© 2011 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10 (4): 333 - 337
ISSN 0717 7917
www.blacpma.usach..cl

Artículo Original | Original Article

Analgesic effect of hydroalcoholic extract of *Cissampelos sympodialis* Eichl leaves

[Efecto analgésico del extracto hidroalcoholico de las hojas de *Cissampelos sympodialis* Eichl]

Walter MENDES DE OLIVEIRA JÚNIOR, Rubens BATISTA BENEDITO, Camila Carolina DE MENEZES PATRÍCIO SANTOS, Luiza Toscano DIAS RODRIGUES, Alexsandro FERNANDES MARINHO, Liana Clébia SOARES LIMA DE MORAIS, Margareth DE FÁTIMA FORMIGA MELO DINIZ & Reinaldo NÓBREGA DE ALMEIDA*

Federal University of Paraíba, Laboratory of Pharmaceutical Technology, João Pessoa, Paraíba, Brazil
***Contactos | Contacts:** **Reinaldo NÓBREGA DE ALMEIDA** ***E-mail address:** reinaldoan@uol.com.br*

Abstract

Cissampelos sympodialis Eichl is a species of the family Meniespermaceae known as "Milona", which extracts showed low toxicity and several pharmacological activities as anti-inflammatory, anti-allergic or anti-depressive. In the present study two classic analgesia models in mice were used to evaluate the hydroalcoholic extract of *C. sympodialis* leaves (HAECs) at doses of 50, 100 and 200 mg/kg, i.p. In the test of writhing by acetic acid HAECs-treatment induced a significant reduction in the number of abdominal contortions in a dose-dependent manner ($p < 0,05$). In the formalin test, HAECs-200 mg/kg induced a significant inhibition of the second phase (15-30 minutes) ($p < 0,05$). Results suggest that the HAECs presented outlying antinociceptive peripheral activity probably related with the alleged anti-inflammatory proprieties of extracts of this plant.

Keywords: *Cissampelos sympodialis* Eichl, Antinociceptive activity, Acetic acid test, Formaldehyde method; Analgesia.

Resumen

Existen varios estudios que evidencian que la *Cissampelos sympodialis* posee atividad anti-inflamatoria, antialérgica, antidepresiva y de baja toxicidad. Este estudio evaluó los efectos de la administración intraperitoneal, del extracto hidroalcohólico de hojas de *cissampelos sympodialis* (EHACS), en las dosis de 50, 100 y 200 mg/Kg, en modelos de analgesia en camumdongos. En el modelo de las contracciones inducidas por el ácido acético (0,85%) fue encontrada una reducción en el número de contracciones proporcional a las dosis usadas ($p<0,05$). En el test de la reacción al formaldeído las dosis utilizadas no presentaron efecto significante en la primera fase del test (0-5 minutos). Sin embargo, la dosis de 200mg/Kg presentó una significante inhibición en la segunda fase (15 – 30 minutos) del test ($p<0,05$). Los resultados mostraron que el EHACS presentó una actividad antinociceptiva periférica probablemente relacionada con la alegada actividad anti-inflamatoria.

Palabras Clave: *Cissampelos sympodialis*, Atividad antinociceptiva, Prueba de ácido acético, Prueba de formalina, Analgésia

Recibido | Received: March 20, 2011.
Aceptado en versión corregida | Accepted in revised form: May 17, 2011.
Publicado en línea | Published online: July 30, 2011.
Declaración de intereses | Declaration of interests: The authors thank to the Brazilian agencies for financial support: FAPESP, CAPES, CNPq.
Este artículo puede ser citado como / This article must be cited as: Walter MENDES DE OLIVEIRA JÚNIOR, Rubens BATISTA BENEDITO, Camila Carolina DE MENEZES PATRÍCIO SANTOS, Luiza Toscano DIAS RODRIGUES, Alexsandro FERNANDES MARINHO, Liana Clébia SOARES LIMA DE MORAIS, Margareth DE FÁTIMA FORMIGA MELO DINIZ, Reinaldo NÓBREGA DE ALMEIDA. 2011. Analgesic effect of hydroalcoholic extract of *Cissampelos sympodialis* Eichl leaves. Bol Latinoam Caribe Plant Med Aromat 10(4): 333 – 337.

List of abbreviations:
LTF – Laboratório de Tecnologia Farmacêutica;
UFPB – Universidade Federal da Paraíba;
CEPA - Ethics Committee guidelines;
HAECs – Hydro alcoholic extract of *Cissampelos sympodialis*;
TNF-∝ - Tumor Necrosis Factor-alpha;
NMDA - N-methyl D-aspartate;
MOR – Morphine.

INTRODUCTION

Cissampelos sympodialis Eichl, (Meniespermaceae), is a plant wild in northeastern and southeastern Brazil. In folk medicine is known as "Milona", "Abuteira" and "Orelha-de-onça", and tea from its roots has been used in folk medicine by natives for the treatment of a variety of pathologies such as inflammatory pain, rheumatic pain, asthma, bronchitis (Corrêa, 1984).

From the chemical point of view, several isoquinoline alkaloids were isolated from *C. sympodialis*, among which are: milonine (Freitas *et al*., 1995), warifteine (Cortes *et al*., 1995), and metilwarifteine laurifoline (Barbosa-Filho *et al*., 1997), and liriodenine roraimine (Lira *et al*., 2002).

In recent works *in vitro* and *in vivo* activities of extracts of this plant, such as antidepressant, anti-inflammatory, anti-spasmodic or anti-allergic were shown, (De Freitas, 1996; Almeida, 1998; Piuvezam, 1999; Batista Lima, 2001; De Freitas, 2000; Diniz, 2000; Santos, 2004; Almeida, 2005; Santos, 2006). It is known that many inflammatory processes are directly or indirectly related to the mechanisms of generation and maintenance of nociceptive stimuli, enabling the use of analgesic drugs as anti-inflammatory (Batista Lima, 2001; Braun, 2004). Studies with extracts from medicinal plants have also shown this effect (Ortiz *et al*., 2009).

For this reason this study investigates the antinociceptive effect of the hydroalcoholic extract of *C. sympodialis* in models of analgesia in mice, considering the anti-inflammatory capacity of *C. sympodialis* described in previous studies.

MATERIAL AND METHODS

Botanic material and preparation of the extract

The botanical identification and morphological description of *C. sympodialis* was achieved by Maria Fátima Agra sector of Botanic of LTF / UFPB. Representative samples are deposited in the Herbarium Lauro Pires Xavier, UFPB under the code AGRA, 1456.

Leaves of *C. sympodialis* were collected 150 days after the first pruning from plants growing in the garden of medicinal plants of LTF-UFPB.

The leaves were dried in an oven with circulating air at 38º C for 72 hours, and then ground in mill kind Harley. Once broken, were macerated with 35% ethanol at room temperature for more than 72 hours. Then the material was filtered and subjected to a new extraction for the same period. The filtrates were concentrated on a rotary evaporator, giving a black material, hygroscopic, called hydroalcoholic crude, which was submitted to lyophilization.

Immediately before testing, doses EHACs were prepared with 0.9% saline and administered at a proportion of 0.1 mL/10g animal.

To verify the presence of the marker warifteine (war) in the hydroalcoholic extract of *C. sympodialis* an identification test was carried out on CCDA (Merck). Were made the following applications: 1) the standard "war", 2) Only the extract (Ext), tested and 3) extract plus warifteine (Ext + W). The solvent system used to elute the samples was $CHCl_3$: MeOH (80:20, v/v) and the plate was revealed with Dragendorff reagent. Warifteine was confirmed in extracts tested. The Rf-values presented for the three samples were Rf = 0.2666.

Animals

Male albino Swiss mice, with 2 to 3 months old, weighing between 25 and 40 g, were maintained under temperature conditions equivalent to 21 ± 1º C with free access to food and water. The animals were kept in a light/dark cycle of 12 hours, consisting of the light phase from 06:00 to 18:00. All experimental procedures were previously approved by CEPA - Ethics Committee guidelines of LTF/UFPB, under protocol number 0108/06.

STUDY OF ANTINOCICEPTIVE EFFECTS

Test of writhing by acetic acid

In this experiment five groups of 08 mice received by i.p. route the following pretreatments: saline 0.9%, 50, 100 and 200 mg/kg HAECs, adding even a group receiving 6 mg/kg morphine.

After 30 minutes of initial treatments, animals were treated with acetic acid 0.85% in distilled water (0.1 mL/10g) by i.p. route and placed in individual polyethylene boxes and after 5 minutes was counted the number of writhing displayed by each animal for 10 minutes (Naraynan et al., 2000; Bastos et al., 2006).

Formalin test

This test is considered a model of persistent pain, having the time of paw licks as an indication of nociceptive response. (Souza *et al*., 2000). Five groups of 08 mice received the following treatments by i.p. route: 0.9% saline, 50, 100 and 200 mg/kg HAECs morphine 10mg/kg. Exactly after 30 minutes was made by intraplantar administration of 20 µl of a 2.5% formalin solution.

Statistical analysis

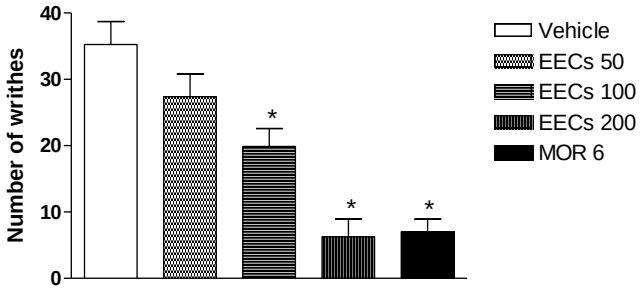
Results were evaluated by analysis of variance (ANOVA) followed by Dunnett's posttest. Numerical data were applied in the software Graph Pad Prism version 3.02. The values were expressed as mean ± standard error of mean (SEM) and were considered significant when the results showed a value p <0.05.

RESULTS

Effect of HAECs testing of writhing induced by acetic acid

This experiment evidenced that in all groups treated with HAECs the number of writhing decreased in a dose dependent effect: control (35.2 ± 3.5s), 50 mg/kg (27.4 ± 3.4s), 100 mg/kg (19.9 ± 2.7s), 200 mg/kg (6.2 ± 2.7s). The latter group presented results very similar to morphine with (7.0 ± 1.9s) contortions, as shown in Figure 1.

Figure 1. Effect of HAECs the writhing test induced by acetic acid in mice

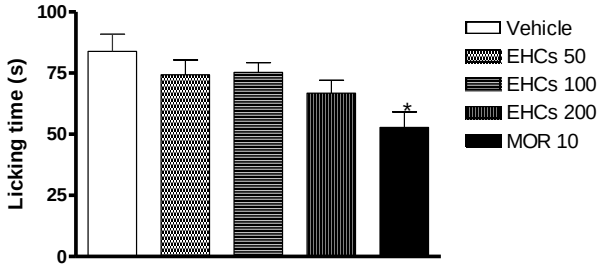


Values are expressed as mean ± e.p.m. (n = 08). ∗ p < 0.05 vs control (ANOVA – Dunnett's test).

HAECs effect in the formalin test

In the 1st phase of formalin test it was observed that mice treated with 50, 100 and 200 mg/kg showed no reduction in HAECs licking times, respectively 74.2 ± 6.1, 75.2 ± 4.1, 66.7 ± 5.3 seconds, compared to control 83.9 ± 7.0 seconds. Licking time of the group treated with morphine was 52.7 ± 6.4 seconds (Figure 2).

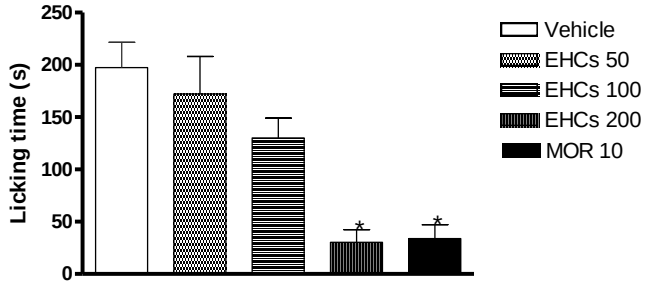
Figure 2. Effect of HAECs in the 1st phase of formalin test in mice treated i.p. route.



Values are expressed as mean ± e.p.m. (n = 08). ∗ p < 0.05 vs control (ANOVA – Dunnett's test).

The 2nd phase of the formalin test (Figure 3) shows a dose dependent reduction of the time of paw licks for doses of 50 mg/kg (172 ± 36s), 100 mg/kg (130 ± 19s) and 200 mg/kg (30.2 ± 12.2s), compared to control (197.4 ± 24.1s). However, only the dose of 200 mg/kg produced a statistically significant decrease when compared with control similar to morphine (33.7 ± 13.4s).

Figure 3. Effect of HAECs in the 2nd phase of formalin test in mice treated i.p. route.



Values are expressed as mean ± e.p.m. (n = 08). ∗ p < 0.05 vs control (ANOVA – Dunnett's test).

DISCUSSION

Analysis of the antinociceptive HAECs was performed using two behavioral models to induce nociception. The test of writhing induced by acetic acid produces chemical noxious stimulation in spinal cord component peripheral level with the formalin test and that is the stimulation of nociceptors so biphasic nociceptive behavior (Almeida and Oliveira, 2006).

The test of writhing induced by acetic acid was selected to evaluate the antinociceptive activity of the substance to be considered a method for easy, reliable and sensitive to drug central and peripheral (Shinde *et al*., 999; Ramezani *et al*, 2001).

The use of HAECs caused a reduction in the number of writhing in three doses, this reduction being

proportional to increased dose of the extract. These results demonstrated the effectiveness of the test HAECs of writhing induced by acetic acid, and allowed us to propose the antinociceptive and/or inhibition in the release of cytokines or inflammatory substances by the HAECs. Unlike the findings of Batista Lima *et al*., (2001) that administered subcutaneously and orally the extract at a dose of 200 mg/kg and received no reduction in the number of writhing.

Possible explanations for the reduction of writhing found in our study are probably the activation of B_2 receptors (receptors of the bradykinin type 2), which has been linked to most effects in vivo of kinins, including inflammation, pain and hyperalgesia (Steranka and Burch, 1991; Hall, 1992; Dray and Perkins, 1993), therefore an effect on these receptors may be involved. Another explanation could be the inhibition of activation of phospholipase A_2, which is directly involved in the cascade of arachidonic acid and prostaglandin formation. Thomas *et al*., in 1997 showed that the extract of *C. sympodialis* inhibited writhing induced by arachidonic acid in smooth muscle of the trachea.

As anti-inflammatory drugs HAECs was only effective inhibiting the second phase of the formalin test, corroborating, in accordance, the findings of the writhing test as a nonspecific test where anti-inflammatory drugs, as well as adrenergic antagonists, H_1 antihistamines, muscle relaxants or neuroleptics, have a reducing effect of contortions. Thus, it is suggested that the role of analgesic HAECs has a major anti-inflammatory component, possibly mediated by the reduction or inhibition of the release of endogenous mediators such as prostaglandins, cytokines, such as interleukins and TNF-α, and the participation of other mechanisms as the route of the NMDA receptor.

The Results showed that HAECs induces peripheral antinociceptive effects, evidenced by the reduction in writhing test, but not central antinociceptive activity, since the first phase of the formalin test failed to produce analgesia; the ability to inhibit the second phase of the formalin test, as well as the reduction of writhing suggests analgesic activity, presumably with anti-inflammatory consequences. Similar effect was obtained with an aqueous ethanolic extract of *Cissampelos pareira* roots, plant of same genus (Amresha *et al*., 2007).

ACKNOWLEDGMENT

The authors thank the José Crispim Duarte for his technical assistance and the Brazilian agencies for financial support: FAPESP, CAPES, CNPq.

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