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Antinociceptive and anti-inflammatory activity of hydroalcoholic extracts and fractions from *Erythrina mulungu*

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Abstract: *Erythrina mulungu* Mart. ex Benth., Fabaceae, popularly known as mulungu, is used for the treatment of insomnia and disorders of the central nervous system. This study examined the antinociceptive effects of the hydroalcoholic extracts (HAE), the ethyl acetate and chloroformic fractions from *E. mulungu* in four experimental models of nociception using laboratory mice. The extracts and fractions were administered orally to mice at doses of 100 mg/kg. Inhibition of abdominal contractions were observed for all the extracts and fractions tested, as compared to controls. All extracts and fractions from *E. mulungu* reduced the nociception activity produced by formalin in the 2nd phase. In the hot plate test no significant effect was observed for any extract or fraction. In the peritonitis test induced by Zymosan, all of the tested extracts and the chloroformic fraction, except for the ethyl acetate phase, reduced cell migration of the peritoneal cavity. We concluded that *E. mulungu* shows antinociceptive effects, which are independent of the opioid system.

Keywords:
Antiimflammatory,
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Introduction

The genus *Erythrina mulungu* Mart. ex Benth., Fabaceae, is widely known. There are more than 130 known species of *Erythrina* in tropical and subtropical areas of the world. Most *Erythrina* species (approximately 70) are native to the Americas (Neill, 1988). *Erythrina* plants are known to produce alkaloids, flavonoids, and terpenes (Mckee et al., 1997). Several species of *Erythrina* are used in folk medicine for to their tranquilizer effects (Garín-Aguilar et al., 2000). Analgesic and anti-inflammatory effects were reported for an aqueous extract from *E. mulungu* and *E. velutina* (Vasconcelos et al., 2003).

Erythrina mulungu popularly known as mulungu, is a medium-sized well-branched tree native to Southern and Northeastern Brazil (Lourenzi, 1992). Popularly, the leaves and bark of the stem decoction of *E. mulungu* are used for treatment of insomnia and disorders of the central nervous system (Rodrigues & Carvalho, 2001) and has been used for a long time in Brazilian traditional medicine by the indigenous populations for the same end. In traditional medicine it is considered an excellent sedative to treat anxiety, nervous coughs and other problems of the nervous system including psychomotor agitation and insomnia, in addition to asthma, bronchitis, hepatitis, hepatic and spleen inflammation and fevers (Almeida, 1993; Anderson et al, 1998; Cruz, 1995). In popular medicine, a tincture prepared from the leaves or bark decoction from *E. mulungu* is used to calm agitation and other nervous system disorders (*e.g.* insomnia and depression). Commercial preparations of the crude extract of *E. mulungu* are available in Brazilian and U.S.A. drugstores as a phytomedicine. The wide use of this plant in home-remedy practices of popular medicine, as well as the phytotherapeutic industry, is enough of a reason for its choice as a theme of study in phytochemistry and pharmacology, seeking to complete its validation as an effective and safe medicine (Lourenzi, 2002).

Material and Methods

Plant material

Erythrina mulungu Mart. ex Benth., Fabaceae (root, root bark, stem and stem bark) was collected in the city of Maceio-AL, Brazil and the exsiccate is deposited in the University of Brasilia Herbarium under the number JEP 3593 (UB).

Extract preparation

For the hydroalcoholic extract preparation, 600 g of each part of the plant (root, root bark, stem and stem bark) were suspended in 90% ethanol and the mixture was left in a percolator for three days, then filtered through gauze and the material submitted to another two extractions under the same conditions. At the end, the crude ethanolic extracts were obtained for the parts of the plant. After a fast filtration of the root's crude extract, the hexanic, chloroformic, ethyl acetate and methanolic fractions were obtained. The chloroformic and ethyl acetate fractions were submitted to pharmacological tests.

Animals

Swiss mice (20-30 g) from the Federal University of Alagoas Biotery were used throughout the experiments. Animals were maintained in plastic cages, and kept in rooms with a controlled 12/12 h light/dark cycle, with 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, (Ethics Committee of UFAL No. 006443/2005-78).

Drugs and reagents

Acetic acid was purchased from Vetec Química Farm. Ltd. and formaldehyde from Reagen Quimibrás Ind. Química S.A. (both companies located in Rio de Janeiro, Brazil). All other drugs were of analytical grade.

Biological activity tests

Acetic acid-induced abdominal contractions

The method of Coolier et al. (1968) was utilized. Mice (n 6-8 per group) were injected with 0.6% acetic acid (10 mL/kg, *i.p.*) and after 5 min the number of contractions were registered for 20 min. The extracts and fractions of *E. mulungu* were administered orally (100 mg/kg) 40 min before the acetic acid administration. In all experiments the controls were injected with saline solution.

Formalin-induced pain in mice

The formalin test was performed according to the method of Hunskaar and Hole (1987). To begin, 20 µL of a 2.5% (v/v) solution of formalin in saline was injected into the sub plantar region of the right hind paw and the amount of time that the animal spent licking the paw during the first 5 min (first phase) and from 15 to 30 min (second phase) of post-injection time was assessed. The test was performed at ambient temperature of 22-26 ºC and care was taken to exclude environmental disturbances (high temperatures, noise and excessive movement) that might interfere with the animal's response. Animals were treated with extracts and phases of *E. mulungu* orally (100 mg/kg), 40 min before formalin administration.

Hot plate test

The hot plate test was used to measure latency response according to the method described by Eddy & Leimbach (1953), with minor modifications. In these experiments, the hot plate apparatus (Ugo Basile, Model-DS 37) was maintained at 55.5±1 ºC. Animals were placed on the heated surface and the time between placement and licking of the paws or jumping was recorded as latency. Latency was recorded for vehicle control groups (10 mL/kg) or groups pre-treated with extracts and phases of *E. mulungu* orally (100 mg/kg), or with morphine (15 µmol/kg, body wt., *i.p.*). The test compounds were administered after animal selection at a time of 30 min. The selection was made on the basis of the reactivity to the test. Pre-treatment times 0 and 30 min were used for assay adaptation and selection of the animals, respectively. Only mice showing a reaction time within the range of 4-10 s. were included in this test. The latency of the reaction to nociception was measured at time 0 and then at 30 min intervals up to the 180th min. The botanical materials were administered at time 30 min and treatment latencies were recorded at times 60, 90, 120 and 150 min.

Peritonitis induced by Zymosan

Peritoneal inflammation was induced as previously described by Doherty et al. (1985) Zymosan A (Sigma Aldrich) was freshly prepared (2 mg/mL) in sterile 0.9% w/v saline, and 0.5 mL was injected *i.p.* Animals were killed by cervical dislocation. The peritoneal cavity was washed with 2 mL of Hanks solution (HBSS, free Ca^{2+} and Mg^{2+}) and after 6 h of

gentle manual massage, the exudate was retrieved, and the volume was measured. The exudate was collected and used freshly for cell counts. The extracts and phases of *E. mulungu* (100 mg/kg) and indometacin (100 μmol/kg), were administered orally 30 min before the Zymosan A injection.

Statistical analysis

The levels of significance between the experimental groups and the control were calculated using ANOVA in the tutorial Prisma®. The values were considered significant when *$p<0.05$. The results were expressed as mean±SEM as indicated in the legends of the figures.

Results

The crude extracts of the stem, the root's bark and the root, as well as the chloroformic and ethyl acetate phases of the root exhibited a significant inhibition of contortions induced by acetic acid with $p<0.01$ (66.78, 68.17, 65.39, 55.72 and 60.90%, respectively), while the crude extract of the stem's bark exhibited an inhibition with $p<0.05$ of 51.83% (Figure 1).

In the first phase of the formalin test no significant effect was observed for the extracts and/or phases (Figure 2). In the second phase, inhibitions were observed for all the extracts and phases. The inhibition percentages were: 55.25% (stem), 76.52% (stem bark), 45.75% (root bark), 80.76% (root), 75.63% (chloroformic fraction) and 64.96% (ethyl acetate fraction).

No significant effect was observed in the hot plate test on mice after treatment with the crude extracts and fractions of *E. mulungu* as compared to the controls. Morphine was used as a positive control in the hot plate test (data not shown).

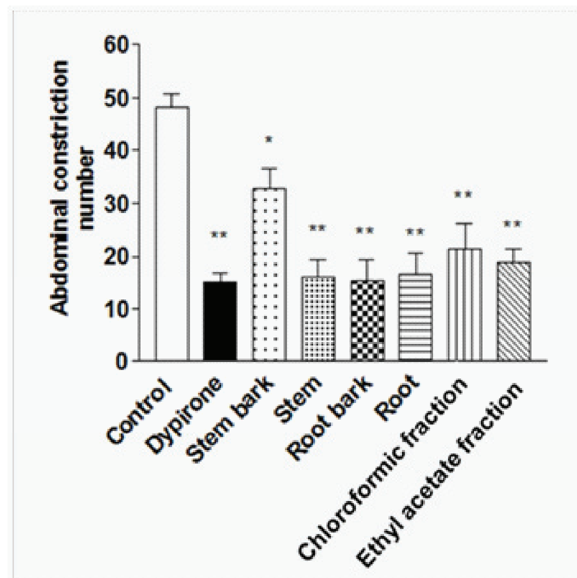
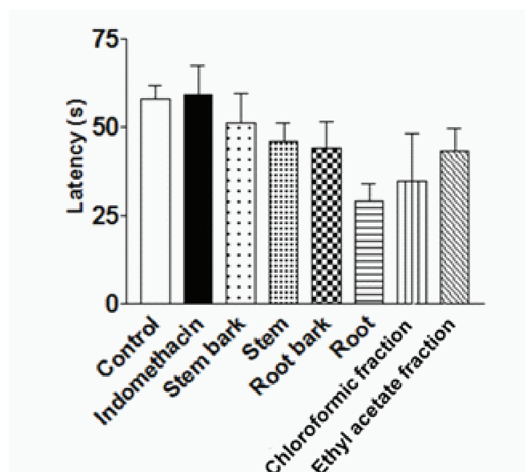


Figure 1. Effect of the crude extracts and fractions (chloroformic and ethyl acetate) obtained from *Erythrina mulungu*, with 100 mg/kg, *p.o.*, in abdominal contractions induced by acetic acid 0.1 M (*i.p.*) in mice (n = 6-8). (*$p<0,05$; **$p<0,01$). ANOVA test, followed by the Dunnet test.

In the peritonitis induced by Zymosan test, except for the ethyl acetate fraction of the root, all of the tested extracts and the chloroformic fraction of the root reduced the cell migration for the peritoneal cavity (Figure 3), giving inhibition percentages of 43.80% (stem), 14.72% (stem bark), 38.76% (root bark), 56.87% (root), and 40.19% (chloroformic fraction).



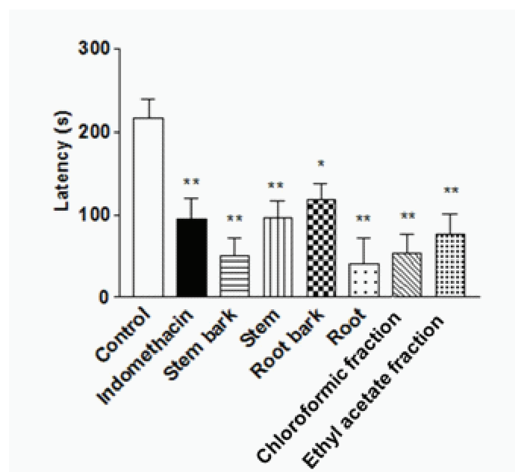


Figure 2. Effect of the crude extracts and fractions (chloroformic and ethyl acetate) obtained from *Erythrina mulungu* at 100 mg/kg, *p.o.* in the neurogenic phase (A) and in the inflammatory phase (B) for formalin-induced nociception in mice (n=6-8). (*$p<0.05$; **$p<0.01$). ANOVA test, followed by the Dunnet test.

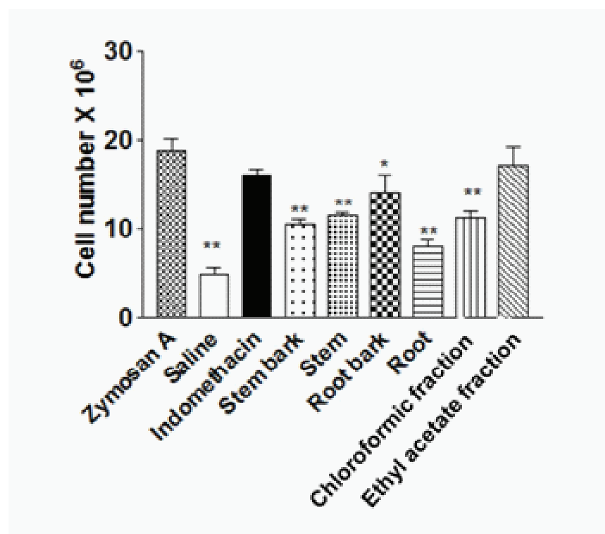


Figure 3. Effect of the crude extracts and fractions (chloroformic and ethyl acetate) obtained from *Erythrina mulungu,* at 100 mg/kg, *p.o.* in peritonitis induced by Zymosan A in mice (n=6-8). (*p<0.05; **p<0.01). ANOVA test, followed by the Dunnet test.

Discussion

Studies on the antinociceptive and anti-inflammatory effects of various species of the genus Erythrina as well as those of their isolated principles have appeared in the literature. Indeed, the most pertinent example being the description by Pillay et al.(2001) for the inhibition of cyclooxygenase activity by different *Erythrina* extracts. The current paper, however, represents the first report on the antinociceptive and anti-inflammatory activity of various extracts and phases of *Erythrina mulungu* Mart. ex Benth., Fabaceae. *E. mulungu* extracts and phases inhibit the number of abdominal contractions induced by acetic acid. Similarly, Vasconcelos et al. (2003) demonstrated a decrease in abdominal contractions after administration of the hydroalcoholic extract from the stem bark of *E. mulungu* (200, 400 mg/kg). In agreement with Coolier et al. (1968) acetic acid acts by inducing, indirectly, the liberation of endogenous mediators such as bradykinin, serotonin, histamine and prostaglandins, that are sensitive to non-steroidal anti-inflammatory drugs and opioids. Deraedt et al., (1980) report that the abdominal constrictions induced by acid acetic cause a sharp inflammatory reaction that is principally related to the increase in the levels of PGE2 and PGF2α in the peritoneal fluid. The formalin test is characterized by showing two different phases of nociception that seem to involve different mediators (Du et al., 2007). The first phase characterized by intense neurogenic pain, starts immediately after the injection, and seems to be caused predominantly by activation of C-fibers subsequent to peripheral stimulation (Shibata et al., 1989). The second phase seems to be caused by tissue and functional changes in a part of the spinal marrow (Hunskaar & Hole, 1987). Vasconcelos et al. (2003) also report that all of the extracts and phases tested reduced the time of latency in the second phase of the test (inflammatory phase), and that there was no activity in the first phase of the test. The hot plate test verifies the possible central action of the extracts and phases. This test is characterized by producing a fast answer to the noxious incentive, mediated by the activation of the nociceptors, leading the pulse to the dorsal part of the spinal marrow and later to cortical centers, in the same way that the opioid agents function (Tjolsen & Hole, 1997). In this study all the extracts and phases showed activity, indicating the absence of central effects. Zymosan induces mastocite degranulation and activates macrophages (Kolaczkowska et al., 2001). When administered in the peritoneal cavity of mice, Zymosan promotes an increase in vascular leakage as one of the first signs of inflammation, followed by a recruitment of migratory cells (Leite et al. 2007). In this test, the results demonstrated that the extracts and the chloroformic fraction from *E. mulungu* significantly reduced the number of recruit cells, indicating that these compounds contain active anti-inflammatory principles. The pharmacological effects with the extracts originating from several parts of the plant, and the chloroformic and ethyl acetate fractions of the root of the plant showed in general, anti-inflammatory effects and outlying antinociceptive activity. However, the extracts and fractions of E. mulungu did not show a central analgesic effect.

In conclusion, this study has shown that all of the extracts and fractions from *E. mulungu* possess significant antinociceptive and anti-inflammatory effects on animals at doses of 100 mg/kg, given orally and that the effects were independent of the opioid system. However, pharmacological and chemical studies are continuing in order to characterize the mechanism(s) responsible for the antinociceptive action and also to identify other active principles present in this species.

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References

Almeida ER 1993. Plantas medicinais brasileiras, conhecimentos populares e científicos. São Paulo. Hemus editora Ltda.

Anderson DC, Siqueira-Batista R, Quintas LEM 1998. Plantas medicinais - do cultivo à terapêutica. Petrópolis. 2nd ed., Editora Vozes.

Coolier HOJ, Dinnen LC, Schneider C 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol Chemother 32*: 295-310.

Cruz GL 1995. *Dicionário das plantas úteis do Brasil*. Rio de Janeiro. 5th ed., Bertrand.

Doherty NX, Poubelle P, Borgeat P, Beaver TH, Westrich GL, Schrader NL 1985. Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptidoleukotrienes and prostaglandin E2. *Prostaglandins 30*: 769-789.

Du J, Yu Y, Ke Y, Wang C, Zhu L, Qian ZM 2007. Ligustilide attenuates pain behavior induced by acetic acid or formalin. *J Ethnopharmacol 112*: 211-214.

Eddy NB, Leimbach D 1953. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutilamines. *J Pharmacol Exp Ther 107*: 385-393.

Garín-Aguilar MA, Luna JER, Soto-Hernández M, Valencia Del Toro G, Vásquez MM 2000. Effect of crude extracts of *Erythrina americana* Mill. on aggressive behavior in rats. *J Ethnopharmacol 69*: 189-196.

Hunskaar S, Hole K 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain 30*: 103-114.

Kolaczkowska E, Seljelid R, Plytycz B 2001. Role of mast cells in zymosan-induced peritoneal inflammation in Balb/c and mast cell-deficient WBB6F1 mice. *J Leukocyte Biol 69*: 33-42.

Leite D F P, Eschevarria-Lima J, Ferreira S C, Calixto J B, Rumjanek V M 2007. ABCC transporter inhibition reduces zymosan-induced peritonitis. *J Leukocyte Biol 82*: 630-637.

Lourenzi H 1992. Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas. São Paulo. Plantarum.

Lourenzi H 2002. Plantas medicinais no Brasil nativas e exóticas. São Paulo. Plantarum.

Mckee TC, Bokesch HR, McCormick JL, Rashid MA, Spielvogel D, Gustafson KR, Alavanja MM, Cardellina JH, Boyd MR 1997. Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine, and microbial organisms. *J Nat Prod 60*: 431-438.

Neill AD 1988. Experimental studies on species relationships in *Erythrina* (Leguminosae: Papilionoideae). *Ann Mo Bot Gard 75*: 886-969.

Pillay CCN, Jäger AK, Mulholland DA, Van Staden J 2001. Cyclooxygenase inhibiting and anti-bacterial activities of South African *Eryhtrina* species. *J Ethnopharmacol 74*: 223-237.

Rodrigues VE, Carvalho DA 2001. *Plantas medicinais do cerrado*. Lavras. Universidade Federal de Lavras.

Shibata M, Ohkubo T, Takahashi H, Inuki R 1989. Modified formalin test: characteristic biphasic pain response. *Pain 38*: 347-352.

Tjolsen A, Hole K 1997. Animal models of analgesia. In: *The Pharmacology of Pain*: Dickenson A, Besson J. Berlin. *Springer Verlag 130*: 1-20.

Vasconcelos SMM, Oliveira GR, Carvalho MM, Rodrigues ACP, Silveira ER, Fonteles MMF, Sousa FCL, Viana GSB 2003. Antinociceptive activities of the hydroalcoholic extracts from Erythrina velutina and *Erythrina mulungu* in mice. *Biol Pharm Bull 26*: 946-949.

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