Antinociceptive effects of an extract, fraction and an isolated compound of the stem bark of *Maytenus rigida*


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Abstract: The antinociceptive activity of the *Maytenus rigida* Mart. (*Celastraceae*) ethanol extract and its ethyl acetate fraction as well as of (-)-4′-methylepigallocatechin (1), a previously isolated compound, was demonstrated in vivo. ED50 for 1 in the writhing test was 14.14 mg/kg. The acetic acid-induced writhing was inhibited by 98.4, 84.4, and 58.3%, respectively, when mice were treated with the ethanol extract, ethyl acetate fraction, and 1. In the hot plate test, mice pretreated with 1 showed significantly increased reaction times (60-89%). Oral administration of 1 significantly inhibited first and second phases of the formalin-induced pain (50 and 26.5%, respectively), whereas indomethacin inhibited only the second phase of the test (41.2%). Ethanol extract and its fraction showed effects on inflammatory pain, while neurogenic and inflammatory pain suppression by 1 is a strong indication of the presence of both central and peripheral effects and suggests its analgesic and anti-inflammatory potential.

Introduction

The enormous floral diversity of Brazil has provided local traditional health practitioners with an impressive array of plant material from which to select ingredients for use in herbal medicines. Over the centuries, this “natural pharmacy” has been developed and refined to treat many human and animal disorders. Even with the enormous technological advances that have been made in conventional modern medicine, nearly 70% of population in Brazil still relies heavily on traditional healing practices and phytomedicines for their daily healthcare needs (Begossi et al., 2002; Lima et al., 2006).
Agar furans (Gonzalez et al., 1993), and a number of phenolic compounds such as flavonoids, xanthones, catechins, leucoanthocyanidins and tannins (Estevam et al., 2009). Some of these compounds are used to treat painful conditions (Mota & Albuquerque, 2002), while others showed antiseptic, antiasthmatic, antitumoral, and fertility-regulating properties (Lee et al., 1982; Kuo et al., 1990; Ghazanfar, 1994; Gonzalez et al., 1996). The book As Muitas Faces da Jurema (The Many Faces of Jurema) describes the use of the bark of *M. rigida* as a component of “jurema-preta” tea, known for its hallucinogenic properties (Mota & Albuquerque, 2002) and a previous study showed its antimicrobial activity (Estevam et al., 2009). Since *M. rigida* reportedly have analgesic activities (Mota & Albuquerque, 2002; Dias et al., 2007), the aim of the present study was better to investigate the antinociceptive (central and peripheral) properties of the ethanol extract of the plant, ethyl acetate fraction, and an isolated compound, using three in vivo mice models, namely, the acetic acid-induced writhing test, the hot plate test, and the formalin-induced nociception test.

**Material and methods**

**Chemicals**

Analytical and spectroscopic grade solvents were obtained from Grupo Química (Rio de Janeiro, Brazil). Acetic acid, indomethacin, and formaldehyde were supplied by Merck (Darmstadt, Germany). Arabic gum and dypirone were purchased from Sigma (St. Louis, MO, USA), while morphine sulfate (Dimorf) was supplied by Cristália (Itapira, SP, Brazil).

**Plant material**

*Maytenus rigida* Mart., Celastraceae, stem barks were collected as previously described by Dias et al (2007) and a voucher specimen was deposited in the herbarium of the Federal University of Sergipe under reference number 007677. Plant extract preparation, fractioning and (−)-4′-methylepigallocatechin (1) isolation were previously described (Dias et al., 2007).

**Animals**

Male Swiss mice obtained from breeding units at the Laboratory of Evaluation and Synthesis of Bioactive Substances, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, and from the Laboratory of Pharmacology and Immunity, Department of Physiology, Federal University of Alagoas, Maceió, Brazil, weighing between 20-30 g each, were used in the antinociceptive tests. The animals were kept at 25-28 °C under a 12 h light/dark cycle with food and water provided *ad libitum*. The study was approved by the Research Ethics Committee of the Federal University of Alagoas (approval protocol UFAL N ° 006443/2005-78) and conducted according ethical guidelines for the care of laboratory animals and investigation of experimental pain in conscious animals (Zimmerman, 1983).

**Writhing antinociceptive test**

The test was carried out using the method proposed by Koster et al. (1959) with modifications. Mice were randomly divided into groups of 10 to be orally pretreated with the crude ethanol plant extract (100 mg/kg), ethyl acetate fraction (100 mg/kg), and compound 1 (0.32-96.1 mg/kg), 60 min before the administration of 0.25 mL of 0.6% acetic acid by intraperitoneal injection. Positive control groups orally received the standard analgesic dypirone (0.1-100 mg/kg) and the standard anti-inflammatory indomethacin (0.036-107.3 mg/kg), while negative control animals received a dose of the vehicle Arabic gum 5% (10 mL/kg) before administering acetic acid. The number of abdominal writhes showed by each mouse was counted starting 10 min after injecting acetic acid, and counting was continued for 20 min. The antinociceptive activity was expressed as the percentage of inhibition of contractions compared with the negative control group.

![Structure of compound 1](image_url)
oral dose) or morphine (4.25 mg/kg, positive control, i.p.). Treatment latencies were recorded at 0, 60, 90, 120 and 150 min.

**Formalin-induced pain test**

The formalin test was performed according to the method of Hunskaar & Hole (1987). Groups of ten mice were orally pretreated with either vehicle 5% arabic gum (10 mL/kg, negative control), compound 1 (32 mg/kg) or indomethacin (12.9 mg/kg, positive control), and 60 min later with 2.5% formaldehyde (20 μL) injected into the subplantar region of the right hind paw. The time each animal spent licking its paws during the first 5 min (first phase) and from 15 to 30 min (second phase) following injection was recorded. The test was performed at 22-26 °C and care was taken to exclude environmental disturbances (high temperature, noise and excessive movement) that might interfere with the natural response of the animal (Tjølsen et al., 1992).

**Data analysis**

Results were recorded as mean values±SEM. The significance of differences between mean values was determined by ANOVA and Dunnett’s test for \( p \leq 0.05 \). The ED50 values were determined by linear regression using Graph Pad Prisma® software.

**Results**

**Writhing test**

The antinociceptive activities of the crude ethanol extract of the stem bark of *Maytenus rigida*, ethyl acetate fraction and 1 were initially evaluated using the acetic acid-induced writhing test. Administration of acetic acid by intraperitoneal injection to a negative control group of mice that had been orally pretreated with vehicle (arabic gum) induced 50.5±5 contortions during the observation period. On the other hand, in the groups of animals pretreated with ethanol extract and ethyl acetate fraction at 100 mg/kg, and 1 at 32 mg/kg, acetic acid-induced writhing was inhibited by 98.4, 84.4, and 58.3%, respectively, all of which are values that differed significantly from that of the control (\( p<0.05 \), Figure 1). The group of animals that received dypirone as a standard antinociceptive drug presented 56% of writhing inhibition compared with the control group.

To further evaluate the effects of 1 on inflammatory pain, the relationship between the inhibition and doses of 1, dypirone (analgesic) and indomethacin (anti-inflammatory) were determined and are shown in Figure 2. The calculated ED50 for 1 was 14.14 mg/kg, a value that is comparable with those of the reference drugs dypirone and indomethacin (Figure 1). These data demonstrated that *M. rigida* possesses a potent anti-inflammatory effect, and suggest that 1 is one of the constituents responsible for this activity. Although no specific test for the toxicity or effect of 1 on motor activity was done, it was observed that treated animals did not show any apparent sign of toxicity such as lethargy, decreased motor activity, morbidity, or mortality, when monitored for 72 h after administration of the extract, fraction, and 1.
Hot plate test

To confirm the antinociceptive effect of 1, and to find out whether the process is mediated by central or peripheral mechanisms, models to investigate the nociceptive activity were employed. In the hot plate test, mice pretreatment with 1 produced a significant increase ($p<0.05$) in the latency time between 60 and 150 min (60-89%; Figure 3). Although the increase in latency induced by 1 following 30 min of post treatment was smaller than the one produced by morphine (70-161%), the effects generated by 1 and morphine were of similar duration and magnitude at 90, 120 and 150 min after pretreatment. These results suggest the antinociceptive of 1 is in part mediated by its central analgesic action.

Formalin-induced pain test

The anti-inflammatory profile of 1 was determined using a classic nociception model based on the two phases of the formalin-induced pain test. As shown in Figure 4, oral administration of 1 induced significant reductions in the licking activity in both phases compared with the control ($p<0.05$) by 50 and 26.5%, respectively, while indomethacin had no effect on the first phase, although it promote a 41.2% reduction in the licking activity in the second phase when compared with the control. These results suggest the antinociceptive activity of 1 is mediated by both central and peripheral mechanisms.

Discussion

Although few studies have focused on the antinociceptive activity of plants in the *Maytenus* genus, a previous report showed antinociceptive effects of the hexane and ethyl acetate extracts of *M. ilicifolia* Mart. ex. Reiss at 320 mg/kg without any toxicity in mice (Jorge et al., 2004). Analgesic properties of *Maytenus rigidia* Mart., Celastraceae, stem bark were previously investigated using the tail flick test, which showed the spinal antinociceptive potential of the plant extract and its ethyl acetate fractions at 100 mg/kg, which were chosen to be used in the present study, and 1. Although that study did not give extensive information on the action mechanism, it showed the involvement of the opioid receptor in the antinociception (Dias et al., 2007).
Compound I was tested at 32 mg/kg in the present study, which is smaller than the 75 mg/kg used by those authors. In the present study, antinociceptive tests were selected so supraspinal antinociception and inflammatory effects could be investigated.

The findings of the present study showed the M. rigida stem bark hydroethanol extract, its ethyl acetate fraction and I obtained from the ethyl acetate fraction (Dias et al., 2007) presented in vivo antinociceptive activity when orally administered to mice. The acetic acid-induced writhing method is widely used to evaluate peripheral antinociceptive activity, which is characterized by inflammation (Gene et al., 1998). The test is very sensitive and is able to detect antinociceptive effects at dosage levels that appear inactive when applied in other methods such as the tail-flick test (Bentley et al., 1981). Local peritoneal receptors are believed to be partly involved in the abdominal constriction response (Bentley et al., 1983), although the acute inflammatory reaction is thought to be related to increased levels of PGE2 and PGF2α, as well as lipoxygenase products in peritoneal fluids (Deraedt et al., 1980). Considering that peripherally acting nonsteroidal drugs, such as dipyrone and indomethacin, inhibit the writhing response in the abdominal constriction model, these compounds were used as standard drugs. Peripheral drugs are cyclooxygenase (COX) inhibitors and show activity in this model (Deraedt et al., 1980; Marchioro et al., 2005). Therefore, it is possible the extract, fraction and I, in mimicking indomethacin, may have a peripherally mediated activity, which may be associated partly with lipoxygenase and/or cyclooxygenase. However, further studies should be conducted to confirm this hypothesis.

(-)-4′-Methylepigallocatechin (I) induced a dose-dependent antinociceptive effect in the acetic-acid writhing model. Thus, its antinociceptive activity was further investigated by the hot plate test and in the formalin-induced pain test. The former test is often employed to evaluate narcotic analgesics and other centrally acting drugs and to investigate their supraspinal action. In the present study, I mimicked the effects of morphine, which indicated supraspinal antinociceptive effect with central activity. In the formalin model, however, there is a distinctive biphasic nociceptive response in which the first (or neurogenic) phase is associated with the irritating effect of formalin on the sensory fibers of type C, while the second phase represents inflammatory pain in response to the release of serotonin, histamine, bradykinin, and prostaglandins and, at least to some degree, central nociceptive neuron sensitization (Marchioro et al., 2005). Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the second phase (Chen et al., 1995; Marchioro et al., 2005). Neurogenic and inflammatory pain suppression, as observed in the present study when I was administered to mice, strongly indicates the presence of both central and peripheral effects.

Conclusions

The present study supports the traditional use of M. rigida to treat painful and inflammatory conditions. To the best of our knowledge, it is the first report on the in vivo anti-inflammatory effects of the stem bark extract of M. rigida, as well as its ethyl acetate fraction. The (-)-4′-methylepigallocatechin (I) isolated of the plant showed antinociceptive activity by inducing both central and peripheral pain relief in mice. Thus, it confirms the pharmacological activities previously described for this species, which include analgesic (Mota & Albuquerque, 2002; Dias et al., 2007), insecticide, antiseptic, antiasthmatic and antitumor activities (Flores, 1998; Ghazanfar, 1994).

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