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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF THE LEAF EXTRACTS OF *SOLANUM TRILOBATUM* LINN

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Abstract

Methanol extracts of *Solanum trilobatum* Linn Leaf were investigated for anti-inflammatory activity with acute and chronic models. In the doses of 100, 200 and 300 mg kg^{-1}exhibited significant ($P < 0.05$) anti-inflammatory activity in all the models tested. The methanol extracts of *Solanum trilobatum* Linn at 300 mg kg^{-1} showed maximum inhibition of 54.44 % in carrageenan-induced rat paw oedema while the standard drug indomethacin was 57.08 % after 3 hrs of carrageenan injection. On the other hand at 100, 200 and 300 mg kg^{-1} inhibited with dextran, histamine and serotonin-induced rat paw oedema significantly and dose-dependently compared with control group. In the chronic inflammatory model, at 200 and 300 mg kg^{-1} inhibited the granuloma weight by 22.65 %, whereas the indomethacin inhibited 28.37 %.

Key words: Anti-inflammatory activity, carrageenan induced rat paw, cotton pellet induced granuloma, *Solanum trilobatum*.

Introduction

The plant *Solanum trilobatum* Linn. (Solanacea) is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka in moist places. This plant is well known in Ayurveda and Siddha system as 'Alarka' and 'Tuduvelai', respectively. The Siddha system of medicine uses a ghee prepared from this plant for treatment of tuberculosis. The decoction of entire plant is has been administered to cases of acute and chronic bronchitis [1]. Roots, berries and flowers are used for cough [2]. Previous reports indicated that some chemical constituent, such as solasodine and β-solamarine have been isolated from whole plant [3].

Pharmacological investigations have demonstrated that *Solanum trilobatum* Linn possess antioxidant, hepatoprotective, anti-inflammatory and analgesics activities [4, 5, 6, 7 and 8]. In the preliminary study, the fraction of methanol extract of *Solanum trilobatum* (MEST) leaves exhibited significant anti-inflammatory activity on carrageenan induced rat paw oedema. Therefore, the present study has been designed to evaluate the anti-inflammatory activity of methanol extract of *Solanum trilobatum* leaves in different experimental models of acute and chronic inflammation.

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Material and Methods

Plant material

The leaves of *Solanum trilobatum* Linn *(*Solanaceae*)* were collected during the month of June 2005 from Tirukovilur, Tamilnadu, India. The plant material was authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai, India. A voucher specimen (PARC/23/06) has been deposited in the Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, India, for future reference.

Preparation of extract

The air-dried powdered leaves of *solanum trilobatum* Linn were defatted with petroleum ether (60-80 ºC) to remove low polar compounds. The defatted material was further extracted with methanol at ambient temperature. The methanol extract was filtered and concentrated to a syrupy mass (Yield 10.2 % w/w) under reduced pressure at 50-55 ºC. The extract was stored in a refrigerator and used for the present study.

Animals

Albino (Wister) rats 180-200 g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum.* The animals were acclimatized for a period of 14 days prior to perform the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 711/02/A/CPESEA).

Acute toxicity study

Acute toxicity study was performed as per OECD-423 guidelines [9]. Swiss albino rats of either sex were used. The animals were fasted for 4 h, but allowed free access to water throughout. The fasted Swiss albino rats were divided into different groups of six animals each. MEST was administered orally at a dose of 5 mg kg^{-1}. The control animals received a similar volume of 2% v/v aqueous Tween 80 solution. Mortality in each group was observed for 7 days. The mortality was not observed, the procedure was repeated at doses 50, 300 and 1000 mg kg^{-1}.

Carrageenan induced paw edema

The rats were divided into six groups, each group consisting of six animals. Edema was induced by subplantar injection of 0.1% freshly prepared carrageenan suspension into the right hind paw of each rat. The paw volume was measured at 0 h and at 3 h after the injection of carrageenan, using a plethysmometer [10]. The methanol extract of *solanum trilobatum* at 100, 200 and 300 mg kg^{-1} doses were administered orally to first three groups of rats. The fourth and fifth groups of rats received 5 ml kg^{-1} of 2% w/v Tween 80 orally as vehicle control or 10 mg kg^{-1} Indomethacin as drug control respectively, for assessing comparative pharmacological significance. Drug pretreatment was given 1 h before the injection of carrageenan.

Dextran-induced rat paw oedema

The paw edema was induced in the right hind paw by subplantar injection of 0.1ml of freshly prepared 1% w/v dextran solution [11]. Paw volume were measured 30 min after dextran injection. The rats were treated as described above. The oedema was expressed as an increase in paw volume due to dextran injection [12].

Histamine-and serotonin-induced rat paw oedema

The paw oedema was produced by subplantar administration of 0.1 ml of a 0.1% freshly prepared solution of histamine or serotonin into the right hind paw of rats. The paw volume was recorded before (0 h) and 1 h after histamine injection [13] or 30 min after serotonin injection [14]. Different groups of animals were pretreated with methanol extract of *solanum trilobatum* at 100, 200 and 300 mg kg^{-1} or with 5ml kg^{-1} 2% v/v aqueous Tween 80 solution (vehicle control) or 10 mg kg^{-1} cyproheptadine (standard drug). The drugs were administered orally 1 h before eliciting paw oedema. The oedema was expressed as an increase in paw volume due to histamine and serotonin injection [12].

Cotton pellet induced granuloma

The five groups of rats, six in each group was included in this study. After shaving off the fur the animals were anaesthetized. Sterile preweighed cotton pellets (50 ± 1 mg) were implanted in the axilla region of each rat through a single needle incision [15]. Methanol extract of *solanum trilobatum* at 100, 200 and 300 mg kg^{-1}, indomethacin at 10 mg kg^{-1} (standard) or 5 ml kg^{-1} of 2%w/v Tween 80 (control) were administered orally to the respective group of animals for seven consecutive days, from the day of cotton-pellet implantation. On the eighth day, the animals were anaesthetized again; the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37 °C for 24 h and dried at 60 °C to constant weight. The increase in the dry weight of the pellets was taken as a measure of granuloma formation.

Statistical Analysis

The results were presented as mean ± SEM. One way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons were used for statistical evaluation. p values less than 0.05 were considered as significance.

Results and Discussion

Acute toxicity studies revealed the non-toxic nature of the methanol extract of *Solanum trilobatum.* After the administration of methanol extract of *Solanum trilobatum* rats were immediately observed for 2 h for behavioral, neurological and autonomic profiles for any changes or lethality for the next 48 h. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. However, at the above mentioned doses, MEST did not show any untoward effects on behavioral response, normal reflexes and so on. According to OECD guidelines for acute oral toxicity, an LD50 dose of 2000 mg kg^{-1} and above is characterized as unclassified and hence the drug is found to be safe.

Interplanetary injection of carrageenan in the hind paw induced gradual increase in the edema paw volume in the control group. Methanol extracts of *Solanum trilobatum* at doses of 100, 200 and 300 mg kg^{-1} significantly ($p < 0.05$) inhibited the edema formation of rat paw at 3 h after carrageenan challenge (Table 1). The reference drug, indomethacin at a dose of 10 mg kg^{-1} markedly reduced the paw edema.

The methanol extracts of *Solanum trilobatum* at oral doses of 100,200 and 300 mg kg^{-1} as well as cyproheptadine exerted significant reduction of the paw oedema at 30 min after dextran challenge. At 10 mg kg^{-1}, the purified compound elicited significant ($P< 0.01$) inhibition of 40.43 % in dextran-induced rat paw oedema while cyproheptadine exhibited 44.55 % of inhibition (Table 2). Administration of methanol extracts of *Solanum trilobatum* at doses of 100, 200 and 300 mg kg^{-1} significantly and dose dependently inhibited the development of paw swelling at 1h after histamine injection (Table 3). The reference drug, cyproheptadine (10 mg kg^{-1}) also produced significant inhibition of the oedema caused by histamine. Methanol extracts of *Solanum trilobatum* at doses (100 mg kg^{-1}) also exhibited maximum inhibition of 48.02 % in serotonin-induced rat paw oedema whereas cyproheptadine produced inhibition of 57.37 % after 30 min of the serotonin injection (Table 2). Animals treated with MEST at doses of 100, 200 and 300 mg kg^{-1} significantly ($p < 0.05$) inhibited the granuloma formation (Table 4). Indomethacin (10 mg kg^{-1}, p.o.) elicited marked reduction in granuloma formation.

Carrageenan induced rat paw oedema is commonly used as an experimental animal model for evaluation of the anti-inflammatory potential of natural products [10] and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenan, a more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents [16, 17].

The extract effectively suppressed the dextran-induced rat paw oedema, but the effect was less than that of cyproheptadine. The dextran-induced oedema is a well-known experimental model in which the oedema is a consequence of liberation of histamine and serotonin from the mast cell [18]. The extract also reduced the oedema produced by histamine and serotonin. The results tend to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine or anti-serotonin activity.

The cotton pellet granuloma bioassay is considered a model for studies on chronic inflammation and considered as a typical feature of established chronic inflammatory reaction [19]. MEST exhibited significant reduction in the granuloma formation in the cotton pellet-induced granuloma in rats. This reflected that MEST may be effective in chronic inflammatory conditions.

Anti-inflammatory activities of many plants have been attributed to their high sterol/ triterpenoid saponins [20]. Though at this stage it is not possible to identify the exact phytochemical constituent(s) responsible for anti-inflammatory activities of *S.trilobatum*, it may be assumed that the effects could be due chemicals present in the methanolic extract examined by qualitative test and these constituents were confirmed using thin-layer chromatography (TLC). The result of present study indicates that methanol extract of *Solanum trilobatum* leaves possess significant anti-inflammatory activity on both acute and chronic inflammation. Further detailed investigation is underway to determine the exact phytoconstituents, which are responsible for the anti-inflammatory activity.

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Table 1. Anti-inflammatory activity of methanol extracts of *Solanum trilobatum* Linn (MEST) on carrageenan induced rat paw oedema.

Treatment	Dose (mg kg^{-1})	% Increase in paw volume	% inhibition
Control	5 ml kg^{-1}	61.22 ± 0.24	-
Indomethacin	10	26.27± 0.23*	57.08
MEST	100	30.76 ± 0.22*	49.75
MEST	200	28.97±0.24*	52.67
MEST	300	27.89 ± 0.19*	54.44

Each value represents the mean ± S.E.M., n = 6,* $P< 0.05$ compared with control, Dunnett's *t*-test after analysis of variance.

Table 2. Anti-inflammatory activity of methanol extracts of *Solanum trilobatum* Linn (MEST) on Dextran-induced rat paw oedema.

Treatment	Dose (mg kg^{-1})	% Increase in paw volume	% inhibition
Control	5 ml kg^{-1}	44.99 ± 0.32	-
Cyproheptadine	10	25.19 ± 0.19*	44.00
MEST	100	35.06 ± 0.25*	22.07
MEST	200	31.17 ± 0.12*	30.71
MEST	300	26.59 ± 0.26 *	40.89

Each value represents the mean ± S.E.M., n = 6. *$P < 0.5$, compared with control, Dunnett's *t*-test after analysis of variance

Table 3. Anti-inflammatory activity of methanol extracts of *Solanum trilobatum* Linn (MEST) on histamine and serotonin-induced rat paw oedema

Treatment	Dose (mg kg^{-1})	% Increase in paw volume	% inhibition
Histamine control	5 ml kg^{-1}	56.62 ± 0.20	-
Cyproheptadine	10	30.43 ± 0.32*	46.25
MEST	100	49.89 ± 0.28*	11.88
MEST	200	43.70 ± 0.22*	22.81
MEST	300	35.69 ± 0.20*	36.96
Serotonin control	5 ml kg^{-1}	47.70 ± 0.21	-
Cyproheptadine	10	20.46 ± 0.21*	57.10
MEST	100	36.37 ± 0.17*	23.75
MEST	200	29.98 ± 0.23*	37.14
MEST	300	24.67 ± 0.26*	48.28

Each value represents the mean ± S.E.M., n = 6. *$P < 0.05$, compared with control, Dunnett's *t*-test after analysis of variance.

Table 4. Anti-inflammatory activity of methanol extracts of *Solanum trilobatum* Linn (MEST) on cotton pellet induced granuloma in rats

Treatment	Dose (mg kg^{-1})	Weight of granulation (mg)	% Inhibition
Control(Tween 80) 2%w/v	5 ml kg^{-1}	85.15 ± 0.23	-
Indomethacin	10	58.60 ± 0.37*	28.37
MEST	100	72.27 ± 0.23*	11.26
MEST	200	69.57 ± 0.23*	15.98
MEST	300	63.30 ± 0.23*	22.65

Each value represents the mean ± S.E.M., n = 6. *$P < 0.05$ compared with control, Dunnett's *t*-test after analysis of variance.

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