Original article

Behavioral study with *Erythrina velutina* fractions

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Abstract

Objective: To study the behavioral effects of semi purified fraction prepared from the stem bark of *Erythrina velutina* on mice. **Methods**: Three chloroformic fractions (CA) at 50 or 100 mg/kg (CA1, CA2 and CA3) were tested in two behavioral tests: plus maze and open field. **Results**: In the plus maze test, it was observed a decrease in the number of entries in the closed arms (NECA) CA2 at both doses as compared to the control group. In the open field test, CA2 and CA3 fractions decreased the number of squares crossed and the time of permanence on the bar, increased the number of falls at both doses in the rota rod test. The CA2 fraction decreased the NECA, but this data did not characterize an anxiolytic effect. It was the consequence from decrease of locomotor activity (LA) observed in the open field test, that the decrease of LA and rearing, the number of falls and the decrease in time of permanence on the bar in rota rod test were observed after treatment with CA2 and CA3 at both doses, suggesting sedative effect. **Conclusion**: The present work demonstrated that CA2 and CA3 fractions have impairing effect on LA and motor coordination.

Keywords: *Erythrina velutina*; Fraction; Locomotor activity; Motor coordination

INTRODUCTION

The *Erythrina* genus, belonging to the Fabaceae family, is used in folk Brazilian medicine against agitation, insomnia and other disorders of the central nervous system [1,2]. This designation includes the species *Erythrina velutina* (*E. velutina*), endemic plant in the plains and river banks of the semi-arid regions in Northeastern Brazil. It is a high tree that shows trunks and branches with red flowers[3].

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This genus is known to produce alkaloids, flavonoids and terpenes [4,5]. These plants represent the main source of tetracyclic alkaloids and have curare-like activity causing muscular paralysis [6]. In phytochemical investigation of non-alkaloidal secondary metabolites of species from the genus *Erythrina*, several isoflavonoids were found[7,8], some of which exhibit anti-inflammatory activity [9]. Rabelo et al. [10] found homohesperetin and phaseollidin in the chemical fractionation of the stem bark from *E. velutina*. The phytochemical analysis of the hydroalcoholic extracts from *E. velutina* has allowed so far the characterization of 5, 7, 3′-trihydroxy-5′-prenyl-6-methoxyisoflavanone, phaseolin, a 1: 1 mixture of β-sitosterol and stigmasterol, erythrodiol and lupeol, besides phaseollidin [11].

Several species of the genus *Erythrina* had pharmacological activities, such as the central nervous

system (CNS) sedative action [12,13]. In studies with hydroalcoholic extracts from the stem bark of *E. velutina*, it was showed antinociceptive properties [14], anti-inflammatory activity [15], anxiolytic potential[16], sedating effect and anticonvulsant action[17]. De Oliveira et al. [18] showed the dose-dependent decrease of locomotor activity after treatment with the aqueous extract from the leaf of *E. velutina*. Their work also suggests the involvement of some bioactive constituent of this species with memory processes.

The objective of the present work was to study the behavioral effects of *E. velutina* with the products of its fractionation emphasizing their activities in the central nervous system. This study intended to contribute to the validation of this species widely used in popular medicine in Brazil.

MATERIALS AND METHODS

Animals

Female 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 30 m^2 rooms with controlled 12 h light/dark cycle, temperature of 25℃, 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. The study was approved by the Research Ethics Committee of the Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará.

Preparation of the fractions

The plant stem bark with the amount of 1 520 kg was crushed, dried, and subjected to cold with hydroalcoholic solvent extraction (ethanol: water = 7: 3 v/v). The solution resulting from the extraction was concentrated under reduced pressure in rotavapor for withdrawal of ethanol, resulting in clear brown solution (aqueous material), which was placed in an Erlenmeyer flask and packaged in refrigerator. There was formation of dark precipitate on the bottom of the Erlenmeyer flask, which was separated from the aqueous solution by filtration, leading to the EVCCHdP fraction. The EVCCHdP fraction was solubilized in ethanol, but it was not completely soluble, presenting a precipitate (EVCCHdP-FU), which was separated by filtration. The ethanolic solution resulting from the filtration was submitted to liquid partition with chloroform and ethyl acetate, resulting in EVCCHdP-Cl and EVCCHdP-A fractions, respectively. The EVCCHdP-Cl fraction was dissolved in methanol and subjected to partition with chloroform, resulting in a new chloroformic fraction (CA) (EVCCHdP-Cl/Cl). The EVCCHdP-Cl/Cl chloroformic fraction was dissolved in methanol and applied to 20 g of Sephadex LH-20. The material was eluted isocratically with methanol. CA1, CA2 and CA3 fractions were obtained. After analysis by RMN ^1H of CA2 and CA3 fractions, it was possible to verify that they were formed basically by terpenes / steroids and flavonoids, respectively. The triterpene erythrodiol and a mixture of β-sitosterol and stigmasterol steroids were isolated from CA2 fraction. Two pterocarpans (phaseollidin and phaseolin) and an isoflavanone (5,7,3′-trihydroxy-5′-prenyl-6-methoxyisoflavanone) were isolated from the CA3 fraction.

Experimental protocol

Animals were treated intraperitoneally with distilled water (controls) or with one of the three fractions (CA1, CA2 or CA3) from *E. velutina* (50 or 100 mg/Kg). Thirty minutes after treatment, each animal was submitted to the test. Firstly, the animal was observed in a free-from-noise room, at constant temperature (23℃ ± 1℃) and poorly illuminated with a 15-V red light. The animal was then placed inside a plus maze apparatus and observed for 5 mins. Immediately after this test, the animal was placed in the open field area for 5 mins. After that, the animal was removed to the rota rod, where it was observed for 1 min.

Elevated plus maze test

The anxiolytic activity of the chloroformic fractions from *E. velutina* was evaluated in the plus maze test[19]. The maze consisted of two perpendicular open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm × 25 cm) also in perpendicular position. The open and closed arms were connected by a central platform (5 cm × 5 cm). The platform and the lateral walls of the closed arms were made of transparent acrylic. The floor was made of black acrylic. The maze was 45 cm above the floor. The parameters observed were number of entries in the open arms (NEOA) and in the closed arms (NECA), time of permanence in each of them, time of permanence in the open arms (TPOA) and in the closed arms (TPCA).

Open field test

The open field area was made of acrylic (transparent walls and black floor, 30 cm × 30 cm × 15 cm), divided into nine squares of equal area. It was used to evaluate the exploratory activity of the animal[20]. The parameters recorded were number of squares crossed (locomotor activity, LA) and number of grooming (number of times the mouse scratched the face with its forepaws) and rearing (number of times the mouse stood completely erect on its hind legs).

Rota rod test

The animal was placed with the four paws on a 2.5 cm diameter bar, 25 cm above the floor, which was at a constant speed of 12 rpm. For each animal, the number of falls (up to three falls) and the time of permanence on the bar for 1 min were registered[21].

Statistical analyses

All results are presented as mean ± SEM. ANOVA was followed by Student-Neuman-Keuls as the *post hoc* test. Results were considered significant at $P < 0.05$.

RESULTS

In the plus maze test, the results of the CA1 and CA3 fractions did not show any significant difference (Table 1). However, the CA2 fraction decreased the number of entries in the closed arms at both doses as compared to control group.

While no behavioral change was observed in LA and rearing (R) tests after treatment with CA1 fraction (Table 1), CA2 and CA3 fractions decreased these behaviors at both doses as compared to control group. With respect to grooming, only the CA3 fraction showed significant decline at both doses as compared to control group.

The animals treated with the CA2 and CA3 fractions decreased the time of permanence (TP) on the bar and increased the number of falls (NF) with both doses as compared to control group. On the other hand, no significant change was observed with CA1 (Table 1).

Table 1 Effects of acute treatment with chloroformic fractions from *Erytrina velutina* in elevated plus maze test, open field test and rota rod test in female mice (mean ± SD).

	Control ($n=10$)	CA1 (mg/kg) ($n=10$)		CA2 (mg/kg) ($n=10$)		CA3 (mg/kg) ($n=8$)	
		50	100	50	100	50	100
TPOA	129.50 ± 10.90	144.00 ± 8.36	143.50 ± 7.29	113.50 ± 9.43	127.90 ± 8.78	127.90 ± 12.80	116.80 ± 9.12
TPCA	154.10 ± 9.24	148.10 ± 7.71	150.20 ± 6.94	165.50 ± 9.49	155.30 ± 9.21	161.60 ± 11.80	170.60 ± 7.43
NEOA	6.50 ± 0.42	7.00 ± 0.36	6.80 ± 0.59	5.50 ± 0.83	5.60 ± 0.42	5.37 ± 0.53	7.12 ± 1.12
NECA	8.00 ± 0.47	8.00 ± 0.51	6.70 ± 0.57	6.30 ± 0.65[a]	5.60 ± 0.30[a]	6.25 ± 0.31	8.12 ± 0.87
LA	66.60 ± 5.40	66.90 ± 3.60	56.60 ± 3.50	47.40 ± 4.50[a]	42.00 ± 3.20[a]	44.00 ± 2.90[a]	49.60 ± 3.90[a]
Rearing	34.80 ± 2.80	32.30 ± 1.90	27.10 ± 2.10	15.60 ± 1.80[a]	15.50 ± 1.30[a]	17.50 ± 1.80[a]	18.20 ± 2.90[a]
Grooming	2.00 ± 0.21	2.30 ± 0.26	1.60 ± 0.16	1.30 ± 0.26	1.50 ± 0.16	1.25 ± 0.16[a]	1.25 ± 0.16[a]
Time of permanence (s)	59.00 ± 0.51	58.80 ± 0.41	58.00 ± 0.59	52.50 ± 2.52[a]	49.60 ± 3.18[a]	56.10 ± 0.95[a]	56.00 ± 0.98[a]
Number of falls	0.30 ± 0.15	0.50 ± 0.16	0.80 ± 0.24	1.70 ± 0.36[a]	1.80 ± 0.32[a]	1.25 ± 0.31[a]	1.25 ± 0.31[a]

a: $P < 0.05$ significance values.

DISCUSSION

This work studied the behavioral alterations caused by the acute intraperitoneally administration of three chloroformic fractions from *E. velutina* in mice. In order to show these alterations, plus maze, open field and rota rod tests were used.

The plus maze is a test used to demonstrate the anxiety of the animal. The open arm is the one responsible for the induction of anxiety. Therefore, an anxiolytic effect is showed by an increase in the NEOA as compared to control[22]. In opposition to what has been observed with other species of the genus *Erythrina*[12,23,24], this study did not show anxiolytic effect with the products of fractionation of *E. velutina*. Although the CA2 fraction decreased the NECA, this data does not characterize an anxiolytic effect but a likely consequence of a decrease in LA observed in the test of open field. Similarly, Vasconcelos et al.[13] showed decreases in the NEOA and NECA of the elevated plus maze only after the administration of the highest dose (800 mg/kg, p.o.) of hydroalcoholic extracts of *E. velutina*, and this effect may also be due to the decrease in LA.

LA is a parameter used to verify if the substance in use causes any alteration in animal capacity to move, due to a sedative effect or an impairment in peripheral nervous system[25]. The decrease of LA and rearing was observed after treatment with CA2 and CA3 at both doses, suggesting sedative effect of

both fractions. Another study using hydroalcoholic extract also demonstrated similar results [13]. The present work still showed decrease in the number of groomings only with the CA3 fraction. This effect suggests effect of this fraction on the dopaminergic system.

The last test used was the rota rod. It is useful to demonstrate impairment on motor coordination, which increases the number of falls and decreases the time spent on the bar. This was demonstrated by the administration of the highest dose of CA2 and of both doses of CA3, supporting a possible mechanism of these fractions involving a sedative effect or a peripheral impairment [25].

In conclusion, the present work demonstrated that the CAs (CA2 and CA3) from *E. velutina* have impairing effect the locomotor activity and the motor coordination. It is important to perform further experiments to prove a specific mechanism of action.

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