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Evaluation of Antinociceptive, Antinflammatory Activities and Phytochemical Analysis of Aerial Parts of *Urtica urens* L.

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The antinociceptive and antiinflammatory activities of the ethanol extract of the aerial part of *Urtica urens* were determined by experimental animal models. *U. urens* extract was found to possess significant antinociceptive activity in chemically induced mouse pain models (ED_{50} 39.3 mg/kg: 17.2–74.5 mg/kg) in the writhing test and 62.8% inhibition of the licking time in the late phase of the formalin test at a dose of 500 mg/kg p.o. and antiinflammatory activity on carrageenan-induced rat hind paw edema (41.5% inhibition at a dose of 300 mg/kg i.p.). The extract displayed activity neither in the thermal model of pain nor in the topical inflammation model. The major component of the extract was determined as chlorogenic acid (670 mg/1000 g dry weight) and could be partly responsible for this activity. **Copyright © 2010 John Wiley & Sons, Ltd.**

Keywords: Urtica urens; antiinflammatory; antinociceptive; chlorogenic acid.

INTRODUCTION

Urtica urens L. (Urticaceae) is known by the common names of ‘ortiga’, ‘ortiga crespa’, ‘ortiga chica’, ‘ortiga negra’, ‘caá poropé’, ‘rupá chico’ (Zuloaga and Morrone, 1999) and ‘dwarf nettle’ and is widely distributed in South America (Bolivia, Brazil, Chile, Uruguay and Argentina), Europe, Africa, Asia and Australia. It is an annual plant, 10–50 cm high (Burkart, 1987) which can be found in different types of grounds (waste land, roadsides, river banks, etc.) (Bombardelli and Morazzoni, 1997). The name *Urtica* of the genus derives from the Latin verb ‘urere’, that means to burn, because of its urticant hairs.

In South America, the aerial parts of *U. urens* are commonly used in popular medicine as a diuretic, depurative, antidiabetic and antirheumatic agent (Domínguez, 1982; Rojas Acosta, 1905; Gonzalez *et al.*, 1928). It has also been used for muscular or minor articulation pain relief. Besides, it is considered nutritious, being included in different food preparations (Martínez Crovetto, 1981; Domínguez, 1982).

The roots of *U. dioica* and *U. urens* have long been used in Europe for self-treatment of benign prostate hyperplasia (EMEA, 2008) and arthritic pain (Randall *et al.*, 2000). Recently, in an experimental model in rat, it was reported that *U. urens* could show potential chemoprotective ability (Ozkarsli *et al.*, 2008). Besides, different species from this genus (*U. dioica, U. macrorrhiza*) have shown high antioxidant activity (Hudec *et al.*, 2007) as well as antiinflammatory and analgesic effects in animal models and *in vitro* assays (Reihemann *et al.*, 1999; Yongna *et al.*, 2005).

The aim of this work was to contribute to the knowledge of the chemical composition of our medicinal flora and validate its ethnomedical use, trying to find a scientific justification for its popular use. In this way, the possible antinociceptive and antiinflammatory activities of the ethanol extract of *U. urens* were studied and a phytochemical analysis was carried out to find agents that could be helpful in the treatment of those pathologies associated with the inflammatory process.

MATERIALS AND METHODS

Plant material. Aerial parts of *U. urens* were collected in Facultad de Agronomía, Universidad de Buenos Aires, and Argentina in November 2007 and identified by Dr G. Giberti. A voucher specimen (BAF N 664) is deposited at the Herbarium of the Museo de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Preparation of plant extract. The dried aerial parts of *U. urens* (20 g) were ground into a fine powder and extracted four times by maceration with 80% ethanol at room temperature for 24 h. The extracts were then pooled, concentrated and lyophilized.

Drugs. Indomethacin, morphine sulfate, naloxone, lambda carrageenan and 12-O-tetradecanoylphorbol-13 acetate (TPA) were purchased from Sigma Chemical

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Co. (St Louis, MO, USA). Acetic acid and formalin were acquired from Merck (Darmstadt, Germany).

Animals. Female Swiss mice weighing 25–30 g and Sprague–Dawley female rats weighing 180–200 g were used taking into account international guiding principles and local regulations concerning the care and use of laboratory animals for biomedical research (ANMAT, 6344/96; 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.

Antinociceptive activity. *Writhing test (chemical stimulus).* The test was performed as described by Collier *et al.* (1968). Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 mL/10 g body weight. One group of mice was pretreated with the *U. urens* extract i.p. (10–500 mg/kg) and orally (p.o.) (250 mg/kg) 30 or 60 min, respectively, before acetic acid injection. Another group was given indomethacin (10 mg/kg i.p.) as a 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 the percentage of inhibition of abdominal writhes.

Formalin test (chemical stimulus). The procedure was described by Hunskaar and Hole (1987) and consisted of 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 *U. urens* extract (30–100 mg/kg, i.p. and 500 mg/kg, p.o.) 30 min and 1 h, respectively before the formalin injection. Indomethacin (30 mg/kg, i.p.) or morphine s.c. (10 mg/kg, s.c.) were used as reference drugs. One group was pretreated with naloxone (5 mg/kg i.p.) 15 min before administration of 100 mg/kg i.p. of *U. urens* extract. Control animals received only the vehicle. The 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 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.

Hot-plate test (thermal stimulus). 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, groups of animals were treated with the *U. urens* extract (250–500 mg/kg i.p.) or morphine (10 mg/kg s.c.) while control animals received the same volume of saline solution (10 mL/kg).

Antiinflammatory activity. *Ear edema in mice.* Ear edema was induced according to the De Young *et al.* (1989) method. The right ear of every mouse received TPA 0.125 μg/μL acetone solution as a topical application (10 μL for each side of the ear). *U. urens* extracts (dissolved in acetone) were applied topically immediately after TPA at doses of 0.8, 2 and 5 mg/ear. The left ear, used as a control, received vehicle. Indomethacin (1 mg/ear/20 μL) was used as a reference drug. After 4 h of TPA administration, the animals were killed and disks of 6 mm diameter were removed from each ear and their weight was determined. The swelling was measured as the difference in weight between the punches from right and left ears, and the percent inhibition of edema was calculated in comparison with control animals.

Carrageenan-induced edema in rats. Paw swelling was induced by subplantar injection of 0.1 mL 1% sterile lambda carrageenan in saline solution into the right hind paw (Winter *et al.*, 1962). *U. urens* extract was administered i.p. 30 min before carrageenan injection in doses of 100 and 300 mg/kg. Indomethacin (10 mg/kg) was used as a reference drug. The control group only received the vehicle (1 mL/kg i.p.). The inflammation was quantified by measuring the volume displaced by the paw, in a plethysmometer (Ugo Basile) at times 0 and 1, 3, 4 and 5 h after carrageenan injection. The difference between the left and the right paw volumes (indicating inflammation) was determined and the edema inhibition percent was calculated in comparison with the control animals.

Effect on the stomach. The extract (100 and 300 mg/kg) was administered i.p. to mice after 24 h fasting. Six hours later, the animals were killed 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 or negative controls, respectively.

Phytochemical analysis. *Total phenolics determination*. The *U. urens* extract total phenol content was determined by the Folin-Ciocalteu colorimetric method described by Singleton *et al.* (1999). The absorbance was measured at 760 nm and compared with a gallic acid calibration curve. The result was expressed as gallic acid equivalents per gram of extract (GAE/g).

HPLC. The HPLC method was developed and validated according Filip *et al.* (2001) and performed with a Varian 9000 instrument using a diode array detector. C18 column (Gemini 5 μm, 150 × 4.6 mm), solvent A: H_2O/AcOH (98:2), solvent B: MeOH/AcOH (98:2). Gradient: 15% B to 40% B, 30 min; 40% B to 75% B, 10 min; 75% B to 85% B, 5 min. Flow rate: 1.2 mL/min. Detection: 325 nm. Rheodyne injector fitted with a 20 μL loop. Quantification was achieved by the external standard method using Carl Roth chlorogenic acid.

Statistical analysis. Data were analysed for statistical significance of differences between treated and control group by analysis of variance followed by Dunnett's or Bonferroni tests. Values were considered to be significantly different when the value of $p < 0.05$. When appro-

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priate, the ED_{50} values were calculated (Litchfield and Wilcoxon, 1949).

RESULTS AND DISCUSSION

Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer and vascular diseases. Inflammatory reactions are not only the response of living tissues to injury and infection, but also are relevant to disease developments, such as asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis. Many natural products are used in traditional medical systems to treat the relief of symptoms from pain and inflammation (Kaplan *et al*., 2007; Ahmed *et al*., 2005).

In the present study, the antinociceptive and antiinflammatory activities of the *U. urens* 80% ethanol extract were investigated, applying experimental animal models, and phytochemical analysis. The results revealed that *U. urens* extract possesses significant antinociceptive activity in the chemically induced mouse pain models and antiinflammatory activity on carrageenan-induced rat paw edema. However, significant antinociceptive activity was found neither in the hot plate test nor in the topical antiinflammatory activity model in the tested conditions.

The potential systemic antiinflammatory activity of the extract was studied using carrageenan-induced paw edema in rats, which represents inflammation during the early stages of the acute process. The carrageenan injection produces a biphasic event. In the first phase, during the first hour, histamine, serotonin and bradykinin are the mediators involved, while prostaglandins are implied in the second phase (3–5 h). The results revealed that the administration of the ethanol extract of *U. urens* at 300 mg/kg showed a significant inhibition of paw edema starting 3 h after carrageenan administration (33% inhibition) with a maximum response at 5 h (41.5% inhibition), meanwhile indomethacin (10 mg/kg) caused a maximum inhibition of 38.4% at 5 h (Fig. 1).

Nevertheless, when the extract was administered topically it was not effective in inhibiting the edema development in the mouse ear edema induced by TPA at 0.8, 2 and 5 mg/ear, although indomethacin (1.0 mg/ear) attenuated significantly, the inflammation response (inhibition of 87.3%). It has been established that the mouse ear edema induced by TPA exerts its inflammatory effect through protein kinase C activation with the subsequent cytosolic phospholipase A_2 stimulation (Nishizuka, 1984), so phospholipase A_2 inhibitors could be effective against the edema induced in this model.

Taking into account that the antiinflammatory activity was observed only in the first model, it is possible to propose that the effect observed may be due to the ability of the extract to inhibit, systemically, the release and/or the activity of the prostaglandins, through the cyclooxygenase but not by lipooxygenase. However it can not be discarded that the absorption of the extract was not sufficient to show topical activity.

On the other hand, three animal models: acetic acid-induced writhing, formalin test and hot plate test, which are useful for screening prospective antinociceptive compounds or botanical extracts, have been used to evaluate the effect of the extract.

In the acetic acid-induced writhing test, local peritoneal receptors are postulated to be partly involved in the abdominal writhing response and the mechanism of the reaction to this nociceptive stimulus seems to be related to the prostanoid system (Nguemfo *et al*., 2007). The effect of the ethanol extract of *U. urens* on the writhing response in mice is shown in Table 1. The extract at doses of 30–500 mg/kg i.p. and 250 p.o. caused an inhibition of the writhing response induced by acetic acid. The maximal inhibition of the nociceptive response was achieved at a dose of 250 mg/kg i.p. The calculated ED_{50} was 39.3 mg/kg (95% confidence limits: 17.2–74.5 mg/kg). The antinociceptive effect might be mediated by its peripheral effects reducing prostaglandin synthesis and interfering with the mechanism of transduction in primary afferent nociceptors.

In order to evaluate different types of pain, the formalin test was used. The nociceptive stimulus produces two distinct phases involving different mechanisms. The first phase corresponds to acute neurogenic pain sensitive to drugs that interact with the opioid system. The second phase results from the action of inflammatory mediators in the peripheral tissues, such as prostaglandins, serotonin, histamine and bradykinin, and is inhib-

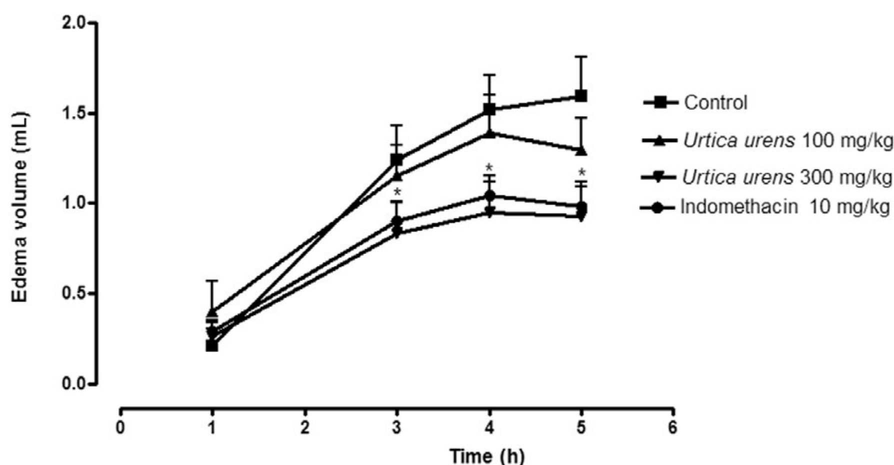

Figure 1. Effect of ethanol extract of *Urtica urens* in the carrageenan test. Results were obtained by i.p. administration of extract and indomethacin. Each value represents the mean ± SEM of results from six rats. Statistical differences from control group were determined by Bonferroni test (*$p < 0.01$ versus control group).

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Table 1. Effect of ethanol extract of *Urtica urens* in the writhing test

Treatment	Number of writhings	Inhibition (%)
Control	29 ± 2	
U. urens 10 mg/kg i.p.	35 ± 4	0
U. urens 30 mg/kg i.p.	12 ± 3[a]	58.6
U. urens 60 mg/kg i.p.	7 ± 4[a]	75.9
U. urens 250 mg/kg i.p.	1 ± 1[a]	96.5
U. urens 250 mg/kg p.o.	14 ± 2[a]	51.7
Indomethacin 10 mg/kg i.p.	13 ± 3[a]	55.2

Results were obtained by administration of extract (i.p. and p.o.) and indomethacin (i.p.). Each value is the mean ± SEM of results from eight mice. Statistical differences from controls were determined by ANOVA followed Dunnett's test ([a] $p < 0.01$ versus control group).

Table 2. Effect of ethanol extract of *Urtica urens* in the formalin test

Treatment	Licking time (s)	
	Early phase	Late phase
Control	62.4 ± 7.5	84.5 ± 11.9
U. urens 30 mg/kg i.p.	41.7 ± 6.5	78.5 ± 16.6
U. urens 100 mg/kg i.p.	36.4 ± 6.7	19.2 ± 8.1[a]
U. urens 500 mg/kg p.o.	44.2 ± 7.5	31.4 ± 16.4[b]
U. urens 100 mg/kg i.p. + naloxone 5 mg/kg i.p.	39.7 ± 6.2	32.1 ± 12.3[b]
Morphine 10 mg/kg s.c.	2.0 ± 0.8[a]	0 ± 0[a]
Indomethacin 30 mg/kg i.p.	50.4 ± 5.5	38.2 ± 5.4[b]

Results were obtained by administration of extract (i.p. and p.o.), morphine (s.c.) and indomethacin (i.p.). Each value is the mean ± SEM of results from 10 mice. Statistical differences from controls were determined by Dunnett's test ([a] $p < 0.01$, [b] $p < 0.05$ versus control group).

ited by opioids and antiinflammatory drugs. Substances that act primarily as central analgesics inhibit both phases, while peripherally acting drugs inhibit only the second phase (Le Bars *et al*., 2001). Therefore, this test can be used to clarify the possible mechanism of the antinociceptive effect of a proposed analgesic. 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 the control group. As shown in Table 2, pretreatment with 100 mg/kg i.p. (30 min) or 500 mg/kg p.o. (60 min) produced a marked reduction of 77.3% and 62.8% of the licking time in the late phase, respectively. These results suggest that the antinociceptive effect observed could be related with peripheral mechanisms. In the same way, indomethacin (10 mg/kg) inhibited pain induced by formalin in the second phase while the positive control drug, morphine (10 mg/kg), significantly attenuated the pain responses of the two phases. Besides, the pretreatment with naloxone, a non-selective antagonist of opioid receptors, could not antagonize the observed *U. urens* effect, which is an indication that the extract does not possess any central activity.

In the same way, 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 *U. urens* extract (250–500 mg/kg) had no effect on thermal pain (time of latency = 8.5 ± 1.8) while the latency time was significantly increased by morphine (10 mg/kg) 30 min after administration (time of latency = control group: 7.6 ± 1.4 s, morphine group: 25.6 ± 1.4 s), suggesting its effect is unrelated to activation of the opioid system.

On the other hand, the *U. urens* extract did not produce any loss of normal morphology, discoloration of mucosa or edema formation in any stomach of any animal. Hemorrhages and petechial points observed in mice treated with *U. urens* 300 mg/kg were of minor magnitude compared with those observed one in animals that received indomethacin 10 mg/kg. The *U. urens* 100 mg/kg group did not show a significant difference compared with the control group, which only received water. Although there was no difference between the stomach weights of the mice treated with the extract and those treated with indomethacin, further histological studies on the stomach must be performed.

Bringing all these results together and taking into account that the injuries induced in the stomach by *U. urens* are similar to those of indomethacin, it could be concluded that the *U. urens* extract shows analgesic and antiinflammatory activities similar to a non-steroidal antiinflammatory drug pattern.

Since the route of administration is one of the most important factors affecting the results of *in vivo* methods, i.p. administration was chosen for primary screening, based on the idea that *in vivo* assays with i.p. administration might be generally more sensitive than those with p.o. administration. According to the results, at the same dose, the activity on antinociceptive models was lower with p.o. administration than i.p. administration. Inadequate absorption and first-pass metabolism may be possible causes. Recently, Ozkarsli *et al.* (2008) observed that *U. urens* produced significant inhibition of 7-methoxyresorufin O-demethylase, depending on cytochrome P450-monooxygenase system, so it may be possible that this effect could modify the bioavailability of the extract administered by the oral route.

According to the phytochemical analysis, the total phenolic content expressed as gallic acid equivalents for the *U. urens* extract is 10.4 GAE/g. Chlorogenic acid was the most abundant phenolic compound in the extract (670 mg/100 g of dry weight). Its presence in *U. urens* extract has been reported previously (Budzianowski, 1991; EMEA, 2008). When the chromatogram was observed at different peaks (254 and 360 nm), no other significant points were found. The corresponding fingerprint chromatogram is shown in Fig. 2.

Phenolic compounds are numerous and ubiquitous in the plant kingdom and exhibit a wide range of biological effects. Beneficial properties such as antioxidant, hypoglycemic, antiviral, hepatoprotective, antiinflammatory and analgesic activities have been attributed to chlorogenic acid (Alonso-Castro *et al*., 2008; Dos Santos *et al*., 2006). Recently, it has been shown that chlorogenic acid suppressed lipopolysaccharide-induced cyclooxygenase-2 expression in RAW cells (Shan *et al*., 2009). This evidence suggests that the observed properties of *U. urens* extract in this study may be explained, at least in

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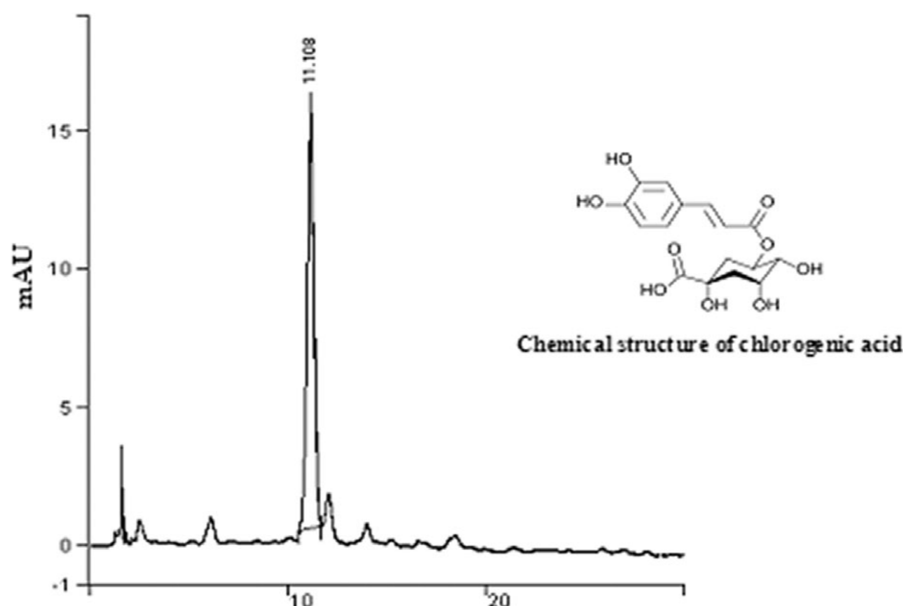

Figure 2. Fingerprint chromatogram of the ethanol extract of *U. urens*. The peak at 11.233 min retention time matches the chlorogenic acid standard.

part, by the presence of chlorogenic acid. Additional experiments are necessary in order to clarify the activity of this compound on the animal models used in this work.

Although nettles have a long history of use as a nutritious food or herbal remedy, further rigorous acute and subacute toxicity studies are required to confirm the safety of this herbal medicine in the treatment of inflammation complaints and pain.

In conclusion, this work reveals that *U. urens* has antinociceptive and antiinflammatory activities and that it would behave as a non-steroidal antiinflammatory drug. Chlorogenic acid, its main component, could be partly responsible for this activity.

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Conflict of Interest

No conflict to disclose.

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