Original Research Article

Acute and Sub-chronic toxicity evaluation of the ethanolic extract of Coula edulis B., (Olacaceae) stem bark

ABSTRACT

The abstract should be concise and informative. It should not exceed 300 words in length. It should briefly describe the purpose of the work, techniques and methods used, major findings with important data and conclusions. Different sub-sections, as given below, should be used. No references should be cited in this part. Generally non-standard abbreviations should not be used, if necessary they should be clearly defined in the abstract, at first use.

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. The aim of this study was to assess the safety of the ethanolic extract of *C. edulis* stem bark (CEE).

Study design:pharmacological study.

Place and Duration of Study: Laboratory of Nutrition and Nutritional sciences and 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 in accordance with guidelines 423 of the Organization for Economic Co-operation and Development. The acute toxicity study consisted of administering orally, to a group of young female rats, a single dose of (2000 mg/kg) of plant extract to be tested. The subacute toxicity of the sample was assessed over a 28 days' period using repeated doses in accordance with OECD Guideline 407.

Results: No cases of death and clinical signs of toxicity were observed in the treated rats in the two studies conducted. 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 haemoglobin concentration decreased at the 300 mg/kg dose. In females, a significant increase in the number of monocytes, red blood cells and haemoglobin 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. However, this plant extract can be safely recommended for therapeutic use.

***Key words**: 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 pharmacopoeia, 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 ethno-botanists 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 pharmacopoeia drugs by the population is without a recommended dose and some of these plants have been shown to be 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 with a view to integrating the observations of short and long-term manifestations of toxicity and establishing 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 25m 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 anaemia, back pain or sore kidneys [10]. In other countries, local populations use this plant in the treatment of gynaecological diseases, indigestion and against trauma [11], but also in the management of gastrointestinal infections, anaemia, diarrhoea, 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, Candidaalbicans*[9], as well as antiplasmodial activity [13].

Despite the widespread use of this plant in nutrition and 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). All animal experiments were conducted in accordance with the ethical guidelines outlined in the Guide to the Care and Use of Laboratory Animals. The practices carried out on animals and the related protocols have been approved by the animal protection and use committee of the institutional ethics committee of the Cameroonian Ministry of Scientific Research and Technological Innovation.

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 Center 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 of (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 in accordance with 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,

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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. Prior to 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, 6 hours after treatment and then daily for a total of 14 days. During this period, changes in behaviour and other parameters such as body weight, urination, water consumption, food intake, convulsions, breathing, lethargy, temperature, constipation, changes in eye and skin colour, 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 anaesthesia (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 a 28 days' period using repeated doses in accordance with 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), 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 in order 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 anaesthesia (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 haematological and biochemical parameters. The heart, liver, kidneys, stomach, spleen and lung were dissected, weighed 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.

2.5. Blood analysis

Part of the blood sample was collected in EDTA tubes for haematological 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.

Haematology analysis of blood samples was performed using a Humacount 30TS automated haematology analyser from Human Diagnostics Worldwide (Wiesbaden, Germany). Among the parameters evaluated were: the number of red blood cells (RBC); haematocrit (Ht); haemoglobin (Hb); mean corpuscular volume (VCM); mean corpuscular haemoglobin (MCH); mean concentration of corpuscular haemoglobin (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 in 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 haematoxylin 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 comparison, 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 AND DISCUSSION

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 colour of the faecal 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).

Table 1: General appearance and behavioural 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
Faecal colour	No effect	Observed
Diarrhoea	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).

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 Figures 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.

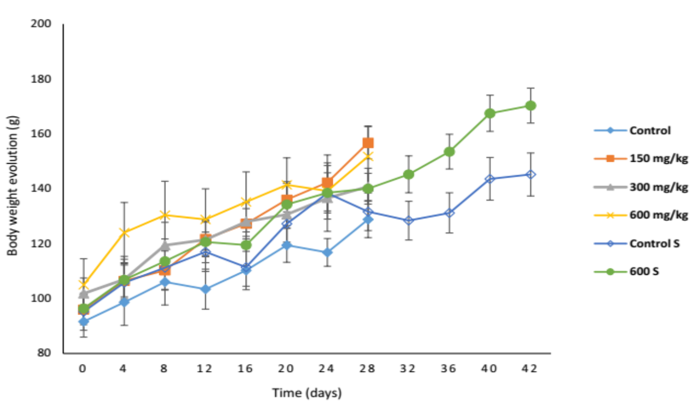

Fig. 1. Body weight evolution of male Wistar rats submitted to a 28-day treatment with an ethanol extract of the stem bark of *C. edulis* at 150, 300 and 600 mg/kg bw (n = 6). Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract.

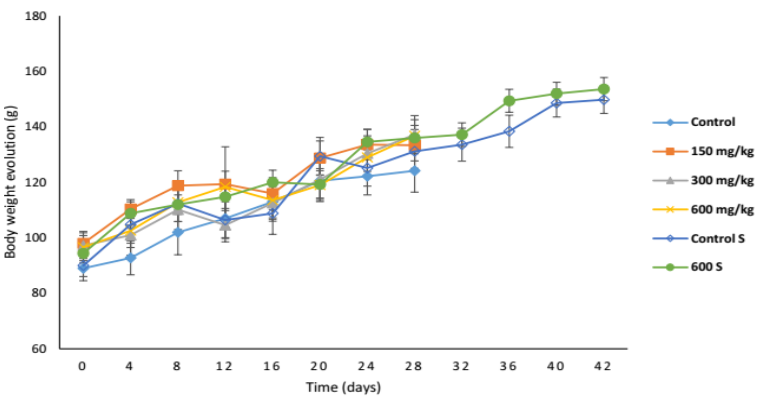

Fig. 2. Body weight evolution of male (A) and female (B) Wistar rats submitted to a 28-day treatment with an ethanol extract of the stem bark of *C. edulis* at 150, 300 and 600 mg/kg bw (n = 6). Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract.

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

		CEE (mg/kg bw)			**Satellite groups**	
	Control	**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-chronic administration of different doses of CEE on relative weight of organs (g/100 g bw) in female Wistar rats.

		CEE (mg/kg bw)			Satellite groups	
	Control	**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.*

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 male and female, at different doses after oral treatment with CEE (Fig. 5 and 6)).

Table5: Effect of the sub-chronic oral administration of different doses of CEE on biochemical parameters of male Wistar rats.

		CEE (mg/kg bw)			Satellite groups	
	Control	**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-chronic oral administration of different doses of CEE on biochemical parameters of female Wistar rats.

		CEE (mg/kg bw)			Satellite groups	
	Control	**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	0.99 ± 0.13	1.28 ± 0.19	1.14 ± 0.26	1.42 ± 0.27	1.24 ± 0.14	1.43 ± 0.19

(mg/dL)						
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).

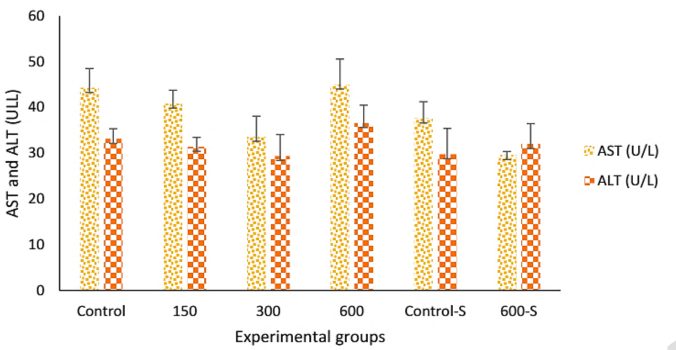

Fig. 3. AST and ALT activities of male Wistar rats submitted to a 28-day treatment with an ethanol extract of the stem bark of *C. edulis* water 150, 300 and 600 mg/kg bw (n = 6). Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract.

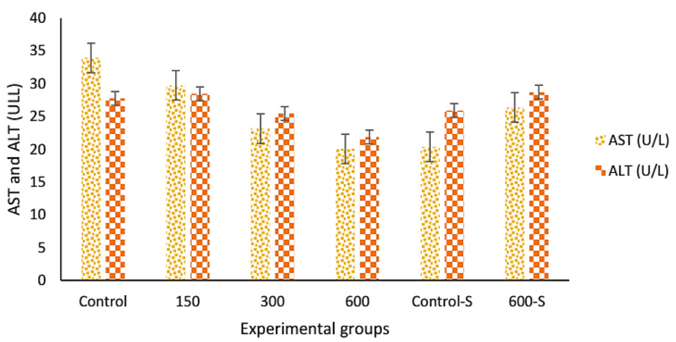

Fig. 4. AST and ALT activities of female Wistar rats submitted to a 28-day treatment with an ethanol extract of the stem bark of *C. edulis* water 150, 300 and 600 mg/kg bw (n = 6). Each point represents the mean ± standard error of the mean (SEM). Control-S: satellite control treated with distilled water, 600-S: satellite of top dose treated with 600 mg/kg of extract.

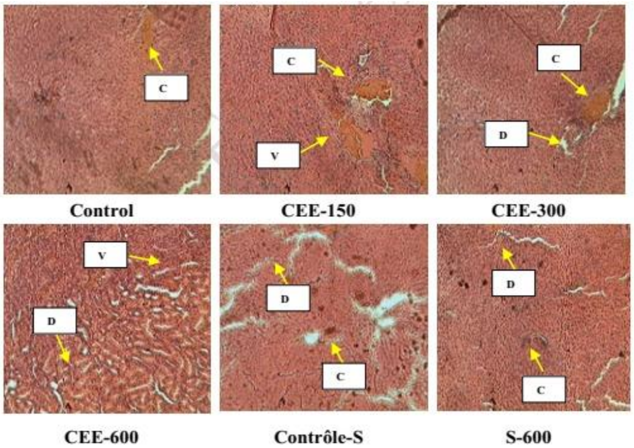

Fig. 5: Microphotograph of the liver tissue of male rats after 28 days of treatment with ethanolic extract of C. *edulis* Baill., [Staining with haematoxylin + eosin (x400)]
*Legend: **C**: Central vein, **D**: Dilated hepatic sinusoids, **V**: Vein*

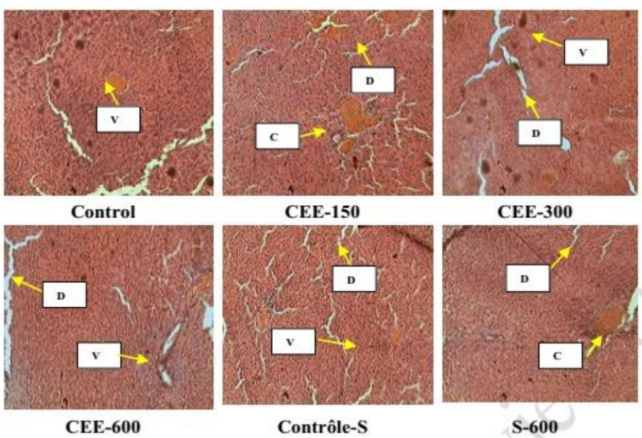

Fig. 6: Microphotograph of the hepatic tissues of rats after 28 days of treatment with ethanolic extract of *C. edulis* Baill., [Staining with haematoxylin + eosin (x400)]
*Legend: **C**: Central vein, **D**: Dilated hepatic sinusoids, **V**: Vein*

Regarding the haematological 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 haemoglobin (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 haemoglobin level was also recorded, at 300 and 600 mg/kg body weight, compared to the control group.

Table 7: Effects of the sub-chronic oral administration of different doses of CEE on haematological parameters of male Wistar rats.

	Control	CEE (mg/kg bw) 150	300	600	Satellite groups 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 haemoglobin, MCHC: mean corpuscular haemoglobin concentration.
**** Significance against Control-S group: $p < 0.001$.*

Table 8: Effects of the sub-chronic oral administration of different doses of CEE on haematological parameters of female Wistar rats.

	Control	CEE (mg/kg bw) 150	300	600	Satellite groups 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 haemoglobin, MCHC: mean corpuscular: haemoglobin concentration.
** Significance against control group: $p < 0.05$. *Significance against Control-S group: $p < 0.05$,*
*** Significance against Control-S group: $p < 0.01$.*

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.

4. Discussion

In view of 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 safety of said plant in order 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 limit 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](Bukola *et al*., 2008). 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] (Bello *et al.,* 2015) Johnnson.

Comment [et5]: review the citation insertion method

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 behaviour and body weight were observed in male and female rats regardless of dose, suggesting that the 28-day treatment did not affect animal growth. Also, we also noted that with the exception of 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. It goes without saying that hypotrophy of the ovaries can be an indicator of adverse effects [22](Tahraoui *et al.,* 2010), correlated with stress and hence a decrease in the activity of the hypothalamo-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 less implications for toxicity due to the ovarian's limited role in removing harmful substances from the body [24][25](Sellers *et al.,* 2007; Greaves, 2011). 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.

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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](Yamthe *et al.,* 2012). 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](Frenzel *et al.,* 2016). The observation of high levels of total protein is frequently observed in situations of chronic inflammation or liver infections [28](Djami *et al.,* 2011).

Levels of transaminases such as AST or ALT can be correlated with a significant increase in tissue damage in an unfavourable environment (Crook, 2006). Plasma activity levels of ALT and AST are frequently used as markers sensitive to possible tissue damage, especially liver toxicity [29] (Ramaiah, 2007). 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](Mistry *et al.,* 2013). 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](Lin *et al.,* 1998).

In the same vein, note that flavonoids and isoflavonoids such as genistein [32] (Huang *et al.,* 2013), quercetin [33](Janbaz et al., 2004) and luteolin [34](Lee *et al.,* 2011) have shown effects protectors on the liver in rodents by significantly reducing or inhibiting the elevation of plasma transaminase levels.

Abnormally elevated plasma creatinine and urea levels are associated with marked impairment of nephron function [35](Lameire *et al.,* 2005), and even renal failure [7](Prasanth *et al.,* 2015). 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, 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](Koba *et al.,* 2006). 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 haematological 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](Chen *et al.,* 2008; Goetz, 2004). In addition, the significant increase in CVD tends to reinforce this observation.

The haematological tests carried out in the females showed a non-significant increase in the level of haemoglobin but also that of the rate of red blood cells. Analysis of the variation in these markers seems to indicate that *C. edulis* extract promotes the production of haemoglobin and its concentration in red blood cells [20][40] Mbaka (Bello *et al*., 2015). This result gives CEEs an interesting potential in the management of anaemia. In other words, *C. edulis* extract improves the oxygen carrying capacity of the blood [1](Adinortey *et al.,* 2012). 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](Nalbantsoy *et al.,* 2012)Ugwah.

Of the remaining hematologic parameters, one can report the relative increases in haematocrit 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 haematocrit, platelet and lymphocyte levels. A high haematocrit level being correlated with a reliable rate of sedimentation and therefore the absence of an inflammatory state [43][44] [45] (Mensah *et al.,* 2019)El-Baz Unuofin. 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 oestrogen in the activity of hematopoietic stem cells.

In view of the liver photographs (Fig 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] Bariweni. Tissues close to the central lobule and the central and hepatic veins have been particularly examined [47](Epoh *et al.,* 2019). 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.

4. 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 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, haemoglobin and RBC in females, oral administration of *C. edulis* did not affect the markers overall. haematological. 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.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

"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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