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Evaluation of acute and sub-acute toxicity of hydro-alcoholic extract of *Capparis tomentosa* Lam. in swiss albino mice

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Abstract

The aim of the present study was to evaluate the toxicity of crude hydro-alcholic extract of *Capparis tomentosa* in Swiss albino mice. In studying the toxicity, Organization for Economic Cooperation and Development (OECD) guidelines was 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, up to 5000 mg/kg crude hydro-alcholic plant extract was given orally using standard intragastric oral gavage. For acute toxicity a single dose was given and gross behavioral change such as inflexibility, irritability, jumping and mortality were recorded. In sub-acute oral toxicity test, *C. tomentosa* crude extract was given to the mice by standard intragastric oral gavage at doses of 500, 750 and 1000 mg/kg body weight every single to 28 days and various hematological parameters were recorded. In acute toxicity, the given dose of the plant extract did not produce significant gross behavioral changes, except slight sleep, inflexibility and sedation at the dose of 3000mg/kg and 5000mg/kgt extracts. However, no death was occurred in the given doses. Therefore, the present study showed that the crude hydro-alcoholic extract of *C. tomentosa* plant did not produce any significant acute and sub-acute toxicity effect in the experimental animals and this may confirm use of the plant in traditional medicines.

Keywords: *Capparis tomentosa, Capparidaceae, acute toxicity, sub-acute toxicity.*

INTRODUCTION

Traditional medicines are mostly considered as non-toxic since they have been used for so long time and are found naturally. However, products of some plants may contain bioactive principles have the potential to cause hazardous effects to human health [1, 2]. Toxicity studies are essential to notice the negative side effects of various products such as medical, pesticides, food additives and other packing materials. According to World health organization (WHO), medicinal herbs are the potential source to obtain wide range of alternative medicines [3]. Studying toxicity is very essential as it indicates whether a new drug can have adverse negative effect or not in clinical use [4]. Toxicity studies can be performed three types such acute, sub-acute and chronic toxicity studies. In acute toxicity test, only a single dose is given to each animal for the determination of gross behavioral change and also Lethal dose 50 (LD 50). In chronic tests, two species, usually one rodent and one non rodent experimental animal are used and they are given doses on daily basis for the period of six months. In sub-acute toxicity tests, experimental animals are given plant extract doses daily, starting at around expected therapeutic level and increasing stepwise every two to three days until toxic signs are recorded [5] *Capparis tomentosa* Lam. is locally known as Gumero in Amharic [6]. It belongs to the family Capparidaceae and found widely in local traditional medicinal use all over Africa [7]. The plant has been traditionally used to treat various diseases such as, fever, asthama, chest pains and pneumonia. The plant was also reported to have notable antifungal effect which could be a potential for discovery of new drug [9]. It has also been reported that the plant has a repellent effects to the malarial mosquito vector, *Anopheles arabiensis* [10]. In studying the toxicity test of medicinal plants, an initial toxic signs needs to be assessed while performing experimental screening of the plant extract. To this end, acute toxicity study test may provide data that serve as the basis for classification and categorizing of the test plant part [11]. Therefore, the present study was aimed to evaluate the toxicity effect of *C. tomentosa* hydroalcholic extract in Swiss Albino mice.

MATERIAL AND METHODS

Collection and identification of plant

The plant materials used in the present study were collected, from their natural habitat of Tolu-kurma Center for Indigenous Trees, which is found 52 km west Addis Ababa, Ethiopia. The selection of plants was done based on its traditional medicinal use as used by local communities [6]. The plant was identified and authenticated by a Botanist, at Addis Ababa University and a voucher specimen of the plant sample was deposited at the National Herbarium of Addis Ababa University.

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 kept clean until extraction. The coarsely powdered plant materials were weighed by sensitive digital weighing balance (Scientech balance). The powdered plant materials were soaked separately in 80% methanol (hydro-alcoholic) for 72 hours by shaking using an orbital shaker at 130 rpm. After 72 hours, the extract was separated from the marc by filtration (Whatman filter paper number 1 with pore size 0.7µm). This procedure was repeated three times to gain more plant extract. Then, the hydro-alcoholic extract filtrate was frozen in 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 4ºC in air tight bottle containers until used for the experiment.

Experimental Animals

Swiss albino mice (25-38 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.*

Toxicity test for the plant materials

The crude plant extracts were evaluated for their toxicity in non-infected male Swiss albino mice aged 6-8 weeks and weighing 23-35 grams as per the guideline of OECD [12]. 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 [1]. 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. 0.2ml of hydro-alcoholic extracts of the selected medicinal plants were given orally in a single dose of 2000, 3000 and 5000mg/kg for the acute toxicity. The mice in the control group received 0.2 ml of vehicle of the extract (dH20). Then, the mice were observed continuously for 1 hour, intermittently for 4 hours and a period of 24 hours for gross behavioral changes such as rigidity, sleep, mortality and other signs of acute toxicity manifestations and followed for 14 days [12]. For sub-acute toxicity test, 0.2ml of hydro-alcoholic extracts of the medicinal plant were given orally in increasing doses of 500, 750 and 1000mg/kg. The mice in the control group received 0.2 ml of respective vehicle of the extract (dH20). 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 PCV, RBC, MCV, MCH, 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-alcoholic extract the plants.

Determination of Body weight Change

The body weights of experimental animals were recorded before the test extracts are given and at the end of each week. Similarly, in case of sub-acute toxicity, it was measured before and after the different doses were given by oral gavages and a sensitive digital weighing balance (Scientech balance) was used to weigh the body of the experimental mice. The percentage of body weight change calculated as given in the equation below [13].

$$\text{Mean body weight} = \frac{\text{Body weight at the end of each week} - \text{initial body weight}}{\text{Initial body weight}}\ 100$$

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 extract given were analyzed among different groups corresponding to each dose levels and vehicle control group at fixed time and overtime (D0 and D4). 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).

RESULT

Acute toxicity test for the plant materials

In the present study, *in vivo* studies on the toxicological effect of hydro-alcoholic extracts from *Capparis tomentosa* was carried out in test mice. Before the experiment was commenced, the mice were fasted overnight (OECD, 2001). The amounts of *Capparis tomentosa* hydro-alcoholic extracts for acute toxicity given were 2000, 3000 and 5000 mg/kg body weight while the negative control group were given dH_2O. At the level of 2000 $mgkg^{-1}$ bwt, no death and other toxicological changes were observed. At the level of 3000 and 5000 $mgkg^{-1}$ bwt slight rigidity and sleepy activities were observed but no death was recorded (Table 1).

Table 1: Acute toxicity of the hydro-alcoholic extracts *Capparis tomentosa* in mice

Doses of extracts				**Negative control**
Signs of toxicity	**2000mg/kg**	**3000mg/kg**	**5000mg/kg**	**dH_2O**
Hyperactivity	0	0	0	0
Twitching	0	0	0	0
Inflexibility	0	+	++	0
Irritability	0	0	0	0
Jumping	0	0	0	0
Sleep	0	+	0	0
Sedation	0	0	++	0
Abnormal secretion	0	0	0	0
Death	0	0	0	0

Key: 0= no sign was observed; += sign was observed and number of mice that showed the sign.

Sub-acute test for plant materials

The hematological status of animals, i.e., levels of red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), of different groups, i.e. 500, 750 and 1000 $mgkg^{-1}$ bwt of hydro-methanol extracts of the plant species were assessed. Control groups were given dH_20 as a vehicle. The sub-acute toxicity studies revealed that, no distinct changes were observed in the *C. tomentosa* hydro-alcoholic extract treated animals. There were no significant changes (P>0.05) in the hematological parameters. No death was recorded in any of the treatment groups. In addition, no significant difference was observed in the body weight change of experimental animals treated with extracts and control groups of the plant extracts (Table2).

Table 2: Sub-acute toxicity of hydro-alcoholic extract of *C. tomentosa* in Swiss Albino mice

Parameters	500mg/kg		750mg/kg		1000mg/kg		NC (dH_2o)	
	Day-0	Day-4	Day-0	Day-4	Day-0	Day-4	Day-0	Day-4
Body w.(g)	38.1±2.6[a]	40±5.1[a]	33.1±0.89[a]	34.47±1.5[a]	30.7±1.0[a]	32.1±7.2[a]	39.1±4.07[a]	40.95±7.4[a]
RBC(x10^6/ul)	4.45±0.15[b]	4.4±1.0[b]	4±0.7[b]	5.0±1.05[b]	4.22±0.77[b]	4.79±0.02[b]	4.33±0.12[b]	4.69±0.8[b]
Hg(g/dl)	4.56±0.15[c]	4.3±1.5[c]	4 ±0.7[c]	3.6±0.7[c]	3.96 ±1.56[c]	5.05 ±2.05[c]	4.43±0.3[c]	4.76±0.32[c]
PCV (%)	51.3±1.65[d]	49.7 ±5.33[d]	49.4±2.04[d]	50.2±1.41[d]	48.3±2.28[d]	46.3±3.8[d]	49.94±1.41[d]	48.1±2.28[d]
MCV(fl)	53.2±0.85[e]	53.85±1.53[e]	48.36±2.13[e]	50±1.55[e]	53.6±2.16[e]	55.8±1.13[e]	49.23±1.83[e]	51.2±3.14[e]
MCH(pg)	15.56±4.07[f]	12.43±4.2[f]	10.73±2.47[f]	15.6±0.98[f]	12.6±5.9[f]	10.6±4.2[f]	18.26±0.61[f]	14.1±2.2[f]
MCHC(g/dl)	29.26 ±7.4[g]	22.6±8.1[g]	22.26 ±5.56[g]	24.25±2.89[g]	23.3 ±10[g]	21.75±7.4[g]	27.16 ±2.3[g]	26.8 ±1.8[g]

Means in the rows followed by the same letter do not differ significantly (P>0.05)
Key=Values are presented as M±SEM; n=5; NC=negative control (0.2ml of dH2O); MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Content; MCV=Mean Corpuscular Volume; PCV=Packed Cell Volume; RBC= Red Blood Cells; Hg=Haemoglobin

DISCUSSION

In the present study, oral administration of the hydro-alcoholic extract of *C. tomentosa* in the dose of 2000 mg/kg for the acute toxicity did not produce any significant changes in hyperactivity, twitching, rigidity, irritability, jumping, sleep, sedation, abnormal secretion, death within 24 hours while in the dose of 3000 mg/kg rigidity and sleep was observed in hydro-alcoholic extract but these signs did not persist and the mice were recovered instantly on the day of plant extracts given. Acute toxicity data are considered as having limited clinical application since cumulative toxic effects can occur even at very low doses. Hence, intensive multiple dose studies are recommended in evaluating the safety profile of phytomedicines [14]. Subsequently, 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. In line with this, when studying toxicity in rodents and non-rodents, evaluating risk for hematological parameters is essential as they are predictive of toxicity in humans [15]. Meanwhile, evaluating blood parameters is important because blood transports different materials including drugs. Hence, blood components are exposed to toxic effect of compounds in drugs and if any adverse effects occurred in the blood, then the body could not function properly [14]. In this regard, the hydro-alcoholic extract of the plant did not produce significant changes in various hematological parameters such as RBC, PCV, body weight, Red blood cell indices that is**;** mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) compared to the control group, the normal value recorded in these parameters were comparable with other study elsewhere [16]. Similar result with the present study was also reported in which *C. tomentosa* displayed no toxic effects on the brine shrimp [10]. However, the present study is contradictory to reports done other study [17] in which *C. tomentosa* had a toxic effect on goats fed with the leave. The contradicting reports might be due to the difference in the composition of the chemical constituents of the root and the leaves of the plant. Hence, the present study may enlighten that crude extract of the plants may not be toxic and does not affect the tested hematological parameters. This could justify the traditional use of this plant by the people of Debrelibanos105 km north Addis Ababa [6] in primary health care.

CONCLUSION

In the present study, hydro-alcoholic extract prepared from *C. tomentosa* was performed by acute and sub-acute oral toxicity test in Swiss Albino mice following OECD guide lines. Since no significant change was observed in behavior of the experimental animals, hydro-alcoholic extract of the plant could be considered as safe up to the given dose. Therefore, the present study confirms, partly, the safety of hydro-alcoholic extract of *C. tomentosa* which is crucial in its application for pharmaceutical uses. However, further chronic toxicity investigations are required to confirm the safe use of the plant extract.

Conflict of Interest

The author declares no conflict of competing interest.

REFERENCES

1. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S. Acute Oral Toxicity of Methanolic Seed Extract of *Cassia fistula* in Mice. *Molecules*. 2011; 16(6):5268-5282.
2. Fabricant DS, Farnsworth NR. The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environ. Health Perspect*. 2001; 109:69-75.
3. Dharmalingam S, Natesan G. Evaluation of acute toxicity of the methanolic extract of *Tanacetum parthenium* L. in albino wistar rats. J Sci Inn Resear 2017; 6(3):113-115.
4. Aneela S, De S, Kanthal L, Choudhury N, Das B, Sagar K. Acute oral toxicity studies of Pongamia Pinnata and Annona squamosa on albino wister rats. Int J Res Pharm Chem 2011; 1(4):820-4.
5. Bhardwaj S, Gupta D. Study of acute subacute and chronic toxicity test. Int J Adv Pharm Biol Sci 2012; 2:103-29.
6. Teklahaimanot T, Giday M, Medhin G, Mekonnen Y. Knowledge and use of medicinal plants by people around Debre-Libanos monastery in Ethiopia. J. Ethnopharmacol 2007; 111:271-283.
7. Akoto O, Oppong IV, Addae-Mensah I, Waibel R, Achenbach H. Isolation and characterization of dipeptide derivative and phytosterol from *Capparis tomentosa Lam. Sci*. *Res. Essays.* 2008; 3:355-358.
8. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agro-fores tree Database: a tree reference and selection guide version 4.0. 2009 (http://www.worldagroforestry.org/af/treedb/)
9. Mirsah SN, Tomar PC, Lakra N. Medicinal and food value of *Capparis*-a harsh terrain plant. Indian J. Trad. Knowl. 2007; 6:230-238.
10. Hurinanthan V. *Immune Modulatory Effect of Dichrostachys cinerea, Carpobrotus dimidiatus, Capparis tomentosa and Leonotis leonurus.* MSc thesis, Durban University of Technology South Africa, 2009.
11. Ukwuani A, Abubakar M, Hassan S, Agaie B. Toxicological studies of hydromethanolic leaves extract of Grewia crenata. Int J Pharm Sci Drug Res, 2012; 4(4):245-9.
12. OECD. OECD guideline for testing of chemicals. Acute Oral Toxicity–Up-and-Down Procedure, 2001.
13. Al-Afifi N, Alabsi M, Bakri M, A Ramanathan A. Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. *BMC Complementary and Alternative Medicine.* 2018; 18:50.
14. Abotsi WKM, Ainooson GK, Gyasi EB. Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) in rodents. West Afr. J. Pharm. 2011; 22:27-35.

15. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al*. Concordance of toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol. 2000; 32:56-67.
16. Sharma V, Pandey D. Beneficial Effects of *Tinospora cordifolia* on Blood Profiles in Male Mice Exposed to Lead. *Toxicol. Int.* 2010; 17:8-11.
17. Ahmed SA, Amin AE, Adam SE, Hapke HJ. Toxic effect of the dried leaves and stem of *Capparis tomentosa* on Nubian goats. *Dtsch Tietarztl, Wochenschr*. 1993; 5:192-194.