Research Article

Acute and chronic toxicity study of ethyl acetate fraction of *Caralluma tuberculata* in mice

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ABSTRACT

Background: *Caralluma tuberculata* (Asclepiadaceae), a succulent perennial herb used as vegetable is reported to treat the diabetes and rheumatism. In the present study the safety profile of *Caralluma tuberculata* (CT) was evaluated by gross behavioral, acute and chronic toxicity study of ethyl acetate fraction of ethanolic extract of CT in Swiss albino mice.

Materials and Methods: For acute toxicity study, mice were divided into seven groups. Normal saline was given by intraperitoneal route to control group and to other six groups, different doses of extract i.e. 0.5, 1, 1.5, 2, 2.5 and 3 g/ kg body weight was given to the mice intraperitoneally. In chronic toxicity study, 100mg/kg body weight was given intraperitoneally route for three months. In acute toxicity mortality rate was determined, while in chronic toxicity changes in body and organ weight, biochemical, hematological parameters in both male and female mice, and spermatotoxic effects in male mice were measured by using standard procedures. Statistical analysis was carried out by student t-test.

Results: Acute toxicity showed that the ethyl acetate fraction was safe up to 3g/kg body weight in mice. In gross behavioral study some signs of passivity and decreased spontaneous activity were observed. No mortality was observed in chronic toxicity study. No detectable alterations were found on weight, vital body organs, biochemical, and hematological parameters; no spermatotoxic effects in treated mice were observed when compared to vehicle control group after three months of chronic treatment.

Conclusion: The results of present study therefore indicated that ethyl acetate fraction of ethanolic extract of *Caralluma tuberculata* was safe in test animals demonstrating no noticeable toxicity.

Key words: *Caralluma tuberculata,* ethyl acetate fraction, gross behavioral, spermatotoxic.

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INTRODUCTION

Compounds derived from plants have nutritional as well as therapeutic values and are used against various animal and human diseases. Different recipes are prepared by practitioners by the combination of two or more plant products, hence used for the treatment of more than one disease condition.[1]

Caralluma tuberculata (Asclepiadaceae) is a succulent perennial herb and is largely grown in Pakistan, India and south east of Egypt.[2] It is used as vegetable and is reported to treat diabetes and rheumatism. It is also used as remedy for snake and scorpion bite, as a blood purifier, hypotensive agent and in leprosy.[3] Locally it is known as chung and pamanke. It is cocked and eaten as a vegetable. Phytochemical studies on this plant have led to the isolation of pregnane, flavones, glycosides [4] β sterol and lupeol.[5] It is also used locally for the treatment of Diabetes. There was no scientific report on acute and chronic toxicity of *Caralluma tubercuata* (CT) and also on the gross behavioral study in the

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literature. The ethyl acetate fraction of this plant has significant anti-diabetic effects in another experiment performed in our lab. Therefore, the current study was planned to evaluate the acute and chronic toxicity of ethyl acetate fraction of CT in mice.

MATERIALS AND METHODS

Plant material

Fresh plant material of CT was purchased from local market and identified by Dr. Qazi Najam-us-saqib professor at CIIT Abbottabad. A voucher specimen of *Caralluma tuberculata* was deposited and preserved at the herbarium of CIIT Abbottabad, KPK, Pakistan.

Preparation ethyl acetate fraction

The fresh plant material (15kg) was minced and extracted with distilled ethanol using soxhlet apparatus. The ethanolic extract was filtered and subsequently evaporated in rotary evaporator, resulting in thick paste. A portion (450g) of the crude extract was dispersed in distilled water (500ml) and shaken with equal volume of ethyl acetate. Total ethyl acetate fraction (EA CT) was evaporated under reduced pressure for dryness.

Animals

Swiss albino mice (*Mus musculus*) weighing 20 - 25 g of male sex, fed on standard diet and access to tap water *ad libitum* were used. They were housed in identical wire-mesh -bottomed stainless-steel cages and maintained in an air-conditioned room at 25 ± 2°C, 50 - 60% relative humidity and artificial illumination between 06:00 and 18:00 h. The animals were kept under controlled condition in accordance with the national institute of health (NIH) guidelines.All procedures concerning animal treatments and experimentations in this study were reviewed and approved(CIIT 0021.11/32) by the Institutional Committee for Ethical Use of Experimental Animals at COMSATS institute of information technology, Abbottabad, Pakistan.

Acute toxicity

The toxicity study was carried out using thirty-five (35) male Swiss albino mice weighing 20 - 25 g each. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum* and were allowed to acclimatize to the laboratory conditions for seven days before the experiment. After overnight fasting, the control group received normal saline and the treated groups received ethyl acetate fraction of CT at the doses of 0.5, 1, 1.5, 2, 2.5 and 3g/kg body weight. The calculated doses were prepared with 0.5% carboxy methyl cellulose (CMC) in distilled water. These doses were given by intraperitoneal route. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering the extract, to observe any death or changes in general behavior and other physiological activities. Acute toxicity and gross behavioral screening were studied.[6]

Chronic Toxicity

Animals were divided into two groups - twenty mice of both sex (10 male and 10 female) were used in each group, one was control and other was test group. Dose of 100 mg/kg body weight/day of EA CT was administered in drinking water to each animal in the test group. This dose was selected on the basis of anti-diabetic effect of the EA CT in the mice in another experiment in our laboratory. The treatment was continued for a period of three months[7] during which the changes in body weight was observed. The male mice were analyzed for spermatotoxicity, while both male and female mice were analyzed for body and organ weight changes, hematological studies, and serum biochemical parameters.

Spermatotoxicity

The chronically treated male animals were analyzed on day 91 for spermatogenic dysfunction using the sperm abnormality test.[8] The caudae epididymides and vas deferens from the same animals were dissected and transferred to a centrifuge tube containing 3ml krebs-Ringer bicarbonate buffer.[9]

The sperm suspension was filtered through 80µm silk mesh tissue fragments and 0.5ml of 1% eosin Y was added to each tube. The contents were thoroughly mixed and slides were prepared by placing one drop of the solution on a slide and spread by three passes of another slide. Coded slides were examined.[10]

Body weight and organ weights

The body weights of mice of each group before and after the chronic treatment were measured. After this period the animals were killed by cervical dislocation and weight of vital organs (Heart, Lungs, Liver, Kidney, Spleen, Testis, Seminal, Caudae epididymis) were measured.

Hematological Studies

The blood was analyzed for WBC, RBC and Hb level by using Contraves Digicell 3100H (Zurich).[11]

Serum Biochemical Parameters

Collected blood from chronically treated animals at fasting, serum was separated and analyzed for aspartate amino transferase (AST), alanine amino transferase (ALT), creatine kinase iso-enzyme MB (CK-MB), glucose, urea and creatinine. These parameters were analyzed on a spectrophotometer (Ultrascope Π, LKB).

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM), and statistical analysis was carried out by student t-test. Differences were considered significant at $p<0.05$.

RESULTS

In acute toxicity and gross behavioral screening of EA CT, the tested animals had no sign of toxicity and mortality up to the dose of 3g/kg body weight. However, during gross behavioral screening some sign of passivity and decrease spontaneous activity were observed in group 3 animals who received 3g/kg body weight of EA CT at 1-4 hour (Table 1).

In chronic toxicity, no symptoms of toxicity were observed upon chronic treatment with EA CT during 0-30 day; however one male and one female mouse died in control group. While in treated group two mice died (one male mouse during 0-30 day and one female mouse died on day 70; Table 2).

After chronic treatment with EA CT, the total body weight gain in post treated group of male mice was significant ($p < 0.05$) as compared to pretreated male mice group. While in post treatment, weight gain in female mice was highly significant ($p < 0.005$) as compared to pretreatment female mice group. Organ weight (per 100 g body weight) in mice after chronic treatment with EA CT (100mg/kg body weight/ day) was normal and comparable to the control (Table 3 & 4).

The sperm toxicity found in treated group was statistically non-significant ($p > 0.05$) and abnormality percentage was less than the control animals (table 5).

While in biochemical parameters, the results showed a significant decrease ($p < 0.05$) in blood glucose level of treated animals as compared to control. There was no significant difference in other parameters studied as compared to the control group except mild reduction in CK-MB and slight rise in serum AST, which was statistically non-significant. Hematological studies revealed no significant difference in level of WBC, RBC and Hb levels in treated animals as compared to control (table 7).

Table 1: Acute toxicity and gross behavioral screening of ethyl acetate fraction of *Caralluma tuberculata*

Time after dosing (hrs)	0hr				1/2hr				1hr				2hr				4hr					8hr				12hr			24hr			
Dose(g/kg)	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3	c	0.5	1	3
Vocalization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stereotype	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irritability	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Passivity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spontaneous activity	++	++	++	++	++	++	++	++	++	++	++	-	++	++	++	-	++	++	++	-	++	++	++	++	++	++	++	++	++	++	++	++
Touch res	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pain res	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staggering gait	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limb tone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Body tone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cornea reflex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Light reflex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ptosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Exophthalmoses	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pilo erection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No of deaths (0 -24hrs)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

c- control, (++) Increase, (+) Decrease, (-) Absent

Table 2: Mortality in chronic toxicity study with ethyl acetate fraction of *Caralluma tuberculata* in mice

Treatment (n=20) (male-10, Female-10)	Death of mice in number (male, female)				Lethality (%)
	0-30 days	31-60 days	61-90 days	Total	
Control	1,1	0, 0	0, 0	2	10
EA CT	1,0	0, 0	1, 0	2	10

EA CT - Ethyl acetate fraction of *Caralluma tuberculata* at the dose of 100mg/kg body weight/day

Table 3: Change in body weight after 90 days treatment of ethyl acetate fraction of *Caralluma tuberculata* in mice

Treatment (n=20) (male-10, Female-10)	Body weight in gram (Mean ± SEM)			
	Pre treatment		Post treatment	
	Male	Female	Male	Female
Control	24.4 ± 1.3	23.9 ± 1.2	30.7 ± 1.2†	29.4 ± 1.0†
EA CT	23.8 ± 1.1	24.2 ± 1.3	27.3 ± 1.5*	28.8 ± 1.2†

EA CT - Ethyl acetate fraction of CT at 100mg/kg body weight/day
* $p < 0.05$ vs. pre treatment, † $p < 0.005$ vs. pre treatment

Table 4: Change in organ weight with 90 days treatment of ethyl acetate fraction of *Caralluma tuberculata* in mice

Organs (n=5)	Organ weight in gram/100g body weight (Mean ± SEM)	
	Control	EA CT
Heart	0.49 ± 0.01	0.50 ± 0.03
Lungs	0.79 ± 0.06	0.82 ± 0.09
Liver	6.00 ± 0.14	6.57 ± 0.24
Kidney	1.61 ± 0.05	1.72 ± 0.04
Spleen	0.52 ± 0.08	0.62 ± 0.04
Testis	0.66 ± 0.02	0.65 ± 0.04
Seminiferous tubules	0.58 ± 0.03	0.56 ± 0.03
Caudae epidedymis	0.23 ± 0.02	0.25 ± 0.02

EA CT - Ethyl acetate fraction of *Caralluma tuberculata* at the dose of 100mg/kg body weight/day

Table 5: Spermototoxic effects of 90 days treatment of ethyl acetate fraction of *Caralluma tuberculata* in mice

Treatment (n=5)	Total spermatozoa (screened)	% abnormality (Mean ± SEM)
Control	5765	1.27 ± 0.05
EA CT	5715	1.25 ± 0.12

EA CT - Ethyl acetate fraction of *Caralluma tuberculata* at the dose of 100mg/kg body weight/day

Table: 6: Effect of 90 days treatment by ethyl acetate fraction of *Caralluma tuberculata* on blood parameters in mice

Parameters	Control (n=5)	EA CT (n=5)
ALT (U/L)	24.63 ± 4.71	27.35 ± 7.61
AST (U/L)	56.88 ± 6.32	57.73 ± 9.50
CK-MB (%)	127.55 ± 16.26	125.71 ± 17.83
Creatinine (µmol/L)	66.14 ± 4.76	65.95 ± 3.39
Urea (µmol/L)	6.71 ± 0.74	6.56 ± 1.52
Glucose (µmol/L)	11.42 ± 0.85	9.14 ± 0.64*
WBC(x 10^3) /mm^3	5.6 ± 0.7	5.7 ± 0.9
RBC (x 10^6) /mm^3	7.9 ± 0.2	6.8 ± 0.3
Hemoglobin(g/dl)	12.4 ± 0.3	13.0 ± 0.4

Values are expressed in mean ± SEM
EA CT - Ethyl acetate fraction of CT at 100mg/kg body weight/day

DISCUSSION

Medicines of herbal origin are nowadays used as alternative to synthetic medicine. Therefore it is essential to carry out the safety and efficacy of these herbal products by experimental studies.[7]

In acute toxicity and gross behavioral study, the dose was safe up to 3g/kg body weight of Swiss albino mice. No sign of toxicity and mortality were observed. During chronic toxicity study there were no toxic symptom during 0-30 days, except two deaths one between 0-30 and other between 61-90 day interval in the treatment group. All the treated male and female mice throughout the study were normal and comparable to the control.

Gaining body weight in both male and female mice indicates that the extract does not interfere with growth processes and may have promoted growth by stimulating the synthesis of body proteins. This increase in weight may be due to increase of appetite by the extract.[12]

Biochemical studies revealed the significant decrease in blood glucose level after chronic oral treatment. Anti-diabetic effect was also shown by the crude extract and ethyl acetate fraction in another experiment performed in our lab. The hypoglycemic action of this fraction might be due to its insulin mimetic action or by some other mechanism. Antioxidant property of the extract in preventing these changes may also be considered to play a vital role.[13]

There were no significant differences in other parameters studied as compared to control except mild reduction in CK-MB and slight rise in serum AST, which was non-significant. Similarly hematological studies revealed no significant difference in the level of WBC, RBC, and Hb levels in treated animals as compared to the control group.

The present acute, gross behavioral and chronic toxicity results of EA CT tend to support the safe folklore use of this plant as a drug. In the light of the results and above discussion, it is concluded that EA CT is safe in Swiss albino mice. It did not affect the biochemical and hematological parameters and also has no effect on the growth of vital body organs. However, further studies are necessary to be carried out about its safety in other species of rodents and humans.

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