Antinociceptive Activities of the Hydroalcoholic Extracts from *Erythrina velutina* and *Erythrina mulungu* in Mice

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This work studied the antinociceptive effects of the hydroalcoholic extracts (HAEs) from *Erythrina velutina* (Ev) and *Erythrina mulungu* (Em) in three experimental models of nociception in mice. The extract was administered intraperitoneally to female mice at the doses of 200 and 400 mg/kg. Inhibitions of abdominal contractions were observed with the doses of 200 (88.6%; 86.8%) and 400 (95.5%; 83.5%) mg/kg of *E. velutina* and *E. mulungu*, respectively, as compared to controls. *E. velutina* and *E. mulungu*, at both doses, reduced the nociception produced by formalin in the 1st and 2nd phases and this effect was not reversed by the pretreatment with naloxone. In the hot plate test an increase of the reaction time was observed only at 60 (Ev=18.0±2.2; Em=20.8±2.52) and 90 min (Ev=20.4±1.71; Em=23.7±2.32) after the treatment with *E. velutina* and *E. mulungu* at the dose of 400 mg/kg as compared to controls (T60=11.1±0.74; T90=11.9±0.86). This effect was not reversed by naloxone. We conclude that *E. velutina* and *E. mulungu* presents antinociceptive effects, which are independent of the opioid system.

Key words *Erythrina velutina*; *Erythrina mulungu*; antinociceptive activity

The genus *Erythrina* (Fabaceae family) is widely known. This designation includes the species *Erythrina velutina* (plant endemic to the plains and river banks of the semi-arid regions in Northeastern Brazil) and *Erythrina mulungu* (plant native to Southern Brazil). It is a high tree that shows trunks and branches not so peaked and with red flowers.

Nowadays, at least 110 species of plants from genus *Erythrina* were determined. Most *Erythrina* species (approximately 70) are native of Americaa and they are known to produce alkaloids, flavonoids and terpenes. It is known that the population use these plants to treat different health problems and pharmacological assays intended to verify the real effects of some species. *Erythrina americana*, for example, has shown anxiolytic-like, hypnotic and analgesic effects. Raw extract from *Erythrina glauca* presented hemagglutinating activity.

The chemical fractionation of the stem bark from *E. velutina* gave homohesperetin and phaseollidin. According to authors this was the first time that homohesperetin has been isolated from a plant of the Fabaceae family. Phaseollidin has previously been isolated from some species of the genus *Erythrina*, but not in the species *E. velutina*. On the other hand, there are no data in the literature concerning the chemical constituents present in *E. mulungu*.

In herbal medicine, leaf or bark decoction or tincture from *Erythrina mulungu* is used to calm agitation and for insomnia and others disorders of the nervous system. Onusic *et al.* showed also anxiolytic-like effects on a specific subset of defensive behaviours after acute treatment with a water–alcohol extract of *E. mulungu*. Other study suggests that the water–alcohol extract from the flowers of *E. velutina* has shown antinociceptive activity.

Because the literature shows only a few works with both *E. velutina* and *E. mulungu* as far as antinociceptive activities are concerned, we decided to study the acute effects of two doses of the hydroalcoholic extracts (HAEs) from *E. velutina* and *E. mulungu* in three pharmacological tests in mice.

MATERIALS AND METHODS

Materials *E. velutina* was collected at the city of Pacoti, state of Ceará-Brazil and the exsicatae is deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará under the number 16046. *E. mulungu* was collected at the city of Rifaina, S˜ao Paulo-Brazil and the exsicatae deposited at the Department of Vegetal Biotechnology of the University of Ribeirao Preto under the code HPM-0032.

**Extract Preparation** For the preparation of the hydroalcoholic extract, 300 g of the plant ground stem bark were suspended in 11 of ethanol: distilled water (3:7) and the mixture was heated for 2 h at 60 °C, filtered through gauze and the material submitted to another extraction at the same condition. The filtrates were added together and heated for the evaporation of ethanol up to half of the original volume and concentration expressed as solid residues per milliliter.

**Animals** Male Swiss mice (20—30 g) from the Animal House of the Federal University of Ceará were used throughout the experiments. Animals were maintained in plastic cages, and kept in rooms with a controlled 12:12 h light/dark cycle, temperature of 25°C and and food and water ad libitum. Experiments were performed according to the guide for the care and use of laboratory animals, from the US Department of Health and Human Services, Institute of Laboratory Animal Resources, Washington DC, 1985.

**Drugs** Acetic acid was purchased from Vetec Quimica Farm. Ltda and formaldehyde from Reagen Quimibrás Ind. Quimica S. A. (both companies from Rio de Janeiro, Brazil). All other drugs were of analytical grade.

**Pharmacological Tests** Acetic acid-induced abdominal contractions: The method of Koster *et al.* was utilized. Mice (*n*=8—50 per group) were injected with 0.6% acetic
produced by drug administration, with cut-off time of 40 s to avoid animal
mg/kg, i.p.) and distilled water (controls). Measurements
For this, 1% formalin was administered in the mouse (n = 7—14 per group) right hind paw and the licking time was imme-
minute for phase 1, neurogenic) and again for 5 min, 20 min after the intraplantar injection of formalin (phase 2, inflammatory). Animals were treated with E. velutina and E. mulungu (200, 400 mg/kg, i.p.), 30 min before formalin administration or morphine 5 mg/kg, i.p. as stan-
standard. In separate experiments, the effect of pretreatment with naloxone (1 mg/kg, s.c.) on the antinociception produced by E. velutina and E. mulungu (400 mg/kg, i.p.) was determined. Morphine was used as standard drug.
Hot plate: For the hot plate test 5 groups of 13—25 mice each were treated with E. velutina and E. mulungu (200, 400 mg/kg, i.p.) and distilled water (controls). Measurements were performed before (0 time) and 30, 60 and 90 min after drug administration, with cut-off time of 40 s to avoid animal lesion. In separate experiments, the effect of pretreat-
with naloxone (1 mg/kg, s.c.) on the antinociception produced by E. velutina and E. mulungu (400 mg/kg, i.p.) was determined. Morphine (5 mg/kg, i.p.) in the absence and presence of naloxone treatment was used as standard drug.

Statistical Analyses All results are presented as mean±S.E.M. ANOVA followed by and Student-Neuman-Keuls as the post hoc test. Results were considered significant at p<0.01 and p<0.05.

RESULTS

E. velutina and E. mulungu at the doses of 200 and 400 mg/kg showed antinociceptive effect in the test of abdominal contractions induced by acetic acid in mice. Inhibitions were observed at the doses of 200 (88.6; 86.8%) and 400 (95.5; 83.5%) mg/kg of E. velutina and E. mulungu, respectively, as compared to controls F(4,87) = 33.585; p<0.0001 (Table 1).

In the formalin test in mice (Table 2) inhibitions of the 1st phase with E. velutina (37, 82%) and E. mulungu (26, 61%) were observed with doses of 200 and 400 mg/kg, respectively, as compared to controls [F(4,50) = 16.22; p<0.0001]. Similar effects were observed in the 2nd phase after the treat-
both E. velutina (96, 98%) and E. mulungu (82, 98%) with doses of 200 and 400 mg/kg, respectively, as com-
pared to controls [F(4,52) = 47.914; p<0.0001].

No significant effect was observed in the hot plate test in mice after the treatment with E. velutina and E. mulungu as compared to controls in time zero (T0 = 13.3±1.01) or 30 min later (T30 = 14.3±0.99). However, an increase was observed in the animals reaction time after the administration of the high dose (400 mg/kg) of E. velutina and E. mulungu at T60 (Ev = 18.0±2.2; Em = 20.8±2.52) [F(4,85) = 4.733; p = 0.0018] and T90 (Ev = 20.4±1.71; Em = 23.7±2.32) [F(4,74) = 3.954; p = 0.0060] as compared to controls (T0 = 11.1±0.74; T30 = 11.9±0.86) (Table 3).

Formalin (Fig. 1) and hot plate (Fig. 2) tests were also performed in the absence and presence of naloxone, an opioid antagonist. In formalin test, while naloxone totally reversed morphine antinociceptive effect in both phases, it did not re-
E. velutina and E. mulungu effects indicating the noninvolvement of the opioid system in the HAEs antinoci-

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of abdominal contractions (20 min)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.0±2.5 (50)</td>
<td></td>
</tr>
<tr>
<td>E. velutina</td>
<td>4.3±0.60 (9)\textsuperscript{a}</td>
<td>88.6</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>1.6±0.65 (13)\textsuperscript{a}</td>
<td>95.5</td>
</tr>
<tr>
<td>E. mulungu</td>
<td>5.0±0.86 (8)\textsuperscript{a}</td>
<td>86.8</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>6.2±0.83 (8)\textsuperscript{a}</td>
<td>83.5</td>
</tr>
</tbody>
</table>

Table 1. Effects of the HAEs from E. velutina and E. mulungu on the Acetic Acid-Induced Abdominal Contractions in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw licking (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.7 (10)</td>
</tr>
<tr>
<td>E. velutina</td>
<td>6.3 (10)</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>4.7 (11)\textsuperscript{a}</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>2.7±0.7 (13)\textsuperscript{a}</td>
</tr>
<tr>
<td>E. mulungu</td>
<td>4.2±0.9 (13)\textsuperscript{a}</td>
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</tbody>
</table>

Table 2. Effects of the HAEs from E. velutina and E. mulungu on Formalin-Induced Nociception in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Reaction time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.3±1.01 (20)</td>
</tr>
<tr>
<td>E. velutina</td>
<td>14.3±0.99 (25)</td>
</tr>
<tr>
<td>200 mg/kg, i.p.</td>
<td>14.7±1.81 (20)</td>
</tr>
<tr>
<td>400 mg/kg, i.p.</td>
<td>14.6±1.45 (17)</td>
</tr>
<tr>
<td>E. mulungu</td>
<td>14.5±0.90 (19)</td>
</tr>
<tr>
<td>200 mg/kg, i.p.</td>
<td>12.3±0.96 (19)</td>
</tr>
<tr>
<td>400 mg/kg, i.p.</td>
<td>12.7±0.84 (17)</td>
</tr>
</tbody>
</table>

Table 3. Antinociceptive effect of the HAEs from E. velutina and E. mulungu on the Hot Plate Test in Mice

Values are reported as means±S.E.M. for the number of animals shown in parentheses. \textsuperscript{a} p<0.05 as compared to controls (ANOVA and Student-Neuman-Keuls as the post hoc test).
ception. Similarly, in hot plate test, naloxone not revert the increase in reaction time observed with *E. velutina* and *E. mulungu* after the 60 and 90 min administration of the HAEs (400 mg/kg).

**DISCUSSION**

The present work showed that *E. velutina* and *E. mulungu* present antinociceptive effects in different experimental models in mice. To the genus *Erythrina* belong medicinal several species of plants largely used in Brazil due to their central and antinociceptive effects.12)

*E. velutina* and *E. mulungu* in both doses inhibit the number of abdominal constrictions induced by acetic acid. Similarly, Arrigoni-Blank *et al.*12) demonstrated a decrease of abdominal contractions after administration of the aqueous extract from leaf of *E. velutina* (300, 600 mg/kg). Collier *et al.*16) postulated that the acetic acid acts indirectly by inducing the release of endogenous mediators sensitive to non-steroidal anti-inflammatory drugs and opioids. Deraedt *et al.*17,18) related that inhibition of the acetic acid writhing in mice causes an acute inflammatory reaction related to the increase in the peritoneal fluid levels of PGE2 and PGF2α.

Our results indicated that *E. velutina* and *E. mulungu* antinociception is not dependent upon the opioid system since the previous treatment with the opioid antagonist naloxone did not reverse the analgesic effect. The formalin test is believed to represent a significant model of clinical pain.19) It is known that the first phase is the result of the direct chemical activation of myelinated and unmyelinated nociceptive affer-
ent fibers while the second phase response is considered a consequence of noxious stimulus-evoked long term changes in the properties of spinal dorsal horn neurons. This is of interest considering that both phases are sensitive to centrally acting drugs such as opioids, but the second phase is also sensitive to non-steroidal anti-inflammatory drugs and corticosteroids.

Also, the results showed that in the formalin test the inhibition was predominantly on the 2nd phase (inflammatory response) with both *E. velutina* and *E. mulungu*. However, the HAEs also significantly inhibited the 1st phase (neurogenic response). From our results we can suggest that the extracts might produce a modulatory influence at the spinal level where they exert antinociceptive effects.

Opioid agents exert their analgesic effects via supra spinal and spinal receptors. The hot plate test is a specific central antinociceptive test. In the present work, the HAE presented antinociceptive effect only at the high dose in the two species and this effect was not reversed by naloxone.

In conclusion we showed that hydroalcoholic extracts of *E. velutina* and *E. mulungu* possess significant antinociceptive effects in mice that supports the folk medicinal use of this plants and this analgesic effect is independent of the opioid system. Further studies currently in progress will enable us to understand the mechanisms of action underlying the effects observed in this investigation.

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**REFERENCES**


