Original Article

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Toxicological Evaluation of *Solanum trilobatum* L. Fruit Extract

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Abstract

Solanum trilobatum L. (Thai name: Mawaeng krueo) is a shrub of the family Solanaceae. Previously, we reported the anti-inflammatory activity of the ethanolic extract of *S. trilobatum* fruit in rodents. The present study was performed to evaluate acute and 28-day repeated dose oral toxicity of this ethanolic extract in rodents. A primary skin irritation test was also examined in rabbits. Oral acute toxicity of the extract at a fixed dose of 5 g/kg body weight was investigated in both sexes of mice and rats. No deaths or abnormalities in clinical signs were observed. There was no significant difference in the body weight between the control and treated animals. Thus, the median lethal dose (LD_{50}) of this extract was greater than 5 g/kg for oral administration in rats and mice. In 28-day repeated administration study, male and female rats were given a daily oral dose of 1.0 g/kg body weight for 28 days. All animals survived throughout the duration of the study, with no evidence of treatment-related toxicity. The analysis of body weight gain, clinical observations, blood chemistry and hematological parameters did not show significant differences between the control and treated groups. Most of the histopathological findings were not statistically significant different from those of the control group. Although, we observed a focal necrosis in the heart of a male rat in the treated group, this was presumed not to be treatment-related pathological change in that animal.

In preliminary irritation study, the ethanolic extract of *S. trilobatum* fruit produced no irritation on the skin in rabbits after immediate application and at 1, 2, 3, 24, 48 and 72 hours.

Keyword: *Solanum trilobatum*, Toxicity, Skin irritation

INTRODUCTION

Solanum trilobatum L. is a shrub of the family Solanaceae and is commonly found in some of the warmer parts of the tropical and subtropical regions. Thai folk medicines have reported that the fruit of this plant is used for the treatment of cough, expectorant, pyrexia and difficult urination[1,2]. Previous study has demonstrated that *S. trilobatum* extract exhibited an antitumor activity[3]. Moreover, there are many pharmacological evaluation of sobatum, the partially purified active fraction of *S. trilobatum*. It was identified as β-sitosterol to be an anticancer substance by *in vitro* and *in vivo* model[3-9].

In clinical study, *S. trilobatum* improved the pulmonary function in patients suffering from asthmatic symptoms and signs[10]. As expectorant, it could increase the sputum volume and decrease the viscosity in these patients[11].

Our previous study revealed that oral administration of the ethanolic and partially purified extracts of *S. trilobatum* fruit in rats significantly inhibited the paw edema induced by carrageenan[12]. Another investigator also reported that *S. trilobatum* possessed anti-inflammatory activity[13]. Some reports have emphasized that an active compound isolated from *S. trilobatum*, sobatum, that has an important role in exhibiting anti-inflammatory[13] and anti-oxidant activities[14]. Moreover, sobatum at a high dose (25 g/kg body weight) did not

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induce any toxicity or change in the anti-oxidant enzymatic system[9]. In addition, another chemical constituent, solasodine, also exerts anti-inflammatory activity via at least partly through the inhibition of cyclo-oxygenase and 5-lipoxygenase pathways[15]. Most of the studies on the pharmacological action of *S. trilobatum* indicated that this plant is useful for the treatment of many pathological conditions. However, there is very little information about the toxic effect of this plant extract. Therefore, the present study was performed to evaluate an acute oral toxicity and a 28-day repeated oral toxicity of *S. trilobatum* fruit extract in rodents. A primary skin irritation test was also examined in rabbits.

MATERIALS AND METHODS

Plant extraction

S. trilobatum dried fruits were purchased from Chantaburi Province, Thailand. Authentication of the fruits of *S. trilobatum* was achieved by comparison with the voucher specimen (PBM#02521) in the Department of Pharmaceutical Botany, Mahidol University, Thailand.

Three hundred grams of ground dried fruits were macerated with 95% ethanol for 1 week. The ethanolic extract was evaporated to dryness by rotary evaporator, yielding approximately 11.5% (w/w)

Chemicals and drug preparation

Dimethyl sulphoxide (DMSO) was purchased from Sigma Chemical Co., St Louis, MO, U.S.A. For oral administration, the ethanolic extract was dissolved in 5% DMSO and the dosing volume was set at 10 ml/kg body weight. While for the topical application, the extract was dissolved in 70% ethanol in a concentration of 400 mg/ml and then 0.5 ml of the extract solution was applied to a 1 inch^2 intact dose site on each rabbit using a micropipette. All drug solutions were freshly prepared before starting the experiments.

Animals

Male and female ICR mice (18-20 g) and both sexes of Wistar rats (180-200 g), from the National Laboratory Animal Centre, Mahidol University were used in oral toxicity studies. Male New Zealand White rabbits (1.8-2.0 kg), from the Faculty of Veterinary Sciences, Chulalongkorn University, were used in a primary skin irritation study. The animals were housed in groups of five per cage for mice and two-three per cage for rats and individual per cage for each rabbit. They were kept in a temperature-controlled room (25±˚C) under 12-hours light/dark cycles for at least one week before the experiments. Standard chow and tap water were supplied *ad libitum.* All experimental protocols were approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University, Thailand.

Oral toxicity study

Acute and 28-day repeated oral toxicity studies were performed following Organization for Economic Cooperation and Development (OECD) test guidelines 423[16] and 407[17], respectively. In acute oral toxicity study, a single oral dose of *S. trilobatum* fruit extract (5 g/kg body weight) was administered to male and female mice and rats (5/sex/species) to assess acute toxicity, as a limit test. The respective control groups (5/sex/species) were administered with the vehicle (5% DMSO) alone. After administrating the extract, the number and time of death within 14 days were observed to determine the median lethal dose (LD_{50})[18,19].

According to OECD 407, no toxic effects of the extract would be expected at a dose of 1g/kg body weight, a limit test. Therefore, in 28-day repeated oral toxicity study, oral administration of the extract (1 g/kg body weight) to five rats of each sex was performed once a day in the morning throughout the 28-day dosing period. The other five rats of each sex were given daily oral administration of 0.5% DMSO and were served as control groups.

The animals were also observed for signs of gross toxicity and behavioral changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes, respiration, circu-

lation, autonomic and central nervous systems, motor activity and behavior pattern. Particular attention was directed to observation of salivation, diarrhea, tremors, convulsions and coma[18,19]. The body weight of all animals was also recorded before the experiment and once a week during the test period.

For the 28-day repeated-dose toxicity study, on the scheduled necropsy's day, rats were sacrificed using overdose of ether inhalation. Thereafter, blood samples were collected by cardiac puncture. For hematology, the erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit value (Hct) and leukocyte count (WBC) were measured with an automated analyzer. Differential leukocyte counts were made with a blood cell automatic analyzer. For blood chemistry determination, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cr), total bilirubin (TB) and direct bilirubin (DB) were examined by an automatic biochemical analyzer.

After the blood sampling, various visceral organs such as heart, lung, thymus gland, liver, kidney, pancreas and gastrointestinal organs were isolated and preserved in modified Millonig's phosphate-buffered formalin. Histopathological examination was performed on internal organs obtained from the rats of the control and treated groups, and on gross lesions of any group. The hematoxylin and eosin straining specimens were prepared according to the standard procedure prior to microscropically examination [20,21].

Primary irritation test

A primary irritation test was examined and evaluated according to the method of Draize *et al*[22,23]. This study was performed in six healthy young adult New Zealand white rabbits. On the day before drug application, hair was removed from the dorsal and trunk area. On the day of experiment, but prior to drug application, the animals were examined for health and the skin was checked for any abnormalities. No pre-existing skin irritation was observed. Then, 0.5 ml of the extract solution (400 mg/ml) or normal saline was directly applied to a 1 $inch^2$ shaved intact skin on each animal using a micropipette. After drug application, each animal was placed in an individual cage for observation of the skin reaction such as erythema and edema at 1, 2, 3 h. Then, both the treated and the control sites are covered by gauze and fixed by using an elastic bandage for 24 h. The animals are returned back to their cages. At the end of this period, the bandage and gauze are removed and the skin reaction is observed. Sign of skin irritation is evaluated again at the end of 48 h and 72 h. Individual evaluation of test dose sites was scored according to Draize Scoring System (Table 1). The degree of irritancy was obtained by calculating the primary irritation index and classified according to the descriptive rating for mean primary irritation index (Table 2).

Statistical Analysis

All values were expressed as mean ± standard error of the mean (S.E.M). Data from treated animals were compared with those of control group. Males and females were evaluated separately and statistical significances between the treated groups and the respective control groups were determined by two-tailed unpaired Student's t-test. Differences with $p < 0.05$ were considered statistically significant.

Table 1. Evaluation of skin reaction

Erythema and eschar formation	Grade	Edema formation	Grade
- No erythema	0	- No edema	0
- Very slight erythema (barely perceptible)	1	- Very slight edema (barely perceptible)	1
- Well-defined erythema	2	- Well-defined edema (edges of are well defined by definite raising)	2
- Moderate to severe erythema	3	- Moderate edema (raised approximately 1 mm)	3
- Severe erythema (beet-redness) to slight, eschar formation (injuries in depth)	4	- Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 2. Descriptive rating for mean primary irritation index

Primary irritation index	Classification
0	non irritant
0.1-2	midly irritant
2.1-5.0	moderate irritant
5.1-6.0	moderate to severe irritant
>6.0	severe irritant

RESULTS AND DISCUSSION

Oral toxicity study

In the present study, the extract, at the limit dose level of 5 g/kg body weight, did not cause any mortality and did not induce any signs of toxicity in the treated animals after administration and during the observation period of 14 days thereafter. The body weight gain of all animals was not significantly different between the treated groups and the respective control groups (Figure 1 and 2). No gross pathological alterations were evident at terminal necropsy in any of the mice and rats. Based on these results and under the conditions of this study, the median lethal dose (LD_{50}) of the extract after single oral administration was greater than 5 g/kg body weight for both sexes of mice and rats. This result suggested that the ethanolic extract of *S. trilobatum* fruit extract was a slightly toxic substance.

In 28-day repeated oral dose toxicity study, all animals survived the scheduled sacrifice, and there were no treatment-related clinical signs. Weekly body weights of rats were summarized in Figure 3. Body weights of treated animals were not significant different from those of the control groups. The hematological results and blood chemistry in males and females were shown in Table 3. Hematological and serum biochemical parameters showed no significant difference between control and treated groups. The histopathological changes in various visceral organs of the treated and the control groups were also examined microscopically. There were no significant histopathological changes in most of visceral organs including lung, liver, kidney and gastrointestinal organs of

the treated animals and those of the control group. Although, we observed a focal necrosis without inflammatory cell infiltration in the heart of a male rat of the treated group but this change was presumed not to be treatment-related pathology in that animal because this histological finding was a spontaneous lesion of the cardiovascular system that was the most commonly finding in the laboratory animals used in safety assessment[24].

These results indicated that there were no changes in clinical signs, body weights, hematology, blood chemistry parameters or histopathological findings in the 28-day repeated oral dose toxicity study. Thus, the extract at a dose of 1g/kg produced no observed adverse effects in both sexes of rats.

Primary irritation test

In preliminary irritation study, the ethanolic extract of *S. trilobatum* fruit had no affect on skin in all rabbits after immediate application and at 1, 2, 3, 24, 48 and 72 h. No erythema or edema was observed. Based on the primary irritation index of the extract was zero, it should be classified as a non irritant agent.

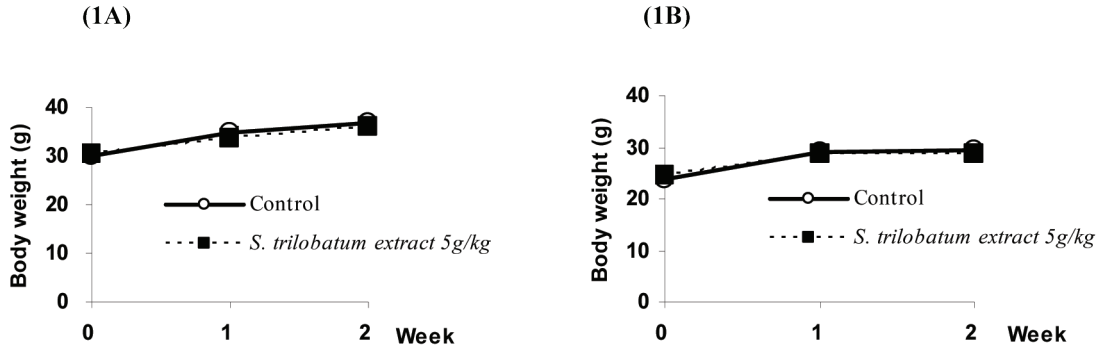

Figure 1. Body weight of male (1A) and female (1B) mice treated with *S. trilobatum* fruit extract at the single dose of 5 g/kg

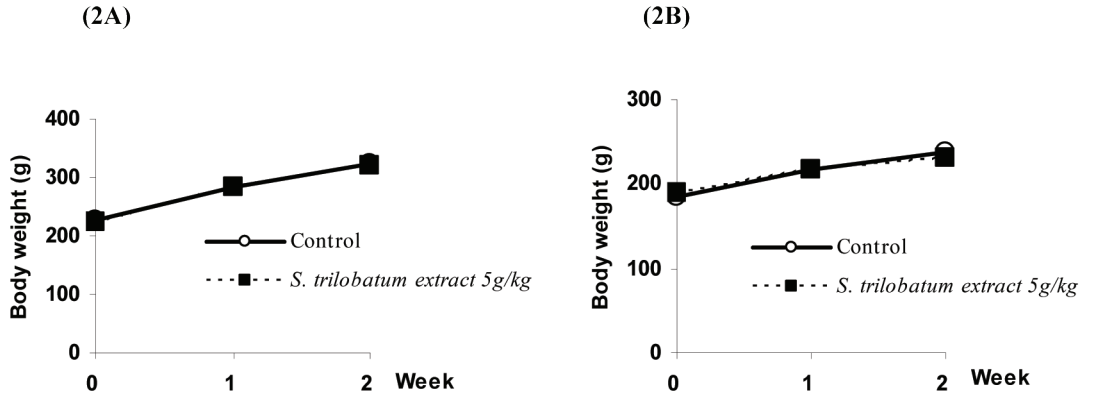

Figure 2. Body weight of male (2A) and female (2B) rats treated with *S. trilobatum* fruit extract at the single dose of 5 g/kg

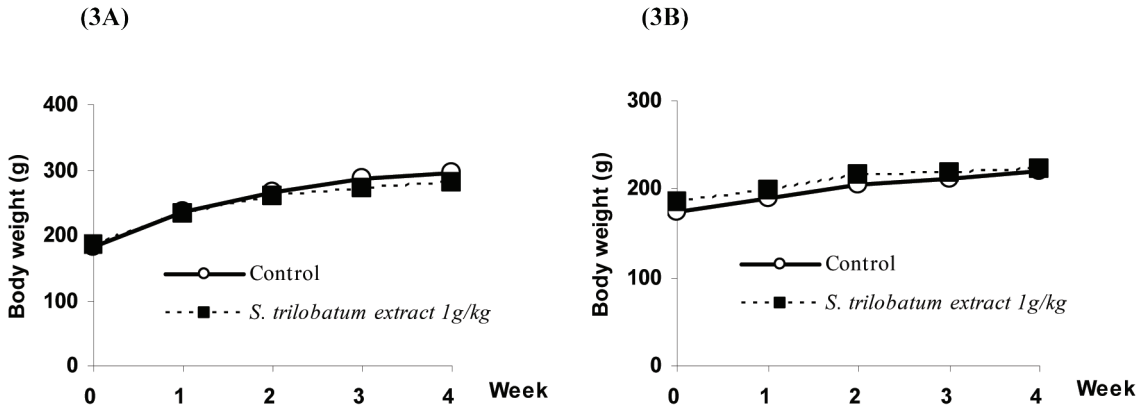

Figure 3. Body weight of male (3A) and female (3B) rats treated with *S. trilobatum* fruit extract at the dose of 1 g/kg for 28 days

Table 3. Results of hematology and blood chemistry in the 28-d repeated oral dose toxicity study (Mean ± S.E.M.)

Parameters	Male		Female	
	Control	*S. trilobatum* extract (1 g/kg)	Control	*S. trilobatum* extract (1 g/kg)
RBC (X10^6 cells/µl)	6.62±0.24	6.22 ±0.88	5.30±0.23	5.26±2.15
Hct (%)	36.80±1.55	34.75±5.25	29.37±1.27	28.20±11.51
Hb (g/dl)	14.48±0.71	14.18±1.87	14.17±0.88	13.08±5.34
WBC (X10^3 cells/µl)	3.90±0.60	3.70±0.60	4.40±0.7	4.40±2.2
Neutrophil (%)	53.75±2.29	51.50±4.05	54.67±1.33	48.00±19.60
Eosinophil (%)	1.75±0.85	2.25±1.11	2.67±0.33	2.17±0.88
Lymphocytes (%)	48.25±4.01	45.25±4.27	41.33±0.88	48.67±19.87
Monocytes (%)	1.25±0.25	1.00±0.01	1.33±0.33	1.17±0.48
AST (U/L)	6.40±0.60	4.67±1.91	6.67±2.72	7.17±2.93
ALT (U/L)	90.40±12.98	100±40.96	83.67±34.16	93.17±38.04
BUN (mg/dl)	18.20±0.73	18.50±7.55	26.33±10.75	26.83±10.95
Cr (mg/dl)	0.56±0.02	0.58±0.24	0.57±0.23	0.58±0.24
Total bilirubin (mg/dl)	0.13±0.03	0.12±0.05	0.13±0.05	0.14±0.06
Direct bilirubin (mg/dl)	0.07±0.01	0.06±0.05	0.10±0.04	0.08±0.03

CONCLUSION

In the present study, the median lethal dose (LD_{50}) of this extract was greater than 5 g/kg for oral administration in mice and rats. No changes in clinical signs, body weights, hematology, blood chemistry parameters or histopathological findings was observed in rats after oral repeated administration of the extract (1 mg/kg) for 28 days. In addition, the extract did not produce the skin reaction such as erythema or edema. Therefore, these results suggest a slightly toxic property of the extract for oral adminis-tration with no toxic effect at the dose of 1 mg/kg in rats. It also is a non irritant substance for topical application to skin.

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