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Antinociceptive activity of ethanolic extract and isolated compounds of *Urtica circularis*

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ABSTRACT

Ethnopharmacological relevance: Urtica circularis (Hicken) Sorarú is a medicinal plant commonly used in traditional medicine to relieve pain in inflammatory processes.
Aim of the study: In the present study, the *in vivo* antinociceptive effect of *Urtica circularis* ethanolic extract and its isolated compounds has been investigated.
Materials and methods: Antinociceptive activity was evaluated through writhing, formalin and hot plate tests in mice. The phytochemical analysis was performed.
Results: The extract produced significant inhibition on nociception induced by acetic acid (ED50: 72.2 mg/kg, i.p.) and formalin (ED50: 15.8 mg/kg, i.p.) administered intraperitoneally and also orally. Atropine diminished the activity of the extract in the acetic acid test. In this model, at dose of 10 mg/kg i.p., vitexin was the most active of the isolated compounds (inhibition of 91%), and chlorogenic acid, caffeic acid and vicenin-2 (6,8-di-C-glucosyl apigenin) produced an inhibition of 72%, 41% and 41%, respectively, whereas apigenin did not show any activity.
Conclusions: These results suggest that *Urtica circularis* extract produced antinociception possibly related to the presence of vitexin, chlorogenic, caffeic acid and vicenin-2. The activation of cholinergic systems seems to be involved in the mechanism of antinociception of the extract.

1. Introduction

Historically, human have used medicinal plants as a traditional way of providing relief to several diseases and it is well known that many plant-derived compounds present significant analgesic properties. Despite the progress that has occurred in the development of therapy, there is still a need of effective and potent analgesic drugs. In this regard, it has been widely shown that many plant-derived substances play a relevant role in the process of development of new strategies to treat complaints related with pain (Calixto et al., 2000).

Urtica circularis (Hicken) Sorarú is an Argentinean native herb and is widely distributed in Paraguay, Uruguay and Brazil. It is known with the common names of "ortiga", "ortiga crespa", "caá poropí" and "urtiginha miúda". Its aerial part is used in folk medicine in alcoholic solution to relieve pain in inflammatory processes (Martínez Crovetto, 1981). It is also indicated as diuretic, antihypertensive agent and against hepatic affections and cough. In addition to its medicinal use, the plant is considered to be highly nutritious for its minerals and vitamins content and it is usually included in different food preparations (Rondina et al., 2003).

There are no previous pharmacological studies that evaluate the antinociceptive potential of *Urtica circularis* but antiinflammatory and analgesic effects in animal models and *in vitro* assays have been reported in other species of the Urticaceae family (*Urtica dioica*, *Urtica urens* and *Urtica macrorrhiza*) (Riehemann et al., 1999; Randall, 2000; Yongna et al., 2005; Hudec et al., 2007; Marrassini et al., 2010). Besides in Europe, traditional herbal medicinal products containing *Urtica dioica* and *Urtica urens* are indicated for relief of minor articular pain (EMEA, 2008).

Taking into account these facts, the aim of this work was therefore to evaluate the possible antinociceptive activity of *Urtica circularis* ethanolic extract, using three classical experimental models of pain. In addition, the phytochemical analysis was performed in order to study the activity of the compounds isolated from the extract.

2. Materials and methods

2.1. Plant material

Urtica circularis (Hicken) Sorarú was collected in Estancia "La Merced", Saladas Department, Corrientes, Argentina and identified

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by Dr Martha Gattuso. A voucher specimen (No. 054) is deposited in Facultad de Ciencias Químicas, Universidad Nacional de Rosario, Argentina.

2.2. Preparation of plant extract

The aerial parts of the plant material were dried and powdered to a fine grade. The extracts were prepared as described below.

Ethanolic extract: Dried plant material (20 g) was extracted by maceration with 80% ethanol (200 ml) at room temperature (seven times). The extract was concentrated and lyophilized (yield: 11.47%, w/w).

Aqueous extract: 20 g of dried plant material with 400 ml of water were boiled during 30 min. The extract was concentrated and lyophilized (yield: 16.70%, w/w).

The extracts were dissolved in water or saline solution for oral (p.o.) or intraperitoneal (i.p.) administration, respectively.

2.3. Phytochemical analysis

The HPLC method was developed and validated according to Filip et al. (2001) and performed with a Varian 9000 instrument using a diode array detector. C18 column (Gemini 5 μm, 150 mm × 4.6 mm), solvent A: H_2O/AcOH (98:2), solvent B: MeOH/AcOH (98:2). Gradient: 15–40% B, 30 min; 40–75% B, 10 min; 75–85% B, 5 min. Flow rate: 1.2 ml/min. Detection: 325 nm. Rheodyne injector fitted with a 20 μl loop. Vicenin-2 (6,8-di-C-glucosyl apigenin) was isolated and purified by different chromatographic systems.

2.4. Animals

Female Swiss mice weighing 25–30 g were used taking into account international guiding principles and local regulations concerning the care and use of laboratory animals for biomedical research (Institute of Laboratory Animal Resources, 1996). The animals had free access to a standard commercial diet and water ad libitum and were kept in rooms maintained at 22 ± 1 °C with a 12-h light/dark cycle. They were fasted overnight before dosing for p.o. administration of antinociceptive test and 24 h before dosing for stomach effect and throughout the experiments.

2.5. Antinociceptive activity

2.5.1. Formalin test

The procedure was described by Hunskaar and Hole (1987) and consisted in the injection of 20 μl of 2.5% formalin solution (0.92% formaldehyde) in phosphate buffer (pH 7.3) into the dorsal surface of the left hind paw of the mice. Groups of animals were treated with the *Urtica circularis* aqueous extract 500 mg/kg and 1000 mg/kg p.o., ethanolic extract 10–100 mg/kg i.p. and 250 mg/kg and 500 mg/kg p.o. 30 min (i.p. administration) and 60 min (p.o. administration) before the formalin injection. Control animals received the same volume of saline solution (10 ml/kg). Animals were placed individually in an observation chamber made of transparent acrylic; beneath the floor, a mirror was mounted at a 45° angle to allow clear observation of the animal's paws. The amount of time the animal spent licking the injected paw, considered as indicative of pain, was recorded for 30 min after formalin injection. The initial nociceptive scores normally peaked 5 min after formalin injection (early phase) and 15–30 min after formalin injection (late phase), representing the neurogenic and inflammatory pain responses, respectively. Indomethacin (i.p.) and morphine subcutaneous (s.c.) administration were used as reference drugs. To assess the possible participation of the opioids system on the antinociceptive effect of *Urtica circularis*, mice were pre-treated with naloxone (5 mg/kg, i.p.) 30 min before *Urtica circularis* extract (100 mg/kg, i.p.). The nociceptive response was evaluated.

2.5.2. Hot-plate test

The hot-plate test was used to measure response latencies according to the method described by Eddy and Leimbach (1953), with minor modifications. The mice were placed on an Ugo Basile hot-plate maintained at 56 °C and the time between placement of the mouse on the platform and shaking or licking of the paws or jumping was recorded as the hot-plate latency. Mice with baseline latencies higher than 10 s were eliminated from the study. Twenty-four hours later and 30 min before the test, a group of animals was treated with the *Urtica circularis* extract (300 mg/kg, i.p.) and another group was given morphine (10 mg/kg, s.c.) while control animals received the same volume of saline solution (10 ml/kg).

2.5.3. Acetic acid-induced abdominal writhing

The test was performed as described by Collier et al. (1968). Nociception was induced by intraperitoneal injection of acetic acid 1.0%, 0.1 ml/10 g body weight. Mice were treated with the *Urtica circularis* extract 30 min (10–300 mg/kg, i.p.) and 60 min (250 mg/kg and 500 mg/kg, p.o.) before acetic acid injection. A group of mice was treated with indomethacin (10 mg/kg, i.p.), used as reference drug. Control animals received a similar volume of saline solution (10 ml/kg). The number of abdominal writhes (full extension of both hind paws) was cumulatively counted over a period of 20 min immediately after the acetic acid injection. The antinociceptive activity was expressed as percentage of inhibition of abdominal writhes.

In order to characterize pharmacologically the antinociception induced by *Urtica circularis*, the following drugs were used: yohimbine (1 mg/kg, i.p.), antagonist of α_2 adrenoceptor; atropine (2 mg/kg, i.p.), a non-selective antagonist of cholinergic muscarinic receptors; L-nitro arginine methyl ester (L-NAME 20 mg/kg, i.p.), a known non-selective oxide nitric synthase inhibitor; glibenclamide (2 mg/kg, i.p.), an ATP-sensitive potassium channel blocker and L-arginine (40 mg/kg, i.p.), oxide nitric synthase substrate. Doses and drug administration schedules were selected based on previous reports and on pilot experiments in our laboratory (Pinardi et al., 2003; Vale et al., 2007; Bezerra et al., 2008; Guginski et al., 2009) and were administered 30 min before *Urtica circularis* extract (100 mg/kg, i.p.) The nociceptive response was evaluated in the acetic acid-induced abdominal writhing test.

2.6. Effect on the stomach

The extract (100 and 300 mg/kg) was administered i.p. to mice. Six hours later, the animals were sacrificed and their stomachs were removed and cut along the greater curvature. The mucosa surface of each stomach was washed with normal saline and observed with a magnifying lens to determine the type and the degree of lesion formation. Indomethacin (10 mg/kg) and water were used as positive and negative control, respectively.

2.7. Acute toxicity

Groups of 10 CF-1 mice, 5 males and 5 females, were used. The control group received only vehicle (water), and the remaining groups received increasing doses up to 3 g/kg (0.5 ml/25 g body weight) of the ethanolic extract, orally, by means of a gastric catheter. Animals were maintained in a cage with free access to a standard diet and water ad libitum and they were observed twice a day, for up to 15 consecutive days. Besides the number of deaths, other parameters such as weight loss, abdominal constrictions, palpebral ptosis, movement, lethargy, stereotypy, ataxia,

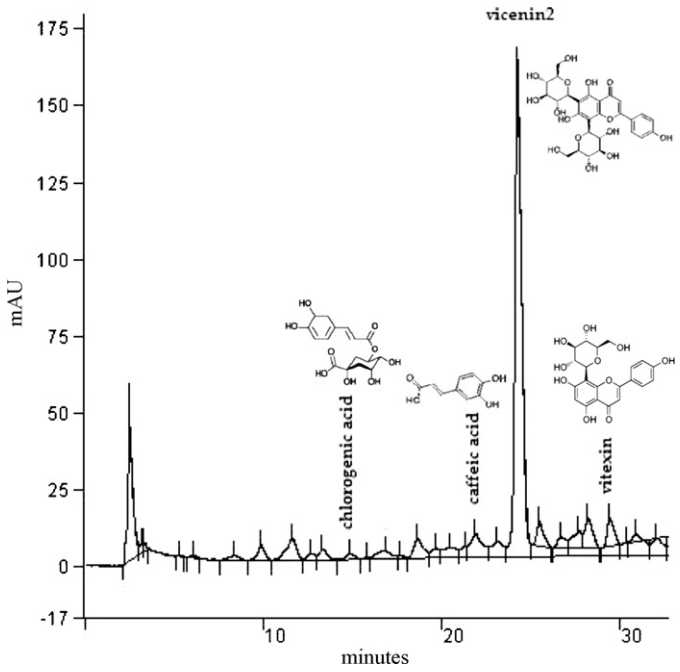

Fig. 1. Fingerprint chromatogram of the *Urtica circularis* ethanolic extract. The peak of 14.798 min. Retention time matches chlorogenic acid, the peak of 21.873 min. Retention time matches caffeic acid, the peak of 24.213 min. Retention time matches vicenin-2, the peak of 29.388 min. Retention time matches vitexin.

tremors, convulsions, diarrhoea and presence of secretions were observed.

2.8. Drugs

Indomethacin, morphine sulfate, glibenclamide, L-arginine, L-NAME, yohimbine, naloxone, atropine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid and formalin were acquired from Merck (Darmstadt, Germany).

2.9. Statistical analysis

The statistical significance of differences between groups was assessed by means of analysis of variance followed by Dunnett's test. *P* values less than 0.05 were considered as indicative of significance. ED50 values were calculated (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Phytochemical analysis

The *Urtica circularis* ethanolic extract HPLC analysis showed the presence of a major compound, a flavone glycoside, vicenin-2 (Tr: 24.213 min) identified by spectroscopy (UV–vis, MS and ^{1}H NMR) by comparison with literature data (Abebe et al., 2005; Jian et al., 2008). Caffeic acid (Tr: 21.873 min), cholorogenic acid (Tr: 14.798 min) and vitexin (Tr: 29.388 min) were identified in comparison with external standards. The corresponding fingerprint chromatogram is shown in Fig. 1.

3.2. Effect of Urtica circularis on the response induced by formalin

As shown in Table 1, the duration of the licking for the early phase (0–5 min) was 62.4 ± 7.5 s and for the late phase (15–30 min) was 84.5 ± 11.9 s in control group. *Urtica circularis* aqueous extract (500 mg/kg and 1000 mg/kg, p.o.) did not produce antinociceptive effect. Nevertheless, the ethanolic extract, 30 and 100 mg/kg i.p. and 500 mg/kg p.o., produced a marked reduction of the licking time in the late phase of 78.5%, 91.0% and 88.5%, respectively, showing an ED50: 15.8 mg/kg (95% confidence limits: 30.3–3.1 mg/kg, i.p.). The positive control drug, morphine (10 mg/kg), significantly attenuated the pain responses of the two phases, whereas indomethacin (10 mg/kg) was only effective in the late phase. Naloxone did not modify the antinociceptive effect of the ethanolic extract.

Table 1
Effect of ethanolic extract of *Urtica circularis* in the formalin test in mice.

Treatment	Licking time (s)	
	1st phase (0–5 min)	2nd phase (15–30 min)
Control	62.4 ± 7.5	84.5 ± 11.9
Aqueous extract		
Urtica circularis 500 mg/kg p.o.	58.6 ± 9.5	79.3 ± 17.5
Urtica circularis 1000 mg/kg p.o.	55.3 ± 8.3	72.3 ± 10.2
Ethanolic extract		
Urtica circularis 10 mg/kg i.p.	61.7 ± 11.1	62.2 ± 21.0
Urtica circularis 30 mg/kg i.p.	25.4 ± 10.3	18.2 ± 12.8*
Urtica circularis 100 mg/kg i.p.	46.0 ± 5.8	7.6 ± 4.2*
Urtica circularis 100 mg/kg i.p. + naloxone 5 mg/kg i.p.	54.0 ± 9.8	18.0 ± 6.5*
Ethanolic extract		
Urtica circularis 250 mg/kg p.o.	93.0 ± 18.0	77.0 ± 5.7
Urtica circularis 500 mg/kg p.o.	73.7 ± 9.1	9.7 ± 9.7*
Morphine 10 mg/kg s.c.	2.0 ± 0.8*	0 ± 0*
Morphine 10 mg/kg s.c. + naloxone 5 mg/kg i.p.	53.2 ± 8.5	73.3 ± 9.1
Indomethacin 10 mg/kg i.p.	50.4 ± 5.5	38.2 ± 5.4*

Results were obtained by oral (p.o.) or intraperitoneal (i.p.) administration of extract, morphine subcutaneous (s.c.) and indomethacin. Aqueous extract are indicated, Each value is the mean ± S.E.M. of results from 10 mice. Statistical differences from the controls were determined by Dunnet's test.

* $P < 0.01$ versus control group.

3.3. Effect of Urtica circularis on the hot plate test

The *Urtica circularis* extract antinociceptive activity was evaluated on the hot-plate test. The ethanolic extract (300 mg/kg, i.p.) did not produce analgesic effect (latency time: 6.1 ± 1.0 s) whereas the latency time was significantly increased by morphine 10 mg/kg s.c. (control: 7.6 ± 1.4 s, morphine: 25.7 ± 1.4 s).

3.4. Effect of Urtica circularis and isolated compounds on the writhing test

The antinociceptive activity of *Urtica circularis* was initially evaluated in the writhing test. The pretreatment of the mice with *Urtica circularis* (10–300 mg/kg, 20 min before stimulus) produced a dose-related inhibition of acetic acid-induced writhing response (ED50: 72.2 mg/kg, 95% confidence limits: 148.4–38.0 mg/kg). The maximal antinociceptive effect 80.6% was obtained at a dose of 300 mg/kg i.p. Also, the administration at a dose of 500 mg/kg p.o. produced a significant inhibition of the writhing response (48.4%) (Table 2).

The mechanism of action of *Urtica circularis* extract was investigated through the pre-treatment of animals with several drugs that interfere in different systems. The results are shown in Fig. 2.

The systemic pre-treatment with yohimbine, L-arginine, L-NAME and glibenclamide did not modify the antinociceptive response elicited by the extract. Only atropine significantly prevented, at least in part, the antinociception caused by *Urtica circularis* (Fig. 2).

On the other hand, the response of the combination of ineffective doses of *Urtica circularis* (50 and 100 mg/kg, p.o.) with

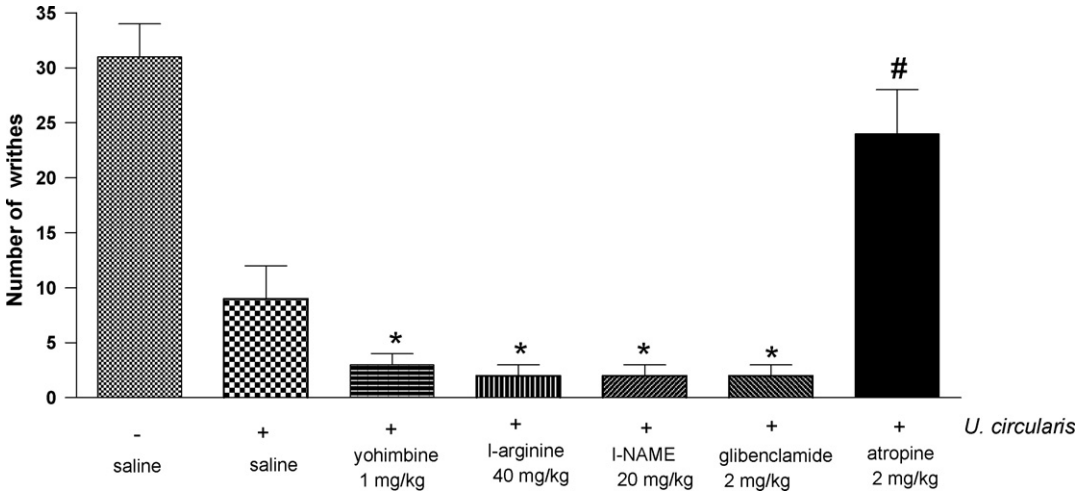

Fig. 2. Influence of several drugs in *Urtica circularis* extract antinociceptive effect. Results were obtained by intraperitoneal (i.p.) administration of *Urtica circularis* ethanolic extract (100 mg/kg) and i.p. administration of different drugs. Each value is the mean ± S.E.M. of results from 8 mice. Statistical differences from the controls were determined by ANOVA followed Dunnett's test. $^{*}P < 0.01$; $^{\#}P < 0.05$ (*Urtica circularis* + atropine versus *Urtica circularis*).

Table 2
Effect of *Urtica circularis* ethanolic extract in the acetic acid-induced abdominal writhing test.

Treatment	Number of writhes	% of inhibition
Control	31 ± 2	
Urtica circularis 10 mg/kg i.p.	31 ± 5	0
Urtica circularis 30 mg/kg i.p.	21 ± 3	32.2
Urtica circularis 100 mg/kg i.p.	9 ± 3*	70.9
Urtica circularis 300 mg/kg i.p.	6 ± 3*	80.6
Urtica circularis 250 mg/kg p.o.	32 ± 10	0
Urtica circularis 500 mg/kg p.o.	16 ± 1*	48.4
Indomethacin 10 mg/kg i.p.	13 ± 3*	58.1

Results were obtained by intraperitoneal (i.p.) or oral (p.o.) administration of ethanolic extract and i.p. administration of indomethacin. Each value is the mean ± S.E.M. of results from 8 mice. Statistical differences from the controls were determined by ANOVA followed Dunnett's test. * $P < 0.01$.

indomethacin (3 mg/kg, p.o.) significantly inhibited the nociceptive response induced by acetic acid (Table 3).

Interestingly, vitexin at 10 mg/kg i.p. produced a significant inhibition (91%) of acetic acid-induced abdominal constrictions in mice, and chlorogenic acid, caffeic acid and vicenin-2 produced an inhibition of 72%, 41% and 41%, respectively, whereas apigenin did not show any activity (Table 4).

Table 3
Effect of *Urtica circularis* ethanolic extract, indomethacin and the association of both in the acetic acid-induced abdominal writhing test in mice.

Treatment	Number of writhes	% of inhibition
Control	34 ± 2	
Urtica circularis 50 mg/kg p.o.	22 ± 1	35
Urtica circularis 100 mg/kg p.o.	23 ± 4	32
Urtica circularis 50 mg/kg + indomethacin 3 mg/kg p.o.	18 ± 5	47
Urtica circularis 100 mg/kg + indomethacin 3 mg/kg p.o.	15 ± 3*	56
Indomethacin 3 mg/kg p.o.	21 ± 3	38

Results were obtained by oral (p.o.) administration of ethanolic extract and indomethacin. Each value is the mean ± S.E.M. of results from 8 mice. Statistical differences from the controls were determined by ANOVA followed Dunnett's test.
* $P < 0.05$.

3.5. Effect on the stomach

There was not any loss of normal morphology, discoloration of mucosa or edema formation in the treated animals. Haemorrhages and petechial points only were observed in mice treated with *Urtica circularis* 300 mg/kg and indomethacin 10 mg/kg. However, the amount of animals which showed lesions and the magnitude of the lesions were of a lesser extent in the animals treated with the extract than the observed in those animals that received indomethacin (animals with lesion: *Urtica circularis* 300 mg/kg: 71%; indomethacin 10 mg/kg: 100%). The *Urtica circularis* 100 mg/kg group did not show a significant difference compared to the control group, which only received water. Although there was no difference between the stomach weights of the mice treated with the extract and the ones treated with indomethacin (control: 232.4 ± 10.3 mg; *Urtica circularis* 100 mg/kg: 249.2 ± 9.7 mg; *Urtica circularis* 300 mg/kg: 255.7 ± 11.3 mg; indomethacin 10 mg/kg: 247.6 ± 25.7 mg), further histological studies on stomach must be perform.

3.6. Acute toxicity

In order to evaluate the *Urtica circularis* extract safety, doses up to 3000 mg/kg were administered orally to mice. The oral LD_{50} is therefore greater than this amount in mice. No significant differences in body weight between the control and any of the treated groups were noted at any time. Besides, the extract did not produce any sign of toxicity at the tested doses during the period of obser-

Table 4
Effect of compounds isolated from *Urtica circularis* extract on acetic acid-induced abdominal writhing test in mice.

Treatment	Number of writhes	% of inhibition
Control	32 ± 4	–
Chlorogenic acid 10 mg/kg i.p.	9 ± 4**	72
Caffeic acid 10 mg/kg i.p.	19 ± 3*	41
Vitexin 10 mg/kg i.p.	3 ± 1**	91
Apigenin 10 mg/kg i.p.	34 ± 3	0
Vicenin-2 10 mg/kg i.p.	19 ± 3*	41
Indomethacin 10 mg/kg i.p.	12 ± 2**	62

Results were obtained by intraperitoneal administration of isolated compounds and indomethacin. Each value is the mean ± S.E.M. of results from 5 mice. Statistical differences from the controls were determined by ANOVA followed Dunnett's test.
* $P < 0.05$.
** $P < 0.01$.

vation and at necropsy no macroscopic changes in organs could be detected in the treated groups.

4. Discussion

The present study was undertaken to evaluate the antinociceptive effect of *Urtica circularis* ethanolic extract on three classical nociception models in mice: the acetic acid-induced writhing test, the formalin test and the hot plate test, which are useful methods for screening prospective antinociceptive compounds or plant extracts.

Although other species of the *Urtica* genus (*Urtica dioca*, *Urtica urens*) are used for pain relief, this is the first time that the antinociceptive effect of *Urtica circularis* in experimental pain models is reported.

Firstly, the aqueous and ethanolic extracts were evaluated, nevertheless only the ethanolic extract showed antinociceptive activity, supporting the traditional use of the plant as alcoholic solution. Therefore, it was decided to continue the study with the ethanolic extract.

On the other hand, since the route of administration is one of the most important factors affecting the results of *in vivo* methods, the i.p. administration was chosen for primary screening, nevertheless, the extract showed activity even if it was administered orally.

The nociception induced by formalin is associated with injured tissue and it is believed that it more closely resembles clinical pain in comparison to other test that employ mechanical or thermal stimuli, so the extract was evaluated in this test, firstly. The subcutaneous injection of formalin in the rat paw induces a biphasic response. The early phase is short-lived and initiates immediately after injection, being characterized by C-fiber activation due to peripheral stimuli. The late phase is a longer, persistent period caused by local tissue inflammation and also by functional changes in the dorsal horn of the spinal cord, therefore, this phase is inhibited both by opioids and antiinflammatory drugs. In this way, substances that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibit only the second phase (Le Bars et al., 2001). The effect observed with the extract suggested that the antinociceptive action would be related with peripheral mechanisms. In the same way, indomethacin inhibited formalin induced pain in the second phase while morphine, significantly attenuated the pain responses in the two phases. Besides naloxone, a non-selective antagonist of opioid receptors, did not change the *Urtica circularis* antinociceptive effect in formalin test, but inhibited the one produced by morphine. Moreover, in the hot plate test, a useful method for the evaluation of central antinociceptive activity, as well as a validated model for opioid-derived analgesic compounds, the *Urtica circularis* extract had no effect; however, the latency time was significantly increased by morphine. These observations together with the lack of activity in the first phase of the formalin test reinforce the notion that the opioid system is not involved in the extract's effect.

In the writhing model, *Urtica circularis* extract produced marked antinociceptive effect. This is a good model of visceral pain and the algesic effects of acetic acid are due to liberation and increased levels of several mediators such as histamine, serotonin, bradykinin, cytokines and eicosanoids in the peritoneal fluid. These mediators are able to increase vascular permeability, reduce the threshold of the nociception and stimulate nociceptive fibers (Deraedt et al., 1980; Ikeda et al., 2001). Additionally, different mechanisms, noradrenergic, cholinergic systems and NO/cGMP, $PKG/_{ATP}$ pathway have been involved in the acetic acid nociceptive response.

It was reported that noradrenergic systems would play a modulatory role in the expression of behavioural effects of this irritant agent (Korzeniewska-Rybicka and Plaźnik, 2001). Bezerra et al. (2008) showed that α2-adrenoceptors agonist significantly reduced the writhing response. Besides, these kinds of agonists are used in clinical practice for the treatment of acute pain events and prevention of postoperative pain (Eisenach et al., 1995). Nevertheless, according to our studies, yohimbine, an antagonist of α2-adrenoceptors, did not modify the extract's effect, discarding that a possible α2 agonism could be involved in its antinociceptive activity.

A growing amount of evidences point out L-arginine-nitric oxide pathway as a relevant factor for antinociceptive effect of many drugs. NO is able to increase the concentration of cGMP which will lead to activation of potassium channels. The opening of these channels induces the membrane hyperpolarization and reduces the depolarization and action potential transmission abilities of neurons, thereby inducing analgesia (Nguelefack et al., 2010). In the same sense, Sachs et al. (2004) and Vale et al. (2007) have described that NO/cGMP, $PKG/_{ATP}$ pathway is an additional mechanism of action of some peripheral analgesics and this events' sequence would be considered an antagonistic mechanism to the hyperalgesic state that occurs in inflamed tissues. Then, to further understand the mechanism of action of the extract and to determine the possible participation of this pathway in the antinociception induced by *Urtica circularis*, its activity was evaluated in presence of L-arginine (an oxide nitric synthase substrate), L-NAME (a non-selective oxide nitric synthase inhibitor) and glibenclamide (an ATP-sensitive K^+ channels blocker). None of these drugs reversed the antinociceptive effect of extract, suggesting a non-participation of this pathway.

Finally, atropine exhibited a significant inhibitory effect of the *Urtica circularis* extract antinociceptive activity, suggesting that muscarinic receptors would be involved in this action. It has been demonstrated that cholinomimetic agents have therapeutic potential for several clinically relevant pain states including inflammatory, neuropathic, visceral pain and arthritis (Pinardi et al., 2003; Wang et al., 2005; Jones and Dunlop, 2007) and several studies have shown that muscarinic agonists can reduce serum proinflammatory cytokines (Borovikova et al., 2000), modulating the pain.

Therefore, from the different mechanisms analyzed, only cholinergic system could be involved in the antinociceptive action of *Urtica circularis* extract.

In the acute pain models, *Urtica circularis* showed similar activity to indomethacin (positive control), a known nonselective COX inhibitor, but unlike this, the extract did not produce mucosal injury on the stomach at the lower dose. Since non-steroid antiinflammatory drugs (NSAIDs) are used for pain related diseases, such as osteoarthritis, but they are usually associated with serious complications (gastrointestinal bleeding and renal failure), the association with the extract could decrease the dose of NSAIDs needed to achieve the same antinociceptive effect. In this way, the coadministration of *Urtica circularis* extract and indomethacin, at doses in which each one of them did not produce any antinociceptive effect, exhibited a significant analgesic activity in the writhing model. In accordance with these findings, it has been reported in a randomized double-blind placebo-controlled clinical trial, that a food supplement containing *Urtica dioica* decreased the need for analgesics and NSAIDs and improved the symptoms of osteoarthritis (Jacquet et al., 2009). So, this association could be a relevant and useful treatment for diseases related with inflammatory pain, although this result must be confirmed in long term studies, in order to test the persistence of this effect.

Based on the pharmacological activity observed and that no scientific references about its toxicity have been described, the safety of the ethanolic extract was evaluated. LD_{50} is higher than 3000 mg/kg, suggesting that the extract has a low toxicity profile.

Finally, aiming to identify the substances that could be responsible for the activity of *Urtica circularis*, the compounds isolated from

the extract (caffeic acid, chlorogenic acid, vitexin and vicenin-2) were analyzed. Apigenin was also evaluated for its structural relation with vitexin and vicenin-2 but it did not produce any effect. Chlorogenic acid showed antinociceptive activity in the writing test, activity that Dos Santos et al. (2006) have previously reported in the formalin-induced pain test. Caffeic acid, a natural compound widely distributed in plants, produced a slight significant activity in this work as well as it was showed by De Campos Buzzi et al. (2009) in several experimental models. Nevertheless, in agreement to our knowledge, this is the first time, that vitexin and vicenin-2 showed a significant antinociceptive effect in an algesic chemical model.

5. Conclusion

The results of the present study demonstrate, for the first time, that the *Urtica circularis* extract has a dose-related antinociceptive action in chemical models of nociception in mice, suggesting that the extract and/or its active principles might represent potential therapeutic options for the treatment of pain related diseases. The antinociceptive action showed in the present study supports the ethnomedical use of this plant and contributes to the knowledge of the chemical composition of our medicinal flora. However, involvement of other components in the plant still has to be determined.

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