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ANALGESIC AND ANTI-INFLAMATORY PROPERTIES OF ETHANOLIC LEAF EXTRACT OF PLANT *SOLANUM TORVUM*

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ABSTRACT

The present study used two models for the investigation of the analgesic effect of *S. torvum* extract. Acetic acid induced writhing and pressure tests were selected to investigate peripheral and central antinociceptive effects of the plant extract. Carrageenan induced paw oedema in rat was selected to represent a model of acute inflammation. It shows that the ethanolic extract of *S. torvum* induced dose dependant analgesic effect against the writhing syndrome indicating its peripheral effect.

KEYWORDS: alkaloids, *S. torvum*, analgesic effect.

INTRODUCTION

The family Solanaceae represent one of the most economically and medicinally important families of angiosperms. The genus *Solanum* is a hyper-diverse taxon of this family. There are about 2000 species of *Solanum* in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas. About 21 species and one variety in this genus are used as herbal medicines. *Solanum torvum* L. is a small solanaceous shrub, distributed widely in India, Pakistan, Malaya, China, Philippines, and tropical America. For many decades, different ethnic groups have used the dried stem and root of this plant for treatment of various ailments. Among the major chemical constituents of *S. torvum* are steroids, steroid saponins, steroid alkaloids, and phenols.

Ethno-pharmacological studies indicate that the leaf and stem and root of *S.torvum* have analgesic, anti-inflamatory, anti-viral, anti-microbial, and other medicinally important effects.

Plant Profile

Taxonomical Classification

Kingdom: Plantae, Plants;

Subkingdom: Tracheobionta, Vascular plants;

Super division: Spermatophyta, Seeds plants;

Division: Angiosperma;

Order: Tubiflorae;

Family: *Solanaceae*;

Genus: *Solanum*;

Species: *torvum*

Description

Evergreen, widely branched, prickly shrub or small tree, to 5 m (16 ft) tall; twigs stellate tomentose; stems armed with stout, and flattened prickles, straight (usually) or slightly curved. Leaves alternate, simple, clearly petioled; blades oval to elliptic, unlobed to strongly lobed, to 25 cm (10 in) long; bases unequal, tips pointed; surfaces densely stellate hairy below, less dense above, with usually a few long prickles on midveins (especially above). Flowers many, in large branched clusters, with simple, mostly glandular hairs on axes; corolla bright white, to 2.5 cm (1 in) across, lobed about 1/3 of its length; lobes not recurved; stamens with prominent anthers. Fruit an erect, subglobose berry, to 1.5 cm (0.6 in) wide, yellow when ripe.

EXPERIMENTAL WORK

Plant material

The fresh leaves of *S. torvum* were collected from Koraput District, Odisha, India and were identified by a scientist of M. S. Swaminathan Plant Research Institute, Jeypore. The leaves were air-dried away from direct sunshine after which they were ground to powder in a food processor. 100g of ground leaves powder were macerated for 2h in 500 ml distilled water at 90oC. After filtration, the aqueous filtrate was concentrated by evaporation at 40°C and yielded 12.8 g of dry powder. Preliminary screening of the aqueous extract of *S. torvum* revealed the presence of sterol, triterpenes, sugars, alkaloids and phenols.

Animals

Wistar rats (weighing 160-220 g) and Swiss mice (weighing 20-30 g) of both sexes were procured and kept in our animal house at room temperature maintaining a 12hr+1 light/dark

cycle and had access to food and water, *ad libitum*. Prior to treatment, the experimental animals were fasted over night but allowed access to drinking water.

Drugs

Aspirin, Indomethacin, Morphine, Tramadol, Formalin, and plant extract were dissolved in distilled water, Carrageenan in physiological saline.

Toxicity Study

Swiss mice of both sexes were taken for this experiment. Animals were divided into six groups and were given different doses of plant extract (250, 500, 1000, 2000, 3000mg/kg,bw) for four consecutive days and their mortality, loss of body weight and general behaviour was recorded from the first dose up to 72 hours after the last administration of plant extract. One group was taken as control group and administered with normal saline.

Extraction of *Solanum Torvum* leaf

Fresh plants were taken, washed properly with clean water then leafs were collected. These leaves were subjected to shade dry properly for 1-2 week and subsequently grinded in to powder. The powdered leaves were packed in to a soxhlet apparatus and was extracted with ethanol for 72 hours. Then the extract was rotary evaporated at 45°C to evaporate the solvent under vacuum. The concentrated ethanolic extract was stored for further investigations. The extract was dissolved in ethyl acetate or 1% phosphate buffer saline (PBS) or both at ratio of 1:1 before performing experiment.

Analgesic Study

Acetic acid-induced abdominal writhing test

30 swiss mice were divided into six groups of five animals each. Each group of animals was treated orally with one of the following: NaCl 90/00, aspirin (100mg/kg), tramadol (25mg/kg), morphine (1.5mg/kg), *S. torvum* (300mg/kg) and (600mg/kg) respectively. One hour after administration of the test drugs, animals were injected intra-peritoneally with 1% acetic acid (1 ml/100 g body weight). The number of writhing responses such as contortions and stretching were recorded for 30 minutes. The results were evaluated by calculating the mean number of contortions per treated group and these results were compared to results obtained from control animals.

Formalin test

Animals were pretreated with either the etanolic extract of *S. torvum* (30 mg/kg, i.p.) or morphine (5 mg/kg, s.c.) 30 min before the administration of 20µL of 1.2% formalin to the right hind paws of the mice. The licking time was determined in two time ranges, from 0 to 5 min (phase 1, neurogenic) and from 20 to 25 min (phase 2, inflammatory), after the intraplantar formalin injection. Control animals were injected with sterile saline (0.5 mL).

Hot plate test

For the hot plate test, mice were submitted to a plate heated to 55-56 °C according to methodology of Eddy & Leinback (1953), with some modifications. Mice were treated with a ethanolic *Solanum torvum* dose of 30 mg/kg (i.p.), and the control group received sterile saline (0.5 mL). Measurements were performed at time zero (0 min) and at 30, 60, 90 and 120 min after the protein fraction administration. The hot plate cut-off time was 45s to avoid animal paw lesions. Morphine (5 mg/kg, s.c.) was used as a reference drug.

Tail Flick Test

The tail flick examination was used to calculate analgesic activity by the method defined by D'amour and Smith 1941, with minor alterations in the procedure. The tail flick method was utilized to study the antinociceptive activity in mice. A radiant heat automatic tail flick analgesiometer was applied to measure reaction latencies. Basal reaction time of animals to radiant heat was recorded by locating the tip (last 1-2 cm) of the tail on radiant heat source. The tail removal from the radiant warmth was taken as end point. The cut off time of 15 seconds was used to avoid tail injury by heat. Mice were divided into five groups (n=6). Mice were treated with morphine (10mg/kg), normal saline, and the extract. The latent period of the tail-flick response was determined by using *S. torvum* (125, 250, 500mg/kg) and administer of drug 45, 60, 75, and 90 minutes after the administration of drugs.

ANTI- INFLAMATORY STUDY

Carrageenan-induced paw edema

Oedema was induced on the right hind paw of rats by a sub-plantar injection of 0.05 ml of a solution of 1% sterile carrageenan in saline(Lanhers et al., 1991). The plant extract (300 and 600 mg/kg) and Indomethacine (10 mg/kg) were administered orally 30 minutes before carrageenan injection while control animals received the vehicle only. The volume of the injected paw was measured before and 1, 2, 3, 4 h after induction of inflammation using a

Plethysmometer. Inflammation was expressed as an increase in paw volume due to carrageenan injection.

RESULTS

Antinociceptive effects

Acetic acid-induced abdominal writhing test

The results presented in Table 1 show that *S. torvum* extract at doses of 300 and 600 mg/kg *po* decreased the number of writhes by 23% and 26% ($p<0.05$) respectively. The reference drugs (Morphine, Tramadol and Aspirin) induced significant ($P<0.01$) reduction of the noted parameters by 61%, 23% and 48% respectively. Pain inhibition by 300mg/kg of the extract was similar to the results obtained with tramadol. Although two doses were used, the higher dose (600mg/kg), did not elicit a greater protection from acetic acid induced abdominal writhing have been expected.

Effect of ethanolic leaf extract of *s. torvum* on acetic acid induced pain

Group	Dose (mg/kg)	Contraction ± SEM	Pain inhibition (%)
Control	-	148 ± 14	-
Aspirin	100	77 ± 8**	48
Tramadol	25	113 ± 10*	23
Morphine	1.5	58 ± 8**	61
S. torvum	300	114 ± 9*	23
S. torvum	600	110 ± 13*	26

Formail Test

The treatment of mice with *S. torvum* resulted in an inhibition of the formalin-induced licking in the early phase and inflammatory pain (late phase) of the formalin test. In the early phase, the maximum analgesia was observed at dose of 500mg/kg of *Solanum torvum leaf* extract ($p<0.01$ compared to control group) and was equal to morphine in dose of 10mg/kg. In the late phase, the dose of 250 mg/kg of *S. torvum* leaf extract showed the most analgesic effects and it was comparable to morphine in dose

Hot Plate Test

The treatment of mice with morphine (10mg/kg i.p.) increased the latency response in the hot plate test from 30 to 120 minutes after treatment. On the other hand, ethanolic leaf extract of *S. torvum* significantly influence the reaction time of the animals to the hot plate at doses of 500mg/kg in 45 and 60 minute.

Pressure test

Aspirin (100 mg/kg) did not show significant antinociceptive effect on mechanical pain. The analgesic effects of the aqueous extract of *S. torvum* leaves (300 and 600 mg/kg *po*) were significant one hour after drug administration, and the results were similar to those obtained with both morphine (1.5 mg/kg) and tramadol (25 mg/kg). Whereas the maximal pain blocking activity of tramadol, morphine and 300mg/kg *S. torvum* extract were observed two hours after drug administration, 600mg/kg of *S. torvum* extract showed maximal activity ($p<0.01$) during the third hour after extract administration (Table 2). The analgesic effects of this extract dose was highest when the analgesic effects of all the other test drugs were already waning out.

Table 2: Effect of the aqueous leave extracts of *S. torvum* on pressure-induced pain. Measurements (in gf (gram force)) were done before the administration of the various drugs and at various time intervals after drug administration. Every animal served as its own control.

GROUP	DOSE (mg/kg)	BEFORE DRUG ADMISTRATION		AFTER DRUG ADMINISTATION	
				1H	2H 3H
CONTROL (0)	-	86±5	80 ± 9 (-7)	92 ± 5 (7)	86 ± 5
ASPIRIN (7)	100	89± 4	105 ±11 (18)	102 ± 6 (15)	95 ± 4
TRAMADOL (33)**	25	78± 5	92 ± 5 (18)*	130 ± 4 (67)**	104 ± 4
MORPHIN (31)*	1.5	81±5	107 ± 7 (32)*	136 ± 2 (68)**	106 ± 10
S.TORVUM (32)*	300	80± 2	107 ± 8 (34)*	113 ± 7 (41)*	105 ± 6
S. TORVUM (61)*	600	82±4	104 ± 4 (27)	117 ± 7 (43)*	132 ± 8

Tail Flick Test

Pretreatment with *S. torvum* 500mg/kg demonstrated a significant and dose dependant antinociceptive activity in the tail flick test(Figure 2). The 500mg/kgbw dose of S. *torvum* etanolic extract increased anantinociceptive activity in 30, 45, 60 minutes after injection that were comparable to the normal saline. This effect was significant in time 45 and 60 minutes after injection for doses 125 and 250mg/kg of *S. torvum* leaf extract. Under similar conditions, treatment with morphine significantly increased latency to thermal stimulation 30 min after administration and the antinociceptive effect was maintained during the entire period of evaluation.

Carrageenan-induced paw edema

Control animals showed progressively increasing paw volume in response to carrageenan injection during the experiment. *S. torvum* leaves ethanolic extract significantly attenuated paw swelling (P<0.05) 2 and 4 hours following oral administration (Table 3). The anti-inflammatory effect of the extracts were however not dose-dependent. Indomethacine had a greater inhibitory effect (P < 0.01) on carrageenan-induced paw oedema compared to *S. torvum.*

Table 3: Anti-inflammatory activity of aqueous extract of *S. torvum* in carrageenan-induced hind paw edema: expressed as a percentage of volume variation (ΔV in mL).

Group	Dose (mg/kg)	1H (% inhibition)	2H (% inhibition)	3H (% inhibition)	4H (% inhibition)
Control	-	0.35±0.06	0.52±0.08	0.61±0.07	0.55±0.05
Indomethacin	10	0.20±0.01 (43)	0.20±0.02(62)**	0.11±0.02(82)**	0.08±0.04(85)**
S. torvum	300	0.24±0.03 (34)	0.36±0.06 (31)	0.37±0.7 (39)*	0.29±0.08 (47)*
S. torvum	600	0.33±0.08 (11)	0.51±0.08 (4)	0.37±0.08 (41)	0.29±0.07 (49)*

Calculation of inhibition

Percentage of inhibition of paw volume for each of biological parameter was calculated using the following formula**:**

$$\frac{(Vt\text{-}Vo)C-(Vt\text{-}Vo)E}{(Vt\text{-}Vo)C}$$

Where

Vt=left hind paw volume at time t,

Vo=left hind paw volume before sub plantar injection,

C=control group,

E=experimental group.

DISCUSSION

The present study, made use of two models for the investigation of the analgesic effect of *S. torvum* extract. Acetic acid induced writhing and pressure tests were selected to investigate peripheral and central antinociceptive effects of the plant extract. Carrageenan induced paw oedema in rat was selected to represent a model of acute inflammation.

The present results showed that the ethanolic extract of *S. torvum* induced dose dependant analgesic effect against the writhing syndrome indicating its peripheral effect. In peripheral tissues, prostaglandins and kinines would seem to play an important role in the pain process

and writhing induced by chemical substances injected intra-peritoneally is said to be the consequence of sensitisation of the chemosensitive nociceptors by prostaglandins. These results suggest that the pain killing effect of this plant may be by the prostaglandins synthesis inhibition. This test also confirms the peripheral action of Aspirin.

The pre-treatment of rats with *S. torvum* extract inhibited pain caused mechanically by a constantly increasing pressure on rat paw by the plunger and plinth of the Analgesy Meter. This system provides a model for the study of non inflammatory pain. The opioid-like analgesic drugs are more effective in inhibiting mechanically induced pain. In this model of pain, the involvement of endogenous substances such as prostaglandins may be minimized. The central protecting effect of *S. torvum* leaves extracts were comparable to tramadol and morphine test results. Tramadol is known to inhibit neuronal re-uptake of serotonin while morphine activity is mediated by μ opioid receptors Aspirin did not show analgesic effect on this model of pain, thus confirming the previous study in which Aspirin and Aspirin like drugs were ineffective against pain due to sensory nerve stimulation.

In the last part of the study, the carrageenan experimental model of inflammation was used to evaluate the anti-inflammatory effect of *S. torvum*. Some authors, think that the initial phase of carrageenan paw oedema is mediated by histamine and serotonin, while the later phase is suspected to be due to arachidonic metabolites (prostaglandins, leukotrienes) producing oedema dependent on mobilisation of neutrophils. Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflammatory process, inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors.

In our experiments, rats pre-treated with *S. torvum* extract showed a significant oedema inhibitory response 2 h following carrageenan injection. This result suggests that *S. torvum* extract may act by suppressing the later phase of the inflammatory process by the inhibition cyclooxygenase, the enzyme involved in the formation of prostaglandins.

The presented data therefore, indicate that the oral administration of *Solanum torvum* extract showed analgesic and anti-inflammatory activities. These properties confirm its use in folk medicine.

CONCLUSION

The experimental procedure developed here is of great importance because it investigates plant species as a source of molecules with specific activities. In the present work, we have confirmed that leaf extract of ***Solanum torvm*** possess anti-inflammatory and antinociceptive properties and such activities seem to be elicited, Our data indicate pharmacological activities through events associated to inhibition of cell migration and release of inflammatory mediators.

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