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ORIGINAL ARTICLE

Psychotria leiocarpa Extract and Vincosamide Reduce Chemically-Induced Inflammation in Mice and Inhibit the Acetylcholinesterase Activity

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Abstract—Species from *Psychotria* are used in folk medicine against inflammatory diseases, respiratory disturbances, and anti-hallucinogenic. In the present study, the compound vincosamide (PL-1) was identified for the first time in methanolic extract of the *Psychotria leiocarpa* (ME-PL) leaves, as well as the anti-inflammatory and anticholinesteric effects in rodents and molecular docking simulations. The fractionation of the chloroform fraction (*CF*-PL) through chromatographic methods afforded the known compound PL-1. The anti-inflammatory activity of the ME-PL (30, 100, and 300 mg/kg) and PL-1 (3, 30, and 100 mg/kg) was analyzed using experimental models: paw edema, pleurisy, and mechanical and thermal hyperalgesia induced by carrageenan. The anticholinesterase activity of the ME-PL (30 and 100 mg/kg) and PL-1 (30 mg/kg) was showed by acetylcholinesterase (AChE) inhibitory in brain structures. The molecular docking simulations were performed using Molegro Virtual Docker v6.0. Overall, the results indicated that ME-PL and PL-1 demonstrated an anti-edematogenic effect in Cg-induced paw edema, leukocyte migration in the pleurisy model, and significantly reduced mechanical hyperalgesia, cold response to acetone in mice. The samples exhibited maximal inhibition of enzyme acetylcholinesterase (AChE) in the frontal cortex. The molecular coupling of PL-1 with the AChE showed significant

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interactions with the catalytic and peripheral site, corroborating the activity presented in the inhibition assay. The acute administration of ME-PL did not cause signs of toxicity in the treated animals. The results showed that *P. leiocarpa* inhibited AChE and anti-inflammatory activity, and alkaloid vincosamide could be responsible, at least in part, for the observed effects, supporting the popular use of this genus.

KEY WORDS: Grandiúva-de-anta; alkaloid; acetylcholinesterase; inflammation; carrageenan; molecular docking.

INTRODUCTION

Grandiúva-de-anta as is known *Psychotria leiocarpa* Cham. & Schlecht. is a small shrub (2 m in height) native of Argentina, Paraguay, and Brazil [1]. Plants in this genus are used in folk medicine against inflammatory diseases, respiratory disturbances, and anti-hallucinogenic [2–5]. Monoterpene indolic alkaloid (*N*-β-glucopyranosyl vincosamide) is the main constituent of the extract from *P. leiocarpa* leaves collected in Morro Santana in Porto Alegre, Brazil [6, 7], along with iridoid glucosides, asperuloside, and deacetylasperuloside [8]. The essential oil of the leaves was characterized exclusively by sesquiterpenes, highlighting bicyclogermacrene, and germacrene D [9]. Pharmacological effects for extract have been reported, such as antioxidant, antimycobacterial on *Mycobacterium bovis* BCG growth [10], and analgesic [11, 12]. Also, anti-inflammatory effects were exhibited presenting dose analgesic activity non-dependent and non-reversible by naloxone, configuring non-specific action in experimental models in rodents [11].

In previous studies with species of *Psychotria*, our group reports the antioxidants activity in different *in vitro* assays, including DPPH, ABTS radicals, and β-carotene bleaching activities of four species of *Psychotria*, among them *P. leiocarpa* [13] and the isolation of a new dimeric tryptamine-related alkaloid, brachybotryne, and the corresponding N-oxide derivative, brachybotryne N-oxide, and bufotenine from *P. brachybotrya* [14].

In this sense, considering the folk use of *Psychotria* genus against process regulated by inflammatory mediators and mental disorders, but without scientific evidence of this potential therapeutic application, prompted research of *P. leiocarpa*. Thus, this study is aimed at evaluating the anti-inflammatory and anticholinesterasic effect in mice of the methanolic extract and alkaloid (vincosamide) obtained from *P. leiocarpa* leaves and molecular docking simulations.

Considering that molecular docking plays an important role in rational drug design, we also conducted a theoretical molecular docking study to evaluate how the alkaloid binds to acetylcholinesterase (AChE). Alzheimer's disease (AD) is a chronic neurodegenerative disease, pathologically associated with a highly atypical inflammatory response, which processes by the activation of the macrophage populations in the brain, characterized by memory impairment, cognitive dysfunction, behavioral disturbances, and deficits in the activities of daily living [15].

MATERIAL AND METHODS

Collection and Plant Identification

Psychotria leiocarpa fresh leaves were collected in Dourados (S 22° 17′ 38.4″, W 54° 95′ 94.2), Mato Grosso do Sul, Brazil. Botanical identification was performed by Profa. Dra. Zefa Valdevina Pereira, and a specimen (DDMS-5007) was deposited in the Herbarium of the Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados - UFGD, Mato Grosso do Sul, Brazil. A scheme of experimental procedure conducted in this work from *P. leiocarpa* leaves is showed Fig. 1.

Isolation and Identification of Alkaloid

The air-dried and powdered leaves of *P. leiocarpa* (560 g) were extracted by maceration with methanol P.A. (2 L) at room temperature for 10 days. After filtration, evaporation of the solvent under vacuum provided the methanolic extract (ME-PL) (41 g). A portion of this extract (25 g) was dissolved in MeOH/H_2O (1:1) and partitioned with *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc) with further evaporation of the solvents resulting in the *n*-hexane (HF-PL; 4.04 g), chloroform (*CF*-PL; 6.45 g), ethyl acetate (EAF-PL; 4.68 g), and aqueous-methanol (AMF-PL; 9.24 g) fractions. Part of the *CF*-PL was fractionated by CC on silica gel (*n*-hexane/EtOAc 10 to 80% and EtOAc/MeOH 10 to 70%), resulting in the subfractions *CF*-PL-1 to *CF*-PL-13. The purification of subfraction *CF*-PL-4 on preparative thin-layer chromatography eluted in EtOAc/MeOH 30% yielded PL-1–labeled sample. **Vincosamide (PL-1)**: ^{1}H NMR (δ_H CD_3OD, 300 MHz): 8.54 (NH), 4.95 (d, $J = 11.5$ Hz, H-3), 2.94

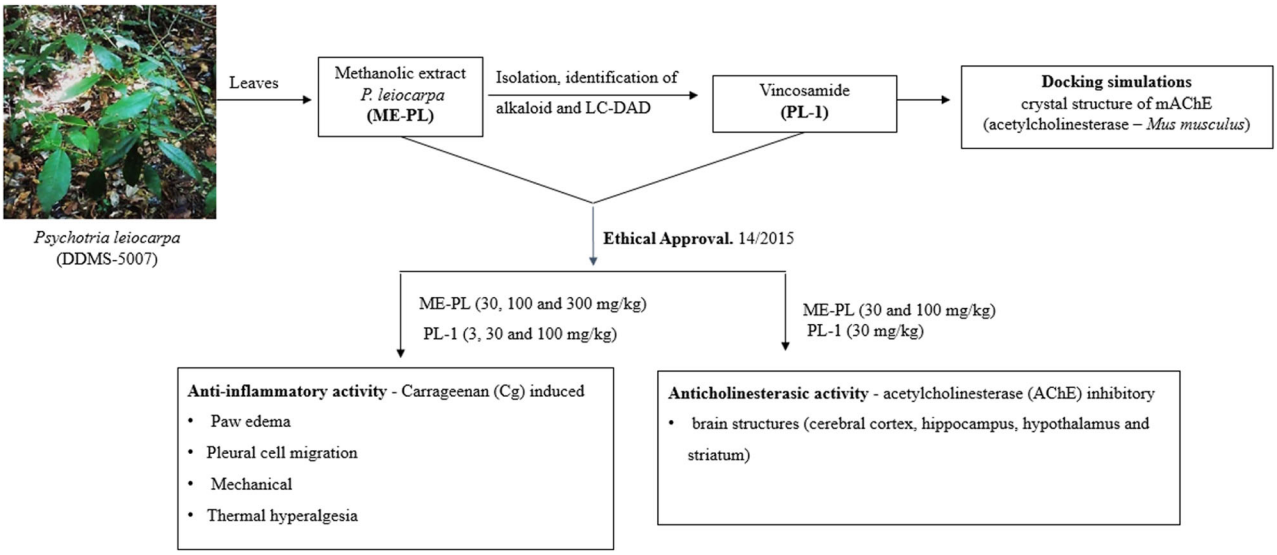

Fig. 1. Experimental procedure from *P. leiocarpa* leaves: Chemistry and biological assay.

(ddd, $J = 13.5$; 11.8; 5.7 Hz, H-5a), 5.05 (ddd, $J = 13.1$; 3.0; 1.2 Hz, H-5b), 2.69 (ddd, $J = 15.2$; 11.8; 3.0 Hz, H-6a), 2.75 (ddd, $J = 15.2$; 5.7; 1.2 Hz, H-6b), 7.40 (d, $J = 7.8$ Hz, H-9), 7.02 (t, $J = 7.8$; 1.2 Hz, H-10), 7.12 (t, $J = 7.8$; 1,2 Hz, H-11), 7.31 (d, $J = 7.8$ Hz, H-12), 1.40 (dddd, $J = 12.9$; 12.9; 11.5; 1.5 Hz, H-14a), 2.48 (dt, $J = 12.9$ Hz, H-14b), 3.23 (m, H-15), 7.44 (d, $J = 2.4$ Hz, H-17), 5.17 (dd, $J = 10.2$; 1.8 Hz, H-18a), 5.28 (dd, $J = 17.1$; 1.8 Hz, H-18b), 5.54 (ddd, $J = 17.1$; 10.2; 1.8 Hz, H-19), 2.71 (m, H-20), 5.50 (d, $J = 1.8$ Hz, H-21), 4.69 (d, $J = 8.1$ Hz, H-1′), 3.23–3.55 (4 H, m, H-2′-H-5′), 3.91 (1H, dd, $J = 12.6$; 1.8, H-6′a), 3.67 (1H, m, H-6′b). ^{13}C NMR (δ_C CD_3OD, 75.5 MHz): δ_C 134.5 (C-2), 54.4 (C-3), 41.2 (C-5), 22.1 (C-6), 109.03 (C-7), 127.9 (C-8), 118.8 (C-9), 120.0 (C-10), 122.5 (C-11), 111.9 (C-12), 138.3 (C-13), 32.6 (C-14), 27.3 (C-15), 109.0 (C-16), 149.0 (C-17), 120.5 (C-18), 133.9 (C-19) 44.5 (C-20), 97.3 (C-21), 166.0 (C-22), 99.6 (C-1′), 74.8 (C-2′), 77.9 (C-3′), 71.5 (C-4′), 78.3 (C-5′), 62.6 (C-6′).

LC Analysis

The ME-PL (10 μg/mL) was sonicated for 15 min and centrifuged at 10.000*g* for 15 min, further filtrated in 0.45 μm, and analyzed by LC. An analytical LC (LC-6AD, Shimadzu, Kyoto, Japan) system with a diode array detector (DAD) monitored at $\lambda = 200$–800 nm. The LC column was a C-18 (25 cm × 4.6 mm; particle size, 5 μm; Luna, Phenomenex, Torrance, CA, USA). In each analysis, the flow rate and the injected volume were set as 0.5 mL min^{-1} and 20 μL, respectively. All chromatographic analyses were performed at 22 °C. Elution was carried out using a binary mobile phase of eluent A: water with 0.05% trifluoroacetic acid and eluent B: acetonitrile; mobile phase gradient was as follows: 15–45% B in 35 min, 45–90% B in 17 min, 90% B for 3 min, and return of initial condition in the 5 min. Vincosamide standard was isolated in the laboratory diluted to 10 μg/mL initial concentration. Sample PL-1 was quantified by external calibration after appropriate dilutions in 0.01–10 μg/mL range.

Animals

The anti-inflammatory and anticholinesterasic assay were conducted using male and female *Swiss* mice (50 days old, 20–30 g, $n = 6$) obtained from the University Federal da Grande Dourados (UFGD). The animals were maintained at a constant temperature (23 ± 1 °C) on a 12-h light/dark cycle with free access to food and water.

Carrageenan (Cg)-Induced Paw Edema

The inhibitory effects on paw edema were evaluated as previously described [16]. For paw edema assay, male mice ($n = 6$) were orally treated with ME-PL (30, 100, and 300 mg/kg) and PL-1 (3, 30, and 100 mg/kg), control (saline solution), or positive control (DEX; 1 mg/kg), and after 1 h, paw edema was induced by injecting of Cg (300 μg/paw, 50 μL insterile 0.9% saline). Edema was

measured after 2, and 4 h with a paw plethysmometer (PANLAB Harvard).

Cg-Induced Pleurisy

The inhibitory effects on pleural cell migration were evaluated as previously described [17]. Female mice (n = 6) were treated with ME-PL (30, 100, and 300 mg/kg, v.o.), PL-1 (3, 30, and 100 mg/kg, v.o.), control (saline solution), DEX (1 mg/kg, v.o), and 1 h before, Cg (300 μg, 0.1 mL in PBS, pH = 7.4) was applied to the pleural cavity [18]. To determine the total number of leukocytes, an aliquot of 20 μL (exudates) was diluted in Turk solution (1:20) in a Neubauer chamber.

Mechanical Hyperalgesia

The mechanical sensitivity of the hind paw was measured by determination of withdraw thresholds. Nociceptive thresholds (g) were estimated using an electronic version of the Von Frey test (Insight ®, EFF 301, Digital analgesymeter). Separate groups of male mice (n = 6) were orally treated with ME-PL (30, 100, and 300 mg/kg) and PL-1 (3, 30, and 100 mg/kg), control or DEX. After 1 h, from respective treatment, an intraplantar injection of the carrageenan (300 μg/paw), in the right paw, was made while in the left paw an injection of saline. Constant pressure was applied to the plantar surface of the right hind paw with the analgesiometer until the mice vocalized or removed the paw, indicating the level of mechanical sensitivity induced by sensitization, 3 and 4 h after carrageenan administration [19].

Cold Thermal Stimulation

The sensitivity to cold was evaluated by the acetone test [20]. The animals were housed in suspended platform, and acetone (20 μL) was distributed in the skin of plantar surface of the right hind paw. The reaction, as indicated by paw licking, shaking, or rubbing the paw, was observed and recorded. The duration of the testing was 30 s.

AChE Assay in Brain Structures

Four groups of six male mice were separately treated by gavage 7 days with ME-PL (30 and 100 mg/kg), PL-1 (30 mg/kg), and control (0.9% saline). On the last day of the experiments, 1 h after samples' last dose, the animals were killed by decapitation and the mice brains were collected and separated into the cerebral cortex, hippocampus, hypothalamus, and striatum and placed in a 10 mM Tris–HCl solution, pH 7.4, on ice. The tissues were homogenized in a glass potter in the Tris–HCl solution at a 1:10 proportion (w/v) and then centrifuged at 3500 rpm for 10 min to yield a supernatant that was used for the enzyme assay. The procedure was performed at 4 °C, and the AChE activity was measured according to the spectrophotometric method previously described [21]. The test medium containing DTNB (1.04 mmol) and potassium phosphate buffer (pH 7.2, 24 mmol) was incubated for 2 min at 30 °C with 25 mL of the sample, and the reaction was initiated by the addition of acetylthiocholine iodide (ACSCh, 0.8 mM). The reaction product was determined at 412 nm for 2 min. The enzyme activity was expressed in μmol ACSCh/h/mg protein. The protein concentration of the homogenized samples was determined by the Coomassie blue method [22] using bovine serum albumin (BSA) as a standard, and protein concentrations were adjusted for each structure: cerebral cortex (0.7 mg/mL), hippocampus (0.8 mg/mL), hypothalamus (0.6 mg/mL), and striatum (0.4 mg/mL).

Docking Simulations

The crystal structure of mAChE (acetylcholinesterase—*Mus musculus*) complexed with choline (code ID: 2HA3) was obtained from the Protein Data Bank, and the molecular docking studies were performed using the Molegro Virtual Docker v6.0 [23]. This program restricts enzyme torsion angles but allows the flexibility of each tested ligand. All water molecules and ions were removed from the structures. The protocol for Molegro v6.0 uses the Moldock Score as a scoring function and the Moldock Optimizer as a search algorithm, with search sphere radiusset to 11 Å around the catalytic site. These protocols restricted the torsion angles of the enzyme, but allowed flexibility for the tested ligand. The results were ranked using the Rerank scores of the ligands, and all other options were set to the default value. Redocking simulations were repeated five times with each program, and the results were reproducible. The structure of vincosamide used in docking calculations was obtained from ChemSpider (http://www.chemspider.com) code id: 8339362.

Acute Toxicity

The acute toxicity study was based on protocol 425 [24, 25]. According to the protocol established, nine animals were used; each received a single oral administration by gavage of the ME-PL. Initially, one of the animals received a dose of 175 mg/kg and was observed at 30 min and 1, 2, 4, 6, 12, 24, and 48 h. After this period,

a second animal received a dose of 560 mg/kg, and after 48 h, the third animal received a dose of 1792 mg/kg. After an additional 48 h, a fourth animal received a dose of 2000 mg/kg. After the last dose had been administered, no deaths were observed, and according to the protocol, four more animals received 2000 mg/kg. The control group received the vehicle used for diluting ME-PA (drops of DMSO + distilled water). The animals were observed for signs of toxicity over 14 days. Behavioral observations (reflexes, tremors, convulsions, lacrimation, cyanosis, salivation, piloerection, muscle tone, and motor coordination) and mortality were analyzed. After 14 days of treatment, the animals were weighed and subsequently euthanized.

Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM). The difference among the groups was determined by analyses of variance (one-way ANOVA) followed by the Newman–Keuls test. $P < 0.05$ was considered to represent a significant difference.

RESULTS

Phytochemical Study

Compound PL-1 was quantified by HPLC resulting in 138.9 ± 0.3 mg/g yield (tr = 24.10 min) (Fig. 2).

The ^{1}H and ^{13}C NMR data of compound PL-1 were characterized by the signals for an indole ring at δ_H 7.40 (d, J = 7.8 Hz, H-9)/δ_C 118.84, 7.31 (d, J = 7.8 Hz, H-12)/δ_C 111.98, 7.12 (ddd, J = 7.8; 7.5; 1.2 Hz, H-11)/δ_C 122.53, and 7.02 (ddd, J = 7.8; 7.5; 1.2 Hz, H-10)/δ_C 120.00 in the region of aromatics. The signals for H-19 at δ_H 5.54 (ddd, J = 17.1; 10.2; 1.8 Hz) and at δ_H 5.17 (dd, J = 10.2; 1.8 Hz, H-18a) and δ_H 5.28 (dd, J = 17.1; 1.8 Hz, H-18b) together with the methylene carbon at δ_C 120.5 (C-18) confirmed the terminal vinylidene unit. The carbonyl group was evidenced by the signal at δ_C 166.05 (C-22). The signal for the β-glucopyranosyl moiety was observed at δ_H 3.23–3.91/δ_C 62.66–78.35 and δ_H 4.69 (d, J = 8.1 Hz, H-1′)/δ_C 99.57 in the ^{1}H and ^{13}C NMR spectra.

Biological Activity

Paw Edema

The ME-PL (300 mg/kg) ($P < 0.05$) and compound PL-1 (100 mg/kg) ($P < 0.01$) presented a decrease in the formation of edema in 1 h, with maximal inhibition of 52.38 ± 2% and 61.30 ± 3%, respectively. In the course of the experiment, ME-PL (100 and 300 mg/kg) ($P < 0.05$) and PL-1 (100 mg/kg) ($P < 0.001$) showed a significant decrease in edema compared to the group control with inhibitions of 40.47 ± 4%, 46.42 ± 3%, and 73.21 ± 2%, after 2 h, respectively, and 35.89 ± 1%, 39.10 ± 2%, and 67.94 ± 2%, after 4 h (Fig. 3). The positive control DEX (1 mg/kg) ($P < 0.001$) significantly reduced edema in 1 h (73.21 ± 2%), 2 h (76.19 ± 4%), and 4 h (77.56 ± 4%) (Fig. 3).

Pleural Cell Migration

It was observed that ME-PL at doses of 30, 100, and 300 mg/kg ($P < 0.001$) inhibited leukocyte migration by 84.76 ± 2%, 86.71 ± 1%, and 88.85 ± 1%, respectively, 4 h after carrageenan injection, when compared to the control group. In relation to PL-1, all doses of 3, 30, and 100 mg/kg demonstrated inhibition of 76.41 ± 1%, 88.35 ± 2%, and 88.51 ± 1%, respectively, (Fig. 4). DEX (91.52 ± 2%) inhibited inflammation, showing effectiveness as anti-inflammatory positive control (Fig. 4).

Anti-Hyperalgesic Effects

The treatment with ME-PL (100 and 300 mg/kg) ($P < 0.05$) and PL-1 (100 mg/kg) ($P < 0.01$) showed significantly anti-hyperalgesic effects, with reduction of 42.50 ± 6%, 55.00 ± 3%, and 70.00 ± 2% in 3 h (Fig. 5a), respectively. At 4 h after carrageenan-treated with maximal inhibition for ME-PL at dose 300 mg/kg ($P < 0.001$) (85.10 ± 3%) and for the PL-1 at dose 100 mg/kg ($P < 0.001$) (90.86 ± 4%), compared with control (Fig. 5b). Treatment with DEX (1 mg/kg) ($P < 0.001$) was able to reduce the mechanical hyperalgesia induced by carrageenan by 97.5 ± 1% after 3 h (Fig. 5a) and by 100% after 4 h (Fig. 5b).

Cold Sensitivity

The ME-PL demonstrated a potential reduction in sensitivity to acetone cold stimulus with a reduction of 63.15 ± 2% evaluated in 3 h (Fig. 6a) and 67.26 ± 2% in 4 h (Fig. 6b) for the doses 300 mg/kg ($P < 0.01$), respectively. PL-1 also presented a potential reduction in the dose evaluated (100 mg/kg) ($P < 0.001$) with 63.81 ± 2% in the time of 3 h (Fig. 6a) and 64.28 ± 2% in 4 h (Fig. 6b). The DEX (1 mg/kg) ($P < 0.001$) control showed high inhibition of 76.97 ± 1% and 85.11 ± 1% when evaluated in 3 h and 4 h, respectively (Fig. 6).

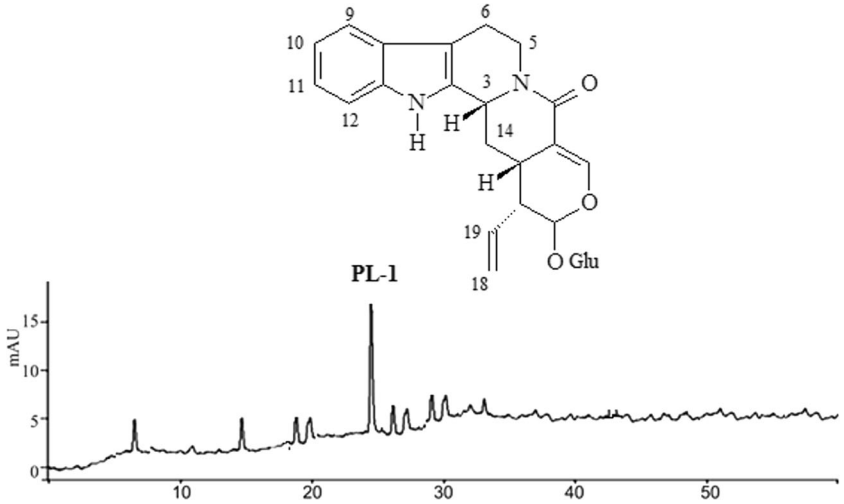

Fig. 2. Chromatogram representative of ME-PL with PL-1 isolated from methanolic extract of *P. leiocarpa* (ME-PL) collected in Dourados-MS.

Anticholinesterase Activity

Results demonstrated that in the oral administration with ME-PL and PL-1, the AChE activity was altered in different brain structures (Fig. 7). Figure 7a shows that in the groups treated with ME-PL (30 and 100 mg/kg) ($P < 0.001$) and PL-1 (30 mg/kg) ($P < 0.001$) acetylcholinesterase inhibitory activity was significantly decreased in the cerebral cortex, 47 ± 3%, 44 ± 1%, and 36 ± 1%, respectively, compared to the control group. In the hippocampus (Fig. 7b), the level of inhibition was also observed in the animals treated with ME-PL at 100 mg/kg ($P < 0.01$) (32 ± 4%), 30 mg/kg (31 ± 3%), and 30 mg/kg (28 ± 3%) doses of PL-1. Moreover, in the hypothalamus, a significant inhibition of 13 ± 1%, 12 ± 1%, and 14 ± 2% at doses of 100 and 30 mg/kg (ME-PL) ($P < 0.05$) and 30 mg/kg (PL-1) ($P < 0.05$), respectively, compared to the control group (Fig. 7c) was observed.

Enzyme-Inhibitor Interactions

Figure 8 shows that PL-1 interacts with both the anionic catalytic (active centre) and peripheral (PAS) sites, and according to the literature [26, 27], the following residues represent these sites in mAChE: His447, Trp86, Glu334, and Ser203; Tyr124, Trp286, and Tyr341.

Figure 9 supplements this information and highlights the hydrogen bonds with His447, Tyr124, and Arg296 that are very important for anchoring the ligand in the active site. In conclusion, vincosamide, a component from the methanolic extract of *P. leiocarpa* (ME-PL) leaves collected in Dourados-MS, is involved with acetylcholinesterase inhibitory activity.

Toxicity

The assessment of acute toxicity was conducted for 14 days to determine the lethal dose (LD_{50}). The animals used in this study were exposed to ME-PL, and no clinical signs of toxicity were observed at any dose. No deaths were reported.

DISCUSSION

Vincosamide (Fig. 2) was isolated from ME-PL collected in Dourados-MS and characterized by NMR spectral data and further evaluated for pharmacological activities. A comparison with the literature spectral data for vincosamide and the epimer strictosamide maintained the relative configuration at α position of H-3 [28]. Structural characterization for PL-1 was consistent with that described in the literature for vincosamide [29] isolated from *Nauclea orientalis* (Rubiaceae). To the best of our knowledge, this is the first time the compound was reported in *P. leiocarpa*. The N-β-glucopyranosyl vincosamide was found in the leaves of *P. leiocarpa* collected in Porto Alegre/Brazil, and vincosamide can be obtained with a small hydrolysis yield [6, 30]. These variations may have relationship to the environmental conditions to which the plant is exposed.

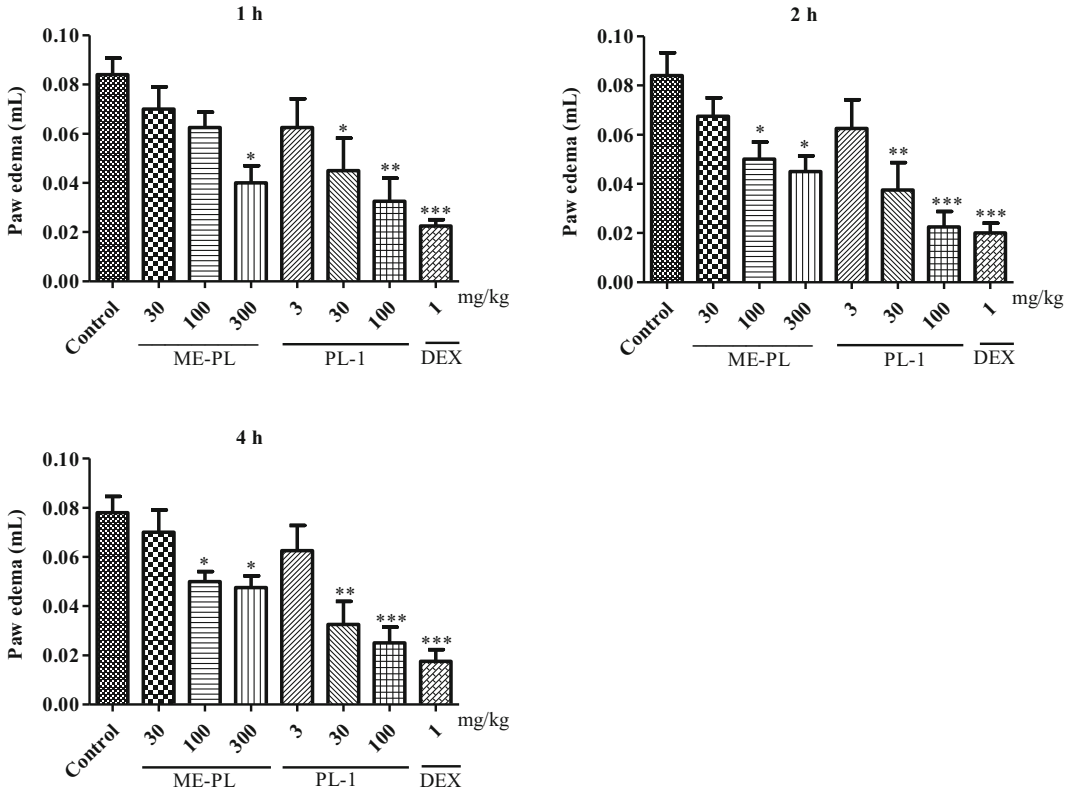

Fig. 3. Effects of ME-PL (30, 100, and 300 mg/kg) and PL-1 (3, 30, and 100 mg/kg), control (0.9% saline), or DEX (1 mg/kg), on paw edema evaluated in 1 h, 2 h, and 4 h after carrageenan induction. The data are represented as the means ± SEM of animals ($n = 6$). The * symbol compared the treated group in relation to the control group: *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, #$p < 0.001$, one-way ANOVA followed by Student–Newman–Keuls.

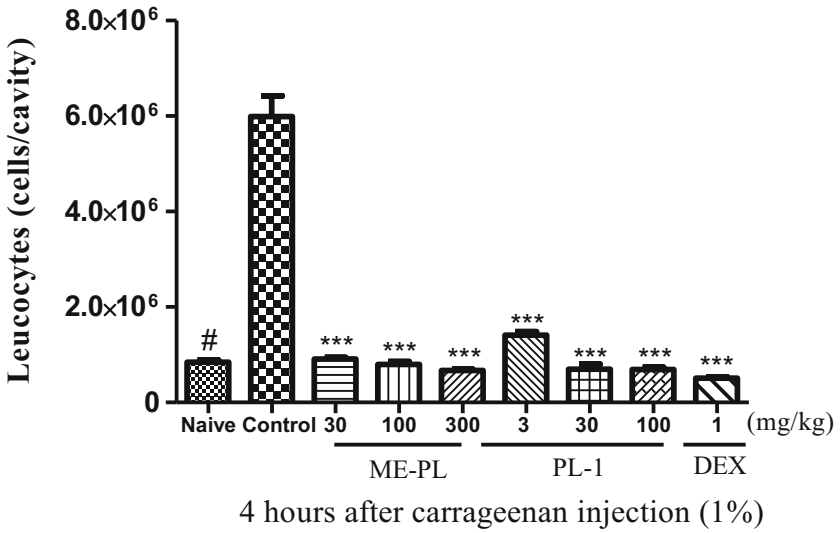

Fig. 4. Effects of ME-PL (30, 100, and 300 mg/kg) and PL-1 (3, 30, and 100 mg/kg), control (0.9% saline), or DEX (1 mg/kg), on total leucocytes induced by carrageenan in the pleural cavity of mice. The data are represented as the means ± SEM of animals ($n = 6$). The # symbol indicates the statistical differences of naïve and control group ($p < 0.001$) while the * compared treated group in relation to control group: *** $p < 0.001$. Differences between groups were analyzed by one-way ANOVA followed by the Newman–Keuls test.

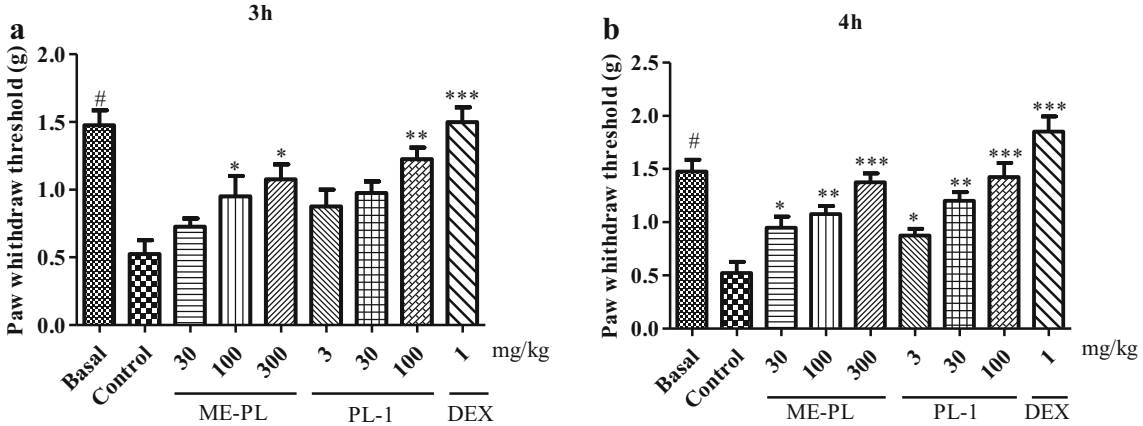

Fig. 5. Effect of oral administration of ME-PL (30, 100, or 300 mg/kg, p.o.), PL-1 (3, 30, or 100 mg/kg, p.o.), on mechanical hyperalgesia in mice. The animals received control (0.9 saline) or DEX (1 mg/kg). In **a,** the mechanical hyperalgesia was measured with a digital analgesy meter for 3, and in **b,** 4 h after carrageenan administration. Each bar represents the mean ± SEM of six animals. Differences between groups were analyzed by one-way ANOVA followed by the Newman–Keuls test, being $*p < 0.05$, $**P < 0.01$, $***P < 0.001$, $\#P < 0.001$ when compared with the control group.

In reviewing the literature, other species of this genus show indole chromophore (tryptamine-iridoid) alkaloids and some of them found in *Psychotria* display a large range of effects on the central nervous system, such as anxiolytic, antidepressant, and analgesic effects, as well as the impairment of learning and memory acquisition [18, 31–40].

The present study represents the first research into the anti-inflammatory and anticolinesterasic effects of the ME-PL and isolated compounds PL-1 (vincosamide) of leaves from *P. leiocarpa*. The focus of this work in inflammatory process was to widely assay the *P. leiocarpa* extract and isolated compound vincosamide in time response analysis (in paw inflammatory model), dose response aspects, and some inflammatory and nociceptive parameters in two models of *in vivo* inflammation. Anti-inflammatory activity of the ME-PL and PL-1 in acute inflammation was assessed by an induction model with Cg-induced paw edema and pleurisy (Figs. 3 and 4) because in the literature [10] only showed a potential *in vitro* activity of *P. leicocarpa* in nitric oxide activity. ME-PL and PL-1

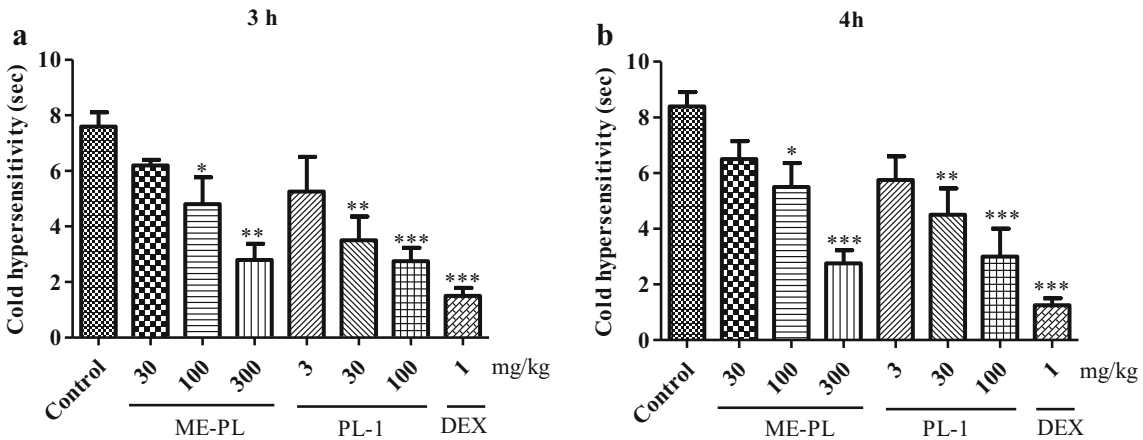

Fig. 6. Effect of oral administration of ME-PL (30, 100, or 300 mg/kg, p.o.), PL-1 (3, 30, or 100 mg/kg, p.o.), on the cold sensitivity induced by acetone in mice. The animal's control (0.9 saline) or DEX (1 mg/kg). The cold sensitivity was measured 3 and 4 h after carrageenan administration. Each bar represents the mean ± SEM of six animals. Differences between groups were analyzed by one-way ANOVA followed by the Newman–Keuls test, being $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $\#P < 0.001$ when compared with the control group.

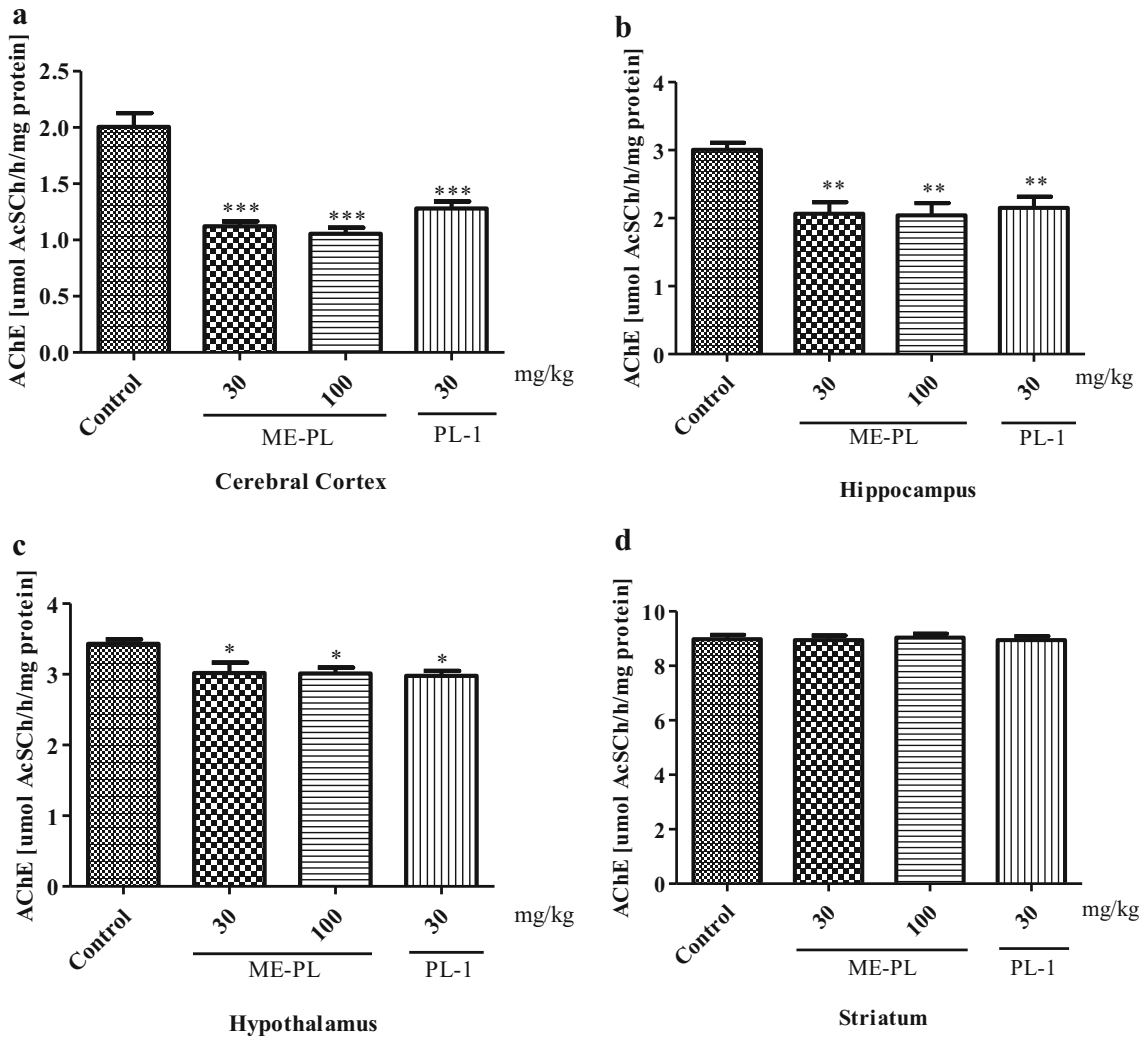

Fig. 7. Effect of the oral treatment with ME-PL and PL-1 on AChE activity in the cerebral cortex (a), hippocampus (b), hypothalamus (c), and striatum (d). Values are expressed as mean ± S. E. M. $n = 6$ observations per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with the control group. One-way ANOVA followed by the Newman–Keuls test.

inhibited significantly (Fig. 3) the carrageenan inflammatory paw in a dose- and time-dependent way showing that ME-PL1 and PL-1 have a specific mechanism of action. In carrageenan paw inflammation, three parameters analyzed such as edema, mechanical hyperalgesia, and cold allodynia was inhibited by ME-PL and PL-1 (Figs. 5 and 6). Acute inflammation is characterized by cardinal signs of inflammation (edema, fever, redness, and pain), being widely evaluated by the described trials and used to analyze the anti-inflammatory agents involved during the inflammatory process [41]. The ME-PL and PL-1 showed significant inhibitory effect for edema formation in 2 h and 4 h, stage correlated with an increased production of prostaglandins, cyclooxygenase-2 (COX-2), and nitric oxide (NO) release in the inflammatory response [42], which confirmed the anti-edematogenic potential of *P. leiocarpa*. Li et al. [43] showed that compounds (including vincosamide) are able to inhibit some mediators (nitric oxide, Il-1β, and TNF) and transcriptional factors of inflammation without inducing cellular death. It is possible to suggest that *P. leiocarpa* acts like the main compound vincosamide. Another point is that ME-PL and PL-1 showed to be so effective as dexamethasone but not so potent but both inhibited all inflammatory parameters analyzed.

The ME-PL and PL-1 also showed inhibition of leukocyte migration above 80% for all doses evaluated and, in

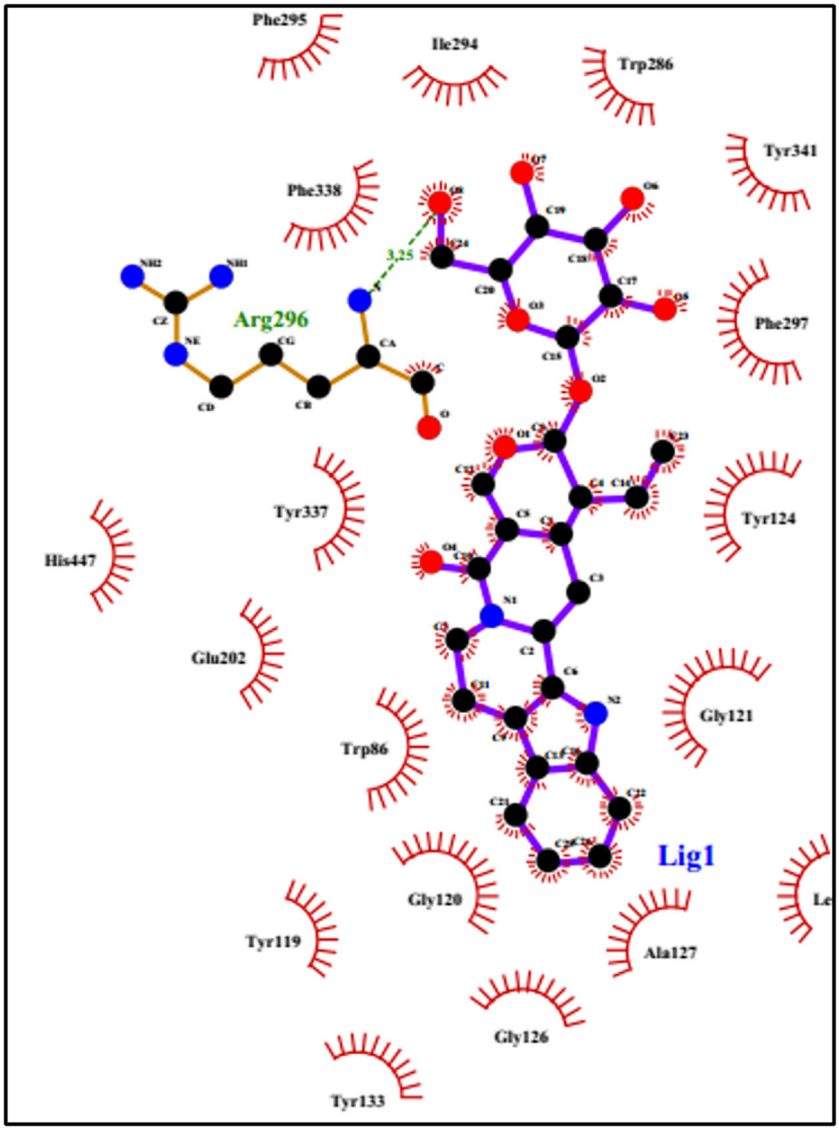

Fig. 8. Docking interactions between the active residues site of the protein with the vincosamide (PL-1) ligand.

the nociception caused by acetone, attenuated the duration of cold sensitivity, which demonstrated its anti-hyperalgesic and antinociceptive potentials. The increase in the pain sensitivity is a common characteristic of the inflammatory response that involves a reduction in the type C nerve fiber activation that is induced by mechanical stimuli [44].

Acetylcholine (ACh) is one of the factors involved in abating the inflammatory response and allowing the recovery of hemostasis and acts by attenuating the secretion of pro-inflammatory cytokines; however, the circulating AChE controls the levels of ACh, suggesting promotion of the inflammatory process under AChE excess [45, 46]. AChE plays an important role in the central and peripheral nervous systems, degrades the neurotransmitter ACh, and subsequently reduces the ACh level in the brain; thus, AChE inhibitor can increase ACh level in cholinergic synapses which have shown to alleviation the disease [47]. Study [48] describes the interaction of many compounds extracted from *Psychotria* with the ability to inhibit AChE, butyrylcholinesterase (BuChE), and monoamine oxidases A and B (MAO-A and MAO-B), highlighting two β-carboline alkaloids, prunifoleine and 14-oxoprunifoleine, and four monoterpene indole alkaloids,

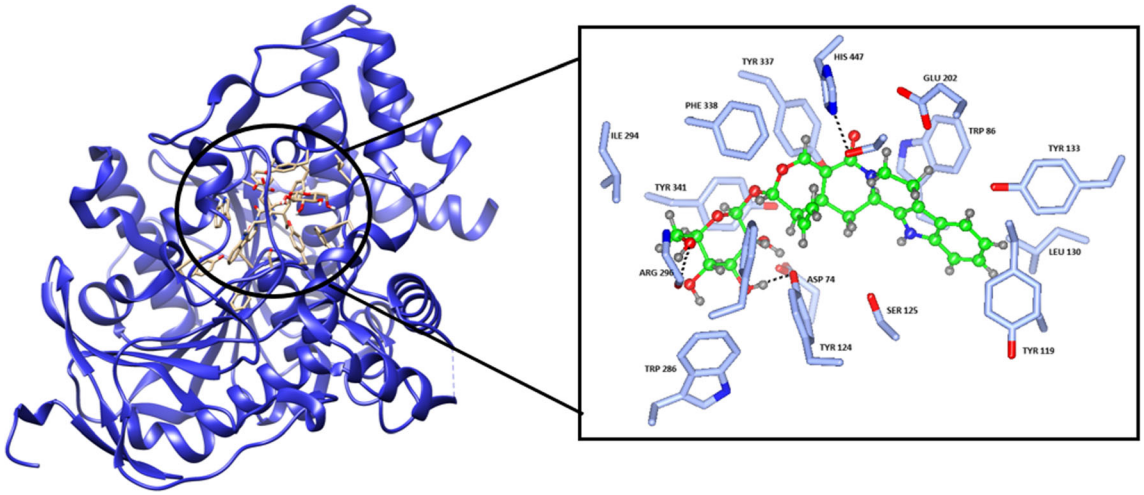

Fig. 9. Overall view of the mAChE subunit complexed with vincosamide (PL-1).

angustine, vallesiachotamine lactone, and E- and Z-vallesiachotamine, which inhibited BuChE and MAO-A. Recent research has increasingly suggested a central role of free radical–induced tissue damage in the pathogenesis of AD [49, 50].

The redocking protocol was evaluated based on the ability of the program to reproduce the choline binding modes observed in the corresponding crystallographic structure; after the validation of the methodology, vincosamide structure (PL-1) was docked in the active site of mAChE, and the protein-ligand interactions were evaluated. The docking study was preferable performed on mAChE, since inhibitory activity data used in the present study were measured on this enzyme [51].

The active AChE center, which consists of the triad Ser203, Glu334, and His447 in mammals [52], is nearly centrosymmetric to the subunit located at the bottom of a narrow gorge that is 20 Å in length. Inhibitors may bind the active site or at a distant allosteric site, the peripheral anionic site (PAS), that is located at the gorge rim [53].

The Trp286 residue plays a very important role in ligand binding with PAS [54]. The ligand interacts with Tyr 337 residue replacing Phe330 residue present in TAChE [53], and this residue belongs to a hydrophobic domain (Phe295, Phe297, Tyr337, Phe338, and Tyr341) that helps anchor the compound. This domain is crucial for enzymatic activity, constituting a difference between AChE and BuChE [55]. In addition, these residues Gly121, Gly126, Ala127, and Tyr119 help in stabilization and hydrophobic interactions. The losses of cholinergic neurons occur primarily in the cortex, the hippocampus, and the brain structures that play important roles in memory and cognitive function [56, 57]. Study has demonstrated that AChE activity is altered in CNS and lymphocytes in many pathological and experimental conditions [58–61]. Thus, synthetic and naturally compounds that may interfere with the activity of this enzyme may be important research targets regarding the treatment of inflammatory, cognitive, and neurochemical dysfunctions. In the present study, we demonstrated that ME-PL and PL-1 exerts an effect in the cholinergic system by altering the AChE activity in the mice' frontal cortex.

In conclusion, we have shown in this study that oral administration of 30 and 100 mg/kg ME-PL and 30 mg/ kg PL-1 inhibited AChE activity in the frontal cortex. Thus, the extract and compound of *P. leiocarpa* showed promising anti-inflammatory activity, in dose-response aspects in inflammatory paw and pleurisy, time curve, and nociceptive parameters. The presence of the alkaloid vincosamide could be responsible, at least in part, for the observed effects. To the best of our knowledge, these experimental trials using this species to evaluate the anti-inflammatory and AChE activity are the first reported in the literature and can contribute to validation of the popular use of this genus. Additional studies must be performed to define the mechanism of action.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no competing interests.

Ethical Approval. All experimental procedures were carried out in accordance with the U.S. National Institutes of Health and were approved by the Animal Ethics Committee from UFGD (Nbr. 14/2015).

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