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Antinociceptive and gastroprotective actions of ethanolic extract from *Pluchea sagittalis* (Lam.) Cabrera

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ABSTRACT

Ethnopharmacological relevance: Pluchea sagittalis, an herbaceous plant widely distributed in South America, is used in folk medicine for the treatment of digestive diseases and inflammation.
Aim of the study: This study was designed to investigate the antinociceptive and gastroprotective effects of the ethanolic extract (EE) of aerial parts from *Pluchea sagittalis* in rodents.
Materials and methods: The antinociceptive effects of EE was evaluated in mice after oral administration in chemical tests (acetic-acid, glutamate and formalin) or by biting behavior following intrathecal administration of cytokines such as interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in mice. Furthermore, rats were treated with EE and subsequently exposed to acute gastric lesions induced by 80% ethanol. Afterwards the gastric lesion extension and the mucus levels of gastric mucosa were measured.
Results: The oral administration of EE showed a dose-dependent inhibition of acetic acid-induced abdominal constrictions and glutamate-induced pain in mice, with ID_{50} values of 624.0 (523.0–746.0) mg/kg and 368.0 (216.0–628.0) mg/kg, respectively. In the formalin test, the EE also produced significant inhibition of the inflammatory phase, with an ID_{50} value of 411.0 (183.0–721.0) mg/kg; however, it was ineffective in the neurogenic phase caused by formalin. In addition, oral treatment with EE caused a significant inhibition of biting behavior induced by i.t. injection of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). The antinociception caused by the EE (300 mg/kg, p.o.) was not reversed by naloxone (1 mg/kg, i.p.) when assessed in the acetic acid writhing test. The EE (300–1000 mg/kg, p.o.) did not affect the motor coordination of animals in an open-field model. Oral treatment with the EE protected rats against gastric lesions induced by ethanol, with an ID_{50} value of 55.0 (46.6–64.9) mg/kg, and increased the mucus levels of gastric mucosa to levels found in the non-lesioned group.
Conclusions: The mechanism by which the extract produced antinociception still remains unclear, but this effect seems to be primarily related to the modulation or inhibition of the action of pro-inflammatory mediators. Furthermore, these data support, at least in part, the ethnomedical use of *Pluchea sagittalis*.
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1. Introduction

The plants belonging to the genus *Pluchea*, a member of the family Asteraceae, consist of approximately 90 species that grow in several countries in South America. These herbaceous plants are widely found in Paraguay, Argentina and Brazil. *Pluchea sagittalis* (Lam.) is popularly known as "Lucera," "Yerba Lucero" or "Quitoc." Extracts or infusions from aerial parts of the plant have been used in different countries in traditional medicine to treat several dis-

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orders, including inflammation, digestive diseases, diarrhea and dolorous processes (Anderberg, 1994).

Some studies have shown that other species of this genus *Pluchea lanceolata* have anti-inflammatory and immunosuppressive activities (Chawla et al., 1991; Bhagwat et al., 2010). In addition, the methanolic fraction of a chloroform extract of roots from *Pluchea indica* showed anti-ulcer and anti-inflammatory activities as well as the inhibition of protein exudation and leukocyte migration through the involvement of the 5-lipoxigenase pathway (Sen and Nag-Chaudhuri, 1991; Sen et al., 1993). Pharmacological studies demonstrated that aqueous and dichloromethane extracts obtained from *Pluchea sagittalis* have a wide spectrum of anti-inflammatory activity, correlating with the reduction of free radicals (Pérez-Garcia et al., 1996). Furthermore, Pérez-Garcia et al. (2001) showed a significant inhibition of reactive nitrogen species (RNS) and heat shock protein (hsp) production in human neutrophils using the dichloromethane extract of *Pluchea sagittalis*. Phytochemical studies have also shown that triterpene taraxasteryl acetate is an important active compound of the extract that has a topical anti-inflammatory effect, similar to the dichloromethane extract of *Pluchea sagittalis.* Furthermore, triterpene taraxasteryl acetate inhibited the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in stimulated human neutrophils (Pérez-Garcia et al., 2004). In addition, studies have demonstrated that the ethanolic extract of *Pluchea quitoc* DC exhibits an anti-inflammatory effect against carrageenan-induced paw edema and croton oil-induced ear edema and produces antinociceptive action against acetic acid-induced visceral nociception and formalin-induced licking as well as in a tail flick test (Barros et al., 2006).

Therefore, considering the therapeutic properties attributed to this plant and the anti-inflammatory effects demonstrated by previous studies, we investigated the antinociceptive action of the EE of aerial parts from *Pluchea sagittalis* on several models of nociception in mice, and we also investigated the possible involvement of pro-inflammatory cytokines, IL-1β and TNF-α, on its antinociceptive action. In addition, we verified the gastroprotective effect of EE on gastric lesions induced by ethanol.

2. Materials and methods

2.1. Preparation of the ethanolic extract of Pluchea sagittalis

Pluchea sagittalis was collected in Asunción, Paraguay, in July 2006. A voucher specimen has been deposited at the Herbarium of the Faculty of Chemical Sciences, Universidad Nacional de Asunción, Paraguay, under number 3464.

After collecting the material, the dry aerial parts of *Pluchea sagittalis* (600 g) were powdered and extracted with ethanol at room temperature for approximately two weeks. After the plant material was filtered and the residue rejected, being the solvent evaporated under reduced pressure to obtain the crude ethanolic extract. Additionally, the *Pluchea sagittalis* sample used in our study was tested for the presence of flavonoids, tannins, saponins and terpenes by thin-layer chromatography using different solvent systems and revealers (Harborne, 1984).

2.2. Animals

Experiments were conducted using adult male Swiss mice (25–35 g) and male Wistar rats (200–250 g) that were housed at 22 ± 2°C under a 12-h light/dark cycle (lights on at 06:00) and with free access to food and water. Animals were habituated to the laboratory conditions for at least two hours before testing. Experiments were performed between 09:00 and 16:00. Each animal was used only once during the study. The experiments were approved by the Ethics Committee for Animal Research of the Federal University of Santa Catarina and were performed in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain on conscious animals (Zimmermann, 1983). The number of animals and the intensity of the noxious stimuli were the minimum necessary to obtain reliable data.

2.3. Abdominal constriction induced by acetic acid

The procedure used for the acetic acid-induced abdominal constriction was similar to one previously described (Santos et al., 2005) and resulted in the contraction of the abdominal muscle together with a stretching of the hind limbs in response to an intraperitoneal injection of acetic acid (0.6%) at the time of the test. Animals were pretreated with EE (3–1000 mg/kg, p.o.) 60 min before testing. Control animals received a similar volume of the appropriate vehicle. After the challenge, the mice were individually placed into glass cylinders of 20 cm diameter, and the abdominal constrictions were cumulatively counted over 20 min.

2.4. Nociception induced by glutamate

In an attempt to provide more direct evidence concerning the possible interaction of EE with the glutamatergic system, we separately investigated whether EE would be able to antagonize glutamate-induced licking in the mouse paw. The procedure used was similar to one previously described (Meotti et al., 2005). A volume of 20 μL of glutamate (20 μmol/paw) was injected intraplantarly in the ventral surface of the right hind paw. Animals were observed individually for 15 min following glutamate injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered to be indicative of nociception. Mice were orally treated with EE (10–1000 mg/kg) or vehicle (10 mL/kg) 60 min before glutamate injection.

2.5. Nociception induced by formalin

The procedure used was essentially the same as previously described (Santos and Calixto, 1997; Santos et al., 1999). Mice received 20 μL of a 2.5% formalin solution (0.92% formaldehyde) in saline and were injected intraplantarly in the ventral surface of the right hindpaw. Animals were observed from 0 to 5 min (neurogenic phase) and 15 to 30 min (inflammatory phase), and the time spent licking the injected paw was recorded with a chronometer and considered to be indicative of nociception. Mice received EE (10–1000 mg/kg, p.o.) 60 min before the formalin injection. Control animals were treated with vehicle (10 mL/kg, p.o.). Following the intraplantar injection of formalin, the mice were immediately placed in a glass cylinder (20 cm diameter), and the time spent licking the injected paw was recorded for both the early and late phase of this model.

2.6. Analysis of the possible mechanism of action of Pluchea sagittalis

2.6.1. Involvement of the opioid system

To investigate the possible participation of the opioid system in the antinociceptive effect of EE, mice were pretreated with naloxone (a nonselective opioid receptor antagonist, 1 mg/kg, i.p.), and after 20 min, the animals received an injection of EE (300 mg/kg, p.o.), morphine (2.5 mg/kg, s.c.) or vehicle (10 mL/kg, p.o.). Other groups were pretreated with vehicle and received EE, morphine, or vehicle after 20 min. After 60 min, they received an acetic acid (0.6%)

injection, and the abdominal constrictions were counted cumulatively over a period of 20 min.

2.6.2. Biting response induced by pro-inflammatory cytokines

We also investigated the properties of EE against pro-inflammatory cytokine (IL-1β and TNF-α)-induced biting behavior in mice. Animals received EE (300 mg/kg, p.o.) or indomethacin (10 mg/kg, i.p.) 1 h or 30 min after they received an intrathecal injection of 5 μL of pro-inflammatory cytokines (TNF-α, 0.1 pmol/site or IL-1β, 1 pmol/site) or vehicle solution. Injections were given according the method described previously (Hylden and Wilcox, 1980) and for a period of 5 s. Animals were observed individually for 15 min following intrathecal injection. The amount of time spent biting the paws, tail and posterior portion of the body was timed and was considered to be indicative of nociception.

2.6.3. Evaluation of locomotor activity

The open-field test was used to exclude the possibility that the antinociceptive action of EE could be related to non-specific disturbances in the locomotor activity of the animals. The ambulatory behavior was assessed in an open-field test as described previously (Rodrigues et al., 2002). The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm. The floor of the arena was divided into 12 equal squares, and the number of squares crossed with all paws crossing was counted in a 6-min session. Mice were treated with EE (300–1000 mg/kg, p.o.) or vehicle 60 min before the test.

2.7. Induction of acute gastric lesions

The possible gastroprotective effects of EE were investigated on acute lesions induced by ethanol in rats. Animals were orally treated with vehicle (0.1 mL/100 g), EE (30–300 mg/kg) or omeprazole (40 mg/kg) 60 min before the administration of 80% ethanol (0.5 mL/200 g, p.o.). Animals were sacrificed by cervical dislocation 1 h after treatment (Robert et al., 1979). The stomachs were removed, and gastric lesion extension was measured as the total injured area (mm^2) = length (mm) × width (mm) of injury as previously described (Baggio et al., 2007). Finally, the glandular mucosa was weighed and used for mucus determination.

2.8. Determination of gastric mucus

The gastric tissues were immediately transferred to 0.1% alcian blue solution prepared in 0.16 mM sucrose and 50 mM sodium acetate (pH 5.0) and stained for 2 h at room temperature. Next, the gastric mucosa was rinsed twice with 250 mM sucrose solution for 15 and 45 min, and the dye complexed with the gastric mucus was extracted with 500 mM magnesium chloride solution for 2 h. The extract was then mixed with an equal volume of diethyl ether and was centrifuged at 3600 rpm for 10 min. Absorbance was determined at 598 nm. The amount of mucus was calculated using standard curves of alcian blue (6.25–100.0 μg) (Corne et al., 1974).

2.9. Drugs

The following substances were used: acetic acid, formalin and morphine hydrochloride (Merck, Darmstadt, Germany); alcian blue, L-glutamic acid, indomethacin and naloxone hydrochloride (Sigma Chemical Co., St. Louis, USA), interleukin-1β (IL-1β) and tumor necroses factor-α (TNF-α) (R&D Systems, Minneapolis, USA) and Tween 80 (Merck, A.G., Darmstadt, Germany). All drugs were dissolved in saline solution (0.9% NaCl) with the exception of ethanolic extract, which was dissolved in saline plus Tween 80, and indomethacin, which was dissolved in saline with 5% DMSO. The final concentration of Tween 80 did not exceed 5% and did not cause any effect *per se*. All control groups of animals received the vehicle used to dissolve the ethanolic extract.

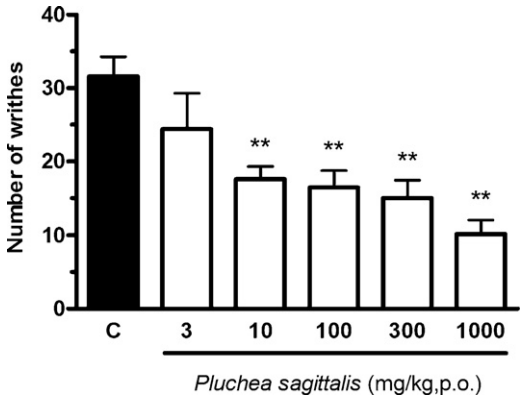

Fig. 1. Effect of EE of *Pluchea sagittalis* (3–1000 mg/kg, p.o.) on abdominal constrictions induced by acetic acid. Each column represents the mean ± S.E.M. (n = 6–8). Control values (*C*) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group; $^{**}p < 0.01$.

2.10. Statistical analysis

The results were presented as means ± S.E.M. except for the ID_{50} values (i.e., the dose of extract necessary to reduce the nociceptive response by 50% relative to the control value), which were reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} value was determined by nonlinear regression from individual experiments using linear regression GraphPad software (GraphPad software, San Diego, CA, USA). The statistical significance of differences between groups was detected by ANOVA followed by Newman–Keuls' test. *P*-values less than 0.05 ($P < 0.05$) were considered to be indicative of significance.

3. Results

3.1. Acetic acid-induced abdominal constrictions

The results depicted in Fig. 1 show that the oral administration of EE 60 min prior to testing produced a dose-related inhibition of acetic acid-induced abdominal constrictions in mice when compared to the control group, with ID_{50} values of 624.0 (523.0–746.0) mg/kg and an inhibition of 67 ± 6%.

3.2. Glutamate-induced nociception

After 60 min, oral treatment with EE caused a significant inhibition of glutamate-induced nociception, with an ID_{50} value of 368.0 (216.0–628.0) mg/kg and an inhibition of 83 ± 4% at dose of 1000 mg/kg orally (Fig. 2).

3.3. Formalin-induced nociception

The administration of EE (10–1000 mg/kg, p.o.) did not inhibit the neurogenic phase (0–5 min) but reduced the inflammatory phase (15–30 min) of formalin-induced licking, with an ID_{50} value of 411.0 (183.0–721.0) mg/kg and an inhibition of 88 ± 12% at dose of 1000 mg/kg orally (Fig. 3A and B).

3.4. Involvement of the opioid system

The results presented in Fig. 4 show that the pre-treatment of mice with naloxone (1 mg/kg, i.p., a non-selective opioid recep-

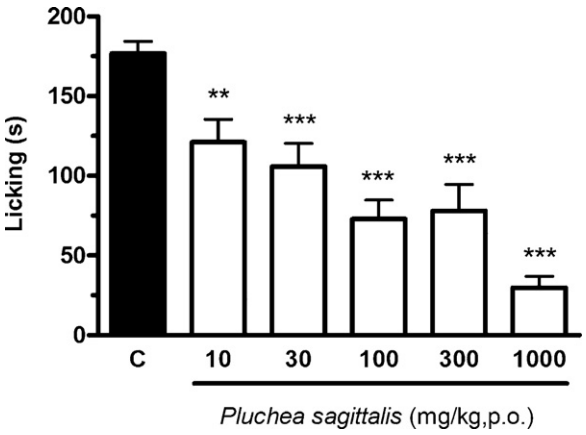

Fig. 2. Effect of EE of *Pluchea sagittalis* (10–1000 mg/kg, p.o.) on glutamate-induced licking in mice. Each column represents the mean ± S.E.M. (n = 6–8). Control values (*C*) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group; $^{**}p < 0.01$ and $^{***}p < 0.001$.

tor antagonist) given 30 min before the test did not reverse the antinociception caused by EE (300 mg/kg, p.o.) but completely reversed the antinociception caused by morphine (2.5 mg/kg, s.c.) when analyzed against acetic acid-induced pain.

3.5. Involvement of pro-inflammatory cytokines

Intrathecal administration of IL-1β and TNF-α caused significant biting behavior in mice. EE (300 mg/kg, p.o.) inhibited the biting responses induced by IL-1β and TNF-α with respective inhibitions of 71 ± 7% and 97 ± 6%. In contrast, indomethacin (10 mg/kg, i.p.) was able to reduce only the nociception caused by IL-1β, with an inhibition of 57 ± 10% (Fig. 5).

3.6. Evaluation of locomotor activity

The treatment with EE (300–1000 mg/kg, p.o.) did not alter the ambulation of mice in the open-field test. The means ± S.E.M. of crossing numbers 1 h after administration were 80.0 ± 6.4, 83.0 ± 7.3 and 64.3 ± 5.6 for the control group and groups receiving 300 and 1000 mg/kg of EE, respectively.

3.7. Effects on gastric injury

The EE treatment (30, 100 or 300 mg/kg, p.o.) caused a dose-related reduction in gastric lesions induced by 80% ethanol, decreasing the ulcer area mainly at doses of 100 and 300 mg/kg (Fig. 6A), with a mean ID_{50} value of 55.0 (46.6–64.9) mg/kg (injured control group value = 150.4 ± 12.8 mm^2) and an inhibition

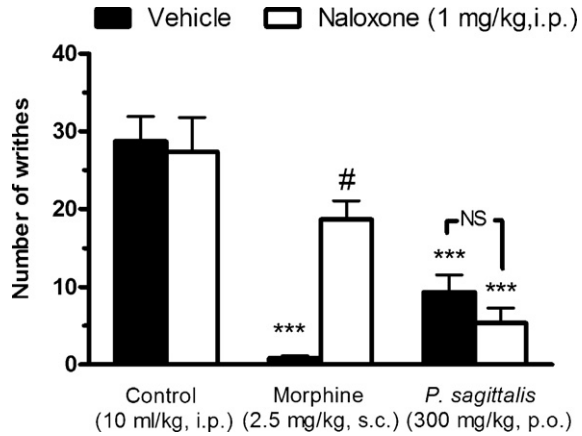

Fig. 4. Effect of pre-treatment of animals with naloxone (1 mg/kg, i.p.) on the antinociceptive profiles of EE of *Pluchea sagittalis* (300 mg/kg) and morphine (2.5 mg/kg, s.c.) against acetic acid-induced writhing in mice. Each column represents the mean ± S.E.M. (n = 6–8). Control values (*C*) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group; $^{***}p < 0.001$; compared to the morphine group, $^{\#}p < 0.05$.

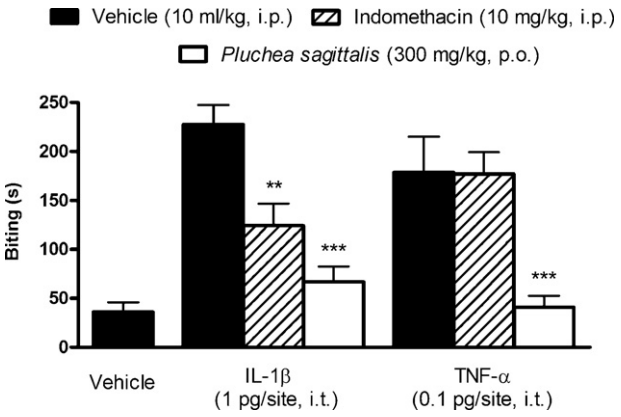

Fig. 5. Effect of EE of *Pluchea sagittalis* (300 mg/kg, p.o.) and indomethacin (10 mg/kg, i.p.) on the biting response caused by intrathecal injection of IL-1β and TNF-α agonists in mice. Each column represents the mean ± S.E.M. (n = 6–8). Control values (*C*) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group; $^{**}p < 0.01$ and $^{***}p < 0.001$.

of 90 ± 2% at 300 mg/kg. The positive control of the test, omeprazole (40 mg/kg, p.o.), reduced the gastric lesions induced by ethanol by 52 ± 8%.

The gastric wall mucus was quantified in the ethanol-induced gastric ulcer model. The amount of gastric wall mucus in the injured control (C) was decreased by 49 ± 5% when compared to the non-

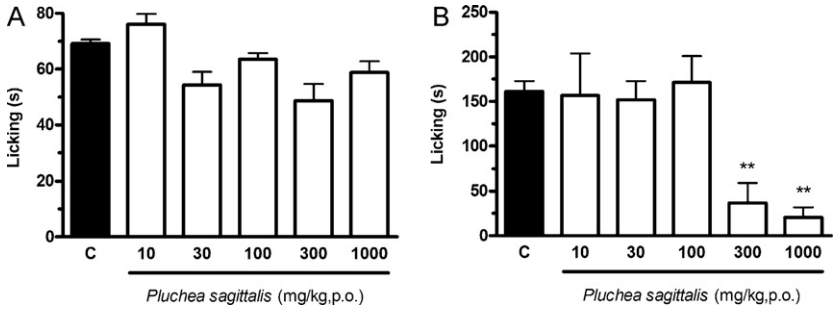

Fig. 3. Effect of EE of *Pluchea sagittalis* (10–1000 mg/kg, p.o.) on the first phase (A) and the second phase (B) of formalin-induced licking in mice. Each column represents the mean ± S.E.M. (n = 6–8). Control values (*C*) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group; $^{**}p < 0.01$.

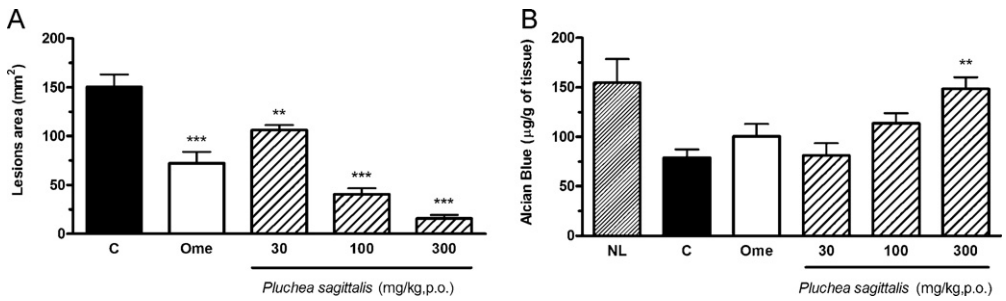

Fig. 6. Effect of EE of *Pluchea sagittalis* (30–300 mg/kg, p.o.) on gastric lesions induced by 80% ethanol (A) and the gastric mucus amount (B) in rats. Each column represents the mean ± S.E.M. (n = 6–8). Control values (C) indicate the administration of vehicle (saline and Tween 80, 10 mL/kg), and the asterisks denote the significance levels when compared with the control group, $^{**}p < 0.05$ and $^{***}p < 0.001$.

lesioned group (NL: 154.6 ± 23.9 μg alcian blue/g of tissue). The administration of EE (300 mg/kg, p.o.) increased the amount of mucus to 148.6 ± 11.7 μg alcian blue/g of tissue when compared to the injured control (C: 78.9 ± 8.4 μg alcian blue/g of tissue) (Fig. 6B).

3.8. Phytochemical analysis

The phytochemical analysis of *Pluchea sagittalis* indicated the intense presence of terpenoids and small presence of flavonoids.

4. Discussion

The plants of genus *Pluchea* are used in folk medicine for the treatment of several conditions, including disorders of the bowel and kidney. In addition to these medicinal uses, there are reports showing anti-microbial and hypoglycemic properties of some *Pluchea* species (Sen et al., 2002).

Chemical studies carried out with some species belonging to the genus *Pluchea* have demonstrated the presence of many constituents, such as stigmasterol, β-amiryn, taraxasteryl, pseudotaraxasteryl, monoterpens, lignin glycosides and flavonoids (Guilhon and Muller, 1996; Vera et al., 2008). Several biological effects of *Pluchea sagittalis* extract and its isolated compound have been demonstrated. Pérez-Garcia and colleagues (2004) demonstrated the anti-inflammatory effects of a dichloromethane extract of *Pluchea sagittalis* and triterpene taraxasteryl acetate in rat hind paw-edema. The anti-inflammatory action of this extract has been attributed to the inhibition of ROS, RNS and Hsp72 synthesis on stimulated neutrophils (Pérez-Garcia et al., 2004).

In accordance with these findings, this work shows for the first time that the EE of *Pluchea sagittalis* when given orally can also present antinociceptive effects. The results demonstrate that the EE produced a dose-related inhibition of the number of abdominal constrictions elicited by acetic acid, a typical model used to search for new drugs with analgesic and anti-inflammatory properties (Koster et al., 1959; Le Bars et al., 2001). Its antinociceptive effects could occur via peripheral or central sites of action. It has been suggested that acetic acid acts by releasing endogenous inflammatory mediators of the nociceptive neurons (Collier et al., 1968), such as bradykinin, prostaglandins and pro-inflammatory cytokines, when injected intraperitoneally (Ribeiro et al., 2000; Ikeda et al., 2001). The main cytokines involved in nociception induced by acetic acid are TNF-α, interleukin-1β and interleukin 8, and they are released from resident peritoneal macrophages and mast cells (Ribeiro et al., 2000). The concentration of glutamate and aspartate in the cerebrospinal fluid was increased after the injection of acetic acid (Feng et al., 2003). Furthermore, this test is sensitive to non-steroidal anti-inflammatory drugs (NSAIDs), narcotics and other centrally acting drugs (Collier et al., 1968; Santos et al., 1998).

Oral administration of the EE also showed antinociceptive effects in the inflammatory phase (late phase) of formalin-induced nociception but did not inhibit the neurogenic phase (early phase). Inflammatory pain is a consequence of the increase in the spinal levels of different mediators, such as PGE_2, excitatory amino acids, nitric oxide, tachykinins and kinins. Furthermore, functional changes in the dorsal horn of the spinal cord are produced by inflammatory pain (Malmberg and Yaksh, 1992; Tjolsen et al., 1992; Santos and Calixto, 1997). The present results demonstrate that systemic treatment of mice with EE causes significant and dose-dependent antinociception against glutamate-induced nociception. It is known that glutamate is the main excitatory amino acid in pain transmission. Therefore, substances capable of blocking either iGluRs (ionotropic glutamate receptors) or mGluRs (metabotropic glutamate receptors) may exhibit antinociceptive effects in several mammalian species (Neugebauer and Carlton, 2002). Here, we observed that EE did not alter locomotor activity in the doses that caused significant antinociception.

The pro-inflammatory cytokines are also involved in the modulation of nociceptive processes. Cytokines such as TNF-α and IL-1β may induce nociceptive behavior when administered by the intrathecal route. As demonstrated by Cumiskey and colleagues (2007), after the injection of TNF-alpha, glutamate may be released by nerve terminals and act on glutamate receptors to induce nociception. In addition, this activation of glutamate receptors increases the calcium influx, activating kinases and mitogen-activated proteins kinases (Pickering et al., 2005). Thus, glutamatergic transmission is involved in nociception induced by pro-inflammatory cytokines. For this reason, we investigated the effect of EE against the TNF-α and IL-1β-induced nociceptive responses in mice (Choi et al., 2003). This study also showed that orally administered EE strongly inhibited the biting response caused by the i.t. injection of cytokines IL-1β and TNF-α. We used indomethacin, a non-steroidal anti-inflammatory drug (NSAID), to compare and evaluate the inhibition of pro-inflammatory cytokines. Our results showed that the EE effect was similar to indomethacin in inhibiting the biting response caused by IL-1β. The binding of IL-1β to its receptor IL-1RI actives tyrosine kinases and is PKC calcium independent (Obreja et al., 2002), which could explain, in part, the antinociception caused by this EE. Another interesting result of this study was that EE completely inhibited TNF-α-induced nociception. At the same time, indomethacin was ineffective against the biting response caused by TNF-α. Previous reports show that TNF-α-induced nociception involves the phosphorylation of the p38 protein (Schäfers et al., 2003). Thus, these results suggested that EE has anti-inflammatory effect more potent than indomethacin in these models.

In the next step, we investigated the possible involvement of the opioid system on the antinociceptive effect of EE, considering that many plants have an antinociceptive activity linked to this system. Our data also demonstrate that the central opioid system is probably not involved in the antinociception produced by EE. This assertion is supported by the demonstration that the treatment of

animals with naloxone (a non-selective opioid receptor antagonist that penetrates the blood–brain barrier) at a dose that produced no significant effect on acetic acid-induced constrictions did not reverse the antinociceptive effect caused by EE.

Conventional NSAIDs inhibit both COX-1 and COX-2 at standard anti-inflammatory doses, and this dual inhibition may lead to a number of side effects, particularly gastrointestinal ulceration (Wallace et al., 2000; Tanaka et al., 2001). For this reason, we tested EE against gastric lesions induced by ethanol and observed that EE possess desirable gastroprotective activity. Ethanol is a well-known necrotizing agent that destroys the mucus barrier (Hirschowitz, 1983), increases vascular permeability (Szabo et al., 1985) and decreases non-proteic sulfhydryl groups (NP-SH) of the gastric mucosa (Repetto and Llesuy, 2002; Siegmund, 2003). The mucus, which continuously coats the gastric mucosa, prevents injuries from luminal acid, bacteria and noxious agents (Chen et al., 2005). In our experiments, we observed that one of these protective factors was enhanced by treatment with EE, which indicates that the mechanism of protection of this extract against ethanol injury may be related to this important mechanism of cytoprotection. In addition, EE is devoid of gastrointestinal adverse effects, and in contrast, seems to exert a protective gastric effect. Thus, it can have considerable advantages compared to indomethacin.

The phytochemical components responsible for *Pluchea sagittalis* antinociceptive effect are unknown, but preliminary studies demonstrated the presence of sesquiterpenoids 3a-(2,3-epoxy-2-methylbutyryloxy)-4a-formoxy-11-hydroxy-6,7-dehydro-eudesman-8-one and 3a-(2,3-epoxy-2-methylbutyryloxy)-4a,7a,11-trihydroxyeudesman-8-one) in this plant (Vera et al., 2008). Moreover, our preliminary phytochemical analysis of *Pluchea sagittalis* indicated the presence of terpenoids and small presence of flavonoids. These data suggest that terpenoids and flavonoids may be contributed to antinociceptive effect caused by EE of *Pluchea sagittalis*.

In conclusion, the present study demonstrates that the EE of *Pluchea sagittalis* exerts dose-related antinociceptive action against acetic acid, formalin and glutamate nociceptive models of pain in mice at a dose that does not interfere with locomotor activity. Additionally, the antinociceptive action of *Pluchea sagittalis* is related to mechanisms that depend on TNF-α and IL-1β and do not depend on the opioid system. The mechanism by which the EE produces antinociception still remains unclear, but we are continuing pharmacological and chemical studies to characterize the mechanism(s) responsible for the antinociceptive action and to identify the effect of the active principles present in *Pluchea sagittalis*. These findings support, at least partially, the use of *Pluchea sagittalis* in traditional medicine.

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