (OECD) guideline 423. Subacute toxicity was carried out according to the OECD Guideline 407 for repeated dose chemical toxicity for 28 days. Hematological and biochemical analyzes of blood were performed after autopsy. An evaluation of the impact of the extract on the blood pressure of rats was performed using the non-invasive method. **Results**: A single oral dose of 2000 mg/kg bw to mice did not cause mortality or clinical signs or symptoms of toxicity during the 14-day study. The FAD was classified in the fifth category of the Harmonized System of Classification of the United Nations and considered practically safe with an estimated 50% lethal dose of 5000 mg/kg bw. Daily gavage of male and female rats with doses of 100, 500 and 1000 mg/kg did not result in mortality or significant adverse effects during the 28 days of experimentation. There were no significant differences in body weight gain, food and water consumption or relative vital organ weights in treated animals. Analysis of the hematological and biochemical parameters of blood serum did not show significant differences between treated and control animals in this study. Additionally, no aberrant changes were found in the systolic and diastolic blood pressures of the test animals during the 28 days of inclusion compared to those of the control group. **Conclusion**: The extract FAD could be considered safe within the doses tested for the results of the toxicological evaluation. However, microscopic, histopathological, and subchronic investigations will have to be carried out to confirm the safety of this extract use.

Keywords: *Flemingia faginea* - Lethal Dose 50 - Acute toxicity - Subacute toxicity - Hypertension

INTRODUCTION

According to the World Health Organization (WHO), traditional medicines of proven quality, safety and efficacy contribute to the goal of ensuring access to health care for all [1]. In fact, medicinal plants are often the main and sometimes the only source of care for millions of people[1]. Therefore, the WHO has strongly encouraged and supported a revival of confidence in herbal medicine through the creation of a WHO Global Centre for Traditional Medicine [1,2], through which it intends to use new technologies to exploit the therapeutic potential of medicinal plants. Furthermore, competent institutions have been established for the scientific validation of traditional medicine deliverables in most WHO member countries. Modern treatment techniques, although they have contributed considerably to improving the quality of human and animal life, have shown their limitations in effectively curbing the resurgence of hypertension (HTA) from which more than one billion people suffer [3]. Based on this observation, medicinal plants are presented as a complementary alternative to the quick fight against the evolution of hypertension [3–6]. However, there is a strong perception that herbal medicine is a major cause of the uncontrolled and autonomous use of herbal products, especially with the advent of COVID-19 [7]. Although natural products are highly appreciated by the population, evaluating of their safety is necessary and even urgent to ensure their safe use. This is the case of *Flemingia faginea*, a

shrub of the Fabaceae family, which was previously used in West Africa, mainly in Burkina Faso, for the traditional treatment of high blood pressure, hemorrhoids, miscarriages, etc. [8–10]. Despite its use for decades, there is very little scientific information on this plant species that validates its safety and potential impact on blood pressure at the preclinical level. Therefore, the aim of the present work is to evaluate the acute and subacute toxicity of the leafy stems aqueous decoctate of *F. faginea* in NMRI mice and its potential effects on arterial parameters in healthy and normotensive Wistar rats by the noninvasive method.

MATERIAL AND METHODS

Material

Collection of plant material

The leafy stems of *F. faginea were* collected in September 2020 in Hêrèdougou, (North: 0480067; West: 1283148), Tuy province, a locality located in the Hauts-Bassins region of Burkina Faso. A herbarium was made and authenticated by a botanical specialist. A sample was deposited in the herbarium of the Centre National des Semences Forestières (CNSF) under the number CNSF-1425. The harvested leafy stems were washed, dried in a ventilated chamber protected from sunlight and dust, and then pulverized with a mechanical grinder (Gladiator Est. 1931 Type BN 1 Mach. 404611083). The dry powder was collected and stored until use.

Laboratory animals

The laboratory animals for this study were provided by the animal house of the Institut de Recherche en Sciences de la Santé/Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST). They consisted mainly of female mice of the NMRI strain (mean weight =23.86±3.04 g) and male and female rats of the WISTAR strain (mean weight =183.13±8.06 g). These animals were acclimatized in an enclosure at a temperature of 25±2 °C with a relative humidity between 50 and 70% and subjected to a cycle of 12 h of light and 12 h of darkness under compatible conditions of their well-being. They had free access to water and food and the experiments were conducted following the procedures of the Guide of Good Practices in Animal Experimentation under the Declaration of Helsinki[11]. Furthermore, all experimental animal procedures have been performed in accordance with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments. The study protocol was approved by the local ethics committee of the University Joseph KI-ZERBO (Protocol number: CE-UOI/2019-04.

Methods

Aqueous decoction preparation

The extract used for the toxicity studies was obtained by an aqueous decoction of the dry powder of the leafy stems of *F. faginea*. Briefly, 100 g of dry powder were dissolved in a flask in a ratio of 1 g to 10 mL distilled water (w/v). The mixture was homogenized and then boiled for 30 min using a hot plate according to the traditional preparation method. After cooling followed by centrifugation (5000 rpm for 5 min) and filtration, the obtained aqueous decoctate was freeze dried (CHRIST® Type ALPHA 1-2, BIO BLOCK SCIENTIFIC) to give an aqueous decoction of leafy stems from *F. faginea* (FAD). The FAD was stored in an anti-adsorbent package against humidity and then used for the experiments.

Determination of acute oral toxicity

The acute oral toxicity assessment was performed according to the Economic Cooperation and Development Organization (OECD) guideline 423 on the acute toxicity class method [12]. *F. faginea* decoctate was tested using a 02-step sequential process using three female mice each. The animals were monitored for signs of intoxication and the absence or presence of mortality related to the administration of FAD was noted.

Briefly, after fasting for 3 h, the mice were divided into two (02) groups of three (03) mice per cage. Lot (I) serving as a negative control received physiological water (NaCl 0.9%, 0.5 mL/kg pc) while a single dose of 2000 mg/kg body weight (bw) of *F. faginea* decoction was administered to the lot (II). Administration was administered orally using a syringe equipped with a gastric tube. The existence of ethological abnormalities or clinical signs of toxicity was routinely investigated during the first 2 h after administration. Subsequently, water and food were made available to the mice with free access. The observational phase extended throughout the study, at least once a day, to record symptoms of intoxication, as well as mortality. In addition, the weight, water and food consumption of the mice were measured with the following frequency: 1st; 2nd; 3rd; 7th and 14th days of the study. At the end of the trial, the mice were sacrificed in a dignified manner and the noble organs were removed, cleaned, weighed and macroscopically observed for any lesions or morphological changes. A cardiac puncture was performed for hematological analysis.

Determination of subacute toxicity

Defined as an assessment of the adverse effects of a substance after repeated or continuous exposure for 28 days, subacute oral toxicity was carried out according to the Organization for Economic Cooperation and Development guideline 407 [13]. Male and female rats were divided into 4 homogeneous groups of 5 animals per cage each. Group I was given physiological water (NaCl 0.9%, 0.5 mL/kg) as a negative control. Groups II, III and IV considered treated groups were gavaged with FAD at doses of 100, 500 and 1000 mg/kg bw respectively. The FAD extract and vehicle (0.9% NaCl) were administered daily to all animals according to their correspondences during the experimental period. Furthermore, signs and symptoms of toxicity were recorded regularly according to their frequency of occurrence throughout the study. Additionally, the daily water consumption, food consumption, and body weight of the different groups of animals were recorded. Specifically, the variations in the systolic and diastolic blood pressure of each rat were determined weekly.

Determination of the effect of FAD on blood pressure

All male and female rats were trained to take systolic and diastolic blood pressure for one week prior to the start of the subacute toxicity test. Noninvasive cuff plethysmography (Blood Pressure Recorder System 58500, Ugo Basile) was used for this purpose [14]. Each rat was placed in a compact thermal chamber (37 °C) with an individual holder for 30 min. After this thermal regulation phase, the plethysmography method of cuffing was applied for the measurement of blood pressure. The average blood pressure value was calculated after at least three valid measurements, while excluding outliers associated with noise or animal agitation.

Biochemical and hematological analyzes

At the end of the treatment, the animals were fasted for 16 h and then humanely sacrificed after ketamine anesthesia (50 mg/kg). Blood was collected by cardiac puncture in dry and K_3EDTA tubes for biochemical and hematological assessment. Blood sera obtained after centrifugation of dry tubes (3000 rpm for 10 min, ROTOFIX-32A, Germany) were analyzed with an automated system (COBAS E411 Roche) for biochemistry.

The biochemical workup involved parameters such as blood glucose (GLU), blood urea (Urea), blood uric acid (AU), blood creatinine (CREAT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), blood chloride (CL), blood sodium (NA), blood potassium (K), blood calcium (CA), blood cholesterol (CHO), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and total protein (TP). Whole blood samples from tubes K_3EDTA were analyzed with a hematology analyzer (XN-1000™ - SYSMEX) for hemogram or complete blood count (CBC). The parameters of red blood cell (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin content (MCHT), and mean corpuscular hemoglobin concentration (MCHC) were determined. Furthermore, leukocytes or white blood cells (WBC), neutrophilic subpopulations (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS) were counted. In addition, the number of thrombocytes or platelets (PLT) was also counted.

Necropsy and weight of noble organs

After dissection in the ulnar position and blood collection, the liver, kidneys, lungs, spleen, heart, and gonads were isolated, cleaned, macroscopically observed for abnormalities in shape, size or color, and then weighed on a Sartorius balance (accuracy=0.10 mg) to determine relative weight.

Statistical analysis

The data recorded during the different tests were classified and processed with Microsoft Excel software. Results were expressed as mean ± standard deviation. Statistical analyzes were conducted by one-way ANOVA followed by Bonferroni's multiple tests to determine the significance of variations between groups. For this purpose, the Graph Pad Prism 8.0.1 (244) software for Windows was used. A p-value *<0.05* was considered statistically significant**.**

RESULTS

Acute oral toxicity

Mortality

The results of this investigation showed that oral administration of *F. faginea* decoction (FAD) at a single dose of 2000 mg/kg bw did not cause mortality or significant clinical signs of toxicity during the 14-day study in the initial trial. Similar results were recorded in the second trial (Table I). Based on the results obtained and following the requirements of the OECD line 423 toxicity class method, the 50% lethal dose (LD_{50}) of FAD would be estimated at 5000 mg/kg bw.

Table I: Mortality ratio in the acute test

Doses	1st Step	2nd step
NaCl 0.9%, (0.5 mL/kg bw)	0/3	0/3
FAD 2000 (mg/kg bw)	0/3	0/3

Water and food intake

Histograms of the average daily food and water consumption of the mice in the study are shown on figures 1A and 1B. Data report that the single dose of 2000 mg/kg contributed to a decreasing in food intake of treated mice compared to the control. The daily amount of food consumed was 5.01±0.75 g and 7.51±0.52 g for treated and control mice, respectively (figure 1A). Statistical analysis showed that there was no significant difference between the differences between the two groups (p=0.05). However, the administration of the extract caused an increase in water consumption in the treated mice, compared to the control group. The mean daily volumes ingested were 7.94±2.43 mL and 5.45±1.55 mL by treated and control mice respectively (figure 1B) with no statistically significant differences (p=0.16).

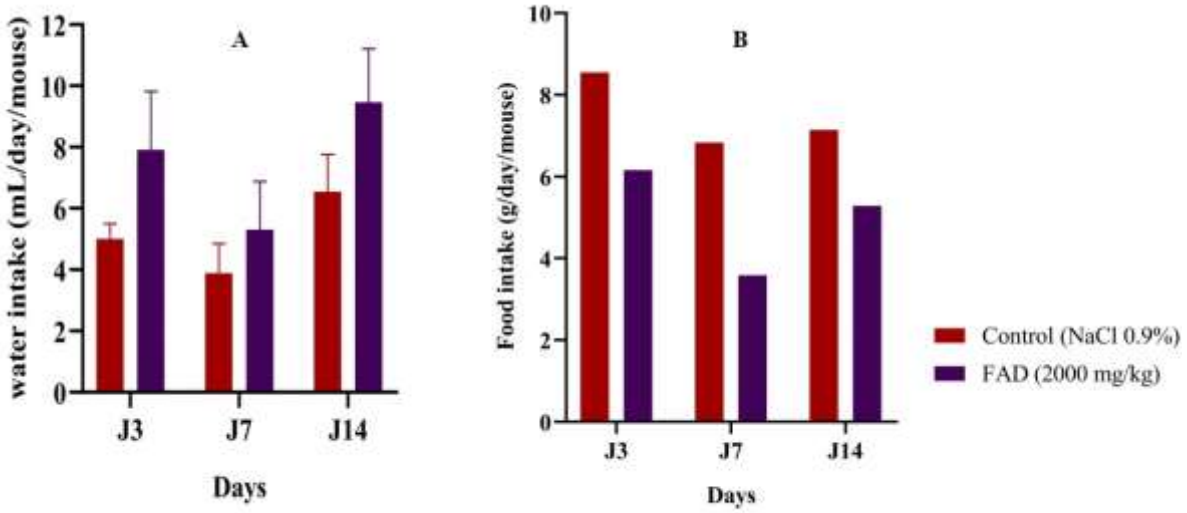

Figure 1: Histogram of mean daily water (1A) and food (1B) intake during acute toxicity

Effect of FAD on weight growth of study animals

Figure 2 shows the weight development of the mice in the study. Analysis shows that mice experienced an increase in body weight during the experiment. A slight but not significant statistical difference was observed in the treated mice from day 3 onwards compared to the control mice. This trend continued until day 14 of the study (figure 2; p=0.08).

Effect of FAD on the relative weight of animal organs

Table II shows the relative organ weights of the mice during the acute toxicity evaluation of the FAD extract. Macroscopic necropsy of noble organs such as the liver, lungs, spleen, heart, and kidneys showed no significant morphological variation or staining detectable by the naked eye. Furthermore, statistical analysis of relative organ weights did not show significant differences between the organs of the treated animals compared to those of the control animals (p=0.15).

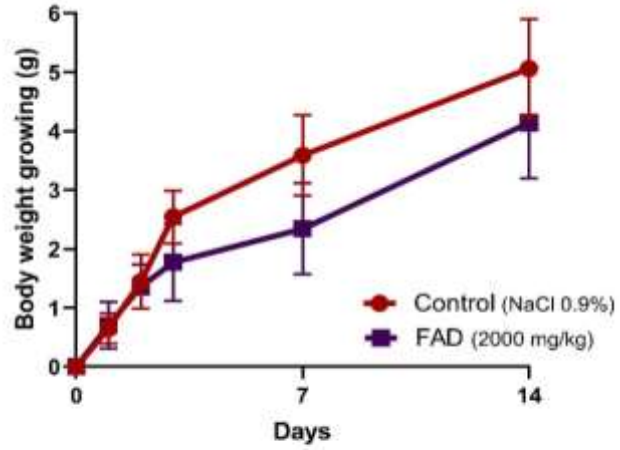

Figure 2: Body weight growth curve of the mice in the study

Abbreviation: FAD: *Flemingia faginea* Aqueous Decoction

Table II: Summary of relative organ weights of mice during acute oral toxicity

	Relative organ weight (mean±SD)				
Substances	**Lungs**	**Liver**	**Heart**	**Spleen**	**Kidney**
NaCl 0.9%, 0.5 mL/kg	0.51±0.05	3.81±0.20	0.41±0.02	0.44±0.03	0.86±0.05
FAD 2000 mg/kg bw	0.65±0.05	4.51±0.88	0.49±0.03	0.50±0.04	0.93±0.10

Effect of FAD on the Blood Count of Study Animals

Table III shows the hematological parameters of the mice in the investigation of acute FAD oral toxicity. Analysis of the results of blood counts did not reveal any morphological or stain abnormalities or anemia, leukopenia, or thrombocytopenia in single-dose 2000 mg/kg bw-treated mice or control mice.

Table III: Summary of hematological parameters of treated mice

Hemogram	**NaCl** 0.9 % (0.5 mL/kg)	**FAD** 2000 mg/kg bw
GR. (10^6 /µL)	8.85±0.45	9.28±0.32
Hb (g/dL)	13.90±1.20	14.70±0.50
HCT (%)	49.66±4.10	51.20±1.10
VGM (fL)	56.00±2.00	55.00±1.00
GB (10^3 /µL)	3.60±0.25	4.86±0.32
NEU (10^3 /µL)	1.45±0.19	1.95±0.12
LYM (10^3 /µL)	1.72±0.16	2.28±0.19
MON (10^3 /µL)	0.33±0.02	0.45±0.06
EOS (10^3 /µL)	0.08±0.01	0.11±0.03
BAS (10^3 /µL)	0.03±0.00	0.04±0.00
PLT (10^3 /µL)	413.60±60.8	561.00 ±16.60

Abbreviations: RBC: Red Blood Cell; Hb: Hemoglobin; MGV: Mean Blood Cell Volume; WBC: White Blood Cell; PLT: Platelet; HCT: Hematocrit; NEU: Neutrophil; LYM: Lymphocyte; MON: Monocytes; EOS: Eosinophil; BAS: Basophil

Determination of the subacute toxicity

Groups of male and female rats were used to evaluate the subacute toxicity of FAD. Daily oral administration of FAD for 28 days at a dose of 100 mg/kg, 500 mg/kg or 1000 mg/kg did not induce any behavioral changes in male and female rats of the corresponding groups. In addition, no clinical signs or symptoms of toxicity were observed throughout the experiment.

Determination of the weight evolution of rats

Figures 3A and 3B show, respectively, the weight growth of male and female rats divided into four groups: group I used as control received NaCl 0.9% (0.5 mL/kg), group II received the dose of 100 mg/kg, group III was gavaged with 500 mg/kg and group IV was administered with 1000 mg/kg during the 28 days of the subacute oral toxicity study of FAD (100; 500; 1000 mg/kg bw). Analysis of the body weight growth curve reported that daily administration of FAD to male and female

rats did not prevent an increase in body weight of treated lots, including group II with 100 mg/kg, group III with 500 mg/kg and finally group IV with 1000 mg/kg. Weight measurements of male rats during the 28 days of the study showed that the weight gain of the control group named lot I was 55.80±2.30 g. Regarding the treated groups II; III and IV, the weight gains were 49.00±2.64 g; 48.00±2.88 g and 35.80±1.74 g respectively. The comparison of weight changes between the different control and treated groups was not statistically significant [(p=0.38 comparing groups I and II); (p-value is >0.99 comparing groups I and III; (p=0.18 comparing groups I and IV)]. In rats, the weight gain for the control group was 30.40±3.64 g and 26.80±1.41 g; 23.60±1.40 g and then 20.00±1.30 g, respectively, for rats given the 100; 500 and 1000 mg/kg bw doses, respectively. No statistically significant differences were found between control and treated mice.

The histograms in figures 4A, 4B, 4C, and 4D represent the average food and drinking water consumption of the study animals. During the 28-day repeated dose toxicity study, daily gavage of FAD doses ingested by the animals did not significantly affect their diet. Nevertheless, a decrease in food consumption was observed for both sexes of treated rats. Analysis of variance did not reveal a statistically significant difference in the amounts of food and water consumed by the treated groups and their respective controls in both males and females.

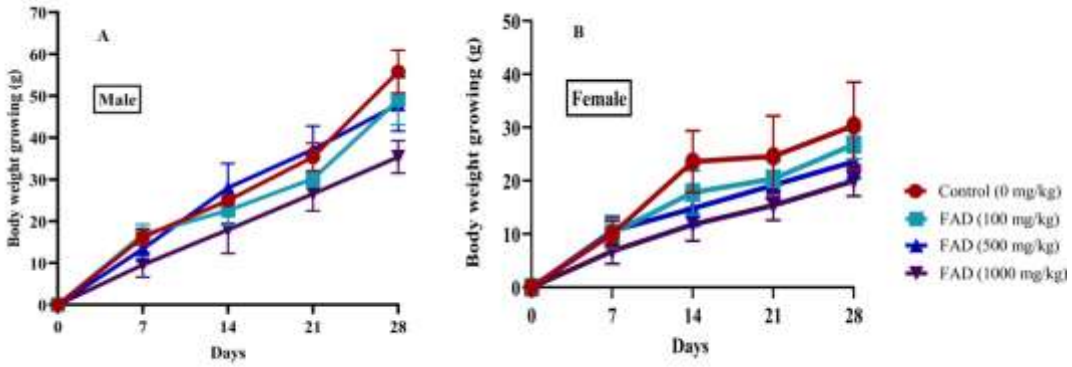

Figure 3: Body weight growth curve of male (4A) and female (4B) rats during subacute toxicity. Feed and water consumption

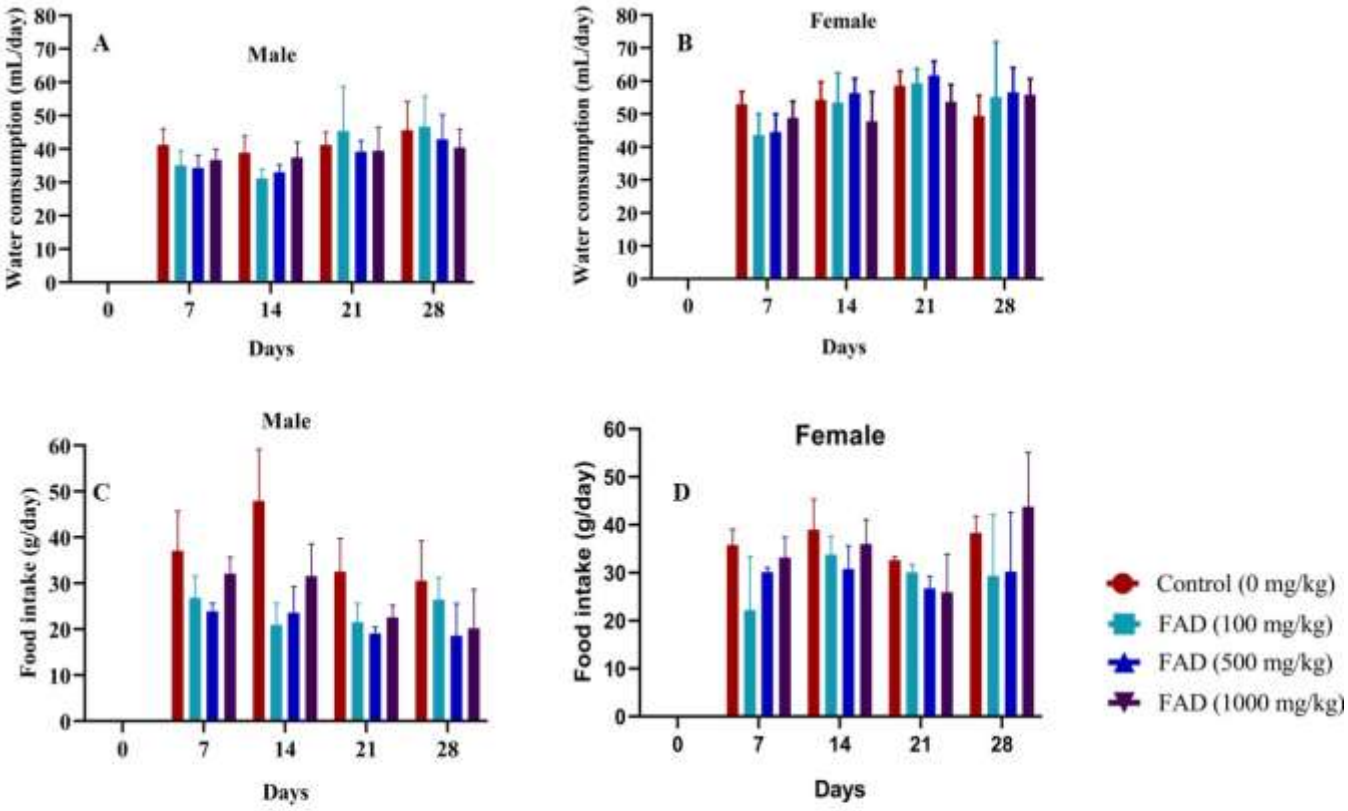

Figure 4: Histogram of the mean of water (4A-4B) and food (4C-4D) intake of male and female rats during the 28-day subacute oral toxicity.
With Bonferroni test; fig 4A: Male rats [(Water: p>0.05 for control versus FAD100; p>0.05 for control versus FAD500; p>0.05 for control versus FAD1000); Fig 4B: Female rats: [(Water: p>0.05 for control versus FAD100; p>0.05 for control versus FAD500; p>0.05 for control versus FAD1000); Fig 4 C: (Food: p>0.05 for control versus FAD100; p>0.05 for control versus FAD500; p>0.05 for control versus FAD1000)]; fig 4D (Food: p>0.05 for control versus FAD100; p>0.05 for control versus FAD500; p>0.05 for control versus FAD1000)]

Evolution of blood pressure during subacute toxicity test

Figures 5A, 5B, 5C and 5D show changes in systolic and diastolic blood pressure of male and female rats in the study. In general, the blood pressure of treated rats did not change significantly during the study. Daily doses of FAD ingested by healthy normotensive male and female rats during the 28-day study did not cause an elevation of their systolic and diastolic blood pressure. No statistical significance was found between the systolic and diastolic blood pressure of the treated groups and their corresponding controls ($p>0.05$).

Analysis of biochemical parameters

The values of the biochemical parameters are summarized in Table IV. Administration of repeated doses of 100 mg/kg, 500 mg/kg and 1000 mg/kg of FAD to healthy male and female rats for 28 days did not result in significant adverse biological effects throughout the study. Thus, no significant changes in serum biochemical parameters were observed during the experiment between treated and control groups. In male rats treated in different groups (II, III, and IV), the analysis of the results of their biochemical balance did not show statistically significant differences in the parameters studied compared to the control group (Table IV, $p>0.05$). In fact, the ALT values of the rats in the treated lots (lot II=39.14±5.59 IU/L; lot III=62.65±17.98 IU/L; lot IV=69.45±17.07 IU/L were slightly higher than those of the control 38.34±6.19 IU/L without being statistically significant. Furthermore, the values of natremia (Na) of the treated groups (lot II=136.90±4.83 mmol/L; lot III=141.73±2.06 mmol/L; lot IV=137.32±3.00 mmol/L) were slightly lower than that of the control group (lot I=145.27±3.86 mmol/L). In contrast, data on aspartate aminotransferase (ASAT), calcium (CA), cholesterol (CHOL), triglycerides (TG), HDL-cholesterol, LDL-cholesterol, potassium (K), gamma-glutamyl transferase (GGT), uric acid (UA), creatinine (CREA), urea and total protein (TP) were nearly similar for control and treated animals. In treated females, there were no pathological changes in serum parameters during the study. However, a slight but significant increase in total protein was observed in 100 and 500 mg/kg bw females with p-values of 0.02 and 0.04, respectively. Indeed, total protein values in female rats from treated lots II (61.33±1.40 g/L) and III (61.84±1.99 g/L) were increased compared to control lot I (53.75±1.73 g/L). However, renal parameters such as creatinine, urea, and uric acid, which are among other products of protein degradation, did not change significantly compared to the control in both sexes of all treated groups ($p>0.05$).

Analysis of hematological parameters

The absolute values of the blood count parameters of male and female rats during the 28 days of subacute toxicity are given in Table V. Clearly, there were no statistically significant changes in the hematological parameters of red blood cells or RBCs, leukocytes or white blood cells, and thrombocytes or platelets after treatment of male and female rats with different repeat doses of 100 mg/kg, 500 mg/kg and 1000 mg/kg FAD. No significant changes were observed in the analysis of red blood cell parameters such as the RBC count (RBC), hemoglobin (Hb), hematocrit (HCT), as well as their mean corpuscular volume (MCV), concentration (MCHC) and mean corpuscular hemoglobin content (MCHT) between control and treated animals. In addition, the analysis of the number of white blood cells (WBC) and their subpopulations of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), basophils (BAS), and eosinophils (EOS) in the blood of treated animals did not show statistically significant difference from those of the control group. However, there was a statistical increase in platelets in male rats of lot III given the daily dose of 500 mg/kg bw ($p=0.01$) compared to the control lot. This variation could not be related to the treatment, as rats in groups II and IV given 100 and 1000 mg/kg bw of FAD, respectively, showed no significant change compared to the control group.

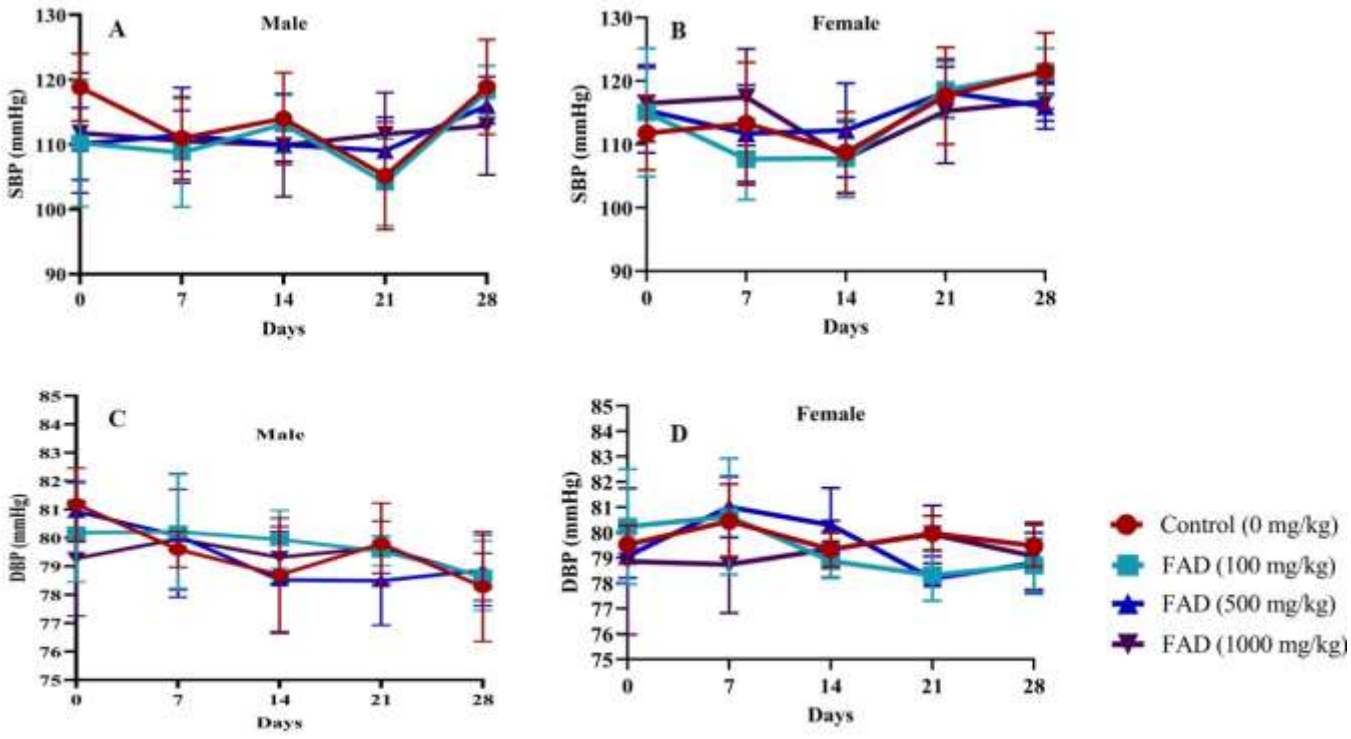

Figure 5: Impact of FAD on systolic (6A-6B) and diastolic (6C-6D) blood pressure of male and female rat during subacute toxicity
Abbreviations: SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure

Table IV: Results of biochemical analyses of serum from male and female rats in the subacute study

	Substances							
	NaCl 0.9% (0.5 mL/kg)		**FAD 100** mg/kg bw		**FAD 500** mg/kg bw		**FAD 1000** mg/kg bw	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
ALT (IU/L)	38.34±6.19	33.46±3.01	39.14±5.59	42.46±3.61	62.65±17.98	43.93±13.43	69.45±17.07	33.21±1.49
AST (IU/L)	161.62±12.60	173.71±24.23	136.83±15.52	188.17±16.77	185.91±32.35	162.38±14.29	156.35±8.64	156.33±4.11
CA (mmol/L)	2.72±0.09	2.64±0.05	2.66±0.09	2.74±0.11	2.79±0.03	2.63±0.11	2.71±0.03	2.70±0.05
CHO (mmol/L)	1.02±0.13	1.63±0.21	1.11±0.06	1.27±0.08	0.97±0.17	1.27±0.22	1.12±0.14	1.20±0.02
CL (mmol/L)	105.83±1.51	105.50±1.48	103.82±2.86	106.53±0.93	106.98±1.18	108.56±1.25	104.92±1.74	103.46±1.78
CREA (µmol/L)	65.08±1.71	73.96±3.52	59.21±4.54	73.70±2.26	85.07±11.66	71.49±0.63	66.11±2.55	72.22±5.12
GLU (mmol/L)	8.42±0.90	5.61±0.67	6.22±0.17	7.51±1.41	9.91±1.70	6.15±0.89	7.69±1.23	6.50±0.64
HDL (mmol/L)	0.52±0.09	0.90±0.15	0.57±0.03	0.80±0.06	0.48±0.10	0.55±0.13	0.65±0.15	0.59±0.03
K (mmol/L)	8.55±1.37	6.81±0.67	5.79±0.71	8.64±1.84	9.64±1.58	6.66±0.26	5.72±0.68	7.25±0.95
Na (mmol/L)	145.27±3.86	135.30±0.82	136.90±4.83	135.95±1.69	141.73±2.06	135.91±1.16	137.32±3.00	133.20±2.02
TG (mmol/L)	0.25±0.04	0.34±0.03	0.15±0.02	0.33±0.05	0.29±0.04	0.32±0.10	0.23±0.01	0.28±0.02
AU (mmol/L)	161.27±34.14	104.31±11.57	178.60±18.76	128.42±28.53	220.66±28.44	89.22±6.55	103.10±14.12	102.85±35.24
UREA (mmol/L)	10.07±0.72	10.39±0.44	10.16±0.52	9.99±0.69	13.82±3.17	10.09±0.92	12.34±2.08	11.92±1.44
TP (g/L)	57.53±2.98	53.75±1.73	54.87±4.86	61.33±1.40*	53.03±4.58	61.84±1.99*	57.81±3.33	57.26±3.08
GGT (IU/L)	7.35±0.92	6.89±0.66	5.78±0.31	8.40±1.02	7.97±1.12	7.13±0.69	7.14±0.94	5.98±1.56
LDL (mmol/L)	0.38±0.05	0.57±0.07	0.47±0.02	0.54±0.06	0.36±0.08	0.57±0.11	0.35±0.09	0.48±0.03

Abbreviations: ALT : alanine aminotransferase; AST: aspartate aminotransferase; CA: calcium; CHO: cholesterol; CL: chloride; CREA: creatinine; GLU: glucose; HDL-C: high-density lipoprotein-cholesterol; K: potassium; NA: sodium; TG: triglycerides; AU: uric acid; UREA: urea; TP: total protein; GGT: gamma-glutamyl transferase; LDL-C: low-density lipoprotein-cholesterol. *p<0.05: Female ((FAD100 and FAD500) vs control)

Table V: Summary of blood count parameters for animals in the subacute study

	Substances							
	NaCl 0.9% (0.5mL/kg)		**FAD 100** mg/kg bw		**FAD 500** mg/kg bw		**FAD 1000** mg/kg bw	
Hemogram parameters	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
RBC (10^6 /µL)	8.53±0.32	8.27±0.18	8.67±0.31	9.14±0.53	9.83±0.59	7.81±0.13	9.83±0.59	7.61±0.18
Hb (g/dL)	15.38±0.51	14.55±0.39	15.39±0.55	16.46±0.80	16.44±0.99	14.16±0.28	16.44±0.99	13.80±0.25
HCT (%)	49.96±1.50	48.84±1.57	50.61±1.58	55.22±3.13	53.51±3.18	46.24±0.92	53.51±3.18	44.56±0.70
VGM (fL)	58.68±1.00	58.74±1.58	59.12±1.63	60.50±1.13	55.78±2.62	59.12±0.81	55.78±2.62	58.58±0.78
MCHT (pg/cell)	18.04±0.31	17.49±0.24	17.99±0.52	18.04±0.19	17.10±0.81	18.14±0.16	17.10±0.81	18.12±0.32
MCHC (g/dL)	30.80±0.38	29.65±0.43	30.77±0.82	30.50±0.49	31.46±1.52	30.68±0.23	31.46±1.52	30.98±0.25
GB (10^3 /µL)	3.86±0.87	2.01±0.38	2.64±0.07	4.64±0.95	1.44±0.17	4.80±0.75	1.44±0.17	5.03±0.67
NEU (10^3 /µL)	1.22±0.42	0.61±0.06	0.77±0.07	1.60±0.39	0.44±0.05	1.51±0.45	0.44±0.05	0.79±0.14
LYM (10^3 /µL)	2.04±0.44	1.20±0.29	1.56±0.08	2.41±0.68	0.85±0.09	2.32±0.24	0.85±0.09	2.96±0.69
MON (10^3 /µL)	0.39±0.08	0.10±0.00	0.16±0.05	0.29±0.06	0.09±0.01	0.88±0.20	0.09±0.01	0.59±0.15
BAS (10^3 /µL)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EOS (10^3 /µL)	0.20±0.06	0.10±0.03	0.11±0.05	0.16±0.05	0.05±0.00	0.09±0.03	0.05±0.00	0.07±0.02
PLT (10^6 /µL)	6.44±0.32	6.09±0.86	7.70±0.25	6.95±0.67	8.17±0.33*	6.74±0.60	8.17±0.33	6.77±0.39

Abbreviations: RBC: Red Blood Cell; Hb: Hemoglobin; MGV: Mean Blood Cell Volume; WBC: White Blood Cell; PLT: Platelet; HCT: Hematocrit; NEU: Neutrophil; LYM: Lymphocyte; MON: Monocytes; EOS: Eosinophil; BAS: Basophil. *p<0.05: Male (Control vs FAD500)

The relative weight of organs

Table VI shows the relative organ weights of the study animals. Macroscopic necropsy showed no pathological changes in the various noble or vital organs related to treatment with repeated doses of FAD of animals in the treated groups compared to controls. Furthermore, statistical analysis could not report significant differences in relative weights could be reported by statistical analysis ($p>0.05$).

Table VI: Relative vital organ weights of male and female rats from the subacute study

	Relative organ weight (mean ±SD)							
	NaCl 0.9% (0.5 mL/kg)		**FAD 100** mg/kg bw		**FAD 500** mg/kg bw		**FAD 1000** mg/kg bw	
Organs	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver	2.68±0.11	2.85±0.08	2.41±0.09	2.91±0.09	2.44±0.14	3.02±0.14	2.18±0.14	3.12±0.32
Heart	0.35±0.01	0.04±0.03	0.38±0.00	0.38±0.02	0.37±0.03	0.40±0.01	0.44±0.00	0.42±0.04
Lungs	0.61±0.01	0.75±0.05	0.61±0.01	0.64±0.03	0.63±0.02	0.71±0.05	0.68±0.03	0.61±0.05
Spleen	0.31±0.03	0.30±0.02	0.25±0.01	0.28±0.02	0.23±0.02	0.26±0.01	0.23±0.01	0.30±0.03
Kidney	0.75±0.02	0.76±0.04	0.74±0.02	0.72±0.04	0.77±0.02	0.84±0.11	0.70±0.03	0.87±0.16
Testicle/Ovaries	1.39±0.23	0.07±0.00	1.28±0.20	0.07±0.00	1.18±0.25	0.06±0.01	1.29±0.17	0.07±0.00

DISCUSSION

Since time immemorial, humans have used plants to feed themselves and to treat several pathologies that threatened their existence. The toxicological evaluation of medicinal plants derived from this ancestral knowledge is a key step in their scientific validation and safety of use [2,15]. Oral administration of a single dose of 2000 mg/kg bw of leafy stems of the *F. faginea* (FAD) aqueous decoction to mice during the two stages of acute toxicity did not produce observable adverse effects until day 14th. No significant toxicity effects were observed on mortality, clinical signs, water and food consumption, and relative vital organ weights of the animals used. The absence of mortality allows the lethal dose of 50% (LD_{50}) to be estimated at 5000 mg/kg bw according to OECD guideline 423 [12]. With such an LD_{50} the decocted FAD could be considered a substance unlikely to be of acute concern to users of the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[16].

These results corroborate those of other authors who have used procedures to estimate the LD_{50} of *Lannea microcarpa* and FACA® syrup at 5000 mg/kg bw [17,18]. In addition, no weight loss was recorded in the mice during this evaluation. Better still, positive weight growth was observed in treated mice, although lower than that in controls. This difference in weight gain is believed to be related to the fact that the FAD caused a slight increase in water consumption by treated mice, so they ate less food than controls. This feeding behavior of the treated mice is correlated with a reduction in weight gain. This reduction in weight gain would be interesting given the harmful effects of excess weight on cardiovascular health. Nevertheless, these non-significant observations could not constitute a proof of toxicity because the relative weight of the vital organs did not vary considerably [19,20]. Furthermore, analysis of hematological parameters through blood count did not reveal significant changes in absolute values of red blood cells, leukocytes, and thrombocytes [21].

This exposure of animals to a high dose of the extract for 14 days is not sufficient to conclude the safety of this product[12,22]. Therefore, daily administration of doses of 100, 500 and 1000 mg/kg of FAD to healthy, normotensive male and female rats for 28 days was conducted. It should be noted that daily gavage of rats with 100; 500; 1000 mg/kg bw did not induce mortality or clinical signs and symptoms during the 4-week oral subacute toxicity evaluation. A slight decrease in body weight growth was observed during the 2nd week, but rapidly overcome by the third week. Thus, the difference in weight development during subacute toxicity was not statistically significant between the control and the groups given 100, 500 and 1000 mg/kg bw of FAD, respectively. The food and water intake of the groups that receiving the extract at different doses did not vary statistically significantly from the control groups. Analysis of relative organ weights is an essential component of the toxicological and risk assessment of chemicals [18,23]. No significance was found in the analysis of relative organ weights of animals treated with repeated doses of FAD. Our results suggest that the FAD extract would not have harmful effects on vital organs, including the liver, kidney, lung, spleen, heart, testicles, and ovary. However, microscopic histopathological sectioning of the various organs is essential to complement and support the conclusions drawn during macroscopic necropsies. Exploring hematologic parameters is of notable importance in detecting hematopoiesis dysfunction and the occurrence of inflammatory reactions [24]. The hematological parameters of the red, white, and platelet lineage of the treated animals did not vary significantly compared to those of the respective controls. No anemia, hypochromic, macrocytosis, hyperleukocytosis, leukopenia, or thrombocytopenia attributable to the FAD extract were diagnosed during the 28-day study. These results would suggest that daily doses of FAD less than or equal to 1000 mg/kg bw would not cause a significant change in the figurative elements of the blood [25].

Repeated oral administration of the extract during the 28-day study did not alter serum levels of liver transaminases, including alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase. These parameters are considered markers of liver integrity [26–28]. These results suggest that the FAD extract would not have hepatotoxic effects at the doses tested [29]. In this study, serum ionic parameters such as natremia, kalaemia, calcaemia, and chloraemia were not affected by FAD. Similarly, markers of kidney integrity, such as creatinine, uremia and uricemia, did not vary significantly in groups of male and female animals treated with 100; 500 and 1000 mg/kg bw. Although serum total protein values were significantly different in the 500 mg/kg bw group, this was not dose dependent. In fact, no significant differences were found with the higher dose of

1000 mg/kg bw. These results would support the absence of nephromegaly during necropsy. These observations suggest that doses of FAD ingested by male and female rats would not have a nephrotoxic effect at the serum level [30,31]. Furthermore, the lipid profile of the treated animals did not change significantly. The cholesterol level and its HDL and LDL fractions, as well as serum triglyceride levels, were not modified by the action of the FAD extract. Thus, FAD doses less than or equal to 1000 mg/kg bw would not modify the lipid profile. Statistical reports suggest that this FAD would not have any negative influence on the metabolism of fatty acids. This is a good thing to know the strong correlation of hypercholesterolemia with the risk of cardiovascular accidents [32,33]. In addition, the animals in the study did not experience a change in their blood sugar levels. These results would suggest that doses of 1000 mg/kg bw or less of FAD would not present a hyperglycemic risk.

Oral administration of FAD doses during toxicological evaluation did not negatively affect systolic and diastolic blood pressure in treated male and female rats. Monitoring of blood pressure is a key indicator of good cardiovascular health in herbal treatments due to the plurality of metabolites they contain. Additionally, FAD extract did not contribute in a dose dependent manner to the variation in SBP and DBP of treated male and female animals. These results could be explained by homeostatic maintenance of the values of natremia, kalaemia, and calcaemia in addition to the absence of cardiomegaly at necropsy. These observations suggest that FAD extract would not be a risk factor for blood pressure in healthy normotensive subjects. Several factors of drug origin have been incriminated in the genesis of secondary hypertension [34,35]. This conclusion is comforting due to of the use of leafy stems of *F. faginea* as tea by certain populations.

CONCLUSION

The toxicological evaluation showed the non-hazardousness of the aqueous decoction of leafy stems of *F. faginea* with an estimated lethal dose of 5000 mg/kg bw. FAD extract did not cause mortality or adverse effects in studies of acute and subacute oral toxicity. No significant detrimental changes in the food and water intake of treated animals were reported, nor in weight growth. Moreover, this extract did not cause of any modification of metabolism through biochemical and hematological balance. However, further investigations, in particular subchronic, histopathological, and cytotoxic, will have to be carried out to confirm the safety and security of the use of FAD for the treatment of arterial hypertension. These preliminary results could be considered as a scientific basis to justify the use of FAD in traditional medicine.

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Conflict of interest

The authors declare that there are no conflicts of interest in the publication of this article.

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