

## Antinociceptive and anti-inflammatory effects of *Lantana camara* L. extract in mice

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**ABSTRACT:** The *Lantana camara* L. belongs to the family Verbenaceae, which contains several active compounds in leaves and roots and which are reported to have medicinal and insecticidal properties. Studies of plants within the same family show the existence of anti-inflammatory activity in paw edema induced by carrageenan, serotonin and histamine and analgesic activity in the acetic acid writhing and tail-flick tests. The present study investigated whether the *L. camara* extract (ACE) also exerts these effects. The ACE toxicity was studied in male mice, and the percentage of mortality recorded 7 days after treatment was assessed. The ACE was evaluated as an antinociceptive agent in the hot plate, tail-flick and acetic acid writhing tests at a nontoxic dose of 1.0 g/Kg. The results showed that 1.5 g/Kg of ACE was not able to cause death, and doses of 3.0 and 4.0 g/Kg caused 50% and 60% death, respectively, in male mice. In all of the antinociceptive tests, 1 g/Kg of ACE markedly reduced responses to pain. Our findings suggest that ACE may have active anti-inflammatory and antinociceptive properties in much smaller doses than toxic.

**Keywords:** *Lantana camara*, hot plate test, tail-flick test, acetic acid writhing test, mice.

### RESUMO: Efeitos antinociceptivos do extrato de *Lantana camara* L. em camundongos.

*Lantana camara* L. pertence à família Verbenaceae, a qual contém muitos princípios ativos em suas folhas e raízes com propriedade medicinais e inseticidas. Estudos com plantas da mesma família mostram a existência de propriedades anti-inflamatórias no modelo de edema de pata induzido pela carragenina, serotonina e histamina, além da atividade analgésica nos testes de contorção induzida pelo ácido acético e da retirada da cauda por estímulo térmico. O presente trabalho investigou os efeitos tóxicos e antinociceptivos do extrato de *L. camara* (ACE) em camundongos. Para tanto, investigou-se a porcentagem de mortes em 7 dias após a administração de diferentes doses do extrato. Avaliou-se também os efeitos antinociceptivos do ACE pelos testes da placa quente, estimulação térmica da cauda e contorções abdominais induzidas pelo ácido acético com a dose não-tóxica [1,0 g/Kg]. Os resultados mostraram que 1,5 g/Kg do ACE não causou mortalidade, enquanto que 3,0 e 4,0 g/Kg promoveram 50 e 60% de mortalidade, respectivamente. Em todos os testes antinociceptivos, a dose de 1,0 g/Kg do ACE reduziu a resposta à dor. Os presentes resultados indicam que o ACE apresenta propriedades anti-inflamatórias e analgésicas em doses muito menores que a tóxica.

**Palavras chaves:** *Lantana camara* L., teste da placa quente, estímulo térmico na cauda, teste de contorções abdominais, camundongos.

### INTRODUCTION

The genus *Lantana* L. is composed of 150 pantropical species, some of which are used as traditional medicines or ornamentals worldwide. *Lantana camara* L. (Verbenaceae) is commonly known as camará, camará chumbinho (Tokarnia et

al., 1984), camará-de-cheiro, camará-de-espinho, camará-miúdo-de-espinho, camará-verdadeiro, camará-vermelho, cambará, cambará-de-duas-cores, cambará-de-folha grande, chumbinho, and chumbinho-roxo (Corrêa, 1984; Braga, 1976). L.

*camara* has several active compounds in its leaves and roots that have both medicinal (Ghisalberti, 2000; Deena & Thoppil, 2000) and toxic insect repellent properties (Seyoum et al., 2002; Dua et al., 1996).

The plant, its essential oil, and its preparations are popularly used for their sudorific, carminative, antiseptic, antispasmodic, and antiemetic effects. Some parts of the plant are used in the treatment of itch, cuts, ulcers, respiratory tract secretions, eczema, malaria, rheumatism, tooth ache, uterine bleeding, colds, flu, asthma, hypertension, tumors, and cancer (Ghisalberti, 2000; Deena & Thoppil, 2000) and as an insecticide (Seyoum et al., 2002; Dua et al., 1996).

*L. camara* has been well studied chemically. Two active toxic principles - lantadene  $\alpha$  and  $\beta$ -are considered the most important, and both target the liver. In addition to lantadenes  $\alpha$  and  $\beta$ , previous reports showed that the plant produces other triterpenes of the lantadene type, such as lantanolic acid, lantic acid, 22- $\beta$ -dimethylacryloyloxylantanolic acid, a mixture of 22- $\beta$ -dimethylacryloyloxylantanolic acid and 22- $\beta$ -angeloyloxylantanolic acid, and lantanolic acid (Barre et al., 1997). The presence of flavonoids, iridoids, oligosaccharides, phenylpropanoids glycosides, and naphthoquinones has been reported (Sharma et al., 2007).

Silva et al. (2005) showed that the *L. trifolia* extract has anti-inflammatory and antinociceptive effects. Ghosh et al. (2010) detected anti-inflammatory and anticancer effects of oleanonic acid from *L. camara*. Forestieri et al. (1996) found anti-inflammatory, antinociceptive, and antipyretic effects of petroleum ether, 95% ethanol, and distilled water extracts of *L. camara*. All of these extracts were effective against carrageenan-induced paw edema and had antipyretic effects in rats. They were also effective in the hot plate and acetic acid writhing tests in mice. Only the petroleum extract showed signs of toxicity. We previously studied the toxicity of the apolar and polar *L. camara* crude extracts in mice. Both extracts exerted similar acute toxicity, suggesting that they share some active toxic principles. The apolar extract was tested in four doses, i.e., 1.5, 3.0, 4.0 and 5.0 g/Kg. The 1.5 g/Kg was the only dose unable to induce mortality (Bevilacqua et al., 2011). Thus in our experiment the 1.0 g/Kg was chosen to perform the antinociceptive studies.

Also, in this study (Bevilacqua et al., 2011), data from the open field test and spontaneous signs of toxicity suggest that these toxic principles have depressive effects on the central nervous system, but not the 1.5 g/Kg. In addition, these mice did not present ataxia. It is known impaired motor coordination has a critical role in the motor

performance of antinociceptive animal models.

## MATERIALS

### Animals

Male and female BALB-c mice, weighing 25-30 g from our colony (Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil) were used. The animals were housed in groups of 15 in polypropylene cages (38 x 32 x 16 cm) with controlled room temperature ( $22 \pm 2^\circ\text{C}$ ), humidity (65-70%), and artificial lighting (12 h/12 h light/dark, lights on at 6:00 a.m.). The animals had free access to Nuvilab<sup>®</sup> rodent chow (Nuvital Company, São Paulo, Brazil) and filtered water. The animals were randomly divided into control and experimental groups. To minimize the influence of possible circadian changes on mice behavior, the animals were observed at the same time each day (2:00-4:00 p.m.). The mice used in this study were maintained in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil.

### Plant collection

The leaves of *L. camara* were collected at Universidade Presbiteriana Mackenzie in May 2004 in São Paulo city, São Paulo state, Brazil. The species was identified by Ph.D. Oriana A. Fávero from Universidade Presbiteriana Mackenzie and Ph.D. Lucia Rossi from Instituto de Botânica, São Paulo. A voucher specimen was deposited at the Herbarium of the Prefeitura Municipal de São Paulo (PMSP; voucher number 8766).

### Extraction

The air-dried plant material (300 g) was individually macerated for 24 h with  $\text{CH}_2\text{Cl}_2$ . After solvent evaporation under reduced pressure, 10 g (3.3%) of the  $\text{CH}_2\text{Cl}_2$  extract (ACE) was obtained. ACE was previously analyzed by TLC  $\text{SiO}_2$  [silicon dioxide] and nuclear magnetic resonance spectroscopy. The presence of steroids, fatty acids, and pentacyclic triterpenoids was detected (Bevilacqua et al., 2011).

### ACE toxicity evaluation

Groups of 10 animals/dose received ACE (1.0, 1.5, 3.0, and 4.0 g/Kg- diluted in almond oil) or 10 ml/Kg almond oil intraperitoneally (maximal volume administered was 0.3 ml/mice). The mice were placed in individual cages, and mortality was assessed for 7 days after ACE administration. Morphine (20 mg/Kg, n= 10) was administered by intraperitoneally as a positive control group. All drugs and the extract were injected intraperitoneally.

## Antinociception evaluation

### Hot plate test

The hot plate test was conducted according to Jacob and Ramabadrán (1978). Each mouse was individually placed on a surface heated to  $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , and the latency for the mouse to withdraw and lick its paw was recorded. The mice were subjected three times to the hot plate test to establish a baseline before exposure to ACE or almond oil treatment. The baseline value was used to calculate the difference in latency before and after treatment. ACE (1.0 g/Kg) or almond oil (10 ml/Kg), were administered 60 min before the hot plate test. An additional group of 10 mice received 20 mg/Kg morphine intraperitoneally, which was used as a positive control.

### Thermal-stimulated tail-flick test

The procedure was similar to Alviano et al. (2004). Noxious thermal stimulation was applied by immersing the tip of the tail in hot water. The latency to lift the tail from the hot water was recorded. Before the treatments were administered, three consistent readings were taken with the distal 5 cm of the tail immersed in  $52 \pm 0.2^{\circ}\text{C}$  water. The cut-off time was 15 s to avoid tail tissue damage. The interval between the three immersions was 30 s. The tail withdrawal latency in seconds (i.e., reaction time) was recorded and used to calculate the baseline, defined as the normal reaction of the animal to the hot water. After the baseline was established, the mice were treated with 1.0 g/Kg ACE or 10 ml/Kg almond oil. The test was conducted 60 min after treatment. The tail-flick antinociceptive index (TFAI) was calculated according to the following formula:  $TFAI = \text{reaction time} - \text{baseline} / \text{cutoff} - \text{baseline}$ .

### Acetic acid writhing test

The acetic acid writhing test was based on the method of Koster et al. (1959). Contortions were induced by intraperitoneal administration of acetic acid (60 mg/Kg; Merck & Co., Inc., Whitehouse Station, New Jersey, EUA). Nine male mice were treated with 1.0 mg/g ACE. The control group ( $n = 8$ ) received vehicle solution (10 ml/Kg almond oil). Sixty minutes after treatment, the mice were injected with the acid acetic solution to induce characteristic writhing behavior. The results are expressed as the number of contortions observed during the 20 min

period after acetic acid injection.

### Statistical analysis

The results are expressed as mean  $\pm$  SEM. Homoscedasticity was verified using the  $F$  test. Normality was verified using the Kolmogorov-Smirnov test. In the hot plate test, one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test was used to analyze the results. Student's  $t$ -test (unpaired, two-tailed) was used for acetic acid writhing and thermal-stimulated tail-flick data. In all cases, the results were considered significant with  $p < 0.05$ .

## RESULTS

Mortality was assessed in male mice that received different doses of ACE. These data were used in the construction of a dose-response relationship with a level of significance of 5%, according to the Trimmed Spearman-Kärber test (Table 1).

The hot plate test results are shown in Fig. 1A. Compared with the control group, animals treated with 1.0 g/Kg ACE or 20 mg/Kg morphine showed a significant increase in latency ( $F_{2,24} = 13.40$ ,  $p < 0.0001$ ; one-way ANOVA followed by Bonferroni test). No differences were detected between ACE- and morphine-treated mice ( $t = 0.48$ ).

Fig. 1B shows the thermal-stimulated tail-flick test data in mice treated with ACE or almond oil. Student's  $t$ -test showed that mice in the ACE group had significantly increased latencies compared with the control group ( $t = 10$ ,  $df = 45$ ,  $p < 0.0001$ ). Interestingly, mice in the control group exhibited hyperalgesia.

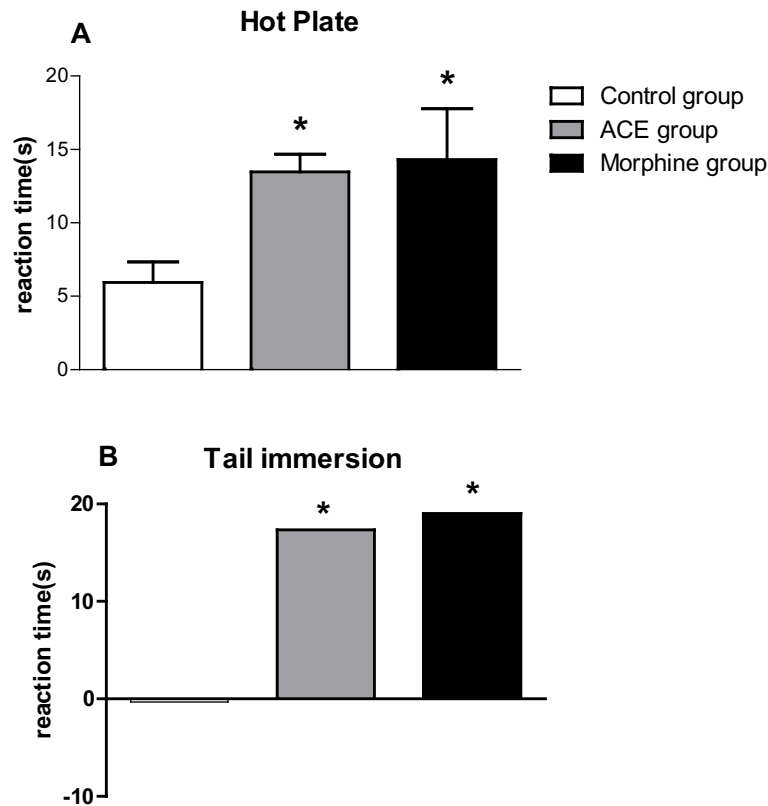
In the acetic acid writhing test, mice treated with ACE did not exhibit contortions, whereas the control group showed a mean of  $40.50 \pm 7.63$  contortions during the 20 min observation period.

## DISCUSSION

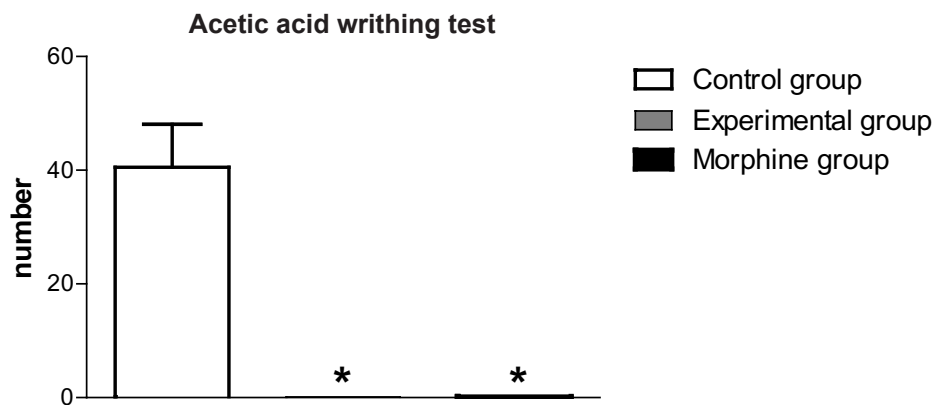
The ACE results are consistent with our previous data, in which mortality occurred at a dose of 3.0 g/Kg. However, even low doses of 1.0 and 1.5 g/Kg induced behavioral signs of toxicity (Bevilacqua et al., 2011). To study the antinociceptive effects of

**TABELA 1.** Mortality of male mice treated with *Lantana camara* L. extract ( $n = 10$ / group).

ACE dose	Mortality number	% of mortality	Death days
0	0	0	-
1.0	0	0	-
1.5	0	0	-
3.0	5	50	2
4.0	6	60	1



**FIGURE 1.** (A) Latency to paw withdrawal in the hot plate and thermal-stimulated tail-flick tests.  $*p < 0.05$ , compared with control group (one-way ANOVA followed by Bonferroni *post hoc* test). (B) Latency to paw withdrawal in mice treated or not with the extract of *Lantana camara* L. or morphine.  $*p < 0.05$ , compared with control group (Student's *t*-test). Data are expressed as mean  $\pm$  SEM. Control group,  $n = 9$ ; ACE group,  $n = 8$ .



**FIGURA 2.** Number of writhings induced by acetic acid in mice treated or not with the extract of *Lantana camara* L. Data are expressed as mean  $\pm$  SEM.  $*p < 0.05$ , compared with control group (Student's *t*-test). Control group,  $n = 9$ ; ACE group,  $n = 8$ .

ACE, we used a dose that was unable to induce toxicity, i.e., 1.0 g/Kg.

The potential antinociceptive effect produced by ACE was assessed using three well-known models of nociception: the acetic acid writhing, hot plate, and thermal-stimulated tail-flick tests. ACE

demonstrated marked antinociceptive properties in both the visceral and central nociceptive mouse models, similar to morphine 20 mg/Kg dose.

The hot plate and thermal-stimulated tail-flick tests are generally considered important models for evaluating central antinociceptive activity

(Chapman et al., 1985). These tests can differentiate between central opioid-like and peripheral analgesics (Asongalem et al., 2004). The hot plate model classically involves activation of central areas related to pain. The results showed that ACE administration increased latencies in this test after intraperitoneal injection. To support our experimental results, morphine was used as a positive control. A dose of 1.0 g/Kg ACE produced similar antinociceptive effects as morphine.

Opiates are generally accepted to act at supraspinal sites or directly on spinal opioid receptors (Picolo et al., 1998; Benedetti, 1987; Lipp, 1991). Opioids activate  $\gamma$ -aminobutyric acid inhibitory interneurons at the supraspinal level, leading to disinhibition/stimulation of descending pathways of pain inhibition, whereas activation inhibits the spinal transmission of stimuli in ascending pathways. The thermal-stimulated tail-flick test predominantly reflects activation of a reflex that occurs at the spinal level because the response persists after a cross-section of the medulla (Dennis et al., 1980; Irwin et al., 1951). Different opioid receptors participate in the mediation of antinociception in this model (Schmauss et al., 1984). Kappa ( $\kappa$ ) and  $\delta$  receptors are sensitive to stimulation by this type of pain (Heyman et al., 1989; Jiang et al., 1990; Patti et al., 2005). Thus, the increased latency in mice treated with ACE in both the hot plate and tail-flick tests suggests that ACE acts at supraspinal sites and directly on spinal opioid receptors.

Intraperitoneal injection of acetic acid induces peritoneal inflammation (i.e., acute peritonitis), which leads to a response characterized by contraction of the abdominal muscle accompanied by extension of the forelimbs and elongation of the body. This writhing response is considered a visceral inflammatory pain model (Koster et al., 1959; Rujjanawate et al., 2003) of disorders of internal organs, such as the stomach and intestines (Al-Chaer and Traub, 2002). This method has been associated with increased prostaglandin levels in peritoneal fluids (Deraedt et al., 1980) and has good sensitivity for weak analgesics. However, it has poor specificity because abdominal constrictions can be suppressed by smooth muscle relaxants, which may lead to discrepant results. This problem can be overcome by using other models of nociception, such as the hot plate test. Human preclinical pain tests have frequently employed heat stimulation for physiological, pathological, and pharmacological assessments (Arendt-Nielsen and Chen, 2003).

Compared with controls, ACE-treated mice did not exhibit a writhing response, indicating that the extract has anti-inflammatory effects. These data are consistent with previous reports that demonstrated the anti-inflammatory effects of *L. camara* extracts.

Forestieri et al. (1996) investigated several plant extracts and showed that *L. camara* extracts are not only effective in anti-inflammatory models but also as analgesics and antipyretics.

Basu and Hazra (2006) reported that the *L. camara* methanol extract exhibited remarkably potent inhibitory activity on nitric oxide (NO) produced *in vitro* from sodium nitroprusside, a key mediator in the phenomenon of inflammation, and lipopolysaccharide-activated murine peritoneal macrophages *ex vivo*. The aqueous and chloroform extracts exhibited only moderate NO-scavenging properties. Furthermore, Western blot analysis showed that inhibition of NO $\cdot$  synthesis correlated with a reduction in iNOS protein expression. Immunoblot analysis confirmed that the modulatory effect of the samples occurred through iNOS protein suppression.

The present results showed an absence of responses in the acetic acid writhing test, suggesting that ACE has a potent active principle with anti-inflammatory properties. A previous analysis of this extract suggested the presence of skeletons of pentacyclic triterpenoids, lantanones, or lantanolic acid derivatives and free sterols, such as stigmasterol and sitosterol (Bevilacqua et al., 2011).

In conclusion, the present findings suggest that ACE may have active principles that have potent anti-inflammatory and antinociceptive properties.

## ACKNOWLEDGMENTS

This research was supported by CNPQ (grant 501147/2004-7).

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