

## Anticonvulsant properties of the total alkaloid fraction of *Rauvolfia ligustrina* Roem. et Schult. in male mice

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**RESUMO:** “Propriedades anticonvulsivantes da fração dos alcalóides totais de *Rauvolfia ligustrina* Roem. et Schult. em ratos machos”. *Rauvolfia ligustrina* Roem. et Schult. (Apocynaceae) é uma planta amplamente distribuída no Nordeste Brasileiro, rica em alcalóides indólicos, conhecida popularmente como “paratudo” e “arrebenta-boi”. O presente estudo buscou avaliar a dose letal 50 % (DL<sub>50</sub>) da fração de alcalóides totais (FAT) das partes aéreas da *R. ligustrina* e a sua possível atividade anticonvulsivantes em roedores. A FAT apresentou uma DL<sub>50</sub> via intraperitoneal (i.p.), de 127,8 (112,5-145,2) mg/kg e foi efetiva, na dose de 20 mg/kg (i.p.), em proteger os animais das convulsões induzidas pelo pentilenotetrazol (PTZ) e picrotoxina (PIC) aumentando significativamente ( $p < 0,05$ ) a latência para o aparecimento das convulsões, sendo um indicativo de um efeito anticonvulsivante.

**Unitermos:** *Rauvolfia ligustrina*, anticonvulsivante, pentilenotetrazol, picrotoxina, eletrochoque auricular.

**ABSTRACT:** *Rauvolfia ligustrina* Roem et. Schult (Apocynaceae), commonly known as “paratudo” and “arrebenta-boi” is a small tree found in Brazilian Northeastern. Previous studies have demonstrated depressant and anticonvulsant properties of the ethanol extract of *Rauvolfia ligustrina*. The aim of the present study was the determination of the lethal dose 50% (LD<sub>50</sub>) and the effects of total alkaloid fraction (TAF) of the aerial parts of *R. ligustrina* in animal models of convulsion. It was found that the acute toxicity of TAF was 127.8 (112.5-145.2) mg/kg (i.p.) in mice. TAF (20 mg/kg, ip) significantly increased ( $p < 0.05$ ) the latencies of clonic seizures induced by pentylenetetrazol (PTZ) and picrotoxin (PIC). However, TAF did not protect the animals in maximal electroshock (MES) induced seizures. These results suggest that TAF of *R. ligustrina* possesses anticonvulsant properties.

**Keywords:** *Rauvolfia ligustrina*, anticonvulsant activity, pentylenetetrazol, picrotoxin, maximal electroshock.

### INTRODUCTION

Bioactive alkaloids have been isolated from several plant species of the Apocynaceae family, including many belonging to the *Rauvolfia* genus. Species of *Rauvolfia* are rich sources of indole alkaloids (Kan et al., 1986; Cancelieri et al., 2002), such as reserpine that was isolated from *Rauvolfia serpentina* Benth. and has attracted interest on account of its pharmacological properties as an antihypertensive, anxiolytic, tranquilizing, inhibitors of the angiotensin converting enzyme and anti-inflammatory activity (Woodson et al., 1957; Neuss, 1970; Lednicer; Mitscer, 1977; Barbosa-Filho et al., 2006a,b). Roots of *Rauvolfia sellowii* are used in folk medicine as an antihypertensive and the analysis of its crude extract

has demonstrated some hypotensive activity in dogs (Batista et al., 1996). The extract from the root bark of *Rauvolfia obscura* showed antiamoebic activity (Tona et al., 1998).

*Rauvolfia ligustrina* Roem et. Schult (Apocynaceae) commonly known as “arrebenta-boi” and “paratudo”, consists of a shrub widely distributed from South Mexico to the Brazilian Northeastern. All parts of the plant are poisonous (Agra et al., 2007). In preliminary behavioral screening by using the method described by Almeida et al. (1999), the ethanolic extract of the roots of *R. ligustrina* showed depressant activity on central nervous system (CNS) (Almeida et al., 2000). Furthermore, recent investigation provided evidence of possible anticonvulsant effect of the ethanol extract of the

roots of *Rauwolfia ligustrina* in mice (Quintans-Júnior et al., 2002). In the present study, we determined the lethal dose (LD<sub>50</sub>) and tested the effects of total alkaloid fraction of the aerial parts of *R. ligustrina* (TAF) in animal models of epilepsy.

## MATERIAL AND METHODS

### Animals

Male Swiss mice (25-30 g), with three months of age, were used throughout this study. The animals were randomly housed in appropriated cages at 25 ± 2 °C on a 12 h light/dark cycle (lights on 06:00-18:00) with free access to food (Purina - Brazil) and water. They were used in groups of ten animals each. Experimental protocols and procedures were approved by the Laboratório de Tecnologia Farmacêutica Animal Care and Use Committee (Certidão/CEPA N° 1105/06).

### Plant material

The aerial parts of *Rauwolfia ligustrina* Roem et. Schult were collected in Santa Rita, Paraíba State in December 1994 and were identified by Dr. Maria de Fátima Agra (NPPN/UFPB). A voucher specimen (Agra-19197) is deposited at the Herbarium Lauro Pires Xavier from Universidade Federal da Paraíba.

### Preparation of the extract

Finely dried and powdered aerial parts of *R. ligustrina* were extracted with 95% aq. EtOH until a negative Mayer test, at room temperature. The EtOH extract was concentrated to dryness under reduced pressure. The resultant dried extract (400 g) was dissolved in 2 L of a 4% acetic acid solution and mechanically agitated during 2 hs. The suspension was filtered and the insoluble resinous matter discarded. The filtrate was partitioned with CHCl<sub>3</sub> to yield, after evaporation to dryness, the acidic fraction (12 g). The acidic water phase was rendered alkaline (pH 9) with NH<sub>4</sub>OH and extracted with CHCl<sub>3</sub>. The organic layers were combined, washed with water and concentrated under vacuum to afford 4g of the total alkaloids fraction of the aerial parts of *R. ligustrina* (TAF).

### Drugs

Pentylentetrazole (PTZ), phenytoin (PHE), picrotoxin (PIC), polyoxyethylene-sorbitan monolated (Tween 80) and Cremophor were purchased from Sigma (USA) and diazepam (DZP) from Roche (Brazil). The vehicle used in protocols was Tween 80 (0.2%) with one drop of Cremophor. Agents were injected intraperitoneally (i.p.) with a dose volume of 1 mL/100g.

### Determination of the lethal dose 50% (LD<sub>50</sub>)

Different doses of TAF (10, 20, 40, 80, 160 and 320 mg/kg) were administered intraperitoneally (i.p.) to groups of male mice (n = 10). The groups were observed for 48 h and at the end of this period mortality was recorded for each group (Dietrich, 1983).

### Pentylentetrazole (PTZ)-induced convulsion

PTZ (60 mg/kg i.p.) was used to induce clonic convulsions (Swinyard et al., 1989; Oliveira et al., 2001). Animals were divided into five groups (n = 10), control group received vehicle and standard group was treated with diazepam (DZP, 4 mg/kg i.p.). The remaining groups were treated with 10, 20 and 40 mg/kg of TEF (i.p.). After 60 min of drug administration, the mice were treated with PTZ at a dose of 60 mg/kg (i.p.). Immediately after the injection of the convulsant, mice were individually placed in plastic polypropylene boxes and observed for the time onset of clonic seizures (latency), percent clonic seizures and deaths. The incidence of deaths was noted until 48 h after the injection of PTZ.

### Picrotoxin (PIC)-induced convulsion

The method has been described previously (Lehmann et al., 1988; Ngo Bum et al., 2001). Animals were divided into five groups (n = 10), control group received vehicle and standard group was treated with diazepam (DZP, 4 mg/kg i.p.). The remaining groups were treated with 10, 20 and 40 mg/kg of TEF (i.p.). After 60 min of drug administration, the mice were treated with PIC at a dose of 8 mg/kg (i.p.). Immediately after the injection of the convulsant, mice were individually placed in plastic boxes and observed for the time onset of clonic seizures (latency), percent clonic seizures and deaths. The incidence of deaths was noted until 48 h after the injection of PIC.

### Maximal electroshock test

Maximal electroshock test (MES) that induces reproducible tonic convulsion characterized by tonic hindlimb extension (THE) was performed (Oliveira et al., 2001). In this experiment, electroconvulsive shock (130 V, 150 pulses/s, 0.5 s) was delivered through auricular electrodes (ECT UNIT 7801- Ugo Basile) to induced THE. Mice were divided into five groups (n = 10), control group received vehicle and standard group was treated with phenytoin (PHE, 25 mg/kg; i.p.). The remaining groups were treated with 10, 20 and 40 mg/kg of TEF (i.p.), similarly before experiment. After 60 min all groups received electroconvulsive shock. The animals that did not exhibit THE were considered protected (Tortoriello; Ortega, 1993).

## Statistical analysis

Calculation of the LD<sub>50</sub> values with 95% confidence limits and comparisons of the results were performed using computerized linear regression analysis, in *GraphPad Prism*, version 3.02, a registered trademark of *GraphPad Software Inc.* The data obtained in experimental models were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. The incidence (%) of clonic or tonic convulsions as well as the mortality were evaluated by Fisher's Exact Test. Differences between means were considered to be statistically significant when  $p < 0.05$ .

## RESULTS

### Determination of the LD<sub>50</sub>

The LD<sub>50</sub> for total alkaloid fraction of the aerial parts of *R. ligustrina* (TAF) with a 95 % confidence limit, after (i.p.) administration was 127.8 (112.5-145.2) mg/kg in mice.

### Effects of TAF on PTZ-induced convulsion

The TAF in dose of 20 mg/kg (ip) increased latency without protecting animals from clonic seizures. The pretreatment with DZP 4 mg/kg (ip) significantly

**Table 1.** Effect of TAF on convulsion induced by pentylenetetrazol.

| Treatment | Dose (mg/kg) | Latency (s) <sup>a</sup>  | % Convulsions   | % Death        |
|-----------|--------------|---------------------------|-----------------|----------------|
| Control   | -            | 109.0 ± 9.0               | 100             | 80             |
| DZP       | 4            | 798.9 ± 73.2 <sup>c</sup> | 10 <sup>d</sup> | 0 <sup>d</sup> |
| TAF       | 10           | 128.9 ± 12.3              | 100             | 70             |
| TAF       | 20           | 329.4 ± 95.6 <sup>b</sup> | 80              | 80             |
| TAF       | 40           | 195.1 ± 73.2              | 90              | 100            |

$n = 10$

<sup>a</sup> Values represent mean ± S.E.M.

<sup>b</sup>  $P < 0.05$  (one-way ANOVA and Dunnett's test), significantly different from control

<sup>c</sup>  $P < 0.01$  (one-way ANOVA and Dunnett's test), significantly different from control

<sup>d</sup>  $P < 0.01$  (Fisher's test), significantly different from control.

**Table 2.** Effect of TAF on convulsion induced by picrotoxin.

| Treatment | Dose (mg/kg) | Latency (s) <sup>a</sup>   | % Convulsions   | % Death        |
|-----------|--------------|----------------------------|-----------------|----------------|
| Control   | -            | 426.0 ± 39.8               | 100             | 100            |
| DZP       | 4            | 1130.9 ± 98.4 <sup>c</sup> | 10 <sup>d</sup> | 0 <sup>d</sup> |
| TAF       | 10           | 527.7 ± 45.5               | 100             | 100            |
| TAF       | 20           | 677.8 ± 81.3 <sup>b</sup>  | 90              | 80             |
| TAF       | 40           | 574.0 ± 67.1               | 100             | 90             |

$n = 10$

<sup>a</sup> Values represent mean ± S.E.M.

<sup>b</sup>  $P < 0.05$  (one-way ANOVA and Dunnett's test), significantly different from control

<sup>c</sup>  $P < 0.01$  (one-way ANOVA and Dunnett's test), significantly different from control

<sup>d</sup>  $P < 0.01$  (Fisher's test), significantly different from control.

( $p < 0.01$ ) prolonged the latency and was effective in preventing clonic seizures induced by PTZ in 90 % of the animals (Table 1)

### Effect of TAF on PIC-induced convulsion

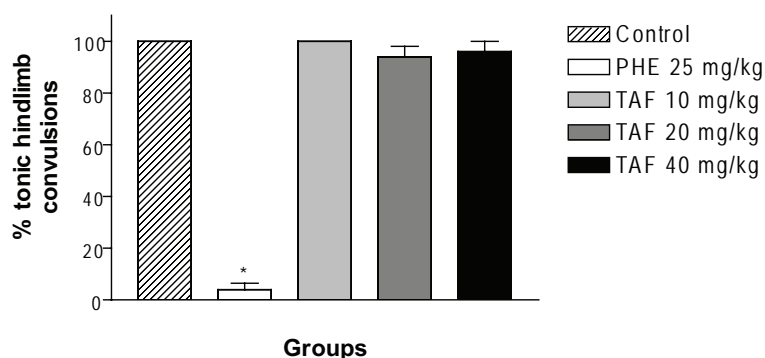
After receiving PIC (8 mg/kg i.p.), all mice of control group exhibited convulsions. The TAF in dose 20 mg/kg (i.p.) failed to inhibit seizures, but significantly ( $p < 0.05$ ) prolonged the latency of the clonic seizures onset induced by PIC. The DZP (4 mg/kg i.p.) which was used as positive control, inhibited seizures in 90 % of the animals (Table 2).

### Effect of TAF on MES

As shown in Fig 1, the treatment of mice with TAF (10, 20 and 40 mg/kg i.p.) did not reduce the percent tonic hindlimb seizures. On the other hand, phenytoin (PHE, 25 mg/kg; i.p.) antagonized MES-induced seizures.

## DISCUSSION

*Rauvolfia ligustrina* (Apocynaceae) is a small tree found in Latin America (Rao, 1956). In preliminary behavioral screening using the method described by Almeida et al. (1999) the ethanol extract of the Roots of



**Figure 1.** Effect of the TAF on MES-induced tonic hindlimb seizures in mice. The figure represents the percentage of animals that exhibit tonic hindlimb seizures induced by MES. \*  $p < 0.05$  (one-way ANOVA and Fisher's test), significantly different from control.

*R. ligustrina* showed depressant activity on the central nervous system (CNS) (Almeida et al., 2000). In addition, Quintans-Júnior et al. (2002) reported evidence of the possible anticonvulsant effect of *R. ligustrina* in mice. The present study has determined the lethal dose ( $LD_{50}$ ) and evaluated the anticonvulsant activity of the total alkaloid fraction of the aerial parts of *R. ligustrina* (TAF) against PTZ-, PIC- and MES-induced seizures in mice.

The respective  $LD_{50}$  of TAF was found to be 127.8 (112.5-145.2) mg/kg in mice. Acute administration of TAF (20 mg/kg, i.p.) in PTZ-induced seizures significantly ( $p < 0.05$ ) increased the latency of clonic seizures onset in comparison with the control group (as shown in Table 1). PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" (Oliveira et al., 2001). PTZ may cause seizures by inhibiting chloride ion channel associated with GABA<sub>A</sub> receptors (Löscher; Schmidt, 1988; Almeida et al., 1998; Ngo Bum et al., 2001). Furthermore, PTZ might induce convulsions by direct excitatory effect upon membranes or by an antagonism of the effect of endogenous benzodiazepine substances (Faingold, 1987). Attenuation of the latency of clonic seizures onset in PTZ-induced seizures TAF (20 mg/kg, i.p.) suggests a possible anticonvulsant effect. The TAF (20 mg/kg, i.p.) also significantly increased ( $p < 0.05$ ) the threshold of clonic convulsions induced by PIC. The PIC induced seizures by blockade of chloride conductance associated to GABA<sub>A</sub> receptors (Faingold, 1987; N'Gouemo et al., 1994). Indeed, the TAF could not totally block seizures, however, the acute anticonvulsant efficacy of a drug does not necessarily predict its antiepileptic activity, which is measured by the ability of the drug to attenuate the epileptogenic process (Starr, 1996; Kasture et al., 2002).

The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" (Löscher, 1998; Oliveira et al., 2001). This model based on observation of the stimulation by repeated

electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity (Quintans-Júnior et al., 2002). The TAF could not demonstrate differences in comparison with the control group. Absence of anticonvulsant activities in the MES test suggest that TAF does not possess effect on this animal model in the used doses level.

Several mechanisms of anticonvulsant action have been proposed, but none enjoys general acceptance, including the increase of the concentration of biogenic amines (Oliveira et al., 2001). It is based on the fact that various anticonvulsants, by nonspecific depression of CNS function, increase the levels of serotonin in the brain (Korolkovas; Burckhalter, 1976; Dailey et al., 1996). Since bioactive alkaloids have been isolated from several species of the *Rauvolfia* genus including sources of indole alkaloids (Cancelieri et al., 2002), and as the 5-HT has indole nucleus in its structure, it might involve serotonergic mechanisms of TAF. Nevertheless, the precise mechanisms underlying the inhibitory effect of TAF are not clear.

In conclusion, agents that have the ability of attenuating the appearance of seizures, might also have anticonvulsant ability. The TAF showed a possible anticonvulsant effect in two animal models of epilepsy. It also suggests that GABAergic and serotonergic systems may be involved.

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