JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 765–771

Received November 26, 2004
Accepted February 4, 2005
DOI 10.1211/0022357056091
ISSN 0022-3573

Antinociceptive effect of proanthocyanidins from *Croton celtidifolius* bark

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Abstract

The chemical composition of the chromatography 63 subfraction (63SF) from the ethyl acetate soluble fraction of the crude extract of *Croton celtidifolius* bark presented a high content of total proanthocyanidins (75.0 ± 2.3%). HPLC analysis of 63SF revealed a dimeric profile (e.g. catechin-(4α→8)-catechin and gallocatechin-(4α→8)-catechin) and polymeric proanthocyanidins. In pharmacological investigations, 63SF administered intraperitoneally exhibited dose-dependent antinociceptive activity against several chemical stimuli, including the intraperitoneal injection of acetic acid (ID50 (the dose of 63SF which was able to reduce the nociceptive response by 50% relative to the control value) = 0.9 (0.5–1.6)) and the intraplantar injection of capsaicin (ID50 = 13.0 (10.0–17.0)), glutamate (ID50 = 4.0 (2.0–7.0)) and formalin (ID50 first phase = 36.0 (24.0–53.0) and late phase = 11.0 (8.0–14.0)). 63SF administered orally exhibited an antinociceptive effect in the formalin test (ID50 first phase = 125.0 (89.0–177.0) and late phase = 65.0 (33.0–95.0)). In the same test, 63SF was effective when given soon after the first phase, as well as exhibiting therapeutic activity. Furthermore, 63SF was effective in models of thermal nociception including tail-flick and hot-plate tests. When the mice were treated in the neonatal period with capsaicin, the antinociceptive effect of 63SF in the first phase of the formalin test was abolished, but pretreatment with naltrexone did not change the antinociceptive effect of 63SF. Together, these results provide evidence that 63SF exerted a pronounced systemic antinociception against chemical (acetic acid, formalin, glutamate and capsaicin tests) and thermal (hot-plate and tail-flick tests) nociceptive models of pain in mice at a dose that did not interfere with the locomotor activity. The mechanism by which this sub-fraction produced antinociception remains unclear, but it is unlikely to involve the activation of the opioid system. However, unmyelinated C-fibres sensitive to treatment with capsaicin are likely to participate in antinociception caused by 63SF.

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Funding: This study was supported by Conselho Nacional de Pesquisa, CNPq, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brazil.

Introduction

Croton celtidifolius is a native plant of the Atlantic Forest regions of Brazil, being frequently found from the state of Rio de Janeiro to Goiás, São Paulo and throughout the southern region of Brazil. The plant has various popular names, depending on the region where it is found, such as Pau-Sangue, Sangue-de-Dragão, and Sangue-de-Adáve (Smith 1988). Published studies devoted to the chemical characterization of the constituents of *C. celtidifolius* are limited. Mukherjee & Axt (1984) determined the presence of cyclitols, including 1L-1-O-methyl-mio-inositol, neo-inositol and sitosterol. The presence of catechins, gallocatechins and proanthocyanidins was observed in fractions obtained from the hydroalcoholic extract of the bark of *C. celtidifolius* (Nardi et al 2003). In addition, other authors have demonstrated the presence of alkaloids and saponins in the bark of this plant (Farnsworth et al 1969; Barnes et al 1980; Amaral & Barnes 1997). In folk medicine, the plant is recommended for the treatment of leukaemia, ulcers, rheumatism, and inflammatory diseases, either by chewing the bark or preparing an infusion of the bark. However, studies on the biological activity of *C. celtidifolius* are still limited. Research in our laboratory carried out with the hydroalcoholic extract, fractions, sub-fractions and isolated compounds

from the bark of this plant has revealed anti-inflammatory, anti-oedematogenic and antioxidant activity, since it was able to reduce paw oedema and protect deoxyribose and rat liver homogenate from oxidative damage, in addition to inhibiting $O_2^{\bullet -}$-induced nitro blue tetrazolium reduction (Nardi et al 2003). Also in our laboratory, experiments with extracts, fractions and sub-fractions of *C. celtidifolius* have demonstrated pronounced antinociceptive activity of this plant in both phases of formalin-induced pain (unpublished data).

In this study, we have extended our investigation of the chemical composition and antinociceptive properties of 63SF, a sub-fraction that presents the most significant antinociceptive activity, in chemical and thermal models of nociception in mice. We have investigated some possible mechanisms of action of this sub-fraction.

Materials and Methods

Plant material

Bark of *Croton celtidifolius* Baill was collected at Orleans City and a voucher specimen (document number 31272) was identified and lodged at the Botany Department, UFSC, and deposited in the authors' laboratory.

Extraction and fractionation procedures

Air-dried bark (154 g) of *C. celtidifolius* was finely milled and extracted with 80% aqueous EtOH at room temperature (3×250 mL) and the combined extracts were filtered and evaporated in a vacuum to give 42.9 g of the crude extract. The residual extract was suspended in H_2O (500 mL) and washed exhaustively with ether before extraction with ethyl acetate (3×100 mL) and n-butanol (3×100 mL) to give ethyl acetate (17.6 g), n-butanol (22.2 g) and aqueous (6.9 g) soluble fractions.

The active ethyl acetate soluble fraction was fractionated on a water (20%) inactivated silica gel column eluted with hexane/ethyl acetate (4:1) and increasing the polarity by gradual addition of ethyl acetate and methanol. After analysis using thin layer chromatography (TLC), four sub-fractions were obtained, named 11SF, 19SF, 35SF and 63SF (Nardi et al 2003).

Determination of the proanthocyanidin content of 63SF

According to the procedure of Hiermann et al (1986), hydrolysis of the dried sub-fraction 63SF (10 mg) was carried out with 6 mL of a solution of n-butanol-HCl 37% (95:5, v/v) in a 20-mL capacity thick-walled, screw-top vial sealed with a Teflon-lined screw cap. The mixture was heated in a water bath at 95.0 ± 0.3°C for 2 h. After cooling, the mixture was diluted to 100 mL with n-butanol and the cyanidin chloride content was measured at 540 nm. The assay was performed in triplicate.

High-performance liquid chromatography (HPLC-UV) analysis of the 63SF

HPLC-UV analysis was performed using a Shimadzu HPLC system (Model LC-10AD two pumps) autosampler equipped with a photo-diode array detector (Model SPD-M10Avp). Separations were achieved at 40°C on an ODS LC-18 column (250×4.6 mm i.d.), particle size 5 μm. The solvent system was a linear gradient using acetonitrile (pump A) and aqueous 0.3% phosphoric acid (pump B) from 10% A to 20% A in 45 min, then to 60% A in 20 min. The flow rate was 1.0 mL min^{-1}, the detector was set at 280 nm and the injection volume was 10 μL. Solvents used were of HPLC grade and were degassed by sonication before use. The 63SF (10 mg) was dissolved in acetonitrile and filtered through 0.45-μm, 13-mm Teflon syringe tip-filters into an autosampler vial. The proanthocyanidins were identified on the basis of their UV spectra and retention times using the reference compounds catechin-(4$\alpha \rightarrow$8)-catechin and gallocatechin-(4$\alpha \rightarrow$8)-catechin previously isolated and characterized in our laboratory.

Animals

Male Swiss mice (25–35 g) were used in the experiments, housed at 22 ± 2°C under a 12-h light/dark cycle and with free access to food and water. The experiments were performed after the protocol was approved by the Institutional Ethics Committee (n° 157/CEUA). Experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann 1983). In all experiments, the control animals received vehicle only (10 mL kg^{-1}).

Abdominal constriction test

Doses of 63SF (0.3–30 mg kg^{-1}, i.p.) were administered 30 min before the intraperitoneal injection of acetic acid solution (0.6%). The number of abdominal constrictions was counted for 20 min immediately after nociception induction (Koster et al 1959; Choi et al 2003).

Formalin test

Animals received 20 μL 2.5% formalin solution (0.92% formaldehyde) dissolved in phosphate-buffered saline (PBS) injected intraplantarly (i.pl.) in the ventral surface of the right hind paw. The time spent licking/flinching and biting the injected paw was measured and considered as an indication of nociception in the first (0–5 min) and second phases (15–30 min) (Hunskaar et al 1985; Santos et al 2003). Five groups of experiments were performed using this test. Two dose–response curves were constructed, in which the animals received increasing doses (1–100 mg kg^{-1}) intraperitoneally 30 min before the test, and orally (30–300 mg kg^{-1}) 60 min before the test. Also a time course in which the animals received ID50 for the late phase (i.p.) determined previously at 0 to 120 min before the test. In the third group, mice were injected with formalin, and received 63SF

(11 mg kg^{-1}, i.p.) immediately after the first phase for assessment against the second phase of nociception.

To investigate the participation of the opioid system in the antinociceptive effect of 63SF, animals were treated with naltrexone (a non-selective opioid antagonist, 1 mg kg^{-1}, i.p.) 15 min before the administration of 63SF (11 mg kg^{-1}, i.p.), morphine (5 mg kg^{-1}, i.p.) or vehicle and 30 min later were submitted to the formalin test. The other groups of animals received only 63SF, morphine or naltrexone 30 min before the formalin injection.

Capsaicin test

Animals were pretreated with 63SF (3–100 mg kg^{-1}, i.p.), administered 30 min before the injection of 20 μL capsaicin (1.6 μg/paw in PBS) intraplantarly and were observed for 5 min following capsaicin injection. The time spent licking/flinching and biting the injected paw was measured and considered as an indication of nociception (Santos & Calixto 1997).

Glutamate test

Animals were pretreated with 63SF (3–100 mg kg^{-1}, i.p.), administered 30 min before the injection of 20 μL of a solution containing 10 μmol glutamate in the ventral surface of the right hind paw and were observed for 15 min following glutamate injection. The time spent licking/flicking and biting the injected paw was measured and considered as an indication of nociception (Beirith et al 2002).

Tail-flick test

The methodology was the same as that described by D'amour & Smith (1941), with minor modifications. The intensities of radiant heat were adjusted so that the animal flicked its tail within 5 to 10 s. The reaction time was recorded before the administration of 63SF (100 or 300 mg kg^{-1}, i.p.) and 30, 60, 90, and 120 min after the treatment. An automatic 20 s cut-off was used to minimize tissue damage. Animals had been selected 24 h previously on the basis of their reactivity in the test, and those animals that were insensitive or too sensitive were excluded from this experiment.

Hot-plate test

The hot-plate (Ugo Basile, model-DS 37) was maintained at 56 ± 1°C. Animals were placed on the heated surface, and the time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency (Eddy & Leimbach 1953). The reaction time was recorded 30 min after administration of 63SF (100 or 300 mg kg^{-1}, i.p.). An automatic 30 s cut-off was used to minimize tissue damage. Animals had been selected 24 h previously on the basis of their reactivity in the test and those animals that were insensitive or too sensitive were excluded from this experiment.

Neonatal capsaicin

New-born mice were treated subcutaneously with 50 mg kg^{-1} capsaicin on the second day of life. Control animals received, by the same route, a similar volume of vehicle used to dissolve the capsaicin (Beirith et al 2003). Two months after the neonatal capsaicin treatment, the animals were treated with 63SF (11 mg kg^{-1}, i.p.) and 30 min later were submitted to the formalin test.

Rota-rod test

Animals were treated with 63SF (11 or 110 mg kg^{-1}, i.p.) or the same volume of PBS (10 mL kg^{-1}, i.p.), 30 min before being tested. The cut-off time used was 60 s (Rosland et al 1990). The animals had been selected 24 h previously by eliminating those mice that did not remain on the bar for 60 s.

Drugs

Formalin and acetic acid were from Nuclear (São Paulo, Brazil). Capsaicin, glutamate, morphine, naltrexone, PBS (NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM), and dimethyl sulfoxide were from Sigma Chemical Co. (St Louis, MO). All drugs and fraction were dissolved in PBS, except capsaicin which was dissolved in PBS containing 1% dimethyl sulfoxide.

Statistical analysis

The results were expressed as mean ± s.e.m. Statistically significant differences between groups were measured using one-way analysis of variance followed by Dunnett's test. $^{*}P < 0.05$ or $^{**}P < 0.01$ was considered statistically significant. The geometric mean ID50 values (the dose of 63SF which was able to reduce the nociceptive response by 50% relative to the control value), accompanied by their respective 95% confidence limits, were determined by non-linear regression from individual experiments using Graph Pad Prism Software.

Results

Characterization and quantification of 63SF

The total proanthocyanidin content of 63SF was 73.0 ± 2.3%. HPLC-UV analysis of the 63SF showed the presence of catechin-(4α→8)-catechin and gallocatechin-(4α→8)-catechin, identified by comparison with authentic samples in terms of retention time and UV spectra (recorded on line by photodiode array detection). The similar chromatography profiles obtained at 280 nm and 220 nm showed a peak at approximately 60 min retention time due to the co-eluted polymeric polyphenols, and separated low molecular weight constituents including dimeric proanthocyanidins.

Pharmacological assays

As shown in Figure 1, 63SF (0.3–30 mg kg^{-1}, i.p.) administered 30 min before, reduced the abdominal constrictions induced by acetic acid in a dose-dependent manner. Similarly, pretreatment of animals with 63SF (1–100 mg kg^{-1}, i.p., 30 min) produced marked and dose-related inhibition of both phases of formalin-induced

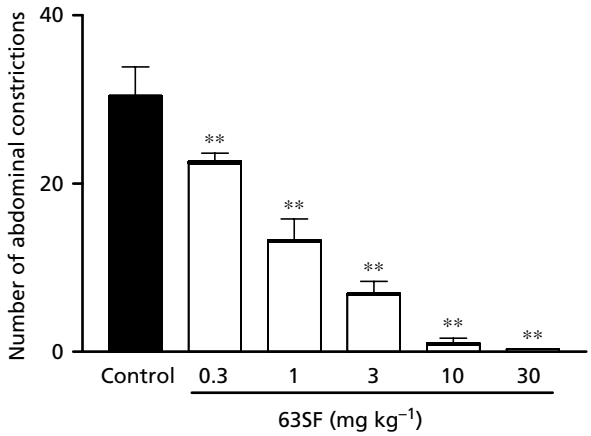

Figure 1 Effects of 63SF (0.3–30 mg kg^{-1}) given intraperitoneally 30 min before the administration of acetic acid to induce abdominal constriction. Each column represents mean ± s.e.m. of six to ten animals. Control values indicate the animals injected with the vehicle and asterisks denote significant differences when compared with control group (**$P < 0.01$).

nociception (Table 1), which was more pronounced against the second phase. Comparable results were obtained in the formalin test when the animals were treated by the oral route. However, doses that exhibited the same effect were four- to sixfold higher (Table 1). The time-course revealed that the 63SF, given intraperitoneally, was effective until 90 min after the treatment, beyond which time the fraction lost its effect in the formalin test (data not shown). In the formalin test, 63SF was effective when given soon after the first phase, inhibiting formalin-induced nociception by 85% in the late phase (control value 201.5 ± 17.02 s). In the capsaicin test, the pretreatment with 63SF gave a dose-related inhibition of nociception, as shown in Figure 2A. Similar results were found in the glutamate test where 63SF (3–100 mg kg^{-1}, i.p.) reduced the nociception in a dose-dependent manner also (Figure 2B). The ID50 values and maximal inhibition of chemical models of nociception induction are given in Table 2 together with their respective confidence intervals. The values obtained demonstrated the potent antinociceptive effect of 63SF in chemical models of pain induction. The inhibition of tail-flick (Figure 3A) and hot-plate paw licking responses (Figure 3B) was expressed as 'percent maximal possible effect (% MPE)', which was calculated as $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 were tail-flick and hot-plate paw-licking latencies before and after the injection of 63SF or vehicle. The cut-off time (T_2) was set at 20 and 30 s for the tail-flick and hot-plate tests, respectively. In the tail-flick test only the highest dose (300 mg kg^{-1}, i.p.) was effective in increasing the latency at all times of pretreatment. 63SF increased the latency in the hot-plate test at both doses tested. When the animals received capsaicin in the neonatal period (second day of life), 63SF lost its antinociceptive effect in the first phase of the formalin test when evaluated in the adult period (Figure 4A), but this effect persisted in the late phase of the same test (Figure 4B). In the same test, the pretreatment of animals with naltrexone, 15 min before injection of morphine, largely reversed the antinociception caused by morphine when analysed against both phases of the formalin test, leaving the antinociceptive effect of 63SF (11 mg kg^{-1}, i.p.) unaffected (results not shown). In addition, the 63SF (11 and 110 mg kg^{-1}, i.p.) had no significant effect in the rota-rod test, dismissing possible unspecific effects. The control response in the rota-rod test was 50.5 ± 9.5 s vs 34.5 ± 11.5 and 34.3 ± 7.4 s in the presence of 11 and 110 mg kg^{-1} of 63SF, respectively (n = 7).

Discussion

We have demonstrated that 63SF possesses a high concentration of proanthocyanidins. Many medicinal plants used for a range of ailments and disorders contain proanthocyanidins as their active principles. De Bruyne et al (1999) reviewed the biological activities of proanthocyanidins, which include antimicrobial, antiviral, enzyme inhibition, antimutagenicity, antitumoral, anti-inflammatory, antioxidant, anti-ulcer, and antidiarrhoeal activities, besides modulation of the cardiovascular system. The activity of these compounds may be explained by some general characteristics of proanthocyanidins. These include: complex formation with metal ions, antioxidant and free radical scavenging and the ability to complex with other molecules, including macro-molecules such as

Table 1 Effects of 63SF (3–100 mg kg^{-1} for i.p. route and 30–300 mg kg^{-1} for p.o. route), against the formalin test in the first (1st) and late (2nd) phase given intraperitoneally and orally in formalin-induced nociception

Route	Phase	Control	Dose (mg kg^{-1})				
			3	10	30	100	300
Intraperitoneal	1st	70.0 ± 3.1	65.8 ± 2.4	54.1 ± 2.8**	37.1 ± 4.5**	11.5 ± 2.2**	–
	2nd	209.1 ± 13.2	208.3 ± 15.7	107.4 ± 12.2**	46.5 ± 16.9**	2.0 ± 0.0**	–
Oral	1st	58.5 ± 2.7	–	–	49.0 ± 3.2*	31.1 ± 1.2**	20.3 ± 1.1**
	2nd	186.0 ± 10.5	–	–	139.5 ± 4.3**	58.6 ± 4.6**	10.0 ± 1.5**

Values represent mean ± s.e.m. of reactivity (s) of six to ten animals. Control values indicate the animals injected with the vehicle and the asterisks denote significant differences when compared with control group (*$P < 0.05$; **$P < 0.01$).

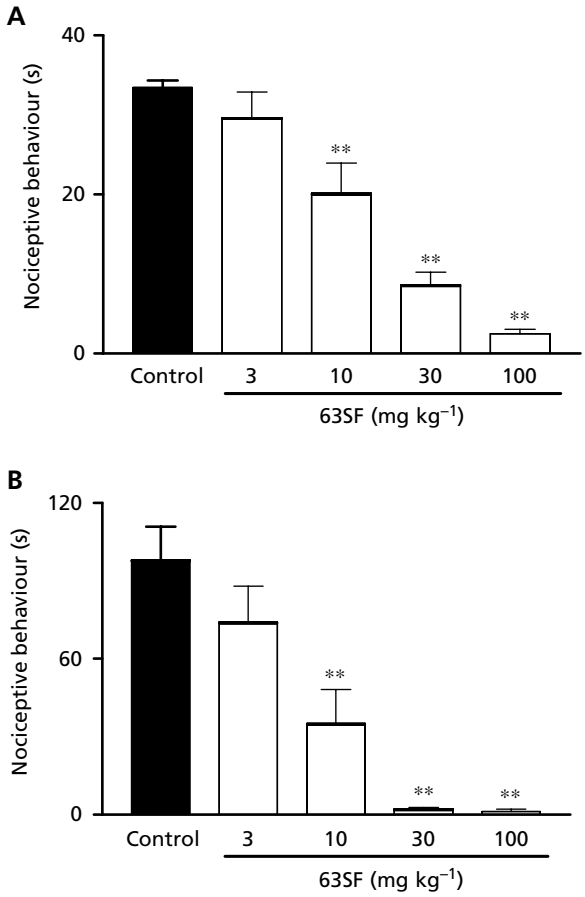

Figure 2 Effects of 63SF (3–100 mg kg^{-1}) given intraperitoneally 30 min before the administration of capsaicin (A) or glutamate (B). Each column represents mean ± s.e.m. of six to ten animals. Control values indicate the animals injected with the vehicle and the asterisks denote significant differences when compared with control group (**$P < 0.01$).

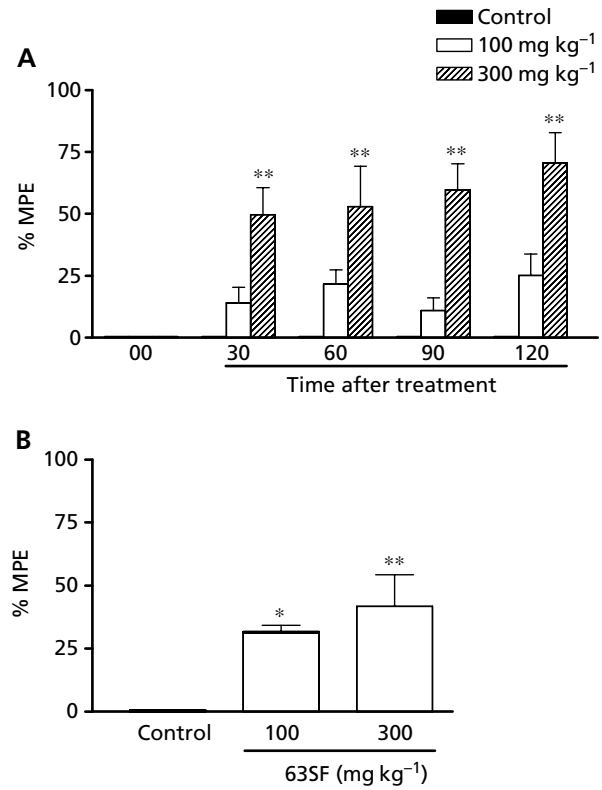

Figure 3 Effects of 63SF (100 or 300 mg kg^{-1}) given intraperitoneally, in the tail-flick (A) and hot-plate (B) tests. In the tail-flick test, the responses were measured before and at 0, 30, 60, 90, and 120 min after the treatment, and in the hot-plate test the responses were measured before and 30 min after the treatment. Control values indicate the animals injected with the vehicle. Each column denotes the mean ± s.e.m. of six to ten animals. *$P < 0.05$, **$P < 0.01$ compared with the baseline.

Table 2 Mean ID50 and maximal inhibition (MI) (%) values obtained for 63SF in different models of chemical nociception

Model	ID50[a] mg kg^{-1}	MI[a] (%)
Abdominal constriction test	0.9 (0.5–1.6)	100 (100–100)
Formalin test (1st phase, i.p.)	36.0 (24.4–53.0)	83 (74.3–93.8)
Formalin test (2nd phase, i.p.)	11.2 (8.8–14.3)	99.5 (99.0–100)
Formalin test (1st phase, p.o.)	125.8 (89.5–177.0)	65 (58.6–71.6)
Formalin test (2nd phase, p.o.)	65.98 (33.3–95.6)	94 (89.7–98.9)
Capsaicin test	13.1 (10.1–17.0)	92 (87.3–96.9)
Glutamate test	4.1 (2.3–7.8)	97.5 (96.6–100.0)

[a]With their respective confidence limits (CL 95%).

proteins and polysaccharides (Santos & Mello 1999). It was assumed that these abilities in some way underlie the biological actions of these compounds.

In pharmacological assays, 63SF extracted from *C. celtidifolius* bark administered systemically to mice produced dose-related antinociception when assessed in chemical models of nociception. Interestingly, 63SF given intraperitoneally caused a significant increase in the latency in hot-plate and tail-flick tests. Also, the antinociception caused by 63SF was unlikely to be secondary to its non-specific muscle relaxant, specific and/or non-specific depressant central effects as revealed by the lack of any detectable result in the rota-rod test (Rosland et al 1990).

Injection of acetic acid produced the peritoneal inflammation that caused a response characterized by contraction of the abdominal muscles accompanying an extension of the forelimbs and elongation of the body (Koster et al 1959; Choi et al 2003). In this study, we clearly showed the antinociceptive effect of 63SF in acetic acid-induced abdominal constriction. Moreover, in the formalin test, 63SF had a dose-related antinociceptive effect in both phases. It is widely agreed that the nociceptive behaviour manifested during the acute first phase is caused by a

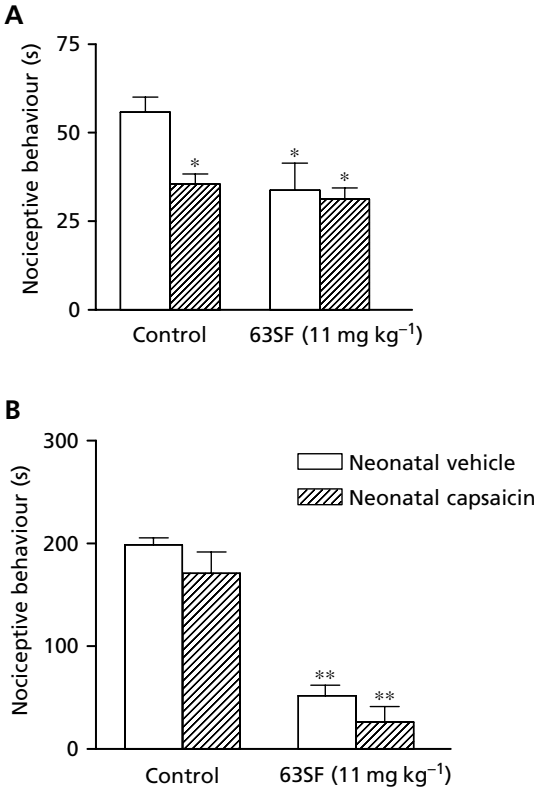

Figure 4 Influence of neonatal treatment of mice with vehicle (1 mL kg^{-1}) or capsaicin (50 mg kg^{-1}, s.c.), on the first (A) and second phase (B) of 63SF (11 mg kg^{-1}, i.p.)-induced antinociception in the formalin test. Each column represents the mean ± s.e.m. of six to ten animals. *$P < 0.05$, **$P < 0.01$ significantly different from the animals neonatally treated with vehicle.

direct effect on peripheral nociceptors, thereby activating the primary afferent fibre. This is followed by the tonic second phase, which represents the inflammatory nociceptive response (Hunskaar & Hole 1987; Choi et al 2001, 2003). In this study, 63SF was significantly more efficacious against the second phase. Moreover, the marked inhibition caused by 63SF when administered after the formalin injection confirmed a prophylactic and a therapeutic effect (Scheidt et al 2002). This may have additional implications for the development of a new phytomedicine to treat inflammatory pain. Another interesting characteristic of 63SF was its marked and dose-related inhibition of the neurogenic pain caused by capsaicin, a substance regarded as a valuable pharmacological tool for studying a subset of mammalian primary sensory C-fibres and Aδ afferent neurons including polymodal nociceptors and warm thermoceptors (Jancso 1992; Holzer 1996). Our results showed that 63SF produced dose-related inhibition of the nociceptive response induced by glutamate. Recently it was reported that the nociceptive response to glutamate appeared to involve peripheral, spinal and supra-spinal sites of action and was largely mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors as well as by the release of nitric oxide or by some nitric oxide-released substance (Beirith et al 2002). In addition, it has been demonstrated that the nociception produced by formalin (first phase), capsaicin and glutamate is quite resistant to the great majority of non-steroidal anti-inflammatory drugs, while it is sensitive to dipyrone, opioid drugs such as morphine and drugs that antagonize substance P or glutamate receptors (Shibata et al 1989; Sakurada et al 1992, 1993). Thus, somehow 63SF could have been playing a role as an antagonist of the vanilloid, kininergic, or glutamatergic receptors. However, this hypothesis could not be assured with these results. It would be necessary to acquire new data from additional experiments.

The tail-flick is a spinal reflex that persists after section or cold block of upper parts of the spinal cord. However, it is possible that the tail-flick is not a purely spinal reflex but is rather more complicated and involves higher neural structures. It might, for example, be mediated by a spinal-bulbo-spinal circuit (King et al 1997). The hot plate, at a constant temperature, produces two behavioural components that can be measured in terms of their reaction times, namely paw licking and jumping, both of which are considered to be supraspinally-integrated responses (Chapman et al 1985; Le Bars et al 2001). Our results demonstrated that the tail-flick and hot-plate response latencies were prolonged by the fraction, indicating an increase of nociceptive threshold. From a pharmacological point of view, there is a consensus that these tests are efficient for revealing the activity of opioid analgesics (Le Bars et al 2001). However, in our study the mechanism underlying the antinociceptive actions of 63SF appeared to be unrelated to activation of the opioid system, since the antinociception caused by 63SF, in contrast to that reported for morphine, was not reversed by naltrexone, a non-selective opioid antagonist.

To investigate some possible mechanisms responsible for the antinociceptive activity of the 63SF, the formalin test was used. In this experiment, the dose of 63SF that was able to reduce the nociceptive response by 50% relative to the control value (ID50) in the late phase of nociception (11 mg kg^{-1} by i.p. route) was used. To evaluate the role played by capsaicin-sensitive C-fibres in the antinociception induced by 63SF, mice were treated in the neonatal period with capsaicin, a neurotoxin known to irreversibly delete approximately 90% of C-fibres and 40% of Aδ-fibres (Khasar & Levine 1996). This treatment also causes the depletion of neuropeptides including substance P and calcitonin gene-related peptide (Buck & Burks 1986; Hua et al 1997) leading to a reduction in the sensitivity of the animals to potentially harmful stimuli (Buck & Burks 1986; Khasar & Levine 1996), including formalin (Hua et al 1997). The results showed a change in the response of these animals to the nociceptive stimulation of the formalin test, in agreement with other reports in the literature. Moreover, it could be seen that the antinociceptive activity of the 63SF in the first phase of formalin-induced nociception was modified by the neonatal treatment of the animals with capsaicin, suggesting that the 63SF acted, at least in part, through mechanisms that involved capsaicin-sensitive C-fibres.

Conclusions

The 63SF obtained from *C. celtidifolius* bark exerted a pronounced antinociceptive effect when assessed in chemical and thermal models of nociception in mice. The involvement of capsaicin-sensitive C fibres was clearly seen in the first phase of formalin-induced nociception, but the opioid system did not appear to participate in the antinociception. These effects appeared to be due to the presence of proanthocyanidins in this fraction and, although the mechanism underlying their action is incompletely understood, it would appear to be a non-opioid effect.

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