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Investigations into the antinociceptive activity of *Sapindus trifoliatus* in various pain models

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Abstract

The effect of the aqueous extract of *Sapindus trifoliatus* (ST) on chemical, thermal-induced pain, nitroglycerin-induced hyperalgesia and pain on inflamed tissue was investigated. The extract (20 and 100 mg kg^{-1}, i.p.) significantly inhibited acetic-acid-induced abdominal constrictions, formalin-induced pain licking and hotplate-induced pain in mice. Furthermore, the extract significantly increased the response latencies of nitroglycerin-induced hyperalgesia by the tail-flick method and mechanical pain on carrageenan-induced inflamed paw in rats. The data suggest that ST has an inhibitory activity on both peripheral and central pain mechanisms and has a modulatory role in NO-mediated nociceptive transmission.

Introduction

Sapindus trifoliatus (ST) is a medium-sized deciduous tree found growing wild in south India that belongs to the family Sapindaceae. It is known locally as the soapnut tree, Aristha (Hindi). The plant has been reported for its high content of saponins and sugars. The saponin moiety is characterized by the hederagenin group of glycosides. The pericarp is reported for various medicinal properties. It is regarded as a tonic, stomachic, spermicidal and for the treatment of hemicrania (migraine pain), etc. A thick watery solution of the pericarp is used for the relief of hemicrania, hysteria or epilepsy (Kritikar & Basu 1999). However, there has been no published preclinical/clinical report available for its possible analgesic properties. It is evident from the literature that plants like feverfew (*Tanacetum parthenium*), which is known for migraine prophylaxis (Johnson et al 1985), exhibit antinociceptive and anti-inflammatory effects (Jain & Kulkarni 1999) in animal models of pain and inflammation. Since *Sapindus trifoliatus* (as an intranasal application) has been quoted for its use in hemicrania and epilepsy in traditional folk medicine, the objective of the present study was to investigate its possible analgesic potential in both peripheral and centrally mediated experimental models of pain.

Material and Methods

Chemicals

Pentazocine lactate (Fortwin, Ranbaxy, India) and nitroglycerin were obtained as injections from the local market. Indometacin was purchased from Sigma (St Louis, MO). Pentazocine was diluted with saline and indometacin was dissolved in 0.3% Tween 80 in saline. Nitroglycerin was diluted with 10% polyethyleneglycol 400 in saline.

Plant material and extraction procedure

Pharmacognostically identified dried pericarps of fruits of *Sapindus trifoliatus* Linn, family Sapindaceae, were collected from the local market and authenticated by Dr A. M. Mujumdar, Agharkar Research Institute (Pune, India). One hundred grams of the pericarp was soaked in 400 mL of distilled water for 16 h. The percolate was then decanted, centrifuged and filtered through Whatman (No.1) filter paper to obtain a clear

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extract (300 mL). This process of extraction was repeated again with the same volume of distilled water. The percolates were pooled and lyophilized to give a brown-coloured powder (68% yield). The hederagenin content in the extract was calculated by boiling it with 50% methanolic hydrochloric acid, then evaporating the mixture to dryness. The HPLC estimation was done by reconstitution with methanol and the concentration of hederagenin was found to be between 4 and 8% w/w in the extract, which is in accordance with the literature (Gupta & Ahmed 1990).

Animals

Adult male Swiss albino mice (18–22 g, 10 animals per group per treatment) and Wistar rats (180–220 g, 6–8 animals per group per treatment) were obtained from the Research Animal Facility of Lupin Ltd (Pune, India). On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 °C and relative humidity of 30–70%. A 12:12 light/dark cycle was followed. All animals had free access to water filtered through Aquaguard and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Lupin Ltd (Pune, India).

Nociceptive procedures

Immediately after the administration of ST, the animals were observed for a period of 15 min for gross behavioural abnormalities if any, and then they were subjected to the nociceptive procedures.

Acetic-acid-induced abdominal constrictions in mice

The measurement of the abdominal constrictions resulting from intraperitoneal injection of acetic acid (1%), consisting of constriction of abdominal muscle together with a stretching of hind limbs, was carried out according to the procedures described by Santos et al (1994), Correa et al (1996) and Besra et al (1996). ST (20 and 100 mg kg^{-1}) and positive control indometacin (20 mg kg^{-1}) were administered intraperitoneally 15 min prior to acetic acid injection. The number of writhing movements was counted for 30 min. Antinociception was expressed as the difference in the number of abdominal constrictions between control animals treated with saline and animals pretreated with extract or indometacin.

Formalin-induced paw licking in mice

The procedure was essentially similar to that described by Hunskaar and Hole (1987) and Gorski et al (1993). The formalin test possesses two distinctive phases, possibly reflecting different types of pain. Mice were injected intraperitoneally with ST (20 and 100 mg kg^{-1}) or saline (10 mL kg^{-1}) or indometacin (20 mg kg^{-1}). Fifteen minutes later 20 μL of 1% formalin was injected subcutaneously under the dorsal surface of the hind paw. Mice were observed in the chambers. The number of licks in the injected paw was counted and considered as indicative of pain stimuli. The first phase of the nociceptive response normally peaked 5 min after the formalin injection and the second phase 20–30 min after the formalin injection, representing neurogenic and inflammatory responses respectively.

Hotplate test in mice

The hotplate test was used to measure the latencies according to the method described by Eddy and Leimbach (1953). Animals were placed on a hotplate maintained at 55 ± 1 °C, and the time between placement of animal on the hotplate and the occurrence of licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before ST (20 and 100 mg kg^{-1}, i.p.) or saline (10 mL kg^{-1}, i.p.) or pentazocine (10 mg kg^{-1}, i.p.) distraction (basal) and 30, 60, 90, 120, 180 and 240 min after treatment. The cut-off time for hotplate latencies was set at 20 s.

Nitroglycerin-induced hyperalgesia in rats

Hyperalgesia was assessed using a tail-flick apparatus following the method of D'Amour and Smith (1941) as modified by Gray et al (1970). The rat was placed in the tail-flick unit such that its tail occluded a slit over a photocell. Heat was applied by a 100-W lamp mounted in a reflector. The apparatus was arranged so that when the operator turned on the lamp, a timer was activated. When the rat felt pain and flicked its tail, light fell on the photocell and the timer was automatically stopped. The light intensity was adjusted to give a normal reaction of 8–12 s. A 20 s cut-off time was used in order to prevent tissue damage. After taking the basal reading rats were injected with nitroglycerin intraperitoneally at a dose of 10 mg kg^{-1} (Tassorelli & Joseph 1995; Tassorelli et al 2003; Pardutz et al 2000). Fifteen minutes later, ST (20 and 100 mg kg^{-1}, i.p.) or saline or pentazocine (10 mg kg^{-1}, i.p.) was administered. The response latencies were measured at 30, 60, 90, 120, 180 and 240 min post treatment.

Pain in the inflamed paw

The method of Randall and Selitto (1957) was followed. Assessment of pain consisted of measurement of the threshold stimulus for reaction (escape or paw withdrawal) using a weight (maximum limit of 500 g) applied to the pads of hindpaws. The threshold for pain sensation was measured before (basal) and 30, 60, 90, 120, 180, 240 min after the intraplantar injection of 1% carrageenan (0.2 mL). ST (20 and 100 mg kg^{-1}, i.p.) or saline or indometacin (20 mg kg^{-1}, i.p.) was administered intraperitoneally 15 min prior to carrageenan injection.

Statistical analysis

Values are expressed as mean values ± s.e.m. The statistical significance of differences between the mean values was analysed by ANOVA and Dunnett's test. A P value of < 0.05 was considered to be significant.

Results

Acetic-acid-induced abdominal constrictions in mice

The results of the abdominal constriction test are shown in Figure 1. Administration of 20 and 100 mg kg^{-1} of ST elicited a pronounced antinociceptive response, as demonstrated by inhibition of constrictions in mice receiving acetic acid, with respect to control animals treated with saline. ST produced significant inhibitions of 90.77 and 96.26% ($P < 0.001$) for 20 and 100 mg kg^{-1}, i.p.), respectively, which are comparable to that of indometacin (20 mg kg^{-1}, i.p.).

Formalin-induced paw licking in mice

ST at doses of 20 and 100 mg kg^{-1} i.p. caused a significant ($P < 0.001$) inhibition of the neurogenic (0–10 min) and inflammatory (20–30 min) phases of formalin-induced licking in mice (Figures 2 and 3). The standard drug indometacin (20 mg kg^{-1}, i.p.) also exhibited a statistically significant inhibition of both phases of formalin-induced paw licking.

Hotplate test in mice

Pretreatment of animals with ST extract (20 and 100 mg kg^{-1}, i.p.) increased the pain latency in the hotplate test (Table 1). The increase in latency responses was found to be statistically significant ($P < 0.05$ and 0.001) at 30, 60 and 90 min post treatment. The known centrally acting analgesic pentazocine also increased the response latencies at various time points; however, only at 30 min was the effect statistically significant.

Nitroglycerin-induced hyperalgesia in rats

Nitroglycerin induced a significant decrease in latency of tail flick, compared to that of basal values. Pretreatment with ST (20 and 100 mg kg^{-1}, i.p.) significantly ($P < 0.05$ and 0.001) increased the pain latencies of nitroglycerin-treated rats compared to the saline control (Table 2).

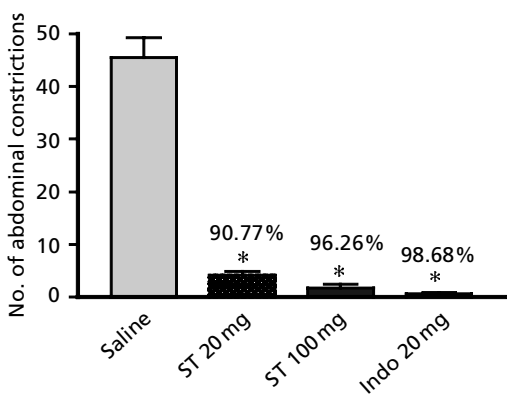

Figure 1 Effect of ST on acetic-acid-induced abdominal constrictions in mice. Compounds were administered intraperitoneally. Bars represent means ± s.e.m. and percentage inhibition from n = 10. *$P < 0.001$ compared with saline treatment. Indo, indometacin.

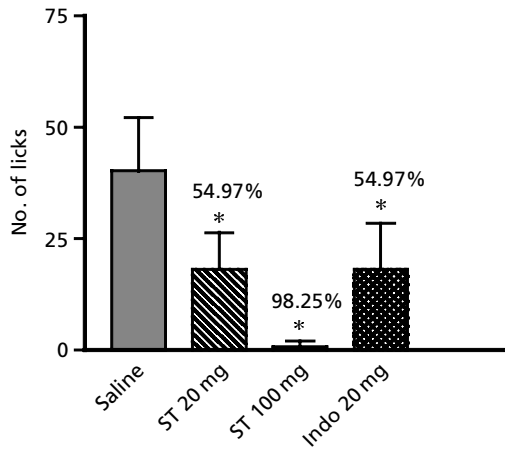

Figure 2 Effect of ST on the neurogenic phase of formalin-induced licks in mice. Compounds were administered intraperitoneally. Bars represent means ± s.e.m. and percentage inhibition from n = 10. *$P < 0.001$ compared with saline treatment. Indo, indometacin.

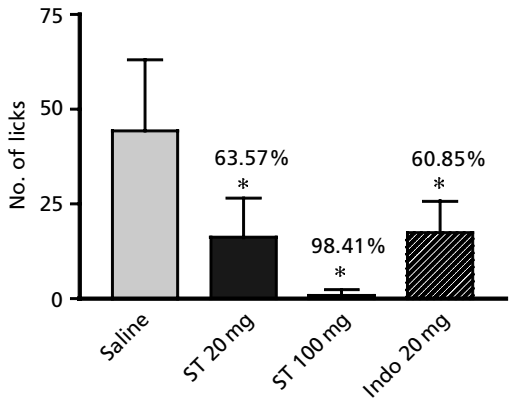

Figure 3 Effect of ST on the inflammatory phase of formalin-induced licks in mice. Compounds were administered intraperitoneally. Bars represent means ± s.e.m. and percentage inhibition from n = 10. *$P < 0.001$ compared with saline treatment. Indo, indometacin.

Pain in inflamed tissue in rats

Intraplantar administration of carrageenan reduced the weight threshold required for the animals' response. ST (20 and 100 mg kg^{-1}, i.p.) significantly ($P < 0.05$ and 0.001) increased the weight threshold compared to the saline-treated animals. The standard drug indometacin (20 mg kg^{-1}, i.p.) at 30, 60 and 90 min post treatment significantly increased the weight threshold ($P < 0.001$) (Table 3).

Receptor radioligand binding studies

The receptor radioligand binding data of ST on selected receptors and enzymes are shown in Table 4. The receptor

Table 1 Effect of the aqueous extract of ST on the hotplate test in mice.

Time points (min post treatment)	Response latencies (s)			
	Saline 10 mL kg^{-1}, i.p.	ST 20 mg kg^{-1}, i.p.	ST 100 mg kg^{-1}, i.p.	Pentazocine 20 mg kg^{-1}, i.p.
Basal	9.32 ± 0.49	9.37 ± 0.66	8.24 ± 0.49	9.35 ± 0.58
30	7.79 ± 0.80	13.61 ± 1.16*	12.86 ± 0.94**	15.6 ± 1.04**
60	8.15 ± 0.61	13.99 ± 0.95*	14.19 ± 1.28**	13.06 ± 0.99
90	8.95 ± 0.60	12.74 ± 1.22	13.75 ± 0.73**	10.93 ± 0.85
120	9.97 ± 1.23	11.28 ± 0.83	10.86 ± 1.14	10.38 ± 1.62
180	7.16 ± 0.65	10.11 ± 1.35	8.34 ± 0.69	7.74 ± 0.92
240	6.49 ± 0.57	8.63 ± 0.70	8.40 ± 1.10	12.11 ± 1.08

Values are expressed as mean ± s.e.m. from n = 10. Significantly different from basal values, *$P < 0.05$, **$P < 0.001$.

Table 2 Effect of aqueous extract of ST on the nitroglycerin-induced hyperalgesia test in rats.

Time points (min post treatment)	Response latencies (s)			
	Saline 10 mL kg^{-1}, i.p.	ST 20 mg kg^{-1}, i.p.	ST 100 mg kg^{-1}, i.p.	Pentazocine 20 mg kg^{-1}, i.p.
Basal	8 ± 2.7	5.8 ± 1.2	7.6 ± 1.2	6.1 ± 1.10
30	5.6 ± 0.10	9.3 ± 1.80	13.8 ± 1.9**	9.3 ± 2.0
60	6.7 ± 0.8	13.6 ± 1.5*	12.4 ± 1.5*	9.6 ± 2.0
90	6.3 ± 0.60	13.3 ± 1.3*	13.9 ± 1.7**	10.6 ± 2.3
120	6.4 ± 0.6	12.6 ± 1.7*	11.3 ± 1.9	6.0 ± 1.2
180	7.2 ± 0.5	15.7 ± 0.2**	8.8 ± 1.9	4.5 ± 0.4
240	8.0 ± 1.5	10.5 ± 2.1	10.5 ± 1.7	7.9 ± 2.3

Values are expressed as mean ± s.e.m. from n = 6–8. Significantly different from saline control, *$P < 0.05$, **$P < 0.001$.

radioligand binding studies were carried out by Novascreen Biosciences Corporation (USA).

Discussion

The animals did not exhibit any remarkable physiological/behavioural abnormalities after the administration of ST.

The aqueous extract of ST was studied for its modulatory affective role in nociception in both peripheral and central algesic models.

In the present study, it was found that the intensity of the analgesic effect of ST was similar to that of indometacin in acetic-acid-induced abdominal constrictions in mice. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas et al 1984) and liberating endogenous substances that excite pain nerve endings (Raj 1996). Non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit cyclo-oxygenase (COX) in peripheral tissues, thus interfering with the mechanism of transduction of primary afferent nociceptors (Fields 1987). The mechanism of analgesic effect of ST is probably due to a blockade of this effect or the release of endogenous substances that excite pain nerve endings similarly to indometacin and other NSAIDs.

ST exhibited a comparable potency of indometacin for inhibiting neurogenic (first phase) and was more efficacious against the inflammatory (second phase) pain stimuli caused by formalin. The formalin test is used to evaluate the mechanism by which an animal responds to moderate continuous pain generated by the injured tissue (Tjolsen et al 1992; Abbot et al 1995). This test is characterized by two phases. The early phase (immediately after injection) seems to be caused by C-fibre activation due to the peripheral stimulus. The late phase (starting approximately 20 min after formalin injection) appears to depend on the combination of an inflammatory reaction, activation of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, and the nitric oxide (NO) cascade (Davidson & Carlton 1998; Omote et al 1998, 2000) in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (Abbot et al 1995). Both these functional changes appear to be initiated by the C-fibre barrage during the early phase and to be related to excitatory amino acid release in the spinal cord and activation of NMDA receptor subtypes (Coderre & Van Empel 1994). The formalin test has been used to evaluate the antinociceptive effects of competitive and non-competitive NMDA receptor antagonists administered intrathecally and systemically (Eisenberg et al 1993). In a recent study CGP 37849, memantine, ketamine and dextromethorphan were reported to have antinociceptive activity in the formalin test (Berrino et al 2003). Linalool, a competitive antagonist of NMDA receptors, is found to be

Table 3 Effect of aqueous extract of ST on pain in inflamed tissue (Randall and Selitto) test in rats.

Time points (min post treatment)	Response latencies (g • 20)			
	Saline 10 mL kg^{-1}, i.p.	ST 20 mg kg^{-1}, i.p.	ST 100 mg kg^{-1}, i.p.	Indometacin 20 mg kg^{-1}, i.p.
Basal	7.9 ± 1.10	7.2 ± 1.20	7.6 ± 0.90	6.9 ± 1.10
30	6.7 ± 0.70	18.2 ± 2.7**	18.4 ± 1.9**	23.1 ± 1.7**
60	3.5 ± 0.6	19.6 ± 2.6**	20.5 ± 1.7**	21.2 ± 2.5**
90	4.0 ± 0.40	20.6 ± 1.8**	23.8 ± 0.60**	12.1 ± 2.7**
120	5.4 ± 0.7	8.6 ± 0.9	19.0 ± 2.7**	7.8 ± 1.2
180	3.9 ± 0.4	10.4 ± 2.9	15.9 ± 2.1*	11.3 ± 3.3
240	4.5 ± 0.9	12.2 ± 3.2	12.4 ± 2.2	8.4 ± 2.1

Values are expressed as mean ± s.e.m. from n = 6–8. Significantly different from saline control, *$P < 0.05$, **$P < 0.001$.

Table 4 Selected receptor ligand binding data of ST.

Receptor/enzyme	Source	Ligand	% inhibition at 250 μg mL^{-1}
Glutamate NMDA-glycine (strychnine insensitive site)	Rat hippocampus	[^{3}H]AMPA	85.33
Glutamate AMPA site	Rat hippocampus	[^{3}H]AMPA	87.36
Glutamate NMDA (agonistic site)	Rat forebrain	[^{3}H]CGP39653	98.14 IC_{50}: 140 μg mL^{-1}
Glutamate kainate site	Rat forebrain	[^{3}H]Kainic acid	87.29
Sigma non-selective	Guinea pig whole brain	[^{3}H]DTG	50.36
NOS (constitutive neuronal)	Rat cerebellum	[^{3}H]Arginine	84.10
COX-1	Bovine seminal vesicle	Arachidonic acid	29.0
COX-2	Bovine seminal vesicle	Arachidonic acid	34.0
LTB_4	Guinea pig spleen	[^{3}H]LTB_4	79.0

The radioligand binding studies were carried out by NovaScreen Biosciences Corporation, USA.

antinociceptive in animal models of nociception (Peana et al 2003). In this context it is quite appropriate to state here that in binding studies ST exhibited an affinity to various glutamate-NMDA receptors (Table 4), which suggests the possible involvement of NMDA receptors in its antinociceptive action.

The aqueous extract of ST produced antinociception against thermal-induced pain stimuli in mice at various time points post treatment. The hotplate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species (Janssen et al 1963; Besra et al 1996). ST also exhibited binding affinity towards non-selective sigma receptors, which may also support this finding (Table 4).

In the present study ST significantly inhibited nitroglycerin-induced hyperalgesia. Several reports have suggested that NO has a hyperalgesic effect (Hoheisel & Mense 2000) and experimental studies have shown that some NO donors can intensify ongoing pain (Kitto et al 1992; Sousa & Prado 2001). Masue et al (1999) and Tassorelli et al (2003) described thermal hyperalgesia following intrathecal and systemic administration of nitroglycerin in rats. It has been proved that systemic nitroglycerin induces neuronal activation in the nucleus trigeminalis caudalis (Tassorelli & Joseph 1995), a very important structure of nociceptive transmission. It has been speculated that nitroglycerin-mediated hyperalgesia may be related to a NO-mediated facilitation of nociceptive transmission in both peripheral tissue and the spinal cord.

Nitroglycerin-induced hyperalgesia is considered to be a model of migraine headaches, and nitroglycerin dose-dependently produces headache in normal volunteers and migraine sufferers (Krabbe & Olesen 1980; Iverson et al 1989; Olesen et al 1993). Nitroglycerin is the most suitable substance for experimental studies of NO-induced headache as it is well tolerated and diffuses freely across membranes because of its lipid solubility. It may thus deliver NO in several tissues, including those protected by the blood–brain barrier (Tassorelli et al 2003). It was observed that at a concentration of 250 μg mL^{-1}, ST has an 84.10% inhibition of NO synthase (constitutive-neuronal) in radioligand binding studies. It is also postulated that glutamate-induced activation of NMDA receptors leads to NO formation (Bruyn 1996). Incidentally, ST has shown affinity to glutamate NMDA agonistic sites (Table 4). Since ST has been traditionally used for the treatment of hemicrania (Kritikar & Basu 1999), the current finding could be a possible explanation for its NO-mediated mechanism of action.

In the other set of experiments, ST dose-dependently increased the response latencies for the mechanical pain stimuli in the Randall and Selitto test. It is appropriate to state that prostaglandins (PGs) are well established as mediators of several components of inflammatory responses. In particular, the oedema resulting from increased microvascular permeability is a consequence of the vasodilator effect of PGs, potentiating the microvascular effect of other mediators, such as bradykinin, substance P and histamine (Williams & Moreley 1973; Williams & Peck 1977). These mediators also induce pain in inflammatory sites and this component is also potentiated by PGs (Ferreira 1972). Radioligand binding studies of ST revealed that at a concentration of 250 $\mu g\,mL^{-1}$ it has an inhibitory effect on COX-1 (29% inhibition), COX-2 (34% inhibition) and leukotriene B_4 (LTB_4) (79% inhibition). These data support the analgesic/anti-inflammatory effect of ST in the Randall and Selitto test for pain on inflamed tissue.

Conclusion

In conclusion, ST administered intraperitoneally exhibited antinociceptive activity in various models of pain. The results suggest that ST has both central and peripheral analgesic mechanisms. Saponins, particularly the hederagenin type of saponins, are known to have antinociceptive and antirheumatic activities (Suh et al 2000; Choi et al 2002), and it is therefore probable that the saponin component of the extract may contribute in part to the observed pharmacological activities. The present data support the ethno-medical application of ST in the treatment of hemicrania. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by ST.

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