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# Antianxiety and antidepressant effects of riparin III from *Aniba riparia* (Nees) Mez (Lauraceae) in mice

F.C.F. Sousa[a,*], C.T.V. Melo[a], A.P. Monteiro[a], V.T.M. Lima[a], S.J.C. Gutierrez[b], B.A. Pereira[a], J.M. Barbosa-Filho[b], S.M.M. Vasconcelos[a], M.F. Fonteles[a], G.S.B. Viana[a]

[a]*Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Rua Cel. Nunes de Melo 1127, 60431-270, Fortaleza, Brazil*
[b]*Laboratory of Pharmaceutics Technology, Federal University of Paraíba, João Pessoa, Brazil*

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**Abstract**

This work presents behavioral effects of methyl ethers of *N*-(2,6-dihydroxybenzoyl) tyramine (riparin III) isolated from the unripe fruit of *Aniba riparia* on the open field, elevated plus maze (EPM), rotarod, hole board, barbiturate-induced sleeping time, tail suspension and forced swimming tests in mice. Riparin III was administered intraperitoneally to male mice at single doses of 25 and 50 mg/kg. The results showed that riparin III with both doses had no effects on spontaneous motor activity in mice or in the rotarod test, but decreased the number of grooming and rearing. At the dose of 50 mg/kg, riparin III increased the number of entries in the open arms of the EPM test as compared with control. Similarly, in the hole-board test, both doses increased the number of head dips. There was a reduction on the sleeping latency with both doses and a prolongation of the pentobarbital-induced sleeping time with the dose of 25 mg/kg. In the tail suspension test, similar to imipramine (30 mg/kg), riparin III at the dose of 50 mg/kg presented a reduction in the immobility time. In the forced swimming test, both doses of riparin III decreased the immobility time. These results showed that riparin III potentiated the barbiturate-induced sleeping time and presented antidepressant- and anxiolytic-like effects.


*Keywords:* *Aniba riparia*; Riparin III; Anxiolytic and antidepressant effects

## 1. Introduction

*Aniba riparia* (Nees) Mez, from the Lauraceae family, is popularly known in Brazil as "louro." The plant belongs to a genus mainly found in Central Amazonia and Guiana which comprises approximately 40 species of lowland shrubs and trees (Barbosa-Filho et al., 1987; Castelo-Branco et al., 2000). From the unripe fruit of *A. riparia*, were isolated three substances with broad spectrum antimicrobial activity: methyl ethers of *N*-benzoyl tyramine (riparin I), *N*-(2-hydroxybenzoyl) tyramine (riparin II) and *N*-(2,6-dihydroxybenzoyl) tyramine (riparin III; Fig. 1). Recently, it was reported that one of the above compounds (*O*-methyl-)-*N*-(2,6-dihydroxybenzoyl) tyramine (riparin III) obtained synthetically (Barbosa-Filho et al., 1990) has potent smooth muscle relaxant activity (Castelo Branco et al., 1991; Castelo Branco, 1992). Thus, in concentrations from 8 to 30 μM, riparin III antagonized acetylcholine- and histamine-induced contractions of guinea-pig ileum, and oxytocin- and bradykinin-induced contractions of the rat uterus. Furthermore, in the guinea-pig trachea, riparin III inhibited the spontaneous tone ($IC_{50}$ 7.7 μM) and carbachol-induced contractions ($IC_{50}$ 10 μM). The spasmolytic effect of riparin III was investigated by Thomas et al. (1994) concerning the involvement of the compound in relation to $Ca^{2+}$ metabolism. It was demonstrated that riparin III produces an inhibition of $Ca^{2+}$ influx and release of intracellular $Ca^{2+}$. These results lead to the reduction of intracellular $Ca^{2+}$ concentration and possibly contribute to the drug spasmolytic effect.

The interest of our group to study biologically active compounds obtained from Brazilian plants led us to perform a broad spectrum pharmacological screening of riparin III on the central nervous system, considering that as far as we know, there are no studies in the literature on the central

* Corresponding author. Tel.: +55-85-288-8337; fax: +55-85-288-8333.
*E-mail address:* clea@ufc.br (F.C.F. Sousa).

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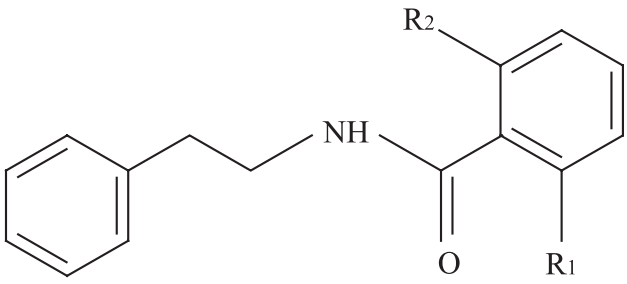


Riparin I (R1=R2=H)

Riparin II (R1=OH, R2=H)

Riparin III (R1=R2=OH)

Fig. 1. Structure of riparin (I, II and III).

actions of riparin III. In this way, the objectives of the present work were to study effects of riparin III on animal models of locomotor, anxiolytic, myorelaxant and depressant activities.

## 2. Materials and methods

### 2.1. Animals

Male Swiss mice (30 g) were used in each experiment and animals were maintained at a controlled temperature (23 ± 1 °C) with a 12-h dark/light cycle and free access to water and food. Animals were treated in accordance to the current law and the NIH Guide for Care and Use of Laboratory Animals.

### 2.2. Drugs

Riparin III was emulsified with 2% Tween 80 (Sigma, USA). Animals were treated with the substance at doses of 25 and 50 mg/kg ip, 30 min before the experiments. Controls received 2% Tween 80 (Sigma) dissolved in distilled water at the same volume as the treated groups (10 ml/kg). Diazepam (DZP) 1 and 2 mg/kg (União Química Brazil) and imipramine (IMP) 10 and 30 mg/kg (Geigy) were used as standards.

### 2.3. Experimental protocol

The animals were tested during the light period and were observed in a closed room at constant temperature (23 ± 1 °C) and poorly illuminated with a 15-V red light, except in the forced swimming test which was illuminated with normal light. Thirty minutes after the treatment, the open-field and rotarod tests were performed with the same animals in the manner described below: Firstly, the animal was placed in the open-field area for 5 min. Immediately after the open-field test, the animal was removed to the rotarod where it was evaluated for 1 min. All the other tests were performed in different days with other groups of animals.

### 2.4. Open-field test

The open-field area was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the animal (Archer, 1973). The observed parameters were as follows: number of squares crossed (with the four paws) and number of grooming and rearing. The animals were divided into four groups with 7–12 animals per group.

### 2.5. Rotarod

In the rotarod test, the method of Dunham and Miya (1957) was used. The animals divided into four groups with 7–16 mice per group were placed with the four paws on a 2.5-cm-diameter bar, 25 cm above the floor, which was turning at 12 rpm. For each animal, the time of permanence on the bar for 1 min was registered.

### 2.6. Elevated plus maze test

The elevated plus maze (EPM) for mice (Lister, 1987) consisted of two perpendicular open arms (30 × 5 cm) and two closed arms (30 × 5 × 25 cm) also in perpendicular position. The open and closed arms were connected by a central platform (5 × 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. After treatment, the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence measures the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the animal's aversion to the open arms and promotes the exploration thereof. On the other hand, the forced or voluntary passages of the animal into the open arms of the EPM are associated with hormonal and behavioral changes indicative of increased anxiety. The animals were divided into four groups with 8–12 per group.

### 2.7. Hole-board test

The hole-board test for exploratory behavior in mice was used as described previously by Clark et al. (1971). The apparatus used was an Ugo Basile of 60 × 30 cm with 16 evenly spaced holes with built-in infrared sensors. In brief, adult male mice were randomly divided into four groups with 7–10 mice per group. Two groups received graded

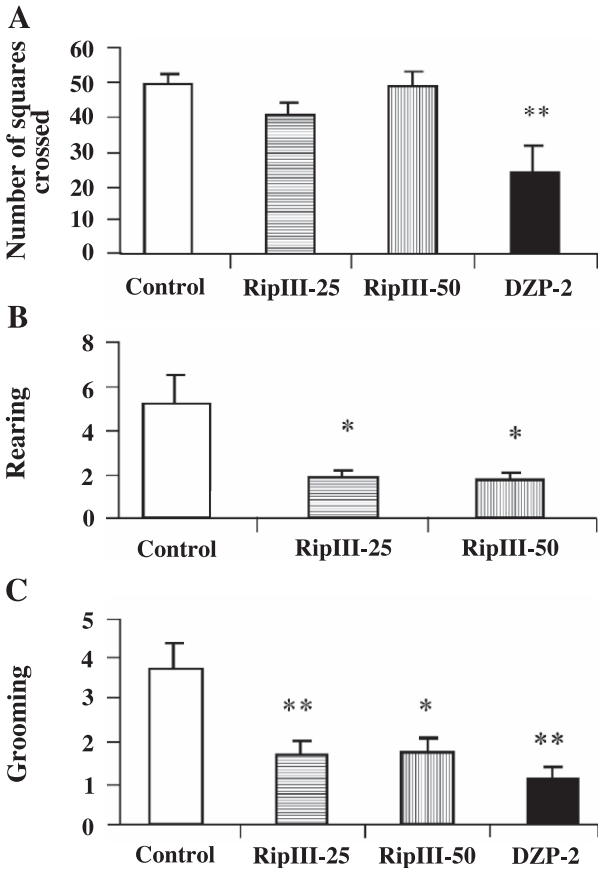


Fig. 2. Open-field test of groups of mice which received vehicle, riparin III (25 and 50 mg/kg) and DZP (2 mg/kg). (A) Number of squares crossed. (B) Rearing. (C) Grooming. The results are presented as mean ± S.E.M. Significant difference compared with control (* $P<.05$; ** $P<0.01$). ANOVA and Tukey as the post hoc test.

doses of riparin III (25 and 50 mg/kg ip). One group received DZP (1 mg/kg ip) and the remaining group received vehicle to serve as control. Thirty minutes later, the number of head dips into the holes was counted for each animal for 5 min.

### 2.8. Pentobarbital-induced sleeping time

Thirty minutes after intraperitoneal injection of riparin III (25 and 50 mg/kg), vehicle (control) or DZP (1 mg/kg), all four groups, with 12–17 animals per group, received sodium pentobarbital (40 mg/kg) intraperitoneally. The time since the injection up to the loss of the righting reflex is recorded as sleeping latency and the time elapsed between the loss and voluntary recovery of the righting reflex is recorded as sleeping time (Wambebe, 1985; Rolland et al., 1991).

### 2.9. Forced swimming test

The Porsolt et al. (1978) swimming test includes two exposures to a water tank, spaced 1 day apart. For these experiments, the tank sizes were 22 cm in diameter and 40 cm in height. The tank had a rounded lid and contained 20-cm-high fresh water at 25 °C. During the first exposure, mice not yet treated were placed in the tank and left there for 15 min. During the second exposure (test session), 30 min after the treatment, mice were placed in the tank and left there for 5 min during which their immobility time was observed. A mouse was considered immobile when it remained floating in the water, without struggling, making only very slight movements necessary to keep its head above the water. The animals were divided into four groups with 8–15 per group. Each animal was used only once.

### 2.10. Tail suspension test

The tail suspension test has been described by Steru et al. (1985). Male Swiss mice were housed in plastic cages in a 12-h light cycle with food and water freely available. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. For the test, the animals were divided into four groups with 10 animals per group. Thirty minutes after the injection, they were suspended by the tail on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 6 min.

### 2.11. Statistical analyses

The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Tukey's post hoc test. Results were considered significant at $P<.05$.

## 3. Results

### 3.1. Open-field test

Fig. 2 shows that riparin III 25 mg/kg (ripIII-25), 50 mg/kg (ripIII-50) and DZP 2 mg/kg (DZP-2) decreased the number of grooming [control: 3.8 ± 0.7; ripIII-25: 1.7 ±0.3,

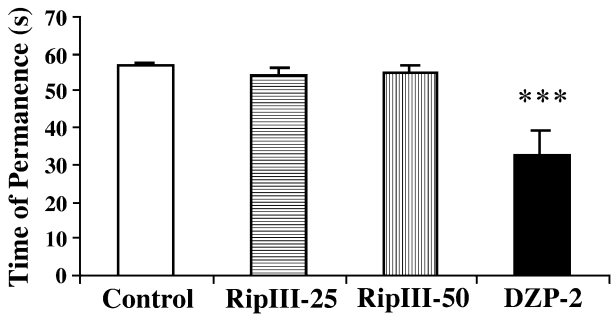


Fig. 3. Rota rod test of groups of mice which received vehicle, riparin III (25 and 50 mg/kg) and DZP (2 mg/kg). The figure shows time of permanence (s). The results are presented as mean ± S.E.M. Significant difference compared with control (*** $P<.001$). ANOVA and Tukey as the post hoc test.

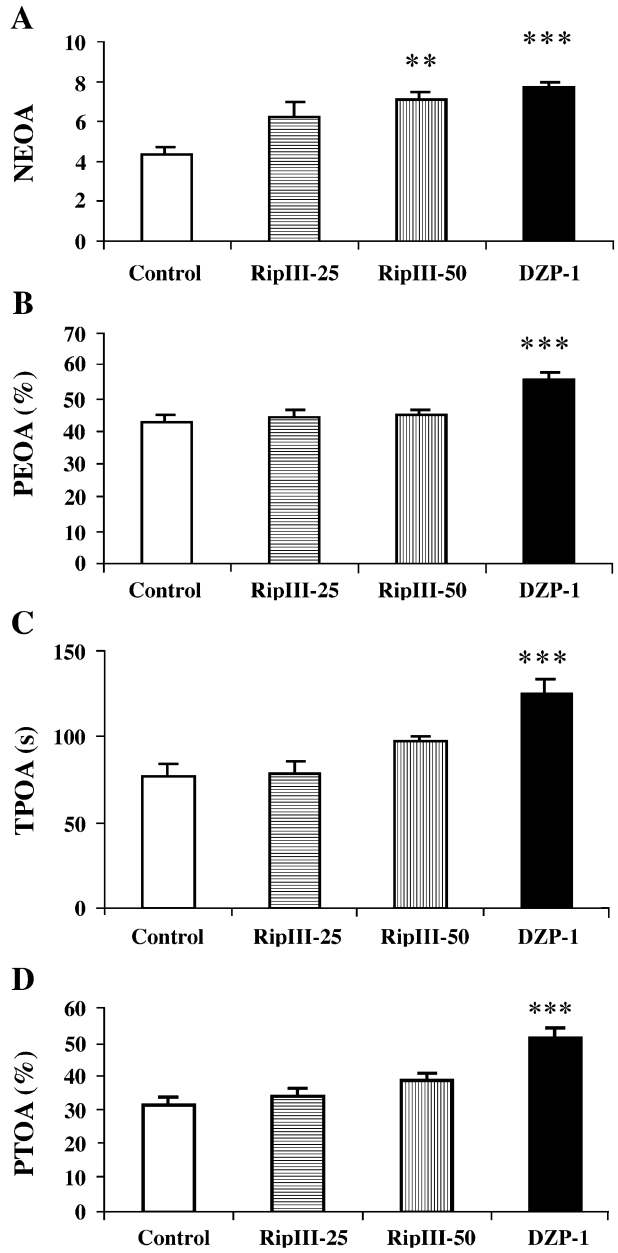


Fig. 4. Plus-maze test of groups of mice which received vehicle, riparin III (25 and 50 mg/kg) and DZP (1 mg/kg). (A) NEOA: number of entries in the open arms; (B) PEOA (%): percentage of entries in the open arms; (C) TPOA (s): time of permanence in the open arms; (D) PTOA (%): percentage of time of permanence in the open arms. The results are presented as mean ± S.E.M. Significant difference compared with control (** $P<.01$; *** $P<.001$). ANOVA and Tukey as the post hoc test.

$F(3,35)=7.224$, $P<.01$; ripIII-50: $1.8\pm0.4$, $F(3,35)=7.224$, $P<.05$; DZP-2: $1.1\pm0.3$, $F(3,35)=7.224$, $P<.001$] and riparin 25 and 50 mg/kg decreased the number of rearing [control: $5.2\pm1.4$; ripIII-25: $1.9\pm0.4$, $F(2,27)=4.697$, $P<.05$; ripIII-50: $1.8\pm0.4$, $F(2,27)=4.697$, $P<.05$]. DZP (2 mg/kg) treatment did not alter the number of rearing but decreased the number of crossings [control: $50.7\pm2.9$; DZP-2: $24.3\pm7.6$, $F(3,40)=6.936$, $P<.01$] as compared to controls.

### 3.2. Rotarod test

No alteration was observed in the time of permanence on bar with both doses of riparin III and, unlikely, DZP (2 mg/kg) which decreased this parameter [control: $57.1\pm0.6$; DZP-2: $32.4\pm7.0$, $F(3,47)=15.308$, $P<.001$] as presented in Fig. 3.

### 3.3. EPM test

In this test (Fig. 4), only riparin III 50 mg/kg significantly increased the number of entries in the open arms (NEOA) [control: $4.4\pm0.4$; ripIII-50: $7.1\pm0.4$, $F(3,40)=8.979$, $P<.01$]. Neither dose of riparin III altered the percentage of entries in the open arms (PEOA), the time of permanence in the open arms (TPOA) nor the percentage of time of permanence in the open arms (PTOA). DZP (1 mg/kg) treatment increased significantly all the observed parameters: NEOA [control: $4.4\pm0.4$; DZP-1: $7.7\pm0.3$, $F(3,40)=8.979$, $P<.001$]; PEOA [control: $42.9\pm2.1$; DZP-1: $55.8\pm1.9$, $F(3,39)=9.950$, $P<.001$]; TPOA [control: $77\pm7.4$; DZP-1: $125.6\pm8.6$, $F(3,40)=10.502$, $P<.001$] and PTOA [control: $31.9\pm2.4$; DZP-1: $51.8\pm3.3$, $F(3,39)=11.439$, $P<.001$].

### 3.4. Hole-board test

Similar to DZP (1 mg/kg), riparin III at both doses (Fig. 5) increased the number of head dips [control: $34.2\pm2.5$; ripIII-25: $45.1\pm1.4$, $F(3,32)=7.811$, $P<.05$; ripIII-50: $48.8\pm3.3$, $F(3,32)=7.811$, $P<.001$; DZP-1: $46.1\pm1.5$, $F(3,32)=7.811$, $P<.01$] as compared to controls.

### 3.5. Pentobarbital sleeping time

The absolute values of the sleeping latency and sleeping time are presented in Fig. 6. The intraperitoneal treatment with riparin III (25 and 50 mg/kg) and DZP (1 mg/kg)

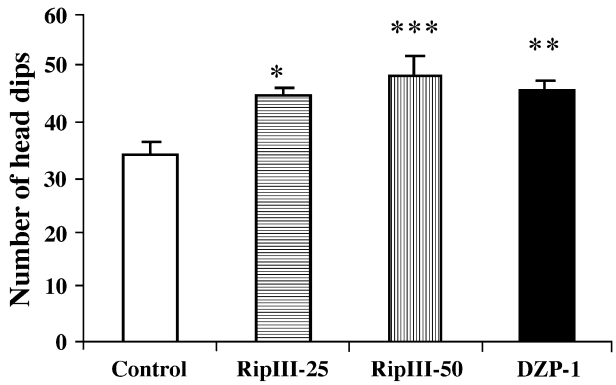


Fig. 5. Hole-board test of groups of mice which received vehicle, riparin III (25 and 50 mg/kg), and DZP (1 mg/kg). The figure shows the number of head dips. The results are presented as mean ± S.E.M. Significant difference compared with control (* $P<.05$; ** $P<.01$; *** $P<.001$). ANOVA and Tukey as the post hoc test.

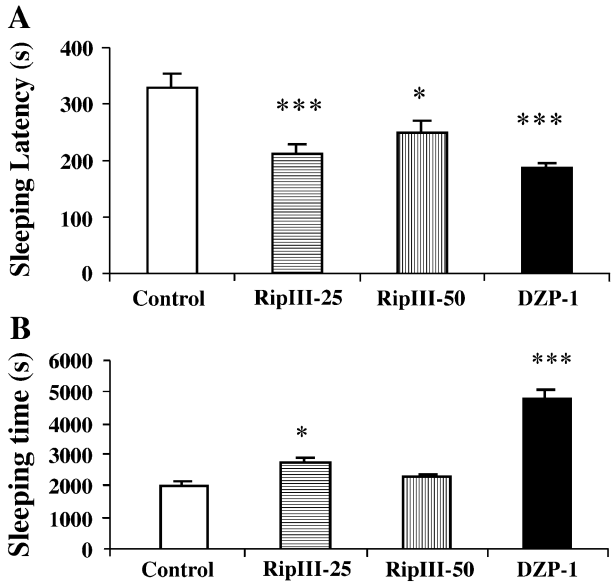


Fig. 6. Effects of mice treatment with riparin III (25 and 50 mg/kg ip) on sleep latency time (A) and sleeping time (B) caused by pentobarbital (40 mg/kg). The results are presented as mean ± S.E.M. Significant difference compared with control (* $P<.05$; *** $P<.001$). ANOVA and Tukey as the post hoc test.

resulted in a statistically significant decrease in sleeping latency [control: 328.7 ± 24.5; ripIII-25: 210.5 ± 16.8, $F(3,53)=9.879$, $P<.001$; ripIII-50: 248.6 ± 20.4, $F(3,53)=9.879$, $P<.05$; DZP-1: 187.6 ± 9.2, $F(3,53)=9.879$, $P<.001$] and increase in sleeping time [control: 2001 ± 185.3; ripIII-25: 2756.5 ± 147.2, $F(3,48)=37.453$, $P<.05$; DZP-1: 4769.2 ±340.4, $F(3,48)=37.453$, $P<.001$].

### 3.6. Forced swimming test

In this test (Fig. 7), animals treated with both doses of riparin III showed decreases in their immobility times [control: 96.8 ± 6.9; ripIII-25: 66.6 ± 6.8, $F(3,40)=23.912$, $P<.05$; ripIII-50: 51.3 ± 7.2, $F(3,40)=23.912$, $P<.001$].

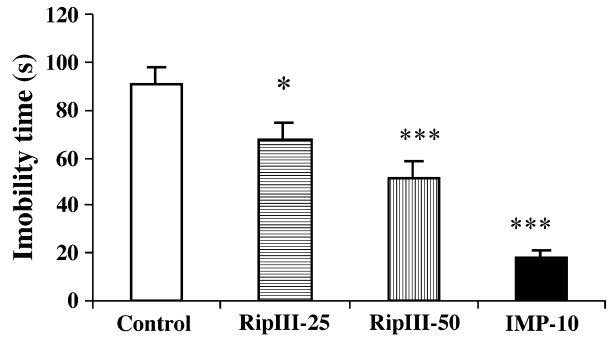


Fig. 7. Forced swimming of groups of mice which received vehicle, riparin III (25 and 50 mg/kg) and imipramine (10 mg/kg). The figure shows immobility time (s). The results are presented as mean ± S.E.M. Significant difference compared with control (* $P<.05$; *** $P<.001$). ANOVA and Tukey as the post hoc test.

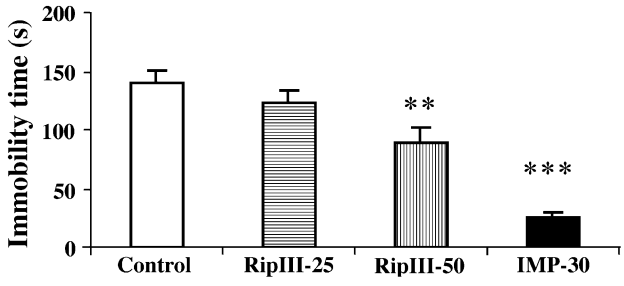


Fig. 8. Tail suspension test of groups of mice which received vehicle, riparin III (25 and 50 mg/kg) and imipramine (30 mg/kg). The figure shows immobility time (s). The results are presented as mean ± S.E.M. Significant difference compared with control (** $P<.01$; *** $P<.001$). ANOVA and Tukey as the post hoc test.

Similarly, animals treated with imipramine 10 mg/kg, as expected, showed a decrease in the immobility time [IMP-10: 18.1 ±2.7, $F(3,40)=23.912$, $P<.001$].

### 3.7. Tail suspension test

Fig. 8 shows that riparin III 50 mg/kg reduced in 64% the immobility time [control: 140 ± 10.8; ripIII-50: 90.2 ± 11.0, $F(3,39)=27.494$, $P<.01$] similar to imipramine 30 mg/kg [IMP-30: 26.2 ± 4.6, $F(3,39)=27.494$, $P<.001$], as compared to control.

## 4. Discussion

In this work, the effects of riparin III from *A. riparia* were studied in several animal models, such as open-field, rotarod, EPM, hole board, barbiturate-induced sleeping time, tail suspension and forced swimming tests to evaluate its possible central activity. These tests are classical models for screening central nervous system actions providing information about psychomotor performance, anxiety, myorelaxant activity and depression.

The effects of riparin III in the central nervous system were evaluated by the potentiation of sodium pentobarbital sleeping time. Decrease in sleeping latency and increase in sleeping time are classically related to central nervous system depressant drugs (Willianson et al., 1996). However, this test is not specific because compounds that interfere with biotransformation of pentobarbital by cytochrome *P*450 complex can show the same effects as central nervous system depressant drugs (Goloubkova et al., 1998). Our results showed that riparin III, in both doses, decreased the sleeping latency time and with the dose of 25 mg/kg, increased the sleeping time, suggesting a potentiation of pentobarbital sleeping time. Although riparin III 50 mg/kg presented lower absolute value as compared to the dose of riparin 25 mg/kg, there was no significant difference between them. Thus, we assume that the maximum effect of riparin III on the sleeping time was already produced by the dose of 25 mg/kg.

The EPM test is the most popular test to search for new benzodiazepine-like anxiolytic agents (Pellow et al., 1985; Rodgers et al., 1997). Similar to DZP, animals treated with riparin III (50 mg/kg) increased the number of entries in the open arms, reducing the animal's aversion to the open arms and promoting the exploration thereof, indicating anxiolytic effect. This effect was confirmed in the hole-board test which measures exploratory behavior (Crawley, 1985). An agent that decreases this parameter reveals a sedative behavior (File and Pellow, 1985). Anxiolytics have been shown to increase the number of head dips (Takeda et al., 1998). Our results showed that riparin III with both doses increased the number of head dips, indicating anxiolytic effect.

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. Although the relationship between immobility (a posture thought to reflect a state of "behavior despair" in which animals have given up the hope to escape) and depression remains controversial (Gardier and Bourin, 2001), it is well demonstrated that drugs with antidepressant activity reduce the time during which the animals remain immobile (Porsolt et al., 1977; Borsini and Meli, 1988). In our results, a significantly reduced immobility time for mice in both tests was observed, although to a smaller extent, in the tail suspension test. These behavior effects are similar to those that we and other investigators have observed after treatment with a classical antidepressant drug, such as imipramine (Porsolt et al., 1977; Borsini and Meli, 1988).

Data in the literature demonstrated that drugs that alter general motor activity may give false-positive/negative results in the forced swimming test. In this way, we decided to study the effects of riparin III in the open-field test, a classical animal model used to evaluate autonomic effects of drugs and general activity of animals (Novas et al., 1988). Our findings show that riparin III (25 and 50 mg/kg) and DZP (1 mg/kg) did not change the locomotor activity, different from DZP at the dose of 2 mg/kg, which decreased the number of crossings. Thus, the present study demonstrated that riparin III, at doses which produced antidepressant- and anxiolytic-like effects, did not significantly change motor activity in naive mice. Therefore, it is unlikely that these effects of riparin III observed in the forced swimming and plus-maze tests are based on the stimulation of general motor activity. Furthermore, this compound did not alter the time of permanence on the bar in the rotarod test, differently from DZP (2 mg/kg), which decreased this parameter. The failure of riparin III in the motor coordination test is suggestive that the activity of the substance might not be exerted through peripheral neuromuscular blockage, but rather, elicited centrally. Thus, together, these experiments support the idea that riparin III plays a major role in depression and anxiety with antidepressant- and anxiolytic-like properties.

As far as we know, there are no studies in the literature on the central actions of riparin III. However, riparin III is an *N*-(2,6-dihydroxybenzoyl) tyramine, and previous data showed that the spectrum of tyramine actions are similar to those of norepinephrine. In fact, some reports related that tyramine-rich foods when ingested by individuals taking antidepressant drugs, such as monoamino oxidase inhibitors, might result in a prolonged increase in blood pressure (Potter and Hollister, 1998). Another work reported that *p*-synephrine has an antidepressant-like activity in murine models of forced swimming and tail suspension tests (Kim et al., 2001). Furthermore, these points must be taken into consideration concerning the antidepressant effects of riparin III.

In conclusion, we showed that acute treatment with riparin III (25 and 50 mg/kg) potentiated the barbiturate-induced sleeping time and presents antidepressant-like effect as demonstrated in the forced swimming and tail suspension tests and also presents anxiolytic-like effect in the plus-maze and hole-board tests.

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