20(2): 180-185, Abr./Mai. 2010
Received 3 November 2008; Accepted 24 November 2008

Artigo

Antinociceptive activity of *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae

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RESUMO: "Atividade antinociceptiva de *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae". A espécie vegetal *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, é usada popularmente para tratar inflamação, inchaço e feridas, bem como para tratar dor após o parto e dor estomacal. A administração do extrato etanólico e das frações lipídica e aquosa de *I. imperati* (300, 100 e 200 mg/kg) inibiu significativamente as contrações abdominais em camundongo induzidas por ácido acético, aumentou o tempo de sono evocado por pentobarbital sódico e mostrou significativa atividade inibitória sobre o edema de pata de camundongo induzido por formalina. As mesmas doses de *I. imperati* (300, 100 e 200 mg/kg) também elevou a latência de todos os tempos observados no teste da placa quente. O pré-tratamento de animais com naloxona (5 mg/kg, i.p) sugere a participação do sistema opioide no efeito anti-nociceptivo de *Ipomoea imperati.*

Unitermos: *Ipomoea imperati,* Convolvulaceae, dor, atividade analgésica, sistema opioide.

ABSTRACT: *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, is used in folk medicine for the treatment of inflammation, swelling and wounds, as well as to treat pains after childbirth and for stomach problems. Administration of ethanol extract, lipid and aqueous fraction of *I. imperati* (300, 100 and 200 mg/kg) significantly inhibited the abdominal constriction in mice induced by acetic acid; increased the sleeping time evoked by pentobarbital sodium and showed a significant activity by inhibiting formalin-induced paw edema in mice. The same dose of *I. imperati* also raised the pain of mice in the hot-plate test and increased the latency at all observation times. The pre-treatment of the animals with naloxone (5 mg/kg, i.p.) suggested the participation of the opioid system in the antinociceptive effect of *Ipomoea imperati.*

Keywords: *Ipomoea imperati,* Convolvulaceae, pain, analgesic activity, opioid system.

INTRODUCTION

Many species of Ipomoea are still used in folk medicine in different parts of the world (Austin, 1975). *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, grows well on sandy seashores in tropical climates, especially in Atlantic coastal areas and it is used in traditional medicine for the treatment of inflammation, swelling and wounds, as well as to treat pains after childbirth and for stomach problems (Fosberg & Sachet, 1977). Pharmacological studies on extracts of many species of *Ipomoea* have reported anti-inflammatory, antimicrobial, analgesic, spasmogenic, anti-spasmolytic, hypotensive, psychotomimetic and anticancer effects (MacLeod et al., 1997). Chemical investigations have shown that indole alkaloids and resin glycosides are the most common biologically active constituents in the Convolvulaceae (Noda et al., 1994).

In previous studies, *I. imperati* inhibited the topical and systemic inflammation in a concentration-dependent manner. *I. imperati* had a significant inhibitory activity against phospolipase A2 enzyme from bee venom (Paula et al., 2003).

Souza et al. (2000) related that other species of the Convolvulaceae family, such as *Ipomoea pes-caprae*, has showed an antinociceptive effect against two classical models of pain in mice. These findings supported the popular use of *I. pes-caprae* to treat dolorous processes. It was also related that *I. pes-caprae* showed anti-inflammatory, anti-spasmodic and anti-hemolytic properties (Pongprayoon et al., 1992).

Chemical studies of *Ipomoea cairica* led to isolation of the caffeoylquinic acid, that could explain, at least in part, its antinociceptive effect. This compound reduced the release of pro-nociceptive mediators unrelated to edema, such as histamine (Ferreira et al., 2006).

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ISSN 0102-695X

In the present study we have examined the antinociceptive activity of ethanolic extract, lipidic and aqueous fraction of *Ipomoea imperati* in abdominal writhing and hot plate animal model. Additionally, formalin-induced paw edema in mice was used to evaluate the antiedematogenic activity. The abdominal writhing animal model also investigated the action mechanism involved in the antinociceptive plant effect.

MATERIAL AND METHODS

Drugs

Acetic acid (Merck, Brazil), pentobarbital sodium (Merck, Brazil), dipyrone sodium (Clariant-Hoechst, Brazil), morphine hydrochloride, naloxone hydrochloride, diazepam and indomethacin (Sigma Chemical Co., St Louis, Mo) were used in this work. All drugs were dissolved in 12% tween solution just before use. Indomethacin was prepared in sodium bicarbonate (5%). All reagents used were of a high grade of purity.

Animals

All experiments were performed on male Swiss mice from the Central Animal House of the Universidade do Sagrado Coração (USC) weighing 25±5 g. The animals were fasted before all assays as the standard drugs were always orally administered, except for morphine, pentobarbital sodium and naloxone. The animals were fed a certified Nuvilab CR-a® (Nuvital) diet with free access to tap water and were kept in the animal house under standard conditions of 12 h dark 12 h light, humidity (50%) and temperature (24±1 °C). The experimental protocols were approved by the Ethics Committee of USC and were conducted according to the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993). All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

Plant

The leaves of *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, were collected by one of the authors (A.C.B. Paula Zurron) along the seashore of Boracéia, São Paulo State, Brazil, in January and February of 2006 and 2007. The specimens were identified by Rosangela Simão Bianchini and deposited in the Botanical Institute of São Paulo under voucher number SP 351848.

Preparation of the aqueous and lipidic fractions of *I. imperati*

The air-dried powdered plant material (800 g) was extracted at room temperature with ethanol during 7 d (repeated three times). After filtration, ethanol was removed under reduced pressure, producing 260 g (7.8%) of dry extract. The ethanolic extract of *Ipomoea imperati* (EtOH-Ipi, 21 g) was partitioned with water:dichloromethane (1:1, v/v, 26 g) three times, and provided both lipidic (LpF) and aqueous fractions (AqF). The pharmacological analysis was performed using the ethanolic extract (EtOH-Ipi), lipidic (LpF, 7.78 g) and aqueous fractions (AqF, 11.42 g).

Abdominal writhing induced by intraperitoneal injection of acetic acid

The response to intraperitoneal injection of a 0.6% acetic acid solution induced according to procedures described by Koster (1959), was a contraction of the abdominal muscle and stretching of the hind limbs. Animals were pre-treated with EtOH-Ipi, LpF and AqF (300, 100 and 200 mg/kg, *p.o.* respectively). The negative control animals received a similar volume of 12% tween (10 mL/kg, *p.o.*). Positive control mice received dipyrone (200 mg/kg, *p.o*). The drugs were administered 30 min before injection of 0.6% acetic acid. After challenge, pairs of mice were placed in separate transparent boxes and the number of abdominal writhes was counted over a period of 6 to 21 min. The antinociceptive activity was expressed as the reduction of the number of abdominal writhes.

Hot plate test

The hot plate test was used to measure response latency according to the method described by Eddy & Leimback (1953). In these experiments, the hot plate apparatus (Ugo Basile, Model-DS 37) was maintained at 56±1 °C. Animals were placed in a 24 cm diameter glass cylinder on a heated surface, and the time between placement and licking of the paws or jumping was recorded as latency. The latency times were recorded for control mice (pre-treated by oral route with vehicle, 12% tween solution 10 mL/kg), for animals pre-treated by subcutaneous route with morphine (10 mg/kg, *s.c*) used as positive control, and for animals pre-treated by oral route with EtOH-Ipi, LpF and AqF of *Ipomoea imperati* (300, 100 and 200 mg/kg, *p.o.* respectively). All substances were administered 30 min before the beginning of the experiment. Animals were selected 24 h before on the basis of their reactivity in the test. Only animals showing a reaction time within the range of 3.9-6.9 s were selected. All animals were observed before (0) and 30, 60 and 90 min after drug administration. A latency period of 30 s was defined as complete analgesia.

Pentobarbital-induced sleep

Thirty minutes after oral administration of

EtOH-Ipi, LpF and AqF of *Ipomoea imperati* (300, 100 and 200 mg/kg, *p.o.* respectively), all mice received an intraperitoneal dose of pentobarbital sodium (40 mg/kg). The period between loss and subsequent recovery of the righting reflex was taken as the sleeping time and recorded for animals pre-treated with the negative control 12% tween or with the positive control diazepam (5 mg/kg, *p.o*.) (Pieretti et al., 1992).

Analysis of the analgesic action mechanism of *Ipomoea imperati*

The possible participation of the opioid system in the antinociceptive effect of *I.imperati* was investigated (Trentin et al., 1997). To analyze this mechanism we also used the model of acetic acid-induced abdominal writhing in mice, with some modifications. Animals were pre-treated by intraperitoneal route with naloxone (5 mg/kg, *i.p*.) 15 min before oral administration of EtOH-Ipi, LpF and AqF (300, 100 and 200 mg/kg, respectively) and subcutaneous administration of morphine (10 mg/kg, *s.c*.). The negative control animals received by oral route a similar volume of 12% tween solution (10 mL/kg).

Formalin-induced paw edema in mice

The method used was similar to that described by Henriques et al. (1987). Group of male animals were treated by subcutaneous route with indomethacin (30 mg/kg) used as positive control or pre-treatment by oral route with EtOH-Ipi, LpF and AqF (300, 100 and 200 mg/kg, *p.o*. respectively). *Ipomoea imperati* was administered 30 min before the injection of 1% formalin (20 µL) into the subplantar area of the left hind paw. The paw volume was measured 4 h after formalin injection. Edema was calculated as the difference (µL) between injected and control paw. The area under the curve (AUC) time versus Δ paw volume was calculated for each animal and the edema was expressed as the mean±S.E.M of AUC.

Statistical analysis

Results are presented as mean±standard error of the mean (SEM) and were statistically analyzed by variance analysis (ANOVA) followed by the Dunnet pair wise test. P values of less than 0.05 were considered significant.

RESULTS

In the acetic acid-induced abdominal writhing model in mice, *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, (ethanolic extract, lipidic and aqueous fraction) significantly inhibited abdominal writhing in mice in 64.7, 83.8 and 72.7% (with an ED_{50} of 200, 60 and 50 mg/kg, respectively) when compared with the negative control (Table 1). The positive control Dipyrone also produced a significantly antinociceptive activity in this experiment ($p<0.001$).

The results presented in Table 2 show that the oral administration of *I. imperati* significantly increased the latency time at 30, 60 and 90 min after treatment with ethanolic extract ($p<0.05$), lipidic and aqueous fraction ($p<0.001$) of I. imperati, in the hot plate test. Morphine, used as a reference drug, also produced a significant antinociceptive effect during all the observation times when compared with control values ($p<0.001$).

In addition, the administration of ethanolic extract, lipidic and aqueous fractions 30 min before an intraperitoneal dose of pentobarbital sodium, increased the sleeping time in 25, 53 and 41% respectively, when compared with the negative control (Table 3, $p<0.05$). Diazepam used as positive control increased significantly the sleeping time in 94% ($p<0.001$).

The results listed in Table 4 showed that the pre-treatment of the animals with naloxone (5 mg/kg, *i.p*., 15 min before the acetic acid injection) significantly inhibited the analgesic effects of the morphine, ethanolic extract, lipidic and aqueous fraction in 45.0, 26.5, 36.2 and 31.3% respectively, on acetic acid induced abdominal writhing ($p<0.05$).

When tested on the classical mouse paw edema induced by formalin (1%, 20 µL), *I. imperati* at the dose of 300, 100 and 200 mg/kg *p.o.* (ethanolic extract, lipidic and aqueous fraction, respectively) significantly decreased the paw swelling in 40, 56 and 43% (figure 1, $p<0.05$). The positive control indomethacin inhibited around 80% of the mouse paw edema induced by formalin (Figure 1, $p<0.001$).

Table 1. Effect of the *Ipomoea imperati* aerial parts ethanolic extract (EtOH-Ipi) and its lipidic and aqueous fraction (LpF and AqF, respectively) on acetic acid-induced abdominal writhing in mice[a]

Treatment (*p.o*.)	Dose (mg/kg)	Number of abdominal writhes	% of inhibition
Control	-	38.50±1.22	-
Dipyrone	200	3.92±0.46**	90
EtOH-Ipi	300	13.50±0.67*	64.7
LpF	100	6.23±0.89**	83.8
AqF	200	10.50±0.67*	72.7

[a]each value is the mean±S.E.M for ten animals. Significantly different compared to the respective control value. ANOVA $F_{(4,45)} = 35.0$; *$p<0.05$, **$p<0.001$, Dunnet´s Test.

Table 2. Effect of the *Ipomoea imperati* aerial parts ethanolic extract (EtOH-Ipi) and its lipidic and aqueous fraction (LpF and AqF, respectively) on mice submitted to the hot-plate test[a].

Observation Time (min)	Control (*p.o.*)	Latency (seg)			
		Morphine (*s.c*)	EtOH-Ipi (*p.o.*)	LpF (*p.o.*)	AqF (*p.o.*)
0	5.00±0.23	5.00±0.30	5.10±0.46	5.00±0.28	5.00±0.37
30	5.20±0.30	16.00±0.80**	9.00±0.53*	14.80±0.46**	12.00±0.33**
60	5.28±0.36	18.30±0.45**	11.00±0.57*	16.70±0.50**	14.30±0.36**
90	6.30±0.50	21.00±1.00**	13.00±0.97*	18.00±0.80**	16.80±0.42**

[a]each value is the mean±S.E.M. for eight animals. Significantly different compared to the respective control value. ANOVA $F_{(4,35)}$; 0 min = 0.17 (p<0.05); 30 min = 43.8 (p<0.05); 60 min = 71.3 (p<0.05); 90 min = 13.4 (p<0.05). *p<0.05 and **p<0.001 (Dunnet´s Test)

Table 3. Effect of the *Ipomoea imperati* aerial parts ethanolic extract and its lipidic and aqueous fraction (LpF and AqF, respectively) on the sleeping time induced by pentobarbital sodium in mice[a].

Treatment (*p.o.*)	Dose (mg/kg)	Sleep time (min)	% of action drug
Control	-	32.30±1.84	-
Diazepam	5	62.60±2.32**	94
EtOH-Ipi	300	40.40±0.63*	25
LpF	100	49.40±1.67*	53
AqF	200	45.70±1.29*	41

[a]each value is the mean±S.E.M for ten animals. Significantly different compared to the respective control value. ANOVA $F_{(4,45)}$ = 37.0 (p<0.05); *p<0.05, **p<0.001, Dunnet´s Test.

Table 4. Effect of the *Ipomoea imperati* aerial parts ethanolic extract (EtOH-Ipi) and its lipidic and aqueous fraction (LpF and AqF, respectively) on acetic acid-induced abdominal writhing in mice pretreated with naloxone (5 mg/kg, *i.p.*) [a].

Treatment (*p.o.*)	Dose (mg/kg)	Number of abdominal writhes	% of inhibition
Control	-	40.00±1.20	-
Morphine	10	0**	100
Naloxone and morphine	10	22.00±1.05*	45
Naloxone and EtOH-Ipi	300	25.50±0.89*	26.5
Naloxone and LpF	200	27.50±0.98*	31.3

[a]each value is the mean± SEM for nine animals. Significantly different compared to the respective control value. ANOVA $F_{(5, 48)}$ = 38.6 (p < 0.05); *p < 0.05, **p< 0.00, Dunnet´s Test.

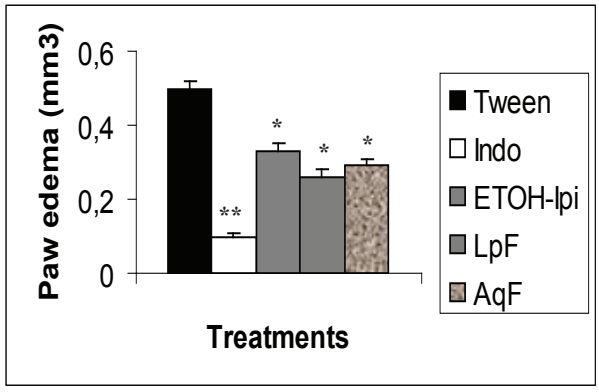

Figure 1. Effect of the ethanolic extract from *Ipomoea imperati* and its lipidic and aqueous fraction (300, 100 and 200 mg/kg. *p.o.*, respectively) in Formalin-Induced Mouse Paw Edema. Each value is the mean±S.E.M. for nine animals. Significantly different compared to the respective control value, ANOVA $F_{(4, 40)}$ = 34.6 (p<0.05). *p<0.05 and **p<0.001 (Dunnet´s Test).

DISCUSSION

In the present study, the analgesic activity of *Ipomoea imperati* (Vahl) Griseb., Convolvulaceae, was evaluated by the acetic acid induced writhing and hot plate tests. These tests analyzed the peripheral and centrally mediated antinociceptive responses. However, *I. imperati* action mechanism was evaluated only by abdominal writhing test.

The first indication of the analgesic property of *Ipomoea imperati* was observed in the acetic acid-induced abdominal writhing model in mice. Dypirone, ethanolic extract, lipidic and aqueous fraction of *I. imperati*, significantly inhibited abdominal writhing in mice (Table 1).

The mouse-writhing model is well known for the antinociceptive activity bioassay. It involves different nociceptive mechanisms, such as the sympathetic system (biogenic amines release), cyclooxygenases (COX) and their metabolites (Duarte et al., 1988) and opioid

mechanisms (Collier et al., 1968). Acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons sensitive to NSAIDs (non-steroidal anti-inflammatory drugs) and/or opioids (Collier et al., 1968).

Hiruma-Lima et al. (2000) related that there is an evident link of bradykinin, prostaglandin E2, leukotriene B4, PAF, interleukin 1, 5-hydroxytryptamine and histamine to the pathophysiological processes that accompany tissue damage and inflammation, especially pain and hyperalgesia.

Other model used to characterize the analgesic activity of *I. imperati* was the hot plate test. The results in Table 2 showed that *I. imperati* significantly increased the latency of the jumping response without affecting the ability to detect the thermal pain (licking response). Morphine, used as a reference drug, also produced a significant antinociceptive effect during all the observation times when compared with control values. The hot plate test is considered to be selective for opioid-like compounds in several animal species (Jansen et al., 1963), although other centrally acting drugs, including sedatives and muscle relaxants, have also shown activity in this test (Eddy & Leimback, 1953).

The fact that many neurosedative drugs tend to increase sleeping time, led us to assay the effect of *I. imperati* on the sleeping time induced by pentobarbital sodium in mice (Costa-Lotufo et al., 2004).

Administration of *I. imperati* before an intraperitoneal injection of pentobarbital sodium, significantly increased the sleeping time in mice when compared with the control animal group (Table 3). These data raised the possibility that the reduction in acetic acid-induced writhing and protection on the hot plate by *I. imperati* was a result of its sedative property. Many hypnotic, antianxiety and anti-epilepsy drugs prolong pentobarbital-induced sleeping time (Ma et al., 2007).

Changes in pentobarbital-induced sleep time can be a useful tool for examining stimulatory or inhibitory effects on the CNS, in particular for investigating influences on the opyoid system (Pan et al., 1999). The same authors related that opioid receptor mediates the actions of morphine and most clinical analgesic agents, as well as drugs of abuse.

The results listed in Table 4 showed that pre treatment of the animals with naloxone significantly inhibited the analgesic effects of morphine, ethanolic extract, lipidic and aqueous fraction of Ipomoea imperati (300, 100 and 200 mg/kg, p.o.) on acetic acid-induced abdominal writhing, when compared to control values. Naloxone, a classical morphine receptor antagonist, was able to modify the analgesia induced by *I. imperati* treatment. This effect results from an action on opioid receptors or the release of endogenous opioid substances (Gracioso et al., 1998; Wolfe 1944), suggesting that *I. imperati,* like morphine, is effective in abolishing acetic acid induced pain in an opioid way.

Thus, these data, taken together with those obtained against a thermal stimulus (hot plate test); revealed the existence of a possible central analgesic property of *I. imperati*. Nociception and drug-induced antinociception are experimentally estimated in animal models by monitoring behavioral motor responses resulting from nociceptive stimuli (Le Bars et al., 2001).

The formalin test is believed to represent a clinical pain model and can be used to clarify the possible mechanism of antiniciceptive effect of a test compound (Amanlou et al., 2005). The intraplantar injection of diluted formalin causes a characteristic response initiated by direct stimulation of nociceptors leading to activation of C fibers (Shibata et al., 1989). In this study, *I. imperati* showed a significant antiedematogenic activity in mouse paw edema induced by formalin ($p < 0.05$, Figure 1). The results are not necessarily related to the antinociceptive activity once the two phases of formalin's response were not evaluated.

It is well documented the involvement of histamine in formalin-induced nociception (Ferreira et al., 2006). Usually, the edema involves the release of several mediators such as 5-HT, histamine, bradykinin and prostaglandins, and active compounds that may act on one or several steps of the inflammatory process (Di Rosa, 1971). According to Ferreira et al. (1973), the edema also depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors, including prostaglandins (PGs).

The isolated caffeoylquinic acids could explain the antinociceptive effect of *Ipomoea cairica* polar extract (Ferreira et al., 2006). Preliminary results of chemical investigation of *Ipomoea pes caprae* showed that the extract contains phenolic and terpenoid substances (Pongprayoon et al., 1991a). *In vitro* assay detected 2-hydroxy-4,4,7-trimethyl-1(4H)-naphthalenone, mullein, eugenol and 4-vinylguaiacol isolated from *I. pes caprae* that had inhibitory effect on the synthesis of prostaglandin (Pongprayoon et al., 1991b). New experiments may be assayed to purify and identify the structure of the active principle (s) present in the *Ipomoea imperati* extract. The chemical constituents discovered in species like *I. pes caprae* and *I. cairica*, could be found in *I. imperati* and explain, at least in part, its antinociceptive effect.

In conclusion, our study clearly indicated the analgesic and antiedematogenic properties of ethanolic extract, lipidic and aqueous fraction obtained from Ipomoea imperati. Additionally, the analgesic effect can be related to the opioid system, showing central analgesic property. However, further research is required to analyze peripherally mediated antinociceptive responses.

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