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Acute and Sub-chronic Toxicity Evaluation of the Ethanolic Extract of *Coula edulis* B., (Olacaceae) Stem Bark

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EB, JFY, and JEO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BGKA, GRT, and JLN managed the analyses of the study. Author FE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Coula edulis* Baill., (Olacaceae) is a non-lignified forest product not well known and widely used in sub-Saharan Africa as a phytomedicine or food additive. However, the toxicity of this plant remains unknown. This study aimed to assess the safety of the ethanolic extract of *C. edulis* stem bark (CEE).

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Study Design: Pharmacological study.
Place and Duration of Study: Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, University of Yaounde 1 (Cameroon), between June 2018 and July 2022.
Methodology: Studies on the assessment of acute and subchronic toxicity were carried out by guidelines 423 of the Organization for Economic Co-operation and Development (OECD). Subacute toxicity of the sample was assessed over 28 days using repeated doses by OECD Guideline 407.
Results: No cases of death and clinical signs of toxicity were observed in the treated rats, suggesting that the LD_{50} of *C. edulis* ethanolic extract is greater than 2000 mg/kg bw. Regarding the subacute toxicity study, the administration of CEE also did not result in any changes in the course of body weight. Only a significant decrease in the relative weight of the ovaries in females at the highest dose of 600 mg/kg was observed. In males and females, CEE did not affect lipid profile markers or transaminase levels (AST, ALT). In addition, a small but non-significant ($p> 0.05$) increase in creatinine was observed without kidney dysfunction. In males, CEE induced an increase in mean corpuscular volume number at 600 mg/kg, while at the same time, mean corpuscular hemoglobin concentration decreased at the 300 mg/kg dose. In females, a significant increase in the number of monocytes, red blood cells, and hemoglobin level were observed. No difference in the levels of urea, glucose, and lipid markers was observed nor histological changes in the organs studied.
Conclusion: As would be expected, exposure to CEE did not cause significant toxic effects in treated rats. Therefore, this plant extract can be safely recommended for therapeutic use.

Keywords: Acute; coula edulis bail; safety; sub-acute; toxicity.

1. INTRODUCTION

The use of traditional medicine remains a common practice in African culture. A complete report on traditional Cameroonian medicinal plants compiled in a book entitled "Traditional Medicine and Pharmacopeia, the contribution of ethnobotanical and floristic studies in Cameroon" [1], presents if it was still needed the extent of the pharmacological potential of Cameroonian plants. This book is a point of reference for phytochemists and ethnobotanists in studies of medicinal plants.

“Indeed, according to the World Health Organization, about 80% of the world's population and a higher rate in developing countries, depend on plants for their primary medical problems” [2]. “This preference for plant-based drugs as a source of drugs is often justified by the accessibility and unwanted side effects of synthetic drugs, which are believed to be suitable for chronic treatment” [3]. “In addition, several studies on the therapeutic potential of plants suggest that the latter could provide new compounds, able to overcome the high cost and toxic effects of current drugs, which would be a boon for many rural populations in developing countries” [4]. However, the use of traditional pharmacopeia drugs by the population is without a recommended dose and some of these plants are highly toxic [5]. Also, traditional medicines are often combined with pharmaceutical medicines, which could lead to cases of overdose and other cases of toxicity [6]. The lack of evidence on their quality, safety, or even their effectiveness [7]. tends to reinforce this reality. Hence the interest of deepening research on phyto-drugs and phytochemicals to integrate the observations of short and long-term manifestations of toxicity and establish effective communication before a possible prescription [8].

The African hazelnut (*Coula edulis* Baill.) is a little-known non-timber forest product. These almonds are produced by an evergreen tree, medium in size but up to 25 m tall. This plant species belonging to the Olacaceae family, is widely distributed in the forest areas of West and Central Africa [9]. In these regions, nuts are used as food additives. They can be eaten raw, roasted, or boiled, while the rest of the plant is used in the prevention and treatment of various disorders. In Ivory Coast, for example, a decoction of the bark of *C. edulis* is used for purging and treating anemia, back pain, or sore kidneys [10]. In other countries, local populations use this plant in the treatment of gynecological diseases, indigestion, and trauma [11], but also in the management of gastrointestinal infections, anemia, diarrhea, and anti-inflammatory and antimicrobial healthcare [12]. Previous studies have proven the antimicrobial activity of the ethanolic extract of the stem bark of *C. edulis*

against *Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans* [9], as well as antiplasmodial activity [13].

Despite the widespread use of this plant in nutrition and as herbal medicine, the ability of extracts from this plant to improve the health of populations without causing harmful side effects remains to be demonstrated [14]. The present study evaluated the possible toxic effects of the ethanolic extract of the stem bark of *C. edulis* after acute and sub-acute oral administration in male and female Wistar rats.

2. MATERIALS AND METHODS

2.1 Animals

Young male and female Wistar strain rats, aged 8 to 10 weeks, were acquired from the Animal Physiology Laboratory of the University of Yaoundé I (Cameroon).

2.2 Plant Material, Extraction, and Composition of the Extract

C. edulis stem barks were collected in July 2014 in Mbalmayo, a locality located in the central region of Cameroon. The plant was identified and authenticated by Mr. Victor Nana, botanist at the Cameroon National Herbarium in Yaoundé where a voucher specimen was deposited under number 46305 HNC. The dried and pulverized stem bark (1 kg) was extracted with 95% ethanol at room temperature (4 L of solvent x 3.48 hrs per extraction). The combined solutions were evaporated under reduced pressure at a temperature of 40°C. At the end of the said procedure, 73 g of extract (a yield of 7.3%) named CEE were obtained. The extract was stored between 4 and 8°C. Distilled water was used as the dissolving solvent before administration.

2.3 Acute Oral Toxicity

The ethanolic extract of *C. edulis*, the sample whose acute toxicity was to be assessed was administered orally using the acute toxicity classes (ATC) method by guideline 423 of the Organization for Cooperation and Development. Economic Development (OECD) [15]. Exclusively female rats were used for this initial experiment. Previous studies have reported that females are slightly more sensitive [16], when testing for the lethal dose 50 (LD_{50}) of a sample, effectively supporting the OECD recommendation to use female animals during acute toxicity studies. Six healthy rats were divided into two groups of 3 animals each. The first group received the 2000 mg/kg bw dose of freshly prepared CEE in distilled water. At the same time, rats in the second group received a comparable volume of distilled water by gavage. The administration volume of the extract or distilled water solution was 1 ml/150 g bw. Before administration, animals were acclimatized, weighed, stained, and fasted overnight. After administration, food was suspended for an additional 3-4 hours, and animals were observed individually for the first 30 minutes, then 2, 4, and 6 hours after treatment, and then daily for a total of 14 days. During this period, changes in behavior and other parameters such as body weight, urination, water consumption, food intake, convulsions, breathing, lethargy, temperature, constipation, changes in eye and skin color, etc., were noted. The experiment was repeated with the same dose and the same number of animals according to the flow charts of the OECD [15]. At the end of the fourteenth day, the animals were sacrificed by decapitation under light anesthesia (10 mg/kg bw of diazepam and 50 mg/kg bw of ketamine ip), and the liver, kidneys, lungs, heart, stomach, spleen, and adrenal glands were collected, observed and weighed.

2.4 Sub-Acute Oral Toxicity

The subacute toxicity of the sample was assessed over 28 days using repeated doses by OECD Guideline 407, adopted October 3, 2008 [17] and OECD, 2008, reported by [18]. However, sixty Wistar strain rats were divided into 6 groups of 10 animals each (5 females and 5 males). The first group received vehicle (distilled water), and groups 2 to 4 received CEE at doses of 150, 300, and 600 mg/kg body weight respectively. Groups 5-6 served as satellite groups for the control group (Control-S) and the highest dose group (600-S). The animals received daily at 8 h a dose of treatment by gavage for 28 days. These animals were also observed once a day to detect possible signs of toxicity. The administered volume of extract solution or distilled water was 1 ml/200 g bw.

“After 28 days of treatment, the satellite groups were followed for a further 14 days without treatment to detect late-onset or persistence of underlying toxic effects. Animals were weighed every 4 days throughout the study. Twenty-four hours after the last administration (for groups 1 to 4) and after post-treatment follow-up (for satellite groups), the animals were sacrificed by

decapitation under light anesthesia (10 mg/kg bw of diazepam and 50 mg/kg bw ip ketamine) after a 12-hour night fast. Blood samples were taken for analysis of hematological and biochemical parameters. The heart, liver, kidneys, stomach, spleen, and lung were dissected, weigand hed, and their relative weight was evaluated according to the following formula (organ weight g/100 g bw). Liver sections were fixed in 10% formaldehyde for histological analyses" [3].

2.5 Blood Analysis

Part of the blood sample was collected in EDTA tubes for hematological analysis and the other in dry tubes for serum separation and related biochemical analyses. Blood samples in dry tubes were centrifuged at 1500g (15 min at 4°C) and the supernatant (serum) was collected and placed in new tubes. The contents of triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL), alanine transaminase (ALT), aspartate transaminase (AST), creatinine, and total proteins were determined using reagent kits by Fortress Diagnostics Limited (Muckamore, UK). The content of low-density lipoproteins (LDL) was calculated using Friedewald's formula.

Hematology analysis of blood samples was performed using a Humacount 30TS automated hematology analyzer from Human Diagnostics Worldwide (Wiesbaden, Germany). Among the parameters evaluated were: the number of red blood cells (RBC); hematocrit (Ht); hemoglobin (Hb); mean corpuscular volume (VCM); mean corpuscular hemoglobin (MCH); mean concentration of corpuscular hemoglobin (MCHC); platelets (PLT); white blood cell count (WBC); as well as the count of granulocytes, lymphocytes and monocytes.

2.6 Histopathology Analysis

The fixed tissues were dehydrated in a series of increasing alcohol baths, thinned in xylene, and embedded in paraffin wax melting at 60 C. Serial sections (5 mm thick) were obtained by cutting the included tissue with a microtome. They were then mounted on induced slides of 3-aminopropyl triethsilane coated and dried for 24 h at 37°C [19]. Sections mounted on the slides were dewaxed with xylene and hydrated in a series of descending alcohol baths. They were then stained with hematoxylin and Mayer's eosin stains, dried and mounted on a light microscope (x 40, 100, and 200).

2.7 Statistical Analysis

Results were presented as means ± standard deviation. The comparison of the means of the parameters studied between the different groups was made by the ANOVA test followed by the Tukey test, a post hoc test for multiple comparisons, using the Graph-Pad Prism software (version 5.00 for Windows, Graph Pad Software, San Diego, CA). The p values <0.05; 0.01 and 0.001, were considered significant.

3. RESULTS

3.1 Acute Toxicity Study

Administration of a single oral dose of CEE 2000 mg/kg did not produce any treatment-related mortality or evidence of toxicity in animals during the entire observation period (14 days). However, some minor changes were noted, in particular in the color of the fecal matter (Table 1). In addition, the autopsy revealed no macroscopic pathological signs and no significant difference in the relative weight of the organs studied (Table 2). Therefore, the acute toxic class method, following the flow chart of the LD50 cut-off, confirmed the ethanolic stem bark of *C. edulis* as a category 5 substance in the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

3.2 Sub-Acute Oral Toxicity

Animals in the 150, 300, and 600 mg/kg per day extract groups survived the 28 days. Furthermore, no outward sign of toxicity was observed in the treated groups compared to the control groups. As shown in Figs 1 and 2, no significant difference was observed in the evolution of the weight of the animals between the control groups and the treated groups during the treatment period and in the satellite groups during the period of 14 days without treatment.

Regarding the relative organ weights of male and female rats treated with CEE orally during the experimental phase between the control group and the groups treated at doses of 150, 300, and 600 mg/kg in the male, no significant difference was found. Similar results were observed in female rats except for the ovaries at doses of 300 and 600 mg/kg bw, where there was a significant reduction in relative organ weight. The relative organ weights of male and female rats treated with CEE orally for 28 days are summarized in Tables 3 and 4.

Table 1. General appearance and behavioral observations of acute toxicity study for control and treated groups

Observation	Control group	2000 mg/kg
Digestion	Normal	Normal
Body weight	Normal	Not change
Food intake	Normal	Normal
Urination	Normal	No effect
Rate of respiration	Normal	No effect
Change in skin	No effect	No effect
Drowsiness	No effect	No effect
Sedation	No effect	Observed
Eye colour	No effect	No effect
Fecal color	No effect	Observed
Diarrhea	Not present	Not present
General physique	Normal	Normal
Coma	Not present	Not present
Death	Alive	Alive

Table 2. Effect of single oral administration of 2000 mg/kg bw of CEE on relative weight of organs (g/100 g bw)

Organs weight (g)	Control	CEE
Liver	2.163 ± 0.156	2.331 ± 0.157
Kidneys	0.544 ± 0.012	0.566 ± 0.014
Adrenals	0.024 ± 0.009	0.028 ± 0.006
Heart	0.312 ± 0.012	0.317 ± 0.015
Spleen	0.214 ± 0.035	0.225 ± 0.012
Ovary	0.071 ± 0.015	0.069 ± 0.012
Pancreas	0.302 ± 0.045	0.297 ± 0.052
Stomach	0.751 ± 0.044	0.722 ± 0.023
Lungs	0.552 ± 0.045	0.558 ± 0.045

Data are expressed as mean ± SEM (n = 6)

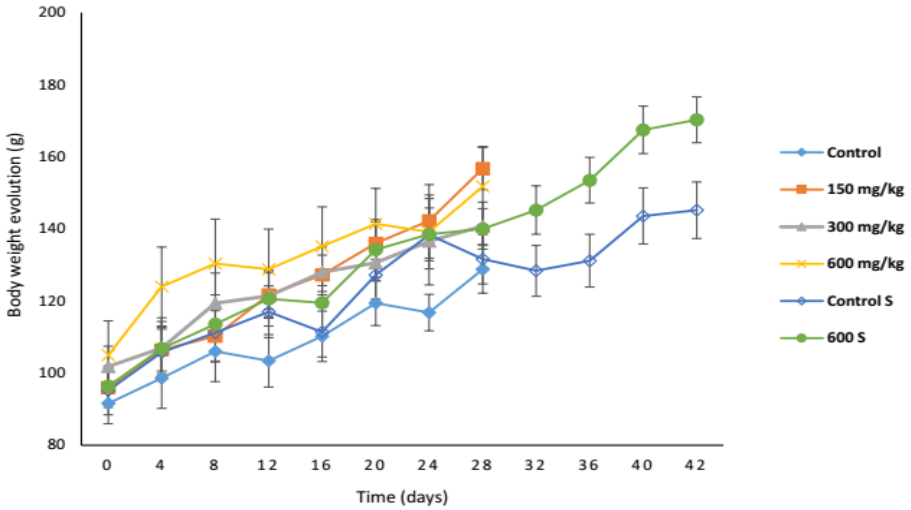

Fig. 1. Body weight evolution of male Wistar rats submitted to 28-day treatment with an ethanol extract of the stem bark of *C. edulis* at 150, 300, and 600 mg/kg bw (n = 6)

Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract

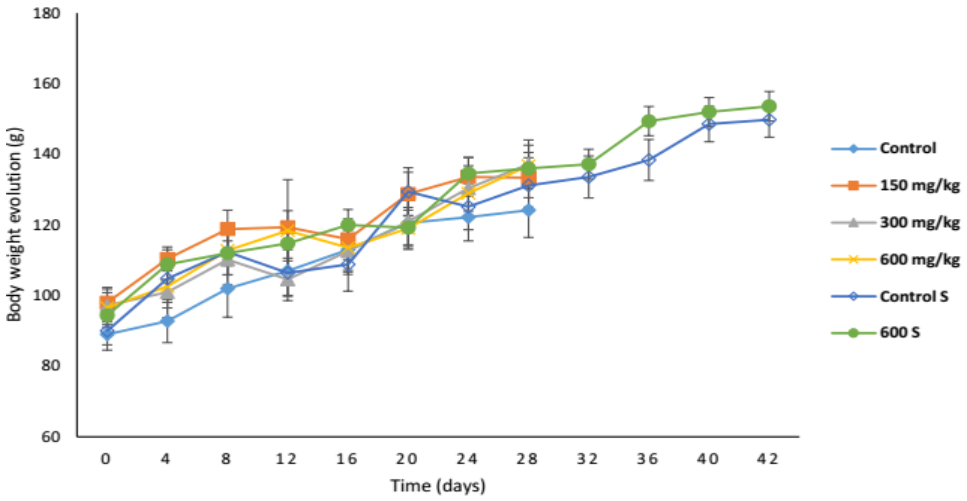

Fig. 2. Body weight evolution of male (A) and female (B) Wistar rats submitted to 28-day treatment with an ethanol extract of the stem bark of *C. edulis* at 150, 300, and 600 mg/kg bw (n = 6)

Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract

Biochemical analysis showed that most of the parameters remained unchanged. Most of the results show a non-significant change following oral administration of CEE (Tables 5 and 6; Fig. 3 and 4). The results of the various biochemical tests on the treated animals are summarized in Table 5 and Table 6. Only a significant increase ($p < 0.05$) in the total protein content in the male rats treated with CEE at the dose 600 mg/kg bw was observed while at the same time, no effect was recorded at doses 150 and 300 mg/kg (Table 5). In male and female rats, a slight but non-significant increase in urea and creatinine levels was observed at 300 and 600 mg/kg and after post-treatment follow-up (Table 5 and Table 6). Both males and females did not show significant changes in lipid profile markers and the appearance of the liver tissues of males and females, at different doses after oral treatment with CEE (Fig. 5 and 6).

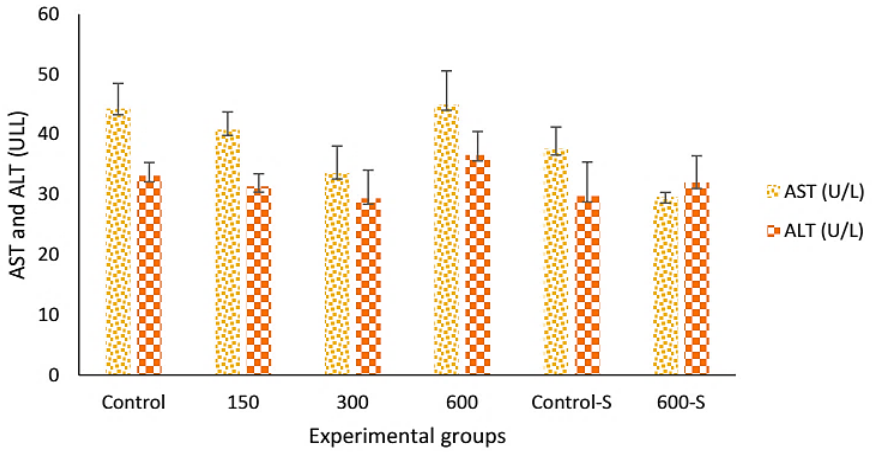

Fig. 3. AST and ALT activities of male Wistar rats submitted to 28-day treatment with an ethanol extract of the stem bark of *C. edulis* water 150, 300, and 600 mg/kg bw (n = 6)

Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract

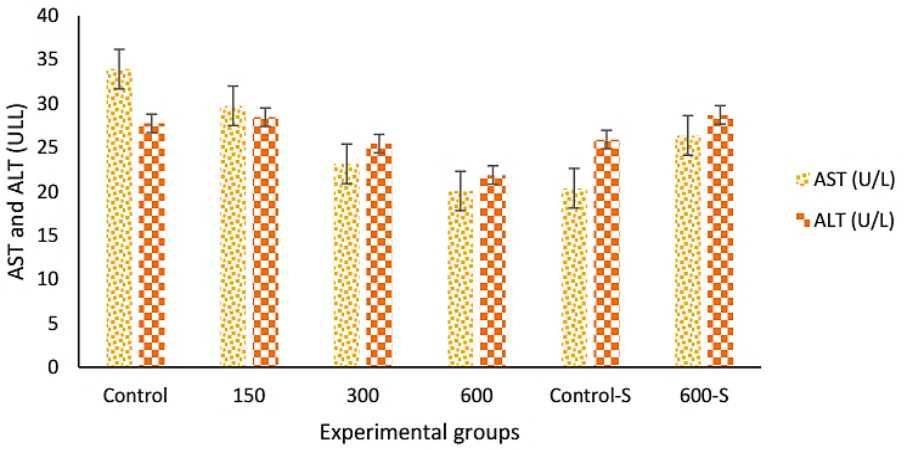

Fig. 4. AST and ALT activities of female Wistar rats submitted to 28-day treatment with an ethanol extract of the stem bark of *C. edulis* water 150, 300, and 600 mg/kg bw (n = 6)

Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract

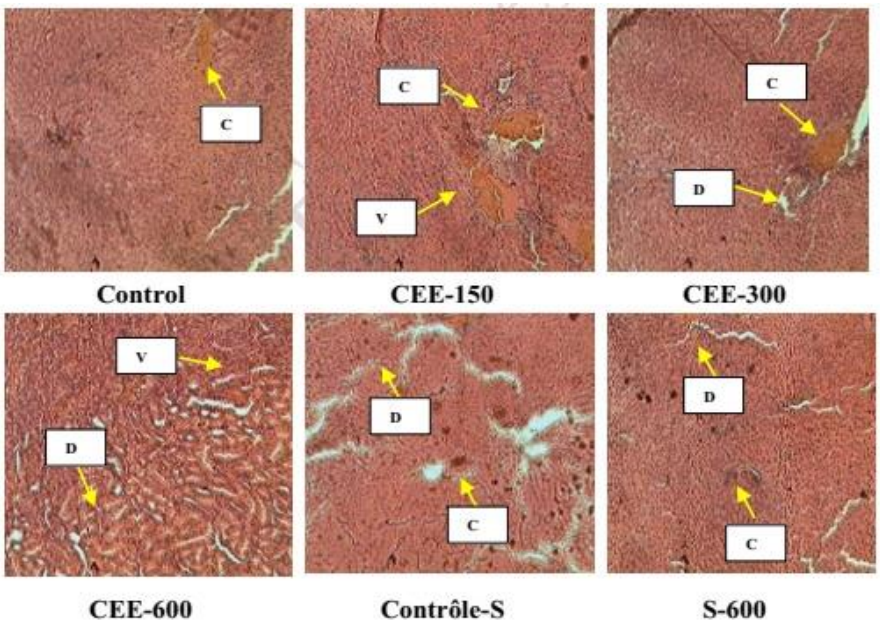

Fig. 5. Microphotograph of the liver tissue of male rats after 28 days of treatment with ethanolic extract of C. *edulis* Baill., [Staining with hematoxylin + eosin (x400)]

Legend: C: Central vein, D: Dilated hepatic sinusoids, V: Vein

Regarding the hematological parameters evaluated in male and female rats (Tables 7 and 8), subacute oral treatment with CEE did not induce any significant difference between the control and treated groups. However, some statistically significant differences were noted.

In male rats, we observed a significant increase in the volume of concentrated cells (MCV) at 600 mg/kg bw. Similarly, a significant decrease in mean corpuscular hemoglobin (MCHC) concentration was observed at 300 mg/kg bw ($p < 0.001$). A significant decrease in the mean number of monocytes and red blood cells at 600 mg/kg bw was noted in females receiving the CEE extract. A significant dose-dependent increase in hemoglobin level was also recorded, at 300 and 600 mg/kg body weight, compared to the control group.

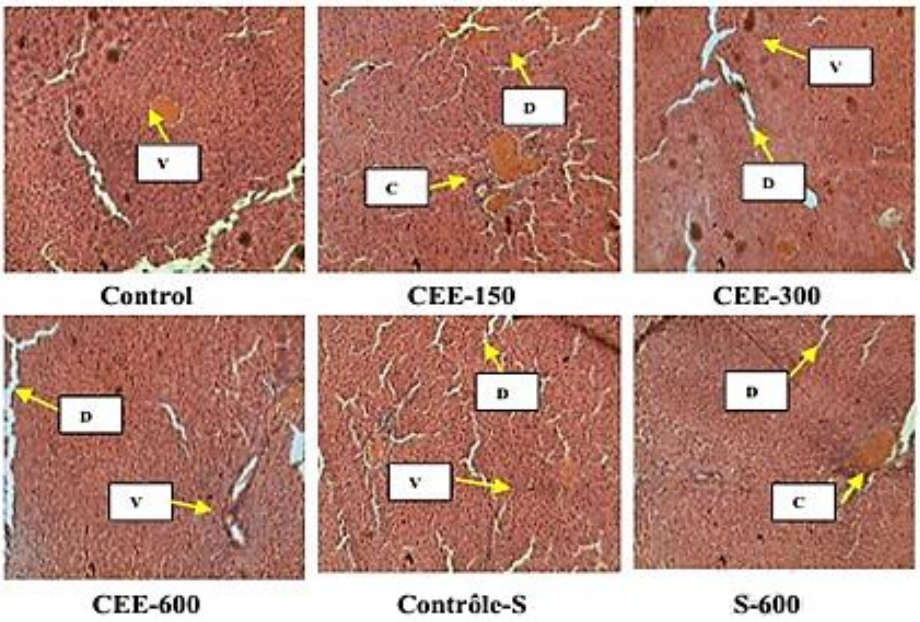

Fig. 6. Microphotograph of the hepatic tissues of rats after 28 days of treatment with ethanolic extract of *C. edulis* Baill., [Staining with hematoxylin + eosin (x400)]

Legend: C: Central vein, D: Dilated hepatic sinusoids, V: Vein

4. DISCUSSION

Given the therapeutic potential of *C. edulis*, in particular, through its ability to constitute an effective alternative for a significant number of diseases and infections [9], it seemed relevant to establish a profile of the safety of said plant to regulate its use in herbal preparations. This should help avoid exposing human subjects to potential risks of toxicity-related health problems when using *C*. *edulis*.

In the acute toxicity study, oral administration at a single dose of 2000 mg/kg bw of CEE did not induce mortality or real toxicological symptoms in animals throughout the observation phase. The approximate lethal dose of the extract would a priori be much greater than 2000 mg/kg bw. This finding therefore suggests that the extract at the limited dose tested is essentially non-toxic and harmless after oral administration. According to previous reports, CEE would be considered a practically non-toxic extract [12]. The absence of manifestations indicative of toxicity during acute oral administration of CEE may be correlated with poor absorption of the extract in the gastrointestinal tract, or high metabolic activity during the first pass through the liver, during this passage the toxic components would have been converted into slightly toxic derivatives [20] [21].

The subacute toxicity assessment study of CEE was performed in male and female rats to partially identify differences based on sex. Accordingly, no changes in animal attitudes and body weight were observed in male and female rats regardless of dose, suggesting that the 28-day treatment did not affect animal growth., we also noted that except for the ovary in the female rats treated at the highest dose (300 and 600 mg/kg bw), no significant difference was found in the weight of the organs of the treated rats compared to the control groups. Histology of the ovary was not performed. Hypotrophy of the ovaries can be an indicator of adverse effects [22], correlated with stress and hence a decrease in the activity of the hypothalamic-pituitary complex. Indeed, the ovaries are target organs of gonadal stimulating hormones because of their essential functions in the body's reproductive processes. The delayed decrease/increase in relative ovarian weight observed after the 28-day oral treatment suggests ovarian dysfunction [23] (Wilcox, 2005). However, changes in ovarian weight have fewer implications for toxicity due to the ovarian's limited role in removing harmful substances from the body [24] [25]. Therefore, it could be safely said that the liver and kidneys are the primary target organs in investigations related to the subacute oral toxicity of a plant extract.

Table 3. Effects of sub-acute oral administration of different doses of CEE on the relative weight of organs (g/100 g bw) in male Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
Liver	2.820 ± 0.186	2.865 ± 0.155	2.761 ± 0.199	2.857 ± 0.121	2.718 ± 0.119	2.600 ± 0.084
Kidneys	0.676 ± 0.016	0.643 ± 0.039	0.586 ± 0.031	0.646 ± 0.026	0.598 ± 0.026	0.596 ± 0.015
Adrenals	0.031 ± 0.010	0.029 ± 0.020	0.029 ± 0.004	0.027 ± 0.002	0.026 ± 0.003	0.025 ± 0.003
Heart	0.376 ± 0,024	0.362 ± 0.015	0.339 ± 0.026	0.355 ± 0.014	0.278 ± 0.015	0.321 ± 0.019
Spleen	0.238 ± 0.036	0.233 ± 0.008	0.189 ± 0.010	0.218 ±0.011	0.160 ± 0.016	0.228 ± 0.015
Testis	1.548 ± 0.029	1.503 ± 0.076	1.447 ± 0.055	1.535 ± 0.05	1.259 ± 0.095	1.260 ± 0.063
pancreas	0.329 ± 0.045	0.278 ± 0.049	0.210 ± 0.037	0.276 ± 0.046	0.217 ± 0.012	0.246 ± 0.037
Stomach	0.941 ± 0.059	0.694 ± 0.036	0.796 ± 0.040	0.822 ± 0.044	0.812 ± 0.119	0.776 ± 0.029
Lungs	0.601 ± 0.021	0.641 ± 0.042	0.597 ± 0.058	0.717 ± 0.058	0.610 ± 0.083	0.596 ± 0.057

Data are expressed as mean ± SEM (n = 6)

Table 4. Effect of the sub-acute administration of different doses of CEE on the relative weight of organs (g/100 g bw) in female Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
Liver	3.148 ±0.199	2.867 ± 0.122	2.838 ± 0.147	3.008 ± 0.169	2.849 ± 0.151	2.822 ± 0.063
Kidneys	0.629 ± 0.026	0.638 ± 0.018	0.598 ± 0.015	0.620 ± 0.063	0.665 ± 0.024	0.593 ± 0.016
Adrenals	0.029 ± 0.003	0.035 ± 0.007	0.083 ± 0.046	0.033 ± 0.003	0.036 ± 0.004	0.037 ± 0.001
Heart	0.389 ± 0.025	0.375 ± 0.047	0.373 ± 0.022	0.538 ± 0.148	0.359 ± 0.010	0.328 ± 0.010
Spleen	0.315 ± 0.079	0.235 ± 0.026	0.247 ± 0.021	0.231 ± 0.041	0.233 ± 0.026	0.253 ± 0.004
Ovary	0.078 ± 0.011	0.048 ± 0.012	0.056 ± 0.009 *	0.108 ± 0.049 *	0.068 ± 0.008	0.102 ± 0.012
pancreas	0.314 ± 0.054	0.345 ± 0.038	0.249 ± 0.022	0.432 ± 0.124	0.351 ± 0.139	0.192 ± 0.046
Stomach	1.093 ± 0.088	0.927 ± 0.055	0.936 ± 0.066	0.903 ± 0.094	0.846 ± 0.042	0.923 ± 0.034
Lungs	0.662 ± 0.032	0.545 ± 0.136	0.720 ± 0.045	0.793 ± 0.072	0.780 ± 0.157	0.693 ± 0.052

Data are expressed as mean ± SEM (n = 5)

** Significance against Control-S group: $p < 0.05$*

Table 5. Effect of the sub-acute oral administration of different doses of CEE on biochemical parameters of male Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
Glucose (mg/dL)	49.75 ± 3.47	48.00 ± 3.39	46.25 ± 6.13	56.75 ± 1.93	44.50 ± 4.02	49.25 ± 1.77
Triglycerides (mg/dL)	129.70 ± 17.34	133.60 ± 14.09	140.30 ± 20.12	129.30 ± 3.54	121.90 ± 13.67	128.30 ± 14.00
TC (mg/dL)	165.00 ± 16.68	161.60 ± 18.22	154.50 ± 16.97	176.00 ± 17.22	135.10 ± 10.94	148.90 ± 14.74
HDL (mg/dL)	92.22 ± 13.00	75.24 ± 7.76	50.16 ± 13.98	80.49 ± 16.42	75.64 ± 5.69	58.45 ± 6.57
LDL (mg/dL)	46.86 ± 11.08	63.01 ± 15.00	76.29 ± 10.44	69.60 ± 3.57	35.08 ± 6.79	49.86 ± 19.09
Creatinine (mg/dL)	1.20 ± 0.15	1.10 ± 0.07	1.23 ± 0.16	1.34 ± 0.14	1.04 ± 0.09	1.11 ± 0.14
Urea (mg/dL)	2.56 ± 0.13	2.45 ± 0.13	2.23 ± 0.14	2.17 ± 0.10	2.50 ± 0.11	2.20 ± 0.11
Total protein (mg/mL)	46.14 ± 2.56	48.20 ± 1.89	49.06 ± 3.52	60.66 ± 3.72 **	50.11 ± 1.51	50.56 ± 2.08

Data are expressed as mean ± SEM (n = 5)
*** Significance against Control-S group: p < 0.01*

Table 6. Effect of the sub-acute oral administration of different doses of CEE on biochemical parameters of female Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
Glucose (mg/dL)	49.25 ± 1.28	50.00 ± 2.47	45.75 ± 3.51	47.25 ± 1.157	47.50 ± 2.84	46.75 ± 2.35
Triglycerides (mg/dL)	141.10 ± 12.40	132.20 ± 16.82	135.60 ± 19.10	160.60 ± 18.39	157.50 ± 20.23	150.60 ± 13.66
TC (mg/dL)	164.40 ± 13.46	168.50 ± 14.15	168.10 ± 30.74	196.40 ± 34.01	173.90 ± 8.59	188.30 ± 23.37
HDL (mg/dL)	75.64 ± 5.69	74.63 ± 2.72	72.40 ± 7.27	71.19 ± 11.53	96.67 ± 11.05	83.73 ± 13.50
LDL (mg/dL)	60.57 ± 13.64	67.40 ± 12.91	68.55 ± 23.21	93.08 ± 24.57	45.76 ± 10.05	57.93 ± 15.43
Creatinine (mg/dL)	0.99 ± 0.13	1.28 ± 0.19	1.14 ± 0.26	1.42 ± 0.27	1.24 ± 0.14	1.43 ± 0.19
Urea (mg/dL)	2.24 ± 0.19	1.97 ± 0.06	2.16 ± 0.09	2.48 ± 0.13	2.19 ± 0.17	2.29 ± 0.13
Total protein (mg/dL)	55.56 ± 5.77	54.93 ± 2,18	55.36 ± 2.97	58.99 ± 2.25	56.93 ± 2.63	57.21 ± 0.92

Data are expressed as mean ± SEM (n = 6)

Table 7. Effects of the sub-acute oral administration of different doses of CEE on hematological parameters of male Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
WBC ($x10^3$ μL^{-1})	6.25 ± 0.49	7.69 ± 0.76	7.22 ± 0.69	6.51 ± 1.03	8.24 ± 0.63	8.61 ± 0.51
Lymphocytes (%)	62.73 ± 2.20	66.00 ± 1.21	65.03 ± 1.04	62.88 ± 2.63	54.40 ± 2.44	65.30 ± 3.84
Monocytes (%)	1.60 ± 0.57	0.93 ± 0.21	1.53 ± 0.45	1.68 ± 0.55	8.18 ± 5.29	1.18 ± 0.33
Granulocytes (%)	33.08 ± 1.31	31.05 ± 0.95	30.10 ± 0.61	32.40 ± 2.05	34.80 ± 6.29	31.35 ± 3.56
RBC ($x10^6$ μL^{-1})	9.58 ± 0.31	9.18 ± 0.08	9.81 ± 0.21	9.51 ± 0.39	9.47 ± 0.13	8.87 ± 0.19
Haematocrit (%)	51.65 ± 1.86	51.95 ± 0.47	54.50 ± 1.63	52.03 ± 1.51	53.68 ±.1.01	52.50 ± 1.41
Haemoglobin (g/dL)	16.65 ± 0.39	15.63 ± 0.12	15.98 ± 0.35	15.95 ± 0.55	15.68 ± 0.16	15.18 ± 0.46
MCV (fL)	53.93 ± 0.63	56.58 ± 0.16	55.53 ± 0.66	54.85 ± 1.16 ***	56.70 ± 0.58	59.23 ± 0.71
MCH (pg)	17.40 ± 0.24	17.05 ± 0.16	16.30 ±.0.10	16.75 ± 0.14	16.60 ± 0.19	17.10 ± 0.35
MCHC (g/dL)	32.33 ± 0.59	30.1±0.24	29.38 ± 0.32 ***	30.63 ± 0.55	29.23 ± 0.4386	28.93 ± 0.28
Platelets ($x10^3$ μL^{-1})	778.3 ± 78.03	660.3±130.30	921.30 ± 95.78	852.00 ± 48.72	1041.00 ± 25.61	1030.00 ± 102.80

Data are expressed as mean ± SEM (n = 5). WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration
**** Significance against Control-S group: p < 0.001*

Table 8. Effects of the sub-acute oral administration of different doses of CEE on hematological parameters of female Wistar rats

	Control	CEE (mg/kg bw)			Satellite groups	
		150	300	600	Control-S	600-S
WBC ($x10^3$ μL^{-1})	5.88 ± 1.49	9.79 ± 1.76	6.51 ± 0.45	5.43 ± 0.95	6.01 ± 0.94	8.48 ± 0.91
Lymphocytes (%)	62.00 ± 7.56	50.98 ± 3.84	61.23 ± 2.01	65.25 ± 5.18	64.98 ± 0.84	46.53 ± 4.77
Monocytes (%)	0,83 ± 0,09	2.08 ± 0.69	1.13 ± 0.27	4.03 ± 1.22 *	1.62 ± 0.55	4.10 ± 0.54
Granulocytes (%)	33.13 ± 6.91	41.20 ± 2.36	34.00 ± 1.843	27.70 ± 5.82	30.30 ± 0.68	46.20 ± 5.06
RBC ($x10^6$ μL^{-1})	7,43 ± 0.84	8.59 ± 0.64	9.38 ± 0.16	9.95 ± 0.15 **	9.28 ± 0.18	8.71 ± 0.33
Haematocrit (%)	12,30 ± 1.28	14.85 ± 0.78	15.93 ± 0.20	16.48 ± 0.15	15.83 ± 0.18	14.95 ± 0.69
Haemoglobin (g/dL)	12,30 ± 1.27	14.85 ± 0.78	15.93 ± 0.20 *	16.48 ± 0.15 **	15.83 ± 0.18	14.95 ± 0.69
MCV (fL)	55,48 ± 2.02	58.50 ± 0.88	56.68 ± 0.52	55.63 ± 0.31	59.23 ± 0.30	56.63 ± 1.03
MCH (pg)	16,70 ± 0.25	17.53 ± 0.59	16.98 ± 0.11	16.58 ± 0.21	17.30 ± 0.20	17.13 ± 0.27
MCHC (g/dL)	30,08 ± 0.78	29.93 ± 0.60	29.95 ± 0.15	29.63 ± 0.43	28.80 ± 0.21	30.35 ± 0.89
Platelets ($x10^3$ μL^{-1})	662,80 ± 73.41	906.30 ± 18.69	865.50 ± 105.00	769.50 ± 55.79	910.30 ± 52.29	1074.00 ± 192.20

Data are expressed as mean ± SEM (n = 6). RBC: red blood cells, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular: hemoglobin concentration
** Significance against control group: p < 0.05. *Significance against Control-S group: p < 0.05*
*** Significance against Control-S group: p < 0.01*

The analysis of the biochemical parameters combined with the evaluation of the toxic effects of a substance on specific tissues, such as the kidneys and the liver, can provide useful information about the mechanisms of toxicity of a sample [26]. One of the important findings of biochemical analyses was an increase in the plasma total protein content in male rats. One of the most common markers used to assess hepatocellular damage is total protein content. This parameter may reflect a subject's nutritional status, and to some extent may be a nonspecific marker of kidney damage, liver disease, and other chronic conditions [27]. The observation of high levels of total protein is frequently observed in situations of chronic inflammation or liver infections [28].

Despite being the largest of the glands, the liver is one of the organs most affected by a substance's toxicity. By acting as a censor for any molecule absorbed from the intestinal lumen, the liver is exposed to a daily alteration that is sometimes irreversible. That said, it is therefore logical that the liver tissue samples were selected as part of the histological analyses. Although the observation of the histological sections of the liver showed minor changes in the number and the staining of the hepatic sinusoids, it goes without saying that it is more the absence of lesions at the level of the hepatic tissues of the exposed rats to treatment that commands attention.

Levels of transaminases such as AST or ALT can be correlated with a significant increase in tissue damage in an unfavorable environment (Crook, 2006). Plasma activity levels of ALT and AST are frequently used as markers sensitive to possible tissue damage, especially liver toxicity [29]. In addition to accounting for 80% and 20% of the total intracellular enzymes of hepatic mitochondria and hyaloplasmique, respectively, these enzymes are also found in the heart, skeletal muscles, kidneys, brain, pancreas, and blood cells [30]. Making transaminases significant indicators of peripheral toxicity. In this study, the differences in transaminase activities observed after CEE administration for 28 days were not significant compared to the control group. This implies that the CEEs administered are hardly hepatotoxic at the doses administered. The slight but insignificant decrease in blood levels of AST and ALT could probably be due to the different active ingredients present in the extract. Indeed, many studies have reported that polyphenols such as punicalagin and punicalin protect the liver of rats against liver damage, an effect marked by the decrease in plasma levels of AST and ALT [31].

In the same vein, note that many bioactive compounds such as flavonoids have shown effects protectors on the liver in rodents by significantly reducing or inhibiting the elevation of plasma transaminase levels [32] [33] [34].

Abnormally elevated plasma creatinine and urea levels are associated with marked impairment of nephron function [35], and even renal failure [7]. Regarding our results, urea and creatinine levels were slightly altered in male and female-treated rats compared to their respective controls. However, the values obtained remained within the recommended range for each of these parameters, effectively excluding the possibility of CEE to induce renal dysfunction. In other words, these results suggest that subacute administration of CEE did not affect renal function. Oral administration of CEE at rates up to 600 mg/kg/day for 28 days was not associated with any biologically significant adverse effects as illustrated by analysis of several biochemical and physiological parameters.

Plasma levels of CT, and TG may under certain circumstances be markers of impaired liver function. Our results suggest that the subacute administration of CEE insignificantly altered lipid indices, including TG, TC, and LDL levels. Increases in LDL cholesterol are often associated with slight decreases in HDL cholesterol and our results tend to follow this rule. HDL cholesterol is known to be an excellent reverse predictor of the development of cardiovascular disease. Although the observed increase in HDL cholesterol content is not significant, the increase in this parameter has been identified as a key factor in the etiology of coronary heart disease [36]. Also, the TC, TG, and glucose levels were not significantly altered in animals treated with CEE. All these results which, by following in close continuity with similar studies carried out in the past such as the work of [37] Ekpo and Eddy in 2005, seem to confirm the lipotropic nature of CEE.

Proteins from organ damage combined with the release of inflammatory mediators very often modify hematological variables, making blood one of the major target tissues for the expression of a substance's toxicity. The results obtained show that most of the values observed in the treated groups were normal in comparison with

the control group. However, some values were significantly different from those of the control group. This is the case with MCV and MCHC in males. Although our results suggest a significant decrease in the MCHC variable, the absence of a significant change in the total number of red blood cells suggests that CEE-based treatment induced the differentiation of hematopoietic cells by stimulating the differentiation of lymphocyte subpopulations, without increasing the rate of cell divisions. According to previous reports, immunostimulating plants generally induce B lymphocyte maturation and blood cell proliferation [38] [39]. In addition, the significant increase in CVD tends to reinforce this observation.

The hematological tests carried out in the females showed a non-significant increase in the level of hemoglobin but also the rate of red blood cells. Analysis of the variation in these markers seems to indicate that *C. edulis* extract promotes the production of hemoglobin and its concentration in red blood cells [20] [40]. This result gives CEE an interesting potential in the management of anemia. In other words, *C. edulis* extract improves the oxygen-carrying capacity of the blood [1]. A significant increase in the level of monocytes was also recorded at 300 mg/kg suggesting that at this dose the administration of CEE improves the production of monocytes by stimulating their amplification. These results, in line with previous data, show that *C. edulis* contains bioactive compounds such as saponins, a family of secondary metabolites endowed with immunostimulatory properties [41] [42].

Of the remaining hematologic parameters, one can report the relative increases in hematocrit and platelet counts and relative reductions in MCH levels in the male test groups. The same trend was observed in the groups of female rats treated for hematocrit, platelet, and lymphocyte levels. A high hematocrit level is correlated with a reliable rate of sedimentation and therefore the absence of an inflammatory state [43] [44] [45]. Although not significant for the most part, the results obtained show more pronounced effects in treated female rats compared to males. Results that would justify the involvement of estrogen in the activity of hematopoietic stem cells.

Given the liver photographs (Figs. 5 and 6) obtained after histopathology analyses, it appears that the appearance of the liver tissues of male and female rats did not show a real difference with the control groups regardless of the dose [46]. Tissues close to the central lobule and the central and hepatic veins have been particularly examined [47]. Due to their proximity to the vascular network, these sites often constitute in many ways a prime point of leukocyte infiltration and thus the starting point for hepatitis.

5. CONCLUSION

This being said a single administration at a dose of 2000 mg/kg bw by the oral route of ethanolic extract of *C. edulis* stem bark did not induce convincing signs of toxicity in the organs studied, let alone of the deceased. Daily oral administration of *C. edulis* extract for 28 days resulted in minor increases in urea and creatinine levels, but no real changes in transaminase activity or lipid markers. Although minor effects were recorded for MCV, MCHC in males, monocytes, hemoglobin, and RBC in females, oral administration of *C. edulis* did not affect the markers overall. hematological. The absence of cases of toxicity in both males and females would be the result of low involvement of reproductive hormones. The richness of CEE in secondary metabolites would support the good character of the results obtained.

CONSENT

It is not applicable.

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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