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Short Communication

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Antinociceptive Activity of *Malva sylvestris* L.

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SUMMARY. The antinociceptive activity of *Malva sylvestris* (Malvaceae) aqueous extract (10 mg/kg, i.p.) was evaluated against classical models of pain in mice, indicating promising results. It showed significant antinociceptive activity in writhing test (76.4% of inhibition) and also inhibited the neurogenic (61.8%) and inflammatory (46.6%) phases of the formalin model. When analysed against capsaicin-induced pain model, the aqueous extract was also effective with inhibition of 62.9%, but it did not cause significant activity against hot-plate model. The results suggest that the antinociception caused by aqueous extract is related to the inhibition of prostaglandins synthesis pathway cyclooxygenase and unrelated to the stimulation of the opioid receptors.

INTRODUCTION

Malva sylvestris L. has been used in folk medicine of Brazil and other countries for the treatment of colitis and stomatitis, in cases of chronic bronchitis, against furuncle and abscess, contusions and haemorrhoids as well as other dolorous and inflammatory processes [1,2]. Previous pharmacological investigations have shown that extracts from this plant exhibit effects against haemorrhoids [3] and inflammations [4], as well as exerted antibacterial [5] and antioxidant activities [6,7]. Previous studies reported the isolation of several constituents, such as essential oils, terpenes, aromatic compounds, anthocyanins, mucilage, tannins and vitamins A, B, C [1,8].

In this study, we have evaluated the antinociceptive potential of aqueous extract obtained from *M. sylvestris* leaves against several models of pain in mice.

MATERIALS AND METHODS

Plant material and extract

Leaves of *Malva sylvestris* L. were collected in Turiaçú, Rio de Janeiro State, Brazil, in April 2004. The plant was identified by Dr. Massimo Bovini of Botanic Garden Research Institute, Rio de Janeiro, Brazil and a voucher specimen (nº RFA-31056) was deposited at Herbarium of Botanic Department of University Federal of Rio de Janeiro, Brazil.

The infusion (10% w/v) was realized with 160 g of fresh M. sylvestris leaves in 1600 ml of water. After, the extract was filtered, frozen with liquid nitrogen and then lyophilized yielding 2.4% of aqueous extract.

Animals

Male Swiss mice (25-30 g) were used. The animals were kept in automatically controlled temperature conditions (23 ± 2°C) with a 12-h light-dark cycle and had free access to food and water. The animals remained in the Bioterium of UNIVALI until the experiments.

Pharmacological assays

Writhing test

Abdominal constriction was induced in mice by intraperitoneal injection of acetic acid (0.6%), as described by Collier *et al.* with minor modifications [9]. The animals were pre-treated intraperitoneally with the studied extract (10

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mg/kg, 30 min before). The control animals received a similar volume of saline solution (10 mL/kg). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions between the control animals and the mice pretreated with the compounds.

Formalin-induced pain

The observation chamber was a glass cylinder of 20 cm in diameter, equipped with a mirror placed at a 45° angle to allow clear observation of the animals' paws. The mice were treated with 0.9% saline solution *(i.p.)* or extract (10 mg/kg, *i.p.*) 30 min before formalin injection. Each animal was placed in the chamber for 5 min before treatment, in order to allow acclimatization to the new environment. The formalin test was carried out as described by Hunskaar et al., with minor modifications [10]. A 2.5% formalin solution (0.92% formaldehyde, 20 µL) in 0.9% saline solution were injected intraplantarly into the right hind paw. The animal was then returned to the chamber and the amount of time spent licking the injected paw was considered as indicative of pain. Two distinct phases of intensive licking activity were identified: an early acute phase and a late or tonic phase (0–5 and 15–30 min after formalin injection, respectively).

Capsaicin-induced pain

The procedure used was similar to that described previously [11]. After the adaptation period capsaicin (20 µL, 1.6 µg/paw) was injected intraplantarly into the right hindpaw. The animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were treated with the extract via *i.p.* (10 mg/kg) 30 min prior to capsaicin injection, respectively. The control animals received a similar volume of saline, intraperitoneally.

Hot-plate test

The hot-plate test was used to measure response latencies, according to the method described by Eddy & Leimback [12]. The mice were treated with saline solution, morphine (10 mg/kg, *s.c.*) or extract (10 mg/kg, *i.p.*), and placed individually on a hot plate maintained at 56 ± 1 °C. The time between placing the animal on the hot plate and the occurrence of either the licking of the hind paws, shaking the paw or jumping off the surface was recorded as response latency. Mice with baseline latencies of more than 20 s were eliminated from the study and the cut-off time for the hot-plate latencies was set at 30 s. The animals were treated 30 min before the assay.

RESULTS AND DISCUSSION

The aqueous extract from *M. sylvestris* revealed significant antinociceptive effect against acetic acid-induced abdominal constrictions, causing 76.4% of inhibition. Although the writhing test is a non-specific model, because anticholinergic and antihistaminic as well as other agents can also indicate activity in this test, the significant reduction of the number of writings induced by the 0.6% acetic acid solution suggests an antinociceptive potential of this plant, once it is widely used for analgesic screening and involves local peritoneal receptors (cholinergic and histamine receptor) and the mediators of acetylcholine and histamine [13]. The aqueous extract was more efficacious than aspirin, a non-steroidal anti-inflammatory and analgesic drug used for comparison, which caused inhibition of 35%, in the same model and dose (Fig. 1).

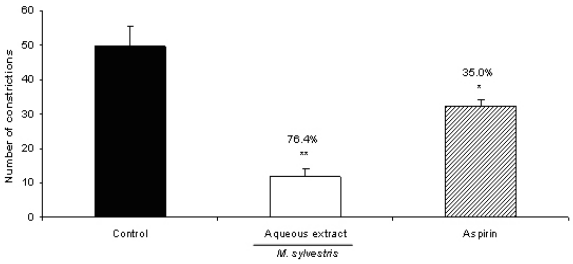

Figure 1. Effect of aqueous extract of *M. sylvestris* and Aspirin, both at a concentration of 10 mg/kg, administrated intraperitonealy, against acetic acid-induced abdominal constrictions in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$ and * $p < 0.05$.

The aqueous extract of *M. sylvestris* was also analyzed in the formalin-induced pain test, a reported behavior model characterized by first phase (neurogenic), which is evoked by the direct formalin stimulation of the nerve endings followed by substance P release and the second phase mainly due to subsequent inflammation reaction in peripheral tissue [10]. The aqueous extract inhibited both phases of pain, neurogenic (61.8%) and inflammatory (46.6%), with greater activity against the neurogenic phase, suggesting an involvement at both central and peripheral levels. Aspirin was inactive against the first phase and inhibited 39% the late phase (Fig. 2).

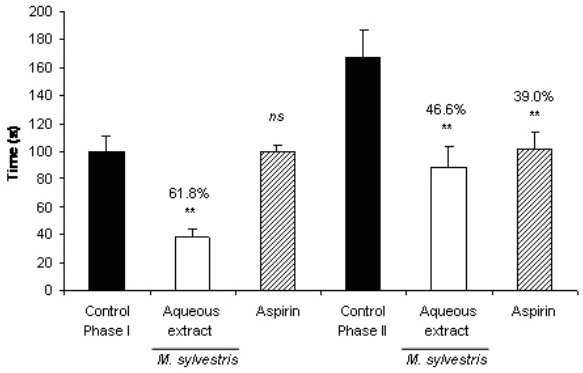

Figure 2. Effect of aqueous extract of *M. sylvestris* and Aspirin at a concentration of 10 mg/kg, administrated intraperitonealy, against formalin-induced pain in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$, * $p < 0.05$ and ns not significant.

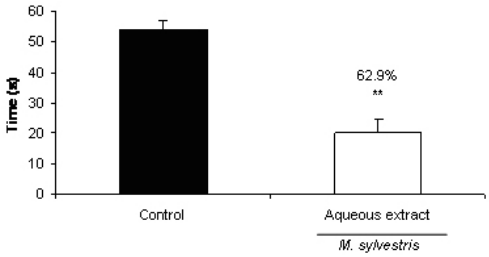

Figure 3. Effect of aqueous extract of *M. sylvestris,* at a concentration of 10 mg/kg, administrated intraperitonealy, against capsaicin-induced pain in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$.

When analyzed against capsaicin-induced pain model, the aqueous extract was also effective with inhibition of 62.9%, confirming direct evidence of the antinociceptive effect of this extract on neurogenic pain (Fig. 3). The hot plate test was undertaken to verify if the aqueous extract could show any central analgesic effect, but the results obtained did not show any significant activity at 10 mg/kg, *i.p.* when compared to morphine, a well-known opioid drug used for comparison (Fig. 4). Such results suggest that the antinociception caused by aqueous extract is related to inhibition of prostaglandins synthesis pathway cyclooxygenase and unrelated to the stimulation of the opioid receptors.

CONCLUSIONS

The results suggest that *M. sylvestris* possesses interesting substances which act as antinociceptive agents, encouraging phytochemical studies to determine the active principles of this plant.

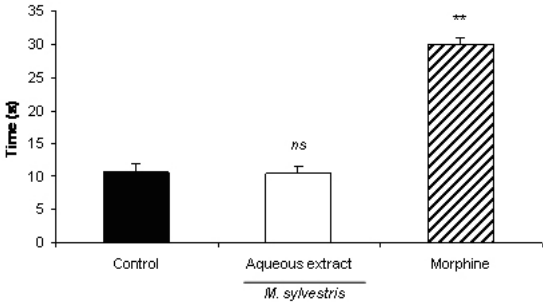

Figure 4. Effect of aqueous extract of *M. sylvestris,* at a concentration of 10 mg/kg, administrated intraperitonealy and Morphine, at a concentration of 5 mg/kg, administrated subcutaneous, at hot plate test in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$ and ns not significant.

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