Cameroon Journal of Experimental Biology (2024) Vol. 18, N°01, 32-40. DOI: https://dx.doi.org/10.4314/cajeb.V18i1.5

CAFOBIOS Cameroon Forum for Biological Sciences

Cameroon Journal of Experimental Biology

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Acute and sub-chronic toxicity screening of aqueous and ethanolic extracts of *Corynanthe pachyceras* K. Schum (Rubiaceae), a Sub-Sahara African aphrodisiac plant

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Keywords

Corynanthe pachyceras;
Acute toxicity;
Sub-chronic toxicity;
Mice, rats.

Historic

Received : 27 January 2024
Received in revised form : 12 March 2024
Accepted : 04 May 2024

Abstract

Objective: This study investigated the acute and sub-chronic toxicities of the aqueous (AE) and ethanolic extract (EE) from *Corynanthe pachyceras*. Methods: Acute and sub-chronic toxicity tests were carried out following the 425 and 407 Organization for Economic Cooperation and Development (OECD) guidelines respectively. For the acute toxicity study, mice were randomly divided into groups treated with a single dose of distilled water (10 ml/kg) or the single reference dose (2000 mg/kg) of aqueous or ethanolic extract of *C. pachyceras*. Lethal dose 50 (LD50) was determined after 24h. Mice were individually checked for any toxicity sign and mortality over a 14-day period. For the sub-chronic toxicity study, young rats orally received repeated doses of each extract (50, 150 or 450 mg/kg) during 28 days. Body weight was taken every 2 days. After sacrifice, organ weights, hematological, biochemical and histopathological markers were measured. Results: The LD50 of *C. pachyceras* extracts was found to be greater than 2000 mg/kg in the acute toxicity study. In the sub-chronic toxicity test, males receiving the high dose (450 mg/kg) of both extracts significantly showed an increase ($P<0.05$-0.001) in immune cells. In rats treated with the low (50 mg/kg) and medium (150mg/kg) doses of the ethanolic extract of *C. pachyceras*, activity of ALAT and, concentrations of direct bilirubin, creatinine and total cholesterol were significantly increased ($P<0.05$-0.0001) when compared with control rats. *C. pachyceras* extracts did not alter the histology of the vital organs studied. Conclusion: *C. pachyceras* can be considered as non-toxic, but low doses are recommended to avoid possible chronic side effects.

1. Introduction

Traditional medicines or therapies are widely used in African countries to meet healthcare needs, especially in rural regions. According to the World Health Organization, approximately 80% of the population of developing countries rely on traditional medicine for the management of several health problems [1]. Health issues include among others, diabetes mellitus, hypertension or erectile dysfunctions. *Corynanthe pachyceras* is a medicinal plant used in Sub-Sahara Africa for the treatment of genitourinary infections. This plant is moreover thought to exhibit aphrodisiac effects [2]. If medicinal plants are by far the first source of drugs to local populations and pharma industries due to their diverse active molecules and large action spectrum, some of these plants possess noticeable toxic adverse effects [3,4,5].

Toxicity studies are essential to guarantee the safety of any product used for health purposes. Thus, toxicity studies of medicinal plants are essential to prevent any unwanted/side effects to human or animals [6]. Reports have evidenced that some medicinal plants can cause anemia (*Pteriddium aquilinum*), respiratory distress and coma (*Arisaema eunaephyllum)* or abortion and congenital malformations (*Nicotina tabacum)* in pregnant women [7].

Corynanthe pachyceras (Rubiaceae) is a lower-storey forest tree growing in Sub-Sahara Africa. It is used in the Centre Region of Cameroon as local anesthetic, for sexual vitality and libido enhancement or against erectile dysfunctions and genito-urinary infections (Adu *et al.*, 2009). Previous scientific studies have also shown that this plant is effective in the treatment of boils, chronic wounds and possessed anti-infective potentials [2]. To the best of our knowledge, no reference study on the toxicological profile of extracts from the barks of *C. pachyceras* is available. Present study was therefore aimed at evaluating the safety potential of the aqueous and ethanolic extracts of *C. pachyceras* using mice *and rats.*

2. Materials and Methods

2.1. Plant collection

Fresh barks from the trunk of *Corynanthe pachyceras* K. Schum (Rubiaceae) were harvested in May 2020 at Ngoumou in the Mefou-Akono Dvision, Centre Region of Cameroon. The plant sample was authenticated at the Cameroon National Herbarium in comparison to the specimen deposited under the voucher number 105025Rf cam. The plant material was then shade-dried and grinded prior to extract preparations.

2.2. Preparation of the aqueous and ethanolic extracts

The aqueous extract of *C. pachyceras* was prepared by macerating 250 g of powder in 1000 mL of distilled water for 72 h with occasional stirring. After filtration using a fine cotton thread and Whatman paper N°4, the filtrate was oven-dried (56°C) to obtain 21 g of aqueous extract (AE), giving an extraction yield of 8.4%.

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The ethanolic extract (EE) was obtained after maceration of 250 g of *C. pachyceras* powder in 1000 mL ethanol for 72 hours at room temperature. After filtration, the filtrate was evaporated using a rotary evaporator (75°C) under reduced pressure and 21.49 gr of ethanolic extract (EE) were obtained (8.6%).

2.3. Animals

Apparently healthy Swiss albino mice (25-30 g) and young male and female (nulliparous) Wistar rats (60-90 g), aged 6-7 weeks, were obtained from the animal house of the Department of Animal Biology, Faculty of Science of the University of Dschang, Cameroon. They were housed separately in plastic cages (4/cages) at room temperature under natural light/dark cycle, with free access to food and water.

2.4. Acute oral toxicity study

The acute oral toxicity of *C. pachyceras* extracts was assessed according to the Organization for Economic Cooperation and Development, guideline test No 425 [8].

A total of 30 healthy Swiss albino mice, consisting of 15 males and 15 females were randomly divided into 3 groups of 10 animals each (5 males and 5 females). Group 1 served as control and received distilled water at 10 ml/kg; groups 2 and 3 were the test groups receiving *per os* the limit dose of 2000 mg/kg of the aqueous or ethanolic extract of *C. pachyceras*. The treatment was first administered to two animals of either sex in each group after six hours of fasting. All treated animals were closely followed during six hours post-treatment. In case of no death recorded, the remaining mice were equally given a single dose of the corresponding treatment and the entire pool was followed for fourteen days during which toxicity signs such as changes in eye colour, abnormal food and water consumptions, stool condition (granular or liquid) and skin colour were noted. Body weight changes were also recorded daily.

2.5. Sub-chronic toxicity study

Sub-chronic toxicity was conducted following OECD guideline test No 407 [9]. The aim of this test was to evaluate the adverse effects associated to repeated oral administration of the plant extracts.

In accordance with the guideline, seventy rats (70), including 35 males and 35 females, were divided into 7 groups of 10 rats each (5 males and 5 females). Group 1, the control group, was treated with distilled water at 10 ml/kg. Groups 2-4 were orally treated with the aqueous extract at 50, 150 or 450 mg/kg, respectively. Similarly, groups 5-7 received the ethanolic extract at 50, 150 or 450 mg/kg. All animals were daily treated for 28 days through oral gavage during which signs of toxicity were inspected and body weight monitored. At the end of the treatment period, animals were sacrificed and samples were collected for weight, histological and haematological assessments.

Doses of plant extract were chosen based on our preliminary study from which the therapeutic dose in rats was found to be 50 mg/kg for each extract (Unpublished data). From there, two other doses (150 and 450 mg/kg) were selected for this study.

2.5.2. Sacrifice and sample collection

Twenty-four hours after the last treatment, animals were sacrificed under diazepam (10 mg/kg) / ketamine (50 mg/kg) anaesthesia. Blood was collected by abdominal artery catheterization and kept in ethylenediamine tetra acetic acid (EDTA) and heparinized tubes. Heparinized blood was centrifuged (3,000×g for 10 min) to obtain plasma and stored at −20°C for analysis of [alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatinine, albumin, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), proteins, total and direct bilirubin]. The liver, kidneys, heart, and spleen were also collected, cleaned of adherent tissues, rinsed in saline (NaCl, 0.9%) weighed and kept in 10% formaldehyde solution for histopathological examinations.

2.5.3. Measurement of hematological and biochemical parameters

Red Blood Cell (RBC) and White Blood Cell (WBC) counts, Hemoglobin (Hgb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets (PLT), Mean Platelet Volume (MPV), Lymphocyte count (LY), Granulocyte count (GRA), Percentage of Lymphocytes (LY), Granulocytes (GRA) and Platelets (PLT) were estimated using an automated hemocytometer (PCE 210N PLC from ERMA Inc, Tokyo).

Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), albumin, total and direct bilirubin, triglycerides ("SGM Italia"), creatinine, total cholesterol HDL cholesterol, LDL cholesterol, total proteins (LABKITS) were measured in plasma using corresponding manufactured kits. The procedure was done according to the manufacturers' instructions.

2.5.4. Histopathological analysis

Kidney, liver, heart and spleen samples were used for histopathological examinations. Briefly, tissues were washed in normal saline and fixed immediately in 10% for at least 24h, then dehydrated with alcohol and embedded in paraffin. Pieces of 5 μm thick sections were prepared and stained with hematoxylin and eosin for nucleus and cytoplasm coloration respectively before microscopic analysis.

2.6. Data analysis

Data were presented as mean ± SEM (standard error of the mean). Weight and biochemical parameters were analyzed using one-way ANOVA, followed by Tukey HSD post-test to separate means. Statistical analysis was performed using GraphPad Prism software, Version 8.4.2. Values of $P<0.05$ were considered significant.

3. Results

3.1. Acute toxicity

Effects of treatments on general appearance and behavioral changes

Results for the acute toxicity are summarized in Table 1. No mortality, morbidity, unusual behavior or adverse clinical signs after the standard single oral dose application of both extracts tested of each extract was observed.

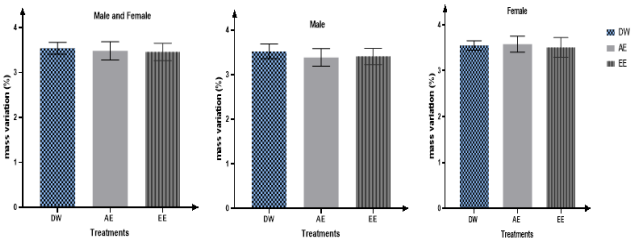
Table 1: General observations after acute administration of 2000 mg/kg of aqueous and ethanolic extracts of *C. pachyceras* in mice

Parameters	Gender	Distilled Water (10 ml/kg)	Aqueous Extract (2000 mg/kg)	Ethanolic Extract (2000 mg/kg)
Diarrhea	M	Not present	Not present	Not present
	F	Not present	Not present	Not present
Change in skin	M	No effect	No effect	No effect
	F	No effect	No effect	No effect
Eye color	M	No effect	No effect	No effect
	F	No effect	No effect	No effect
Death	F	NO	NO	NO
	M	NO	NO	NO
Food intake	F	Normal	Normal	Normal
	M	Normal	Normal	Normal

M: Male; **F:** Female; Number of animals per gender: 5; NO =Not observers.

Effects of treatments on body mass change

In mice receiving the single dose of 2000 mg/kg of aqueous and ethanolic extracts from *C. pachyceras*, the body mass remained unchanged when compared with controls (P>0.05, Figure 1).



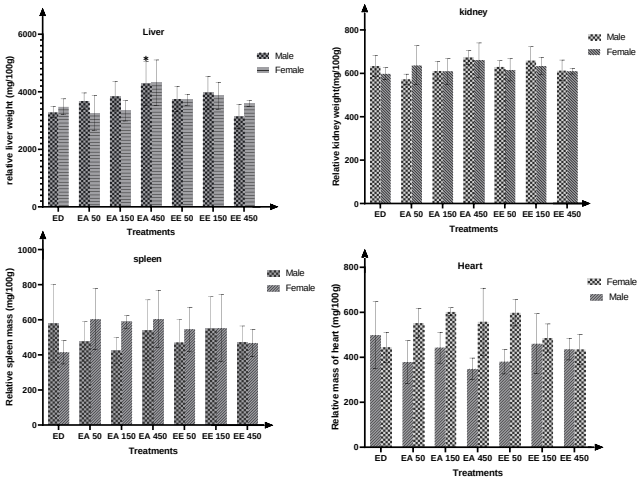
Values are presented as means ± SEM (standard error of the mean); number of animals per group and sex = 5. DW: Distilled Water (10 ml/kg); AE 2000: Aqueous Extract (2000 mg/kg); EE 2000: Ethanolic Extract (2000 mg/kg).

Figure 1: Effects of single administration of aqueous and ethanolic extracts of *C. pachyceras* on body mass in mice.

3.2. Sub-chronic toxicity

Effects of treatments on liver, kidneys, spleen and heart weight

No significant difference (P>0.05) was observed in the relative weights of kidney, spleen and heart of animals from different groups. In male rats, an increase (P<0.05) was recorded in the liver relative mass (aqueous extract, 450 mg/kg) whereas a downward trend was noticed in that of the heart (ethanolic extract, 50 mg/kg). At equal doses, the relative heart mass of females was generally greater than that of males (Figure 2).



Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10). *P < 0.05 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 2: Effects of aqueous and ethanolic extracts of *C. pachyceras* on the relative masses of liver, kidney, heart and spleen.

Effects of treatments on hematological parameters

The effects of aqueous and ethanolic extracts of *C. pachyceras* on hematological parameters in male and female rats treated for 28 days are shown in Tables 2 and 3, respectively.

In males, a tendency to increase the white blood cells (most marked effect in group EE 50 with 31%), granulocytes (most marked effect in group AE 150 with 143%), monocytes (most marked effect in group EE 150 with 165%), hematocrit (most marked effect in group AE 450 with 17%), and platelets (most marked effect in group AE 450 with 20%) was observed. These increases were significant for white blood cells with EE 450 (P<0.05), granulocytes with AE 150 (P<0.001) and AE 450 (P<0.01), and hemoglobin with AE 450 (P<0.001). On the other hand, the opposite effect was observed with lymphocytes, where there was a significant drop with the aqueous extract at 150 mg/kg (P<0.05).

In females, there was no significant difference between these markers except for the number of white blood cells, particularly the increase in platelets in animals receiving AE 50 mg/kg (P<0.001), EE 150 (P<0.01) and EE 50 (P<0.05).

Effects of treatments on biochemical markers

Effects on ALAT and ASAT activities

In males, a significant (P<0.05) increase in ALAT activity at 50 mg/kg was observed (Figure 3A). In both males and females, there was no significant (P>0.05) variation in ASAT (Figure 3B) activities between the plant extract-treated groups and the control.

Effects on Bilirubin level

There was no significant (P>0.05) variation in total bilirubin in extract-treated animals compared with the distilled water group (Figure 4 A). On the contrary, a significant increase (P<0.001) was observed in the direct bilirubin level at 50 mg/kg of ethanolic extract compared with control. This difference was more pronounced in females (Figure 4 B).

Effects on total proteins concentration

After 28 days of treatment, no significant (P>0.05) variation in protein levels between the plant extract and control groups was observed (Figure 5).

Effects on HDL, LDL, total cholesterol and triglycerides levels

The effects of aqueous and ethanolic extracts of *C. pachyceras* on HDL and LDL cholesterol levels in rats are summarized in the figures below. There was no significant difference (P>0.05) in HDL, LDL cholesterol and triglycerides between the plant extract and control groups (Figures 6 A, B and 8). However, a significant increase in total cholesterol was observed not only in the grouped analysis (P< 0.05) but also in females (P<0.01) receiving the ethanolic extract at 150 mg/kg (Figure 7).

Effects on creatinine and albumin levels

There was no significant (P>0.05) variation in albumin concentrations between the plant extract and control groups (Figure 9A). A significant increase in creatinine concentration was observed in males given the 50 mg/kg aqueous extract (P<0.001) and the 150 mg/kg ethanolic extract (P<0.01) (Figure 9B).

Effects of treatments on histopathological sections.

Figures 10, 11, 12 and 13 show the results of histological sections of liver, kidney, spleen and heart respectively. Treatment of male and female rats with aqueous and ethanolic extract of *C. pachyceras* at doses 50, 150 and 450 mg/kg had no histological abnormality on the vital organs. The liver exhibited normal hepatocellular architecture, normal central vein and no sign of apoptosis or inflammation (Figure 10). The kidney revealed regular renal corpuscles, glomerulus tubules and collecting ducts (Figure 11). The spleen showed normal red and white pulps (Figure 12). The heart myocardium revealed intact muscle bundles with normal nucleus (Figure 13).

Table 2: Effects of repeated doses of aqueous and ethanolic extracts of *C. pachyceras* on hematological parameters in rats

Parameters	Male						
	DW 10 ml/kg)	AE 50 mg/kg	AE 150 mg/kg	AE 450 mg/kg	EE 50 mg/kg	EE 150 mg/kg	EE 450 mg/kg
RBC (10^{12}/L)	9.28 ±0.26	10.1 ± 0.39	9.06 ± 0.26	10.67 ± 0.45	9.6 ±0.3	9.54 ± 0.43	11 ± 0.46*
WBC (10^9/L)	6.16 ±1.20	7.76 ±1.03	5.55 ±0.49	7.56 ±0.78	8.11 ±1.28	8.10 ±1.53	6.57 ± 0.99
LY (%)	77.7 ± 5.02	81.34 ± 4.48	60.42±1.25*	67.95± 7.51	81.41±3.26	69.62 ± 5.83	70.01 ± 4.94
Hb (g/dl)	16.48 ±0.40	17.36 ±0.42	16.20 ±0.36	20 ± 0.49***	16.84 ±0.43	16.86 ± 0.76	18.38 ± 0.55
HCT (%)	57.44 ±2.33	61.82 ±1.65	53.44 ±1.33	67.36 ± 1.79*	57.17 ±1.30	62.25 ± 2.02	65.48 ± 2.78
GRA (%)	14.14 ±2.19	9.76 ±2.73	34.40****±3.52	22.26** ± 3.67	10.94 ± 2.59	12.72 ± 1.67	14.78 ± 2.51
PLT (10^9/L)	486 ±14.80	505. 75 ±23.72	499 ± 38.82	586.4 ± 32.53	493.25 ± 25.97	539.6 ± 19.67	463.4 ± 82.79
VGM (fl)	61.2 ±1.11	61.2 ±1.74	59	60.6 ± 0.81	61.8 ± 0.73	61.4 ± 1.33	59.6 ± 0.68
TCMH (pg)	17.6 ± 0.21	17.34 ± 0.37	17.78 ± 0.27	17.6 ± 0.23	18.06 ± 0.20	17.14 ± 0.20	17.46 ± 0.19
MCHC (g/l)	28.76 ± 0.60	26.58 ± 1.59	30.36 ± 0.29	27.82 ± 0.95	29.08 ± 0.40	27.14 ± 1.08	29.60 ± 0.23

Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10). *p < 0.05, **p < 0.01, ***p < 0.001 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Parameters	Female						
	DW 10 ml/kg)	AE 50 mg/kg	AE 150 mg/kg	AE 450 mg/kg	EE 50 mg/kg	EE 150 mg/kg	EE 450 mg/kg
RBC (10^{12}/L)	9.51 ±0.35	12.65 ±2.29	9.50 ±0.30	9.08 ± 0.17	8.76 ±0.63	10.23 ± 0.98	9.27 ± 0.42
WBC (10^9/L)	5.89 ±1.01	11.97 ± 2.31***	5.74 ± 0.44	6.94 ±0.97	6.90 ±1.07	9.94 ± 0.76	5.84 ± 1.33
LY (%)	68.46 ±2.31	64.62 ±8.68	74.18 ±4.59	68.84 ±4.29	72.30 ±2.24	72.83 ± 2.14	74.75 ± 3.20
Hb (g/dl)	17.26 ±0.62	17.28 ±0.48	16.8 ±0.44	14.66 ±0.41	15.66 ±1.12	16.70 ± 0.49	16.46 ± 0.64
HCT (%)	56.12 ±1.71	60.31 ±2.33	56.95 ±1	51.30 ±2.60	54.20 ±1.72	62.06 ± 4.57	53.77 ± 2.21
GRA (%)	19.95 ±1.78	24.10 ±8.80	13.90 ±1.50	15.74 ±3.30	19.86 ±3.86	14.86 ± 1.25	12.94 ± 1.12
PLT (10^9/L)	447.4 ±21.28	543.6 ±32.86	499.8 ± 21.17	663.2 ± 51.01**	598.8 ± 31.30*	565.6 ±23.97	501.8 ± 33.09
VGM (fl)	59 ±0.71	57.4 ±0.60	61 ±1.38	56.2 ±2.46	57.6 ±1.29	62.8 ± 1.53	61.4 ± 0.75
TCMH (pg)	17.68 ± 0.25	16.92 ± 0.52	17.18 ± 0.59	16.78 ±0.6	17.56 ±0.32	16.88 ±1.25	17.86 ± 0.39
MCHC (g/l)	29.96 ± 0.29	25.66 ±2.79	28.22 ±1.24	30.18 ±0.28	30.32 ±0.49	28.48 ±0.52	29.2 ±0.38

Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10). *p < 0.05, **p < 0.01*** p < 0.001 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

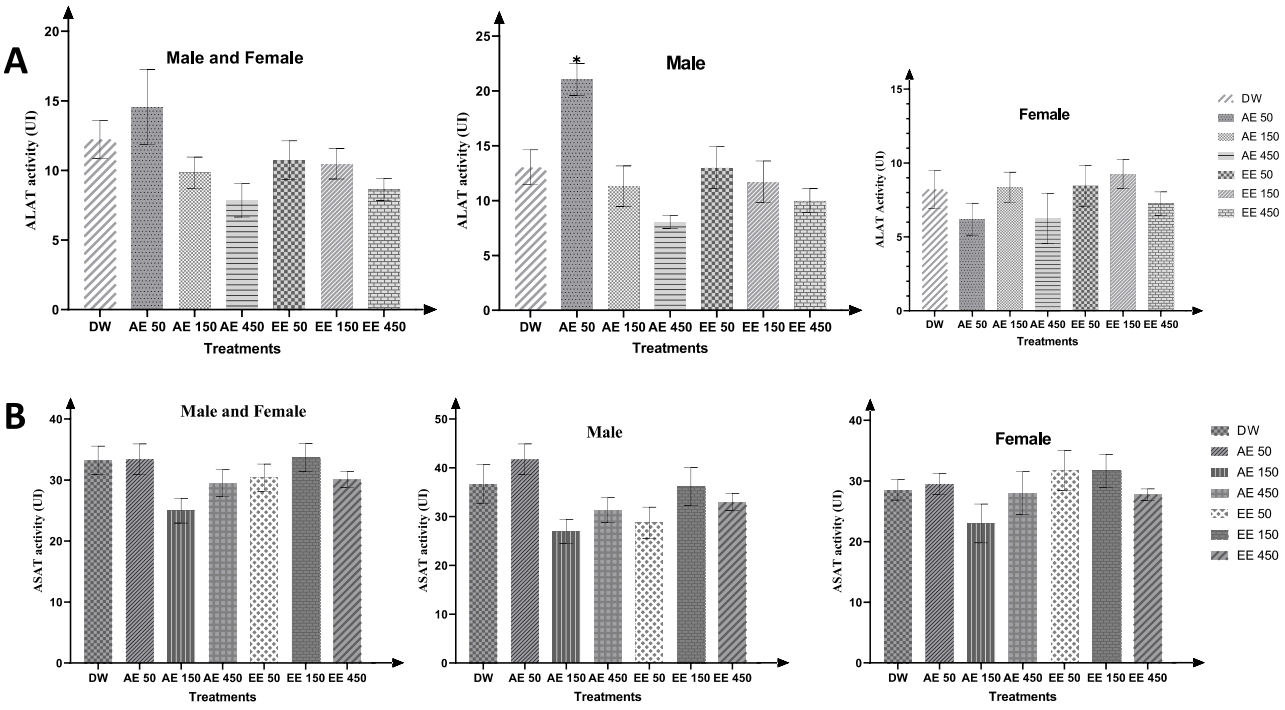


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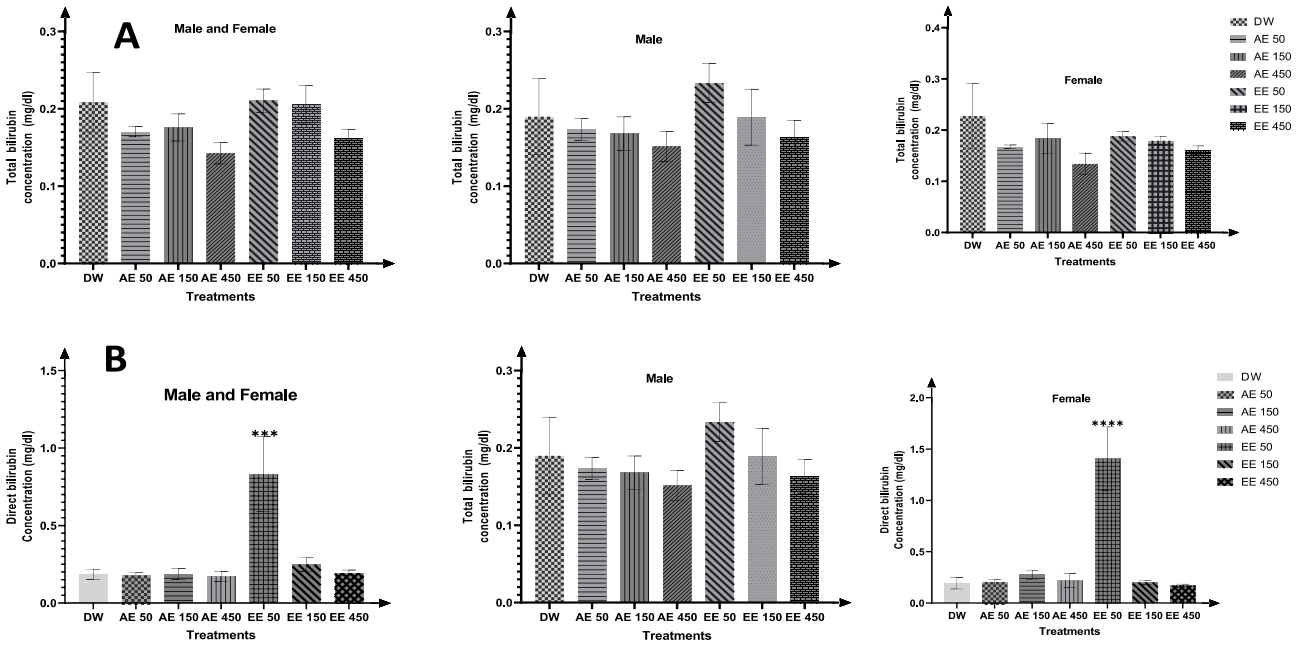
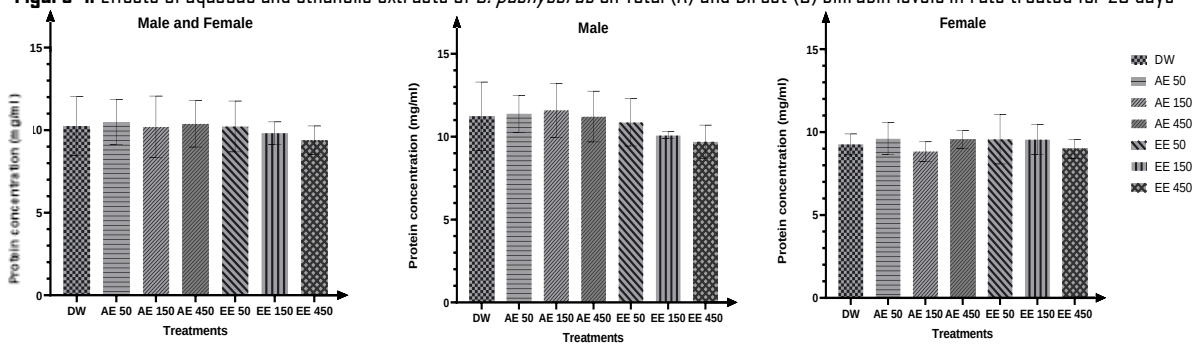
Figure 3: Effects of aqueous and ethanolic extracts of *C. pachyceras* on ALAT (A) and ASAT (B) **activities** in rats treated for 28 days.



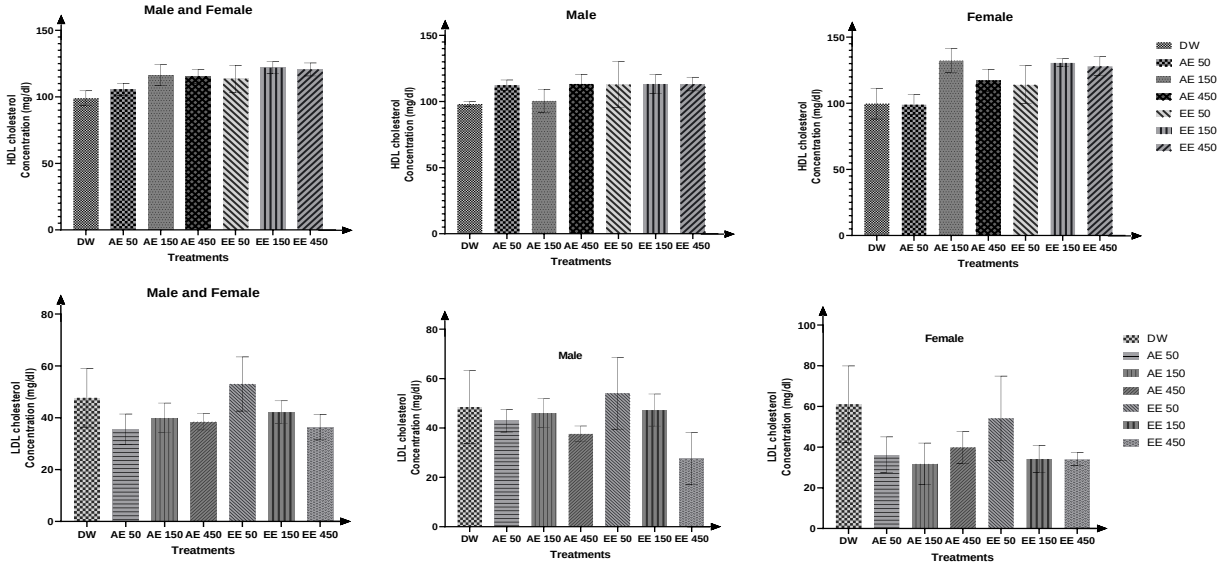
Values are presented as means ± SEM (standard error of the mean); number of animals per group= 10. ***p < 0.001, ****P < 0.0 001 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 4: Effects of aqueous and ethanolic extracts of *C. pachyceras* on Total (A) and Direct (B) bilirubin levels in rats treated for 28 days



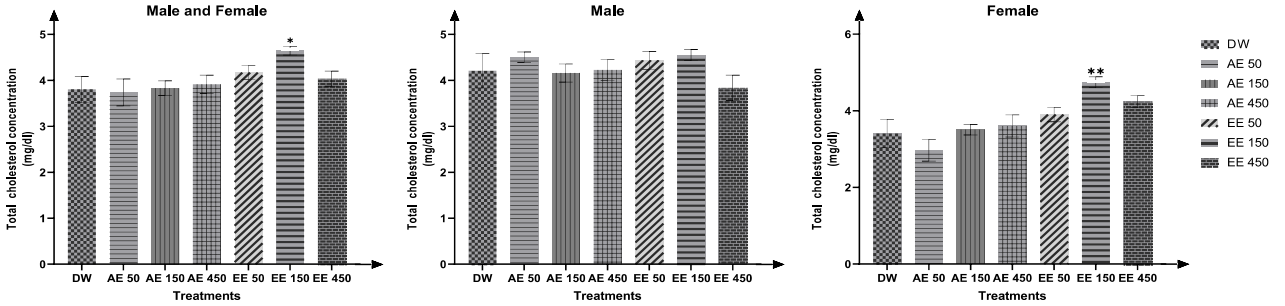
Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 5: Effects of aqueous and ethanolic extracts of *C. pachyceras* on protein concentration in rats treated for 28 days



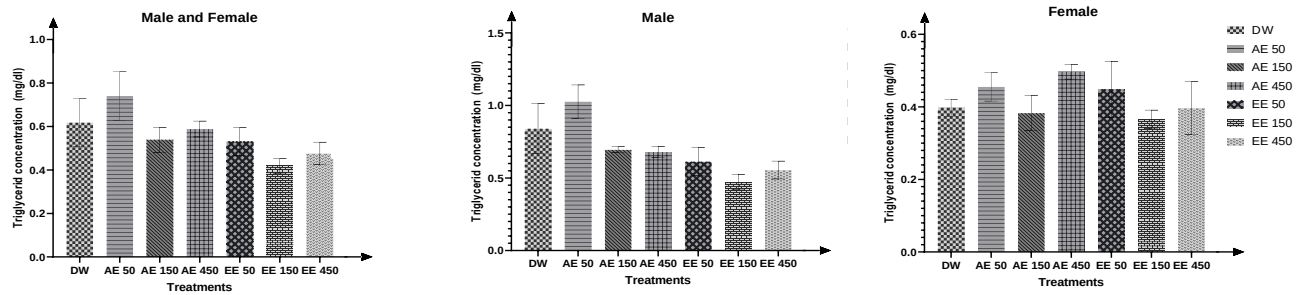
Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 6: Effects of aqueous and ethanolic extracts of *C. pachyceras* on HDL (A) and LDL (B) Cholesterol levels in rats treated for 28 days



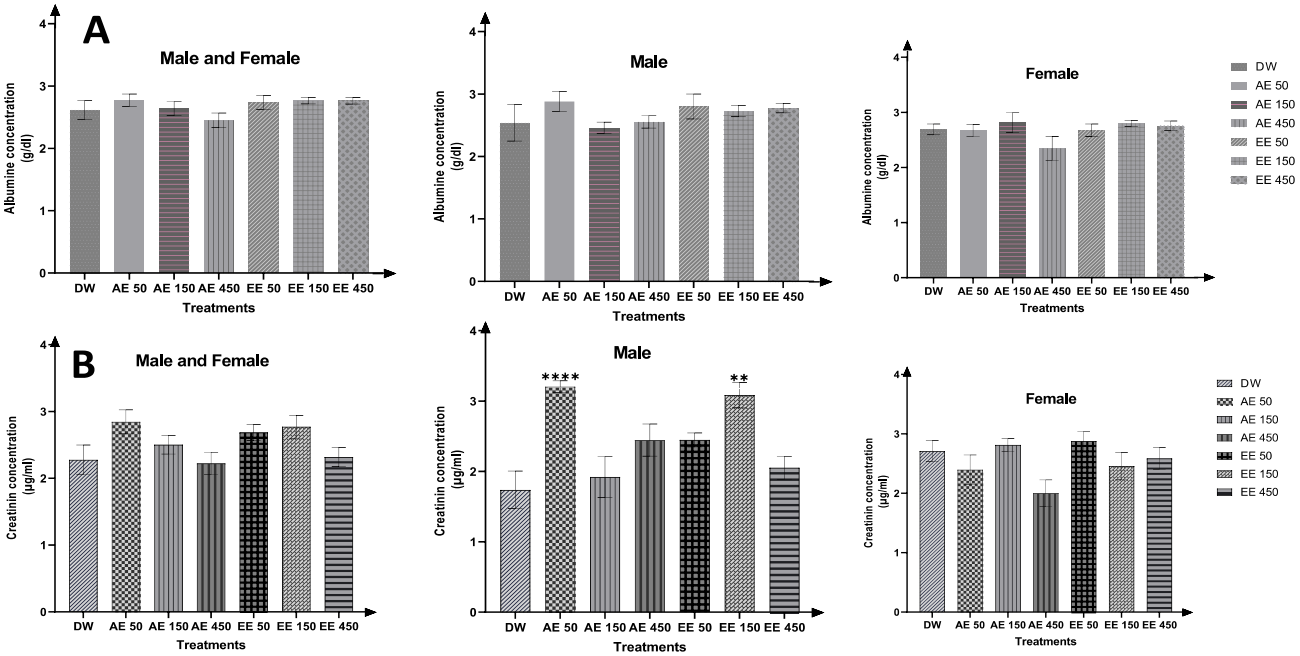
Values are presented as means ± SEM (standard error of the mean); number of animals per group = 10. *p < 0.05, **p < 0.01 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 7: Effects of aqueous and ethanolic extracts of *C. pachyceras* on Total cholesterol in rats treated for 28 days



Values are presented as means ± SEM (standard error of the mean); number of animals per group and sex = 5. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 8: Effects of aqueous and ethanolic extracts of *C. pachyceras* on triglycerides levels in rats treated for 28 days



Values are presented as means ± SEM (standard error of the mean); number of animals per group= 10. **p < 0.01, ****p < 0.0001 indicate significant changes in comparison with the normal control. DW: Distilled water (10 ml/kg); AE 50: Aqueous extract (50 mg/kg); AE 150: Aqueous extract (150 mg/kg); AE 540: Aqueous extract (450 mg/kg); EE 50: Ethanolic extract (50 mg/kg); EE 150: Ethanolic extract (150 mg/kg); EE 450: Ethanolic extract (450 mg/kg).

Figure 9: Effects of aqueous and ethanolic extracts of *C. pachyceras* on albumin (A) and creatinine (B) levels in rats treated for 28 days

Effects of treatments on histopathological sections.

Figures 10, 11, 12 and 13 show the results of histological sections of liver, kidney, spleen and heart respectively. Treatment of male and female rats with aqueous and ethanolic extract of *C. pachyceras* at doses 50, 150 and 450 mg/kg had no histological abnormality on the vital organs. The liver exhibited normal hepatocellular architecture, normal central vein and no sign of apoptosis or inflammation (Figure 10). The kidney revealed regular renal corpuscles, glomerulus tubules and collecting ducts (Figure 11). The spleen showed normal red and white pulps (Figure 12). The heart myocardium revealed intact muscle bundles with normal nucleus (Figure 13).

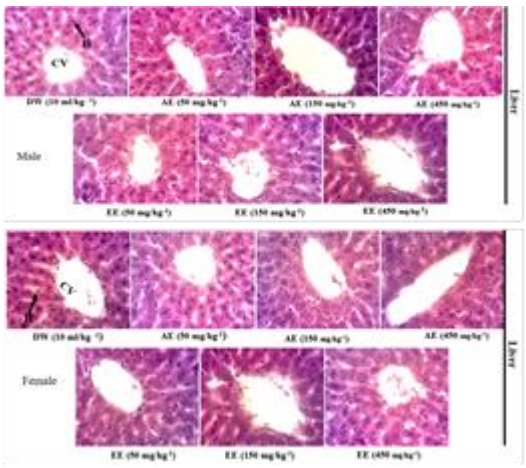
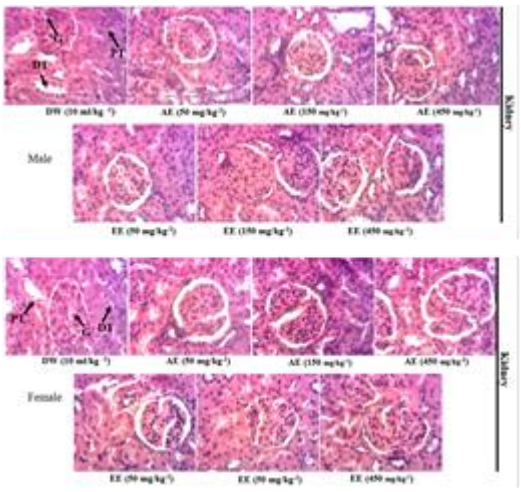
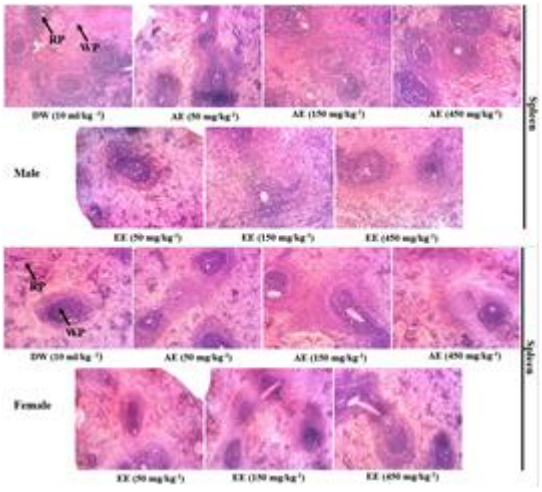


Figure 10: Histological sections of liver of the rats in 28-days sub-chronic toxicity study, (HE × 250).



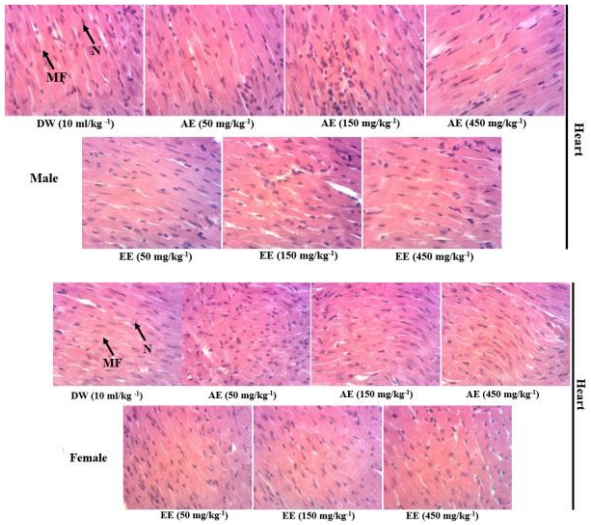
G=Glomerulus, DT = Distal Convoluted Tube, PT = Proximal Convoluted Tube

Figure 11: Histological sections of kidneys of the rats in 28-days sub-chronic toxicity study, (HE × 250).



RP= Red Pulp, WP=White pulp.

Figure 12: Histological sections of spleen of the rats in 28-days sub-chronic toxicity study, (HE × 250).



MF= Muscular Fiber, N=nucleus.

Figure 13: Histological sections of heart of the rats in 28-days sub-chronic toxicity study, (HE × 250).

4. Discussion

Corynanthe pachyceras bark is used in traditional medicine to treat disorders of the male reproductive system, although no scientific data on its toxicity profile has yet been provided. As toxicological profiling is a crucial tool for assessing the safety of drugs or plants [10], the aim of this study was to evaluate the acute and sub-chronic toxicological profile of *C. pachyceras* aqueous and ethanolic extracts.

In the acute toxicity study, the aqueous and ethanolic extracts of *C. pachyceras* were administered to mice at a single dose of 2000 mg/kg for each extract. No changes were observed in the skin and hair, mucous membranes or eyes of animals during the study. Mortality is a critical parameter in toxicity studies [4,11]. Aqueous and ethanolic extracts of *C. pachyceras* did not induce death or toxic syndromes in mice. Consequently, the LD50 of the aqueous and ethanolic extracts of *C. pachyceras* was more than 2000 mg/kg in accordance with the OECD guidelines. Similar results were obtained with *G. tessmanni* that did not produce any abnormal behavior response or death in male and female mice during the 14 days of follow-up [12]. Administering a single high dose of *C. pachyceras* extract did not show any toxic effects. Therefore, there is also a need to investigate the effects of repeated administration at a low dose over a period of 28 days.

Relative organ weights in the sub-chronic toxicity studies are observed as a relatively sensitive indicator for particular organs. That is, toxicity can be defined as significant changes observed in these particular organs [13]. In this study, no significant changes in heart, spleen, kidney or liver relative weights were observed except at 450 mg/kg aqueous extract in males (significant increase, $P<0.05$), suggesting that administration of *C. pachyceras* extracts in the sub-chronic study had no adverse effect on the health of the animals.

Results of this study revealed that essential organs such as heart, liver, spleen and kidneys, were not adversely affected and showed no clinical signs of toxicity throughout treatment. It could be therefore suggested that, at the doses used, the extracts from *C. pachyceras* were not toxic to the target organs.

Hematological analyses are essential for evaluating the toxicity of substances in humans and animals [14]. Any variation in these hematological parameters is considered as a strong indicator of the harmful potential of a substance. For this sub-chronic toxicity study, significant differences were found between the groups administered the high-dose of plant extracts (RBC : EE 450 ; Hg : AE 450 and Gra : AE 150, AE

450 in male ; PTL : AE 450, EE 50 in females). These findings clearly indicate that consumption of *C. pachyceras* extracts at high-dose could weaken the immune system.

Plasmatic clinical biochemistry analyses were carried out in order to assess the possible alterations in hepatic, renal and cardiovascular functions due to plant extracts. Analysis of liver, kidney and heart functions is very important in assessing the toxicity of drugs and plant extracts, as they are necessary for the survival of an organism [15]. The liver function can be assessed by measuring the levels of protein, bilirubin, and liver enzymes. Elevated activities of ALAT, ASAT and alkaline phosphatase are reported in liver disease or hepatotoxicity [16]. The absence of significant changes in ALAT, ASAT, total proteins and total bilirubin concentrations in male and female rats at all doses suggest that the sub-chronic administration of *C. pachyceras* extracts did not affect the hepatocytes and biliary function in rats. However, the significant increase observed in direct bilirubin at EE 50 mg/kg in females suggests a toxic sign in these animals. On the other hand, the lack of change in total protein levels is indicative of no change in synthetic liver function, or no alteration in hepatocellular function. Thus, these results generally confirm that *C. pachyceras* does not damage the hepatocellular or secretory liver functions, whatever the dose tested.

Increased blood urea nitrogen, albumin, bilirubin, total protein and creatinine levels may indicate impaired renal function [17]. In the present study, although urea and ion levels were not measured, albumin and protein values related to renal function were within the normal range and did not differ significantly in all animals. However, the significant increase in creatinine levels only in males at doses of 50 mg/kg for the aqueous extract and 150 mg/kg for the ethanolic extract could indicate nephrotoxicity. Histological analysis showed no structural abnormalities.

Concerning the cardiovascular function, HDL and LDL cholesterol and triglyceride levels were kept normal, therefore suggesting that *C. pachyceras* extracts did not alter the cardiovascular function in rats. It is generally believed that significant increase in HDL and LDL cholesterol and triglycerides denotes cardiovascular risks [18]. The noteworthy increase in the total cholesterol level observed in females treated with EE at the dose of 150 mg/kg suggests that the ethanol extract of *C pachyceras* at high dose contains compounds capable of increasing the cholesterol concentration.

Histological analysis makes it possible to better explain organ's integrity. It serves as supporting evidence for hematological and biochemical analyzes [19]. In this study, all vital organs (liver, kidney, spleen and heart) showed normal architecture, compared with control, allowing to validate that the aqueous and ethanolic extracts of *C. pachyceras* are non-toxic.

Additional studies of genotoxicity, carcinogenicity, teratogenicity and chronic toxicity studies are also required as they will strengthen the safety profile of *C. pachyceras* extracts.

5. Conclusion

Findings from this study indicate that the LD50 was greater than 2000 mg/kg. *C. pachyceras* extracts showed no mortality and no behavior-related toxicity in the acute toxicity study. However, *C. pachyceras* extracts at high doses may influence the immune system as well as renal and hepatic functions. It is therefore recommended that *C. pachyceras* extracts be administered safely at low doses for a short duration of less than 28 days to avoid harmful effects. The findings of this study contribute to the existing scientific knowledge on the safety profile of *C. pachyceras* extracts, particularly about their traditional medicinal uses for genito-urinary infections, sexual vitality, and libido enhancement. Importantly, this study addresses a gap in the literature by providing new insights into the toxicological aspects of *C. pachyceras* extracts, which can inform future research and the development of safe and effective medicinal products.

Funding: Not applicable

Statement and declarations

Competing interests: The authors declare no conflict of interest

Financial interests: The authors have no relevant financial or nonfinancial interests to disclose.

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