

# Anti-inflammatory activities of the hydroalcoholic extracts from *Erythrina velutina* and *E. mulungu* in mice

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**Abstract:** This work studied the anti-inflammatory activities of the hydroalcoholic extracts (HAEs) from *Erythrina velutina* Willd. (Ev) and *E. mulungu* Mart. ex Benth. (Em) in the carrageenan- and dextran-induced mice hind paw edema models. These medicinal plants belonging to the Fabaceae family are used in some Brazilian communities to treat pain, inflammation, insomnia and disorders of the central nervous system. In the present work, the extracts were administered orally in male mice at the doses of 200 or 400 mg/kg. In the carrageenan-induced test, only Em showed anti-inflammatory activity, decreasing the paw edema, at the doses of 200 and 400 mg/kg. No effect was observed with Ev in this model. On the other hand, in the dextran model, Ev demonstrated anti-inflammatory effect, showing decrease of the paw edema at the 1, 2, 3, 4 and 24<sup>th</sup> h. Em (200 or 400 mg/kg) presented anti-inflammatory effect at the 2, 3 and 4<sup>th</sup> h after administration of dextran, as compared to control. In conclusion, the work showed that Ev and Em present anti-edematous actions, which possibly occurs by distinct mechanisms. While Ev seems to interfere especially in inflammatory processes in which mast cells have an important role, Em exerts greater activity in the inflammatory process that depends mainly on polymorphonuclear leucocytes. However, further studies are needed to determine the exact mechanism of action of the species investigated.

## Introduction

Many species of the genus *Erythrina* are used in Brazilian folk medicine to treat central nervous system diseases (Marchioro et al., 2005). Among these, there are *Erythrina velutina* Willd. and *E. mulungu* Mart. ex Benth., classified in the Fabaceae family. The first one is a tree measuring from 6 to 10 m and it is very common at the coast region of all north and northeast Brazilian states (Dantas et al., 2004). In the other hand, *E. mulungu* is a medium-sized well-branched tree native to Southern Brazil (Lorenzi, 1992).

Some researches already showed many effects of these species in rodents, like the sedative and neuromuscular blocking actions from the crude extract from the leaves of *E. velutina* (Dantas et al., 2004), the antinociceptive and the depressant activities of the hydroalcoholic extracts (HAE) from the stem bark of

both species (Vasconcelos et al, 2003; 2004; 2007) and the anxiolytic effect of the HAE from the inflorescence of *E. mulungu* and from the stem bark of *E. velutina* (Onusic et al., 2003; Sarragiotto et al., 1981).

The plants from the genus *Erythrina* are known to produce alkaloids, flavonoids and terpenes. These plants represent the main source of alkaloids tetracyclics and have curare-like activity causing muscular paralysis (Armer et al., 1991). In phytochemical investigation of non-alkaloidal secondary metabolites of species from the genus *Erythrina*, several isoflavonoids were found (Deshapande et al., 1977; Kôbaiashi et al., 1997), some of which exhibiting anti-inflammatory activity (Hedge et al., 1997)

The chemical fractionation of the stem bark from *E. velutina* gave homohesperetin and phaseollidin (Rabelo et al., 2001). The last one has previously been isolated from other species of the genus *Erythrina*, but

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not of *E. velutina*. On the other hand, it was the first time that homohesperetin has been isolated from a plant of the Fabaceae family (Dagne et al., 1993; Nkengfak et al., 1994; Tanaka et al., 1997). It was also already isolated five major alkaloids from the methanol extract of *E. mulungu* flowers. Among these are erysotrine, erythartine, hypaphorine, that are common constituents of *Erythrina* species (Sarragiotto et al., 1981).

In view of all observations cited above, the present work investigated the anti-inflammatory activities of the HAE from the stem bark of *E. velutina* and *E. mulungu* in carrageenan and dextran-induced paw edema models in mice.

## Materials and Methods

### Plant material

*Erythrina velutina* Willd. was collected at the city of Pacoti, state of Ceará-Brazil and the exsicate deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará under the number 16046. *E. mulungu* Mart. ex Benth., Fabaceae, was collected at the city of Rifaina, São Paulo-Brazil, and the exsicate deposited at the Department of Vegetal Biotechnology of the University of Ribeirão Preto under the code HPM-0032.

### Preparation of the extract

For the preparation of the hydroalcoholic extract, 300 g of the plant grounded stem bark were suspended in 1 L 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 mL.

### Animals

Male Swiss mice (20-30 g), in groups of 9 to 17 animals, from the Animal House of the Federal University of Ceará were used throughout the experiments. Animals were maintained in plastic cages, and kept in 30 m<sup>2</sup> rooms with a controlled 12/12 h light/dark cycle, temperature of 25 °C, 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.

### Measurement of paw edema

The different groups were pretreated orally with HAE from *E. velutina* or *E. mulungu* (200 or 400 mg/kg), indomethacin (10 mg/kg), cyproheptadine (10 mg/kg) or vehicle (distilled water 10 mL/kg, control group) 1 h before receiving the injection of 0.05 mL of carrageenan (1% w/v) or dextran (12% w/v) dissolved in distilled water into the subplantar area of the right hind paw. Indomethacin and cyproheptadine are the positive control groups of carrageenan- and dextran-induced paw edema model, respectively. The volume of the paw was determined with a plethysmometer (Ugo Basile, Italy), as described by Winter et al. (1962), 1, 2, 3, 4 and 24 h after the administration of pro-inflammatory substances. The edema was reported as the difference between the final and the initial volumes of the paw, in  $\mu\text{L}$ .

All experiments were conducted in a quiet room at a constant temperature of 25 °C. The HAE were administered orally at a volume of 0.1 mL/10 g. Controls received distilled water.

### Statistical analysis

The results are expressed as mean+SEM. ANOVA followed by Student-Neuman-Keuls as the *post hoc* test. Results are considered significant at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ .

## Results

The injection of carrageenan into the subplantar area of the right hind paw induced a progressive edema with the peak in the 4<sup>th</sup> h. The pretreatment with the HAE from *E. mulungu*, at the dose of 200 mg/kg, decreased significantly the paw edema induced by carrageenan at all the times analyzed, but the reduction was more important at the 3<sup>rd</sup> h as compared to control (48.5%). The highest dose also reduced the edema at the 3 and 4<sup>th</sup> h, but it was a slighter reduction (19.7 and 23.3%, respectively). The HAE from *E. velutina* at the doses of 200 and 400 mg/kg, did not interfere significantly with the edema induced by carrageenan as compared to control (Table 1).

On the other hand, the injection of dextran into the subplantar area of the hind paw induced a rapid and almost constant edema until after the 4<sup>th</sup> h. The administration of the HAE of *E. velutina* (200 and 400 mg/kg) caused a reduction in the paw edema induced by dextran ranging from 33.0 to 53.1% at the 1, 2, 3, 4 and 24<sup>th</sup> h as compared to control. The highest decrease of the paw edema induced by dextran was observed at doses of 200 and 400 mg/kg at the 4<sup>th</sup> h with a reduction of 53.1 and 51.3%, respectively. In dextran model with *E. mulungu*, the lowest dose demonstrated significantly

**Table 1.** Effects of HAE of *Erythrina velutina* (Ev) and *E. mulungu* (Em) leaves on carrageenan induced paw edema in mice.

Group (n)	Dose (mg/kg)	T1 (µL)	T2	T3	T4	T24
Control (16)		105.9±5.8	168.8±7.4	201.6±7.3	207.8±10.1	110.3±8.9
Indomethacin (8)		110.0±4.2	128.5±5.0 <sup>a***</sup>	134±5.1 <sup>a***</sup>	130±5.0 <sup>a***</sup>	126,5±6,0
Ev (10)	200	103.5±9.2	176.0±10.5	218.5±8.3	212.0±6.4	90.5±9.8
Ev (11)	400	110.0±7.2	180.0±9.9	213.2±7.8	220.5±8.1	135.0±12.5
Em (9)	200	71.1±5.8 <sup>a</sup>	98.8±7.7 <sup>a***</sup>	103.9±13.1 <sup>a***</sup>	136.1±11.9 <sup>a**</sup>	62.2±10.4 <sup>a*</sup>
Em (10)	400	103.0±12.2	142.5±11.9 <sup>b*</sup>	162.0±15.0 <sup>a**b**</sup>	159.5±22.3 <sup>a*</sup>	99.0±9.7

<sup>a</sup> versus control; <sup>b</sup> versus HAE of Em (200 mg/kg); \**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001 (ANOVA and Student-Neuman-Keuls as the post hoc test).

**Table 2.** Effect of HAE of *Erythrina velutina* (Ev) and *E. mulungu* (Em) leaves on dextran induced paw edema in mice.

Group (n)	Dose (mg/kg)	T1 (µL)	T2	T3	T4	T24
Control (17)		164.1±9.7	188.8±	195.6±17.4	201.2±14.8	110.3±12.9
Indomethacin (8)		116.2±4.5 <sup>a**</sup>	118.2±4.7 <sup>a**</sup>	111.1±3.1 <sup>a**</sup>	116.5±2.2 <sup>a***</sup>	100.0±2.5
Ev (10)	200	83.0±9.3 <sup>a***</sup>	112.0±9.3 <sup>a***</sup>	99.5±7.3 <sup>a***</sup>	94.5±11.8 <sup>a***</sup>	64.0±7.0 <sup>a**</sup>
Ev (10)	400	110.0±10.5 <sup>a**</sup>	113.0±11.7 <sup>a***</sup>	109.5±13.3 <sup>a***</sup>	98.0±17.2 <sup>a***</sup>	67.0±5.9 <sup>a*</sup>
Em (10)	200	132.0±8.9	131.5±6.1 <sup>a*</sup>	122.0±8.8 <sup>a*</sup>	136.0±8.8 <sup>a**</sup>	74.0±5.1
Em (10)	400	134.0±7.7	147.0±11.4	136.5±10.8 <sup>a*</sup>	125.5±9.1 <sup>a**</sup>	72.5±5.9

<sup>a</sup> versus control; <sup>b</sup> versus HAE of Em (200 mg/kg); \**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001 (ANOVA and Student-Neuman-Keuls as the post hoc test).

alterations at the 2, 3 and 4<sup>th</sup> h as compared to control (reduction of 30.4, 37.6 and 32.4%, respectively). The highest dose also showed anti-inflammatory activity, but only in the 3 and 4<sup>th</sup> h (decrease of 30.2 and 37.6%, respectively, Table 2).

## Discussion

The HAE of *E. velutina* and *E. mulungu* showed in the present work anti-inflammatory activities in carrageenan or dextran-induced mice paw edema. Carrageenan induces paw edema characterized by an early phase (1-2 h) caused by the release of histamine, 5-hydroxytryptamine and bradykinin, followed by a late phase (3-4 h) mainly sustained by prostaglandin release. Endogenous nitric oxide and myeloperoxidase are also involved in this type of acute inflammation (Ialenti et al., 1992; Posadas et al., 2004).

*E. mulungu* decreased paw edema induced by carrageenan, especially after the 2<sup>nd</sup> h of experiment. The HAE of this plant at the dose of 200 mg/kg showed significant inhibition from the 1<sup>st</sup> to the 24<sup>th</sup> h, while the dose of 400 mg/kg only demonstrated a significant reduction of the paw edema at the 3 and 4<sup>th</sup> h. This result can suggest that Em exhibit its effect by inhibiting the migration and activation of polymorphonuclear cells (PMN), predominantly neutrophils, which plays an important role in the inflammatory response induced by carrageenan. In addition, the HAE from Em may also modulate the synthesis, release or action of several inflammatory mediators, such as prostaglandins, nitric oxide and cytokines (IL-1, TNF- $\alpha$  and others) from

resident cells (DiRosa et al., 1971; Vinegar et al., 1969; 1982; Wedmore & Williams, 1981). The fact that the lowest dose exerts greater reduction in paw edema suggests that HAE does not act in a dose-dependent manner. This can be explained by a possible alteration in the pharmacokinetics patterns of the extracts (that are influenced by the dose).

While carrageenan edema is characterized by massive infiltration of PMN and the participation of several inflammatory mediators (Di Rosa et al., 1971; Di Rosa & Willoughby, 1971), the edema induced by dextran is also produced by several mediators, with mast cell degranulation playing a major role (Lo et al., 1982). In the present study the treatment with HAE of Ev did not interfere in carrageenan-induced paw edema in mice. However, Ev was able to inhibit significantly the dextran-induced paw edema, showing, at both doses, the better results (reduced the paw edema significantly from the 1<sup>st</sup> to the 24<sup>th</sup> hour). Similar results with the leaves of Ev were also demonstrated by other research (Marchioro et al., 2005). Taken together these results suggest that the mast cells and inflammatory mediators, such as histamine and serotonin, seem to play an important role in the anti-inflammatory effects of HAE of Em.

In conclusion, we showed anti-inflammatory activities from the HAE of *E. mulungu* and *E. velutina* in different mice paw edema models. The first one seems to modulate mainly inflammatory process where PMNs has an important role, while in the last one the mast cells possibly seem to account for its antiedematogenic effect. However, considering that it was a preliminary study about the anti-inflammatory potential of the

HAE, additional studies are required to determine the precise mechanism of action of these.

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