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Anti-Inflammatory and Antinociceptive Activity of *Urera aurantiaca*

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***Urera aurantiaca* Wedd. (Urticaceae) is a medicinal plant commonly used in traditional medicine to relieve pain in inflammatory processes. In the present study, the *in vivo* anti-inflammatory and antinociceptive effects of *U. aurantiaca* methanolic extract and its possible mechanisms of action were investigated. The extract showed anti-inflammatory activity in the ear edema in mice test (34.3% inhibition), myeloperoxidase (MPO) activity was markedly reduced in animals administered with the extract: within 49.6% and 68.5%. In the histological analysis, intense dermal edema and intense cellular infiltration of inflammatory cells were markedly reduced in the ear tissue of the animals treated with the extract. In the carrageenan-induced hind paw edema in rats assay the extract provoked a significant inhibition of the inflammation (45.5%, 5 h after the treatment) and the MPO activity was markedly reduced (maximum inhibition 71.7%), The extract also exhibited significant and dose-dependent inhibitory effect on the increased vascular permeability induced by acetic acid. The extract presented antioxidant activity in both 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azinobis 3-ethylbenzothiazoline 6-sulfonic acid tests and its total phenol content was 35.4 ± 0.06 mg GAE/g of extract. Also, the extract produced significant inhibition on nociception induced by acetic acid (ED_{50}: 8.7 mg/kg, i.p.) administered intraperitoneally and orally. Naloxone significantly prevented this activity. Copyright © 2014 John Wiley & Sons, Ltd.**

Keywords: Urera aurantiaca; anti-inflammatory activity; antinociceptive activity; *in vivo*; antioxidant activity.

INTRODUCTION

Inflammation is the initial response of the body to tissue damage caused by mechanical, chemical, or microbial stimuli. The main cells involved in the inflammatory response are monocytes/macrophages, polymorphonuclear leucocytes, and endothelial cells. When these cells become activated, they aggregate and infiltrate tissues where they undergo a respiratory burst, increasing their oxygen use and production of cytokines, reactive oxygen species (ROS), and other mediators of inflammation. These events can initiate and also perpetuate inflammatory cascades and cause subsequent tissue damage (Kaplan *et al*., 2007).

Inflammation and pain are usually managed with non-steroidal anti-inflammatory drugs (NSAIDs). However, in chronic pain conditions NSAIDs are used for prolonged time which ultimately leads to severe toxicities including gastric and renal adverse effects (Sheeba and Asha, 2009). Many natural products are used in traditional medical systems to treat pain and inflammation symptoms (Kaplan *et al*., 2007; Ahmed *et al.,* 2005). Investigation of natural remedies is required to efficiently control the pain and inflammation with least side effects.

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Urera aurantiaca Wedd. (Urticaceae), commonly known as 'ortiga colorada', 'pino guasú', and 'pica pica', is an Argentinean native herb also distributed in Paraguay, Uruguay, Bolivia, and Brazil (Martínez Crovetto, 1981; Rondina *et al*., 2003). This bush can reach more than 60 cm high and is usually found in lowland riverside forests and hygrophilerain forests (Burkart, 1987). *U. aurantiaca* is well known by rural people of these countries for its medicinal properties: it is popularly used for rheumatic pain, for the treatment of varicose vein, furuncles, bruises, and inflammation (Martínez Crovetto, 1981). It is also used for tooth pain, skin issues, and trauma (Rondina *et al*., 2003).

Some pharmacological activities related to inflammatory processes have been reported for other species that belong to this family: *Urtica dioica*'s activity in the treatment of arthritic pain (Randall *et al*., 2000), anti-inflammatory and analgesic effects of *U. dioica* and *Urtica macrorrhiza* in *in vitro* and *in vivo* assays (Riehemann *et al*., 1999; Yongna *et al*., 2005), and anti-inflammatory and antinociceptive activities of *Urtica circularis* and *Urtica urens* reported by our investigation group (Marrassini *et al*., 2010; Gorzalczany *et al*., 2011; Marrassini *et al*., 2011). In Europe, *U. dioica* and *U. urens*' extracts are recommended for the treatment of benign prostate hyperplasia (European Medicines Agency (EMEA), 2008),

Nevertheless, despite its folkloric use, no scientific evaluation of this plant related to the inflammatory process has been carried out to date. Therefore, the present study aims to examine the effects of *U. aurantiaca* on inflammatory and nociceptive models in *in vivo* assays.

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Attempts have been made to further investigate some of the possible mechanisms that underlie the pharmacological activity of the extract.

MATERIALS AND METHODS

Plant material. *U. aurantiaca* Wedd. (Urticaceae) was collected in Parque Nacional Cerro Corá, Naranja-hai (22° 38′S; 58° 36′W, 288-m elevation, 2.5-m alt.) Depto. Amambay, Paraguay, in March 2008 and identified by Dr R. H. Fortunato. A voucher specimen (no. 9339) is deposited at Museo de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Extraction. The dried aerial parts of *U. aurantiaca* were ground into a fine powder and extracted sequentially by maceration with dichloromethane, ethyl acetate, and methanol. The methanol extract was concentrated and lyophilized and used for all the experiments (yield: 1.81%).

Phytochemical analysis. Total phenol content of *U. aurantica* extract was determined by 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 mg of gallic acid equivalents per gram of extract (GAE/g).

Drugs. Indomethacine, naloxone, yohimbine, atropine, glibenclamide, N ω -Nitro-L-arginine methyl ester hydrochloride (l-NAME), ondansetron, methylene blue, carrageenan, 12-O-tetradecanoylphorbol-13-acetate (TPA), Evans blue, hexadecyltrimethylammonium bromide, *O*-dianisidine dihydrochloride, thiobarbituric acid, and 1,1,3,3-tetramethylpropan were purchased from Sigma Chemical Co. (St. Louis, MO., USA). Ethanol, formaldehyde and acetic acid were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade.

Animals. Female Sprague–Dawley rats (180–200 g) and female Swiss mice (25–30 g) were obtained from Animal House of the Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires. The experiments were carried out 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, Commission on Life Sciences, National Research Council, 1996). The experiments were approved by the local Ethics Committee (Exp-FyB: 0738658/2011). 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.

Ear edema in mice test. Ear edema was induced according to Carlson *et al.* (1985). Groups of six mice each were used. The right ear of each mouse received a topical application of 2.5 μg of TPA in 0.125 μg/μl acetone solution (10 μl to each side of the ear). *U. aurantiaca* extract dissolved in EtOH 80% at doses of 0.1, 0.3, or 1 mg/ear, was topically applied immediately after TPA. The left ear, used as control, received the vehicle (acetone). Indomethacin (0.5 mg/ear/20 μl) was used as the anti-inflammatory reference drug. After 4 h, animals were sacrificed, and disks of 6 mm diameter were removed from each ear and their weights determined. The swelling was measured as the difference in weight between the punches from right and left ears, and the percentage inhibition of edema was calculated in comparison to control animals.

Histological study. Samples of ears were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5-μm thickness were prepared and stained with hematoxylin and eosin and examined under a light microscope.

Myeloperoxidase (MPO) activity. MPO activity, as an indicator of polymorphonuclear leukocyte accumulation, was determined by Paiva *et al.*, 2003 method. A punch of each ear from extract, indomethacin, or saline treated animals was homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 50 mM potassium phosphate buffer (pH 6), before sonication in an ice bath for 10 s. The homogenates were freeze-thawed three times, repeating the sonication, and then they were centrifuged for 20 min at 20,000 rpm at 4 °C. The level of MPO activity was measured spectrophotometrically. Of the material to be measured, 0.1 ml was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was then measured for 5 min using a Metrolab spectrophotometer (Metrolab 325 BD). Myeloperoxidase activity was expressed in optic density (DO) × 100/mg of tissue.

Carrageenan-induced hind paw edema in rats assay. Paw swelling was induced by sub-plantar injection of 0.1 ml of 1% sterile lambda carrageenan in saline solution into the right hind paw (Winter *et al.*, 1962). Groups of six animals were used for each treatment. The extract (dissolved in water) was administered i.p. 30 min before carrageenan injection at doses of 100 and 300 mg/kg. Indomethacin (3 mg/kg, i.p.) was used as the anti-inflammatory positive control drug. The control group received only 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. Also, area under the time-course curve ($AUC_{0–5}$) was calculated.

Thiobarbituric acid-reactant substances measurement. Thiobarbituric acid-reactant substances measurement, which is considered a good indicator of lipid peroxidation,

was determined in extract, indomethacin, and saline-treated paws, collected 5 h after carrageenan injection according to Mariotto *et al.* (2008). Briefly, tissues were homogenized, and aliquot of the homogenate was added to a reaction mixture containing sodium dodecyl sulfate, acetic acid and thiobarbituric acid. Samples were then boiled for 1 h at 95 °C. The optical density at 532 nm was measured using Metrolab 325 BD spectrophotometer. Thiobarbituric acid-reactant substances were calculated by comparison with standard solutions of 1,1,3,3-tetramethylpropan. The paw MDA (malondialdehyde) level was expressed as nmol/mg protein.

Vascular permeability. According to Yu *et al.* (2012), animals were pretreated with *U. aurantiaca* extract, (10, 30, 100, and 300 mg/kg i.p.) or the vehicle (10 ml/kg, i. p.). 30 min after, each mouse received an intravenous injection of 0.5% Evans blue solution (*w/v*, in 0.9% of saline, 0.1 ml/10 g), and then a 0.8% acetic acid solution i.p. injection (0.2 ml/mouse), to induce inflammation. Twenty minutes after acetic acid administration, the animals were sacrificed, and the peritoneal cavity was washed with 6 ml of cold normal saline (divided into several washings). With a gentle manual massage, the exudates were retrieved, and their volume was added to 10 ml of saline, followed by centrifugation for 15 min at 3000 rpm. The supernatant optical density was measured at 590 nm in a Metrolab 325 BD spectrophotometer. Dyed extravasations were quantified from the standard curve.

Acetic acid-induced abdominal writhing test. The test was performed as described by Collier *et al.* (1968). Nociception was induced by intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 ml/10 g body weight. Mice were treated with *U. aurantiaca* extract 3–30 mg/kg, i.p. or 100–1000 mg/kg, p.o. for 30 min or 60 min, respectively, 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 writhes (full extension of both hind paws) were cumulatively counted over a period of 20 min immediately after the acetic acid injection. Doses of extract were selected based on pilot experiments in our laboratory. A significant reduction in the number of abdominal contractions between the control animals and pretreated animals was considered as indicative of antinociceptive activity.

Evaluation of the mechanism of action of *U. aurantiaca* extract antinociceptive activity. To assess the possible participation of different systems in the antinociceptive effect of *U. aurantiaca* extract, mice were pre-treated with naloxone (1 mg/kg i.p.), a non-selective antagonist of opioid receptors; 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; glibenclamide (10 mg/kg i.p.), an ATP-sensitive potassium channel blocker; ondansetron (0.2 mg/kg i.p.), a 5-HT_3 receptor antagonist; methylene blue, an inhibitor of nitric oxide-stimulated guanylate cyclase activity (20 mg/kg i.p.); and l-NAME (30 mg/kg i.p.), a non-selective inhibitor of nitric oxide synthase, 30 min before *U. aurantiaca*'s extract (10 mg/kg i.p.). Doses and drug administration schedules were selected based on previous reports and on pilot experiments in our laboratory (Abdel-Salam, 2006; De Mattos *et al.*, 2007; Spindola *et al.*, 2011; Vidyalakshmi *et al.*, 2010). The nociceptive response was evaluated in the acetic acid-induced abdominal writhing test.

Assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The scavenging activity of the extract on the stable free radical DPPH was assayed using the modified Blois (1958), in which the bleaching rate of DPPH is monitored at a characteristic wavelength in the presence of the sample. A volume of 0.1 ml of an aqueous dilution of the extract was mixed with 0.5 ml of a 500 µM DPPH solution in absolute ethanol and 0.4 ml of a 0.1 M Tris–HCl buffer, pH 7.4. The mixture was kept for 20 min in the darkness, and then the absorbance was read at 517 nm. The percentage of decrease of DPPH absorbance was calculated by measuring the absorbance of the sample and applying the following equation: % of inhibition = $[1 - (As/A_0)] \times 100$, where As is absorbance of sample and A_0 is the absorbance of the DPPH solution. Different concentrations of ascorbic acid were used as positive controls for antioxidant activity.

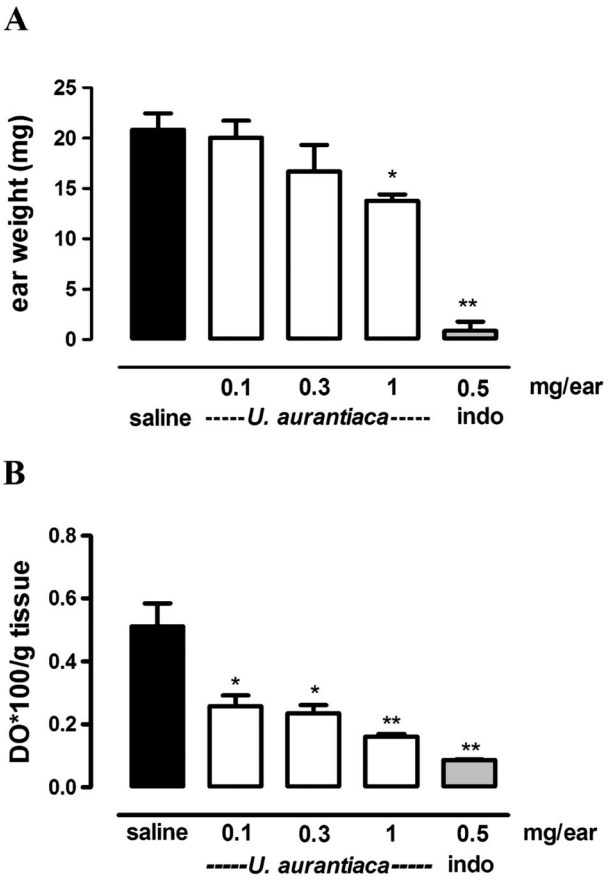

Figure 1. Effects of *U. aurantiaca* extract and indomethacin (indo) on ear weight (A) and MPO activity (B) in ear edema induced by TPA. Each value is the mean ± SEM of results from six mice. Statistical differences from saline group were determined by ANOVA followed by Dunnett's test. ** $P < 0.05$; * $P < 0.01$.

Assessment of 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid (ABTS) radical scavenging activity. The scavenging activity of the extract on the free radical ABTS was measured using Re *et al.* (1999) with slight modifications. Briefly, a volume of 10 µl of an ethanol dilution of the extract was mixed with 990 µl of an ABTS solution in absolute ethanol. The absorbance was read at 734 nm within 6 min. This activity was compared to the one presented by TROLOX® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

Statistical analysis. The pharmacological results are expressed as means ± SEM (standard error of the mean). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by the Dunnet or Bonferroni's tests. *P* values lower than 0.05 were considered to be statistically significant (Graph Pad Prism 5, version 5.03 for windows). ED_{50} values were calculated (Litchfield and Wilcoxon, 1949).

RESULTS

The extract's topical anti-inflammatory activity was tested in the ear edema in mice assay, and the results are shown in Fig. 1A. The maximal anti-inflammatory activity (inhibition of 34.3%) was obtained at a dose of 1 mg/ear. Meanwhile, the anti-inflammatory control drug, indomethacin (0.5 mg), exhibited anti-inflammatory activity with an inhibition of 95.7%.

The inflammation caused by TPA was associated with an increase in MPO activity. MPO activity was markedly reduced in animal groups that received either *U. aurantiaca* extract or indomethacin. The inhibitions were within 49.6% and 68.5% for the extract and 83.2% for indomethacin, respectively (Fig. 1B).

Increased skin thickness is often the first hallmark of skin irritation and local inflammation. This parameter is an indicator of a number of processes that occur during skin inflammation. In this sense, microscopic analysis of the sections of mice ears 4 h after TPA application showed intense dermal edema and increased inflammatory cells infiltration and epidermal thickness as compared with the control group which showed a normal tissue histoarchitecture. Ear tissue of the animals treated with *U. aurantiaca* extract and indomethacin showed a significant reduction of TPA induced leukocyte infiltration as well as dermal edema (Fig. 2).

The local injection of carrageenan induced a gradual increase in the hind paw edema volume in the control group. The effect was evident from the first hour after the phlogistic agent injection and persisted even 5 h later. Indomethacin treatment (positive control) exhibited a

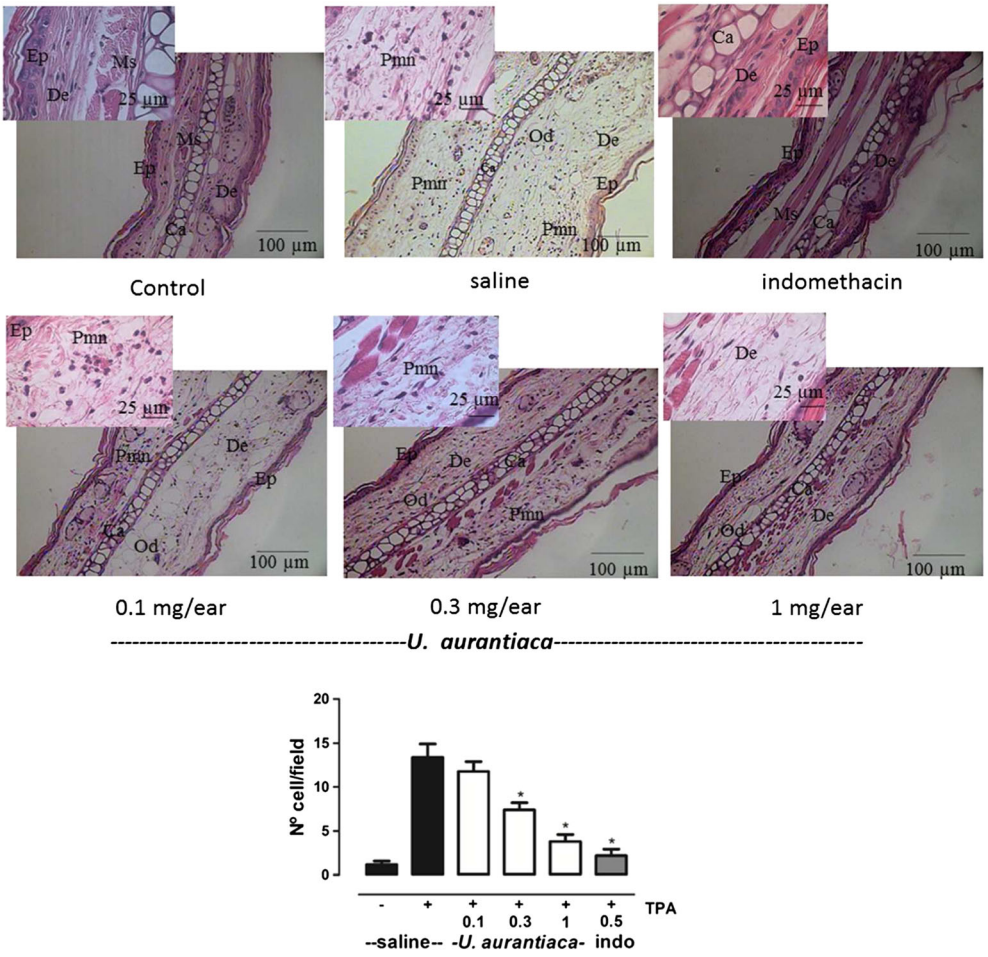

Figure 2. Representative photomicrographs of transversal cuts of mice ears (HE-20× and 100×) 4 h after TPA application. Ep: epidermis, De:dermis, Od: oedema, Ca: cartilage, Pmn: polymorphonuclear. Ms: muscle. Number of polymorphonuclear cell per field in 100×. Each value is the mean ± SEM of results from six mice. Statistical differences from saline group were determined by ANOVA followed by Dunnett's test. * $P < 0.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

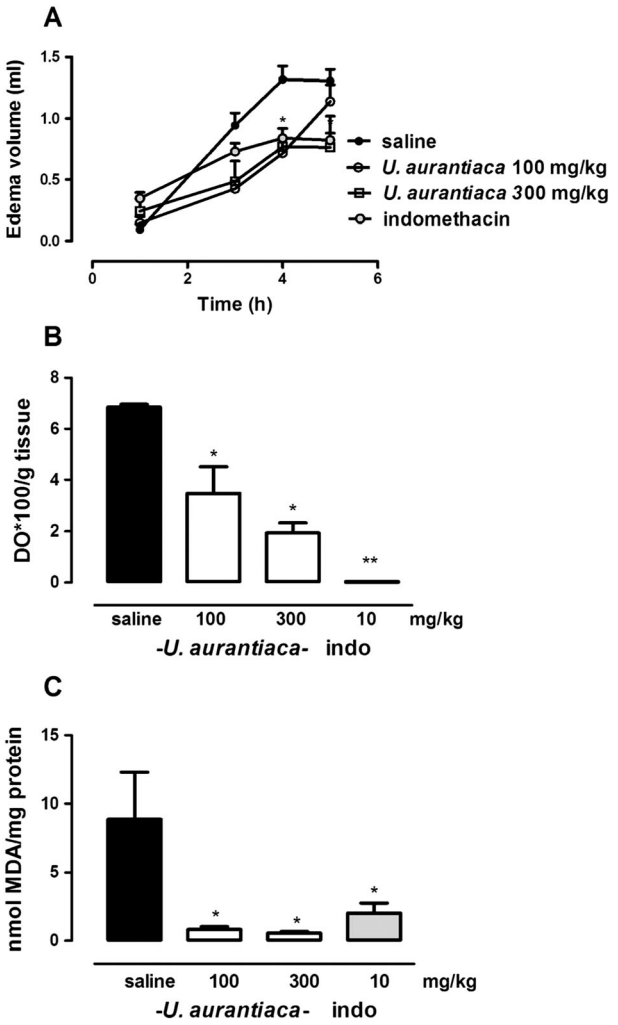

Figure 3. Effects of *U. aurantiaca* extract and indomethacin (indo) in volume (A), MPO activity (B), and MDA quantification (C) in rat paw edema induced by carrageenan. Each value is the mean ± S.E.M. of results from six rats. Statistical differences from the saline group were determined by ANOVA followed Bonferroni test (volume) or Dunnett test (MPO and MDA). $*P < 0.05$, $**P < 0.01$.

significant inhibitory effect on paw swelling (inhibition: 27–45%; Fig. 3A). The extract induced a significant anti-inflammatory activity with an inhibition of 41.6 and 45.5% at 4 and 5 h, respectively, after administration of 300 mg/kg. However, the dose of 100 mg/kg produced an inhibition of 45.7% at 4 h, which was reduced the next hour (inhibition of 12.7%). Besides, total oedema effect calculated as AUC_{0-5}, indicated a significant reduction in *U. aurantiaca* 100 mg/kg (2.07 ± 0.34), *U. aurantiaca* 300 mg/kg (2.12 ± 0.44) and indomethacin (2.69 ± 0.22) groups compared with saline group (3.48 ± 0.28).

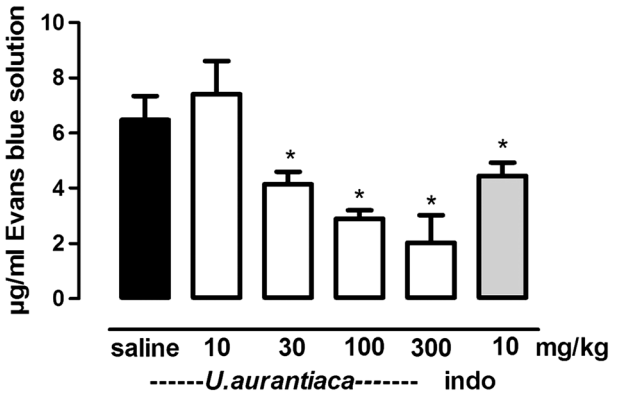

Figure 4. Effects of U*. aurantiaca* extract and indomethacin (indo) on the enhancement of vascular permeability induced by acetic acid. Each value is the mean ± S.E.M. of results from six mice. Statistical differences from saline group were determined by ANOVA followed by Dunnet's test $*P < 0.05$.

The inflammation caused by carrageenan was associated with an increase in MPO activity. In the groups treated with *U. aurantiaca* extract, the MPO activity was significantly decreased (Fig. 3B) with a maximum inhibition of 71.7%, at dose of 300 mg/kg; meanwhile, the control group, treated with indomethacin, produced an inhibition of 99% of the MPO activity.

Also, MDA level was decreased significantly by treatment with *U. aurantiaca* extract at doses of 100 and 300 mg/kg i.p, as well as 10 mg/kg of indomethacin (Fig. 3C). As shown in Fig. 4, the extract at 30, 100, and 300 mg/kg exhibited significant and dose-dependent inhibitory effect on the increased vascular permeability induced by acetic acid in mice. The anti-inflammatory control drug, indomethacin (10 mg/kg, i.p.), also reduced the dye leakage considerably with an inhibition of 31.2%.

U. aurantica extract (3–30 mg/kg i.p.) produced a dose-related inhibition of acetic acid-induced writhing response (ED_{50}: 8.7 mg/kg, 95%: 14.7–4.7 mg/kg; Fig. 5A). The highest antinociceptive effect, 76.0%, was obtained at a dose of 30 mg/kg, i.p. In addition, 300 mg/kg, p.o., produced a significant inhibition of the writhing response (35.7%). The mechanism of action of *U. aurantica*'s extract was investigated through the pre-treatment of animals with several drugs that interfere in different systems. The pre-treatment with yohimbine, atropine, glibenclamide, methylene blue, l-NAME, and ondansetron did not modify the antinociceptive response elicited by the extract.

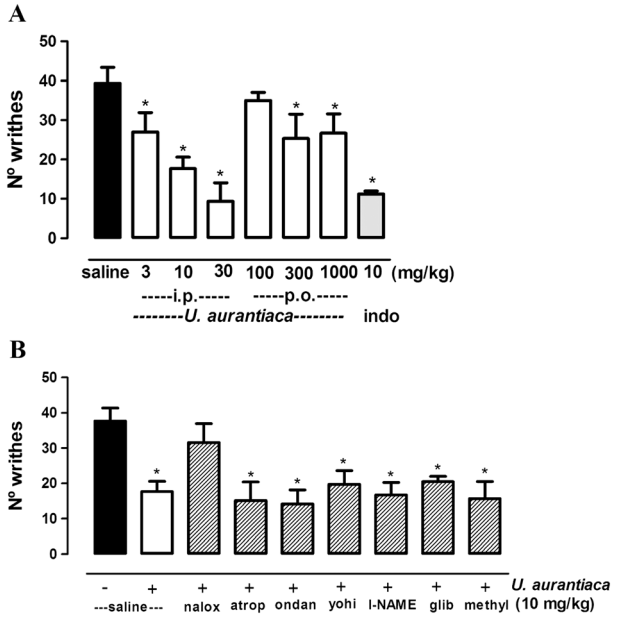

Figure 5. A. Results were obtained by oral (p.o) or intraperitoneal (i.p) administration of *U. aurantiaca* extract and indomethacin (indo) in writhing test. B. Results were obtained by i.p. administration of extract and different drugs (naloxone, atropine, ondansetron, yohimbine, l-NAME, glibenclamide, methylene blue) in writhing test. Each value represents mean ± S.E.M. of six mice. Statistical differences from the controls were determined by Dunnet's test $*P < 0.05$ versus saline group.

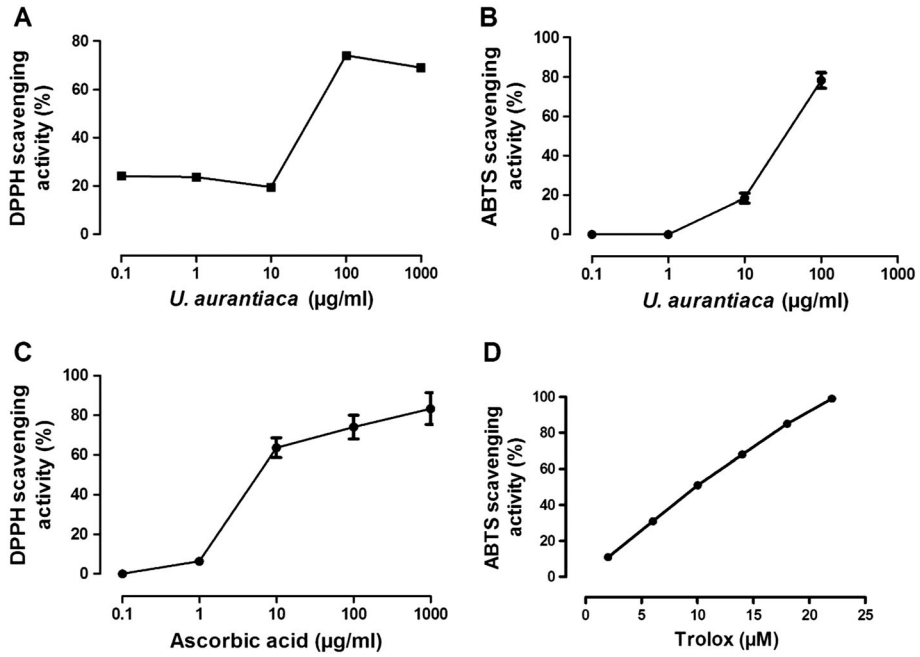

Figure 6. Antioxidant activity of *U. aurantiaca* extract. A. DPPH scavenging activity of the extract. B. ABTS scavenging activity of the extract. C. DPPH scavenging activity of ascorbic acid. D. ABTS scavenging activity of trolox.

Nevertheless, naloxone significantly prevented the antinociceptive activity of *U. aurantica* extract (Fig. 5B).

The phytochemical analysis showed that the total phenol content as mg of GAE/g of extract was *U. aurantiaca* 35.4 ± 0.06 mg GAE/g (data not shown in any figures).

The scavenging activity of the extract was concentration dependent as shown in Fig. 6A. The results indicate that *U. aurantiaca*'s extract shows high antioxidant activity reaching 74% of DPPH scavenging activity at 100 µg/ml. The ascorbic acid (control antioxidant drug) showed a similar activity at the same concentration (Fig. 6C). In the same sense, when reacted with ABTS, the extract showed the highest scavenging activity at 100 µg/ml (Fig. 6B), although this activity was smaller than the activity produced by Trolox, the reference drug (Fig. 6D).

DISCUSSION

Natural products derived from plants have been used in folk medicine to treat different diseases related to inflammatory processes. In this sense, with the objective to contribute to the knowledge of the medicinal flora, considering the popular use of this plant, and taking into account the studied activities of different species from this family, a pharmacological study of the methanol extract of the dried aerial parts of *U. aurantiaca* was done. To our knowledge, this is the first time that the anti-inflammatory, antinociceptive, and antioxidant activities of *U. aurantiaca* in experimental models are reported.

The extract's anti-inflammatory activity was demonstrated using two classical *in vivo* models with different mechanisms. On the one hand, the extract showed a systemic anti-inflammatory activity in carrageenan-induced hind paw edema model. On the other hand, *U. aurantiaca* extract demonstrated to possess topical antiedematous effect in the ear edema in mice test induced by TPA. In the carrageenan model the early phase of the inflammation starts with the release of histamine, serotonin and similar compounds, whereas, the second phase is due to the activation of prostaglandins. In the ear edema test, TPA exerts its inflammatory effect through proteinkinase C activation with the phospholipase A2 stimulation, which leads to the release of arachidonic acid and the biosynthesis of prostaglandins and leukotrienes (Carlson *et al.*, 1985). These tests represent acute inflammation models, and since the extract showed to be active in both, it can be suggested that blockade of the cyclooxygenase pathway which is common to both models, could be involved in the extract's anti-inflammatory activity.

MPO is a member of the haem peroxidase-cyclooxygenase superfamily found primarily in azurophilic granules of neutrophils and is used as a marker for tissue neutrophil content (Prokopowicz *et al.*, 2012). In inflamed tissues, MPO activity is significantly increased, indicating that neutrophils accumulation is a critical event in the pathogenesis of inflammation. In the last years, there has been an important development of therapeutic intervention strategies aiming at an efficient MPO inhibition (Malle *et al.*, 2007). The MPO decrease induced by *U. aurantiaca* extract in carrageenan and TPA tests offers an attractive explanation for its mechanism of action and suggests it could represent a therapeutically useful MPO inhibitor.

Also, during inflammation, neutrophils migrate to sites of trauma and release not only tissue destructive enzymes such as MPO, but superoxide anions that can exacerbate the inflammatory response. Neutrophils are armed with a variety of potent systems, including NADPH oxidase that is capable of generating vast amounts of ROS and has long been considered responsible for tissue damage and the initiation and propagation of the inflammatory process (Björkman *et al.*, 2008). The role of oxidative stress is considered as a remarkable point which is frequently reported in the signalling cascade of inflammation as well as in chemoattractant production and there

are sufficient evidences suggesting its involvement in pathology and complications of many clinical disorders. Medicinal plants have been traditionally employed for the treatment of diseases that recently appeared to be associated with oxidative stress (Saeidnia and Abdollahi, 2013). The extract showed an antioxidant activity in DPPH and ABTS tests, which have been widely used for evaluating antioxidant properties of natural products. In these tests, antioxidants intercept the free radical chain oxidation by donating hydrogens from the phenolic hydroxyl groups, thereby forming stable end products, which do not initiate or propagate further oxidation. MDA, one of the most important markers of free radical generation, was reduced by the extract *in vivo*, indicating that the extract has a beneficial effect on lipid peroxidation induced by oxidants on the polyunsaturated fatty acids of the membranes. So, *U. aurantiaca* extract showed a significant antioxidant activity both *in vitro* and *in vivo*, and these effects could play an important role in the mechanisms underlying its pharmacological activity: acting as an initiating radical species scavenger or altering peroxidation kinetics, decreasing the fluidity of the membrane that could sterically restrict peroxidative reactions (El-Beltagi and Mohamed, 2013). Finally, both mechanisms protect tissues from damage induced by oxidants.

It is known that alterations in ROS level contribute to characteristic microvascular inflammation responses, such as leukocyte recruitment and increased vascular permeability. A transient increase in vascular permeability is also an important characteristic of many disease states, where inflammatory mediators (cytokines, prostaglandins) and vascular endothelial growth factor are involved (Goddard and Iruela-Arispe, 2013). The present study provides evidence that *U. aurantiaca* extract has a protective effect on acetic acid stimulated vascular permeability *in vivo*, collaborating in the recovery of the highly coordinated process that regulates the passage of solutes between the blood and tissues, maintaining the vascular integrity, crucial for tissue homeostasis and essential for the functioning of normal tissues.

Pain is a hallmark of inflammation process, so the evaluation of the activity induced by the extract in inflammatory pain is an interesting point to be evaluated. The acetic acid-induced writhing test is used for screening peripheral antinociceptive effects of test compounds and is considered to be a visceral pain model where the stimulation of nociceptive neurons by liberation of mediators such as histamine, serotonin, cytokines, and eicosanoids in the peritoneal fluid produce writhing responses related to the development of peripheral inflammation. This test is a classical model widely used to screen new agents with potential analgesic activity where both neurogenic and/or inflammatory pains are involved. In addition to the anti-inflammatory effect induced by the extract, *U. aurantiaca* extract showed antinociceptive effect in the writhing test. 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 when it was administered orally. Pain transmission is a complex mechanism that involves interaction of peripheral and central structures and different modulatory pathways (Ossipov *et al*., 2010), in order to elucidate possible mechanisms by which *U. aurantiaca* exerted its analgesic effect, different neurotransmitter systems, such as cholinergic, catecholaminergic, Opioid, and serotoninergic systems, were evaluated. Also, because NO-cGMP-K^+ channels pathway are involved in the modulation of pain perception, this route was tested as well. However, only the Opioid system seemed to be involved in the antinocipective activity of the extract. Additionally, taking into account the ability of *kappa*-opioids to act at multiple sites in the inflammatory cascade (Walker, 2003), it could be possible to include this system as responsible, at least in part, of the anti-inflammatory activity of the extract.

Finally, it is known that phytochemicals such as phenolics and flavonoids have antioxidant properties and play a protective role in oxidative stress related disorders including pain and inflammation (Murugan and Parimelazhagan, 2013). Since the extract total phenol content is considerable, these compounds could be implicated in the extract's activity.

In conclusion, this work reveals, for the first time, that *U. aurantiaca* methanolic extract has antinociceptive and anti-inflammatory effects, suggesting that the extract might represent a potential therapeutic option for the treatment of inflammation processes and inflammation-related pain syndromes. The Opioid system implication, the reduction in MPO activity and in the vascular permeability, and the antioxidant activity exerted by the extract, could explain, at least in part, the underlying mechanism involved in its protective effects on the acute inflammation process.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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