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Antinociceptive activity of *Mirabilis jalapa* in mice

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ABSTRACT

Ethnopharmacological relevance: The infusion or decoction of *Mirabilis jalapa* leaves is used in traditional medicine in Brazil to treat inflammatory and painful diseases.
Aim of the study: The present study examined the antinociceptive effect of *Mirabilis jalapa* extracts from leaves and stems in models of pain in mice.
Materials, methods and results: The crude hydroethanolic extract from leaves (CrdL) was more potent than the crude extract from stems (CrdS) to inhibit abdominal constrictions induced by acetic acid, with ID_{50} values of 5.5 (2.3–13.1) and 18.0 (11.3–28.5) mg/kg, respectively. Among the fractions tested, the Eta fraction from leaves (Eta) was more effective (maximal inhibition of 83 ± 8%) and potent (ID_{50} of 1.1 (0.6–2.1) mg/kg) to induce antinociception. Eta and CrdL also possessed an antinociceptive effect in the tail-flick test. Pre-treatment with naloxone did not modify the antinociceptive effect of Eta, but co-administration with atropine completely prevented it. This suggests that the antinociceptive effect might depend on the cholinergic system. Instead, Eta was not able to alter the acetylcholinesterase activity in blood or spinal cord. Concerning side effects, Eta did not alter locomotor activity, body temperature, gastrointestinal transit and did not produce gastric lesions.
Conclusion: Our results demonstrate that *Mirabilis jalapa* presents antinociceptive activity in mice, which supports its folkloric use as an analgesic.

1. Introduction

Over the years, natural products have contributed to the development of important therapeutic drugs used currently in modern medicine. The study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy, in the search for new analgesic drugs and pain mechanisms (Calixto et al., 2000). *Mirabilis jalapa* belongs to the family Nyctaginaceae and is known as 'maravilha' or 'bonina' in Brazil and 'four o'clock', 'marvel-of-Peru', 'belle de nuit' or 'dondiego de noche' in different countries (Corrêa, 1984). In spite of its wide cultivation in several countries as a decorative plant, it is native of tropical America (Lorenzi and Souza, 1999).

Mirabilis jalapa has been well characterized with respect to its chemical components. Several compounds have been isolated from its aerial parts, such as β-sitosterol, stigmasterol, ursolic acid, oleanolic acid and brassicasterol (Siddiqui et al., 1990, 1994), and *Mirabilis* antiviral protein has been isolated from its roots (Kataoba et al., 1991). Regarding its biological activity, this plant has antibacterial and antiviral activities (Kusamba et al., 1991; Kataoba et al., 1991; Dimayuga et al., 1998). Cosmetic or dermo-pharmaceutical compositions containing *Mirabilis jalapa* are claimed to be useful against inflammation and dry skin (Linter, 2002). Moreover, methanolic extracts from the flowers, stems and leaves induced contractions of isolated jejunum muscle, and the most effective was the flowers extract (Cortés et al., 2004). Another recent study has shown the involvement of adrenergic and serotoninergic mechanisms in the inhibitory effect of *Mirabilis jalapa* flower extract on smooth muscle contractility (Aoki et al., 2008).

Mirabilis jalapa leaves are used in traditional folk medicine in the South of Brazil to treat inflammatory and painful diseases and as a laxative (Corrêa, 1984; Siddiqui et al., 1990; Somavilla and Canto-Dorow, 1996). As a laxative, 2–4 g of root powder in water are indicated for adults (Quer, 1962). For analgesic purposes, the infusion or decoction of leaves is popularly used (Somavilla and

Abbreviations: CrdL, crude extract from leaves; CrdS, crude extract from stems; Hex, *n*-Hexane fraction; Eta, ethyl acetate fraction; But, *n*-Butanol fraction; Dich, dichloromethane fraction.

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Canto-Dorow, 1996). Thus, the major aim of this study is to evaluate the possible antinociceptive effect of *Mirabilis jalapa* extracts, in models of visceral and thermal pain, in mice. We also investigated some possible mechanisms of action and adverse effects caused by *Mirabilis jalapa*.

2. Materials and methods

2.1. Plant material

The plant was collected in March 2006, in Santa Maria, in the State of Rio Grande do Sul, Brazil. A voucher specimen number SMDB 10.077 was deposited at the Herbarium of the Botany Department, Federal University of Santa Maria (UFSM), Brazil.

2.2. Extraction and fractionation

Fresh leaves and stems were dried in a ventilated oven (45 °C). Previously powdered dried leaves and stems were macerated with ethanol (70%) at room temperature. The crude hydroethanolic extracts from leaves (CrdL) or from stems (CrdS) were concentrated until dryness in a rotary evaporator. The dry hydroethanolic extract from leaves was dissolved in methanol (10%) and partitioned with *n*-hexane (Hex), dichloromethane (Dich), ethyl acetate (Eta) or *n*-butanol (But), respectively. The yields of CrdL and CrdS are 14,25 and 22,45% (w/w), respectively. The yields of these fractions in relation to the CrdL were 23, 6, 4 and 35% (w/w) for Hex, Dich, Eta and But, respectively.

2.3. Animals

Adult male albino Swiss mice (25–35 g) bred in our animal house were used. Animals were housed at controlled temperature (22 ± 2 °C) with a 12 h light/dark cycle and with standard lab chow and tap water *ad libitum*. The animals were habituated to the experimental room for at least 2 h before the experiments. Each animal was used only once. All protocols employed have been approved by the Local Ethic's Committee (process number: 23081.010134/2007-66) and are in accordance the US guidelines for the care and use of Laboratory animals (NIH publication #85-23, revised in 1985). The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.4. Visceral pain model

To investigate the possible antinociceptive effect of *Mirabilis jalapa*, leaf or stem crude extracts (1–100 mg/kg, p.o.) were tested in the writhing test induced by intraperitoneal injection of acetic acid 0.6% (Ferreira et al., 2000). Morphine (10 mg/kg, p.o.) was used as a positive control. We also tested the effect of some fractions from the leaf extract and compared their actions with the CrdL. The doses tested (10, 2.3, 0.6, 0.4 and 3.5 mg/kg, p.o., for CrdL, Hex, Dich, Eta or But fractions, respectively) were based on the fraction yield (see above). The animals were placed in glass chambers for 30 min before the experiment for habituation. One hour after oral treatment, the animals received an intraperitoneal injection of acetic acid solution, and the number of writhes was assessed during 30 min after the injection. The decrease in the number of writhes was considered the antinociceptive index. In this same manner, we evaluated the dose–response and time-course of antinociceptive effects of *Mirabilis jalapa* CrdL and Eta or CrdS (0.1–100 mg/kg, p.o.) in other groups of animals.

2.5. Investigation of some mechanisms of action

In order to investigate the participation of the muscarinic or opioid system in the antinociceptive effect of *Mirabilis jalapa* leaf Eta, mice were pre-treated with atropine (5 mg/kg, s.c.), naloxone (2 mg/kg, i.p.) or vehicle 1 h before the experiment. Eta (10 mg/kg, p.o.) or pilocarpine (1 mg/kg, s.c., used as positive control for atropine pre-treatment), morphine (10 mg/kg, p.o., used as positive control for naloxone pre-treatment) or vehicle was administered and 1 h later the animal was exposed to the acetic acid writhing test as described above. The choice of the dose of agonists and antagonists and their treatment times were based on previous data described in the literature (Sheardown et al., 1997; Yue et al., 2007).

2.6. Thermal pain model

Another test used to evaluate the possible antinociceptive effect of *Mirabilis jalapa* was the tail-flick test, which consists of measuring the reaction time to tail-flick after the tail was immersed in a bath heated to 48 °C (D'Amour and Smith, 1941). Before the test, the baseline latency (6–7 s) was determined. At 0.5, 1, 2 and 4 h after the administration of vehicle (10 mL/kg, p.o.), Eta or CrdL (10 mg/kg, p.o.) tail-flick latency was reassessed, and differ from the control and test were calculated. An 18 s maximum latency was employed to avoid tissue damage. Antinociception was expressed as percentage of maximum possible effect calculated as: $\%MPE = [(test - baseline)/(18 - baseline)] \times 100$.

2.7. Acetylcholinesterase activity

For the assay, animals were treated with vehicle, Eta or CrdL (10 mg/kg, p.o.) and then euthanized by pentobarbital overdose (10 mg/kg, i.p.). Blood was then immediately collected from the hepatic vein, using a heparinized syringe. The sample was diluted 1:100 in phosphate buffer (0.1 M, pH 7.4) with 0.03% Triton X-100. Samples of the spinal cord were also collected from the same animals by mechanical extrusion using cold saline. These samples were homogenized in 1 mL of potassium phosphate buffer (0.1 mM, pH 7.4) and diluted 1:5 in the same buffer. After preparation, both samples were frozen until analysis. For the assay, 500 μL of sample were added to 1 mL of phosphate buffer (blood) or potassium buffer (spinal cord), 50 μL of DTNB 10 mM and 25 μL of acetylthiocholine (ATC; 28.4 mM). The tubes were incubated in a water bath at 37 °C during 3 min. Then, 10 μL of ethopropazine (3 mM), an inhibitor of butyrylcholinesterase, were also added. The development of color was measured at 435 nm for blood (Worek et al., 1999) and 412 nm for spinal cord (Pereira et al., 2004). Results were expressed in μM ATC hydrolysed/hour/mg protein.

2.8. Gastrointestinal transit

As *Mirabilis jalapa* is popularly used for its laxative effect, we also decided to test the possible effect of *Mirabilis jalapa* CrdL and Eta in the gastrointestinal transit. In this study, mice were fasted for 18–24 h (water *ad libitum*) before the gastrointestinal transit analyses, as described previously (Milano et al., 2008). The animals were treated with Eta (10 mg/kg, p.o.), CrdL (10 mg/kg, p.o.), castor oil (10 mL/kg, p.o., used as positive control) or vehicle (10 mL/kg, p.o.) and 55 min later, a standard charcoal meal (5% charcoal, 20% Arabic gum, 0.3 mL) was given to mice by gavage. Five minutes after administration of the charcoal meal, the animals were euthanized and their stomachs and small intestines were removed to measure the length of the intestine (from the pyloric sphincter to the ileum–caecal junctions, the total gut length) and the distance traveled by the charcoal meal. Propulsive activity of the gut was

determined by the percentage of gastrointestinal traveled charcoal, calculated as: traveled% = 100 × (charcoal traveled distance/total gut length).

2.9. Ulcerogenic activity

To evaluate the gastric tolerability of animals after oral administration of *Mirabilis jalapa*, mice were fasted for 18 h prior to drug exposure (water *ad libitum*). The animals were treated with Eta, CrdL (10 mg/kg, p.o.), indomethacin (100 mg/kg, p.o., used as positive control) or vehicle (10 mL/kg, p.o.). Four hours later, animals were euthanized and the stomachs were opened by cutting along the greater curvature, washed with saline 4 °C. Immediately after that, the development of lesions was assessed with support of a magnifying glass. The quantification of gastric mucosal lesions was scored according to their number and size in a scale from 0 up to 8 points, adapted from Magistretti et al. (1988), as follows: (0) without injury, (1) color modification, (2) few petechia/alterations of villous, (3) 1–3 small injuries (≤1 mm length), (4) 1–3 big injuries (≤1 mm length), (5) 1–3 big injuries (>1 mm), (6) more than three small injuries, (7) more than three big injuries and (8) more than three deep injuries.

2.10. Rectal temperature

To evaluate whether Eta or CrdL was able to change the body temperature, rectal temperature was determined before and 0.5, 1, 2 and 4 h after drug administration (10 mg/kg, p.o.), as previously described (Otuki et al., 2001). The difference between pre-injection and post-injection values was calculated (Δ°C).

2.11. Locomotor activity

In order to investigate the possible effect of Eta or CrdL over the forced and spontaneous locomotor performance was tested in the rotarod and open-field tests, respectively (Milano et al., 2008). Twenty-four hours before the tail-flick test, all animals were trained in the rotarod (3.7 cm in diameter, 8 r.p.m.) until they could remain in the apparatus for 60 s without falling. On the day of the experiment, right after the measurement of tail-flick latency, each mouse was tested in the rotarod. The latency to fall and the number of falls from the apparatus were recorded with a stopwatch for up to 4 min. The open-field test was performed in another group of animals. The apparatus was a rectangular arena (28 cm × 18 cm × 12 cm) with the floor divided into 18 equal squares. The number of areas crossed with all paws and number of rearing responses were recorded.

2.12. Acute toxicological evaluation

To assess the acute toxicity of *Mirabilis jalapa*, we attempted to determine the LD_{50} value from the Eta and CrdL using the up-and-down method as described by Bruce (1985). After the administration of one single dose of *Mirabilis jalapa* (10, 30, 100, 300, 1000, 3000 mg/kg, p.o.), the survival of animals was observed during 24 h. If an animal survived at any given dose, the dose for the next animal was logarithmically increased; if it died, the dose was decreased. Doses above 3000 mg/kg were not tested due to solubility problems.

2.13. Drugs and reagents

All extracts were prepared in the Laboratory of Pharmacognosy from UFSM, diluted in 5% Tween 80, 20% polyethylenoglycol and 75% saline and administered orally. Morphine sulphate (Cristália, Brazil), indomethacin (Sigma) and pilocarpine (Merck) were also diluted in the same vehicle and administrated by oral route. Naloxone sulphate and atropine sulphate were purchased from Sigma and diluted in saline solution for intraperitoneal administration. 5,5′-Dithiobis(2-nitro-benzoic acid) (DTNB), ethopropazine and acetylthiocholine were purchased from Sigma. Arabic gum, activated charcoal, acetic acid and Triton X-100 were purchased from VETEC (Rio de Janeiro, Brazil).

2.14. Statistical analysis

The results of antinociceptive and side effects were expressed as means ± S.E.M., and the gastric lesion scores were expressed as median, and ID_{50} values (i.e. the dose of compounds that reduces nociceptive responses to the order of 50% relative to the control value), which were reported as geometric means accompanied by their respective 95% confidence limits. Data were analyzed by one or two-way analysis of variance (ANOVA), or *t*-test when appropriate. Post hoc tests (Student–Newman–Keuls test-SNK) were carried out when appropriate. Non-parametric Kruskal–Wallis followed by Dunn's test was used to analyze gastric lesion scores. The ID_{50} values were determined by non-linear regression analysis using a sigmoidal dose–response equation of individual experiments using GraphPad Software 4.0 (GraphPad, USA).

3. Results

3.1. Antinociceptive effect of Mirabilis jalapa extracts and its fractions

Firstly, we verified the effect of CrdL and CrdS on the nociception induced by acetic acid. Both CrdL and CrdS treatments (1–100 mg kg, p.o.) reduced the number of abdominal writhes induced by acetic acid (Fig. 1A and B). CrdL and CrdS had similar efficacy to produce antinociception (maximal inhibitions of 65 ± 13 and 77 ± 6%, respectively), but CrdL was more potent than CrdS with calculated ID_{50} values of 5.5 (2.3–13.1) and 18.0 (11.3–28.5) mg/kg, respectively. Thus, we choose the CrdL to conduct further studies. The antinociceptive effect produced by CrdL was induced quickly, being observed as early as 30 min after oral treatment, but it was short-lasting, not lasting more than 1 h (Fig. 1C). Next, we assessed the effect of several fractions from CrdL in the same nociception test. When tested in doses based on their yields in CrdL, only treatment with the Eta produced antinociceptive action (Fig. 1D).

The antinociceptive effect of Eta was of the same magnitude as that produced by CrdL. For this reason, the Eta was chosen for dose–response and time-course studies (Fig. 2A and B). The Eta had an antinociceptive effect from 0.5 up to 2 h after treatment, with an ID_{50} value of 1.1 (0.6–2.1) mg/kg and maximal inhibition of 77 ± 8%. The antinociceptive effect of Eta was of the same magnitude as that produced by morphine (10 mg/kg, p.o.).

To confirm their antinociceptive effect, we also investigate Eta and CrdL action in the tail-flick test. In this model, both extracts also showed significant antinociceptive effect (10 mg/kg; p.o.) (Fig. 3A and B). The antinociceptive effect observed for CrdL started at 0.5 and lasted up to 1 h after treatment, while the Eta antinociceptive effect lasted up to 2 h.

3.2. Side effects investigation

Firstly, we evaluated the alteration in gastrointestinal transit produced by *Mirabilis jalapa*. In this study, Eta did not change gastrointestinal motility. On the other hand, CrdL or castor oil increased the gastrointestinal transit in treated mice (Fig. 4).

We also evaluated the possible ulcerogenic activity of *Mirabilis jalapa*. Neither Eta nor CrdL (10 mg/kg, p.o.) were induced ulcero-

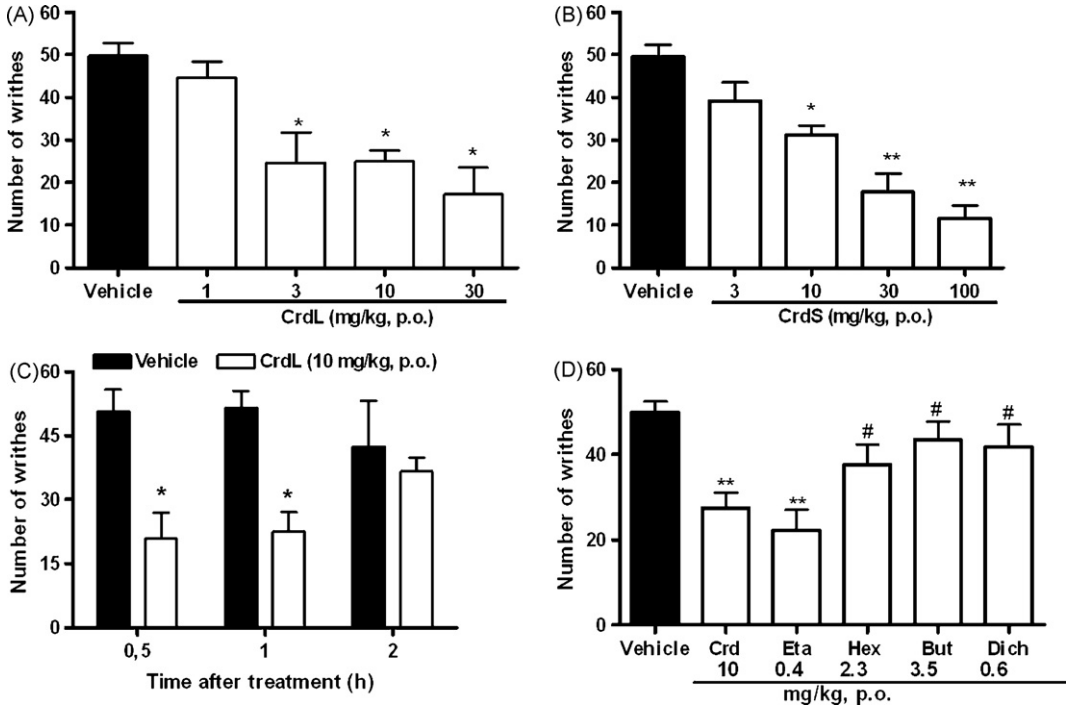

Fig. 1. (A and B) Dose–response curves for the antinociceptive effect of leaf (CrdL, A) and stem (CrdS, B) crude extracts of *Mirabilis jalapa* (p.o.) administered 1 h before acetic acid test. (C) Time-course for the antinociceptive effect of leaf crude extract (CrdL, 10 mg/kg, p.o.) of *Mirabilis jalapa*. (D) Effect of the oral treatment with leaf crude extract from *Mirabilis jalapa* (CrdL) or *n*-hexane (Hex), dichloromethane (Dich), ethyl acetate (Eta) or *n*-butanol (But) fractions on acetic acid-induced nociception. Data are expressed as means ± S.E.M. of number of writhes observed 30 min after the administration of acetic acid (0.6%, 10 mL/kg, i.p.) in mice ($n = 7–8$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle group; $^{\#}P < 0.05$ compared with Eta group; one-way ANOVA followed by SNK test.

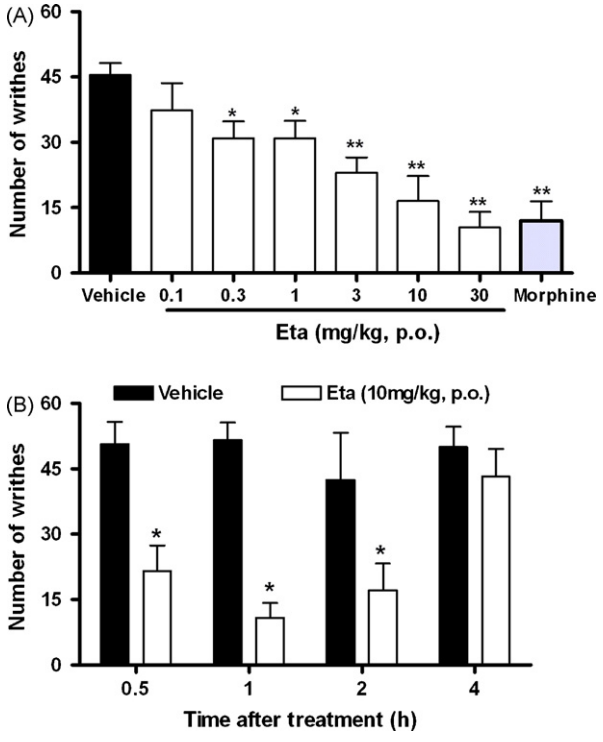

Fig. 2. (A) Dose–response for the antinociceptive effect of leaf ethyl acetate (Eta) fraction (0.1–30 mg/kg, p.o.) of *Mirabilis jalapa* administered 1 h before acetic acid test. (B) Time-course for the antinociceptive effect of Eta (10 mg/kg, p.o.) of *Mirabilis jalapa* against acetic acid-induced abdominal constriction test. Data are expressed as means ± S.E.M. of number of writhes observed 30 min after the administration of acetic acid (0.6%, 10 mL/kg, i.p.) in mice ($n = 7–8$). $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$, one-way ANOVA followed by SNK test (A) or two-way ANOVA followed by Bonferroni test (B).

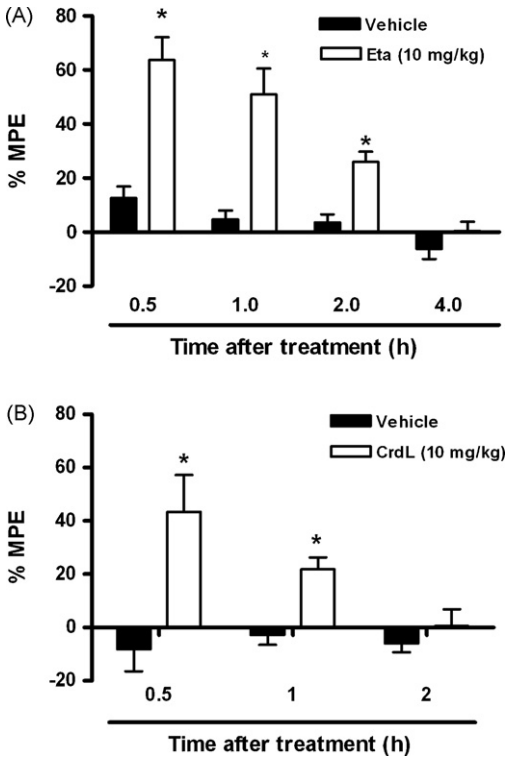

Fig. 3. Time-course of antinociceptive effect of leaf ethyl acetate fraction (Eta) fraction (A) or crude hydroethanolic extract (B) from (10 mg/kg, p.o.) of *Mirabilis jalapa* in the tail-flick test in mice ($n = 7–8$). Data are expressed as means ± S.E.M. $^{*}P < 0.05$ and $^{***}P < 0.001$, two-way ANOVA followed by Bonferroni post hoc test.

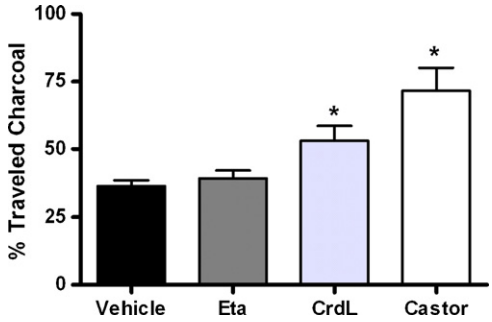

Fig. 4. Effect of castor oil, leaf ethyl acetate (Eta) fraction and hydroethanolic crude (CrdL) extract (10 mg/kg, p.o.) from of *Mirabilis jalapa* in the gastrointestinal transit. Data are expressed as means ± S.E.M. $^{*}P < 0.05$, one-way ANOVA followed by SNK post hoc test.

genic activity, while indomethacin (100 mg/kg, p.o., positive control) induced the formation of gastric lesions [the medians (25–75 percentiles) with lesion scores of 0.5 (0–1.5), 5.0 (4.5–5.5), 2.0 (1.5–2.0) and 0 (0–0.5) for vehicle, indomethacin, CrdL and Eta, respectively].

We also investigated the effect of Eta or CrdL on motor performance and body temperature. Neither Eta nor CrdL (10 mg/kg, p.o.) altered body temperature or forced and spontaneous locomotion, assessed in the rotarod and open-field tests, respectively (data not shown). Finally, we assessed acute toxicity after Eta or CrdL administration. Treatment with either Eta or CrdL up to 3000 mg/kg did not cause mortality in any of the animals.

3.3. Some possible mechanisms of action

In order to investigate some possible mechanisms of action of Eta antinociceptive action, animals were pre-treated with opioid or muscarinic receptor antagonists. Treatment with naloxone (2 mg/kg, i.p.) did not change the antinociceptive effect of Eta (Fig. 5A), but greatly reversed the antinociception produced by morphine. On the other hand, pre-treatment with atropine (10 mg/kg, i.p.) completely prevented the antinociceptive effect of Eta (Fig. 5B) and pilocarpine (positive control). This result suggests that the muscarinic system, rather than the opioid system, might be involved in the antinociceptive effect of Eta.

3.4. Acetylcholinesterase activity

As the muscarinic system is involved in the Eta antinociceptive effect, we investigated the possible action of Eta on acetylcholinesterase activity in blood and spinal cord. We observed that Eta treatment *in vivo* (10 mg/kg, p.o.) did not alter acetylcholinesterase activity in blood or spinal cord of treated mice (acetylthiocholine hydrolyzed per hour per mg protein of 21.7 ± 5.6 and 17.9 ± 5.9 or 0.30 ± 0.07 and 0.28 ± 0.04 in blood or spinal cord after treatment with vehicle and Eta, respectively).

4. Discussion

Mirabilis jalapa is a native plant of tropical America that currently is widely cultivated in several countries as a decorative plant (Lorenzi and Souza, 1999). Moreover, different parts of *Mirabilis jalapa* have medicinal uses in several regions of the world, such as Latin America, South Africa, Zaire, Madagascar, India and Pakistan, where they are used as a laxative and to treat infections, inflammation, and allergic and painful conditions (Kusamba et al., 1991; Somavilla and Canto-Dorow, 1996; Dimayuga et al., 1998; Cortés et al., 2004). *Mirabilis jalapa* is used as an analgesic in Madagascar and Mexico to treat several painful conditions, including intestinal pain

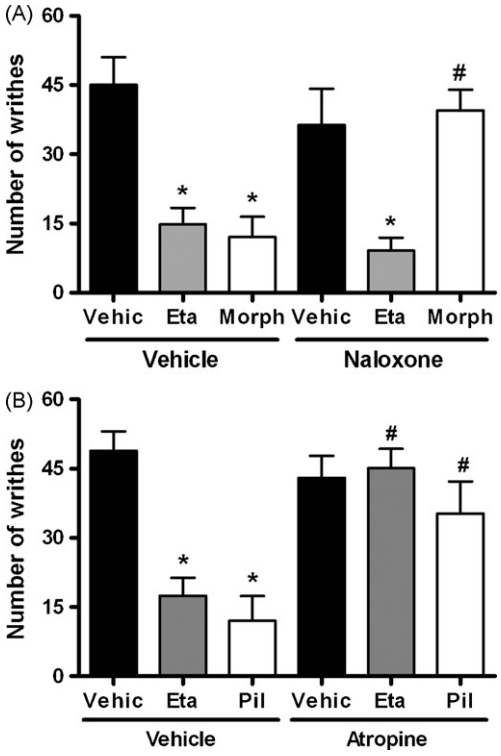

Fig. 5. Effect of naloxone (A, 2 mg/kg, i.p.) or atropine (B, 5 mg/kg, s.c.) on the antinociceptive effect caused by leaf ethyl acetate fraction (Eta, 10 mg/kg, p.o.), morphine (morph, 10 mg/kg, p.o.) or pilocarpine (Pil, 1 mg/kg, s.c.) in acetic acid constriction test in mice. Data are expressed as means ± S.E.M. $^{***}P < 0.001$, as compared with vehicle plus vehicle group, $^{\#}P < 0.05$, compared with vehicle plus morphine or pilocarpine groups, one-way ANOVA followed by SNK test.

and that produced by scorpion and bee stings (Kusamba et al., 1991; Dimayuga et al., 1998). Furthermore, in the state of Rio Grande do Sul in the South of Brazil, where the current study was carried out, the leaves of *Mirabilis jalapa* are popularly used as an analgesic and anti-inflammatory agent (Somavilla and Canto-Dorow, 1996). However, to our knowledge, there is no study confirming the analgesic activity of *Mirabilis jalapa* in laboratory conditions. However, it has been demonstrated that some other plants of the Nyctaginaceae family, such as *Boerhaavia diffusa* and *Bougainvilla spectabilis*, presented analgesic activity (Hiruma-Lima et al., 2000; Malairajan et al., 2006). Here, we have shown that crude extracts from leaves and stems of *Mirabilis jalapa* as well as the Eta from leaves have antinociceptive activity in mice. Moreover, the antinociceptive effect of *Mirabilis jalapa* seems to be related to stimulation of the muscarinic system, but not related with alterations of motor ability, body temperature, gastrointestinal transit and stomach integrity.

One of the most used tests for screening possible analgesic compounds is the writhing test using diluted acetic acid (Negus et al., 2006). This test revealed that crude extracts from leaves and stems of *Mirabilis jalapa* exhibited antinociceptive activity. However, the leaf extract was about three-fold more potent to produce antinociception than the stem extract. Among all fractions tested from the leaf extract, Eta was the only one that produced antinociceptive action in a dose compatible with its yield in the crude extract. Moreover, Eta was about five times more potent than the CrdL in the writhing test and displayed a longer time of action. Of note, the efficacy and potency of Eta was similar to morphine in this test. Although the acetic acid test is good for screening studies, it can be subject to false-positive results (Franklin and Abbott, 1989). Thus, we confirmed our results by using the tail-flick test. In this test, it was observed that both Eta and CrdL produced an antinociceptive

effect with the same duration of action. The tail-flick test is insensitive to non-steroidal anti-inflammatory drugs, but is sensitive to centrally acting analgesics, such as opiates (Franklin and Abbott, 1989).

Thus, we first investigated the role of the opioid system on the antinociceptive effect of the Eta. Pre-treatment with naloxone did not reverse the antinociceptive effect induced by Eta in conditions where it greatly prevented the antinociceptive effect of morphine. This result suggests that the opioid system did not participate in the antinociceptive effect of *Mirabilis jalapa*. Besides the opioid system, the cholinergic system, especially through muscarinic receptors, is an important modulator of pain in the central nervous system (Wess et al., 2007). Our present study has shown that pre-treatment with atropine completely prevented the antinociceptive effect of Eta, demonstrating a critical role of the cholinergic muscarinic system in Eta antinociception. Cholinesterase inhibitors have been shown to have an antinociceptive effect, which is mediated through spinal cord cholinergic receptors, mainly muscarinic (Yoon et al., 2003). However, we have found that Eta did not alter acetylcholinesterase activity in the blood or spinal cord of treated mice. This result suggests that the antinociceptive action of the Eta from *Mirabilis jalapa* did not involve acetylcholinesterase inhibition. Thus, other targets of the cholinergic system could be altered by *Mirabilis jalapa*, such as a direct receptor interaction or a stimulation of acetylcholine release. However, further studies must be carried out to elucidate this point.

Although it demonstrated involvement in the cholinergic muscarinic system to induce antinociception, Eta did not modify the gastrointestinal motility in mice. It has been demonstrated that non-selective muscarinic agonists produced increases in mice gastrointestinal transit, while M1, M2 or M3 selective agonist receptors failed to produce any significant changes (Williams et al., 1992). On the other hand, CrdL increased gastrointestinal transit. This last result agrees with the popular use of *Mirabilis jalapa* as a laxative. Furthermore, the fraction of the crude extract from leaves with ethyl acetate seems to reduce the amount of active substances responsible for gastrointestinal motility interference.

A major concern in experiments designed to evaluate the analgesic action of new agents is whether pharmacological treatment causes other behavioral alterations, such as altering motor coordination, causing sedation or producing hypothermia, which could be misinterpreted as analgesia (Negus et al., 2006). Treatment with the Eta did not cause any alteration in the rotarod and open field tests, nor in rectal temperature. Different from non-steroidal anti-inflammatory drugs, neither the Eta nor the CrdL produced ulcerogenic activity. This result reinforces the idea that the leaf Eta induces antinociception without significant side effects.

Furthermore, we have found that the acute treatment of mice with the Eta and CrdL from *Mirabilis jalapa* (up to 3000 mg/kg, p.o.) did not cause death of any animal. On the other hand, Rocha (2006) found that high doses of the hexanic fraction from *Mirabilis jalapa* leaf methanolic extract (1000–3000 mg/kg) administered by intraperitoneal route induced acute toxicity in female mice with a LD_{50} of 2009 mg/kg. Several differences between our study and that of Rocha (2006) may explain these discrepant findings, including the kind of fraction used (ethyl acetate vs. hexanic), the route of administration (oral vs. intraperitoneal) and the gender of mice (male vs. female). However, more studies must be carried out to further assess the toxicity of *Mirabilis jalapa*.

5. Conclusion

Taken together, the results presented in the current study show that the CrdL or Eta from *Mirabilis jalapa* administered orally to mice, produces antinociceptive action. Thus, the present study confirms the efficacy of this plant used in traditional folk medicine.

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