# Effects of Erythrinian Alkaloids Isolated from *Erythrina mulungu* (Papilionaceae) in Mice Submitted to Animal Models of Anxiety

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**The effects of acute oral administration of erythrinian alkaloids, *i.e.* (+)-$\alpha$-hydroxy-erysotrine, erythravine and (+)-11$\alpha$-hydroxy-erythravine isolated from the flowers of *Erythrina mulungu* were investigated in two animal models of anxiety in mice—the light–dark transition model (LDTM) and the elevated plus-maze (EPM). In the LDTM, erythravine (3, 10 mg/kg) and (+)-11$\alpha$-hydroxy-erythravine (10 mg/kg) increased the time spent by the animals in the illuminated compartment and (+)-11$\alpha$-hydroxy-erythravine (3 mg/kg) increased the number of transitions between compartments of the LDTM, suggesting an anxiolytic-like effect of these erythrinian alkaloids. Nevertheless, the third alkaloid studied, (+)-$\alpha$-hydroxy-erysotrine, did not change any behavioral response with the range of doses used (3—10 mg/kg). Since the oral administration of the crude extract of *E. mulungu* (EM) (100—400 mg/kg) did not modify the conventional measures of anxiety in the EPM, this animal model was not chosen to evaluate the anxiolytic properties of the isolated alkaloids. These results suggest that the alkaloids erythravine and (+)-11$\alpha$-hydroxy-erythravine are responsible for the anxiolytic effects of the crude extract of *E. mulungu*.**

**Key words** *Erythrina mulungu*; alkaloid; anxiety; medicinal plant; central nervous system; animal model

In popular medicine, a tincture prepared from the leaves or barks decoction from *Erythrina mulungu* is used to calm agitation and other nervous system disorders (*e.g.* insomnia and depression).[1] Commercial preparations of the crude extract of *E. mulungu* are available in Brazilian and U.S.A. drugstores as a phythomedicine. However, there are a lack of studies demonstrating its safety, efficacy and mechanism of action. In addition, many studies have investigated the potential anxiolytic of the crude extract only, not emphasizing, for instance, which constituents of the *E. mulungu* alter this emotional state.

For instance, it has been demonstrated that acute and chronic treatments with hydroalcoholic extracts from *E. mulungu* produce anxiolytic-like effects on a specific subsets of defensive behavior in rats exposed in the elevated T-maze (ETM) and in the light–dark transition model (LDTM). The anti-anxiety effects were similar to that provoked by the well-known anxiolytic compound diazepam (DZP).[2,3]

Recently[4] we have reported that a new erythrinian alkaloid, (+)-11$\alpha$-hydroxy-erythravine (OH-Ery), and the two known erythrinian alkaloids, erythravine (Ery) and (+)-$\alpha$-hydroxyerysotrine (Eryso), isolated from *E. mulungu* impaired the inhibitory avoidance acquisition of the open arms in the ETM, an animal model of anxiety validated for rats[5,6] and mice.[7] Interestingly, neither crude extract nor erythrinian alkaloids changed locomotion in the arena, suggesting a selective anxiolytic-like effect of these erythrinian compounds.[4]

Although the ETM has been validated as an animal model of anxiety for rats[5,6] and mice[7] it is relevant to investigate the anxiolytic properties of new compounds by using different anxiety tests. In the present study we investigated the effects of the crude extract of *E. mulungu* (EM) and its alkaloids, the Eryso, Ery and OH-Ery in two widely used tests of anxiety, the light dark transition model (LDTM)[8] and the elevated plus-maze (EPM).[9,10]

## MATERIAL AND METHODS

**Plant Material** The inflorescence was used since the procedure of scraping the bark out of the tree often damages the plant. Inflorescences of *Erythrina mulungu* Mart. were collected at Rifaina, São Paulo State, and exsiccate was deposited at the Department of Vegetal Biotechnology of University of Ribeirão Preto under the code HPM-0032.

**Extraction and Isolation** The erythrinian alkaloids Eryso (36 mg, 0.03%), Ery (95 mg, 0.08%) and OH-Ery (48 mg, 0.04%) were isolated from the hydroalcoholic crude extract (120 g) as previously reported.[4]

**Pharmacological Essay. Animals** Male Swiss mice (São Paulo State University/UNESP, SP, Brazil), weighing 25—35 g, were housed in groups of 10 per cage (41 cm×34 cm×16 cm) and maintained under a normal 12-h light cycle (lights on 07:00 h) in a temperature/humidity controlled environment (23±1 °C/55±5%). Food and water were freely available. All mice were experimentally naive. Experimental procedures were in compliance with the US National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Local Ethical Committee (UNESP–Araraquara/SP, nr 35/2004).

**Compound Administration** EM and alkaloids were suspended in saline (NaCl 0.9%) and diazepam (DZP) in saline+2% Tween 80. Animals were orally treated with EM (100, 200, 400 mg/kg),[2,3] and with a range of doses (3—10 mg/kg) of each isolated alkaloid,[4] or vehicle (saline). These doses were based on a previous study.[4] A positive control group receiving intraperitoneal injection of diazepam (DZP, 2 mg/kg) was also included in the study. All compounds were administered 30 min before the experimental session.

**Apparatus** Light–Dark Transition Model (LDTM): The LDTM was a plexiglas box (44.5×37×25 cm) divided by a

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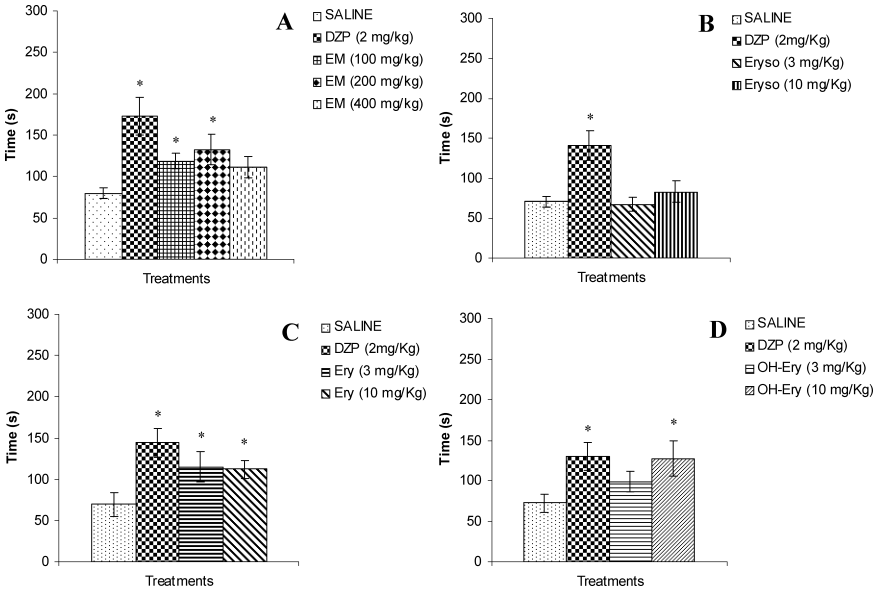


Fig. 1. Effects (Mean±S.E.M.) of the EM (A), Eryso (B), Ery (C) and OH-Ery (D) ($n$=9—17) on the Time Spent by the Mice in the Illuminated Compartment of the Light Dark Transition Model

*$p \leq 0.05$ *vs.* control group (Duncan *post hoc* test).

barrier with a door way (7×7.5 cm) through which mice could cross between two chambers, one made by white walls (27×37×25 cm) and illuminated by a white light (1320 lux, illuminated compartment) and one made by black walls (17×37×25 cm) and illuminated by a red light (35 lux, dark compartment).

Elevated Plus-Maze (EPM): The EPM comprised two open arms (30×5 cm) and two closed arms (30×5×15 cm) that extended from a common central platform (5×5 cm). To avoid falls, the open arms were surrounded by Plexiglas rim 0.25 cm high. The apparatus was constructed from glass (clear walls) and wood (floor) and elevated 38.5 cm above floor level.

**Procedure** Initially animals were treated with crude extract of EM (0, 100, 200, 400 mg/kg; *p.o.*) and then individually exposed in the LDTM or EPM. When crude extract of EM produced anxiolytic-like effect the experiment was followed with treatment of each isolated alkaloid (for instance, 3 and 10 mg/kg of Eryso; 3 and 10 mg/kg of Ery; 3 and 10 mg/kg of OH-Ery). At each stage, a positive-control group was added to the study with animals receiving diazepam (2 mg/kg; i.p.), a classical anxiolytic drug.

LDTM: Each animal was placed at the center of the illuminated compartment and, after the first passage to the dark one, the behavior of the animal was video recorded for 5 min to further analysis of the number of transition between compartments and the time spent in the illuminated compartment. Entry into a compartment was recorded when the animal crossed with all four paws the line that separated the two compartments.[8)]

EPM: Each animal was placed in the central square facing a closed arm, and allowed to freely explore the EPM. The following spatio-temporal measures were recorded during a 5-min test: number of open and closed arm entries (arm entry=all four paws into an arm), % open entries [(open/total)×100], and % open time [(time open/300)×100]. Percentage open arm entries and percentage open arm time were used as anxiety indices, and frequency of closed arms entries was used as locomotor activity.[9,10)]

**Statistics** All results were initially submitted to Levene's test for homogeneity of variance. Where this test yielded significance, results were log transformed before being submitted to one-way ANOVA, followed by Duncan's Multiple Comparison Test when significant. In all cases a $p$ value $\leq 0.05$ was considered significant.

## RESULTS

**Light Dark Transition Model** Figure 1 shows the effects of oral administration of crude extract of EM (A), Eryso (B), Ery (C) and OH-Ery (D) on time spent by animals in the illuminated compartment of the LDTM. One-way ANOVA revealed significant differences of treatment in all experiments (A—D), as follow: EM [$F(4,43)=4.82$; $p<0.01$], Eryso [$F(3,49)=3.66$; $p<0.01$], Ery [$F(3,39)=4.27$; $p=0.01$] and OH-Ery [$F(3,38)=3.14$; $p<0.05$]. Posterior comparisons ($p \leq 0.05$) revealed that treatment increased time spent in the illuminated compartment of the LDTM when compared to the control group with the following doses: EM (100, 200 mg/kg, Fig. 1A); Ery (3, 10 mg/kg, Fig. 1C); OH-Ery (10 mg/kg, Fig. 1D). Although one-way ANOVA had revealed significant effect of Eryso, *post hoc* analysis showed that only diazepam treated animals spent more time in the illuminated compartment. This effect of diazepam was also present in A, C and D.

As shown in Fig. 2, ANOVA did not reveal significant differences of treatments on the number of transitions between both compartments of the LDTM for EM [$F(4,43)=1.34$; $p=0.26$; Fig. 2A], Eryso [$F(3,49)=1.19$; $p=0.31$; Fig. 2B], Ery [$F(3,39)=1.13$; $p=0.34$; Fig. 2C]. However, one-way ANOVA revealed effect of OH-Ery [$F(3,38)=3.36$; $p<0.05$; Fig. 2D]. As shown in Fig. 2D, the dose of 3 mg/kg of OH-Ery increased the number of transitions between both compartments ($p \leq 0.05$).

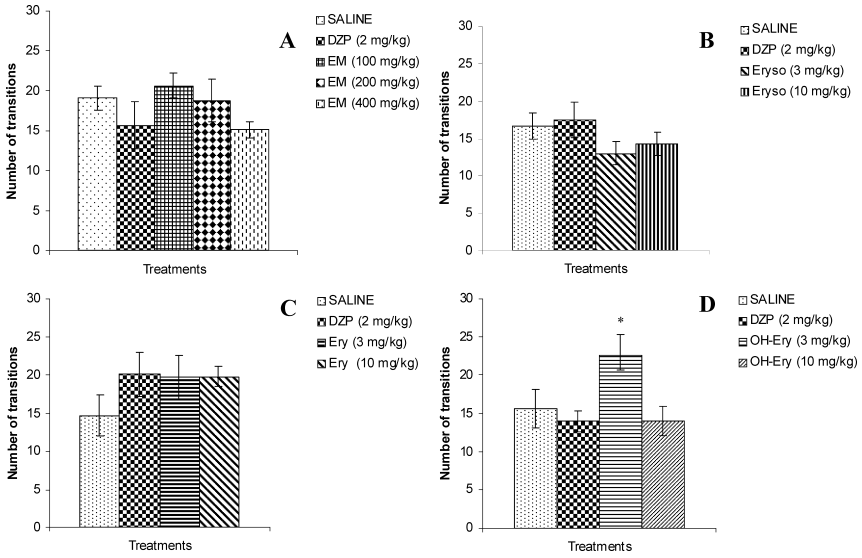


Fig. 2. Effects (Mean±S.E.M.) of the EM (A), Eryso (B), Ery (C) and OH-Ery (D) ($n$=9—17) on the Number of Transitions between Compartments of the Light Dark Transition Model

∗ $p \leq 0.05$ *vs.* control group (Duncan *post hoc* test).

Table 1. Effects (Mean±S.E.M.) of the Crude Extract from *Erythrina mulungu* and DZP in Mice ($n$=8—9) Submitted to the Elevated Plus-Maze

| | % open arm entries | % open time | Closed arm entries |
|---|---|---|---|
| Saline | 17.27±4.23 | 6.59±2.20 | 11.67±0.88 |
| DZP (2 mg/kg) | 39.41±3.21* | 29.62±3.25* | 16.14±2.25 |
| EM (100 mg/kg) | 21.98±5.18 | 12.30±4.47 | 12.44±1.41 |
| EM (200 mg/kg) | 25.14±4.51 | 12.56±2.95 | 12.22±1.37 |
| EM (400 mg/kg) | 17.73±3.20 | 7.74±2.24 | 10.78±1.36 |

∗$p \leq 0.05$ (Duncan *post hoc* test).

**Elevated Plus-Maze** Table 1 shows the effects of EM and DZP on anxiety indices (% open arms entries and % open arms time) and locomotor activity (closed arms entries) in the elevated plus-maze. ANOVA revealed significant differences of treatment on % open arms entries [$F(4,38)=4.00$; $p<0.01$] and % open arms time [$F(4,38)=8.95$; $p<0.001$]. Duncan's test revealed that these differences were produced by DZP treatment in both parameters ($p \leq 0.05$). Neither DZP nor EM significantly changed frequency of closed arms entries [$F(4,38)=1.48$; $p=0.22$].

## DISCUSSION

This study demonstrated that acute *p.o.* treatment with EM and the alkaloids, erythravine and (+)-11$\alpha$-hydroxy-erythravine produced anxiolytic effects in a classical animal model of anxiety, the LDTM. Similarly to the reference drug, the benzodiazepine DZP, EM (100, 200 mg/kg), erythravine (3, 10 mg/kg), and (+)-11$\alpha$-hydroxy-erythravine (10 mg/kg) increased the time spent by the animals in the illuminated compartment. In addition, (+)-11$\alpha$-hydroxy-erythravine (3 mg/kg) increased the number of transitions between compartments of the LDTM. This seems to be a selective anxiolytic-like effect since we have recently demonstrated that (+)-11$\alpha$-hydroxy-erythravine did not alter the locomotor activity in the arena test in mice.[4]

Unexpectedly, the present study failed to demonstrate anxiolytic effects of the alkaloid (+)-$\alpha$-hydroxy-erysotrine in the LDTM. Such failure contrasts with recent results,[4] in which this erythrinian alkaloid (3, 10 mg/kg) impaired inhibitory avoidance acquisition in the elevated T-maze, an anxiolytic-like effect. Thus it is not clear why (+)-$\alpha$-hydroxy-erysotrine did not alter anxiety in the LDTM. Further studies involving the use of higher doses of (+)-$\alpha$-hydroxy-erysotrine could contribute to compare its potency to that of other erythrinian alkaloids (*e.g.* erythravine and (+)-11$\alpha$-hydroxy-erythravine) in attenuating anxiety in the LDTM.

Taken together, present results with LDTM corroborate previous studies that have reported anxiolytic effects produced by acute (100 mg/kg) and chronic (50 mg/kg) treatment with EM *p.o.* in rats in the LDTM.[2,3] In addition, present results also support a recent study using the elevated T-maze (ETM) as an animal model of anxiety in mice.[4] In that study we have demonstrated that the alkaloids present in the hydroalcoholic extract of *E. mulungu* are responsible, at least in part, for its anxiolytic effects recorded in the ETM. Briefly, acute *p.o.* treatment with erythravine (3, 10 mg/kg), (+)-11$\alpha$-hydroxy-erythravine (3, 10 mg/kg) and (+)-$\alpha$-hydroxy-erysotrine (3, 10 mg/kg) impaired the inhibitory avoidance acquisition from the open arms of the ETM for mice, suggesting an anxiolytic effect of these erythrinian compounds.[4]

In the present study EM failed to alter anxiety indices in the EPM in mice. This lack of effects has been previously demonstrated.[11] Vasconcelos S. M. M. *et al.* (2003) demonstrated that neither intraperitoneal (200, 400 mg/kg) nor oral (200, 400, 800 mg/kg) administration of the hydroalcoholic extract of both *E. mulungu* and *E. velutina* alter the anxiety indices in the EPM in female mice.[11] Since crude extract of *E. mulungu* did not modify the conventional measures of anxiety in the EPM, we did not continue to use this animal model to evaluate the anxiolytic properties of the isolated alkaloids. It has been emphasized that the EPM is an animal model of anxiety useful to predict the anxiolytic effects of benzodiazepines. However, many inconsistent results have been demonstrated with other classes of clinically effective

drugs in anxiety disorders (*e.g.* buspirone, antidepressants).[12] For instance, serotonin (5-HT) reuptake blockers and 5-$HT_{1A}$ receptor agonists produce anxiolytic and anxiogenic effects in the EPM.[8] There are also reports showing that these compounds fail to alter anxiety in the rodent EPM.[8] Thus, the failure of effects of *E. mulungu* on anxiety in the EPM does not necessarily exclude its potential as an anxiolytic natural product.

The underlying mechanisms involved in the anxiolytic effects of the erythrinian alkaloids was not the scope of the present study and remain to be determined. Hypothetically, erythrinian alkaloids may act at GABA/benzodiazepine receptor complex. It has been reported that the alkaloids present in several species of *Erythrina* affect GABAergic neurotransmission.[15] However, since GABA/benzodiazepine receptor complex agonists usually produce motoric disruption (*i.e.* moderate doses increase locomotion and high doses impair locomotion) and crude extract of EM as well as its isolated alkaloids failed to alter locomotor behavior, it is unlikely that the anti-anxiety effects provoked with *E. mulungu* (mainly with higher doses) involve GABA/benzodiazepine complex mechanisms. It has been demonstrated that the crude extract of *Erythrina vespertilio* inhibited platelet 5-HT release.[13] Platelets have been used as a useful model for studying $Ca^{2+}$-dependent 5-HT release,[13] the main action of serotonin at 5-$HT_3$ receptors. In addition, it has been demonstrated the dihydro-$\beta$-erythroidine, an erythrinian alkaloid, antagonizes the effects of 5-HT in eliciting currents related to 5-$HT_3$ receptor stimulation.[14] In view of this evidence, it is tempting to speculate that the anxiolytic profile obtained in the present study can be attributed to an antagonist action of erythrinian alkaloids at $5HT_3$ receptors. Although attractive, such hypothesis remain to be empirically tested *e.g.* with *in vitro* and *in vivo* methods.

Corroborating our previous study,[4] the present results strongly suggest that the erythrinian alkaloids isolated from *E. mulungu* are markedly involved in the anxiolytic effects of the crude extract, and support the popular use of a tincture prepared from the leaves or barks decoction from *E. mulungu* as an anxiolytic medicine.

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