

Involvement of 5-HT₂ receptors in the antinociceptive effect of *Uncaria tomentosa*

Sofia Jürgensen^a, Sílvia DalBó^a, Paul Angers^b, Adair Roberto Soares Santos^c, Rosa Maria Ribeiro-do-Valle^{a,*}

^aDepartamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

^bDepartment of Food Science and Nutrition, Université Laval, Sainte-Foy, Québec, Canada

^cDepartamento de Ciências Fisiológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

Received 24 November 2004; received in revised form 22 March 2005; accepted 15 April 2005

Available online 23 May 2005

Abstract

Uncaria tomentosa (Willd.) DC (Rubiaceae) is a vine that grows in the Amazon rainforest. Its bark decoctions are used by Peruvian Indians to treat several diseases. Chemically, it consists mainly of oxindole alkaloids. An industrial fraction of *U. tomentosa* (UT fraction), containing 95% oxindole alkaloids, was used in this study in order to characterize its antinociceptive activity in chemical (acetic acid-induced abdominal writhing, formalin and capsaicin tests) and thermal (tail-flick and hot-plate tests) models of nociception in mice. UT fraction given by the i.p. route dose-dependently suppressed the behavioural response to the chemical stimuli in the models indicated and increased latencies in the thermal stimuli models. The antinociception caused by UT fraction in the formalin test was significantly attenuated by i.p. treatment of mice with ketanserin (5-HT₂ receptor antagonist), but was not affected by naltrexone (opioid receptor antagonist), atropine (a nonselective muscarinic antagonist), L-arginine (precursor of nitric oxide), prazosin (α₁-adrenoceptor antagonist), yohimbine (α₂-adrenoceptor antagonist), and reserpine (a monoamine depletor). Together, these results indicate that UT fraction produces dose-related antinociception in several models of chemical and thermal pain through mechanisms that involve an interaction with 5-HT₂ receptors.

© 2005 Elsevier Inc. All rights reserved.

Keywords: *Uncaria tomentosa*; Antinociception; 5-HT₂ receptors; Mechanism of action

1. Introduction

Uncaria tomentosa (Willd.) DC (Rubiaceae) is a woody liana, indigenous to the Amazon rainforest. It is commonly known as cat's claw or *uña de gato* due to its morphological aspects. South American Indians have reportedly used decoctions of the barks in folk medicine as a "miraculous" remedy (Keplinger et al., 1999). Its applications include the treatment of arthritis, rheumatism, abscesses, inflammations, fever, allergy, asthma, cancer, gastric ulcer, contraception, menstrual irregularity, recov-

ery from childbirth, and skin impurities (Laus et al., 1997).

Many biological activities have been described for numerous extracts of *Uncaria tomentosa* (UT). The anti-inflammatory activity has been assayed in vitro and in vivo. It was demonstrated that an aqueous extract of UT was able to minimize rat mucosal injury in the indomethacin-induced intestinal inflammation, deplete peroxynitrite and attenuate peroxynitrite-induced cell death, prevent the activation of the transcription factor NF-κB, inhibit the expression of inducible genes associated with inflammation—specifically negating the expression of inducible nitric oxide synthase (iNOS) and thereby attenuating NO production (Sandoval-Chacón et al., 1998; Aguilar et al., 2002; Akesson et al., 2003). Extracts and fractions of UT were also bioassayed by the carrageenan-induced oedema test in rat paw, and the

* Corresponding author. Centro de Ciências Biológicas-Bloco D, caixa postal 476, Campus Universitário-Trindade, Florianópolis, SC, Brazil, CEP 88049-900. Tel.: +55 48 331 9491; fax: +55 48 3375479.

E-mail address: ribeiro@farmaco.ufsc.br (R.M. Ribeiro-do-Valle).

most active fractions were isolated and analysed for their chemical constituents; however, all the resulting compounds were found to be inactive in this model at the tested doses, which suggests that the strong anti-inflammatory action of UT observed in other studies was due to a combination of compounds (Aquino et al., 1991). Decoctions of the bark of UT showed an antimutagenic effect in vitro, and were able to inhibit the epithelial cell death in response to oxidant stress (Rizzi et al., 1993; Miller et al., 2001). Antioxidant properties have also been described (Sandoval et al., 2000). Furthermore, it was found that UT extracts can have an immunostimulant action and enhance DNA repair capability (Lemaire et al., 1999; Sheng et al., 2000a,b).

Phytochemical analyses of *Uncaria tomentosa* root bark have revealed the presence of triterpenes, quinovic acid glycosides, some minor constituents and alkaloids (Aquino et al., 1989, 1997). Alkaloids are usually divided into classes according to their main chemical structure. The indole family is the most numerous plant alkaloid class, and oxindole alkaloids represent one of the major subgroups of this class (Martin and Mortimore, 1990). Six main pentacyclic oxindole alkaloids are found in UT: pteropodine and isopteropodine as major alkaloids, together with mitraphylline, isomitraphylline, speciophylline and uncarine F (Stuppner et al., 1992a,b; Laus and Keplinger, 1994; Ginkel, 1996). Other isomers – uncarines C, D and E – were also reported in lower quantities (Muhammad et al., 2001). In vitro cytotoxic activity of isolated oxindole alkaloids was observed against four human cell lines (Muhammad et al., 2001). In addition, antiviral activity of *U. tomentosa* has also been described, as some quinovic acid glycosides found in UT barks have shown an antiviral effect against cell infection with DNA viruses (Aquino et al., 1989).

Alkaloid compounds, such as morphine, have been extensively used in human history to alleviate pain. Mitragynine, an indole alkaloid from the Thai medicinal plant *Mitragyna speciosa*, had its antinociceptive activity described by Matsumoto et al., (1996, 2004), and there is an extensive literature describing the antinociceptive activity of other alkaloids (Elisabetsky et al., 1995; Ameri, 1998; Küpeli et al., 2002; Verotta et al., 2002). However, no previous correlation between the alkaloids of *Uncaria tomentosa* and antinociceptive activity has been reported so far. In this study, we have examined in detail the antinociceptive properties of UT in chemical and thermal models of nociception in mice and also some of the mechanisms that might potentially underlie this activity.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 35–40 g were used in all experiments. Animals were housed under standard light/

dark cycle (12 h each) and temperature (22 ± 2 °C) and acclimatized to the laboratory for at least an hour before the tests. Food and water were available ad libitum. The experiments were performed after approval of the protocol by the Institutional Ethics Committee and were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals (Zimmermann, 1983). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.2. Drugs

Formalin (Nuclear, SP, Brazil), acetic acid (Cinética Química, SP, Brazil), capsaicin (8-methyl-*N*-vanillyl-6-nonamide; Calbiochem, San Diego, USA), atropine sulphate, chlorimipramine hydrochloride, clonidine hydrochloride, ketanserin, L-arginine (L-ARG), N^G-nitro-L-arginine (L-NOARG), naltrexone hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, reserpine, R(-)-DOI (R-[-]-2,5-Dimethoxy-4-iodoamphetamine), yohimbine (Sigma Chemical Co., St. Louis, MO, USA), ascorbic acid and morphine hydrochloride (Merck AG, Darmstadt, Germany) were dissolved in PBS (NaCl 137 mM, KCl 2.7 mM and phosphate buffer 10 mM; Sigma Chemical Co., St. Louis, MO, USA). Capsaicin was dissolved in 1% dimethylsulphoxide (Sigma Chemical Co., St. Louis, MO, USA) and PBS. Reserpine was dissolved in 2% ascorbic acid solution. Prazosin was dissolved in 0.2% dimethylformamide (Sigma Chemical Co., St. Louis, MO, USA) solution.

An industrial standardised *Uncaria tomentosa* fraction (UT fraction) containing 95% oxindole alkaloids was used in all experiments. Plant material was collected in Pozuzo area, in Peru. The following stereoisomers were identified by HPLC method: speciophylline, uncarine f, mitraphylline, isomitraphylline, pteropodine, rhynchophylline, isorhynchophylline and isopteropodine, accounting for 95% of the sample (m/m). In order to be administered by i.p. route, the fraction was dissolved in Tween-80 (Fischer Scientific International) and then diluted with PBS in order to obtain a 10% Tween-80 solution. The final concentration of Tween-80 did not exceed 10% and did not cause any “per se” effect. Control animals received vehicle only (10 mL/kg i.p.).

2.3. The abdominal writhing test

The writhing test was carried out according to the method previously described by Koster et al. (1959). Animals were pre-treated intraperitoneally (i.p) with the UT fraction at the following doses: 3, 10, 30 and 100 mg/kg administered 30 min before the induction of nociception. 0.6% acetic acid solution was injected i.p. (10 mL/kg) and the animals were placed in an acrylic observation chamber (12 × 12 × 25 cm). The number of writhing responses

(abdominal cramps) was counted in 20 min after the injection of acetic acid.

2.4. The capsaicin test

The procedure used was similar to that described previously (Santos and Calixto, 1997). The noxious stimulus consisted of 20 μ L of a capsaicin solution (1.6 μ g/paw prepared in PBS) injected i.pl. in the ventral surface of the right hind paw. Immediately after the injection, animals were placed in an acrylic observation chamber and observed for 5 min. The time spent licking and biting the injected paw was measured with a stopwatch and considered as an indication of nociception. Animals were pre-treated with doses of the UT fraction (30, 100 and 300 mg/kg, i.p.), administered 30 min before the induction of nociception.

2.5. The formalin test

The procedure used was essentially the same as that previously described by Hunskar et al. (1985) with minor modifications. A 20- μ L aliquot of 2.5% formalin solution (0.92% formaldehyde) made up in PBS was injected intraplantarly (i.pl.) in the right hind paw of the animal. Subsequently to the formalin injection, animals were placed in an acrylic observation chamber, and the time spent licking or biting the injected paw was measured with a stopwatch and considered as a quantitative indication of nociception. The early phase of the nociceptive response normally peaks from 0 to 5 min and the late phase from 15 to 30 min after formalin injection, which represents the direct effect of formalin on nociceptors and inflammatory nociceptive responses, respectively (Hunskar and Hole, 1987; Tjølsen et al., 1992). Animals were pre-treated intraperitoneally (i.p.) with the UT fraction (10–300 mg/kg) administered 30 min before the formalin injection.

2.6. The tail-flick test

A radiant heat tail-flick analgesiometer was used to measure response latencies according to the method described previously by D'Amour and Smith (1941), with minor modifications. In this model, animals respond to a focused heat stimulus by flicking or removing their tail, exposing a photocell that is situated immediately below it. The intensity of radiant heat was adjusted so that the animal flicked its tail within 5 to 10 s (20 W). The reaction time of each animal was recorded 30 min before the administration of UT fraction (100 and 300 mg/kg, 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 were selected 24 h previously on the basis of their reactivity in the test to ensure that all those used exhibited responses within the normal range.

2.7. The hot-plate test

The hot-plate test used to measure response latencies was carried out according to the method described by Eddy and Leimbach (1953), with minor modifications. In this experiment, the hot-plate (Ugo Basile, model-DS 37) was maintained at $56 \pm 1^\circ\text{C}$. Animals were placed in a glass cylinder of 24-cm diameter 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. The reaction time was recorded 30 min after administration of UT fraction (30, 100 and 300 mg/kg, i.p.) or morphine (10 mg/kg, s.c.). An automatic 30 s cut-off was used to minimize tissue damage. Animals were selected 24 h previously on the basis of their reactivity in the test to ensure that all those used exhibited responses within the normal range.

2.8. Assessment of locomotor activity

In order to investigate the possible non-specific muscle relaxant or sedative effects of UT fraction, mice were tested on the rota-rod (Rosland et al., 1990). The apparatus consisted of a bar with a diameter of 2.5 cm, subdivided into four compartments. The bar rotated at a constant speed of 22 revolutions per minute and animals were evaluated for the time spent to fall from the bar. The animals were selected 24 h previously by eliminating those mice that did not remain on the bar for 60 s. Animals were treated with UT fraction (100 and 300 mg/kg, i.p.) or the same volume of vehicle (10 mL/kg, i.p.), 30 min before being tested. Results are expressed as the times for which animals remained on the rota-rod bar. The cut-off time used was 60 s.

2.9. Investigation of the mechanism of action

To address some of the mechanisms by which UT fraction causes antinociception in the formalin-induced nociception, animals were treated with different drugs through several 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 (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).

In order to investigate the participation of the opioid system in the antinociceptive effect of UT fraction, mice were pre-treated with naltrexone (1 mg/kg, i.p., a non-selective opioid receptor antagonist), and after 15 min, the animals received an injection of UT fraction (100 mg/kg, i.p.), morphine (5 mg/kg, i.p., used as positive control) or vehicle. Another group of animals was pre-treated with vehicle and after 20 min received UT fraction, morphine, or vehicle, 30 min before formalin injection.

Table 1

Comparison between ID₅₀ values, and their respective 95% confidence limit, of pre-treatment (i.p.) with UT fraction in three chemical models of nociception in mice

Assay	ID ₅₀ value and 95% confidence limit (mg/kg)
Abdominal writhing test	12.78 (5.80–19.76)
Capsaicin test	68.5 (44.97–92.03)
Formalin test–1st phase	107.11 (88.12–126.10)
Formalin test–2nd phase	95.25 (71.33–119.17)

To investigate the possible involvement of the muscarinic system in the antinociceptive action of UT fraction, mice were pre-treated with atropine (a nonselective muscarinic antagonist, 0.1 mg/kg, i.p.), and after 15 min, they received UT fraction (100 mg/kg, i.p.) or vehicle injection, before being subjected to the formalin test 30 min later. Another group of animals was pre-treated with vehicle and after 20 min, they received UT fraction or vehicle, 30 min before formalin injection.

To examine the possible participation of the adrenergic system in the antinociceptive effect of UT fraction, animals were pre-treated with prazosin (an α_1 -adrenoreceptor antagonist, 0.15 mg/kg, i.p.) or with yohimbine (an α_2 -adrenoreceptor antagonist, 1.0 mg/kg, i.p.), and after 15 min, the animals received UT fraction (100 mg/kg, i.p.), phenylephrine (an α_1 -adrenoreceptor agonist, 10 mg/kg, i.p.), clonidine (an α_2 -adrenoreceptor agonist, 0.1 mg/kg, i.p.), or vehicle injection. Other groups of animals were pre-treated with vehicle (10 mg/kg, i.p.), and after 20 min received UT fraction, phenylephrine, clonidine, or vehicle injection 30 min before formalin injection.

To investigate the role played by the nitric oxide-L-arginine pathway in the antinociception caused by UT fraction in the formalin test, mice were pre-treated with L-arginine (L-ARG; 600 mg/kg, i.p., a nitric oxide precursor) and after 20 min, they received UT fraction (100 mg/kg, i.p.), N^G-nitro-L-arginine (L-NOARG, 75 mg/kg, i.p., a nitric oxide synthase inhibitor) or vehicle. Another group of animals was pre-treated with vehicle and after 20 min received UT fraction, L-NOARG, or vehicle, 30 min before the formalin test.

We next investigated the possible role played by reserpine in the antinociceptive effect of UT fraction in the formalin test. For this purpose, mice were pre-treated with reserpine (5 mg/kg, i.p., a catecholamine depleter), and after 24 h, the animals received an injection of UT fraction (100 mg/kg, i.p.), chlorimipramine (1.0 mg/kg, i.p., a monoamines reuptake inhibitor) or vehicle. Another group of animals was pre-treated with vehicle and after 24 h received UT fraction, chlorimipramine, or vehicle, 30 min before formalin injection.

Finally, to explore the possible participation of the serotonergic system in the antinociceptive action of UT fraction, mice were pre-treated with ketanserin (a 5-HT₂ receptor antagonist, 1.0 mg/kg, i.p.), and after 20 min, the animals received UT fraction (100 mg/kg, i.p.), DOI (a

selective 5-HT_{2A} receptor agonist, 1.0 mg/kg, i.p.), or vehicle. Other groups of animals were pre-treated with vehicle, and after 20 min received UT fraction, DOI, or vehicle injection 30 min before formalin injection.

2.10. Statistical analysis

The results were expressed as mean \pm standard error of mean (SEM). Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA) followed by Dunnett's test or Student–Newman–Keuls' test. The geometric mean ID₅₀ values (Table 1), accompanied by their respective 95% confidence limits, were determined by non-linear regression from individual experiments using “Graph Pad Prism Software.” Unpaired Student's *t* test with Welch correction was used in the analysis of rota-rod data.

3. Results

3.1. The abdominal writhing test

The pre-treatment of animals with UT fraction caused a significant reduction in the number of abdominal writhing

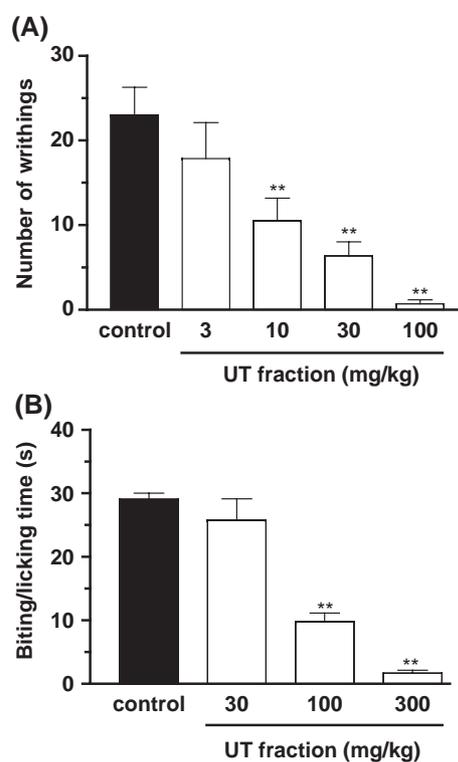


Fig. 1. (A) Effect of pre-treatment with UT extract at the doses of 3, 10, 30 and 100 mg/kg, i.p. in the abdominal writhing test induced with i.p. (10 mL/kg) injection of 0.6% acetic acid solution. (B) Effect of pre-treatment with UT extract at the doses of 30, 100 and 300 mg/kg, i.p. in the capsaicin test. Each column represents the number of cramps or the reactivity time (time spent licking/biting injected paw) of six animals per group as mean \pm standard error of mean (SEM). ***p* < 0.01.

responses of approximately 54, 72 and 97%, at the doses of 10, 30 and 100 mg/kg i.p., respectively, when compared to vehicle-treated control (23 ± 3.3 writhing responses) (Fig. 1A). ANOVA found extremely significant effects between groups [$F(4,43)=10.175$, $P<0.0001$].

3.2. The capsaicin test

In the capsaicin test, animals that had been pre-treated i.p. with UT fraction at the doses of 30, 100 and 300 mg/kg (Fig. 1B) showed a statistically significant reduction in the response to the noxious stimulus of approximately 11, 66 and 94%, respectively, when compared to vehicle-treated control response (29 ± 1.0 s). ANOVA considered extremely significant effects between groups [$F(3,23)=48.019$, $P<0.0001$].

3.3. The formalin test

The pre-treatment of animals with varying doses of UT fraction significantly reduced the time spent licking or biting the injected paw in the formalin test. The percentages of inhibition for the doses of 10, 30, 100, 150 and 300 mg/kg in the early phase (Fig. 2A) were 8, 30, 33, 65 and 94%, while the corresponding values for the second phase were 28, 36, 41, 94 and 100%, respectively (Fig. 2B). Control values for the early and late phases were 71.1 ± 2.8 s and

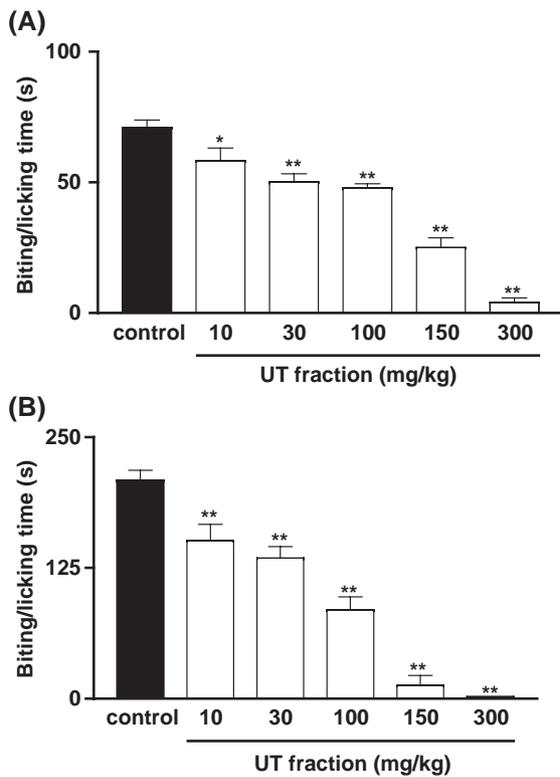


Fig. 2. Effect of pre-treatment with UT extract at the doses of 10, 30, 100, 150 and 300 mg/kg, i.p., in both phases of the formalin test. Each column represents reactivity time (time spent licking/biting injected paw) of 6–10 animals per group as mean \pm SEM. * $p<0.05$ and ** $p<0.01$.

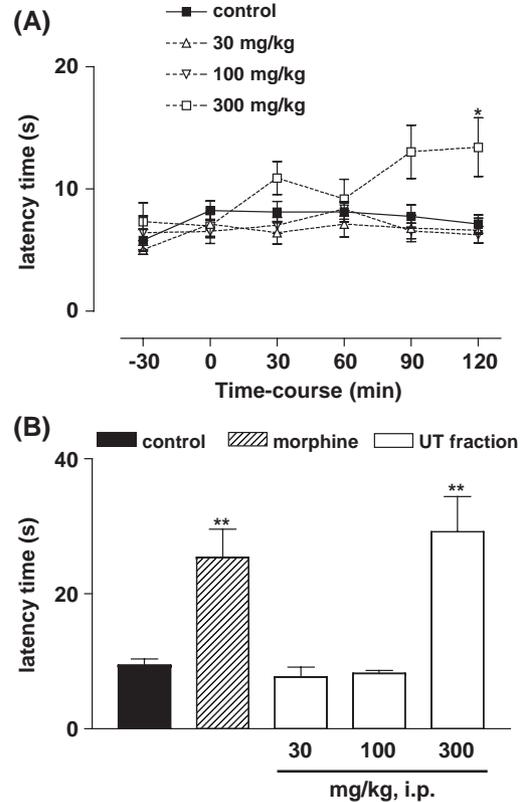


Fig. 3. Effect of pre-treatment with UT extract at the doses of 30, 100 and 300 mg/kg, i.p., or morphine (10 mg/kg, s.c.) in the tail-flick test (A) or in the hot-plate test (B). Each column represents the latency(s) expressed as mean \pm SEM of six animals per group. ** $p<0.01$.

208.6 ± 9.7 s, respectively. ANOVA considered extremely significant effects between groups in both phases [$F(5,50)=45.716$ (first phase) and $F(5,59)=48.208$ (second phase), $P<0.0001$].

3.4. Tail-flick and hot-plate tests

In the tail-flick test, the treatment of animals with UT fraction at the dose of 300 mg/kg caused a significant increase of 192% in the latency, 120 min later (Fig. 3A). ANOVA considered very significant effects between groups [$F(3,26)=6.243$, $P=0.0029$]. Control latency was 7.3 ± 1.5 s. Similarly, treatment with UT fraction at the dose of 300 mg/kg increased the latency in the hot-plate test by about 311% (Fig. 3B). ANOVA considered extremely significant effects between groups [$F(4,30)=12.976$, $P<0.0001$]. Control value was 9.4 ± 1.0 s.

3.5. Assessment of locomotor activity

Treatment of animals with UT fraction at the doses of 100 and 300 mg/kg, given by i.p. route, did not significantly affect the motor response of animals. The mean performance in the rota-rod test was 36.2 ± 9.5 s (control value 50.5 ± 9.5 s) and 43.4 ± 5.6 s (control value 57.0 ± 2.3 s) in the presence of the 100 and 300 mg/kg doses of the tested fraction,

respectively ($n=10$ each group; data not shown). The two-tailed P values were 0.3134 and 0.0659 for the groups treated with 100 and 300 mg/kg of UT fraction, respectively.

3.6. Analysis of possible mechanism of action of UT fraction

3.6.1. Opioids

The results presented in Fig. 4 show that the pre-treatment of mice with naltrexone (a nonselective opioid antagonist, 1.0 mg/kg, i.p.), given 15 min earlier, did not significantly modify the antinociception caused by UT fraction against either phase of formalin-induced nociception. However, under the same conditions, naltrexone completely reversed the antinociception caused by morphine (5 mg/kg, s.c.) in the formalin test (Fig. 4). ANOVA considered extremely significant effects between groups in both phases [$F(5,53)=21.931$ (first phase) and $F(5,55)=52.801$ (second phase), $P<0.0001$].

3.6.2. Cholinergic mechanisms

The previous treatment of animals with atropine (a nonselective muscarinic antagonist, 0.1 mg/kg, i.p.), given 20 min earlier, did not significantly modify the antinociception caused by UT fraction in either phase of formalin-

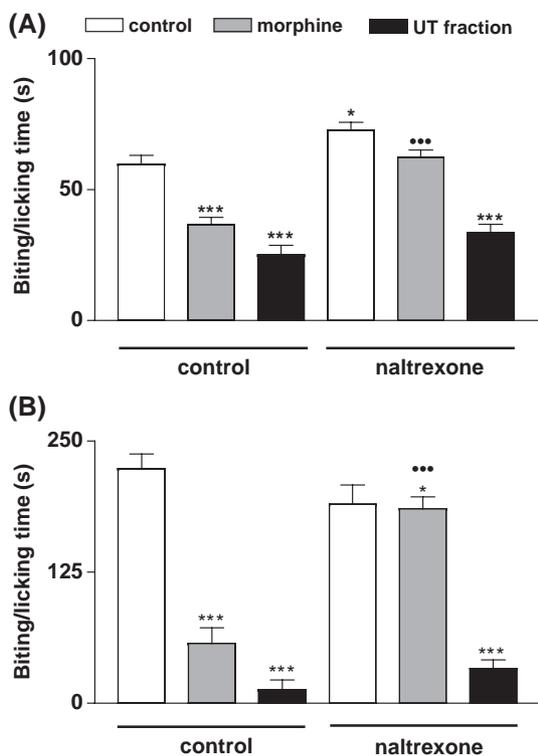


Fig. 4. Influence of the pre-treatment of animals with naltrexone (1 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or morphine (5 mg/kg, i.p.) in phases 1 and 2 of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. * $p<0.05$ and *** $p<0.001$ represent the statistical differences between treated groups and control group (vehicle only), while *** $p<0.001$ represents the statistical difference between groups treated with morphine in the absence and presence of naltrexone.

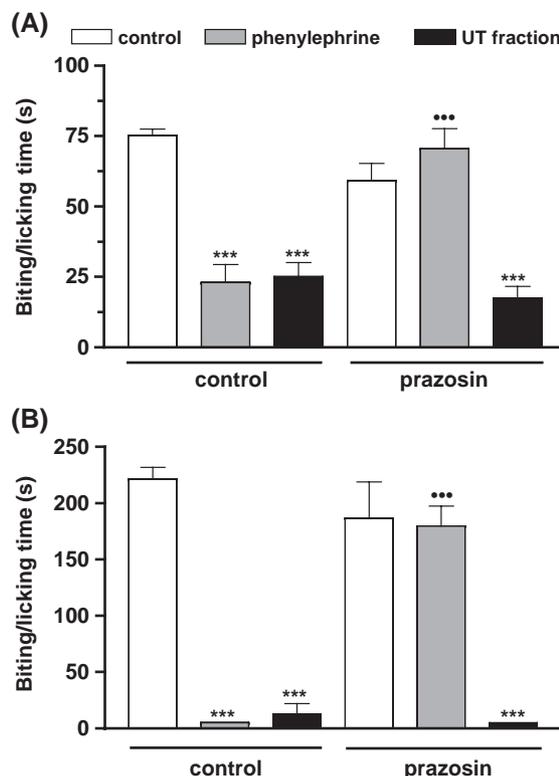


Fig. 5. Influence of the pre-treatment of animals with prazosin (0.15 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or phenylephrine (10 mg/kg, i.p.) in phases 1 and 2 of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. *** $p<0.001$ represents the statistical difference between treated groups and control group (vehicle only), while *** $p<0.001$ represents the statistical difference between groups treated with phenylephrine in the absence and presence of prazosin.

induced nociception (results not shown). ANOVA considered extremely significant effects between groups in both phases [$F(3,26)=19.165$ (first phase) and $F(3,23)=40.077$ (second phase), $P<0.0001$].

3.6.3. α -Adrenergic mechanisms

The treatment of mice with prazosin (an α_1 -selective antagonist, 0.15 mg/kg, i.p.) or yohimbine (an α_2 -selective antagonist, 1.0 mg/kg, i.p.), 20 min before, significantly reversed the antinociception caused by phenylephrine (an α_1 -selective agonist, 1.0 mg/kg, i.p.) or clonidine (an α_2 -selective agonist, 0.1 mg/kg, i.p.), but did not significantly change the antinociception caused by UT fraction in both phases of the formalin test (Figs. 5 and 6). ANOVA considered extremely significant effects between groups in both phases for α_1 [$F(5,38)=21.207$ (first phase) and $F(5,43)=48.955$ (second phase), $P<0.0001$] and α_2 [$F(5,35)=29.286$ (first phase) and $F(5,35)=46.997$ (second phase), $P<0.0001$].

3.6.4. NO system

The results presented in Fig. 7 show that the pre-treatment of mice with the nitric oxide precursor L-ARG

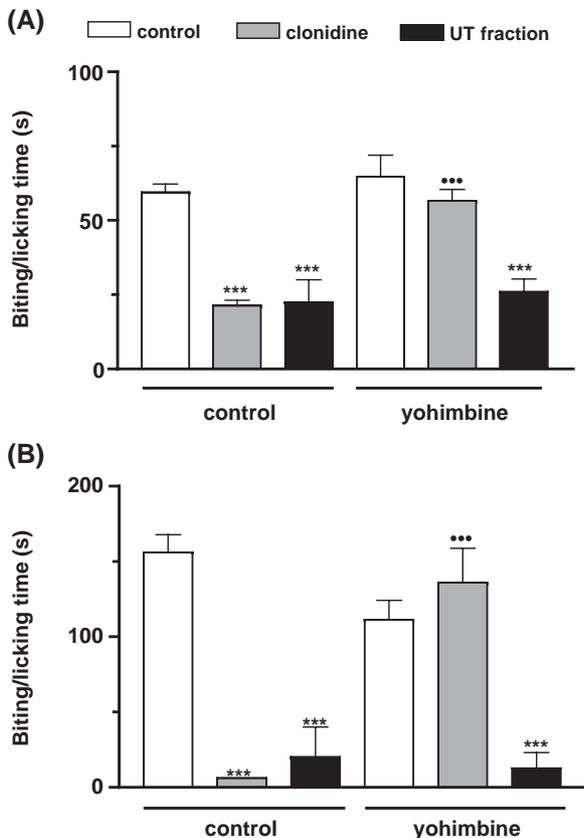


Fig. 6. Influence of the pre-treatment of animals with yohimbine (1.0 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or clonidine (0.1 mg/kg, i.p.) in the first and second phases of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. * p < 0.05 and *** p < 0.001 represent the statistical differences between treated groups and control group (vehicle only), while * p < 0.05 and *** p < 0.001 represent the statistical differences between groups treated with clonidine in the absence and presence of yohimbine.

(600 mg/kg, i.p.), given 20 min earlier, completely reversed 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 UT fraction in the formalin test (Fig. 7). ANOVA considered extremely significant effects between groups in both phases [$F(5,38)=17.121$ (first phase) and $F(5,37)=30.777$ (second phase), $P < 0.0001$].

3.6.5. Biogenic amines

Fig. 8 shows that the pre-treatment of animals with reserpine (5 mg/kg, i.p.), 24 h before, caused a significant inhibition of chlorimipramine (10 mg/kg, i.p.)-induced antinociception when assessed against both phases of formalin-induced pain. Under the same conditions, reserpine did not significantly modify the antinociception caused by UT fraction in the formalin test. ANOVA considered extremely significant effects between groups in both phases [$F(5,36)=19.280$ (first phase) and $F(5,38)=116.68$ (second phase), $P < 0.0001$].

3.6.6. 5-HT systems

Finally, the treatment of animals with ketanserin (1.0 mg/kg, i.p.) given 20 min before, completely reversed the antinociception caused by DOI (1.0 mg/kg, i.p.) against formalin-induced licking (Fig. 9). Under the same conditions, ketanserin treatment significantly antagonised the antinociceptive action of the UT fraction in the formalin test (Fig. 9). ANOVA considered extremely significant effects between groups in both phases [$F(5,40)=19.001$ (first phase) and $F(5,39)=20.991$ (second phase), $P < 0.0001$].

4. Discussion

The present study demonstrates that systemic (i.p.) administration of UT fraction elicits a dose-dependent inhibition of the nociceptive behavioural response in mice submitted to chemical and thermal pain-inducing stimuli. The most relevant findings in the work are that (1) i.p. administration of UT fraction causes significant inhibition against both phases of the pain response to the intraplantar injection of formalin, and against the nociception caused by activation of vanilloid receptors by capsaicin in the mouse

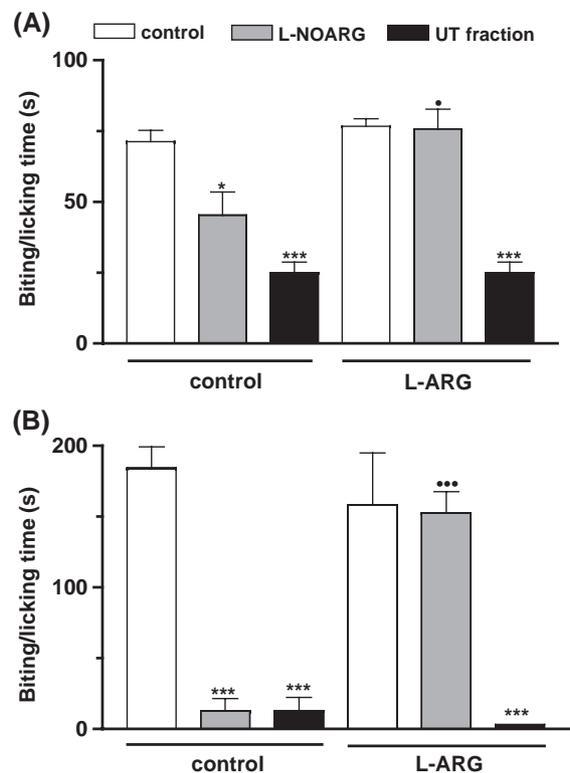


Fig. 7. Influence of the pre-treatment of animals with L-ARG (100 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or L-NOARG (75 mg/kg, i.p.) in phases 1 and 2 of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. * p < 0.05 and *** p < 0.001 represent the statistical differences between treated groups and control group (vehicle only), while * p < 0.05 and *** p < 0.001 represent the statistical differences between groups treated with L-NOARG in the absence and presence of L-ARG.

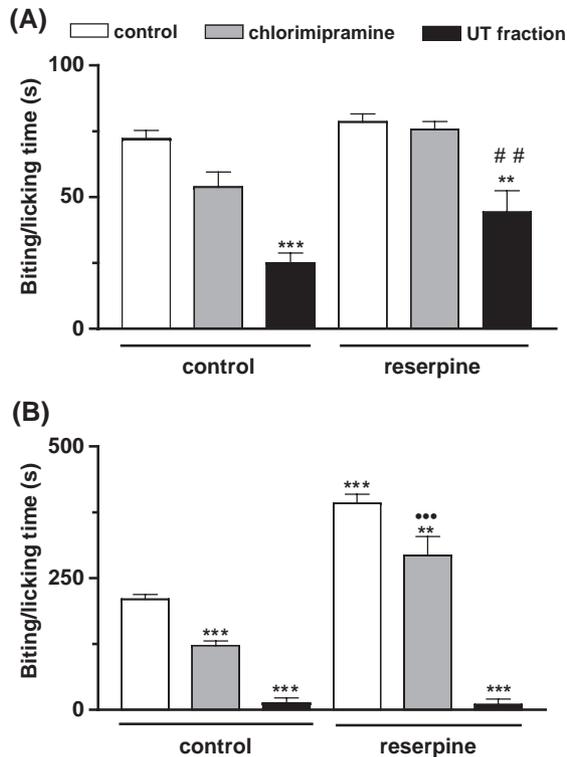


Fig. 8. Influence of the pre-treatment of animals with reserpine (5 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or chlorimipramine (10 mg/kg, i.p.) in phases 1 and 2 of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. ** p < 0.01 and *** p < 0.001 represent the statistical differences between treated groups and control group (vehicle only), while *** p < 0.001 represents the statistical difference between groups treated with chlorimipramine in the absence and presence of reserpine, and ## p < 0.01 represents the statistical difference between groups treated with UT extract in the absence and presence of reserpine.

paw; (2) the antinociceptive action of UT fraction in the formalin test was significantly reversed by i.p. pre-treatment of animals with ketanserin, but not naltrexone, atropine, prazosin, yohimbine, L-arginine, or reserpine; (3) UT fraction was effective at increasing the response latency of animals in both thermal nociceptive models (tail-flick and hot-plate tests); and (4) the dose of UT fraction that caused significant antinociception did not produce any statistically significant motor dysfunction or any detectable side effect (see data).

4.1. Profile of antinociceptive activity

A considerable number of studies have suggested that extracts or isolated compounds obtained from *Uncaria tomentosa* have a broad spectrum of biological activities, including anti-inflammatory, antioxidant, antiviral, antimutagenic, anticancer, and immunomodulatory activities (Miller et al., 2001; Sandoval-Chacón et al., 1998; Sandoval et al., 2000; Akesson et al., 2003). In addition, it has been shown that *Uncaria tomentosa* is a remarkably potent inhibitor of NF- κ B and is an effective inhibitor of TNF- α

gene expression in vitro and in vivo (Sandoval-Chacón et al., 1998; Akesson et al., 2003). However, in spite of the considerable amount of data regarding the anti-inflammatory effects of UT, the putative antinociceptive activity of UT as well as its mechanisms of action in rodents had not been reported.

The results reported here indicate that i.p. administration of UT fraction, at doses that did not produce any statistically significant motor dysfunction or any detectable side effect, produced marked and dose-related antinociception when assessed in acetic acid-induced visceral nociception. The acetic acid-induced writhing test in mice produces a type of nociceptive response that can be considered a visceral inflammatory pain reflex, and has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents (Vyklícky, 1979; Tjølsen and Hole, 1997). At the cellular level, protons depolarize sensory neurones by directly activating a non-selective cationic channel localized on cutaneous, visceral and other types of nociceptive peripheral afferent C-fibres

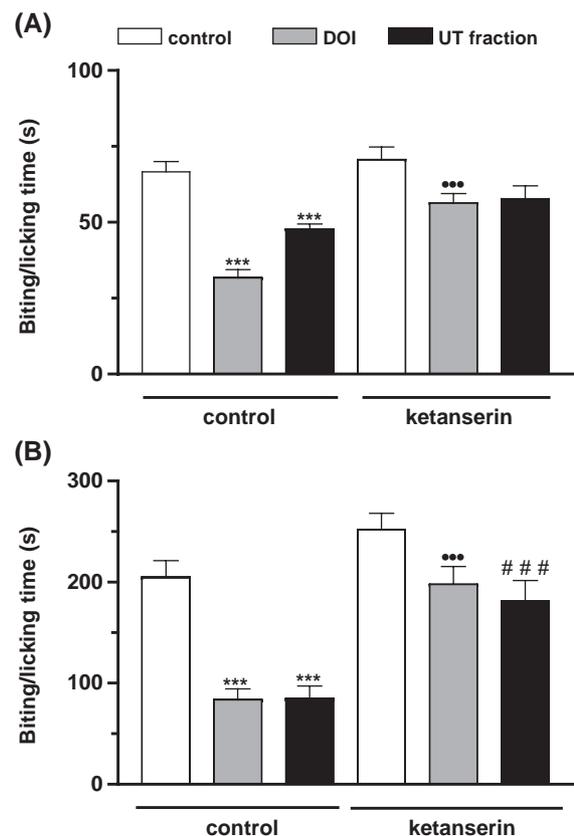


Fig. 9. Influence of the pre-treatment of animals with ketanserin (1 mg/kg, i.p.) on the antinociception caused by UT extract (100 mg/kg, i.p.) or DOI (1 mg/kg, i.p.) in phases 1 and 2 of the formalin test. Each column represents the reactivity time of 6–10 animals per group as mean \pm SEM. *** p < 0.001 represents the statistical difference between treated groups and control group (vehicle only), while *** p < 0.001 represents the statistical difference between groups treated with DOI in the absence and presence of ketanserin, and ### p < 0.001 represents the statistical difference between groups treated with UT extract in the absence and presence of ketanserin.

(Reeh and Kress, 2001; Julius and Basbaum, 2001). This method shows good sensitivity, as it allows for the effects of weak analgesics, but shows poor specificity because the abdominal writhing response may be suppressed by muscle relaxants and other drugs, leaving scope for the misinterpretation of results (Le Bars et al., 2001). This can be avoided by complementing the test with other models of nociception and a motility test. For this reason, UT fraction was examined for its inhibitory action on motility in the rota-rod test. It showed no statistically significant interference in motility patterns at a dose that produced complete suppression of the writhing response.

Another important finding is the demonstration that i.p. administration of UT fraction produces a dose-dependent antinociceptive effect on the capsaicin-induced paw licking response, which is very similar to the abdominal writhing test. Capsaicin is regarded as a valuable pharmacological tool for studying a subset of mammalian primary sensory C-fibres and A δ afferent neurones, including polymodal nociceptors and warm thermoceptors (for review, see Holzer, 1991; Jancso, 1992). In addition, it has been proposed that capsaicin-induced nociception is brought about by activation of the capsaicin receptor, also known as the vanilloid receptor (VR), termed VR subtype 1 (VR1), which is a ligand-gated nonselective cation channel in primary sensory neurones (Caterina et al., 1997; Tominaga et al., 1998). Studies have shown that, in the periphery, capsaicin evokes the release of neuropeptides, excitatory amino acids (glutamate and aspartate), nitric oxide and pro-inflammatory mediators that transmit nociceptive information to the spinal cord (Sakurada et al., 1992, 1996, 2003). Therefore, the suppression of the capsaicin-induced acute pain response and of the acetic acid-induced abdominal writhing response, caused by treatment with UT fraction, is complementary indication that the antinociceptive action of this plant is strongly observed when the noxious stimulus is conducted mainly by C-fibres.

In the formalin test, UT fraction caused significant and dose-related antinociception when administered by i.p. route against both early and late phases of the pain response in mice. These two phases of manifested behavioural response differ in origin of the neuronal impulse: the first is acute and represents the direct chemical stimulation of nociceptors, while the second is tonic and may result from the peripheral inflammatory process (Hunskar and Hole, 1987) possibly associated with a centrally-mediated facilitation (Martindale et al., 2001). Experimental data indicate that formalin predominantly evokes activity in C-fibres (Tjølsen et al., 1992), although in the first phase of pain A δ -fibres are thought to be responsible for fast nociceptive transmission (Julius and Basbaum, 2001). The major transmission pathway for inflammatory pain has been documented as that comprising peripheral polymodal receptors around small vessels that signal to the CNS via afferent C-fibres entering the dorsal horn (Kumazawa et al., 1996). Recently, Ribeiro et al. (2000) demonstrated that the nociceptive activity of

acetic acid may be due to the release of cytokines, such as TNF- α , interleukin-1 β and interleukin-8, by resident peritoneal macrophages and mast cells. Thus, the antinociceptive action of UT may be due, at least in part, to inhibition of the release of TNF- α and other cytokines by resident local cells. However, this possibility, in the case of UT fraction, remains to be tested in future studies. The formalin test is a satisfactory and comprehensive model for evaluating the antinociceptive activity of drugs, and since UT fraction was effective at inhibiting both phases of pain, it can be suggested that it has great potential as an analgesic drug.

Another interesting result of the current study was the fact that i.p. administration of UT fraction produced a significant antinociception in thermal models of nociception: the tail-flick and the hot-plate tests. We observed a significant increase in the reaction time of animals in both models, but only for the group treated with the highest dose of 300 mg/kg, while in the nociception caused by acetic acid and formalin, ten times-lower doses were able to efficiently inhibit the nociceptive response with the same statistical significance. This disparity, however, was expected, due to a similar dose-profile of other analgesics in these models in mice. It is known, for example, that thermal stimuli models are less sensitive to morphine than other models such as the acetic acid-induced writhing test and, therefore, the effective dose of morphine in the former is sixteen times greater than in the latter one. Morphine depresses responses in the dorsal horn, produced by C-fibres, more easily than it depresses those produced by A δ -fibres (Le Bars et al., 1976, 2001), hence, drugs with similar profiles can be compared with morphine on the basis of the selectivity of fibres on which they act.

The tail-flick is considered to be a spinal reflex, but the mechanism of response could also involve higher neural structures (Jensen and Yaksh, 1986). The hot-plate test produces, at constant temperature, two kinds of behavioural response, which are paw licking and jumping. Both of these are considered to be supraspinally-integrated responses (Chapman et al., 1985).

4.2. Mechanisms of action

Further experiments were undertaken to elucidate the molecular mechanism by which UT fraction exerted its antinociceptive activity. The present results lead to the conclusion that the opioid system is unlikely to be involved. This is drawn from the fact that pre-treatment of animals with naltrexone, a nonselective opioid receptor antagonist, completely inhibited the antinociceptive effect of morphine but not the action of UT fraction. Furthermore, the results of the present study provide consistent evidence to suggest that the L-arginine nitric oxide pathway is unlikely to be involved in the antinociceptive action of UT fraction. This evidence derives from the fact that pre-treatment of animals with the substrate for NOS, L-arginine, significantly reversed the antinociception caused by L-NOARG (a known

inhibitor of NOS), but not the antinociceptive action of UT fraction, in the formalin test.

Furthermore, the results of the present study provide consistent evidence that antinociception elicited by the UT fraction is independent of the activation of important endogenous analgesic systems, namely cholinergic and noradrenergic systems. In fact, the treatment of animals with atropine, a nonselective muscarinic antagonist, failed to interfere with UT fraction-induced antinociception when assessed in the formalin model of pain. Moreover, the α_1 and α_2 -adrenoceptors appear unlikely to be involved in the antinociceptive action of UT fraction, evidenced by the fact that selective antagonists of these receptors failed to alter the antinociception caused by treatment with UT fraction, in conditions where they produce significant inhibition of the antinociception caused by the respective selective agonists.

It is well known that serotonin (5-HT) pathways within the CNS arise from a series of nuclei situated in the midline of the brain stem, the raphe nuclei, which represent the richest source of neuronal 5-HT synthesised in the mammalian brain (Fields et al., 1991; Millan, 2002). In addition, several studies have shown that the bulbospinal serotonin system may suppress incoming noxious input to the spinal cord and inhibit pain transmission (Basbaum and Fields, 1984; Alhaider, 1991). Descending serotonergic pathways modulate the activity of projection neurons directly, as well as via interneurons (Alhaider et al., 1991). The multiple 5-HT receptor types within the spinal cord appear to fulfil different roles in the control of nociception, reflecting their contrasting patterns of coupling to intracellular transduction mechanisms (Millan, 1995; Bardin et al., 2000). The activities of 5-HT receptors are complex and sometimes even contrasting, and can depend on (1) the receptor subtype being activated, (2) the relative contributions of pre-versus postsynaptic actions of receptors, and (3) the nociceptive paradigm in terms of quality and intensity of stimulus (Sawynok and Reid, 1996; Millan, 2002), and (4) the dose-related effect, which can be pro- or antinociceptive, of agonists and antagonists of serotonergic receptor subtypes (Hylden and Wilcox, 1983). Several pieces of evidence point to 5-HT₂ and 5-HT₃ receptors modulating nociceptive transmission, as activation of these receptors in the spinal cord produces antinociception in the formalin test and other models (Bardin et al., 2000; Sasaki et al., 2001; Millan, 2002). Furthermore, the results of the present study show that UT fraction produces antinociception that appears to be mediated by an interaction with 5-HT₂ receptors. This assertion is supported by the demonstration that a selective antagonist of 5-HT_{2A} receptors, namely ketanserin, consistently reversed the antinociception caused by both systemic administration of UT fraction and DOI, a 5-HT₂ receptor agonist, when analysed in the formalin test in mice. In addition, another important finding regarding the activity of UT alkaloids on serotonergic receptors is our result showing that pre-treatment of mice with reserpine, 24 h prior to the treatment with UT fraction, was not able to prevent the

antinociceptive effect of the latter. Reserpine depletes monoamines stores, which, in normal conditions, exacerbates nociceptive behaviour in formalin-induced nociception. Therefore, it is likely that these alkaloids exert their action independently from endogenous 5-HT. Kang et al. (2002) had previously demonstrated an interaction between oxindole alkaloids from *Uncaria tomentosa* and 5-HT₂ receptors. That study showed that pteropodine and isopteropodine, both pentacyclic oxindole alkaloids identified and isolated from this plant (Laus et al., 1997), markedly enhanced the current responses evoked by serotonin on 5-HT₂ receptors expressed in *Xenopus* oocytes. Thus, the present findings extend literature data and show that UT fraction may be able to directly interact with 5-HT₂ receptors, rather than releasing endogenous 5-HT from nerve terminals of the descending monoaminergic neurones, and this results in blockade of the nociceptive information transmission in the spinal cord.

Kitajima et al. (2002) developed a study of the chemical synthesis of a monoterpene glucoindole alkaloid 3,4-dehydro-5(S)-5-carboxystrictosidine, which was isolated from Peruvian *Uncaria tomentosa*. The structure of the compound was established by the synthesis from secologanin and L-tryptophan through a Bishler–Napieralski reaction. It has been long known that L-tryptophan is the natural precursor for the biosynthesis of indolamine 5-HT, or serotonin, as well as being the precursor molecule for the plant biosynthesis of all indole alkaloids. All of these pieces of evidence lead to a possible relationship between the chemical structure of compounds and biological activity.

It is important to state that the total pentacyclic oxindole alkaloid concentration of UT fraction is 95% (m/m) and this percentage contains the six main oxindole alkaloids described by other authors as characteristic of *Uncaria tomentosa* (Laus and Keplinger, 1994). We believe that it represents a special opportunity for characterizing its biological activity and diminishes the possibility of interference from other chemical substances.

In conclusion, the data from the present study show that UT fraction exerts pronounced systemic antinociception in chemical (acetic acid-, formalin-, and capsaicin-induced pain) and thermal (hot-plate and tail-flick test) models of nociception in the mouse at a dose that does not interfere with the locomotor activity of animals. In addition, antinociceptive effect of UT fraction involves an interaction with serotonergic (specifically 5-HT₂) systems, but not with opioidergic nor noradrenergic systems nor the L-arginine nitric oxide pathway. Furthermore, the antinociceptive action demonstrated in the present study supports, at least partly, the ethnomedical uses of this plant.

5. Conclusions

Uncaria tomentosa is a very important plant from the ethnopharmacological point of view, since it has spread

from therapeutic and prophylactic application by native South American Indians to commercial use around the world. The characterization of its biological activities is, therefore, of major importance. This study reveals a potent antinociceptive activity of an alkaloid-rich fraction of this plant and an involvement of serotonergic mechanisms in this activity.

Acknowledgement

The authors would like to thank CNPq–Conselho Nacional de Pesquisa for financial support.

References

- Aguilar JL, Rojas P, Marcelo A, Plaza A, Bauer R, Reininger E, et al. Anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiaceae). *J Ethnopharmacol* 2002;81:271–6.
- Akesson C, Lindgren H, Pero RW, Leanderson T, Ivars F. An extract of *Uncaria tomentosa* inhibiting cell division and NF- κ B activity without inducing cell death. *Int Immunopharmacol* 2003;3:1889–900.
- Alhaider AA. Antinociceptive effect of ketanserin in mice: involvement of supraspinal 5-HT₂ receptors in nociceptive transmission. *Brain Res* 1991;543:335–40.
- Alhaider AA, Lei SZ, Wilcox GL. Spinal 5-HT₃ receptor-mediated antinociception: possible release of GABA. *J Neurosci* 1991;11:1881–8.
- Ameri A. The effects of Aconitum alkaloids on the central nervous system. *Prog Neurobiol* 1998;56:211–35.
- Aquino R, De Simone F, Pizza C. Plant metabolites, structure in vitro antiviral activity of quinovic acid glycosides from *Uncaria tomentosa* and *Guettarda platypoda*. *J Nat Prod* 1989;52:679–85.
- Aquino R, De Feo V, De Simone F, Pizza C, Cirino G. Plant metabolites, new compounds and anti-inflammatory activity of *Uncaria tomentosa*. *J Nat Prod* 1991;54:453–9.
- Aquino R, De Tommasi N, De Simone F, Pizza C. Triterpenes and quinovic acid glycosides from *Uncaria tomentosa*. *Phytochemistry* 1997;45(5):1035–40.
- Bardin L, Lavarenne J, Eschalier A. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. *Pain* 2000;86:11–8.
- Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 1984;7:309–38.
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–24.
- Chapman CR, Casey KL, Dubner R, Foley KM, Graceley RH, Reading AE. Pain measurement: an overview. *Pain* 1985;22:1–31.
- D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J Pharmacol Exp Ther* 1941;72:74–9.
- Eddy NB, Leimbach D. Synthetic analgesics: II. Dithienylbutenyl and dithienylbutylamines. *J Pharmacol Exp Ther* 1953;107:385–93.
- Elisabetsky E, Amador TA, Albuquerque RR, Nunes DS, Carvalho ACT. Analgesic activity of *Psychotria colorata* (Willd ex R and S) Muell Arg alkaloids. *J Ethnopharmacol* 1995;48:77–83.
- Fields HL, Heinricher MM, Mason P. Neurotransmitters in nociceptive modulatory circuits. *Annu Rev Neurosci* 1991;14:219–45.
- Ginkel A. Identification of the alkaloids and flavonoids from *Uncaria tomentosa* bark by TLC in quality control. *Phytother Res* 1996;10:18–9.
- Holzer P. Capsaicin as a tool for studying sensory neuron functions. *Adv Exp Med Biol* 1991;298:3–16.
- Hunnskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 1987;30:103–14.
- Hunnskaar S, Fasmer OB, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. *J Neurosci Methods* 1985;14:69–73.
- Hyliden JL, Wilcox GL. Intrathecal serotonin in mice: analgesia and inhibition of a spinal action of substance P. *Life Sci* 1983;33:789–95.
- Jancso G. Pathobiological reactions of C-fibre primary sensory neurones to peripheral nerve injury. *Exp Physiol* 1992;77:405–31.
- Jensen TS, Yaksh TL. Examination of spinal monoamine receptors through which brainstem opiate-sensitive systems act in the rat. *Brain Res* 1986;363:99–113.
- Julius D, Basbaum AI. Mechanisms molecular of nociception. *Nature* 2001;413:203–10.
- Kang T, Matsumoto K, Tohda M, Murakami Y, Takayama H, Kitajima M, et al. Pteropodine and isopteropodine positively modulate the function of rat muscarinic M1 and 5-HT₂ receptors expressed in *Xenopus* oocyte. *Eur J Pharmacol* 2002;444:39–45.
- Keplinger K, Laus G, Wurm M, Dierich MP, Teppner H. *Uncaria tomentosa* (Willd) DC—ethnomedicinal use and new pharmacological, toxicological and botanical results. *J Ethnopharmacol* 1999;64:23–34.
- Kitajima M, Yokoya M, Takayama H, Aimi N. Synthesis and absolute configuration of a new 3,4-dihydro-beta-carboline-type alkaloid, 3,4-dehydro-5(S)-5-carboxystricoidine, isolated from Peruvian *Una de Gato* (*Uncaria tomentosa*). *Chem Pharm Bull* 2002;50:1376–8.
- Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. *Fed Proc* 1959;18:412.
- Kumazawa T, Mizumura K, Koda H, Fukusako H. EP receptor subtypes implicated in the PGE₂-induced sensitization of polymodal receptors in response to bradykinin and heat. *J Neurophysiol* 1996;75:2361–8.
- Küpel E, Koşar M, Yeşilada E, Başer KHC, Başer C. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish *Berberis* species. *Life Sci* 2002;72:645–57.
- Laus G, Keplinger D. Separation of stereoisomeric oxindole alkaloids from *Uncaria tomentosa* by high performance liquid chromatography. *J Chromatogr A* 1994;662:243–9.
- Laus G, Brössner D, Keplinger K. Alkaloids of Peruvian *Uncaria tomentosa*. *Phytochemistry* 1997;45:855–60.
- Le Bars D, Guilbaud G, Jurna I, Besson JM. Differential effects of morphine on responses of dorsal horn lamina V type cells elicited by A and C fiber stimulation in the spinal cat. *Brain Res* 1976;115:518–24.
- Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev* 2001;53:597–652.
- Lemaire I, Assinewe V, Cano P, Awang DVC, Arnason JT. Stimulation of interleukin-1 and -6 production in alveolar macrophages by the neotropical liana, *Uncaria tomentosa* (Uña de gato). *J Ethnopharmacol* 1999;64:109–15.
- Martin SF, Mortimore M. New methods for the synthesis of oxindole alkaloids Total synthesis of isopteropodine and pteropodine. *Tetrahedron Lett* 1990;31:4557–60.
- Martindale J, Bland-Ward PA, Chessell IP. Inhibition of C-fibre mediated sensory transmission in the rat following intraplantar formalin. *Neurosci Lett* 2001;316:33–6.
- Matsumoto K, Mizowaki M, Suchitra T, Takayama H, Sakai S, Aimi N, et al. Antinociceptive action of mitragynine in mice: evidence for the involvement of supraspinal opioid receptors. *Life Sci* 1996;59:1149–55.
- Matsumoto K, Horie S, Ishikawa H, Takayama H, Aimi N, Ponglux D, et al. Antinociceptive effect of 7-hydroxymitragynine in mice: discovery of an orally active opioid analgesic from the Thai medicinal herb *Mitragyna speciosa*. *Life Sci* 2004;74:2144–55.
- Millan MJ. Serotonin (5-HT) and pain: a reappraisal of its role in the light of receptor multiplicity. *Semin Neurosci* 1995;7:409–19.

- Millan MJ. Descending control of pain. *Prog Neurobiol* 2002;66:355–474.
- Miller MJS, Angeles FM, Reuter BK, Bobrowski P, Sandoval M. Dietary antioxidants protect gut epithelial cells from oxidant-induced apoptosis. *BMC Complement Altern Med* 2001;1:11.
- Muhammad I, Dunbar DC, Khan RA, Ganzera M, Khan IA. Investigation of uña de gato 7-deoxyloganic acid and ¹⁵N NMR spectroscopic studies on pentacyclic oxindole alkaloids from *Uncaria tomentosa*. *Phytochemistry* 2001;57:781–5.
- Reeh PW, Kress M. Molecular physiology of proton transduction in nociceptors. *Curr Opin Pharmacol* 2001;1:45–51.
- Ribeiro RA, Vale ML, Thomazzi FM, Paschoalato ABP, Poole S, Ferreira SH, et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur J Pharmacol* 2000;387:111–8.
- Rizzi R, Re F, Bianchi A, De Feo V, De Simone F, Bianchi L, et al. Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts. *J Ethnopharmacol* 1993;38:63–77.
- Rosland JH, Hunskaar S, Hole K. Diazepam attenuates morphine antinociception test-dependently in mice. *Pharmacol Toxicol* 1990;66:382–6.
- Sakurada T, Katsumata K, Tan-no K, Saturada S, Kisara K. The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. *Neuropharmacology* 1992;31:1279–85.
- Sakurada T, Sugiyama A, Sakurada C, Tanno K, Sakurada S, Kisara K, et al. Involvement of nitric oxide in spinally mediated capsaicin- and glutamate-induced behavioural responses in the mouse. *Neurochem Int* 1996;29:271–8.
- Sakurada T, Matsumura T, Moriyama T, Sakurada C, Ueno S, Sakurada S. Differential effects of intraplantar capsaizepine and ruthenium red on capsaicin-induced desensitization in mice. *Pharmacol Biochem Behav* 2003;75:115–21.
- Sandoval M, Charbonnet RM, Okuhama NN, Trentacosti AM, Miller MJS. Cat's claw inhibits TNF α production and scavenges free radicals: role in cytoprotection. *Free Radic Biol Med* 2000;29:71–8.
- Sandoval-Chacón M, Thompson JH, Zhang XJ, Liu X, Mannick EE, Sadowska-Krowicka H, et al. Anti-inflammatory actions of cat's claw: the role of NF- κ B. *Aliment Pharmacol Ther* 1998;12:1279–89.
- Santos ARS, Calixto JB. Ruthenium red and capsaizepine antinociceptive effect in formalin and capsaicin models of pain in mice. *Neurosci Lett* 1997;235:73–6.
- Sasaki M, Ishizaki K, Obata H, Goto F. Effects of 5-HT₂ and 5-HT₃ receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test. *Eur J Pharmacol* 2001;424:45–52.
- Sawynok J, Reid A. Interactions of descending serotonergic systems with other neurotransmitters in the modulation of nociception. *Behav Brain Res* 1996;73:63–8.
- Sheng Y, Pero RW, Wagner H. Treatment of chemotherapy-induced leukopenia in a rat model with aqueous extract from *Uncaria tomentosa*. *Phytomedicine* 2000a;7:137–43.
- Sheng Y, Bryngelsson C, Pero RW. Enhanced DNA repair, immune function and reduced toxicity of C-MED-100™, a novel aqueous extract from *Uncaria tomentosa*. *J Ethnopharmacol* 2000b;69:115–26.
- Stuppner H, Sturm S, Konwalinka G. HPLC analysis of the main oxindole alkaloids from *Uncaria tomentosa*. *Chromatographia* 1992a;34:597–600.
- Stuppner H, Sturm S, Konwalinka G. Capillary electrophoretic analysis of oxindole alkaloids from *Uncaria tomentosa*. *J Chromatogr* 1992b;609:375–80.
- Tjølsen A, Hole K. Animal models of analgesia. In: Dickenson A, Besson J, editors. *The pharmacology of pain (Handbook of experimental pharmacology)*, vol. 30/I. Berlin: Springer Verlag; 1997. p. 1–20.
- Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain* 1992;51:5–17.
- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998;21:531–43.
- Verotta A, Orsini F, Sbacchi M, Scheidler MA, Amadorc TA, Elisabetsky E. Synthesis and antinociceptive activity of chimonanthines and pyrrolidinoindoline-type alkaloids. *Bioorg Med Chem* 2002;10:2133–42.
- Vyklicky L. Techniques for the study of pain in animals. *Adv Pain Res Ther* 1979;3:727–45.
- Zimmermann P. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;16:109–10.