J. Pharm. Pharmacol. 1997, 49: 823–827
Received December 9, 1996
Accepted April 7, 1997

Antinociceptive Effect of the Hydroalcoholic Extract of *Bauhinia splendens* Stems in Mice

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Abstract

The analgesic effect of the hydroalcoholic extract of the stems of *Bauhinia splendens* (Leguminosae) has been investigated in chemical and thermal models of nociception in mice.

The hydroalcoholic extract of *B. splendens*, 3–60 mg kg^{-1} intraperitoneally or 50–400 mg kg^{-1} orally, caused dose-related, and long-lasting (up to 3 h) inhibition of acetic acid-induced abdominal constriction in mice, with ID50 values of 3·2 and 177·6 mg kg^{-1} and maximum inhibition of 95 ± 2 and $61 \pm 6\%$, respectively. In the formalin test, the extract given intraperitoneally (1–60 mg kg^{-1}) or orally (50-400 mg kg^{-1}) caused graded inhibition of both phases of formalin-induced pain, being about 5- to 6-fold more potent in attenuating the second phase of pain. The calculated mean ID50 values for the first and the second phases were 11·5 and 2·5 mg kg^{-1}, respectively, for intraperitoneal administration and > 200 and 70 mg kg^{-1}, respectively, for oral administration; the percentages of maximum inhibition for the first and the second phases were 68 ± 6 and 99 ± 1, respectively, for intraperitoneal administration and 37 ± 6 and 69 ± 9, respectively, for oral administration. However, at the same doses the extract did not significantly affect the oedematogenic response induced by formalin. The treatment of animals with naloxone (5 mg kg^{-1}, i.p.) completely reversed the analgesic effect caused by morphine (5 mg kg^{-1}, s.c.), but had no effect against the antinociceptive effect of the hydroalcoholic extract of *B. splendens* (60 mg kg^{-1}, i.p.) when assessed against acetic acid-induced abdominal constrictions. Furthermore, the extract, in contrast with morphine, had no analgesic effect in the hot-plate test.

These data show that the hydroalcoholic extract of *B. splendens* has significant analgesic action when assessed against several models of pain. The mechanism underlying its analgesic effect still remains unknown, but seems to be unrelated to interaction with opioid systems.

Bauhinia splendens (Leguminosae) is a native plant widely-distributed in Brazil, being popularly known as 'cipó escada', 'cipó unha de boi', 'escada de jaboti' or 'escada de macaco'. Its leaves and stem bark have been used as traditional remedies in folk medicine for the management of several diseases, e.g. infections, inflammatory processes, diabetes and infections of the urinary tract, among others (Pio Correia 1984; Cirilo 1993). Pharmacological pre-clinical studies have confirmed that some plants belonging to genus *Bauhinia* exert significant effects against diabetes (Miyake et al 1986) and against fungi (Maillard et al 1991); these studies have extended our knowledge of these species. Phytochemical studies of this genus have demonstrated the presence of steroidal glycosides, triterpenes, lactones and flavonoids (Gupta et al 1980; Okwute et al 1986; Achembach et al 1988; Iribarren & Pomilio 1989).

Laux et al (1985), reported the isolation of β-sitosterol, stigmasterol, stearic acid and a new flavone, denoted bausplendin, from *B. splendens* bark.

Previous studies conducted by our group have demonstrated that some extracts obtained from different parts of this plant have antibacterial activity (Savi et al 1997). In addition, we have demonstrated that these extracts contain flavonoids and tannins which have antinociceptive action against the acetic acid-induced abdominal constrictions in mice (Cechinel Filho et al 1995).

In this study we have extended our previous findings, evaluating the antinociceptive action of the hydroalcoholic extract obtained from stems of *B. splendens* on chemical and thermal models of nociception in mice.

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Materials and Methods

Preparation of the crude extract

Botanical material was collected in Urussanga, State of Santa Catarina, Brazil, in June 1995, and classified by Professor Leila da Graça Amaral (UFSC). A voucher specimen was deposited in the Flor Herbarium (Department of Botany, Federal University of Santa Catarina). The stems of *Bauhinia splendens* were minced and extracted with 50% ethanol-water in the proportion 1:3 (w/v) while being 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 −20°C. The extracts were dissolved in 0·9% NaCl (saline) solution at the desired concentration just before use.

Drugs

Acetic acid, formalin and morphine hydrochloride were from Merck (Darmstadt, Germany) and naloxone hydrochloride from Dupont (Garden City, USA). All reagents were of a high grade of purity. All drugs and extracts were dissolved in saline or in phosphate-buffered solution just before use.

Animals

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, were

used for all of the experiments. Animals were acclimatized in the laboratory for at least 1 h before testing and were used once throughout the experiments. 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 dilute acetic acid

The response to intraperitoneal injection of acetic acid (0·6%), contraction of the abdominal muscle and stretching of the hind limbs, was performed according to procedures described previously (Santos et al 1994). Animals were pre-treated with the hydroalcoholic extract intraperitoneally (3–60 mg kg^{-1}) or orally (50–400 mg kg^{-1}) 30 or 60 min before injection of the acetic acid. 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 over a period of 20 min was counted. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions between control animals (saline pre-treated) and mice pre-treated with hydroalcoholic extract.

In an attempt to investigate the participation of the opioid system on the analgesic effect of the extract, separate groups of mice were treated with naloxone (5 mg kg^{-1}, i.p.) 15 min before administration of the extract (60 mg kg^{-1}, i.p.) or with morphine (5 mg kg^{-1}, s.c.), which was used as positive control.

Formalin-induced pain

The procedure was similar to that described previously (Santos et al 1994, 1995). Formalin (0·92% formaldehyde) diluted 40-fold with phosphate buffer was injected (20 μL) under the surface of the right hind paw by use of a microsyringe with a 26-gauge needle. Two mice (control and treated) were observed simultaneously for 0 to 30 min after formalin 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 (first phase) and 15–30 min after formalin injection (second phase), representing the tonic and inflammatory pain responses respectively (Hunskaar & Hole 1987). Animals were treated with the hydroalcoholic extract of *B. splendens* intraperitoneally (1–60 mg kg^{-1}) or orally (50–400 mg kg^{-1}), or with saline (10 mL kg^{-1}), 30 or 60 min 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 was timed with a chronometer and 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 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 cut at the knee joint and weighed on an analytical balance.

Hot-plate test

The hot-plate test was used to measure response latencies according to the method described by Eddy & Leimback (1953), with minor modifications. In these experiments the hot-plate was maintained at 56 ± 1°C. Animals were placed in 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 (positive control) or pre-treated with the hydroalcoholic extract of *B. splendens*. Animals were selected 24 h previously on the basis of their reactivity in the test. A latency period of 30 s was defined as complete analysis. Animals were treated with the hydroalcoholic extract (up to 100 mg kg^{-1}, i.p.) or with morphine (10 mg kg^{-1}, s.c.) 30 min before experiments. Control animals received the same volume of vehicle (10 mL kg^{-1}).

Statistical analysis

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

Results

Intraperitoneal injection of animals with the hydroalcoholic extract of *B. splendens* (3–60 mg kg^{-1}, i.p.) caused dose-related inhibition of the acetic acid-induced abdominal constriction response in mice (Table 1). The mean ID50 (and the 95% confidence limit) was 3·2 (2·0–5·0) mg kg^{-1} and maximum inhibition was 95·0 ± 2·0%. Given orally (50–400 mg kg^{-1}), the extract also caused significant inhibition of acetic acid-induced abdominal constrictions; the ID50 was 177·6 (129·4–243·8) mg kg^{-1} and maximum inhibition was 61·0 ± 6·0%. However, it was about 5- to 60-fold less potent than when given intraperitoneally (Table 1). The antinociceptive profile of the hydroalcoholic extract given intraperitoneally or orally was long-lasting (3 to 4 h) against acetic acid-induced abdominal constriction (Table 2).

In the formalin test, the hydroalcoholic extract of *B. splendens* (1–60 mg kg^{-1}, i.p.) caused marked and dose-related inhibition of both phases of formalin-induced persistent pain (Table 3); the analgesic effect was more pronounced against the second phase of the pain model. The ID50 values for the first and second phases were 11·5 (8·3–15·9) and 2·5 (1·6–3·8) mg kg^{-1}, respectively, and maximum inhibition was 68·0 ± 6·0 and 99·0 ± 1·2%, respectively (Table 3). Given orally (50–400 mg kg^{-1}), the extract resulted in dose-related inhibition of the response to both phases of formalin-induced nociception, but was approximately 50- to 60-fold less potent than when given intraperitoneally. However, the hydroalcoholic extract failed to inhibit the oedematogenic response associated with the second phase of the formalin test in mice (Table 3).

Table 1. Effect of the hydroalcoholic extract of *B. splendens* on acetic acid-induced abdominal constrictions in mice.

	Intraperitoneal dose (mg kg^{-1})	Number of abdominal constrictions	Oral dose (mg kg^{-1})	Number of abdominal constrictions
	0	43·3 ± 2·5	0	55·7 ± 1·6
	3	22·0 ± 1·7**	50	43·7 ± 6·3*
	10	14·4 ± 1·9**	100	33·5 ± 1·4**
	30	4·8 ± 1·4**	200	21·5 ± 3·5**
	60	2·0 ± 0·9**	400	23·7 ± 4·3**
Extract dose reducing pain responses by 50% relative to the control value (mg kg^{-1})		3·2 (2·0–5·0)†		177·6 (129·4–243·8)†
Maximum inhibition (%)		95·0 ± 2·0		61·0 ± 6·0

Each value is the mean ± s.e.m. of results from 8 to 10 animals. *$P < 0.05$, **$P < 0.01$, significantly different compared with the respective control value.† Values in parentheses are the 95% confidence limits.

Table 2. Time-course of the antinociceptive effect of hydroalcoholic extracts of *B. splendens* given either intraperitoneally or orally against acetic acid-induced abdominal constrictions in mice.

Time (h)	Number of abdominal constrictions	
	Intraperitoneal (10 mg kg^{-1})	Oral (100 mg kg^{-1})
0	44·9 ± 2·3	47·2 ± 4·6
0·5	13·6 ± 2·1**	–
1·0	7·7 ± 1·4**	25·0 ± 4·0**
2·0	10·0 ± 2·6**	25·3 ± 3·7**
3·0	–	38·0 ± 5·6*
4·0	24·5 ± 3·4**	52·7 ± 2·2
6·0	43·3 ± 0·9	–

Each result is the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.05$, **$P < 0.01$, significantly different compared with the control value.

Table 4 shows that hydroalcoholic extract administered intraperitoneally (up to 100 mg kg^{-1}) was virtually ineffective against the hot-plate test, under conditions where morphine (10 mg kg^{-1}, s.c.) caused a marked increase in pain latency.

The analgesic effects of morphine (5 mg kg^{-1}), but not those of the hydroalcoholic extract of *B. splendens* (60 mg kg^{-1}), were fully reversed by previous treatment of animals with naloxone (5 mg kg^{-1}, i.p.) when analysed against acetic acid-induced abdominal constriction.

Discussion

Studies by our group have recently demonstrated that some extracts obtained from different parts of *Bauhinia splendens* have a significant antinociceptive effect when tested against acetic acid-induced abdominal constriction in mice (Cechinel

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

	Dose	Licking (s)		Change in paw
	(mg kg^{-1})	0–5 min	15–30 min	weight (mg)
Intraperitoneal				
	0	69·2 ± 6·9	167·8 ± 22·4	67·0 ± 4·6
	1	56·4 ± 1·7	103·9 ± 16·4*	62·1 ± 5·5
	3	34·0 ± 5·3*	39·9 ± 9·2*	56·0 ± 4·7
	10	26·0 ± 2·6*	3·0 ± 2·0*	57·0 ± 6·9
	30	22·3 ± 4·4*	5·8 ± 5·8*	58·1 ± 5·5
	60	22·7 ± 2·6*	–	–
Extract dose reducing pain responses by 50% relative to the control value (mg kg^{-1})		11·5 (8·3 – 15·9)†	2·5 (1·6–3·8)†	
Maximum inhibition (%)		68·0 ± 6·0	99·0 ± 1·0	
Oral				
	0	65·4 ± 5·9	154·5 ± 5·7	62·8 ± 4·0
	50	59 ± 4·9	106·8 ± 6·3*	59·1 ± 7·1
	100	41·3 ± 2·3*	66·8 ± 13·2*	63·0 ± 5·7
	200	41·2 ± 4·2*	47·3 ± 14·4*	57·0 ± 7·0
	400	42·2 ± 2·4*	48·6 ± 17·0*	58·2 ± 6·2
Extract dose reducing pain responses by 50% relative to the control value (mg kg^{-1})		> 400	91·7 (58·2 – 144·6)†	
Maximum inhibition (%)		37·0 ± 6·0	69·0 ± 9·0	

Each value is the mean ± s.e.m. of results from 6 to 14 animals. *$P < 0.01$, significantly different compared with the respective control value. †Values in parentheses are the 95% confidence limits.

Table 4. Effect of morphine and the hydroalcoholic extract of *B. splendens* on the hot-plate test in mice.

Drug	Dose (mg kg^{-1})	Hot-plate latency (s)
Control	0	4·1 ± 0·3
Morphine	10	20·0 ± 1·7*
B. splendens	100	4·2 ± 0·5

Each value is the mean ± s.e.m. of results from 6 to 10 animals. *$P < 0.01$, significantly different compared with the control value.

Filho et al 1995). We have also demonstrated that these extracts have a potent antibacterial effect (Savi et al 1997). In the current study we have confirmed and also extended these initial observations by demonstrating that the hydroalcoholic extract of the stems of *Bauhinia splendens* had a potent and dose-related analgesic effect when given either intraperitoneally or orally to mice. In addition, their analgesic effects were long-lasting and occurred rapidly after their systemic administration. Interestingly, the hydroalcoholic extract was capable of attenuating, in a dose-related fashion, both neurogenic and inflammatory phases of formalin-induced persistent pain. Moreover, these extracts, even in higher doses, failed to inhibit the paw oedema associated with the second phase of the formalin test.

Recent studies have shown that formalin releases several inflammatory mediators (Hunskaar et al 1986; Hunskaar & Hole 1987; Murray et al 1988; Corrêa & Calixto 1993). However, the second (but not the first) phase (neurogenic pain) of the formalin test can be attenuated in a dose-dependent fashion by drugs, such as aspirin, paracetamol or indomethacin, known to inhibit cyclooxygenase activity (Gorski et al 1993; Cechinel Filho et al 1996; Vaz et al 1996). In the second phase of the formalin response, the hydroalcoholic extract given intraperitoneally was approximately 2- to 15-fold more potent at the ID50 level than some non-steroidal anti-inflammatory drugs such as aspirin, paracetamol or indomethacin (Gorski et al 1993; Cechinel Filho et al 1996; Vaz et al 1996). In contrast, the principle present in the hydroalcoholic extract of *B. splendens* was also quite effective at attenuating the neurogenic component of formalin-induced licking. In addition, morphine potently inhibits both phases of formalin-induced pain (Gorski et al 1993; Vaz et al 1996).

The mechanisms underlying the antinociceptive action of the hydroalcoholic extract of *B. splendens* seem to be unrelated to activation of the opioid system. The antinociceptive action of the extract, in contrast with that reported for morphine, was not reversed by naloxone, an opioid antagonist. Secondly, the hydroalcoholic extract was completely devoid of analgesic action when tested against radiant heat in the hot-plate test, under conditions where morphine had a marked analgesic effect.

Although the hot-plate test is commonly used to assess narcotic analgesics, other centrally acting drugs, including sedatives and muscle relaxants or psychotomimetics have shown activity in this test (Woolfe & MacDonald 1944; Eddy & Leimback 1953; Knoll 1967; Vaz et al 1996; this study). However, abdominal constriction elicited by acetic acid has also been used to assess the potential analgesic activity of drugs. Collier et al (1968) postulated that the acetic acid acted indirectly by releasing endogenous mediators that stimulated the nociceptive neurons. It is sensitive to non-steroidal anti-inflammatory drugs and to narcotics and other centrally acting drugs (Collier et al 1968; Miguel et al 1996; Vaz et al 1996).

Chemical studies of *B. splendens* have enabled the isolation and identification of some phenolic compounds, such as quercetin, rutin and gallic acid ethyl ester (Cechinel Filho et al 1995). However, these compounds have already been isolated from other plants. In addition, we have recently reported that such compounds, isolated from plants of the genus *Phyllanthus* (Euphorbiaceae), had a significant antinociceptive effect in mice (Miguel et al 1995, 1996; Cechinel Filho et al 1996). Another interesting aspect of the current study was that β-sitosterol and the new flavone bausplendin present in the bark of *B. splendens* (Laux et al 1985) were not observed in this investigation. This suggests that the plant is affected by different environmental factors.

Pharmacological and chemical studies are continuing to characterize the mechanism(s) responsible for the antinociceptive action, and also to identify other active compounds present in *B. splendens*.

Acknowledgements

The authors are indebted to Mr Emilio Cecconi and Homero De Bona Filho, for collection of *B. splendens*, to Professor Leila da Graça Amaral, for botanical classification, and to Joanésia Maria Junkes Rotnstein of the Central Bioterio of Universidade Federal de Santa Catarina, for donating the animals. This study was supported by grants from ProBIC/Pro-PPEx/UNIVALI and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

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