J. Pharm. Pharmacol. 1997, 49: 567–572
Received November 6, 1996
Accepted January 27, 1997

Mechanisms Involved in the Antinociceptive Effect in Mice of the Hydroalcoholic Extract of *Siphocampylus verticillatus*

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Abstract

The antinociception caused by the hydroalcoholic extract of *Siphocampylus verticillatus* (Campanulaceae) has been investigated in chemical and thermal models of nociception in mice. We have also assessed some of the mechanisms underlying the antinociceptive effect of the extract.

The hydroalcoholic extract of *S. verticillatus* (60–1000 mg kg^{-1}, i.p. or p.o.) produced dose-related, significant and long-lasting (6 to 8 h) inhibition of acetic acid-induced abdominal constriction in mice, with ID50 values of 204 and ~1000 mg kg^{-1}, respectively. In the formalin test, the extract (100–1000 mg kg^{-1}), given either intraperitoneally or orally, resulted in graded inhibition of both phases of formalin-induced pain, being about 2- to 4-fold more potent in attenuating the second phase of the pain. The calculated mean ID50 (mg kg^{-1}) values for the earlier and the later phases were: 491 and 186 and 640 and 441, respectively. In addition, the extract (60–1000 mg kg^{-1}, i.p. or p.o.) caused marked and dose-related inhibition of capsaicin-induced neurogenic pain with mean ID50 values of 420 and 485 mg kg^{-1}, respectively. The hydroalcoholic extract, at the same doses, did not significantly affect the performance of animals in the rota-rod test, nor did it have any analgesic effect in the tail-flick or hot-plate tests. The treatment of animals with naloxone (5 mg kg^{-1}, s.c.) significantly reversed the analgesic effect of both morphine (5 mg kg^{-1}, s.c.) and the extract (300 mg kg^{-1}, i.p.) when assessed against acetic acid-induced abdominal constrictions. The treatment of animals with L-arginine (600 mg kg^{-1}, i.p.) significantly attenuated the antinociceptive effects of N^G-nitro-L-arginine (L-NOARG) (75 mg kg^{-1}, i.p.), of the hydroalcoholic extract (600 mg kg^{-1}, i.p.) or of morphine (5 mg kg^{-1}, s.c.), when analysed against the formalin test. In addition, adrenalectomy of animals 7 days before the tests significantly reversed the antinociception caused by the hydroalcoholic extract (300 mg kg^{-1}, i.p.) in the formalin-induced pain.

These data show that the hydroalcoholic extract of *S. verticillatus* has significant and long-lasting oral antinociception when assessed against both neurogenic and inflammatory models of nociception in mice. The precise mechanism responsible for the analgesic effect of the extract still remains unclear, but a great part of this effect seems to be partly related to an opioid-like action and involvement of the L-arginine-nitric oxide pathway. Finally, the antinociception caused by the hydroalcoholic extract of *S. verticillatus* is modulated by adrenal hormones.

The plant family Campanulaceae consists of approximately 29 genera and more than 1200 species that are widely distributed in tropical and sub-tropical countries (Wimmer 1968). *Siphocampylus verticillatus*, a member of this family, is a native plant which grows abundantly in the South of Brazil. Among the main traditional medicinal uses of this plant is its reported beneficial action in the treatment of asthma (Garello 1950). Earlier chemical studies have demonstrated the presence of several classes of compounds in *S. verticillatus*; these include alkaloids, tannins, phytosterols, flavonoids and phenols (Garello 1950; Corrol et al 1970; Moreira et al 1984). No pharmacological studies have, however, yet been performed, either with the extract or with the principles isolated from this plant, to confirm its medicinal use reported in folk medicine.

This study aims, firstly, to examine the antinociceptive action of the hydroalcoholic extract of *S. verticillatus* on chemical and thermal models of nociception, and, secondly, to investigate some the mechanisms underlying the antinociceptive effect of the hydroalcoholic extract of *S. verticillatus* by use of several in-vivo pharmacological procedures and selective antagonists and enzyme inhibitors.

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Materials and Methods

Drugs

Acetic acid, formalin and morphine hydrochloride were from Merck (Darmstadt, Germany), N^G-nitro-L-arginine (L-NOARG) and L-arginine from Sigma (St Louis, MO), capsaicin from Calbiochem (San Diego, CA) and naloxone hydrochloride from Dupont (Garden City, USA). Other reagents used were of a high grade of purity. All drugs and extract were dissolved in 0.9% NaCl solution or in physiological buffer solution just before use.

Preparation of the crude extract

Botanical material was collected in the São José dos Pinhais district, Curitiba, Paraná, Brazil, in January 1993, and classified by Dr Gerdt Hatschbach, Botanical Garden of Curitiba-PR. A voucher specimen (no. 68920) was deposited in the Herbarium Botanical Garden at Curitiba. The dried stems and leaves were minced and extracted with 50% ethanol–water in

the proportion of 1:3 (w/v), while being stirred and macerated, at room temperature (21 ± 3°C) for 15 days. The ethanol was evaporated and the extract was concentrated to the desired level and stored under refrigeration at − 4°C until use. The extract was dissolved in 0·9% NaCl solution at the desired concentration just before use.

Pharmacological procedures

Animals. All experiments were performed with Male Swiss mice, 25–35 g, housed at 22 ± 2°C under a 12-h light-dark cycle, and with free access to water and food. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for one experiment only. All experiments were performed in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann 1983).

Abdominal constriction response caused by intraperitoneal injection of acetic acid. Abdominal constrictions resulting from intraperitoneal injection of acetic acid (0.6%), consisting of contraction of the abdominal muscle together with stretching of the hind limbs, were investigated according to the procedures described previously (Santos et al 1994). Animals were pre-treated with the hydroalcoholic extract (60–600 mg kg^{-1}, i.p.) 30 min before, or orally (100–1000 mg kg^{-1}) 0·5 to 10 h before acetic acid injection. Control animals received a similar volume of 0·9% NaCl (10 mL kg^{-1}). After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociception was expressed as the reduction of the number of abdominal constrictions of mice pre-treated with hydroalcoholic extract compared with control (saline pre-treated) animals.

Formalin-induced nociception. The procedure was similar to that described previously (Hunskaar et al 1985, 1986; Murray et al 1988; Corrêa & Calixto 1993; Santos et al 1995a; Corrêa et al 1996). A solution of 2.5% formalin (0·92% formaldehyde) was prepared in phosphate buffer solution and 20 μL was injected under the surface of the right hind paw using a microsyringe with a 26-gauge needle. Two mice (control and treated) were observed simultaneously for 0 to 30 min after injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. The initial nociceptive scores normally peaked 5 min after formalin injection (earlier phase) and 15 to 30 min after formalin injection (later phase), representing the neurogenic and inflammatory pain responses respectively (Hunskaar & Hole 1987). Animals were treated intraperitoneally or orally with saline (0.9%; 10 mL kg^{-1}) or with the hydroalcoholic extract of *S. verticillatus* (100–1000 mg kg^{-1}) 30 min and 4 h before formalin injection. After intraplantar injection of formalin the animals were immediately placed into a glass cylinder 20 cm in diameter and the time spent licking the injected paw, timed with a chronometer, was considered as indicative of pain. To investigate whether the antinociceptive activity of hydroalcoholic extract in formalin-induced pain was associated with anti-oedematogenic activity, we measured the paw oedema by comparing the difference in weight of the formalin-treated paw and the weight of the control paw (treated with saline). For this purpose animals were killed by cervical dislocation 30 min after formalin injection and the paw was amputated at the knee joint and weighed on an analytical balance.

Capsaicin-induced nociception. To assess the possible analgesic effect of the hydroalcoholic extract of *S. verticillatus* on neurogenic pain we also investigated whether extract antagonizes capsaicin-induced pain in the mouse paw. Animals were placed individually into transparent glass cylinders 20 cm in diameter, serving as observation chambers. After a period of acclimatization 20 μL capsaicin (1·6 μgl paw) was injected under the skin of the dorsal surface of the right hind paw using a microsyringe with a 26-gauge needle. The procedure used was similar to that described previously (Sakurada et al 1992), with minor modifications. The animals were observed individually for 5 min after capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered to be indicative of pain. Animals were treated with the hydroalcoholic extract of *S. verticillatus* intraperitoneally (100–1000 mg kg^{-1}) 30 min before, or orally (100–1000 mg kg^{-1}) 4 h before, capsaicin injection. Control animals received a similar volume of NaCl solution (0·9%; 10 mL kg^{-1}) either intraperitoneally or orally.

Tail-flick test. A radiant heat tail-flick analgesiometer was used to measure response latencies according to the method described by D'Amour & Smith (1941), with minor modifications. Animals responded to a focused heat-stimulus (90 W) by flicking or removing their inflicted tail, exposing a photocell in the apparatus immediately below. The reaction time was recorded for control mice and for animals pre-treated with morphine or with the extract. The animals were selected 24 h previously on the basis of their reactivity to the model, by eliminating those which remained on the apparatus for up to 8 s. A latency period of 20 s was defined as complete analgesia. The animals received the hydroalcoholic extract of *S. verticillatus* administered intraperitoneally (up to 300 mg kg^{-1}), or morphine, used as positive control (10 mg kg^{-1}, s.c.), 30 min before experiments. Control animals received the same volume of vehicle (10 mL kg^{-1}).

Hot-plate test. The hot-plate test was used to measure response latencies according to the method described by Eddy & Leimback (1953). In these experiments the hot-plate (Ugo Basile, Model-DS 37) was maintained at 56 ± 1°C. Animals were placed into a 24-cm diameter glass cylinder on the heated surface and the time (s) between placement and shaking or licking of the paws or jumping was recorded as response latency. The reaction time was recorded for control mice and for animals pre-treated with morphine (used as positive control) or pre-treated with the hydroalcoholic extract of *S. verticillatus*. Animals were selected 24 h previously on the basis of their reactivity in the model. A latency period of 30 s was defined as complete analgesia. Animals were treated with the extract (up to 300 mg kg^{-1}, i.p.) or with morphine (10 mg kg^{-1}, s.c.) 30 min before the experiments. Control animals received the same volume of vehicle (10 mL kg^{-1}, i.p.).

Rota-rod test. In order to assess the possible non-specific muscle-relaxant or sedative effects caused by the hydroalcoholic extract, the mice were tested on the rota-rod, as described previously (Santos et al 1995a; Vaz et al 1996). The apparatus

(Ugo Basile, Model-DS 37) consisted of a bar, diameter 2·5 cm, subdivided into six compartments by disks 25 cm in diameter. The bar rotated at a constant speed (22 rev. min^{-1}. Animals were selected 24 h previously by eliminating those mice which did not remain on the bar for two consecutive periods of 60 s. Animals were re-tested 4 h after oral treatment with NaCl (10 mL kg^{-1}) or with the hydroalcoholic extract of *S. verticillatus* (up to 600 mg kg^{-1}). Results are expressed as the time (s) during which animals remained on the rota-rod. The cut-off time was 60 s.

Analysis of the mechanism of action of the hydroalcoholic extract of S. verticillatus

To assess the possible participation of the opioid system in the antinociceptive effect of the hydroalcoholic extract 30 min before acetic acid injection animals were pre-treated with naloxone (5 mg kg^{-1}, i.p.) 15 min before administration of the extract (600 mg kg^{-1}, i.p.) or of morphine (5 mg kg^{-1}, s.c.). The other groups of animals received the naloxone, the extract, morphine or the vehicle (10 mL kg^{-1}, i.p.) 30 min before the acetic acid injection.

In separate experiments, we investigated the possible participation of the nitric oxide L-arginine pathway in the antinociceptive effect of the extract. For this purpose, animals received the hydroalcoholic extract (600 mg kg^{-1}, i.p.), morphine (5 mg kg^{-1}, s.c.) or N^G-nitro-L-arginine (L-NOARG, a nitric oxide inhibitor; 75 mg kg^{-1}, i.p.) 30 min before formalin injection. The other groups received the L-arginine (a nitric oxide precursor; 600 mg kg^{-1}, i.p.) 15 min before administration of the extract, morphine, L-NOARG or vehicle injection (10 mL kg^{-1}, i.p.), 30 min before formalin injection as described before (Vaz et al 1996).

In other experiments we investigated the possible role of endogenous corticosteroids in the antinociceptive effect caused by the hydroalcoholic extract. To this end, animals were anaesthetized with ether and both adrenal glands were removed by dorsal incision, as described previously (Vaz et al 1996). After surgery, animals were returned to their cages, with free access to food and drink, but water was substituted by saline (0·9% NaCl) to maintain physiological sodium plasma concentration. Another group of animals was sham-operated and allowed free access to water and food. After 2 weeks, animals received hydroalcoholic extract (300 mg kg^{-1}, i.p.) or vehicle (10 mL kg^{-1}, i.p.) 30 min before injection of formalin.

Statistical analysis

Results are presented as mean ± s.e.m., except the ID50 values (i.e. the dose of extract that reduced pain responses by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance between groups was calculated by means of analysis of variance then Dunnett's multiple comparison test or Newman Keuls test. P values less than 0.05 ($P < 0·05$) were considered as indicative of significance. When appropriate, the ID50 values were determined by graphical interpolation from individual experiments.

Results

The results presented in Table 1 show that when assessed against acetic acid-induced abdominal constrictions the hydroalcoholic extract of *S. verticillatus* resulted in significant and long-lasting (6 and 8 h) antinociception when given intraperitoneally (300 mg kg^{-1}) or orally (600 mg kg^{-1}) 0·5 to 8 h before injection of acetic acid. The extract (60–600 mg kg^{-1}, i.p.) or (100–1000 mg kg^{-1}, p.o.) resulted in dose-related and significant inhibition of acetic acid-induced abdominal constriction response in mice (Table 2). The calculated mean ID50 (mg kg^{-1}) values and the respective 95% confidence limits for these effects were 204·1 (140·0–300·0) and ~1000, and the maximum inhibition (%) was 78·0 ± 4·0 and 49·0 ± 2·0, respectively.

When assessed in the formalin test, the hydroalcoholic extract produced in marked and dose-related inhibition of both phases (neurogenic and inflammatory) of formalin-induced pain when given either intraperitoneally or orally (100–1000 mg kg^{-1}) 0·5 or 4 h, respectively, before injection of formalin (Table 3). The hydroalcoholic extract was, moreover, approximately 2- to 4-fold more active against the second phase of the pain model, depending on the route of administration. The estimated mean ID50 (mg kg^{-1}) values for the first phase were 491·0 (283·5–853·5) and 640·0 (355·0–1150·0) and the maximum inhibition values (%) were 61 ± 5 and 55 ± 3, respectively. The mean ID50 values (mg kg^{-1}) for the second phase were 186·0 (103·6–332·3) and 441·0 (26·0–735·0), and the maximum inhibition values (%) were 87 ± 5 and 68 ± 6, respectively, for intraperitoneal or oral administration (Table 3). Given orally, the hydroalcoholic extract was about 2- to 5-fold less potent than when given intraperitoneally. It failed, however, to affect the oedematogenic response associated with second phase of the formalin test (Table 3).

When tested in the capsaicin algesic model, the hydroalcoholic extract from *S. verticillatus* resulted in marked and dose-related inhibition of capsaicin-induced neurogenic pain when given either intraperitoneally or orally (100–1000 mg kg^{-1}), 0.5 and 4 h before capsaicin (Table 4). Interestingly, the hydroalcoholic extract was equally potent at inhibiting capsaicin-induced nociception irrespective of its route of administration. The estimated mean ID50 (mg kg^{-1}) values were 420·0 (357·3–492·0) and 485·0 (372·7–633·3), and the maximum inhibition values (%) were 67 ± 4 and 54 ± 6, respectively (Table 4).

Table 1. Time-course of antinociceptive effect of hydroalcoholic extracts of *S. verticillatus* given either intraperitoneally or orally against acetic acid-induced abdominal constrictions in mice.

Time (h)	Number of abdominal constrictions	
	Intraperitoneal (300 mg kg^{-1})	Oral (600 mg kg^{-1})
0	42·5 ± 1·9	44·6 ± 2·7
0·5	8·6 ± 1·5**	–
1·0	5·0 ± 2·2**	–
2·0	10·7 ± 2·4**	28·1 ± 1·7**
4·0	17·0 ± 4·9**	23·4 ± 3·8**
6·0	27·2 ± 2·0**	23·4 ± 3·5**
8·0	35·1 ± 1·8	25·0 ± 2·6**
10·0	41·7 ± 2·0	30·3 ± 2·6*
12·0	–	38·7 ± 2·1

Each group represents the mean ± s.e.m. of results from 4 to 10 animals. *$P < 0·05$, **$P < 0·01$, significant compared with control values.

Table 2. Effect of the hydroalcoholic extract of *S. verticillatus* on acetic acid-induced abdominal constrictions in mice.

Treatment	Number of abdominal constrictions			
	Dose (mg kg^{-1})	Intraperitoneal	Dose mg (kg^{-1})	Oral
S. verticillatus	0	40·1 ± 2·8	0	49·2 ± 3·2
	60	32·0 ± 3·3	100	37·5 ± 4·1
	100	24·8 ± 4·7*	300	33·0 ± 5·7
	300	8·5 ± 1·5**	600	25·0 ± 1·0
	600	13·3 ± 2·0**	1000	25·2 ± 1·1
Dose reducing pain by 50%†		204·1 (140·0–300·0)		~1000
Maximum inhibition (%)		78·0 ± 4·0		49·1 ± 1·1

Each group represents the mean ± s.e.m. of results from 6 to 8 animals. *$P < 0.05$, **$P < 0.01$, significant compared with respective control values. †(mg kg^{-1}) with 95% confidence limits.

Table 3. Effect of hydroalcoholic extract of *S. verticillatus* against the first phase, 0 to 5 min, and the second phase, 15 to 30 min, in the formalin test on mice.

	Dose (mg kg^{-1})	Amount of licking (s)		Δ Paw weight (mg)
		0–5 min	15–30 min	
Intraperitoneal				
	0	63·3 ± 2·6	186·0 ± 8·0	74·1 ± 4·9
	100	46·1 ± 6·2*	130·2 ± 9·4**	77·0 ± 6·6
	300	33·5 ± 4·7**	76·6 ± 11·0**	74·4 ± 4·3
	600	24·8 ± 2·9**	23·5 ± 9·7**	79·5 ± 5·0
	1000	24·4 ± 2·8**	28·5 ± 13·6**	78·5 ± 5·5
Dose reducing pain by 50%†		491·0 (283·5 – 853·5)	185·6 (103·7 – 332·3)	
Maximum inhibition (%)		61·0 ± 5·0	87·0 ± 5·0	
Oral				
	0	62·1 ± 2·5	164·8 ± 17·4	71·2 ± 3·3
	100	43·8 ± 1·9**	127·4 ± 9·0**	75·6 ± 4·2
	300	34·6 ± 3·7**	73·3 ± 9·0**	74·9 ± 4·9
	600	30·7 ± 1·7**	63·5 ± 7·8**	76·5 ± 3·5
	1000	27·3 ± 1·0**	57·8 ± 9·5**	69·1 ± 2·5
Dose reducing pain by 50%†		640·0 (355·0 – 1150·0)	440·8 (226·0 – 735·0)	
Maximum inhibition (%)		55·0 ± 2·4	68·0 ± 5·7	

Each group represents the mean ± s.e.m. of results from 6 to 14 animals. *$P < 0.05$; **$P < 0.01$, significant compared with respective control values. †(mg kg^{-1}) with their respective 95% confidence limits.

Table 4. Effect of the hydroalcoholic extract of *S. verticillatus* on capsaicin-induced neurogenic pain in mice.

Treatment	Amount of licking (s)			
	Dose (mg kg^{-1})	Intraperitoneal	Dose (mg kg^{-1})	Oral
S. verticillatus	0	42·1 ± 2·5	0	41·0 ± 2·8
	60	32·2 ± 2·8	100	24·4 ± 3·0*
	100	24·9 ± 3·0*	300	21·4 ± 2·3*
	300	18·6 ± 1·5*	600	18·2 ± 2·6*
	600	13·6 ± 2·0*	1000	17·3 ± 2·2*
	1000	15·0 ± 2·1		
Dose reducing pain by 50%†		420·0 (357·3 – 492·0)		485·0 (372·7 – 633·3)
Maximum inhibition (%)		67·0 ± 4·3		54·3 ± 6·1

Each group represents the mean ± s.e.m. of results from 6 to 8 animals. *$P < 0.01$, significant compared with respective control values. †(mg kg^{-1}) with their respective 95% confidence limits.

The results listed in Table 5 show that the pre-treatment of animals with naloxone (5 mg kg^{-1}, i.p., 10 min before) significantly inhibited the analgesic effects of morphine (5 mg kg^{-1}, s.c.) or the hydroalcoholic extract (300 mg kg^{-1}, i.p.) when analysed against acetic acid-induced abdominal constriction. The treatment of animals with L-arginine (a precursor of nitric oxide synthase; 600 mg kg^{-1}, i.p.) significantly reversed the antinociceptive effect of L-NOARG (75 mg kg^{-1}, i.p.; 30 min before) against the early and late phase of the formalin test. The same treatment with L-arginine, however, significantly reversed the antinociceptive action of the hydroalcoholic extract (600 mg kg^{-1}, i.p.) or of morphine (5 mg kg^{-1}, s.c.) when assessed against the late (but not the early) phase of the formalin test (Table 6).

Bilateral adrenalectomy of the animals two weeks before the experiments significantly attenuated the antinociceptive effect

Table 5. Effect of naloxone on the antinociception induced by morphine and the hydroalcoholic extract of *S. verticillatus* against acetic acid-induced abdominal constrictions in mice.

Drug	Dose (mg kg^{-1})	Number of abdominal constrictions
Control	0	41·2 ± 2·0
Naloxone (i.p.)	5	43·1 ± 2·6
Morphine (s.c.)	5	0 ± 0*
S. verticillatus (i.p.)	300	9·5 ± 1·6*
Naloxone + *S. verticillatus*	5 + 300	20·1 ± 3·9†
Naloxone + morphine	5 + 5	27·2 ± 4·6†

Each group represents the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.01$ significant when compared with control value; †$P < 0.01$ significant when compared with agonist plus antagonists against agonist alone.

Table 6. Effect of L-arginine on antinociception induced by L-NOARG, morphine and the hydroalcoholic extract of *S. verticillatus* against formalin-induced pain.

Drug	Dose (mg kg^{-1})	Amount of licking (s)	
		0–5 min	15–30 min
Control	0	56·0 ± 2·1	189·2 ± 18·8
L-arginine (i.p.)	600	59·1 ± 5·0	160·0 ± 15·0
L-NOARG (i.p.)	75	36·3 ± 3·2*	32·3 ± 10·8*
Morphine (s.c.)	5	20·3 ± 1·6*	31·1 ± 9·3*
S. verticillatus (i.p.)	600	32·2 ± 2·7*	10·5 ± 10·5*
L-arginine + *S. verticillatus*	600 + 600	36·3 ± 3·0	104·2 ± 13·3†
L-arginine + L-NOARG	600 + 75	54·0 ± 4·3†	144·4 ± 22·3†
L-arginine + morphine	600 + 5	22·1 ± 2·3	141·1 ± 20·6†

Each group represents the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.01$, significant when compared with control value; †$P < 0.01$, significant when compared with agonist plus antagonists against agonist alone.

of the hydroalcoholic extract of *S. verticillatus* against the late (but not the early) phase of formalin-induced pain (Table 7).

Table 8 shows that the extract (up to 300 mg kg^{-1}, i.p.) was virtually ineffective against the tail-flick and hot-plate tests, in conditions where morphine (10 mg kg^{-1}, s.c.) caused a marked increase in the pain latency in both algesiometer assays. When submitted to the rota-rod test, the hydroalcoholic extract of *S. verticillatus* (600 mg kg^{-1}, p.o.), at a dose which consistently produced antinociception, did not cause any significant effect in the performance of the animals (control value of 60 s against 60 s for treated animals, n = 7).

Discussion

In this study, we have investigated the antinociceptive effect of the hydroalcoholic extract of *S. verticillatus* on chemical and thermal models of nociception. We also used selective antagonists of several mediators involved in the nociception in

Table 8. Effect of morphine and the hydroalcoholic extract of *S. verticillatus* in the hot-plate and tail-flick tests in mice.

Drug	Dose (mg kg^{-1})	Latency (s)	
		Tail-flick	Hot-plate
Control	0	5·7 ± 0·6	5·8 ± 0·4
Morphine	10	19·4 ± 0·6*	23·9 ± 1·5*
S. verticillatus	300	6·1 ± 0·4	6·5 ± 0·5

Each group represents the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.01$, significant when compared with control value.

an attempt to provide some insight into the mechanisms involved in its antinociceptive properties. Our results show that the active principle(s) present in the stems and leaves of *S. verticillatus*, given either intraperitoneally or orally, produce dose-related and long-lasting (up to 8 h) antinociception in the mouse when assessed in inflammatory and neurogenic models of nociception, i.e. against acetic acid-, formalin- (both phases) and capsaicin-induced pain. When assessed in two algesiometric tests, the tail flick and hot-plate assays, however, the hydroalcoholic extract of *S. verticillatus* did not result in antinociception. When tested against two models of neurogenic pain in the formalin and capsaicin assays, the hydroalcoholic extract of *S. verticillatus* produced significant antinociception when administered either intraperitoneally or orally, being only approximately 2- to 4-fold less potent than when given orally. Interestingly, when compared with some standard non-steroidal anti-inflammatory drugs such as aspirin, indomethacin and paracetamol, among others, the antinociceptive action of the extract seems to be relevant when it is taken into consideration that these drugs are largely ineffective in preventing neurogenic nociception in both the formalin (early phase) and capsaicin models (Santos et al 1995b; Corrêa et al 1996; Vaz et al 1996). When compared with the second phase of the formalin response, the extract, given orally, was about 2- to 5-fold less potent at the ID50 level when compared with some non-steroidal anti-inflammatory drugs such as aspirin, paracetamol and indomethacin, but its duration of action was significantly greater than that of these drugs (Corrêa et al 1996; Vaz et al 1996).

The mechanisms by which the hydroalcoholic extract of *S. verticillatus* produced antinociception in the three models of nociception studied are not completely understood. Our results show, however, that the extract acts partially by interaction with the opioid system, because the non-selective opioid antagonist naloxone, at a dose that consistently reversed morphine-induced nociception, partially (but significantly) attenuated extract-induced antinociception when assessed against acetic acid-induced pain. Curiously, the extract, at the same dose, was virtually unable to cause antinociception in

Table 7. Effect of adrenalectomy on the antinociception induced by the hydroalcoholic extract of *S. verticillatus* against formalin-induced pain.

Drug	Dose (mg kg^{-1})	Amount of licking (s)			
		0–5 min Operated	Sham-operated	15–30 min Operated	Sham-operated
Control	0	52·8 ± 2·0	59·7 ± 2·2	170·6 ± 23·0	183·7 ± 26·1
S. verticillatus	300	56·6 ± 5·2*	42·6 ± 6·0	114·0 ± 16·9**	16·2 ± 7·0

Each group represents the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.05$, **$P < 0.01$, significant when compared with sham-operated

two opioid-sensitive algesiometer models, i.e. the tail-flick and hot-plate assays. The reason for these discrepancies remains unclear and was not investigated further in this study. The antinociception effect of the hydroalcoholic extract of *S. verticillatus* seems, furthermore, not to be associated with its anti-oedematogenic properties, because it failed to interfere with the paw oedema associated with the later phase of the formalin response, a test demonstrated to be very sensitive to the action of non-steroidal anti-inflammatory drugs (Corrêa & Calixto 1993; Gorski et al 1993; Cechinel Filho et al 1996).

The nitric oxide-L-arginine pathway seems, however, to be involved in the antinociception effect of the hydroalcoholic extract of *S. verticillatus*, because previous treatment of the animals with L-arginine, a precursor of nitric oxide biosynthesis, at a dose which has been previously demonstrated to reverse L-NOARG- (a nitric oxide inhibitor) or morphine-induced antinociception (Kawabata et al 1993; Moore et al 1993; Przewlocka et al 1994; Santos et al 1995b, Vaz et al 1996 and this study), partially but significantly reversed the antinociception resulting from the extract of *S. verticillatus* when assessed against the late phase in the formalin test. The antinociceptive action of the hydroalcoholic extract appears, furthermore, to be modulated partly by the adrenal glands, because bilateral adrenalectomy performed two weeks before the experiments significantly attenuated the antinociceptive action of the hydroalcoholic extract when assessed against the late phase of the formalin test. The results obtained in the rota-rod test, on the other hand, clearly show that the antinociception resulting from the active principle(s) present in the extract is unlikely to be associated with non-specific central or peripheral motor dysfunction, because the dose that resulted in consistent antinocipetion failed to interfere with motor coordination of animals.

The active principle(s) responsible for the antinociceptive effects of the hydroalcoholic extract of *S. verticillatus* is not known, but much of the action could be related with the presence of the novel alkaloid, *cis*-8,10-di-*n*-propyllobelidiol hydrochloride dehydrate, recently isolated and identified by X-ray crystallography (Miguel et al 1996). Preliminary pharmacological studies revealed that this alkaloid resulted in oral and intraperitoneal antinociception. Pharmacological, biochemical and chemical studies are in progress in order to characterize the mechanism(s) responsible for the antinociceptive action of this alkaloid and also to isolate other active principles present in this plant.

Acknowledgements

The authors are indebted to Dr Gerdt Hatschbach for botanical classification of *S. verticillatus*. This study was supported by grants from Financiadora de Estudos e Projetos (FINEP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), Brazil. A. P. Trentin is an undergraduate medical student receiving a grant from CNPq. A. R. S. Santos and O. G. Miguel are supported by grants from CNPq, Brazil.

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