Behavioral effects of aqueous and dichloromethane extracts of *Erythrina speciosa* Andrews, Fabaceae, leaves in mice

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RESUMO: “Efeitos comportamentais do extrato aquoso bruto e fração diclorometano das folhas de *Erythrina speciosa* Andrews, Fabaceae, em camundongos”. *Erythrina speciosa* Andrews, Fabaceae, é usada na região Sul do Brasil como sedativa e tranquilizante. Neste estudo, uma possível ação central do extrato aquoso (EA) e fração diclorometano (DCM) das folhas da *E. speciosa* foi avaliada em camundongos machos submetidos a testes comportamentais 1 h após o tratamento (gavage). Também foi avaliada a dose letal 50% (DL50) como indicativa da toxicidade aguda desta planta. O EA diminuiu a locomoção (50, 100 e 400 mg/kg) e o levantar (50 e 400 mg/kg) no teste de campo aberto mas não alterou nenhum dos comportamentos avaliados nos testes de labirinto em cruz elevado, rotarod e sono induzido. A DCM não alterou nenhum dos comportamentos avaliados. A DL50 de ambos os extratos foi estimada como sendo >2000 mg/kg. Os resultados sugerem ausência de efeito ansiolítico e depressor do Sistema Nervoso Central das folhas de *E. speciosa*. Entretanto, como mecanismos serotonérgicos podem estar envolvidos na ação farmacológica de plantas do gênero *Erythrina* e o teste de labirinto em cruz elevado não é adequado para avaliar o efeito de drogas serotonérgicas, nossos resultados não invalidam o uso desta planta na medicina popular, mas apontam a necessidade de se investigar o mecanismo de ação envolvido no possível efeito central de plantas do gênero *Erythrina*.

**Unitermos:** *Erythrina speciosa*, campo aberto, labirinto em cruz elevado, rotarod, sono induzido, dose letal.

ABSTRACT: *Erythrina speciosa* Andrews, Fabaceae, is used in the South of Brazil as sedative and tranquilizers. In this study, behavioral effects of aqueous (AE) and dichloromethane (DCM) extracts of *E. speciosa* leaves were evaluated in male mice, as well as their lethal dose 50% (LD50). The extracts were administered by gavage in doses ranging from 50 to 400 mg/kg 1 h before the behavioral tests. AE decreased ambulation (50, 100 and 400 mg/kg) and rearing (50 and 400 mg/kg) in the open-field but did not influence elevated plus maze, rotarod and pentobarbital-induced sleep tests. No behavioral effects were observed after DCM administration. LD50 for both extracts were higher than 2000 mg/kg. The results showed that AE and DCM extracts of *E. speciosa* leaves do not produce anxiolytic effect in the elevated plus maze nor seems to depress the Central Nervous System. However, since serotonergic mechanisms may be involved in the pharmacological action of *Erythrina* plants and the elevated plus maze test is not adequate to evaluate serotonergic drugs, our results do not invalidate the use of this plant in folk medicine but suggest that the mechanism involved in a possible central action of *Erythrina* needs to be clarified.

**Keywords:** *Erythrina speciosa*, open-field; elevated plus maze; rotarod; pentobarbital-induced sleep; lethal dose.
INTRODUCTION

Several species of Erythrina, Fabaceae, are reported in folk medicine as tranquilizers and sedatives. In Brazil three species have been used: E. velutina, E. mulungu and E. speciosa (Lorenzi, 1992). Despite of its common use, there are few pharmacological studies on this genus. The curare-like effect has long been known (Lehman, 1937). Regarding central nervous system activity, sedative, anticonvulsant and anxiolytic effects have been reported with extracts from stem bark, flowers or leaves of E. mulungu or E. velutina (Onusic et al., 2002; Dantas et al., 2004; Flausino et al., 2007; Vasconcelos et al., 2007; Raupp et al., 2008).

Considering the folk use of Erythrina speciosa Andrews in the South of Brazil and the lack of pharmacological studies with this species, we investigated, in mice, possible central effects of aqueous extract from leaves. Moreover, since alkaloids have been suggested as the central active components of Erythrina (Flausino et al., 2007) and dichloromethane was suggested to be the best extractor for erythrinan alkaloids in flowers (Silva, 2003), the dichloromethane extract was also investigated. As an indicative of acute toxicity, lethal dose 50% (LD50) was estimated.

MATERIALS AND METHODS

Plant material

The plant (Erythrina speciosa Andrews, Fabaceae) was collected at the Universidade Estadual de Londrina (UEL), Paraná, Brazil on March, 2007. A voucher specimen was deposited in the herbarium of UEL (FUEL 35133) and authenticated by M.R.C. Paiva.

For the aqueous extract (AE) preparation, fresh leaves were washed and dried. Powdered leaves were extracted with distilled water (1 g/10 mL) at 100 °C for 30 min. The AE was filtered, lyophilized and stored at -20 °C. For the dichloromethane (DCM) extraction, 3 g of powdered leaves and 60 mL of solvent were used and after 24 h the material was filtered. The solvent was evaporated under reduced pressure and the w/w extraction yield was 24%.

Animals

Male Swiss mice (25-35 g) from the institutional colony were acclimatized for seven days in a temperature- and light- controlled room under a 12 h light/12 h dark cycle (lights on at 6:00 am). They had free access to tap water and food, except for a fasting period of 3 h before the experiments. The experimental procedures were approved by the UEL Animal Ethics Committee (protocol number CEEA 54/06). All mice were experimentally naive and were used only once.

Extract administration

AE was dissolved in distilled water and DCM in Cremophor® (Basf) 5% (v/v) immediately before experiments. Control groups were treated with these solvents (vehicle, VEH). Both extracts and VEH were administered orally (p.o., gavage) 1 h before the behavioral evaluation and the doses were selected based on studies conducted with other species of Erythrina (Onusic et al., 2002; Flausino et al., 2007; Vasconcelos et al., 2007).

Behavioral evaluation

In order to avoid the unnecessary use of animals, a tier approach was adopted, i.e., the behavioral tests as well as the doses evaluated in each test were chosen accordingly to the results obtained in previous steps. Open-field and elevated plus maze sessions were recorded by a video camera, linked to a monitor in an adjacent room and videotapes were analyzed blindly. After each evaluation, the apparatuses were cleaned with ethanol 5%.

Open-field test

Mice treated with 0, 50, 100, 200 and 400 mg/kg of AE or 0, 50, 100 and 200 mg/kg of DCM were submitted to the open-field test as described elsewhere (Lisboa et al., 2007). Ambulation, rearing and grooming were recorded during a 3-min session.

Elevated plus-maze test

Mice treated with 0 and 200 mg/kg of AE or 0, 50, 100 and 200 mg/kg of DCM were submitted to the elevated plus maze as described elsewhere (Lisboa et al., 2007). The number of entries into, and the time spent in the open and closed arms were registered for 5 min. The percentage of entries in the open arms was obtained dividing the number of entries in open arms by the total number of entries. The percentage of time spent in the open arms was obtained by dividing the time spent in the open arms by total time spent in both arms.

Pentobarbital-induced sleep

Mice treated with 0, 50, 100, 200 and 400 mg/kg of AE were injected intraperitoneally (i.p.) with 50 mg/kg of sodium pentobarbital (Cristália). The latency and duration of sleep (loss of the righting reflex) were recorded (Wambebe, 1985). The time limit for the duration of sleep was fixed at 2 h.
Rotarod test

Mice were trained on a rotarod (Insight) at 4 rpm for 2 min, three times on the day prior to the experiment. On the test day, mice that could not retain their balance on the accelerating bar (6 to 12 rpm) for 2 min were discarded. Mice were treated with 0, 50 and 400 mg/kg of AE and after 1 h they were placed on the accelerating bar (6 to 16 rpm in 3 min). The times that the mice remained on the rotating rod were recorded. The time limit was fixed at 3 min.

LD50

Oral LD50 was estimated in male mice following the guideline 423 from the Organization for Economics Cooperation and Development (OECD, 2001). The starting dose was 2000 mg/kg.

Statistical analysis

Data were analyzed by ANOVA followed by Student-Newman-Keuls (SNK), except for AE in the elevated plus-maze, which data were analyzed by Student’s t test. The level of significance was set at 5%.

RESULTS

Behavioral evaluation results of AE are presented in Table 1. We started evaluating the general activity of animals in the open-field and observed that, compared to VEH, AE significantly decreased both ambulation (50, 100 and 400 mg/kg) and rearing (50 and 400 mg/kg) behaviors. Since the elevated plus-maze test is only valid to evaluate anxiolytic effect if motor function is not altered (Dawson et al., 1995), only the dose of 200 mg/kg of AE was tested in this model. Student’s t test showed no difference between AE and VEH both in percentage of open arm entries and of time spent in the open arms. To evaluate if the decreased ambulation and rearing observed in the open-field could be due to AE-induced motor coordination disturbance, mice administered with doses that compromised both of those behaviors in the open-field (50 and 400 mg/kg) were tested in the rotarod. No statistical difference was detected between treated and control groups. Finally, to evaluate possible central nervous system depressant action of the AE extract, pentobarbital-induced sleep was conducted with mice previously treated with AE but none of the doses evaluated (0, 50, 100 and 400 mg/kg) potentiated the pentobarbital effect.

As can be seen in Table 2, DCM did not induce behavioral effects either in the open-field or the elevated plus maze tests. In this way, pentobarbital-induced sleep and rotarod test were not conducted.

Regarding the LD50 estimation, we followed a guideline from OECD that employs a validated methodology to estimate LD50 with a few number of animals. We started administering 2000 mg/kg of AE or DCM for three animals and observing them for two weeks. Since no death or sign of toxicity were observed, the same procedure was repeated with another set of three animals for each extract. Again, no death was observed and, following the guideline, the evaluation was ended and the LD50 was estimated as being higher than 2000 mg/kg.

<table>
<thead>
<tr>
<th>Table 1. Behavioral evaluation of the AE from Erythrina speciosa Andrews, Fabaceae, leaves.</th>
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<td>Open-field</td>
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<td>Ambulation (count)</td>
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<td>Rearing (count)</td>
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<td>Grooming (s)</td>
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<td>Elevated plus maze</td>
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<td>% open arm entries</td>
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<td>% open arm duration</td>
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<td>Pentobarbital-induced sleep</td>
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<td>Latency to sleep (s)</td>
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<td>Duration of sleep (s)</td>
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<td>Rotarod</td>
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<td>Time in the rotating bar (s)</td>
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Data are means±SEM. Number in parentheses represent the number of animals evaluated. VEH: distilled water. *p<0.05 and #p<0.001 compared to VEH (ANOVA complemented with SNK); s: seconds.
Table 2. Behavioral evaluation of the DCM from *Erythrina speciosa* Andrews, Fabaceae, leaves.

<table>
<thead>
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<th></th>
<th>VEH</th>
<th>50</th>
<th>100</th>
<th>200</th>
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<tr>
<td><strong>Open-field</strong></td>
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<tr>
<td>Ambulation (count)</td>
<td>73.0±6.9 (10)</td>
<td>71.7±6.1 (10)</td>
<td>69.7±6.2 (10)</td>
<td>75.7±5.6 (9)</td>
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<tr>
<td>Rearing (count)</td>
<td>15.9±1.6 (10)</td>
<td>19.3±1.0 (10)</td>
<td>14.4±1.3 (10)</td>
<td>14.0±2.1 (10)</td>
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<tr>
<td>Grooming(s)</td>
<td>5.8±1.5 (10)</td>
<td>4.4±1.4 (10)</td>
<td>3.2±1.0 (10)</td>
<td>3.6±1.0 (9)</td>
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<tr>
<td><strong>Elevated plus maze</strong></td>
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<tr>
<td>% open arm entries</td>
<td>0.20±0.03 (10)</td>
<td>0.22±0.04 (11)</td>
<td>0.19±0.02 (11)</td>
<td>0.21±0.02 (9)</td>
</tr>
<tr>
<td>% open arm duration</td>
<td>0.18±0.02 (10)</td>
<td>0.12±0.02 (11)</td>
<td>0.16±0.02 (10)</td>
<td>0.15±0.03 (10)</td>
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Data are means±SEM. Number in parentheses represent the number of animals evaluated. VEH: Cremophor® 5%; s: seconds.

**DISCUSSION AND CONCLUSIONS**

In the present study, possible central nervous system effects of AE and DCM from *E. speciosa* leaves were evaluated in male mice submitted to behavioral tests. In the open-field, AE decreased both ambulation and rearing and did not alter grooming, suggesting decreased motor activity in the absence of emotionality alteration (Broadhurst, 1960). In order to determine if the decreased motor activity was due to central or peripheral action, AE was evaluated in the pentobarbital-induced sleep, which screens for central depressants, and rotarod test, which evaluates motor coordination and may be disturbed by drugs that act centrally and/or peripherally. The lack of AE effect in the rotarod test corroborates studies conducted with other *Erythrina* species (Vasconcelos et al., 2004) and discard the involvement of a neuromuscular blockade on the effect observed in the open-field. On the other hand, the lack of AE effect in the pentobarbital-induced sleep was surprising since potentiation of pentobarbital-induced sleep have been described for AE from *E. velutina* leaves (10, 100, 300 and 400 mg/kg, i.p.) and hydroalcoholic extract from *E. mulungu* stem bark (200 mg/kg, i.p.) (Dantas et al., 2004; Vasconcelos et al., 2007).

Considering the folk use of *Erythrina* as an anxiolytic plant and that this effect has been described in experimental studies with different *Erythrina* species (Onusic et al., 2002; Dantas et al., 2004; Ribeiro et al., 2006; Flausino, 2007) in this work we investigated if AE and DCM of *E. speciosa* would induce anxiolytic effect in the elevated plus maze, the most classic test to evaluate anxiety experimentally. No alteration in the percentage of open arm entries and time spent in the open arms was observed for both AE and DCM, suggesting that this species would not be anxiolytic. Interestingly, despite the fact that Dantas et al. (2004) described anxiolytic effect for AE from *E. mulungu* leaves (20 mg/kg, i.p.) in the elevated plus maze, other studies with *E. mulungu* and *E. velutina* failed to demonstrate such an effect in this test (Vasconcelos et al., 2004; Flausino et al., 2007). Recently, Raupp et al. (2008) also failed to show anxiolytic effect after acute administration of the hydroalcoholic extract of the stem bark of *E. velutina* but they did detect an anxiolytic effect after a 23-26 days repeated administration of the 100 mg/kg dose.

Some studies have suggested that the central effects of *Erythrina* could be mediated by the neurotransmitter GABA (Onusic et al., 2002; Vasconcelos et al., 2007) based on the observation that the efficacy of the extracts was similar to the efficacy of benzodiazepine drugs used as positive controls or reference drugs. This suggestion could be corroborated by an in vitro study with guinea-pig ileum that showed, among other mechanisms of action, GABAA receptor activation in response to AE from *E. velutina* leaves (Carvalho et al., 2009). However, we could not find behavioral studies that investigated this proposed mechanism of action through the concomitant use of antagonists of gabaergic and/or benzodiazepine receptors. It is noteworthy to mention that the elevated plus maze test is very sensitive to detect anxiolytic effect induced by benzodiazepine-like drugs (Pinheiro et al., 2007) and the failure of this test to reveal the anxiolytic effect of *Erythrina* not only in the present study but also in previous ones raises a red flag on a possible gabaergic-benzodiazepine mechanism of action for the central effects of *Erythrina* species.

Another neurotransmitter that has been suggested to be involved in the anxiolytic effect of *Erythrina* is serotonin (Flausino et al., 2007). The crude extract of *E. vespertilio* inhibited platelet serotonin release, which is a model for studying calcium-dependent serotonin release (Rogers et al., 2001). In addition, the alkaloid β-erythroidine seems to be an antagonist of 5-HT3 receptors (Eiselé et al., 1993). A serotonergic action could explain many of the results that have been observed in the pharmacological screening of plants from the genus *Erythrina* because: i) it is classically recognized the false negative results of anxiolytic serotoninergic drugs in the elevated plus maze test after acute administration (Handley & Macblane, 1993); ii) serotoninergic drugs are more likely to induce anxiolytic effect in the elevated plus...
maze after repeated treatment (Silva & Brandão, 2000), which has been observed by Raup et al. (2008) with the hydroalcoholic extract of E. mulungu; iii) similarly to the results obtained in the present study, serotonergic anxiolytic drugs, like buspirone, do not potentiate the effect of hypnotics but do decrease motor activity in the open-field (Andreatini et al., 2001; Shireen & Haleem, 2005; Vadiya et al., 2005). Despite those evidences, the serotonergic involvement on the Erythrina central action is so far just a speculation and future studies should specifically address this issue.

It was somewhat surprising the complete lack of behavioral effects of the DCM extract because DCM has been suggested as the best extractor for erythrinan alkaloids in flowers (Silva, 2003) and alkaloids have been claimed as the central active components of Erythrina (Flausino et al., 2007). One possibility, is that since alkaloids have low stability and are sensitive to light, pH and some organic solvents, including chloroform and DCM (Henriques et al., 2004), the extraction conducted in our study may have resulted in the loose of pharmacological activity of the alkaloids.

Regarding the acute toxicity of E. speciosa, the result obtained in this study suggests it as having low acute toxicity since the estimated LD50 was higher than 2000 mg/kg. The OECD guideline discourage the testing of doses higher than 2000 mg/kg and recommends that this should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health (OECD, 2001). This was not the case in the present study because two other studies conducted with E. velutina described the oral LD50 of the AE from leaves of this species as being higher than 5000 mg/kg (Bonfim, 2001; Craveiro et al., 2007). Taken together the LD50 results suggest that species from the genus Erythrina found in Brazil may have low acute toxicity. However, it is important to recognize that having low acute toxicity does not mean that those plants lack toxicity and a complete toxicity evaluation following national and international guidelines is necessary before attesting the safety of Erythrina species as phytomedicines.

In conclusion, the results showed that the only behavioral effect induced by AE extract of E. speciosa leaves was a decreased motor activity in the open-field test. However, the lack of effect in the elevated plus maze test does not invalidate the use of this plant in folk medicine as anxiolytic since this test is not adequate to evaluate drugs with mechanisms of action differently from the gabaergic-benzodiazepine one. Future studies are necessary to clarify the mechanism of action of E. speciosa and validate its folk use as anxiolytics.

ACKNOWLEDGEMENTS

This study was supported by Fundação Araucária.

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