Objective: The objective of the present research 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 28d 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 extract. 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*
For sub-acute toxicity test, 0.2ml of hydro-methanolic extracts of the individual identification. All mice were maintained on a 12-h throughout the experiment, except for the short fasting period. Drinking water and food were provided. Cooperation and Development (OECD) [17]. The mice were housed closely observed for one month to see the mortality effect of the given case of chloroform extract of C. spinarum (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 the erythrocytes were measured and PCV was calculated as:

\[
\text{PCV} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100
\]

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).

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 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 (dH₂O). At the level of 2000, 3000 up to5000 mg/kg 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 content (MCHC), of different groups, that is, 500, 750 and 1000 mg/kg bw of hydro-methanol extracts of the plant species were assessed. Control groups were given dH₂O/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

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 weeks weighing 23-35 grams. The crude extracts for sub-acute toxicity study crude extracts of the plant species were assessed. Control groups were given distilled water (dH₂O) and the volume of erythrocytes were measured and PCV was calculated as:

\[
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\]

Determination of packed cell volume

The packed cell volume (PCV) of each mouse was measured before infection and on day-4 at an infectious dose (RBC). Blood was collected from tail of each mouse in heparinized microhematocrit capillary tubes up to 3/4th of their length. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettichhaaratemokrit) 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 the erythrocytes were measured and PCV was calculated as:

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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
Gebrehiwot
Int J Pharm Pharm Sci, Vol 11, Issue 6, 62-65

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

| Parameters | 500 mg/kg | 750 mg/kg | 1000 mg/kg | NC(dH2o) |
|------------|-----------|-----------|------------|----------|
|            | Day-0     | Day-4     | Day-0      | Day-4    |
| Body weight (g) | 38.0±2.26 | 35.6±3.3  | 33.2±4.5   | 34.4±2.14 |
| RBC(x10^12/ul) | 4.4±1.21  | 5.1±0.87  | 4.7±0.79   | 3.6±0.17  |
| Hg(g/dl)     | 4.0±0.1   | 6.6±1.96  | 4.0±0.79   | 3.5±0.21  |
| PCV (%)      | 48.8±2.04 | 45.8±5.01 | 51.9±1.65  | 51.9±2.72 |
| MCV(FL)      | 49.0±2.9  | 51.6±1.2  | 54.3±1.12  | 51.6±1.53 |
| MCHC(g/dl)   | 9.4±3.46  | 10.3±3.9  | 13.2±3.95  | 9.2±0.14  |
| MCHC(g/dl)   | 29.2±3.4  | 29.9±7.39 | 24.5±7.73  | 26.7±2.1  |
| Mean % change | -1.9±5.2 | 31.4±13   | 32.8±2.5   | 4.45±0.5  |

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.

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 drug 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’uet el 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 body weight and PCV in sub-acute toxicity for chloroform root extract treated groups in the doses of 200, 600 and 1000 mg/kg bw of Carissa spinarum (table 2).

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

| Parameters | 500 mg/kg | 750 mg/kg | 1000 mg/kg | NC(dH2o) |
|------------|-----------|-----------|------------|----------|
|            | Day-0     | Day-4     | Day-0      | Day-4    |
| PCV (%)    | 29.9±0.1  | 51.6±1.53 | 54.3±3.36  | 59.4±1.3 |
| MCHC(g/dl) | 9.4±3.46  | 10.3±3.9  | 13.2±3.95  | 9.2±0.14  |
| MCHC(g/dl) | 29.2±3.4  | 29.9±7.39 | 24.5±7.73  | 26.7±2.1  |
| Mean % change | -1.9±5.2 | 31.4±13   | 32.8±2.5   | 4.45±0.5  |

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.

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 safety profile of phytomedicines [25]. Consequently; the acute and sub-acute toxicity test should also be done.

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AUTHORS CONTRIBUTIONS

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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