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Original Article

IN VIVO ACUTE AND SUB-ACUTE TOXICITY STUDY OF ROOT EXTRACT OF *CARISSA SPINARUM* LINN. IN SWISS ALBINO MICE

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ABSTRACT

Objective: The objective of the present research work had been done to evaluate the toxicity of crude extract of *Carissa spinarum* in Swiss albino mice.

Methods: In studying the toxicity, the Organization for Economic Cooperation and Development (OECD) guidelines were used. Experimental animals (mice), five mice in each, were grouped into four groups; three experimental groups and one negative control. In studying the acute toxicity, 2000, 3000 up to 5000 mg/kg crude plant extract was given orally using standard intragastric oral gavages. For acute toxicity, a single dose was given and gross behavioral changes were recorded. In sub-acute oral toxicity test, *Carissa spinarum* crude extract was given to the mice by standard intragastric oral gavages at doses of 500, 750 and 1000 mg/kg body weight of hydro-methanolic extract and 200, 600 and 1000 mg/kg of body weight of chloroform extract in every single to 28 d and various hematological and physical parameters were recorded.

Results: In acute toxicity, the given dose of the plant extract did not produce significant physical and behavior changes up to the dose of 5000 mg/kg extracts. In addition, no death was occurred in the given doses. In sub-acute toxicity studies of the hydro-methanolic and chloroform extracts, there was no recorded significant change ($p>0.05$) of hematological and physical parameters in the treated groups when compared to the control groups.

Conclusion: from the present study it was revealed that the crude extract of the plant did not produce any significant toxicological effect in the experimental animals and this supports the use of the plant in folk medicines.

Keywords: *Carissa spinarum*, Apocynaceae, Acute toxicity, Sub-acute toxicity, *In vivo*

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INTRODUCTION

Carissa spinarum Linn. (Formerly *Carissa edulis*) belongs to the family Apocynaceae [1, 2]. It is shrubby species found commonly growing in the forests and wastelands up to elevations of 1,500 meters. It is highly drought-resistant plant. It bears small fruits which are edible and are also offered for sale at manyplaces. The plant is native in the following countries: Australia, Botswana, Cambodia, Cameroon, Eritrea, Ethiopia, Ghana, Guinea, Japan, Kenya, Myanmar, Namibia, Nigeria, Papua, Saudi Arabia, Senegal, South Africa, Sudan, Tanzania, Thailand, Uganda, Vietnam and Yemen [3]. *C. spinarum* L. is one of the main African ethnomedicine; it is one of themost prevalent traditional cures for a myriad of diseases. All the plantparts, roots, barks, leaves, and even the fruits are used to treat manydiseases [4]. As a multipurpose medicinal tree, some communities across Africa refer to *C. spinarum* as the "magic herb" [2] as it is used to treat various diseases including headache, chest complaints [5], rheumatism [5-7], gonorrhea, syphilis, rabies, herpes, malaria [8], sickle-cell anemia, hernia, edema, toothache, cough, ulcer, worm infestation [9] and as a diuretic, also for the treatment of typhoid fever, jaundice [10], sexual asthenias in males, measles, and as a cough expectorant [11]. The plant is also useful in the treatment of chickenpox and other skin diseases [12]. The decoction from the pounded root is also administered to treat epilepsy in some communities. In some cases, the patient is made to inhale the vapors coming from the root infusion to treat epilepsy. The traditional birth attendants use the decoction from dried leaves to increase labor and bring about quick child delivery especially during difficult labor. Like the roots, a decoction from the leaves and bark of *C. spinarum* is used in many societies in Africa in the treatment and management of breast cancer, headache, chest pains, gonorrhea, lowering blood pressure, rheumatism, syphilis, rabies, immune booster, fever, edema, cough, ulcer, malaria [13], and to relieve toothache. Roots and root barkare used as anti-venom and snake repellent [14, 15]. It pharmacological activities have been also evaluated by different researchers. The ripe fruits are eaten as snacks to treat and manage dysentery. *C. spinarum* is known to possess an extensive range of phytochemicals in its leaves, roots, barks, as well as fruits that impart enormous medicinal value to the plant. These active constituents offer medicinal value to the plant. Pharmacological importance of the plant fruits has been evaluated by several researchers through *in vitro* and *in vivo* advances. These activities of *C. spinarum* have been reported from the crude extract and their different fractions and isolates from fruit, leave, and root [4]. However, there are no extensive researches studies on the possible toxicity effects of the root part of the plant. Therefore, with the above mentioned extensive usage of the plant in folk medicine, it is imperative to study the toxicological effect of the plant. To this effect, the present study was aimed to evaluate the oral acute and sub-acute effect of the *C. spinarum* in Swiss albino mice.

MATERIALS AND METHODS

Plant material collection

Plant samples

The plant materials used in the present study were collected from their natural habitat. The selection of plants was done on the basis of traditional reputation of particular plants for efficacy in the treatment and management of various diseases as used by traditional health practitioners and the local communities. The plant was identified and authenticated by a botanist, at Addis Ababa University and a voucher specimen of plant samples were deposited at the National Herbarium of Addis Ababa University with voucher number SG45/2016.

Preparation of crude plant extracts

The plant materials were cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered using a grinding mill (IEC,158 VDE 066, Germany). The powdered plant materials were kept clean until they are processed for

extraction. The coarsely powdered plant materials were weighed by sensitive digital weighing balance (Scientech balance) and repeatedly extracted in hydro-alcoholic and alcoholic solvents in maceration flasks (Erlenmeyer flask). The powdered plant materials were soaked separately in alcoholic (non-polar) and 80% methanol (hydro-methanolic) for 72 h by shaking using an orbital shaker at 130 rpm. After 72 h, the extract was separated from the marc by filtration (Whatman filter paper number 1 with pore size 0.7 µm). This procedure was repeated three times. In the non-polar extracts, the solvents were removed by evaporation under reduced pressure by rotary evaporator (Buchi Rotavapor, TRE 121, Switzerland) in distillation flask at 45 rpm and temperature 450C to obtain the crude extracts of each plant. The extract was further concentrated to dryness in a water bath. While the hydro-methanolic extracts filtrate was frozen in the refrigerator overnight and then it was further frozen and dried in a lyophilizer (CHRIST, 3660 Osterode/ harz/France) at-40oC and vacuum pressure to obtain a freeze-dried product. Lastly, the semi-solid crude extracts were then stored in a refrigerator at 4 °C in airtight bottle containers until used for the experiment.

Experimental animals

Swiss albino mice (25-35 grams), 6-8 weeks of age obtained from, Addis Ababa University, were used for the study. They were given a standard diet and tap water ad libitum. The animals were handled according to the international guidelines for the use and maintenance of experimental animals [16]. Ethical clearance was also obtained from University review board for ethical issues of Addis Ababa University.

***In vivo* toxicity study crude extracts of the plant**

The crude extracts of *C. spinarum* were evaluated for their toxicity in naive Swiss albino mice aged 6-8 w and weighing 23-35 grams according to the guideline of Organization for Economic Cooperation and Development (OECD) [17]. The mice were housed in cages and randomly selected ones were marked on the tail for individual identification. All mice were maintained on a 12-h light/dark cycle at room temperature. They were allowed to acclimatize to laboratory conditions for a week before starting the experiment. Drinking water and food were provided *ad libitum* throughout the experiment, except for the short fasting period where the drinking water was still in free access but no food supply was provided 12 h prior to treatment [18]. For the test of each plant extract, a total of 20 mice were selected and randomly divided into four groups of five mice per cage; one control group and three test groups. 0.2 ml of hydro-alcoholic extracts of the selected medicinal plants were given orally in a single dose of 2000, 3000 and 5000 mg/kg for the acute toxicity. The mice in the control group received 0.2 ml of the vehicle of the extract (dH20). Then, the mice were observed continuously for 1 hour, intermittently for 4 h and a period of 24 h for gross behavioral changes such as rigidity, sleep, mortality and other signs of acute toxicity manifestations and followed for 14 d [17].

For sub-acute toxicity test, 0.2ml of hydro-methanolic extracts of the medicinal plant were given orally in an increasing doses of 500, 750 and 1000mg/kg; and for chloroform extract 200, 600 and 1000 mg/kg doses were given for the mice in group one, two and three respectively for 28 consecutive days. The mice in the control group received 0.2 ml of respective vehicle of the extract, distilled water (dH20) and Dimethyl sulfoxide (DMSO). Body weight and hematological parameters were measured before and after treatment for sub-acute toxicity studies. Data were recorded on day 0 and day 4 (after 12 hours of the last dose was given) in terms of body weight loss, and reduction in Packed Cell Volume (PCV), Red Blood Cells (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Content (MCHC) and the mice were closely observed for one month to see the mortality effect of the given extracts. In measuring the hematological parameters, calibrated automatic analyzer (Surmax, K-800, plus Auto Hematology Analyzer, Germany) was used in the hydro methanolic extract of the plant but in case of chloroform extract of *C. spinarum* only PCV and body weight were measured.

Determination of packed cell volume

The packed cell volume (PCV) of each mouse was measured before infection and on day 4 after infection. For this purpose, blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes up to 3/4th of their length. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettichhaematokrit) with the sealed ends outwards. The blood was centrifuged at 12,000 rpm for 5 min. The volume of the total blood and the volume of erythrocytes were measured and PCV was calculated as:

$$\text{PCV} = \frac{\textit{Volume of erythrocytes in a given volume of blood}}{\textit{Total blood volume}} X\ 100$$

Determination of body weight change

The body weight of each mice in all the groups was measured before infection (day 0) and on day-4 in case of treatment, in the same fashion in case of sub-acute toxicity, it was measured before and after the different doses were given by a sensitive digital weighing balance (Scientech balance).

$$\text{Mean body weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}}$$

Data analysis

Results were presented as a mean plus or minus standard error of the mean (M±SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) using SPSS version 20 for windows software. The data obtained from sub-acute toxicity, mean PCV and body weight before and after treatment were analyzed among different groups corresponding to each dose levels and vehicle control group at fixed time and overtime. Mean PCV and body weight before and after infection and treatment were compared by two-tailed paired t-test. To observe any significance differences in the parameters across the two time periods, the average of both parameters was calculated and compared using one way ANOVA followed by Tukey-multiple comparison test. The result was considered statistically significant at 95% confidence level (P-value<0.05).

RESULTS

In the present study, *in vivo* studies on the toxicological effect of hydro-alcoholic and chloroform extracts from *C. spinarum* were carried out in test mice. Before the experiment was commenced, the mice were fasted overnight [17]. The amounts of the *C. spinarum* extracts for acute toxicity given were 2000, 3000 and 5000 mg/kg body weight while the negative control group was given distilled water (dH_2O). At the level of 2000, 3000 up to5000 mgkg-1 body weight, toxicological changes such as hyperactivity, twitching, rigidity, irritability, jumping, sleeping, sedation and abnormal secretions were not observed. Moreover, no mortality was recorded at the given doses.

Sub-acute test for plant materials

The hematological status of animals, i.e., levels of red blood cells (RBC), hemoglobin (Hg), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), of different groups, that is, 500, 750 and 1000 $mgkg^{-1}$bwt of hydro-methanol extracts of the plant species were assessed. Control groups were given dH_20/20%DMSO as a vehicle. The sub-acute toxicity studies revealed that no distinct clinical changes were observed in the *C. spinarum* and. There were no significant changes (P>0.05) in the abovementioned hematological parameters. No mortality was observed in any of the treatment groups. There were no significant differences observed in the body weight of animals treated with extracts and control groups of the plant extracts (table 1).

DISCUSSION

Traditional medicinal plants are usually considered as nontoxic as they are believed to be "natural", nevertheless, some products that contain bioactive principles have the potential to cause adverse

effects [18-20]. Experimental screening method is, therefore, important in order to ascertain the safety and efficacy of traditional plant products and establish the active component of the plant product [21].

Table 1: Sub-acute toxicity of hydro-alcoholic extract of *Carissa spinarum* in mice

	500 mg/kg		750 mg/kg		1000 mg/kg		NC(dH_2o)	
Parameters	**Day-0**	**Day-4**	**Day-0**	**Day-4**	**Day-0**	**Day-4**	**Day-0**	**Day-4**
Body weight (g)	38.06±2.26	35.63±6.3	33.26±4.5	34.46±1.4	28.2±4.09	31.93±3.39	34.6±7.77	40.85±5. 4
RBC(x10^6/ul)	4.48±1.22	5.15±0.87	4.7±0.79	3.64±0.17	4.98±106	3.80±0.9	4.89±0.95	4.25±0.59
Hg(g/dl)	4.0±0.4	6.6±1.96	4.07±0.79	3.5±0.21	4.26±0.46	3.76±0.92	4.8±2	4.4±0.2
PCV (%)	48.87±2.04	45.8±5.01	51.97±1.65	51.19±2.72	47.8±3.8	44.3±2.55	49.3±1.65	48.5±5.33
MCV(fl)	49.06±2.9	51.63±1.29	54.13±1.12	54.85±1.62	51.16±1.53	53.4±3.36	54.93±1.3	55.56±1.95
MCH(pg)	9.46±3.46	10.3±3.9	13.23±3.95	9.2±0.14	14.13±2.0	12.03±2.2	16.1±4.1	14.8±0.5
MCHC(g/dl)	29.2±3.4	29.9±7.39	24.5±7.73	26.7±0.21	27.56±3.23	26.63±4.76	23.2±2.2	26.73±1.19

Key= Values are presented as M±SEM; n=5; NC = negative control (0.2 ml of dH2O), MCHC=Mean Corpuscular Hemoglobin Content; MCV=Mean Corpuscular Volume; PCV=Packed Cell Volume; RBC= Red Blood Cells; Hg=Hemoglobin,

In addition, there were no significant (P>0.05) changes in body weight and PCV in sub-acute toxicity for chloroform root extract treated groups in the doses of 200, 600 and 1000 $mgkg^{-1}$bwt of *Carissa spinarum* (table 2).

Table 2: Sub-acute toxicity of chloroform extract of *Carissa spinarum* in mice

	PCV			Body weight		
Dose mg/kg extract	**Day-0**	**Day-28**	**Mean % change**	**Day-0**	**Day-28**	**Mean % Change**
NC	51.58±2.58	50.61±1.41	-1.91±5.2[a]	31.4±3.13	32.8±2.5	4.45±0.5[a]
200	52.17±2.90	52.83±0.72	1.26±0.43[a]	28.62±2.37	30.24±1.44	5.66±1.88[a]
600	54.62±0.97	52.42±1.99	-4.19±1.13[a]	31.24±2.59	30.18±3.21	-3.39±0.61[a]
1000	50.44±1.36	48.45±2.58	-4.10±.47[a]	29.78±1.41	27.56±2.3	-7.45±6.4[a]

Means in a column followed by the same letter do not differ significantly (P>0.05), **Key** =Values are presented as M±SEM; n=5; NC= negative control (0.2 ml of 20%DMSO);

Both acute and sub-acute toxicity were carried out in the present study. According to the Center for Drug Evaluation and Research (CDER) [22] and OECD [17], acute toxicity is a toxicity produced by a pharmaceutical when administered in one or more doses within a period not exceeding 24 h. When studying acute toxicity, the oral route administration is the most convenient and commonly used one. The absorption might be slow, but this method costs less and is painless to the animals. Since the crude extract is administered orally, the animals should be fasted before taking the dose because food and other chemicals in the digestive tracts may affect the reaction(s) of the compound [18].

In the present study, oral administration of the hydro-alcoholic and chloroform extract of *C. spinarum* in the dose of 5000 mg/kg for the acute toxicity did not produce any significant physical and behavioral changes and no death was recorded in the extract of *C. spinarum* within 24 h. The plants can be considered as safe according to the Organization for Economic Cooperation and Development [17]. On other settings, the water and methanol plant extract of *C. spinarum* showed insignificant cytotoxicity *in vitro* [23]. Moreover, the present acute toxicity study is comparable with the study by Ya'u*et al.* [24]. In which no death was documented up to the dose of 5000 mg/kg following oral administration of root bark of *C. spinarum*. Acute toxicity data are of limited clinical application as cumulative toxic effects do occur even at very low doses. For this reason, multiple dose studies are almost always invaluable in evaluating the safety profile of phytomedicines [25]. Consequently; sub-acute toxicity was carried out in the present study at doses of 500, 750 and 1000 mg/kg of hydro-alcoholic extract of the plant. Similarly, sub-acute toxicity for the chloroform extract of *C. spinarum* was also tested at doses of 200, 600 and 1000 mg/kg to ensure that the given extracts, for four days, are safe for the experiment done. In line with this, analysis of blood parameters is relevant for risk evaluation as the hematological system has a higher predictive value for toxicity in humans when assays involve rodents and non-rodents [26].

Blood forms the main medium of transport for many drugs in the body and for that matter components of the blood such as red blood cells and hemoglobin are at least initially exposed to significant concentrations of toxic compounds. Damage to and destruction of the blood cells is inimical to normal functioning of the body [25]. Meanwhile, the hydro-methanolic extract of the plant species showed no significant changes in various hematological parameters such as Red blood cells (RBC), packed cell volume (PCV), body weight, Red blood cell indices that is**;** mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) concentration compared to the control group, the normal value gained in these parameters were comparable with the study by Sharma and Pandey [27]. In addition, the chloroform extract of *C. spinarum* did not show any significant change on the bodyweight and packed cell volume (PCV) measurement between day-0 and day-4. Hence, the present study may enlighten that crude extract of the plant may not be toxic and does not affect circulating red blood cells, or hematopoiesis. Therefore, the present study results contribute in the approval of the use of the plant in folk medicines.

CONCLUSION

The acute and sub-acute test on the plant extracts was not toxic to the mice at the tested doses of the extracts. Hence, the lack of toxicity of the extracts found in the present study may confirm the claim by traditional practitioners for the use of the plant against different diseases and suggest its ethno-pharmacological usefulness. To ensure the safety of the plant in long term, further chronic toxicity test should also be done.

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AUTHORS CONTRIRBUTIONS

The author designed and performed the experiment, analyzed data and prepared the manuscript.

CONFLICT OF INTERESTS

The author declares no conflict of competing interest

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