Behavioral tests and oxidative stress evaluation in mitochondria isolated from the brain and liver of mice treated with riparin A

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Aims: This study aimed at evaluating the oxidative stress in mitochondria isolated from the brain and liver of mice treated with riparin A, as well as the locomotor activity and myorelaxant effect of this compound. Behavioral models of rota rod and open field tests were used for locomotor activity and myorelaxant effect evaluation.

Main methods: The animals were divided into five groups (n = 8), which were treated with: diazepam (1 mg/kg, i.p.), riparin A (5, 10, and 20 mg/kg, o.r.) or vehicle (0.9% saline, o.r.). The oxidative stress evaluation was carried out in mitochondria isolated from the brain and liver of mice from five experimental groups (n = 8), which were treated with: ascorbic acid (250 mg/kg; positive control), vehicle (0.9% saline; negative control) and riparin A (5, 10 and 20 mg/kg).

Key findings: In an open field and rota rod test a significant difference in the number of crossings, in time of permanence on the swivel bar and in the number of falls in riparin A treated animals (5, 10 and 20 mg/kg) was not observed, when compared with negative control (vehicle) (p > 0.05). In comparison to the negative control, there was a reduction of lipid peroxidation levels and nitrite content in mice treated with riparin A (p < 0.05). Reduced glutathione (GSH) levels (p < 0.05), superoxide dismutase (SOD) and catalase activities increased in the brain (rip A 5 mg/kg; p < 0.05), while in the liver SOD remained unchanged (p > 0.05) and catalase activity (p < 0.05) was reduced.

Significance: Riparin A was presented as a bioactive molecule devoid of adverse effects of alteration of motor activity.

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Introduction

Oxidative stress is often defined as an imbalance between pro-oxidants and antioxidants [42]. Reactive oxygen species (ROS), whether produced endogenously as a consequence of normal cell function or provoked by external interference, represent a constant threat to living cells because in an aerobic environment, ROS can cause damage to DNA, protein and lipids. Cells contain a series of antioxidant defenses to minimize the fluctuations of ROS, but the generation of ROS frequently exceeds the cell antioxidant capacity, resulting in a condition defined as oxidative stress. It can be observed in several diseases, such as neurodegenerative diseases [3,15,44].

The mitochondrion is an organelle that, with use of oxygen from cellular respiration, converts food energy into chemical energy (ATP), which is vital to cell activities. A small fraction of oxygen we breathe turns, in respiratory chain of mitochondria in reactive oxygen, a type of free radical. The primary production of ROS occurs in the mitochondrial making it vulnerable to oxidative damage, and playing an important role in controlling neuronal excitability [46].

Mitochondrial dysfunction can result in inhibition of complex I of the mitochondrial respiratory chain, which may impair the efficiency of ATP production by oxidative phosphorylation activity, resulting in increased production of the superoxide molecule with consequent mitochondrial cell death [25,38]. Changes in energy metabolism, and oxidative stress are directly linked to chronic diseases such as cardiovascular and neurological diseases, diabetes and cancer.

The process of discovering and developing new drugs includes various strategies and a combination of methods and drug design, beginning with the understanding that chemical systems have a decisive role in the modulation of biological systems [4], and that understanding stimulates research and development of novel antioxidant drugs for...
These riparin analogs were previously submitted to in vitro assays to investigate their antioxidant potential. It was found that these compounds are very promising in antioxidant activity, mainly riparin A, which was capable of decreasing the levels of free radicals even in low concentrations [30]. Riparin A is constituted by a chemical structure that is the basic structure of the other synthesized molecules of this group and by the analysis of its molecular structure it is probable that the antioxidant potential observed in vitro is related to the presence of unstable hydrogen that is captured by the free radicals, inactivating them.

The structurally suggested antioxidant potential for riparin A and its results in the evaluation of in vitro antioxidant activity are a stimulus to the research of its pharmacological activity and safety, prospecting a new active principle for the development of drugs with greater safety and fewer side effects than those currently marketed.

This study aimed at evaluating the effects of riparin A against the oxidative stress in samples of mitochondria isolated from the brain and liver of mice, as well as the preclinical evaluation of the locomotor activity and myorelaxant effect of this compound.

Material and methods

Riparin A obtention

Riparin A (N-phenethylbenzamide) was obtained by a simple and safe synthesis route, using the Schotten–Baumann reaction, described previously in literature [20]. The substances were provided by Laboratory Chemistry of Bioactive Natural and Synthetic Products, Federal University of Piauí, Teresina, PI, Brazil (Scheme 1).

The analysis of the compound obtained was made through thin-layer chromatography (TLC), which revealed a Riparin A 93.33% pure, with yield of 84%. When subjected to the trial with layer chromatography (TLC), which revealed a Riparin A 93.33% pure, 40.90 (CH₂, C-8'), 166.18 (C = O).

Scheme 1. General reaction for the synthesis of riparin A.
step was repeated discarding the supernatant, the pellet resuspension and centrifugation (precipitation of mitochondria).

The pellet was resuspended supplementing the volume to 4 mL of homogenization solution and finally the samples were homogenized with a brush. Samples of the liver and brain were used to determine the protein concentration applying the protocol method [24] and mitochondria oxidative stress (lipid peroxidation level, nitrite formation, reduced glutathione content, as well as catalase and superoxide dismutase activities).

**Evaluation of oxidative stress**

The samples of isolated mitochondrial of mice treated with riparin A (5, 10 and 20 mg/kg) and with vehicle were subjected to tests of oxidative stress, as described below. The same parameters were measured for the group treated with ascorbic acid 250 mg/kg, which was the positive control. The evaluation of oxidative stress was performed with five experimental groups (n = 8): a group treated with ascorbic acid (250 mg/kg; positive control), one group treated with vehicle (saline; negative control) and the groups treated with riparin A (5, 10 and 20 mg/kg).

**Determination of lipid peroxidation levels**

Lipid peroxidation levels in mice treated with riparin A (n = 8) at doses 5, 10 and 20 mg/kg, ascorbic acid 250 mg/kg (n = 8) and vehicle (n = 8) groups were analyzed by measuring the thiobarbituric acid reactive substances (TBARs) in 10% (w/v) homogenates isolated mitochondrial of mice hepatic and brain, as previously described [19]. Brieﬂy, the samples were mixed with 1 mL 10% trichloroacetic acid and 1 mL 0.067% thiobarbituric acid. They were then heated in a boiling water bath for 15 min and butanol (2:1, v/v) was added to the solution. After centrifugation (800 × g; 5 min), thiobarbituric acid-reacting substances were determined from the absorbance at 535 nm. Results were expressed as nmol of malondialdehyde (MDA)/g wet tissue [32].

**Determination of nitrite content**

To determine nitrite contents in mice treated with riparin A at doses 5, 10 and 20 mg/kg (n = 8), ascorbic acid 250 mg/kg (n = 8) and vehicle (n = 8) groups, 10% (w/v) homogenates isolated mitochondrial of hepatic and brain from mice were centrifuged (800 × g; 10 min), supernatants were collected and nitric oxide production was determined based on Griess reaction [18]. Brieﬂy, 100 μL of the supernatant was incubated with 100 μL of Griess reagent at room temperature for 10 min. Absorbance was measured using a microplate reader at 550 nm. Nitrite concentration was determined from a standard nitrite curve produced using NaNO2. Results were expressed as μM [32].

**Determination of reduced glutathione (GSH) concentration**

Reduced glutathione concentration was measured in mice treated with riparin A at doses 5, 10 and 20 mg/kg (n = 8), ascorbic acid 250 mg/kg (n = 8) and vehicle (n = 8) groups by calculating the content of non-protein sulphydryl groups by technique previously described in the literature [39]. 400 μL of the homogenate was added to 320 μL of distilled water and 80 μL of trichloroacetic acid at 50%, the mixture was centrifuged for 15 min at 3000 rpm and then 400 μL of the supernatant was taken and added to 800 μL of Tris–HCl buffer (0.4 M, pH 8.9) and 20 μL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) 0.1M and after 1 min the reading was made at 412 nm. The concentration of GSH was expressed in nanogram per gram of tissue [32].

**Determination of catalepsy activity (CAT)**

Catalase activity was measured in mice treated with riparin A at doses 5, 10 and 20 mg/kg (n = 8), ascorbic acid 250 mg/kg (n = 8) and vehicle (n = 8) groups. The activity was measured by the progression of the reaction of degradation of H2O2 in H2O and O2 [9]. The standard assay substrate mixture contained 0.30 mL H2O2 in 50 mL 0.05 M sodium phosphate buffer, pH 7.0. The sample aliquot (20 μL) of 10% (w/v) homogenates isolated mitochondrial of hepatic and brain from mice was added to 980 μL of the substrate mixture. The initial absorption was recorded after 1 min, and the final absorption after 6 min. The reaction was followed at 230 nm. A standard curve was established using purified catalese (Sigma, St Louis, MO, USA) under identical conditions. All samples were diluted with 0.1 mmol/L sodium phosphate buffer (pH 7.0) to induce a 50% inhibition of the diluent rate (i.e. the uninhibited reaction). Results were expressed as mmol/min/mg of protein [32].

**Determination of superoxide dismutase (SOD) activity**

Homogenates were centrifuged (800 × g; 20 min), and the supernatants were used to assay superoxide dismutase (SOD) activity in mice treated with riparin A at doses 5, 10 and 20 mg/kg (n = 8), ascorbic acid 250 mg/kg (n = 8) and vehicle (n = 8) groups was assayed by using xanthine and xanthine oxidase to generate superoxide radicals [12]. Radicals react with 2,4-iodophenyl-3,4-nitophenol-5-phenyltetrazolium chloride to form a red formazan dye. The degree of inhibition of this reaction was measured to assess SOD activity. The standard assay substrate mixture contained 3.0 mL xanthine (500 μM), 7.44 mg cytochrome c, 3.0 mL KCN (200 μM), and 3.0 mL EDTA (1 mM) in 18.0 mL 0.05 M sodium phosphate buffer, pH 7.0. The sample aliquot (20 μL) was added to 975 μL of the substrate mixture plus 5 μL xanthine oxidase. After 1 min, the initial absorption was recorded and the timer was started. The final absorption after 6 min was recorded at 550 nm. SOD activity in samples was determined from this curve and results were expressed as U/mg of protein [32].

**Statistical analysis**

The results were expressed as mean ± SEM (Standard Error of the Mean). For statistical analysis, nonparametric results were analyzed by analysis of variance (ANOVA) for multiple comparisons and Student–Newman–Keuls as post hoc test by GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego California USA. Copyright© 1994–1999 by GraphPad software. Differences were considered statistically significant from p < 0.05.

**Results**

In this study a significant difference in the number of crossings in riparinA treated animals (5,10 and 20 mg/kg, respectively) was observed when compared to the negative control (vehicle) (p < 0.05; Fig. 1). There was a decrease in squares crossed in the group treated with diazepam (1 mg/kg; 28.25 ± 2.21; positive control) when compared with vehicle (56.25 ± 2.71) (p < 0.05; Fig. 1), due to the sedative side effect of this benzodiazepine [13]. There was a significant statistical difference between groups treated with riparin A (5, 10 and 20 mg/kg) and the positive control (p < 0.05; Fig. 1), which demonstrates that riparin A has no significant effect on locomotor activity.

In the rota rod test the groups treated with riparin A did not present significant difference in the time of permanence on the swivel bar [5 mg/kg (163.6 ± 6.56); 10 mg/kg (164.5 ± 6.00); 20 mg/kg (157.36 ± 5.93)] or in number of falls [5 mg/kg (1.87 ± 0.29); 10 mg/kg (2.00 ± 0.26); 20 mg/kg (2.00 ± 0.37); p > 0.05] when compared with vehicle (time of permanence: 171.40 ± 4.05; number of falls: 1.87 ± 0.29), whereas when compared to the group treated with diazepam, significant alterations (time of permanence: 130.80 ± 6.60; number of falls: 3.62 ± 0.37; p < 0.05) were observed (Fig. 2). The diazepam group had an increase of falls and a decrease in the time of permanence on the swivel bar (time of permanence: 130.80 ± 6.60; number of falls: 3.62 ± 0.37) compared to vehicle (time of permanence: 171.40 ± 4.05; number of falls: 1.87 ± 0.29; p < 0.05), which demonstrates the myorelaxant effect of diazepam, whereas groups treated with riparin A did not present impaired motor coordination.
The oxidative stress evaluation was performed in mitochondria isolated from mice exposed to riparin A (5, 10 and 20 mg/kg) regarding lipid peroxidation levels, nitrite content, reduced glutathione (GSH) levels, catalase activity and superoxide dismutase (SOD) activity.

Fig. 3 presents the evaluation of oxidative stress regarding lipid peroxidation levels and nitrite content.

In hepatic mitochondria, the lipid peroxidation levels decreased by 45.1; 46.3; 36.4 and 34.3%, respectively, in animals treated with ascorbic acid (250 mg/kg; positive control) and with riparin A at doses 5 mg/kg (14.03 ± 1.28 nmol of MDA/g wet tissue) and with riparin A at doses of 5 mg/kg (14.03 ± 1.28 nmol of MDA/g wet tissue), 10 mg/kg (16.59 ± 1.37 nmol of MDA/g wet tissue) and 20 mg/kg (17.15 ± 1.31 nmol of MDA/g wet tissue) when compared to vehicle (negative control; 26.10 ± 1.83 nmol of MDA/g wet tissue; p < 0.05; Fig. 3).

With regard to hepatic mitochondria, when compared to the group treated with ascorbic acid riparin A did not present a significant difference in decreasing nitrite levels. On the other hand, brain mitochondria with riparin A at dose 20 mg/kg presented a decrease (75.46%) which was higher than the mean obtained with the group treated with ascorbic acid (47.01%; positive control; p < 0.05; Fig. 3).

Fig. 4 presents the evaluation of oxidative stress in relation to reduced glutathione levels.

The levels of reduced glutathione had a decrease in brain mitochondria of groups treated with riparin A 5 mg/kg (73.48 ± 3.92 ng/g protein), 10 mg/kg (76.30 ± 4.25 ng/g protein) and 20 mg/kg (61.78 ± 10.24 ng/g protein) corresponding to 34.88; 31.96 and 44.90%, respectively, when compared to group treated with negative control (112.1 ± 5.81 ng/g protein; p < 0.05) and 49.25; 47.30 and 57.33%, respectively, when compared with the group treated with ascorbic acid (250 mg/kg; 144.8 ± 7.77 ng/g protein). The reduction was also observed in the levels of GSH in hepatic mitochondria with riparin A 5 mg/kg (47.69 ± 7.85 ng/g protein), 10 mg/kg (48.80 ± 9.87 ng/g protein) and 20 mg/kg (50.03 ± 9.22 ng/g protein), corresponding to 32.87; 31.59 and 29.59%, respectively, when compared to negative control (71.06 ± 3.38 ng/g protein; p < 0.05) and 36.80;
Fig. 3. Evaluation of oxidative stress in mitochondria isolated from mice exposed to riparin A (5, 10 and 20 mg/kg) in lipid peroxidation levels and nitrite content; results were expressed as mean ± SEM (n = 8 per group) (ANOVA and t-Student–Newman–Keuls as post hoc test). *p < 0.05, when compared with negative control (vehicle); †p < 0.05, when compared with ascorbic acid group; ‡p < 0.05, when compared with riparin A (5 mg/kg); §p < 0.05, when compared with riparin A (10 mg/kg).

Fig. 4. Evaluation of oxidative stress in mitochondria isolated from mice exposed to riparin A (5, 10 and 20 mg/kg) in relation to reduced glutathione levels; results were expressed as mean ± SEM (n = 8 per group) (ANOVA and t-Student–Newman–Keuls as post hoc test). *p < 0.05, when compared with negative control (vehicle).
Oxidative stress can modify neuronal and hepatic functions and has been linked to neurochemical alterations observed during the onset of neurodegenerative and hepatic diseases [19,22].

Oxidative stress is particularly facilitated in the brain due to the high oxygen utilization, generation of free radicals, insufficient antioxidant defense mechanisms, high lipid content and excitotoxicity [31]. The liver is an organ with high metabolic activity and, therefore, is constantly submitted to high concentrations of metabolites of drugs as well as reactive oxygen species from the electron transport chain in mitochondria. Also, these organs (the brain and liver) have a high rate of blood oxygen utilization, generation of free radicals, insufficient antioxidant defense systems such as superoxide dismutase and catalase [27].

Moreover, recent evidence suggests that ROS can also initiate signaling cascades that respond to stress and modify specific redox-sensitive areas as well a regulatory mechanism [47]. Thus, an antioxidant action that promotes a reduction of deleterious effects and a control of oxidative stress becomes imperative in the search of pharmacologically active compounds.

Diazepam was used as positive control for its known sedative and muscle relaxant effects and, despite the well documented effects of benzodiazepines, their side effects dominate, including sedation, muscle relaxation, anterograde amnesia and physical dependence [23]. The comparison between the effect of riparin A and diazepam is important to evaluate the safety and side effects of riparin A at the central nervous system (CNS). Diazepam has been widely used as a positive control in studies investigating the effect of drugs on CNS [14,28,33,36].

The open field and rota rod tests, conducted with mice treated with riparin A, showed that, despite the substance’s power to cross the blood brain barrier, it does not produce a sedative effect, loss of motor coordination or muscle relaxation; that is, riparin A is devoid of side effects on the CNS, possibly because it does not interact with the GABAergic system as diazepam.

To evaluate the oxidative stress, mitochondria from the liver and brain of mice treated with riparin A were used. The mitochondrion is the main source of free radicals, producing them as the action of cytochrome oxidase controls the generation of free radicals, preventing their excessive generation in mitochondria. However, nearly 2% to 5% of the oxygen metabolized in mitochondria is diverted to another metabolic route and reduced to univalent form, giving rise to free radicals [5].

The thiobarbituric acid reactive substance (TBARS) levels in mice treated with riparin A were higher than the levels found in the group treated with ascorbic acid, but lower than the ones found in mice treated with vehicle, suggesting a potential of riparin A to avoid the oxidation of polyunsaturated fatty acids, decrease the lipid peroxidation and protect from oxidative stress. The decrease of TBARS levels in mice treated with vehicle, suggesting a potential of riparin A to avoid the oxidation of polyunsaturated fatty acids, decrease the lipid peroxidation and protect from oxidative stress. However, the comparison between the effect of riparin A and diazepam did not present a dose-dependent response, indicating that even with increased dose tested, reduction of TBARS was significant (p < 0.05), maintaining an average (40.26 ± 1.96%) and indicating a possible saturation of the effects of riparin A.

Reactive species of oxygen (ROS) are a part of normal human metabolism. When ROS are produced in excess, they can cause an increase in nitrite content [21,45]. The quantification in mice brain tissue treated with riparin A showed a lower nitrite content compared with vehicle, indicating that riparin A promotes the decrease of this reactive nitrogen probably by blocking the mitochondrial disfunction and by inactivating the enzyme nitric oxide synthase. These effects are dose-dependent, as
an increase in the dose of 20 mg/kg riparin A caused a reduction of nitrite 55.52% greater than that obtained with the dose of 5 mg/kg.

The nitrite content decreased in brain mitochondria from mice treated with ascorbic acid and riparin A when compared with negative control. The effect of riparin A in liver mitochondria was not dose-dependent.

The possible mechanisms for the antioxidant activity of riparin A molecule were proposed (Scheme 2), relating the chemical structure of the molecule with the potential to capture free radicals and inactivate them. It was found that these compounds are very promising in antioxidant activity, mainly riparin A, which was capable of reducing in vitro the levels of free radicals even in low concentrations [30].

The riparin A by its chemical structure can exercise primary antioxidant activity, promoting the removal or inactivation of free radicals of reaction by donating hydrogen atoms, similar to tocopherol. The ascorbic acid, its isomers and derivatives are the best examples of removers of oxygen compounds that act by capturing oxygen present in the medium, through stable chemical reactions making them, therefore, unavailable to act as propagators of autoxidation. Ascorbic acid can also act as a synergist in regeneration of primary antioxidants [7].

Other substances are mentioned in the literature for their antioxidant potential due to their ability to reduce lipid peroxidation level and nitrite content, suggesting an antioxidant role in vivo since they are able to reduce the formation of reactive species derived from oxygen and nitrogen [1].

The lipid peroxidation and the increase in nitrite contents caused by oxidative stress are toxic reactions that can cause partial or total destruction of cell membranes and cause significant biochemical changes, resulting in behavior and/or motor alterations [34,37]. There was no correlation between the locomotor activity and the decrease in the TBARS levels produced by riparin A ($r^2 = 0.180, p = 0.670$). The correlation between the locomotor activity and nitrite content was also negative ($r^2 = 0.419, p = 0.301$), suggesting that reduction in lipid peroxidation levels and nitrite content by the treatment with riparin A may have contributed to the preservation of locomotor activity.

Substances evaluated in previous studies by similar parameters showed an antioxidant potential similar to that obtained with riparin A as regards the reduction of nitrite and TBARS levels. They differed, however, in the actions exerted on the central nervous system, when a positive correlation between the reduction of nitrite and locomotor activity was observed. In the study mentioned, the antioxidant potential was associated with a simultaneous presence of sedative effect while with riparin A the promising antioxidant activity is devoid of sedative and muscle relaxant effects [29].

The repair of lipid peroxidation is performed by a complex process and occurs through the action of various reducing agents of glutathione peroxidase and glutathione reductase [40]. The decrease in TBARS production and consequent decrease in lipid peroxidation may be related to the decrease of GSH, without, being associated with reduction in antioxidant capacity of the substance tested. Reduced GSH can be related to a possible measure to maintain the redox balance of the organism.

SOD activity in brain tissue was increased by the dose of 5 mg/kg but remained unchanged at other doses (10 and 20 mg/kg). In liver tissue SOD activity remained unchanged in all doses tested (5, 10 and 20 mg/kg). The superoxide dismutase, the first enzyme in removing the superoxide anion, is found in mitochondria in MnSOD isoform, connected to manganese ion. The SOD activity is also regulated by reactive species and depends on the levels of reduced glutathione, once the synthesis of the enzyme occurs by this substance [8]. Reduction of GSH can have culminated in the non-activation of SOD activity that remained unchanged in the brain (10 and 20 mg/kg) and liver mitochondria.

Catalase activity in brain mitochondria of mice treated with riparin A was increased mainly by the dose 5 mg/kg but was not significantly changed by the other doses (10 and 20 mg/kg), while in hepatic mitochondria, catalase activity decreased when compared with negative control, regardless of the dose, suggesting that the raise of the dose cannot produce an increase of catalase enzymatic activity.

Catalase activity is carried through the use of reduced glutathione (GSH), in a reaction catalyzed by glutathione peroxidase. After donating electrons, GSH turns to its oxidized form (GSSG), but it can be regenerated by glutathione reductase by transference of hydrogen through the reduction of nicotinamide adenine dinucleotide phosphate (NADPH) [35]. The reduced levels of hepatic and brain GSH in mice treated with riparin A could be related to the reduced catalase activity, while increased brain enzymatic activity at the dose of 5 mg/kg can result from immediate activation of antioxidant enzyme systems.

According to previous studies [15,17], an increase in free radical formation is accompanied by an immediate compensatory increase in catalase activity, which may be a long-term compensatory mechanism including enzymatic modulation of GSH.

Considering that there was no increase of reactive oxygen and nitrogen species in treatment with riparin A, the antioxidant enzyme modulation may not have been activated, or the pro-oxidant alterations (decrease of GSH levels and CAT activity) or not antioxidants (SOD and CAT activities unchanged compared with negative control), can mean a possible modulation of enzymatic activity mediated by riparin A for CAT and SOD, altering the antioxidant defenses, which require complementary studies to be better elucidated.

In contrast to antioxidant activity demonstrated by riparin A treatment, through the decreasing in TBARS levels and nitrite content, there is the oxidant alterations and/or the inactivation of antioxidant activity demonstrated by the reduction in GSH levels associated with a decrease of catalase activity and the unchanged superoxide dismutase enzymatic activity. This suggests that the inactivation of antioxidant activity might be a response to promote the redox balance of the organism, since the negative changes of the enzymatic antioxidant system were not significant as compared to the negative control, which could not establish oxidant levels which were able to suggest hepatotoxicity or neurotoxicity.

Conclusions

The dichotomies established in the evaluation of riparin A effect on mice cerebral and hepatic mitochondria can be related to the structure–activity relationship of the substance, which needs to be further elucidated.
There is a structurally antioxidant potential defined by its capacity to capture free radicals. This capacity is related to the presence of unstable hydrogen in their molecules, which may have favored the reduction of lipid peroxidation levels and nitrite content. This signals an antioxidant potential of riparin A, coexisting with a response to promote the redox balance of the organism, observed by negative change like the increase of GSH, characterized by absence of enzymatic changes, or no significant change compared with negative control, suggesting a possible mechanism of pharmacological activity of riparin A.

The riparin A presents a conceivable neuroprotective effect with possible involvement of NO pathway. Therefore, our study suggests that enhancement of in vivo antioxidant defenses and improvements in the cellular antioxidant status might be an important mechanism underlying the neuroprotective action present, making riparin as bioactive molecule which does not produce a sedative effect, loss of motor coordination or muscle relaxation.

Conflict of interest statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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