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Analysis of the antinociceptive effect of the proanthocyanidin-rich fraction obtained from *Croton celtidifolius* barks: Evidence for a role of the dopaminergic system

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Abstract

In a previous study, we demonstrated the antinociceptive effect of 63SF, a proanthocyanidin-rich fraction obtained from *Croton celtidifolius* barks, in chemical and thermal behavioural models of pain in mice. The current study now investigate the possible mechanisms underlying the antinociceptive activity of 63SF in the formalin test, by using drugs which interfere with systems that are implicated in descending control of nociception. The antinociceptive effect of 63SF (11 mg/kg, i.p., given 30 min prior to 2.5% formalin) was not altered by pre-treatment of animals 45–50 min beforehand with either prazosin (α_1-adrenergic antagonist; 0.15 mg/kg, i.p.), yohimbine (α_2-adrenergic antagonist; 0.15 mg/kg, i.p.), ketanserin (5-HT_{2A}-receptor antagonist; 1.0 mg/kg, i.p.), or L-arginine (substrate for NO synthase, 600 mg/kg, i.p.). On the other hand, treatment with sulpiride, an antagonist of dopaminergic D_2-receptors (1.0 mg/kg, i.p., 45 min of pre-treatment), reversed the antinociceptive activity of 63SF. Pre-treatment of animals with reserpine (5 mg/kg, i.p., 24 h beforehand) did not alter the antinociceptive effect of 63SF. The current results support the view that the 63SF exerts antinociceptive effects by enhancing the activity of descending control, possibly by direct stimulation of dopaminergic D_2 receptors.

Keywords: *Croton celtidifolius*; Antinociception; Proanthocyanidins; Formalin test; Dopaminergic receptors

1. Introduction

Croton celtidifolius (Euphorbiaceae), known under various popular names, such as "Pau-Sangue", "Sangue-de-dragão", "Sangue-de-Adáve", is a tree which occurs in regions of the Atlantic Forest, being frequently found in the Southern region of Brazil (Smith et al., 1988). In folk medicine, its bark is either chewed or taken as an infusion for the treatment of inflammatory and ulcerative diseases.

Chemical and pharmacological studies on this tree remain limited. Some authors have demonstrated the presence of cyclitols, including 1L-1-*O*-methyl-mio-inositol, neo-inositol and sitosterol (Mukherjee and Axt, 1984). In addition, the presence of catechins, gallocatechins and proanthocyanidin have been detected in fractions obtained from the hydroalcoholic extract of the barks of the *C. celtidifolius* (Nardi et al., 2003). Other researchers have identified the presence of alkaloids and saponins in the barks of this plant (Farnsworth et al., 1969; Barnes et al., 1980; Amaral and Barnes, 1997). Some biological activities of *C. celtidifolius* have been described, including antiedematogenic and antioxidant activity (Nardi et al., 2003), anti-inflammatory effects in the pleurisy model and modulation of superoxide dismutase enzyme activity (Nardi et al., 2006). The proanthocyanidin-rich fraction 63SF, obtained from the barks of *C. celtidifolius*, has also been shown

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to display opioid independent antinociceptive effect in several chemical and thermal models of nociception. This fraction contains a rich mixture (75%) of different dimeric profile (e.g. catechin-(4α→8)-catechin and gallocatechin-(4α→8)-catechin) and polymeric proanthocyanidins (DalBó et al., 2005). Thus, we have proposed that the observed antinociceptive action is due to the proanthocyanidin content of the fraction (DalBo et al., 2005).

In the current study, we have extended on our previous findings by investigating in greater detail the mechanisms that might be involved in the antinociceptive action of the proanthocyanidin-rich 63SF fraction obtained from *C. celtidifolius* barks.

2. Material and methods

2.1. Plant material

Bark of *C. celtidifolius* Baill. was collected from the forest surrounding the city of Orleans (State of Santa Catartina, Brazil) and a voucher specimen (document number 31272) was identified and deposited both at the Department of Botany, UFSC, and also in the author's laboratory.

2.2. 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 under 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 thin layer chromatography (TLC) analysis, four sub-fractions were obtained and named 11SF, 19SF, 35SF and 63SF (Nardi et al., 2003). The chemical composition of the chromatography 63SF of the *C. celtidifolius* bark presented a high content of total proanthocyanidins (75±2%). Furthermore, HPLC analysis of 63SF revealed a dimeric profile (e.g. catechin-(4α→8)-catechin and gallocatechin-(4α→8)-catechin) and polymeric proanthocyanidins (DalBo et al., 2005). However, all these attempts have failed to isolate one single active compound present in the fraction (63SF). For this reason, it was decided to carry on the study using the fraction, instead of an isolated compound.

2.3. Animals

Male Swiss mice (25–35 g) were used in the experiments, housed at 22±2 °C under a 12 h light/12 h dark cycle and with free access to food and water. The experiments were performed after approval of the protocol by the Institutional Ethics Committee (no. 157/CEUA) and 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). The number of animals and intensity of the noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.4. Formalin test

To address some of the mechanisms by which 63SF inhibits formalin-induced nociception, animals were treated with different drugs given via various routes of administration. The choice of the doses of each drug was based on previous data in the literature or on preliminary experiments carried out in our laboratory (data not shown). The formalin test was chosen for this purpose because of the specificity and sensitivity in nociception transmission that this model provides (Le Bars et al., 2001).

The procedure used was essentially the same as that previously described by Hunskaar et al. (1985) with minor modifications. A 20 μL aliquot of a 2.5% formalin solution (0.92% formaldehyde), made up in PBS, was injected intraplantarly (i.pl.) in the right hind paw of the animal. Following the formalin injection, animals were placed in an acrylic observation chamber, and the time spent licking the injected paw was measured continuously during the observation period with a stopwatch and considered as a quantitative indication of nociception. The first

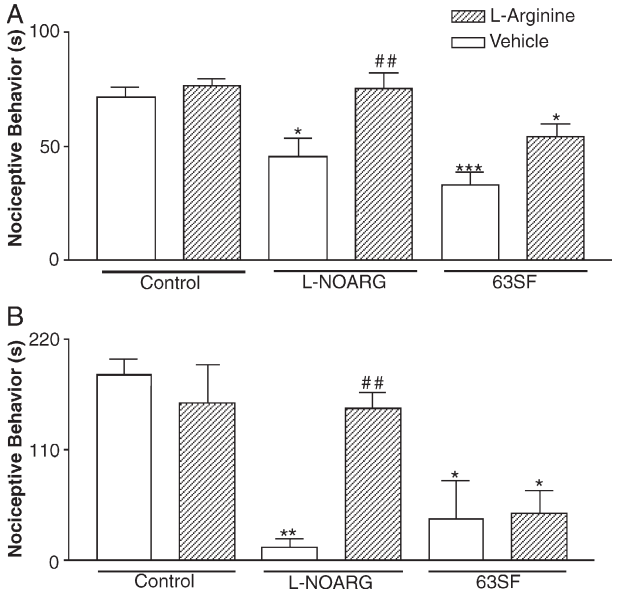

Fig. 1. Influence of L-ARG (600 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or L-NOARG (75 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean±S.E.M. of the reactivity time of 6–10 animals per group. *$p<0.05$; **$p<0.01$ and ***$p<0.001$ represent the significance of differences between treated groups and control group (vehicle only), while $^{\#\#}p<0.01$ represents the significance of differences between groups treated with L-NOARG in the absence and presence of L-ARG.

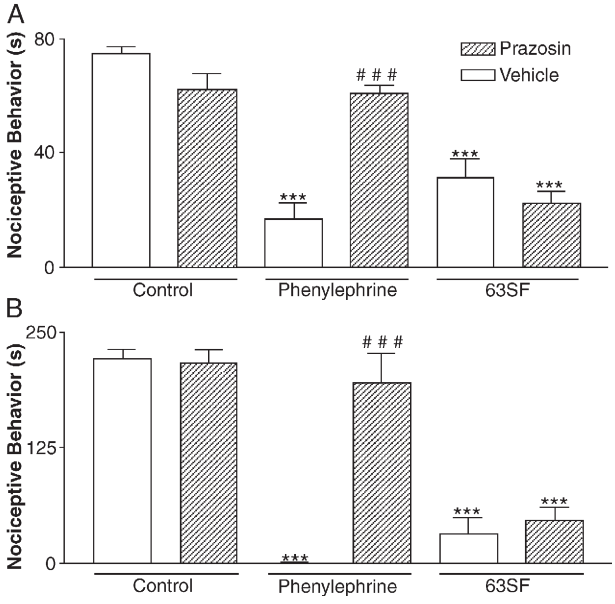

Fig. 2. Influence of prazosin (0.15 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or phenylephrine (10 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean±S.E.M. of the reactivity time of 6–10 animals per group. ***$p<0.001$ represents the significance of difference between treated groups and control group (vehicle only), while $^{\#\#\#}p<0.001$ represents the significance of differences between groups treated with phenylephrine in the absence and presence of prazosin.

phase of the nociceptive response normally peaks between 0 and 5 min and the second phase from 15 to 30 min after formalin injection, which represents the direct effect of formalin on nociceptors and the inflammatory nociceptive responses, respectively (Hunskaar and Hole, 1987; Tjølsen et al., 1992; Choi et al., 2003). The 63SF was administered at the dose that reduces the nociceptive response in the second phase of formalin-induced nociception by 50%, relative to the control value, as described by DalBo et al. (2005).

2.5. Participation of the nitric oxide system

In order to investigate the role played by the L-arginine/nitric oxide pathway in the antinociception caused by 63SF in the formalin test, mice were pre-treated with L-arginine (L-ARG; 600 mg/kg, i.p., the precursor of nitric oxide) or vehicle and 15 min later received 63SF (11 mg/kg, i.p.), N^G-nitro-L-arginine (L-NOARG, 75 mg/kg, i.p., a nitric oxide synthase inhibitor) or vehicle, 30 min before the formalin test (Vaz et al., 1996; Beirith et al., 1998).

2.6. Participation of the adrenergic system

To evaluate the possible participation of the α_1-adrenergic receptors in the antinociceptive effect of 63SF, different groups of animals were pre-treated with either prazosin (an α_1-adrenoceptor antagonist, 0.15 mg/kg, i.p.) or vehicle (10 mL/kg, i.p.) and then, 15 min later, received either 63SF (11 mg/kg, i.p.), phenylephrine (an α_1-adrenoceptor agonist, 10 mg/kg, i.p.), or vehicle, 30 min before formalin injection. In another set of experiments aimed at investigating the role of the α_2-adrenergic receptors in the antinociceptive effect of 63SF, different groups of animals were pre-treated with either yohimbine (an α_2-adrenoceptor antagonist, 0.15 mg/kg, i.p.) or vehicle (10 mL/kg, i.p.) and then, 15 min later, received either 63SF (11 mg/kg, i.p.), clonidine (an α_2-adrenoceptor agonist, 0.1 mg/kg, i.p.), or vehicle, 30 min before formalin injection (Santos et al., 1995).

2.7. Participation of serotonergic system

To explore the possible participation of the serotonergic system in the antinociceptive action of 63SF, mice were pre-treated with either ketanserin (a 5-HT_{2A}-receptor antagonist, 1.0 mg/kg, i.p.) or vehicle and 20 min later were injected with either 63SF (11 mg/kg, i.p.), DOI (a selective 5-HT_{2A}-receptor agonist, 1.0 mg/kg, i.p.), or vehicle 30 min before formalin injection (Kurihara et al., 2003).

2.8. Participation of dopaminergic system

We next investigated the possible participation of the dopaminergic system in the antinociceptive action of 63SF. Mice were pre-treated with sulpiride (a D_2-dopaminergic receptor antagonist, 1.0 mg/kg, i.p.) or vehicle and after 15 min the animals received either 63SF (11 mg/kg, i.p.), apomorphine (a non-selective dopaminergic receptor agonist, 5 mg/kg, i.p.), or vehicle, 30 min before formalin injection

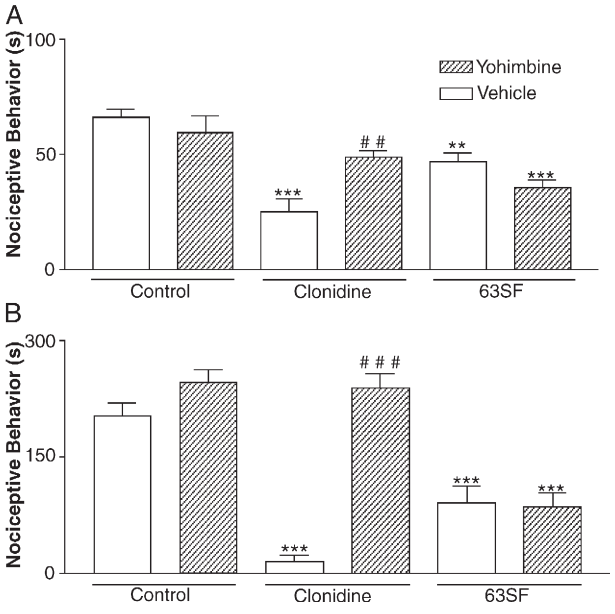

Fig. 3. Influence of yohimbine (0.15 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or clonidine (0.1 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean±S.E.M. of the reactivity time of 6–10 animals per group. **$p<0.05$ and ***$p<0.001$ represent the significance of differences between treated groups and control group (vehicle only), while $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ represent the significance of differences between groups treated with clonidine in the absence and presence of yohimbine.

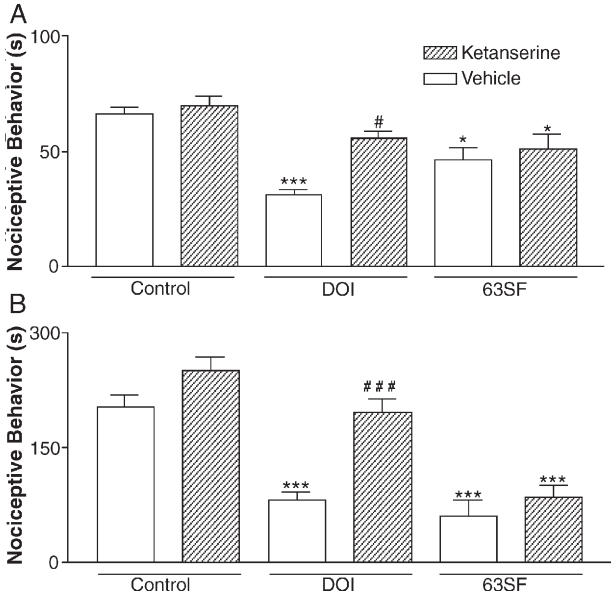

Fig. 4. Influence of ketanserin (1 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or DOI (1 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean±S.E.M. of the reactivity time of 6–10 animals per group. *$p<0.05$ and ***$p<0.001$ represent the significance of differences between treated groups and control group (vehicle only), while $^{\#}p<0.05$ and $^{\#\#\#}p<0.001$ represent the significance of differences between groups treated with DOI in the absence and presence of ketanserin.

(Michael-Titus et al., 1990; Zarrindast and Moghaddampour, 1991).

2.9. Participation of biogenic amines

Finally, we assessed the possible effect of reserpine on the antinociceptive action of 63SF in the formalin test. For this purpose, mice were pre-treated with reserpine (5 mg/kg, i.p., a catecholamine depleter), or vehicle and 24 h later received an injection of either 63SF (11 mg/kg, i.p.), clomipramine (10 mg/kg, i.p., a monoamines reuptake inhibitor) or vehicle, 30 min before formalin injection (Ochi et al., 2002; Giovannoni et al., 2003).

2.10. Drugs

The following substances were used: formaldehyde, dimethylformamide (Nuclear, São Paulo, Brazil), clomipramine hydrochloride, clonidine hydrochloride, apomorphine hydrochloride, ketanserin, L-arginine (L-ARG), N^G-nitro-L-arginine (L-NOARG), phenylephrine hydrochloride, prazosin hydrochloride, reserpine, R-(−)-DOI (R-[−]-2,5-Dimethoxy-4-iodoamphetamine), yohimbine tartarate, sulpiride hydrochloride, PBS (NaCl 137 mM, KCl 2.7 mM and phosphate buffer 10 mM) (Sigma Chemical Co., St. Louis, MO, USA), and ascorbic acid (Merck AG, Darmstadt, Germany). All drugs and 63SF were dissolved in PBS, except reserpine (dissolved in PBS containing 2% of ascorbic acid) and prazosin (dissolved in PBS containing 0.2% of dimethylformamide solution). The doses mentioned refer to the salt form.

2.11. Statistical analysis

The results were expressed as mean±standard error of the mean (S.E.M.). Statistical comparisons between groups were carried out using two-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test. Differences with p-values less than 0.05 ($p<0.05$) were considered statistically significant.

3. Results

The results presented in Fig. 1 show that the pre-treatment of mice with the nitric oxide precursor L-ARG (600 mg/kg, i.p.), given 15 min earlier, fully prevented the antinociception caused by L-NOARG (a nitric oxide synthase enzyme inhibitor, 75 mg/kg, i.p.), when analysed against both phases of the formalin test. However, under the same conditions, L-ARG did not significantly modify the antinociception caused by 63SF in the formalin test (Fig. 1). ANOVA revealed an extremely significant difference between groups in both phases [$F(5,25)=10.745$ (first phase) and $F(5,27)=6.86$ (second phase), $p<0.0005$].

The treatment of mice with prazosin (α_1-selective antagonist, 0.15 mg/kg, i.p.) or yohimbine (α_2-selective antagonist,

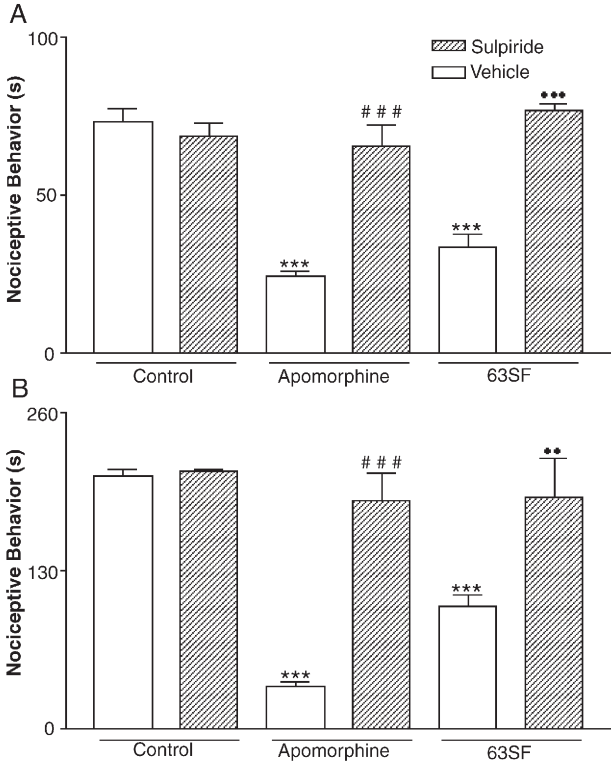

Fig. 5. Influence of sulpiride (5 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or apomorphine (5 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean±S.E.M. of the reactivity time of 6–10 animals per group. ***$p<0.001$ represents the significance of differences between treated groups and control group (vehicle only), while $^{\#\#\#}p<0.001$ represents the significance of differences between groups treated with apomorphine in the absence and presence of sulpiride, and ●●$p<0.01$ and ●●●$p<0.001$ represent the significance of differences between groups treated with 63SF in the absence and presence of sulpiride.

0.15 mg/kg, i.p.), 15 min beforehand, significantly reversed the antinociception caused by phenylephrine (α_1-selective agonist, 10 mg/kg, i.p.) or clonidine (α_2-selective agonist, 0.1 mg/kg, i.p.) respectively, but did not significantly change the antinociception caused by 63SF in both phases of the formalin test (Figs. 2 and 3). ANOVA revealed a highly significant difference between groups in both phases for α_1 [$F(5,39)=24.637$ (first phase) and $F(5,39)=33.410$ (second phase), $p<0.0001$] and α_2 [$F(5,43)=11.418$ (first phase) and $F(5,43)=25.706$ (second phase), $p<0.0001$] selective antagonists.

The results depicted in Fig. 4 show that ketanserin (1.0 mg/kg, i.p.), given 15 min beforehand, completely reversed the antinociception caused by DOI (1.0 mg/kg, i.p.) against formalin-induced licking, but did not significantly change the antinociception caused by 63SF in both phases of the formalin test. ANOVA revealed an extremely significant difference between groups in both phases [$F(5,39)=7.214$ (first phase) and $F(5,39)=22.081$ (second phase), $p<0.0001$].

The treatment of animals with sulpiride (5 mg/kg, i.p.), given 15 min before, completely reversed the antinociception caused by apomorphine (5 mg/kg, i.p.) against formalin-induced licking (Fig. 5). Under the same conditions, sulpiride treatment significantly antagonised the antinociceptive action of the 63SF in the formalin test (Fig. 5). ANOVA revealed a highly significant difference between groups in both phases [$F(5,34)=26.333$ (first phase) and $F(5,34)=17.816$ (second phase), $p<0.0001$].

Finally, Fig. 6 shows that the pre-treatment of animals with reserpine (5 mg/kg, i.p.), 24 h beforehand, caused a marked increase in nociceptive responsiveness to formalin during the second, but not the first phase of the test when compared to control group. This pre-treatment also abolished the antinociceptive effect of clomipramine (10 mg/kg, i.p.) against the first phase and attenuated that observed during the second phase of the response to formalin. Under the same conditions, reserpine did not significantly modify the magnitude of antinociception caused by 63SF in the formalin test. ANOVA revealed a high significant difference between groups in both phases [$F(5,33)=14.180$ (first phase); $F(5,33)=47.440$ (second phase), $p<0.0001$].

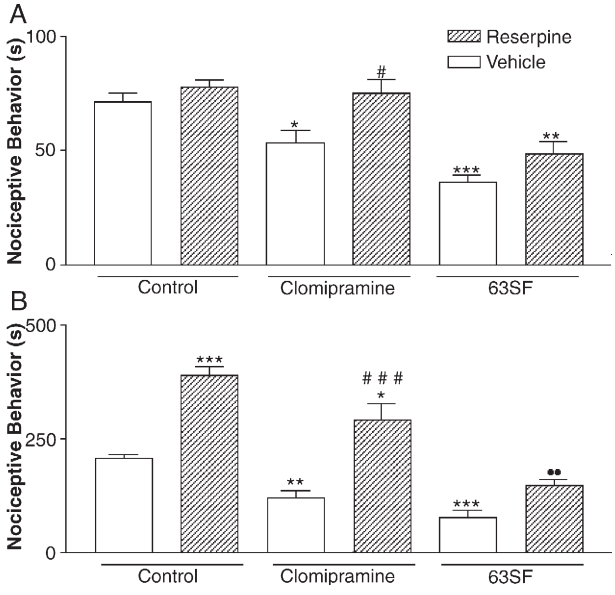

Fig. 6. Influence of reserpine (5 mg/kg, i.p.) pre-treatment on the antinociception caused by 63SF (11 mg/kg, i.p.) or clomipramine (10 mg/kg, i.p.) in first (A) and second (B) phases of the formalin test. Each column represents mean ± S.E.M. of the reactivity time of 6–10 animals per group. $*p<0.05$; $**p<0.01$ and $***p<0.001$ represent the significance of differences between treated groups and control group (vehicle only), while $^{\#}p<0.05$ and $^{\#\#\#}p<0.001$ represent the significance of differences between groups treated with clomipramine in the absence and presence of reserpine, and $\bullet\bullet p<0.01$ represents the significance of differences between groups treated with 63SF in the absence and presence of reserpine.

4. Discussion

In the previous studies, we described the antinociceptive effect of 63SF, a proanthocyanidins-rich fraction obtained from barks of *C. celtidifolius*, in several chemical and thermal behavioural models of pain, and in the first phase of the formalin test, the results demonstrated an involvement of capsaicin-sensitive C-fibres. In some experiments, we found that the opioid system is not involved in the antinociceptive effect of *C. celtidifolius* (DalBo et al., 2005) and we propose that the antinociceptive action of 63SF may be due to the presence in the fraction of these compounds (DalBo et al., 2005). In this study, we extended our previous findings by investigating in greater detail the underlying mechanisms of the antinociceptive action of 63SF.

According to the description by Melzack (1999), in the spinal cord the nociceptive information coming from gut, skin and other organs is submitted to a modulation by a great variety of transmitters that will filter and modulate the transmission of nociceptive impulses to the brain (Besson, 1999; Fürst, 1999; Millan, 2002). These modulating substances are able to act as pro- (descending facilitation) or antinociceptive (descending inhibition), depending on diverse factors, such as the type and intensity of the stimulation, the central region activated, receptor type, and others (Millan, 2002). The neurons projected by the central areas responsible for the control of the perception of pain (descending facilitation and descending inhibition) contain several transmitters, including noradrenaline, serotonin (5-HT), acetylcholine, γ-hydroxy-butyric acid (GABA), nitric oxide (NO), glutamate, dopamine, and others (Fürst, 1999; Millan, 2002).

In this context, we investigated the participation of the L-arginine/nitric oxide pathway in the antinociceptive effect of 63SF. However, the pre-treatment of animals with L-arginine, a nitric oxide precursor, was not able to reverse the antinociception produced by 63SF, suggesting that the L-arginine/nitric oxide pathway does not participate in effect of the fraction. We also investigated the possible involvement of the two most widely studied descending inhibitory pathways, noradrenergic and serotonergic, in the antinociceptive effect of 63SF. The pre-treatment of animals with prazosin, an α_1-adrenoreceptor antagonist, reversed the antinociceptive effect induced by phenylephrine. Similar to these results, yohimbine (an α_2-adrenoreceptor antagonist) was able to reverse the antinociceptive effect of clonidine in the formalin test. However, both pre-treatments were ineffective in reversing the antinociceptive action of 63SF, when evaluated in the formalin test. Similarly,

pre-treatment of animals with ketanserin did not promote any change in the antinociceptive effect of 63SF, but it was efficient in reversing the antinociception caused by DOI, an agonist of 5-HT_{2A}-receptors. These results suggest that 5-HT_{2A}-receptors, as well as α- and α_2-adrenergic receptors do not participate in the antinociceptive effect of 63SF.

Nevertheless, sulpiride, an antagonist of dopaminergic D_2-receptors, reversed the antinociceptive effect provoked by intraperitoneal administration of 63SF and apomorphine in the formalin test. This result discloses a participation of D_2-receptors in the antinociceptive activity of 63SF. Diverse studies have demonstrated that dopamine exerts an important function in nociception control in several models of chronic (Jaaskelainen et al., 2001; Hagelberg et al., 2003) and acute pain (Jensen and Yaksh, 1984; Michael-Titus et al., 1990; Morgan and Franklin, 1991; Zarrindast et al., 1999). When a harmful stimulation occurs, there is an increase in dopamine "turnover" in the dorsal horn of the spinal cord, suggesting an increase in the activity of descending dopaminergic pathways (Millan, 2002). Moreover, the mesolimbic, mesocortical and nigrostriatal dopaminergic pathways are involved in nociception inhibition at the supraspinal level. They are responsible for the central antinociceptive action and for modulating the dopaminergic descending controls, acting on its D_2-receptors. Stimulation of D_2-receptors leads, via $G_{i/o}$, to the inhibition of adenylyl cyclase. Activation of D_2-receptors also suppresses and potentiates Ca^{2+}- and K^+-currents, respectively, promoting a reduction in neuronal excitability (Missale et al., 1998). This supports the idea that the substances present in 63SF may have act by supraspinal level, in agreement with our previous results in the hot plate test (DalBo et al., 2005) in which only supraspinally-acting substances are able to increase the latency of the animals against the thermal stimulation (Le Bars et al., 2001). As reported previously (Gonzales-Rios et al., 1986), apomorphine induced a dose-dependent increase in the jump latency of mice in the hot plate test. Administration of the dopamine D_2-receptor selective agonist RU24926 resulted in a similar analgesia, which was reversed by sulpiride (Euvrard et al., 1980). These data suggest that dopamine D_2-receptors are involved in the control of nociception in the hot plate test, in agreement with our results.

In order to investigate whether the antinociception induced by 63SF is due to a direct activity on the D_2-dopaminergic receptors, animals were pre-treated with reserpine, which provokes a depletion of neuronal monoamine reserves in brain, spinal cord and peripheral nerves (Okubo et al., 1991; Metzger et al., 2002). Animals treated with reserpine only showed an increase in the reactivity in the second phase of formalin-induced nociception. This fact is due to the block by reserpine of the inhibitory control played by the descending pathways, particularly noradrenergic, serotonergic (Giovannoni et al., 2003) and dopaminergic ones (Okubo et al., 1991; Metzger et al., 2002). The same occurred with animals pre-treated with 63SF that had received reserpine. However, when the results of both reserpine-treated and -untreated groups are compared, it can be seen that the antinociception promoted by 63SF in both groups was equivalent. These data suggest that the antinociceptive effect of 63SF may be due to a direct stimulation of D_2-receptors, and not dependent on dopamine reserves.

These results lead us to believe that the proanthocyanidins could be efficient in the treatment of neuropathic pain. Neuropathic pain is caused by lesions or dysfunctions in the nervous system with the primary lesion or dysfunction affecting either the peripheral or central nervous system. Therefore, the scope of neuropathic pain is broader than the classical peripheral and central neuropathic pain conditions. Neuropathic pain conditions are most often chronic in nature and represent a genuine challenge in clinical practice due to their frequency, severity and the limited number of effective treatment options. Some studies have shown that dopaminergic agonists and tricyclic antidepressants, which inhibit the degradation or reuptake of dopamine, are efficient in the treatment of experimental neuropathic pain (Sindrup et al., 2005). Taking into account the results presented in this work and those described previously (DalBo et al., 2005), we believe that these compounds may be effective in providing antinociception in models of neuropathic pain; however, such a possibility requires further investigation.

5. Conclusions

In summary, a previous study (DalBo et al., 2005) demonstrated that the 63SF, a proanthocyanidin-rich fraction obtained from *C. celtidifolius* bark, exerts a pronounced antinociceptive effect in chemical and thermal behavioural models of pain, with the involvement of capsaicin-sensitive C-fibres. In this study, we demonstrated that the treatment with sulpiride, an antagonist of D_2-dopaminergic receptors, reversed the antinociceptive effect of 63SF. However, the pre-treatment of animals with reserpine did not alter this effect, possibly indicating that the latter is due to a direct action of proanthocyanidins on D_2-dopaminergic receptors. Therefore, the study shows that 63SF causes pronounced antinociception that appears to involve a direct action on the dopaminergic pathway.

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