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Antinociceptive activity of a hydroalcoholic extract obtained from aerial parts of *Sebastiania schottiana* (Euphorbiaceae)

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This study analyzed the antinociceptive effects of a hydroalcoholic extract obtained from the aerial parts of *Sebastiania schottiana,* a Brazilian medicinal plant used to treat various painful diseases. For this purpose, the writhing test, capsaicin and formalin induced-pain in mice were used. The results showed that the hydroalcoholic extract exhibited considerable antinociception in all the models studied, being more potent than aspirin.

1. Introdution

The genus *Sebastiania* consists of a group of plants which are used in several parts of the Brazil and South America to treat different diseases, especially as a remedy for kidney bladder calculi and dolorous process [1,2]. Biological studies have confirmed that these plants produce active principles which exert antispasmodic, fungicidal and fungiostatic [3, 4] activities. *S. schottiana* (Euphorbiaceae) grows abundantly in the South of Brazil, being known as "sarandi negro", "branquicho" or "branquilho"[5]. No reports have been found regarding antinociceptive properties of extracts and compounds of the aerial parts, although we have previously shown the presence of triterpenes with this activity from roots [6].

The aim of our research group is to discover naturally occurring active constituents and in recent years many plants which grow in our region have been investigated. Some of them have shown promising pharmacological results *in vitro* or *in vivo* [7–12]. As part of our continuing research in this field and based on the scarcity of studies about its pharmacological properties, we initially prepared a hydroalcoholic extract from the aerial parts of *S. schottiana*, and evaluate it as antinociceptive in mice.

2. Investigations, results and discussion

Considering that a hydroalcoholic extract of the aerial parts of this plant inhibit acetic acid induced abdominal constrictions in mice we studied its activity in two other models using formalin and capsaicin. Aspirin as reference drug was included for comparison.

In the writhing test, the hydroalcoholic extract, when given intraperitoneally, caused graded and dose-dependent inhibition of abdominal constrictions, with ID_{50} (mg/kg) of 6.3 (5.4–7.3). It was about 3 times more potent than aspirin a standard clinically used drug (Table l). Orally, this extract also exhibited activity, but to a lesser extent, with a maximal inhibition of 39% at 400 mg/kg. When analysed against formalin induced pain, a useful technique for obtaining neurogenic and inflammation continuous pain [13–15], the hydroalcoholic extract, administred intraperitoneally, displayed significant and dose-related antinociceptive effects against both phases of pain. However, it was more pronounced in the second phase with ID_{50} (mg/kg) of 5.8 (4.4–7.8), being 4 times more potent than aspirin (Table 2). Given orally, the hydroalcoholic extract showed just weak activity against the first phase of the formalin induced-pain, but considerably inhibited the inflammatory phase, with ID_{50} (mg/kg) of 132.8 (106–165.4). It was about 2 times more potent than the reference drug (Table 2). When tested on capsaicin induced-pain [16], a model which provides evidence of the anti-

Table 1: Effect of the hydroalcoholic extract of *S. schottiana* given either intraperitoneally or orally, against acetic acid-induced abdominal constriction in mice

HE	Dose (mg/kg)	Number of abdominal constrictions		
		intraperitoneally	Dose (mg/kg)	orally
	0	44.3 ± 2.1	0	47.2 ± 4.1
	3	27.5 ± 3.4**	50	44.3 ± 3.5
	10	11.5 ± 4.9**	100	31.7 ± 3.3**
	30	8.3 ± 2.1**	200	30.57 ± 4.9**
	–	–	400	29.0 ± 5.0**
[a] ID_{50}		6.3 (5.4–7.3)		
Maximal inhibition (%)		81.0 ± 5.0		39.0 ± 11.0
Aspirin ([a] ID_{50})		24 (13.1–43.8)	50–300	109 (93–127)

Each group represents mean ± sem of 8 to 10 animals. **$p < 0.01$ when compared with respective control values (ANOVA). [a] (mg/kg) with 95% confidence limits

Table 2: Effect of hydroalcoholic extract of *S. schottiana* given either intraperitoneally or orally against the first phase (0–5 min) or against the second phase (15–30 min) in the formalin test in mice

HE	Dose (mg/kg)	Licking (s)	
		0–5 min	15–30 min
Intraperitoneally	0	65.7 ± 5.1	171.0 ± 21.1
	3	47.4 ± 5.6*	104.4 ± 14.1*
	10	36.0 ± 2.6**	42.9 ± 10.4**
	30	41.0 ± 4.6**	12.2 ± 7.6**
[a] ID_{50}		ND	5.8 (4.4–7.8)
Maximal Inhibition (%)		37.0 ± 7.0	93.0 ± 5.0
Aspirin (ID_{50})		inactive	18.1 (13.6–24.3)
Oral	0	64.3 ± 2.5	129.0 ± 7.4
	50	59.4 ± 3.6	122.4 ± 10.5
	100	55.1 ± 3.6	81.1 ± 14.9*
	200	57.4 ± 4.3	33.0 ± 6.8**
	400	50.5 ± 4.5	49.2 ± 3.4**
[a] ID_{50}		ND	132.8 (106–165.4)
Maximal Inhibition (%)		21.0 ± 7.0	74.0 ± 5.0
Aspirin ([a] ID_{50})		inactive	282 (243–328)

Each group represents mean ± sem of 8 to 10 animals. *$p < 0.05$, **$p < 0.01$ when compared with respective control values (ANOVA). [a] (mg/kg) with 95% confidence limits. ND: not determined

Table 3: Effect of the hydroalcoholic extract of *S. schottiana* given either intraperitoneally or orally, on capsaicin-induced neurogenic pain in mice

HE	Dose (mg/kg)	Licking (s)		
		Intraperitoneal	Dose (mg/kg)	Oral
	0	42.6 ± 1.8	0	43.4 ± 3.8
	3	34.7 ± 5.0	25	35.1 ± 1.5
	10	21.8 ± 2.6**	50	31.2 ± 3.3*
	30	9.7 ± 2.7**	100	29.8 ± 1.7**
	100	6.2 ± 2.6**	–	–
[a] ID_{50}		13.3 (11.4–15.6)		ND
Maximum Inhibition (%)		85.0 ± 6.0		31.0 ± 4.0

Each group represents mean ± sem of 8 to 10 animals. *$p < 0.05$; **$p < 0.01$ when compared with respective control values (ANOVA). [a] (mg/kg) with 95% confidence limits. ND: Not determined

nociceptive effect on neurogenic pain, this extract also showed strong activity when administered intraperitoneally, with ID_{50} (mg/kg) of 13.3 (11.4–15.6), but to a lesser extent than when given orally (ID_{50} > 100 mg/kg (Table 3)).

These results suggest that *S. schottiana* produces different constituents which may interfere with some mediators involved in both neurogenic and inflammatory pain responses. They seem to be poorly absorbed through the gastrointestinal tract. Further investigations are necessary to determine the mechanism of the antinociceptive effects of this extract and of the components of this plant.

Since we previously showed that two triterpenes from the roots of this plant, moretenone and glutinol, exhibited antinociceptive effects in mice [6], we evaluated using TLC whether these compounds are present in the aerial parts of *S. schottiana*. The results indicated that both compounds are also produced by the aerial parts of the plant. For this reason, we partitioned the hydroalcoholic extract to obtain three distinct fractions: hexane, ethyl acetate, and butanol. They were then analysed in writhing test at 10 mg/kg, intraperitoneally, causing inhibitions of 75 ± 6, 59 ± 7 and 95 ± 2%, respectively. This suggests that other polar components, besides triterpenes previously detected in this plant [6], exert antinociceptive effects in mice.

In summary, the results reported in this study show that the hydroalcoholic extract obtained from the aerial parts of *S. schottiana* exhibits pronounced antinociceptive effects in the experimental models used. Our studies confirm the antinociceptive activity of *S. schottiana* and support, at least partially, its use in folk medicine for the treatment of dolorous processes. Phytochemical and pharmacological investigations are currently being carried out to determine other active compound(s) present in this part of the plant as well as to elucidate the mechanism(s) of antinociceptive action.

3. Experimental

3.1. Plant material

Leaves and stems of *S. schottiana* were collected in Apiúna in the State of Santa Catarina, Brazil, in September 1997 and classified by Dr. Ademir Reis (Departamento de Botânica, Universidade Federal de Santa Catarina). A voucher was deposited in the Herbarium FLOR (UFSC) under number 5397.

3.2. Extract preparation

Dried aerial parts of *S. schottiana* (1.6 kg) were powdered and macerated with EtOH/H_2O (50/50) at room temperature for seven days. The extract was then concentrated under reduced pressure to the desired volume and stored at −20 °C. The resulting extract was dissolved in 0.9% NaCl solution to the desired concentration just before use.

3.3. Pharmacological analysis

3.3.1. Writhing test in mice

Abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%) was carried out according to the procedures described previously [9, 13] with minor modifications. Animals were pretreated with the extracts (3–30 mg/kg) or orally (50–400 mg/kg) 30 or 60 min before the acetic acid injection. Control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.). All experiments were carried out at 23 ± 2 °C. After the challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions of the abdominal muscles together with stretching, were cumulatively counted over a period of 20 mim. Antinociceptive activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pretreated with extracts studied.

3.3.2. Formalin-induced pain test

The procedure used was essentially similar to that previously described [14, 15]. The animals were acclimatized to the laboratory for at least 24 h. Animals from the same strain were slightly anesthetized with diethyl ether, except when used to analyze the first phase of formalin-induced pain, and 20 µl of 2.5% of 0.92% formaldehyde made up of PBS (phosphate buffered solution, containing NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM), was injected subcutaneally under the plantar surface of the left hindpaw using a Hamilton syringe. The animals were treated with normal saline (10 ml/kg, i.p.) or with hydroalcoholic extract (3–30 mg/kg, i.p.) or (40–400 mg/kg, v.o.) obtained from *S. schottiana* 60 min before formalin injection. After intraplantar irritant application, the animals were immediately placed into a glass cylinder (20 cm diameter). The time spent by animals licking or biting the injected paw was timed with a chronometer and was considered indicative of pain. Two mice (control and treated) were simultaneously observed from 0 up to 30 mins following formalin injection. The initial nociceptive scores normally peaked 5 min after (first phase, representing the neurogenic pain), and 15–30 min after formalin injection (second phase, representing the inflammatory pain).

3.3.3. Capsaicin-induced pain test

The procedure used was similar to that described previously [16]. The animals were placed individually in transparent glass cylinders. Following the adaptation period, 20 µl of capsaicin (1.6 µg/paw) was injected under the skin of the plantar surface of the right hindpaw, using a microsyringe. The animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were treated with the hydroalcoholic extract (10 mg/kg, i. p.) or saline (10 ml/kg, i.p.) or (25–100 mg/kg, v.o.), 1 hour before administration of capsaicin. Control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.).

3.4. Statistical analysis

The results are presented as mean ± s.e.m, except the mean ID_{50} values (i.e., the dose of drugs or compounds reducing the antinociceptive responses by 50% relative to control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance among groups was first analyzed by ANOVA, followed by Dunnett′s multiple comparison test to check significance between groups. P-values of less than 0.05 were considered significant. The ID_{50} values were determined by graphical interpolation from individual experiments.

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