Anxiolytic-like effect of *Rauvolfia ligustrina* Willd. ex Roem. & Schult., Apocynaceae, in the elevated plus-maze and hole-board tests

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RESUMO: “Avaliação do efeito ansiolítico de *Rauvolfia ligustrina* Willd. ex Roem. & Schult., Apocynaceae, nos testes do labirinto em cruz elevado e placa-perfurada”. *Rauvolfia ligustrina* Willd. ex Roem. & Schult. (Apocynaceae) é conhecida popularmente como “arrebenta-boi” e “paratudo”. Em triagem farmacológica comportamental o extrato etanólico das raízes de *R. ligustrina* (EER) mostrou efeito depressor do SNC e propriedades anticonvulsivantes. O presente estudo buscou avaliar o possível efeito ansiolítico do EER utilizando os testes do labirinto em cruz elevado (LCE) e o teste da placa perfurada (“hole-board”) em roedores. A administração do EER, por via intraperitoneal (i.p.), em diferentes doses (3,9; 7,8 e 15,6 mg/kg) foi capaz de aumentar significativamente o número de entradas (p < 0,05), assim como o tempo despendido nos braços abertos do LCE. Além disso, nos animais tratados com o EER (3,9 e 7,8 mg/kg, i.p.) ocorreu aumento significativo no número de visitas à borda e mergulho com a cabeça no teste da placa perfurada em comparação com os animais do grupo controle. Estes dados sugerem um possível efeito ansiolítico do EER nos modelos animais testados.


ABSTRACT: *Rauvolfia ligustrina* Willd. ex Roem. & Schult. (Apocynaceae), popularly known as “arrebenta-boi” and “paratudo”. In behavioral screening ethanol extract of *R. ligustrina* roots demonstrated depressant effect on the CNS and anticonvulsant properties. The purpose of this study was to characterize the putative anxiolytic-like effects of the ethanol extract of *Rauvolfia ligustrina* roots (EER) using the elevated plus maze (EPM) and the hole-board apparatus in rodents. This extract, administered intraperitoneally, in different doses (3,9, 7,8 and 15,6 mg/kg) was able to increase significantly the number of entries (p < 0,05), as well as the time spent in the open arms of the EPM, indicating an anxiolytic-like effect. Additionally, EER-treated (3,9 and 7,8 mg/kg) increased significantly the number of border visit and head-dipping. This data suggest an anxiolytic effect of EER in animal models of anxiety.

Keywords: *Rauvolfia ligustrina*, Apocynaceae, medicinal plant, anxiolytic, plus-maze, hole board test.

INTRODUCTION

Anxiety disorders are considered the most common psychiatric diagnoses, affecting between 10-30% of the general population (Rice & Miller, 1998; Wittchen & Hoyer, 2001). The development of new anxiolytics has been an area of interest. Recently, various types of herbal medicines have been used as anxiolytic drugs in the world (Rex et al., 2002; Carlini, 2003). Species of *Rauvolfia* are rich sources of indole alkaloids (Cancelieri et al., 2002), such as reserpine that was isolated from *R. serpentina* Benth. and had interest on account of its pharmacological properties as an antihypertensive, anxiolytic and tranquilizing agent (Woodson et al., 1957; Neuss, 1970; Lednicer & Mitscer, 1977). *Rauvolfia ligustrina* Willd. ex Roem. & Schult.

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(Apocynaceae), popularly known as “arrebenta-boi” and “paratudo”, is a plant found in Latin America (Moura & Agra, 1989). Behavioral screening showed that the ethanol extract of *R. ligustrina* roots (EER) has depressants effects on the CNS (Almeida et al., 2000). In addition, recent investigation provided evidence of the possible anticonvulsant effect of total alkaloid fraction of the aerial parts of *R. ligustrina* in mice (Quintans-Júnior et al., 2002, 2007).

The aim of our study was the evaluation the possible anxiolitic effect of the ethanol extract of *Rauvolfia ligustrina* roots (EER) in elevated plus-maze (EPM) and hole-board test in rodents.

**MATERIAL AND METHODS**

**Animals**

Male Swiss albino mice (25-30 g) and male Wistar rats (200-250 g), with two-three months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 23±2 °C on a 12 h light/dark cycle (lights on 06:00-18:00) with free access to food (Purina) and water. They were used in groups of ten animals each (n = 10). Experimental protocols and procedures were approved by the Animal Care and Use Committee from the “Laboratório de Tecnologia Farmacêutica” (CEPA Nº1105/06).

**Plant material**

Roots of *Rauvolfia ligustrina* Willd. ex Roem. & Schult. (Apocynaceae) were collected in Santa Rita, Paraiba State in December 2001 and were identified by Dr. Maria de Fátima Agra (NPPN/UFPB). A voucher specimen (Agra-19197) is deposited at the Herbarium Lauro Pires Xavier of the “Universidade Federal da Paraíba”.

**Preparation of the extract**

Roots of *R. ligustrina* (2.143 g) were oven-dried at 40 °C and pulverized and extracted at room temperature with 95% ethanol in water for 72 h. The extract was dried at 60 °C using rotavapor and the income was approximately 20% for obtaining the ethanol extract of *R. ligustrina* roots (EER) (427 g). The lyophilized extract was suspended in Tween 80 (0.2%) with one drop of Cremophor for experiments.

**Drugs**

Diazepam (DZP), polyoxyethylene-sorbitan monolated (Tween 80) and Cremophor were purchased from Sigma (USA) and diazepam (DZP) from Roche (Brazil). Agents were injected intraperitoneally (i.p.) with a dose volume of 0.1 mL/10 g (mice) and 0.1 mL/100 g (rats).

**Statistical analysis**

The data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Differences were considered to be statistically significant when p < 0.05.

**Elevated plus-maze test (EPM)**

This model of anxiety is based on observation that rats and others rodents avoid open and elevated place. The elevated plus-maze was based on that described by Lister (1987). It is consisted of two opposing open (50 x 10 cm) and two opposing closed arms (50 x 10 x 30 cm). The apparatus was elevated to a height of 70 cm above floor level. Drugs as benzodiazepines significantly increase the time spent and numbers of entries in the open arms. In contrast, the number of entries in the closed arms not change (Graeff, 2000). Rats were divided into five groups (n = 10, each). The first group served as control and received tween 80 (0.2%) and one drop of cremophor (vehicle), while experimental groups received EER at doses 3.9, 7.8 and 15.6 mg/kg. The standard group received DZP at dose 1.5 mg/kg. The rats were treated with intraperitoneal route (i.p.). The time spent and numbers of entries in the open and closed arms was noted until 5 min per animal.

**Hole-board test**

The hole-board apparatus (Ugo Basile, Verese, Italy) consisted of gray perspex panels (40 x 40 cm, 2.2 cm thick) with sixteen equidistant holes 3 cm in diameters on the floor. Photocells below the surface of the holes measured the number of head-dips. The apparatus has not walls and was elevated to a height of 18 cm above floor level. Similarly before experiment, the first group served as control and received tween 80 (0.2%) and one drop of cremophor (vehicle), while experimental groups received EER at doses 3.9, 7.8 and 15.6 mg/kg. The standard group received DZP at dose 1.5 mg/kg. The mice were treated with intraperitoneal route (i.p.). Each animal was placed singly in the center of the board facing away from the observer and its behavior recorded for 5 min: (1) the total number of head-dips, (2) number of border visit, (3) number of head-dips in the central hole, (4) ambulation, (5) grooming.

**RESULTS**

**Effects on elevated plus-maze test**

The results of the EPM are demonstrated in Figures 1 and 2. Data showed an increase of time spent and numbers of entries in the open arms in groups treated with EER using doses 3.9 and 7.8 mg/kg (i.p.). Similar results occur in standard group (DZP 1.5 mg/kg, i.p.).
Nevertheless, the time spent and numbers of entries in the closed arms in experimental groups were unchangeable in comparison with control group.

**Figure 1.** Anxiolytic-like effects of DZP and EER upon entries numbers of open and closed arms on the elevated plus-maze test (n = 10, each). Values represent mean ± S.E.M. Asterisks (*) represent significant difference between vehicle and experimental groups (one-way ANOVA followed by the Student-Newman-Keuls Test, *p < 0.05).

**Figure 2.** Anxiolytic-like effects of DZP and EER upon permanence time of open and closed arms on the elevated plus-maze test (n = 10, each). Values represent mean ± S.E.M. Asterisks (*) represent significant difference between vehicle and experimental groups (one-way ANOVA followed by the Student-Newman-Keuls Test, *p < 0.05).

**Effects on hole-board test**

The effect of EER on the head-dipping behavior in mice is shown in Figure 3 and Table 1. EER-treated mice showed significant increases in number and duration of head-dips at doses of 3.9 and 7.8 mg/kg (i.p.) versus control group.

**Figure 3.** Anxiolytic-like effects of DZP and EER upon visits to board in hole-board test. Value represent mean ± S.E.M of visits to apparatus board (n = 10, each). Asterisks (*) represent significant difference between vehicle and experimental groups (one-way ANOVA followed by the Student-Newman-Keuls Test, *p < 0.05).

**Table 1.** Effect of DZP and EER in hole-board test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dose (mg/kg)</th>
<th>Head-dip counts</th>
<th>Head-dip duration (s)</th>
<th>Ambulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>-</td>
<td>8.2±1.1</td>
<td>14.5±1.6</td>
<td>65.4±6.9</td>
</tr>
<tr>
<td>DZP</td>
<td>10</td>
<td>1.5</td>
<td>19.2±2.4*</td>
<td>23.4±2.8*</td>
<td>39.8±7.2*</td>
</tr>
<tr>
<td>EER</td>
<td>10</td>
<td>15.6</td>
<td>16.6±2.1*</td>
<td>17.7±2.5</td>
<td>59.6±5.9</td>
</tr>
<tr>
<td>EER</td>
<td>10</td>
<td>7.8</td>
<td>17.1±1.9*</td>
<td>21.2±1.7*</td>
<td>64.1±4.7</td>
</tr>
<tr>
<td>EER</td>
<td>10</td>
<td>3.9</td>
<td>17.3±2.3*</td>
<td>19.2±1.7*</td>
<td>61.1±7.5</td>
</tr>
</tbody>
</table>

n: number of mice per group; *Values represent mean ± S.E.M.; *p < 0.05 (one-way ANOVA followed by the Student–Newman–Keuls test), significantly different from control group (vehicle).

**DISCUSSION**

There are a considerable popular interest in the use of the so-called natural medicines, or herbal products, to treat anxiety and depression. Recently, several plants have been reported to have anxiolytic effects through animal models of anxiety (Beaubrum & Gray, 2000; Seo et al., 2007).

*Rauvolfia ligustrina* is used in folk medicine for treatment of pain, convulsion, anxiety and as abortive. Total alkaloid fraction of the aerial parts of *R. ligustrina* has showed depressant activity on CNS and anticonvulsant properties and suggest of the environment of GABAergic system (Quintans-Júnior et al., 2007). Therefore, the present study was designed to investigate the anxiolytic properties of EER in elevated plus maze (EPM) and hole-board tests in rodents.

EPM is one of the most important animals model used in evaluation of anxyolitic effect of drugs (Pellow et al., 1985; Pellow & File, 1986). Moreover, it is known that anxiolytic agents increase the frequency of entries and the time spent in open arms of the EPM (Pellow et al., 1985). Administration of EER (7.8 mg/kg, i.p.) significantly
increased (p < 0.05) the percentage of entries and permanence time into open arms, compared with control group. The effects of EER seem to be more potent than diazepam group in comparison to experimental groups.

The hole-board test, developed by Boissier & Simon (1962, 1964), has gained popularity as model of anxiety, offering “a simple method for measuring the response of an animal to an unfamiliar environment, with advantages that several behaviors can be readily observed and quantified in this test” (Takeda et al., 1998; Silva & Elisabetsky, 2001). Drugs as benzodiazepines significantly increase the number of head-dips in the hole-board test (Takeda et al., 1998). In our study, EER (3.9 and 7.8 mg/kg, i.p.) increased head-dip counts and increased head-dip duration without changing locomotion in the hole-board test. Additionally, EER increased visits numbers to board compared with control group. These results suggest that EER has a significant anxiolytic effect in this paradigm.

Although some mechanisms of anxiolytic action have been proposed, none enjoys general acceptance, including changes in concentration of biogenic amines. Indole alkaloids isolated from species of Rauvolfia, such as reserpine, has attracted interest on account of its pharmacological properties as sedative and tranquilizing agent (Kato et al., 2002). The indole alkaloid alstonine present as major component of a plant-based medicine traditionally used in Nigeria as antipsychotic, showed anxiolytic properties and this effect can be indicative of the involvement of 5-HT$_{2A/2C}$ receptors (Costa-Campos et al., 2004).

Fluoxetine is usually prescribed for the treatment of depression. Recently these compound, in particular drugs belonging to the class of selective serotonin re-uptake inhibitors (SSRIs), was also recommended for the treatment of anxiety in adults and children (Drapier et al., 2007). In some cases of anxiety disorders, SSRIs have been approved for these indications and may be more appropriate than BZDs. Studies with experimental animals suggest that a damage of the serotonergic neurotransmission should be related to the difficulties of adaptation to stress and to the anxiety (Mendonça Netto & Guimarães, 1996; Netto et al., 2002). However, the role of 5-HT in anxiety is complex and may be dependent of many factors, including the 5-HT neurotransmission in multiple brain regions, the type of behavioral paradigms used to evaluate anxiety, and the emotional and cognitive contexts of the tests (Handley, 1991; Handley & McBlane, 1993; Salchner & Singewald, 2002). Therefore, the increased of the biogenic amines, such as indole alkaloids, induced by EER, may be related with its anxiolytic properties. Additionally, Quintans-Júnior et al. (2007) suggest the role of GABAergic system in anticonvulsive property of R. ligustrina.

Our results suggest that EER exhibits anxiolytic effects in the EPM model and in the hole-board test. The pharmacological effects may be mediated by GABAergic and 5-HTnergic systems. However, its mode of action remains to be elucidated.

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