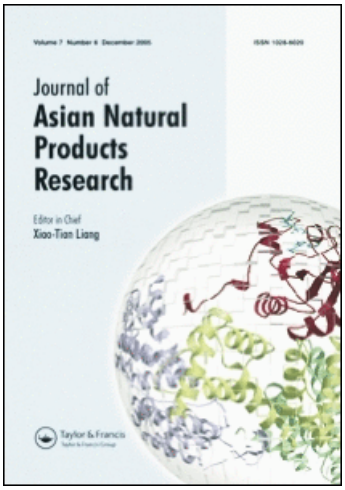

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Antinociceptive activity of steroid alkaloids isolated from *Solanum trilobatum* Linn.

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ORIGINAL ARTICLE

Antinociceptive activity of steroid alkaloids isolated from *Solanum trilobatum* Linn.

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Solasodine (**1**) was isolated for the first time from the roots of *Solanum trilobatum* Linn., a member of the Solanaceae, and assessed for its presumed antinociceptive activity using several experimental murine models, viz. the writhing, formalin, and hot plate tests. When used at doses of 2, 4, and 8 mg/kg, this steroidal alkaloid caused a significant and dose-dependent decrease in the nociception induced by an intraperitoneal injection of acetic acid ($p < 0.001$). It also led to a significant reduction of the painful sensation caused by formalin in both phases of the formalin test ($p < 0.001$). Furthermore, the alkaloid produced a significant increase in the reaction time in the hot plate test ($p < 0.001$). These results suggest that solasodine elicited antinociceptive activity through both central and peripheral mechanisms.

Keywords: solasodine; analgesic activity; formalin test; hot plate test; writhing test

1. Introduction

Solasodine **(1)** (Figure 1), a steroidal glycoalkaloid, is considered as a potential alternative to diosgenin for the synthesis of various steroidal drugs [1]. It can be converted into dehydropregnenolone and shows a close similarity to diosgenin. Compound **1** is widely regarded as a defensive allelochemical agent against a number of pathogens and predators [2]. In recent years, mixtures of solasodine glycosides have been used successfully for the treatment of a variety of cancers [3–5]. Previous studies have reported analgesic and anti-inflammatory activities of this plant using crude extracts of its roots [6], and **1** displayed significant anti-inflammatory activity against carrageenan-induced paw edema in rats [7]. The purpose of this investigation was to determine the analgesic activity of **1** isolated from the root of *Solanum trilobatum* Linn. (Solanaceae) in different animal models.

2. Results

The lethal dose (LD50) of **1** was established to be 30 mg/kg body weight and in the globally harmonized system classes it comes under Class 2 (>5–50 mg/kg).

The effect of alkaloids on writhing response in mice is shown in Table 1. Compound **1** (2, 4, and 8 mg/kg, p.o.) caused an inhibition. Such effects were observed in mice pretreated by indomethacin (74%, $p < 0.001$). The maximal inhibition of the nociceptive response (61%, $p < 0.001$) was achieved at a dose of 8 mg/kg.

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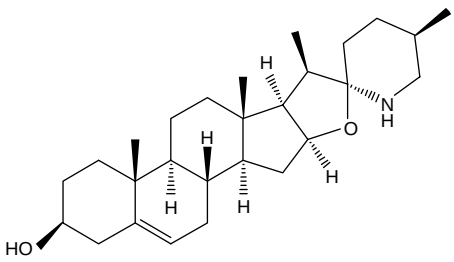

Figure 1. Structure of solasodine (**1**).

Intraplantar injection of formalin (2.5%) evoked a characteristic biphasic licking response. The duration of the early phase (0–5 min) was 44 ± 1 s and for the late phase (15–30 min) it was 174.3 ± 0.7 s in the control groups. As shown in Table 2, pretreatment with different doses (2, 4, and 8 mg/kg, p.o.) of the steroid alkaloid had a significant effect on the duration of licking activity in both phases, whereas a dose of 8 mg/kg, p.o. produced a marked reduction of the licking time by 33 and 66% ($p < 0.001$) of the early and late phase, respectively. Similarly, the treatment of the animals with indomethacin (10 mg/kg, p.o.) caused a significant inhibition (60%; $p < 0.001$) of the late but not the early phase.

The results of the hot plate test are reported in Table 3. The administration of the alkaloid (2 mg/kg, p.o.) and of pentazocine (5 mg/kg, s.c.) significantly ($p < 0.001$) increased the reaction time. The highest nociception of the thermal stimulus was exhibited at a higher dose (8 mg/kg) of the alkaloid (70%), which is comparable to that of pentazocine (75%).

3. Discussion

Solasodine (**1**) inhibited acetic acid-induced writhing in mice. It is therefore suggested that the analgesic effect of **1** is peripherally mediated. Compound **1** produced a reduction in the number of writhes at all three doses used, the most significant being obtained at the dose of 8 mg/kg (Table 1). The mouse-writhing model is well known for the antinociceptive activity bioassay. It involves different nociceptive mechanisms, such as the sympathetic system (biogenic amines release), cyclooxygenases (COXs) and their metabolites [8], and opioid mechanisms [9]. Acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and/or opioids [9]. Thus, **1** might induce antinociception by mechanisms comparable to non-narcotics and/or narcotic drugs, perhaps by blocking the receptor or the release of endogenous substances that excite pain nerve endings [10]. NSAIDs such as indomethacin inhibit COXs in peripheral tissues, thereby reducing prostaglandin E_2 (PGE_2) synthesis and interfering with the mechanism of transduction in primary afferent nociceptors [11]. In the formalin test, the early phase is thought to be induced by direct activation of nociception neurons by formalin, whereas the late phase reflects

Table 1. Effect of solasodine on acetic acid-induced abdominal constrictions in mice.

Treatment	Dose (mg/kg)	No. of abdominal constrictions	% Inhibition
Control (2% w/v aqueous Tween 80)		43.3 ± 1.4	–
Indomethacin	10 mg/kg	11.4 ± 1.5*	74
Solasodine	2 mg/kg	30.8 ± 0.9*	29
	4 mg/kg	21.4 ± 0.8*	51
	8 mg/kg	16.9 ± 1.5*	61

Notes: Data are shown as mean ± SEM, $n = 6$. *$p < 0.001$ significantly different from the control; Dunnet's *t*-test after analysis of variance.

Table 2. Effect of solasodine on formalin-induced licking in mice.

Treatment	Licking time (s) (0–5 min)	% Inhibition	Licking time (s) (15–30 min)	% Inhibition
Control (2% w/v aqueous Tween 80)	44.5 ± 1.1	–	174.3 ± 0.7	–
Indomethacin (10 mg/kg)	39.0 ± 1.0	12	69.3 ± 0.7*	60
Solasodine (2 mg/kg)	35.0 ± 0.9*	21	76.3 ± 0.6*	56
Solasodine (4 mg/kg)	38 ± 1.0*	15	73.7 ± 0.6*	58
Solasodine (8 mg/kg)	29.9 ± 0.8*	33	65.7 ± 0.9*	62

Notes: Data are shown as mean ± SEM, $n = 6$. *$p < 0.001$ significantly different from the control.

pain generated in acutely injured tissues [10]. It is a well-described model of nociception and can be consistently inhibited by typical analgesic and anti-inflammatory drugs, including morphine, indomethacin, and dexamethasone [10]. Centrally acting drugs, such as opioids, inhibit both phases of pain by equally [12] involving the effect produced by prostaglandins released at this level in response to inflammation [10] and by endogenous opioids through their action on the central nervous system. The results depicted in Table 2 show that **1** (8 mg/kg, p.o.) caused a significant inhibition of both neurogenic (0–5 min) and inflammatory (15–30 min) phases of formalin-induced licking. Its antinociceptive effects were significantly more pronounced in the second phase of this model of pain. The antinociceptive effects of the alkaloid suggest an involvement at both the central and peripheral levels; it also suggests that **1** may possess antinociceptive activity. The other algesimetric test used was the hot plate test and is believed to show a central analgesic response. Compound **1** showed a significant increase in the pain threshold, corresponding to a significant increase in the percentage of protection, as compared to the control group (Table 3). Overall, the results from this study suggest that **1** may possess analgesic activity which may be mediated by both central and peripheral mechanisms.

4. Materials and methods

4.1 Plant material

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

Table 3. Effect of solasodine on hot plate-induced pain responses in mice.

Treatment	Dose (mg/kg)	Hot plate reaction time (s)	% Inhibition
Control (saline)	0.5 ml	5.1 ± 0.6	–
Solasodine	2	12.7 ± 0.9*	60
	4	15.0 ± 0.8*	66
	8	17.1 ± 1.0*	70
Pentazocine	5.0	20.6 ± 0.7*	75

Notes: Each value represents the mean ± SEM, $n = 6$. *$p < 0.001$ compared with the control, Dunnett's *t*-test after analysis of variance.

4.2 Extraction and isolation of solasodine

The suspension of dried, finely ground powder of the *S. trilobatum* root (1 kg) in toluene:water:conc. HCl (3:2:1) was refluxed under stirring for 5 h. The reaction mixture was subsequently alkalinized with aqueous sodium hydroxide (40%) and refluxed again by stirring for 2 h. Following phase separation, the upper, pale-yellow toluene layer was separated out, and the remaining dark brown aqueous mixture was extracted 12 times with 100 ml portions of toluene. The combined toluene extracts were clarified with animal charcoal, and then concentrated *in vacuo* to a small volume. The concentrated toluene extracts were re-extracted with equal volume of aqueous acetic acid (25%) by stirring twice for 1 h at room temperature. The aqueous acid extract was separated off from the toluene layer and alkalinized with aqueous ammonia (25%). The mixture was briefly heated, and then cooled to room temperature. The crude alkaloids, which precipitated, were filtered off, washed with cold water, and dried under vacuum to yield crude alkaloid [13]. This was dissolved in a minimum amount of $CHCl_3$–MeOH (1:1) and adsorbed on basic alumina, air-dried, and chromatographed over basic alumina that furnished a colorless residue which crystallized from methanol as needles, mp 198–99°C. The compound was repeatedly crystallized from dry methanol to yield a homogeneous crystalline mass, mp 201–202°C, 95% pure, yield 140 mg (0.019%). Rf 0.19 (*n*-hexane:methanol:acetone, 8:1:1), mp 201–202°C (molecular formula: $C_{27}H_{43}NO_2$). The homogeneity of the alkaloid was ascertained by TLC and NMR spectroscopy [14].

4.3 Animals

Swiss albino mice of either sex weighing 20–25 g were used in this study. The animals were housed in polypropylene cages (38 × 23 × 10 cm), with six animals per cage at 22 ± 2°C, at a relative humidity of 50 ± 5%, with a 12 h light and 12 h dark cycle. All the animals were acclimatized to the laboratory environment for 24 h before the experiment. The animals were fed with standard dry pellet diet (M/s. Hindustan Lever Ltd, Mumbai, India) with water *ad libitum.* The animals were cared for and used in accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines [15] and experimental protocols were approved by the Institutional Animal Ethics Committee (Regn No.: 711/02/A/CPESEA).

4.4 Acute toxicity

The acute toxicity was assessed as per OECD-423 guidelines [16]. The initial dose of the isolated **1** was 5 mg/kg body weight p.o. administered to each mouse using a gastric gavage needle. Dose volume was administered 10 ml/kg body weight to the mice which were fasted overnight with water *ad libitum.* Food was withheld for a further 3–4 h after administration of the drug. The onset of toxicity and signs of toxicity were also observed. The control animals received a similar volume of 2% (v/v) aqueous Tween 80 solution. Mortality was recorded at intervals of 24 h for 3 days.

4.5 Acetic acid-induced writhing test

This test was done using the method as described by Collier *et al.* [9]. The muscular contractions were induced in mice by intraperitoneal injection of 7% solution of acetic acid (10 ml/kg). Immediately after the administration of acetic acid, the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min. Compound **1** (2, 4, and 8 mg/kg) and indomethacin (10 mg/kg) were orally administered 30 min before the acetic acid injection.

4.6 Formalin-induced pain in mice

The method described by Tjolsen *et al.* [17] was used. Animals were injected subplantarly with 20 µl of 2.5% formalin into the right hind paw of the mice. Compound **1** (2, 4, and 8 mg/kg) and indomethacin (10 mg/kg) or vehicle control were orally administered to different groups of mice. Drug pretreatment was given orally 1 h prior to the formalin injection. One mouse per group was observed from 0 to 30 min following the formalin injection. The time (s) used for licking and/or biting the injected paw, indicative of pain, was monitored. The first period (earlier or neurogenic phase) was recorded 0–5 min after the formalin injection and the second period (later or inflammatory phase) was recorded 15–30 min after the injection.

4.7 Hot plate test

The hot plate test described by Turner [18] was used. The mice were first treated with different doses of **1** (2, 4, and 8 mg/kg, p.o.). One hour after drug administration, they were placed on a hot plate maintained at 55 ± 1.0°C. The time taken by the animals to lick the fore or hind paw or to jump out of the place was taken as the reaction time. Pentazocine (5 mg/kg, s.c.) was used as the reference drug.

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