

Acute and Sub-Chronic Toxicity Study of *Trichosanthes dioica* Root in Mice

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Abstract: In the present study, the safety profile of *Trichosanthes dioica* root was evaluated by acute and sub-chronic toxicity study of the hydro alcoholic extract of *T. dioica* root (TDA) in Swiss albino mice. The oral median lethal dose (LD₅₀) of TDA was found to be 2800 mg/kg body weight. For sub-chronic toxicity study TDA was administered at the single daily dose of 10 mg/kg for 28 consecutive days; and at the 29th day, the hematological, serum and liver biochemical parameters were evaluated. No mortality and signs and symptoms of toxicity were observed during the course of whole study period. No significant alterations were found in hematological, serum and liver biochemical parameters in TDA treated mice when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *T. dioica* root extract may be regarded as safe in Swiss albino mice when given orally eliciting no noticeable toxicity.

Key words: Sub-Chronic Toxicity • *Trichosanthes dioica* • Biochemical • Root • Antioxidant • LD₅₀

INTRODUCTION

In evaluation of toxic characteristics of a substance, study of acute oral toxicity is the initial step. Acute oral toxicity is the adverse effect occurring within a short term (like 2-24 h) of oral administration of a single dose of a substance or multiple doses given within 24 h [1]. It provides information about the unwanted or adverse effects likely to arise after the acute exposure and the median lethal dose (MLD or LD₅₀) i.e., the dose which kills 50% of the animals of a particular species. LD₅₀ value is usually determined in a 24 h test using any species [2].

The objective of sub-chronic toxicity study is to determine the effect of a test substance after repeated and short term administration. The design and conduct of such toxicity studies should allow the detection of general physiological toxic effects and exposure related morphological effects. A sub-chronic toxicity study should generate data from which majority of chronic effects can be identified to define long term dose response relationships [3].

Trichosanthes dioica Roxb. (Cucurbitaceae), called pointed gourd in English, *Potol* in Bengali and *Patola* in Sanskrit, is a dioecious climber found wild throughout the plains of North and North-east India from Punjab to Assam and Tripura states. It is also grown and commercially cultivated in India, Pakistan, Bangladesh and Sri Lanka for its fruits, a common culinary vegetable in the Indian subcontinent. In India, all parts of this plant have been traditionally used for medicinal purposes. According to Ayurveda, the traditional system of Indian medicine, its root is a strong purgative. The root has been traditionally used in India as cathartic, tonic, febrifuge; in treatment of jaundice, anasarca and ascites [4-7]. In our previous course of studies, we have reported anthelmintic, antibacterial, antimitotic, antiproliferative, antitumor, analgesic, laxative, cancer chemopreventive, arsenic toxicity ameliorative and antileishmanial activities of the root of *T. dioica* [8-22]. The present study evaluated the hydro alcoholic extract from *T. dioica* root (TDA) for possible acute and sub-chronic oral toxic effects in Swiss albino mice to ascertain its acute and short term safety profile.

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MATERIALS AND METHODS

Plant Material: The mature tuberous roots of *T. dioica* were collected during December 2008 from Majdia, Nadia district, West Bengal, India. The plant species was identified by Dr. M. S. Mondal, at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India and a voucher specimen (CNH/I-I/57/2009/Tech.II/493) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology, Delhi Road, Hooghly 712102, India. Just after collection, the plant material was washed thoroughly with running tap water and shade dried at room temperature (24-26°C) and ground mechanically into a coarse powder.

Preparation of Extract (TDA): The powdered plant material (644 g) was macerated at the room temperature (24-26°C) with 20% ethanol water (950 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 days. The macerates were combined, filtered and evaporated to dryness *in vacuo* (at 35°C and 0.8 MPa) in a Buchi evaporator, R-114. The dry extract (TDA, yield: 12.15%) was kept in a vacuum desiccator until use.

Standardization of TDA: TDA was subjected to preliminary phytochemical analysis that revealed the presence of reducing sugars, amino acids, triterpenoids and steroids in TDA [23]. Presence of cucurbitacin type triterpenoid aglycones in TDA was ascertained by thin layer chromatography on silica gel pre-coated HPTLC plates (Silica gel 60 F₂₅₄ Merck, Germany) detected with vanillin-phosphoric acid reagent [24].

Drugs and Chemicals: Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) from Loba Chemie, Mumbai, India; 5,5'-dithio bis-2-nitro benzoic acid (DTNB), phenazonium methosulphate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially. Doubled distilled water from all-glass still was employed throughout the study.

Experimental Animals: Adult male Swiss albino mice weighing 20 ± 2 g were grouped and housed in a clean polypropylene cage with not more than four animals per cage and maintained under standard laboratory

conditions (temperature $25 \pm 2^\circ\text{C}$ with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All the experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee, Jadavpur University.

Acute Toxicity Study: The acute oral toxicity of TDA in Swiss albino mice was studied by 'Up and Down Procedure' (UDP) and the median lethal dose (LD₅₀ value) was determined as per reported method [25]. The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 h (with special attention given during the first 4 hours) and then for further 24 h, i.e., total 48 h for morbidity or mortality. Additional observations for signs of toxicity included changes in skin, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and behavioral pattern. Attentions were directed for observations of tremors, convulsions, salivations, diarrhea, lethargy, sleep and coma.

Sub-Chronic Toxicity Study: The animals were divided into two groups ($n = 6$). The first group received normal saline (5 ml/kg body weight p.o.) and the second group received TDA at 10 mg/kg body weight p.o. once daily for 28 days. Food and water intake of animals were observed during this period. Twenty four hours after the last dose (i.e. at 29th day), blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of haematological and serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical parameters and organ weights [26].

Observations: The treated mice were observed daily for 28 days to record the sign of any unwanted effects, morbidity and mortality. The sign of unwanted effect include tremor, rigidity, convulsion, salivation, diarrhoea, lethargy, sleep, coma, dyspnoea, nasal bleeding etc. The body weight and food and water intakes were monitored periodically.

Body Weight and Organ Weights: The body weight of mice of each group were measured just before and 28 days after TDA treatment. Heart, lung, liver, kidney and pancreas weights of all mice were measured immediately after post treatment sacrifice.

Hematological Parameters: Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count and total white blood cell (WBC) counts as per the standard methods [27, 28].

Serum Biochemical Parameters: Collected blood was used for the estimation of serum biochemical parameters viz. aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum bilirubin, serum total cholesterol, urea, uric acid, creatinine and non protein nitrogen (NPN) by using commercially available reagent kits (Span Diagnostics, Surat, India). Serum total protein content was measured by previously reported method [29].

Liver Biochemical Parameters: The liver tissue of sacrificed animals was processed and subjected to estimation of lipid peroxidation i.e., thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) as per reported methods [30-33].

Statistical Analysis: All the data are expressed as mean \pm standard error of mean (SEM).

RESULTS

In acute toxicity study the oral LD₅₀ value of the hydro alcoholic extract of *T. dioica* root (TDA) in Swiss mice was found to be 2800 mg/kg body weight. It did not produce any signs and symptoms of adverse effects during the 48 h observation period. In sub-chronic study there were no significant changes in body weights and organ weights of mice of TDA treated group (after 28 days) from saline control group (Table 1). No mortality or moribund animal was evident from the experimental results. The food and water intake of TDA treated group was found comparable to the control group without showing significant alteration in body weight and growth rate. The hematological parameters were found practically unaltered in animals of TDA treated group as compared to the control group (Table 2). After 28 days of treatment no significant alterations were observed in all serum and liver biochemical parameters in animals of TDA treated group when compared with those of control group (Tables 3 and 4). No signs and symptoms of adverse effects were noticed during the observation period of 28 days.

DISCUSSION

The present study was aimed to investigate the possible toxic effects of the hydro alcoholic extract of *T. dioica* root (TDA) in Swiss mice. In any *in vivo* toxicological screening programme acute toxicity studies in mice are usually performed first [2]. In acute toxicity study, the oral median lethal dose (LD₅₀ value) of TDA was determined to be 2800 mg/kg body weight indicating very low acute oral toxicity in mice. It did not produce any signs and symptoms of adverse effects in the acute toxicity study.

In all the previously performed repeated dose pharmacological studies, TDA was administered orally at the doses ranging 2-10 mg/kg body weight demonstrating remarkable activities [17-19, 22]. Therefore, the highest dose among those regimens i.e., 10 mg/kg body weight p.o. was selected for conducting the present sub-chronic toxicity study of TDA.

Various parameters were studied in sub-chronic toxicity study. Body weight is regarded as a non-specific indicator of general wellbeing of animals. Reduction in body weight is an indicator of decline in general health conditions. The body weights, food and water intakes were found to be unaltered during the 28 days of TDA treatment period when compared to control group. Similarly there were no significant changes in different vital organ weights also. No mortality or moribund animal was observed during this period.

Haematological parameters were evaluated to assess haematological toxicity of TDA on long term use. The results showed no deleterious effects on blood cell counts and haemoglobin content thereby suggesting that TDA elicited no toxic effect on blood and haematopoietic system thus maintaining the normal haematological profile in TDA treated mice.

It is well known that liver is the important organ for metabolism and detoxification of xenobiotics. Serum biochemical parameters like AST, ALT, ALP, bilirubin, cholesterol and total protein are known as hepatic function parameters, elevation of which occurs due to impaired hepatic functions [34]. The present study indicated that these hepatic function parameters are not significantly altered in 28 days of TDA treatment as compared to the vehicle control group.

Kidney is the principal excretory organ of mammals. It is also well known that almost all drugs, chemicals, xenobiotics are eliminated through renal excretion hence

Table 1: Effect of TDA on body weight and major organs weights of mice

Treatment	Initial body wt (g)	Final body wt (g)	Heart wt (g)	Lung wt (g)	Liver wt (g)	Kidney wt (g)	Pancreas wt (g)
Normal control (0.9% NaCl)	19.23±0.33	21±0.93	1.42±0.07	2.10±0.03	2.29±0.88	0.68±0.15	0.28±0.07
TDA (10 mg/kg)	20.43±0.80	22±1.05	1.44±0.05	2.09±0.08	2.26±1.04	0.70±0.28	0.27±0.03

Values are expressed as mean ± SEM (n = 6).

Table 2: Effect of TDA on hematological parameters in mice

Treatment	Hemoglobin (g/dl)	RBC (cells×10 ⁶ /mm ³)	WBC (cells×10 ³ /mm ³)
Normal control (0.9% NaCl)	12.16 ± 0.58	6.36 ± 0.45	4.15 ± 0.24
TDA (10 mg/kg)	11.68 ± 0.54	6.59 ± 0.30	4.19 ± 0.45

Values are expressed as mean ± SEM (n = 6).

Table 3: Effect of TDA on serum biochemical parameters in mice

Treatment	AST (IU/dl)	ALT (IU/dl)	ALP (IU/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Total protein (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/ml)	NPN (mg/dl)
Normal control (0.9% NaCl)	40.15±1.09	34.59±1.72	74.29±1.39	1.11±0.51	51.73±2.61	6.63±1.53	32.15±1.18	3.97±1.39	1.08±0.31	12.63±0.36
TDA (10 mg/kg)	41.33±1.20	35.03±1.54	76.32±1.33	1.08±0.21	53.25±3.21	6.59±1.61	33.06±1.34	4.09±1.43	1.02±0.72	13.09±0.72

Values are expressed as mean ± SEM (n = 6).

Table 4: Effect of TDA on liver biochemical parameters in mice.

Treatment	TBARS (µg/g of wet liver tissue)	GSH (µg/g of wet liver tissue)	SOD (IU/mg of wet liver tissue)	CAT (µmoles of H ₂ O ₂ consumed/min/mg of wet liver tissue)
Normal control (0.9% NaCl)	4.29±0.37	23.45±1.48	8.57±0.86	36.01±1.53
TDA (10 mg/kg)	4.08±0.45	24.78±1.16	8.94±0.93	34.91±1.72

Values are expressed as mean ± SEM (n = 6).

it was found necessary to estimate the effects of TDA on kidney functions. Urea, uric acid, creatinine and non protein nitrogen are commonly measured serum biochemical parameters to assess kidney function [35]. The present study demonstrated no significant differences in these renal function parameters with respect to control group animals. Therefore, it can be inferred that TDA did not affect the normal hepatic and renal functions on 28 days treatment.

Uncontrolled production of free radicals or reactive oxygen species (ROS) is regarded to be involved in the pathogenesis of several degenerative diseases [36]. Antioxidants can retard or stop the uncontrolled generation of ROS, thereby help to reduce the oxidative stress-induced diseases [37]. In the present study, hepatic antioxidant parameters viz. lipid peroxidation (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase activities (CAT) were estimated to ascertain the functioning of normal liver antioxidant defense systems and it was found that no significant alterations in these parameters took place thereby implying maintenance of normal hepatic non-enzymatic and enzymatic antioxidant defense mechanisms during TDA treatment.

From the present results it is evident that the 28 days daily repeated oral administration of TDA neither altered hematological and serum as well as liver biochemical

parameters nor produced any signs and symptoms of adverse effects in mice during the observation period.

From the present study, it can be concluded that the hydro alcoholic extract of *T. dioica* root (TDA) exhibited satisfactory acute and short term safety profile in mice as evidenced by acute and sub-chronic oral toxicity studies in Swiss albino mice. Therefore, *T. dioica* root extract may be regarded as safe in Swiss albino mice when administered orally offering no noticeable toxicity. However, further short and long term toxicity studies are necessary with different doses, different routes, different species and different solvent extracts of *T. dioica* root to ascertain the reliable safety of the plant material.

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