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Original Article

Acute and Sub-acute Oral Toxicity Profile of Root Bark Methanol Extract of *Carissa Edulis* Vahl

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ABSTRACT

Background: *Carissa edulis* is widely used in traditional medicine to manage numerous ailments. However, few studies have assessed its toxicity. Therefore, this study aimed to determine acute and sub-toxicity levels of *C. edulis* methanol extract. **Methods:** In the acute toxicity probe, a limit test was conducted whereby the extract was given as a solo dose by gavage. The rats were observed for two weeks. The observations included mortality and changes in the general appearance and behavior of the experimental animals. The body weights of the rats were taken weekly. For the sub-acute toxicity probe, the rats received the extract daily at dosages 300, 520, and 900 mg/kg by gavage for 28 days. Body weights were also taken weekly. On day twenty-nine, the weights of the rats were taken, the rats were sacrificed, and blood was collected for biochemical and hematological analysis. Body organs were harvested, and their weights were taken. **Results:** The results of the acute toxicity probe showed that the extract didn't cause mortality or toxicity signs throughout the study duration. The LD_{50} of the extract was therefore deemed to be above 2,000 mg/kg. The sub-acute toxicity probe results demonstrated that the extract, at all the tested dosages, didn't cause mortality or affect the rats' organ weights, body weights, or hematological and biochemical parameters throughout the study duration. **Conclusions:** In conclusion, the methanol extract of *C. edulis* is not toxic since it didn't cause mortality or toxicity signs in both acute and sub-acute toxicity probes.

Key words: Biochemical parameters, Body weights, Hematological parameters, Organ weights.

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INTRODUCTION

Medicinal plants have been utilized to manage and treat various ailments since time immemorial.[1] The World Health Organization (WHO) reports that most people in the world (80%) depend on traditional herbal remedies for their primary healthcare needs.[1] The increased reliance on herbal medicine is due to the adverse side effects and high costs of conventional drugs.[2] Besides, herbal medicine has been reported to be safer, readily available, and more affordable than conventional drugs.[3]

Diverse biological activities of numerous medicinal plants have been validated scientifically. Medicinal plants are thus potential candidates for drug development.[3] However, safety concerns still arise, especially if the herbal remedies are consumed for a prolonged period.[4] Therefore, evaluation of the safety profiles of medicinal plants is critical in the process of developing drugs from natural products.[5] The safety of medicinal plants is also compromised by unethical and unhygienic preparation, inappropriate dosages, and lack of quality controls.[5] Thus, herbal medicine consumers need to be educated about herbal remedies' safety, proper use, and preparation.[4]

Carissa edulis belongs to the Apocynaceae family.[6] It extensively occurs in Africa.[7] In addition, *C. edulis* is cultivated in Indian Ocean Islands, India, and Thailand.[7] *Carissa edulis* is used as a traditional remedy for diabetes, ulcers, headaches, rheumatism, breast cancer, rabies, HIV and AIDs, syphilis, gonorrhea, epilepsy, inflammation, pain, worm infestation, sickle cell anemia, hernia, fever, among other conditions.[8,9] Various biological activities of *C. edulis* including, antiplasmodial,[10] anti-inflammatory,[11] analgesic,[12] antipyretic,[8] antiviral,[13] anticonvulsant,[14] hypoglycemic,[15] diuretic,[16] antimicrobial,[17] among others, have been reported.

Despite the wide usage of *C. edulis* in traditional medicine, only minimal scientific toxicity data is available. Therefore, this study's objective was to investigate acute and sub-acute toxicity levels of *C. edulis* root bark methanol extract in albino Wistar rats. This study provides crucial information on the safety profile of *C. edulis* that can be leveraged in determining dosages for pre-clinical trials.

MATERIAL AND METHODS

Plant sample

A root bark sample of *C. edulis* was acquired from Kitui County, Kenya (longitude 37.7558° E, latitude 1.3099° S; about 153 km from Nairobi city) in March 2021. A local herbalist helped in the discernment of the plant. The sample was verified at the East African Herbarium, and a sample specimen was deposited there (accession number: JWM001). Sample preparation, extraction, and bioassays were conducted at Kenyatta University.

The sample was chopped up into smaller bits and shade-dried till perfectly dry. Utilizing an electric mill, the sample was reduced into fine powder. The powder was packaged in a khaki bag and labeled accordingly.[18]

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Extraction procedure

One liter of methanol (Xilong Scientific Co., Ltd., Guangdong, China) was carefully poured into a flask containing 300 g of sample powder. The mixture was regularly shaken for two days to increase the extraction surface area.[18] To filter the extract, A VE-11 aspirator pump (DEKKER Vacuum Technologies, US) was used. The extract was concentrated utilizing an R-300 rotary evaporator (Henan Lanphan Industry Company Limited, China) at 40 °C and refrigerated at 4 °C.

Experimental animals

Female Wistar rats aged between 2 to 3 months and weighing from 140 to 160 g were utilized. The rats were provided by the Kenyatta University's animal breeding and experimentation facility. They were given standard rodent food and water was provided *ad libitum*. The rats were given one week to accustom to the laboratory conditions prior to the toxicity studies. This study was sanctioned by the Kenyatta University Animal Care and Use Committee (approval number: PKUA/005/005).

Acute toxicity study of *C. edulis* root bark methanol extract

The study was executed as guided by the OECD guidelines.[19] Aqueous extract of *C. edulis* leaves has been shown to cause no toxicity at a 2,000 mg/kg BW dose.[20] Therefore, a limit test was conducted. Rats were parted into 2 groups, control and test groups, with 5 rats each. Treatments were administered by gavage as a solo dose. Food was withheld from the animals overnight before dosing. After the fasting period, the animals were weighed and treatments were administered. Food was further withheld for 4 h. The control group was given 2.5% DMSO (Wego Chemical Group Inc., Great Neck, NY) in normal saline (Hemant Surgical Industries Ltd. Mulund West, Mumbai), while the test group received the extract at a 2,000 mg/kg BW dose. Observation for mortality and signs of toxicity was done for two weeks. The toxicity signs that were observed included those highlighted in the OECD guidelines for acute toxicity inquiries.[19] Body weights were again taken weekly. Rats were then sacrificed *via* inhalation of diethyl ether in a desiccator, and carcasses were incinerated.

Sub-acute toxicity study of *C. edulis* root bark methanol extract

This study was done following OECD guidelines.[21] Rats were separated into 4 groups. Group A was the control group, and the animals were given 2.5% DMSO in normal saline. Groups B, C, and D got the extract at 300, 520, and 900 mg/kg BW doses, respectively. The treatments were orally administered daily for 28 days, and observations for mortality and signs of toxicity. Body weights are taken weekly. On the 29th day, the experimental animals were sacrificed and blood was drawn through cardiac puncture. Organ weights were also taken and recorded. Relative organ weights were computes as follows:

Relative organ weight = [Absolute organ weight (g) / Body weight of the rat on sacrifice day (g)] * 100

Hematological and biochemical parameters analysis

Blood samples for hematology analysis were obtained and put in EDTA tubes (Ningbo Siny Medical Technology Co., Ltd., Zhejiang, China), while biochemical samples were collected in plain Eppendorf tubes (Ningbo Siny Medical Technology Co., Ltd., Zhejiang, China). To obtain serum for analysis of biochemical parameters, centrifugation of the blood samples was done for 10 min at 3,000 rpm.[22] The hematological and biochemical parameters analyzed involved those listed by the OECD guidlines[21] for repeated dose 28-day oral toxicity study in rodents.

Data analysis

The GraphPad Prism 8 software was utilized for data analysis. A one-way analysis of variance was done to establish differences between groups. Further, Tukey's post hoc test was utilized to separate the mean values. Values of $p < 0.05$ were deemed statistically significantly different.

RESULTS

Acute Toxicity

The root bark methanol extract of *C. edulis* did not affect the body weights, behavior, and general appearance of the rats.

Effect of C. edulis Root Bark Methanol Extract on the Body Weights of Female Wistar Rats

There was no significant difference ($p > 0.05$) between the body weights of extract-treated rats and control rats throughout the study duration (Table 1).

Effect of C. edulis Root Bark Methanol Extract on the Behavior and Overall Appearance of Female Wistar rats

The root bark methanol extract of *C. edulis* administered at 2,000mg/kg BW did not affect the rats' behavior and general appearance (Table 2). No coma, diarrhea, salivation, convulsions, lethargy, sleep, tremors, and changes in the mucous membranes, eyes, fur, and skin was observed in both the extract-treated and control rats.

Sub-acute Toxicity

Carissa edulis root bark methanol extract didn't affect the body weights, organ weights, and biochemical and hematological parameters of the experimental animals.

Effect of C. edulis Root Bark Methanol Extract on the Body Weights of Female Wistar Rats

There was no significant difference ($p > 0.05$) between the body weights of the rats treated with *C. edulis* methanol extract at dosages 300, 520 and 900mg/kg BW and the control rats throughout the test duration (Table 3). Further, the extract at the three tested dosages produced comparable ($p > 0.05$) body weights (Table 3).

Effect of C. edulis Root Bark Methanol Extract on the Organ Weights of Female Wistar Rats

Relative organ weights of the spleen, brain, lungs, heart, kidney and liver obtained from the rats treated with the extract at dosages 300, 520 and 900mg/kg BW were comparable ($p > 0.05$) with those of the control rats (Table 4). In addition, the relative organ weights of rats treated with the extract at the three tested dosages were comparable ($p > 0.05$).

Effect of C. edulis Root Bark Methanol Extract on the Hematological Parameters of Female Wistar Rats

Levels of the assessed hematological parameters were comparable ($p > 0.05$) between the all the extract-treated rats and the control rats (Table 5). Further, the extract at the three tested dosages produced comparable ($p > 0.05$) levels of the examined hematological parameters (Table 5).

Effect of C. edulis Root Bark Methanol Extract on the Biochemical Parameters of Female Wistar Rats

There was no significant difference ($p > 0.05$) in the levels of the assessed biochemical parameters between the rats treated with the extract at

Table 1: Effects of *C. edulis* root bark methanol extract on body weights (g) of female Wistar rats in acute toxicity study.

Days	Normal Control	CM (2000 mg/kg BW)
0	208.4±2.7	205.8±2.8
7	216.6±3.0	213.2±2.6
14	222.4±2.7	219.4±2.5

Statistical comparison was made within a column, and values were expressed as Mean ± SEM. Values were not significantly different by one-way ANOVA ($p > 0.05$). CM = *C. edulis* methanol extract

Table 2: Effect of *C. edulis* root bark methanol extract on the behavior and general appearance of female Wistar rats in acute toxicity study.

Behavior and general appearance	Observation	
	Control	CM (2,000 mg/kg BW)
Coma	-	-
Diarrhea	-	-
Salivation	-	-
Convulsions	-	-
Lethargy	-	-
Sleep	-	-
Tremors	-	-
Changes in the mucous membranes	-	-
Changes in the eyes	-	-
Changes in the fur	-	-
Changes in the skin	-	-

CM = *C. edulis* methanol extract; - denotes absent

Table 3: Effects of *C. edulis* root bark methanol extract on body weights (g) of female Wistar rats in sub-acute toxicity study.

Days		0	7	14	21	28
Treatment	Control	207.4±2.2	215.4±2.4	221.2±3.0	225.6±3.3	229.0±3.2
	CM (300 mg/kg)	208.2±2.9	217.8±3.3	225.4±3.5	230.8±3.3	233.2±3.4
	CM (520 mg/kg)	206.6±2.7	216.0±2.7	223.2±2.4	229.2±2.4	232.0±2.5
	CM (900 mg/kg)	207.2±3.6	218.4±3.6	226.0±3.6	231.0±3.6	234.8±3.5

Values were expressed as Mean ± SEM. Statistical comparison was made a long row, and values were not significantly distinct by one-way ANOVA ($p > 0.05$). CM = *C. edulis* methanol extract

Table 4: Effect of *C. edulis* root bark methanol extract on relative organ weights of female Wistar rats in sub-acute toxicity study.

Organs	Control	CM (300 mg/kg BW)	CM (520 mg/kg BW)	CM (900 mg/kg BW)
Spleen	0.41±0.05	0.40±0.06	0.41±0.04	0.43±0.06
Brain	0.73±0.04	0.73±0.03	0.75±0.02	0.72±0.03
Lungs	0.59±0.03	0.58±0.02	0.57±0.03	0.58±0.03
Heart	0.40±0.01	0.37±0.02	0.39±0.02	0.38±0.02
Kidney	0.64±0.03	0.63±0.02	0.63±0.03	0.64±0.03
Liver	3.53±0.11	3.37±0.12	3.48±0.12	3.34±0.16

Values were expressed as Mean ± SEM. Statistical comparison was made a long row, and values were not significantly distinct by one-way ANOVA. CM = *C. edulis* methanol extract

Table 5: Effect of *C. edulis* root bark methanol extracts on hematological parameters of female Wistar rats in sub-acute toxicity study.

Parameter	Treatment			
	Control	CM (300 mg/kg BW)	CM (520 mg/kg BW)	CM (900 mg/kg BW)
WBCs (10^3/µl)	8.99±0.23	8.86±0.34	9.02±0.22	8.82±0.25
NEUT (10^3/µl)	1.68±0.06	1.60±0.04	1.68±0.05	1.63±0.06
LYMP (10^3/µl)	6.37±0.11	6.32±0.18	6.37±0.21	6.28±0.25
MONO (10^3/µl)	0.85±0.03	0.85±0.05	0.87±0.06	0.82±0.05
EOS (10^3/µl)	0.07±0.004	0.07±0.003	0.07±0.004	0.07±0.004
BAS (10^3/µl)	0.02±0.004	0.02±0.004	0.03±0.002	0.02±0.004
RBC (10^6/µl)	7.72±0.26	7.67±0.24	7.79±0.19	7.32±0.38
HGB (g/dl)	13.79±0.62	13.31±0.79	13.46±0.71	13.52±0.61
HCT (%)	43.10±3.75	45.34±3.49	42.64±2.15	46.26±3.71
MCV (fl)	55.48±3.67	57.08±3.03	56.50±3.52	51.66±4.13
MCH (pg)	21.60±2.66	20.54±1.39	22.90±2.09	21.00±2.28
MCHC (g/dl)	33.86±3.33	37.40±3.27	38.08±3.77	36.16±2.66
RDW (%)	16.12±2.77	16.60±2.11	14.90±1.97	17.02±2.29
PLT (10^3/µl)	728.60±19.50	732.40±29.20	746.40±35.70	738.80±21.10
MPV (fl)	7.62±0.81	7.36±0.79	7.30±0.87	7.59±0.96

Values were expressed as Mean ± SEM. Statistical comparison was made across the rows, and values were not significantly distinct by ANOVA ($p > 0.05$). CM = *C. edulis* methanol extract. NEUT = neutrophils; LYMP = lymphocytes; MONO = monocytes; EOS = eosinophils; BAS = basophils; HGB = Hemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; HCT = Hematocrit; MCH = Mean Corpuscular Hemoglobin; MCV = Mean Corpuscular Volume; MCHC = Mean Corpuscular Hemoglobin Concentration; PLT = Platelets; RDW = Red Cell Distribution Width; MPV = Mean Platelet Volume

Table 6: Effect of *C. edulis* root bark methanol extract on biochemical parameters of female Wistar rats in sub-acute toxicity study.

Parameter	Treatment			
	Control	CM (300 mg/kg BW)	CM (520 mg/kg BW)	CM (900 mg/kg BW)
Na^+ (mmol/L)	141.8±3.5	138.3±5.5	144.8±3.6	137.1±3.8
Cl^- (mmol/L)	105.6±1.2	106.5±5.4	110.2±1.9	109.6±3.1
K^+ (mmol/L)	4.7±0.3	4.8±0.3	4.5±0.2	4.9±0.1
CREA (µmol/L)	51.7±2.4	55.6±2.8	52.8±3.3	50.6±2.5
Urea (mmol/L)	6.2±0.3	6.4±0.2	5.8±0.3	6.5±0.2
AST (U/L)	108.6±7.3	105.4±5.1	105.0±3.8	102.9±4.9
ALT (U/L)	69.3±4.0	71.2±2.8	74.3±3.4	66.6±2.9
ALP (U/L)	132.6±6.7	129.6±4.3	131.2±5.3	134.6±6.6
ALB (g/L)	35.1±2.6	30.7±1.6	35.1±1.9	33.3±2.4
BIL (µmol/L)	2.9±0.1	2.8±0.1	2.7±0.1	3.0±0.1
TP (g/L)	64.0±2.4	63.1±2.6	68.8±2.4	66.4±2.1

Values were expressed as Mean ± SEM. Statistical comparison was made across the rows, and values were not significantly different by one-way ANOVA ($p > 0.05$). CM = *C. edulis* methanol extract; Na^+ = sodium ions; Cl^- = chloride ions; K^+ = potassium ions; CREA = creatinine; AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; ALB = albumin; BIL = bilirubin; GLOB = globulin; TP = total protein

dosages 300, 520 and 900mg/kg BW and the control rats (Table 6). Furthermore, no significant difference ($p > 0.05$) was observed in the levels of the studied biochemical parameters between rats treated with the extract at the three tested dosages (Table 6).

DISCUSSION

The present inquiry investigated the toxicity of *C. edulis* root bark methanol extract through acute and sub-acute probes. Toxicity studies of medicinal plants help in the identification of potential adverse effects of the plants and establishing exposure levels at which the effects are observed.[3] This, in turn, helps in dose determination for pre-clinical studies.[23]

Acute toxicity refers to the detrimental effects that arise over a period of 24 h after taking a single dose of a substance.[24] Our acute toxicity study assessed mortality, changes in body weights, and the general appearance and behavior of the rats. The *C. edulis* methanol extract at 2,000 mg/kg dosage didn't occasion death or toxicity signs throughout the study period. These results show that *C. edulis* methanol extract has a high safety degree with LD_{50} greater than 2,000 mg/kg.[23] Therefore, as per the OECD guidelines under the Globally Harmonized Classification System (GSH) for Chemical Substances and Mixtures, the extract can be placed under the non-toxic category of chemicals (Class 5).[19]

According to Kifayatullah *et al*,[25] plant extracts with high safety profiles in acute toxicity studies may produce a toxic effect on biological systems after sub-acute administration. Therefore, sub-acute toxicity studies are critical when assessing toxicity profiles of medicinal plants. In addition, it is crucial to conduct sub-acute toxicity studies of medicinal plants commonly utilized to manage chronic diseases, including *C. edulis*. This is because daily administration of the treatment may lead to its accumulation in the blood which can, in turn, have a gradual detrimental effect on various body organs and tissues.[26] Further, data obtained from acute toxicity studies have limited application in clinical studies.[3] Therefore, a repeated dose study is crucial in establishing the safety profiles of medicinal plants.

Sub-acute toxicity is the harmful effects that arise from administration of a substance repeatedly for a long period of time.[4] In our sub-acute toxicity study, we assessed organ weights, body weights, and hematological and biochemical parameters. Changes in experimental animals' body weights indicate detrimental effects of the test substance.[27] Loss of experimental animals' body weight exceeding 20% is regarded as a humane end-point.[28] In the present inquiry, the extract-treated rats gained weight normally throughout the acute and sub-acute toxicity probes. This denotes that *C. edulis* methanol extract didn't have an effect on the body weights of the rats hence, it is non-toxic.[29] Changes in organ weights are another indicator of the toxicity of the test substance. Administration of *C. edulis* methanol extract in the sub-acute toxicity probe did not affect the organ weights of the experimental animals. This result indicates that the extract was not toxic.[29]

Detrimental effects that arise from sub-acute administration of plant extracts usually manifest in vital organs, including the kidney and liver, because they are involved in the extract's metabolism .[4] Therefore, it is crucial to evaluate biochemical parameters in repeated dose studies to assess the liver's and kidney's integrity and functionality. The most critical biochemical parameters to evaluate the liver's functionality include serum ALP, ALT, AST, bilirubin, albumin, and TP.[3] These proteins and enzymes are mainly found in the liver, but when a compromise in the liver's integrity occurs, they are released in significant amounts.[30,31] As our results indicated, the methanol extract of *C. edulis* did not alter levels of the assessed liver biochemical parameters. This suggests that the extract didn't prompt necrotic damage or inflammation to the liver; hence, it is not hepatotoxic.[29] Alanine aminotransferase is the utmost appropriate liver toxicity marker compared with AST and ALP; although it is also produced in other body tissues like AST and ALP, its activity is higher in the liver.[3]

Serum urea, creatinine, and electrolyte levels are some of the biochemical parameters used to assess kidney functionality.[3] These biochemical parameters help determine the rate of glomeruli filtration and the diluting and concentrating capability of kidney tubules.[32] Increased levels of these elements in serum indicate renal tubule imparement.[32] Administration of the *C. edulis* methanol extract to rats in the sub-acute toxicity probe did not alter levels of the assessed kidney biochemical parameters suggesting that the extract did not cause kidney toxicity.

Hematological data is also critical in establishing toxicity profiles of herbal products. The hematopoietic system, a carrier of metabolites, nutrients, and genetic material throughout the body, is the most susceptible body system to assaults by noxious substances.[4] In addition, damaged blood cells affect the body's normal functioning in animals and humans.[33] Fortunately, the *C. edulis* methanol extract didn't change levels of the assessed hematological parameters, suggesting that the extract is not toxic.

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated that the methanol extract of *C. edulis* root bark is safe since it didn't prompt death or toxicity signs to the experimental animals in both acute and sub-acute toxicity probes. A limitation of the present investigation is that chronic toxicity of the

extract was not investigated. We, therefore, recommend a chronic toxicity study of the extract.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflicts of interest.

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