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Neurobehavioral and Neuroprotective Effects of the Polysaccharide Extract from Libidibia ferrea Barks in PTZ-Induced Seizures: Involvement of the GABAergic System

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Abstract

This study aimed to investigate neurobehavioral, neurochemical, and histopathological effects of the polysaccharide extract from the barks of Libidibia ferrea (EP-Lf) on the central nervous system in mice. Animals were treated via intraperitoneal (i.p.) with 0.9% saline, EP-Lf (1, 3, 9, or 27 mg/kg), diazepam (1 mg/kg, i.p.), or imipramine (10 mg/kg, i.p) 30 minutes before seizure induction with pentylenetetrazole (PTZ - 85 mg/kg, i.p.) EP-Lf at 27 mg/kg increased seizure latency (112.9 ± 6.87 vs. saline: 61.40 ± 3.55) and death latency (941.6 ± 74.20 vs. saline: 265.5 ± 42.54) and reduced pycnotic nuclei in the hippocampus. The use of flumazenil reversed seizures (76.60 ± 5.70) and death (340.7 ± 30.92) latencies. Moreover, EP-Lf elicited antioxidant effect by the increase in reduced glutathione (GSH) in prefrontal cortex [H = 38.38; p < 0.0001; k = 6], hippocampus [F (5, 52)=15.88; p<0.0001], and corpus striatum [F (5, 52)=25.19; p<0.0001]; and decreased malondialdehyde (MDA) in prefrontal cortex at 9 and 27 mg/kg [H = 35.51; p < 0.0001; k = 6]; hippocampus [F (5, 54)=14.64; p<0.0001] and corpus striatum [H = 35.51; p < 0.0001; k = 6]. In conclusion, EP-Lf has a central inhibitory effect, conferring neuroprotection on mice's PTZ-induced seizures, suggesting a GABAergic effect.

Introduction

Epilepsy is one of the most prevalent neurological disorders (Beghi 2020), described by the International League Against Epilepsy (ILAE) as a brain disorder characterized by an enduring predisposition to generate epileptic seizures, showing potential recurrence and capable of causing neurobiological, cognitive, psychological, and social consequences (Beniczky et al. 2025). Progressive events secondary to epilepsy include cognitive impairment, abnormal behavior (Fisher et al. 2017), and the generation of reactive oxygen species in the brain, considered one of the main causes of generalized epilepsy associated with recurrent seizures (Pearson-Smith and Patel 2017).

The treatment of epilepsy still relies on the use of low-cost traditional antiepileptic drugs, including carbamazepine, phenobarbital, phenytoin, valproic acid, and benzodiazepines (Guerreiro 2016), provided free of charge by Brazil's Unified Health System (SUS). The main issue related to these medicines concerns their side effects and seizure control (Akyüz et al. 2021).

In this context, plant-derived biomolecules may be considered a viable therapeutic alternative for the treatment of this disorder. *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz, formerly classified as *Caesalpinia ferrea* and commonly known as "pau-ferro" or "jucá" (Balbach 1972; Bragança 1996), is predominantly found in the semi-arid regions of northern and northeastern Brazil, being widespread throughout the state of Ceará (Maia 2004). In traditional medicine, *L. ferrea* is used as an antipyretic, antidiabetic, and for the treatment of wounds, bruises, asthma, and chronic cough (Balbach 1972; Braga 1976). Due to its ethnopharmacological relevance, *L. ferrea* has been included in the National List of Medicinal Plants of Interest to the Brazilian Unified Health System (RENISUS) (Brasil 2009).

Experimental studies conducted in our laboratory have demonstrated the anti-inflammatory and antioxidant effects of the polysaccharide extract obtained from *L. ferrea* barks and pods in rodent models, such as paw edema, peritonitis (Pereira et al. 2012; Holanda et al. 2021), cutaneous wounds (Pereira et al. 2016; Mota et al. 2022; Assreuy et al. 2023), and venous thrombosis (de Araujo et al. 2021). Later, the neuroprotective effect of the galactomannan isolated from *C. ferrea* pods was demonstrated in the mouse model of seizures (Craveiro et al. 2025). However, no studies on the polysaccharides from *L. ferrea* barks are described in the literature regarding the central nervous system.

Therefore, this study aimed to investigate the neurobehavioral, neurochemical, and histopathological effects of the polysaccharide extract from *Libidibia ferrea* barks on the central nervous system of mice.

Materials and methods

Obtention and chemical analysis of the polysaccharide extract from the barks of *Libidibia ferrea*

The stem barks of *L. ferrea* (Mart. ex Tul.) L.P. Queiroz var. *Caesalpinia ferrea* were collected in the Custódio District (Quixadá/CE) - latitude (4º 58' 17") and longitude (39º 00' 55"). An exsiccate (no. 46085) of the plant was deposited in the Prisco Bezerra Herbarium at the Federal University of Ceará – UFC.

For the polysaccharide extraction, the barks of *L. ferrea* were washed, dried, macerated, and the powder (5 g) suspended, homogenized (2x), and reduced in a rotary evaporator and centrifuged (3.500 rpm, 30 min). The acidic supernatants (S1) were discarded, and the alkaline (S2 and S3), containing polysaccharides, were centrifuged, and the precipitate collected, dialyzed (15x) (Pereira et al. 2016), and deproteinated (Holanda et al. 2021). The final supernatant was dialyzed (20 x), lyophilized (Zhao et al. 2014), and named EP-Lf. The chemical analysis of EP-Lf was performed by Araujo et al. (2021) for total proteins (Bradford 1976) and carbohydrates (Dubois et al. 1956), uronic acid (Dische 1947), and polyphenols (Khoo et al. 2014).

Animals

The experimental protocols were performed in accordance with the standards recommended by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee for the Use of Animals (CEUA/UECE: nº 510620/2020). Male Swiss mice (25-35 g) were maintained in a light/dark cycle (22-25ºC) at the quarantine room of the Higher Institute of Biomedical Sciences (ISCB-UECE). Animals (N= 111) received water and food *ad libitum* until the experiments were carried out.

The animals were placed in a closed environment, free from external noise, with constant temperature (26±2°C) and low-intensity lighting (15 W red lamp), so that they could be adapted to the environment.

Drugs

Diazepam (DZP) (Roche - Rio de Janeiro - RJ), imipramine (IMP), and flumazenil (FLU) were from União Química - Embu-Guaçu – SP, and pentylenetetrazol (PTZ) from Sigma-Aldrich, St. Louis, MO, USA. The drugs were dissolved in sterile saline (0.9% NaCl), except for DZP, which was dissolved in distilled water.

Experimental design

Animals (n=8-10 /group) were treated via intraperitoneal (i.p.) with EP-Lf (1, 3, 9, 27 mg/kg) or vehicle (0.9% sterile saline). Thirty minutes later, behavioral tests were performed according to the following order: open field, hole-board, EPM, and tail suspension. Diazepam (DZP; 1 mg or 2 mg/kg, i.p.) or imipramine (IMP; 10 mg/kg, i.p.) were used as reference drugs. In each test, the animals were observed for 5 minutes. Before testing each animal, 4% alcohol was used to remove residues and odors. In the Open field, Hole-board, and Elevated Plus Maze tests, the animal movements were captured on video using an Intelbras® Full HD camera for later analysis using the X-Plo-Rat program (Cardenas et al. 2001). Animals were euthanized, and the brain areas (prefrontal cortex-PFC, hippocampus-HC, corpus striatum-CS) were removed for evaluation of oxidative stress markers (reduced-glutathione-GSH, malondialdehyde-MDA).

Another group of animals was assayed for the acute seizures induced by pentylenetetrazole (PTZ; 85 mg/kg, i.p.) 30 min after treatment with EP-Lf (1, 3, 9, 27 mg/kg), DZP (1 mg/kg), IMP (10 mg/kg), or saline. At the most effective dose of EP-Lf, the animal's hippocampus was submitted for histopathological analysis to identify the presence of dark neurons. The brain areas (PFC, HC, CS) were also analyzed for oxidative stress markers (GSH, MDA, nitrite). In this model, the involvement of the GABA pathway in the EP-Lf anticonvulsant effect was also investigated.

Open field Test

To evaluate depressant, stimulant, and exploratory activities (Archer 1973), mice were individually placed in the open-field apparatus consisting of an acrylic box (30 × 30 × 15 cm) with the floor divided into 9 squares. Animals stayed in the apparatus for 5 minutes to quantify the number of crossings, rearing, and grooming.

Hole-Board Test

To evaluate the intensity of exploratory behavior and anxiety (Clark et al. 1971), mice were placed in the Ugo Basile apparatus, composed of 16 evenly spaced holes (60 cm × 30 cm). The number of head dips and the time spent in the holes were counted for each animal for 5 min.

Elevated Plus maze Test

For evaluation of activity and anxiety profiles (Lister 1987), mice were placed in the center of the apparatus, consisting of two opposing open arms (30 x 5 x 25 cm) and two closed arms (30 x 5 x 25 cm). The number of entries and the time spent on the open and closed arms were registered for 5 minutes.

Tail suspension Test

To the neurobehavioral evaluation for depression (Steru et al. 1987), mice were suspended 50 cm from the ground by a tape fixed at 1 cm from the tail tip, and the immobility time was recorded for 5 minutes. The decrease in immobility time reveals antidepressant activity.

Acute seizure model

For evaluation of acute seizures, animals were observed for the latency of seizures (time spent from PTZ administration until the appearance of the first convulsion) and for the latency of death (the occurrence of death in the interval of twenty minutes after the first convulsion) (Czuczwar and Frey 1986).

To investigate the pathway mechanism of the anticonvulsant effect, animals received EP-Lf (at the most effective dose) or vehicle, 30 min before the agonist of GABAA receptors, flumazenil (2.5 mg/kg i.p.), and 15 minutes later, the convulsion was induced by PTZ (Viswanatha et al. 2017)

Assessment of oxidative stress

Prefrontal cortex (PFC), hippocampus (HC), and corpus striatum (STRI) were homogenized in sodium phosphate buffer pH 7.4 to quantify GSH (Sedlak and Lindsay 1968), MDA (Cighetti et al. 1999), and nitrite (Green et al. 1982).

Histopathological analysis

The brain was removed and immersed in 10% buffered formalin for 24 to 48 h, transferred to 70% alcohol, sectioned (5 µm) in the coronal plane (bregma), and embedded in paraffin. This procedure was repeated nine times throughout the entire brain, totaling ten sections of the hippocampus (50 µm between each section). The hippocampal section was stained with hematoxylin-eosin (HE) and analyzed by light microscopy at 40X magnification. Five slides of the hippocampus in the CA1, CA2, and CA4 areas were chosen to count the number of black neurons in the naive saline-treated group or in EP-Lf and saline-treated groups that had been induced to convulsion with PTZ. Neurons were identified by intense staining and morphological changes. The average was calculated per area, generating qualitative and quantitative data (Kherani and Auer 2008; Naseer et al. 2013; Ullah et al. 2013).

Statistical Analysis

Statistical analysis of the data was performed using GraphPad Prism software version 8.0 for Windows (GraphPad Software, San Diego, California, USA. Copyright © 1992–2018 by GraphPad Software, Inc). The Shapiro-Wilk test was used to evaluate the normality $sdates.\ Res\underline{t}swere\exp ressedasMean \pm S\tan dardErr\ \text{or}\ ofthe Mean(SEM),\ \text{and}\ valuesthe\widehat{f}ollowedaparametricdistributionwerean$

s post-test, and the F test was used to verify the difference between means. Non-parametric data were analyzed by Kruskal-Wallis, followed by Dunn`s post-test, and the H statistical rank test was calculated. *P* values less than 0.05 were considered significant.

Results

Behavioral Tests

Open Field

EP-Lf at 1 (31.50 ± 3.77), 3 (30.75 ± 3.23), 9 (20.00 ± 3.27), or 27 (15.75 ± 1.98) mg/kg reduced the n° of crossings when compared to controls that received saline (47.10 ± 4.06) [F (5, 44) =16.58; p<0.0001]. Regarding the n° of rearings, all animals treated with EP-Lf at 1 (10.00 ± 2.00), 3 (5.75 ± 1.70), 9 (2.25 ± 1.03), and 27 (0.50 ± 0.26) mg/kg showed a reduction compared to saline (30.90 ± 2.57) (Fig 1Ab). However, for the n° of grooming, only EP-Lf at 1 mg/kg (7.35 ± 1.28) showed an increase in comparison to saline (3.60 ± 0.63) [F (5, 44)=11.31; p<0.0001] (Fig 1Ac). Diazepam, the standard depressant drug, reduced all behaviors (n° of crossings: 11,63 ± 2.50; n° of rearing: 0.00 ± 0.00; n° of grooming: 0.12 ± 0.12) compared to saline (**Fig. 1A**).

Hole-Board Test

EP-Lf at 27 mg/kg (15.25 ± 2.05) reduced the n° of dives compared to saline (32.25 ± 1.14) [F (5, 42)=10.79; p<0.0001] (Fig 1Ba). Regarding the dive time EP-Lf at 3 (7.87 ± 1.27), 9 (3.50 ± 0.82), and 27 (2.75 ± 0.79) mg/kg decreased these parameters when compared to saline (22.25 ± 2.26) [H = 36.35; p < 0.0001; k = 6] (Fig 1Bb). Diazepam, at the dose that induces anxiety (1 mg/kg), did not alter the number of dives (42.75 ± 3.70) or the dive time (14.75 ± 1.33) compared to saline (**Fig. 1B**).

Elevated Plus Maze Test

EP-Lf reduced the nº of entries into the open arms (NEOA) at 1 (2.75 ± 0.64), 3 (2.75 ± 0.67), 9 (2.75 ± 0.67), and 27 (1.87 ± 0.51) mg/kg compared to saline (7.75 ± 0.55) [F (5, 42)=35.95; p<0.0001]. Regarding the time spent in the open arms (TSOA), EP-Lf at all doses [1 (24.38 ± 6.85), 3 (33.38 ± 8.51), 9 (33.00 ± 9.97), and 27 (16.38 ± 6.60) mg/kg] decreased TSOA compared to saline (84.60 ± 6.53) [F (5, 46)=44.74; p<0.0001]. EP-Lf produced no alterations in the nº of entries into the closed arms (NECA) (3.37 ± 0.62 [F (5, 42)=12.24; p<0.0001]) or the time spent in the closed arms (TSCA) compared to saline (161.6 ± 11.03 [F (5, 42)=11.93; p<0.0001]. In all parameters, DZP increased NEOA (11.63 ± 0.80), TSOA (16.38 ± 6.60), and decreased NECA (0.75 ± 0.25) and TSCA (82.25 ± 12.70) (**Fig. 1C**).

Tail Suspension Test

Animals treated with EP-Lf only at 9 (143.3 ± 9.10) and 27 (163.4 ± 11.03) mg/kg showed increased immobility time in comparison with saline (88.40 ± 7.84). Imipramine (35.00 ± 4.96), an antidepressant control, promoted a decrease in the immobility time when compared to saline [F (5, 44)=19.63; p<0.0001] (**Fig. 1D**).

Per se effect of EP-Lf on Oxidative Stress markers

Reduced Glutathione (GSH)

In the prefrontal cortex, EP-Lf at 9 (636.8 ± 39.33) and 27 mg/kg (671.1 ± 29.77) increased GSH compared to saline (467.9 ± 26.72) [F (4, 35) = 6.899; p=0.0003]. In the hippocampus, EP-Lf at 1 (633.2 ± 32.65), 3 (633.3 ± 51.63), 9 (731.7 ± 57.50), and 27 (663.0 ± 43.09) mg/kg increased GSH content when compared to saline (451.5 ± 24.58) [H = 16.37; p = 0.0026; k = 5]. In the corpus striatum, EP-Lf did not alter GSH content at any dose when compared to saline [F (4. 35) =1.401; p=0.2538] (**Fig. 2A**).

Lipid Peroxidation (MDA)

In the prefrontal cortex, EP-Lf at 27 mg/kg (308.7 ± 25.80) reduced MDA content compared to saline (476.9 ± 43.88) [H = 12.44; p = 0.0144; k = 5]. In the hippocampus, EP-Lf at all doses: 1 (334.4 ± 28.49), 3 (317.2 ± 30.20), 9 (305.2 ± 20.12), and 27 mg/kg (242.0 ± 26.78) decreased MDA content compared to saline (454.0 ± 28.35) [F (4, 35)=8.189; p<0.0001]. In the corpus striatum, EP-Lf at 3 (296.8 ± 30.93), 9 (304.0 ± 15.76), and 27 mg/kg (257.0 ± 13.72) reduced MDA compared to saline (460.2 ± 28.38) [H = 22.21; p = 0.0002; k = 5]. (**Fig. 2B**).

Evaluation of Anticonvulsant Activity and Mechanism of EP-Lf

PTZ (85 mg/kg) induced seizures in all animals. From the animals treated with EP-Lf at 27 mg/kg (n=10), 4 survived, and from those treated with DZP at 1 mg/kg (n=8), 6 survived. EP-Lf at 27 mg/kg increased both seizure latency (112.9 ± 6.87 *vs.* saline: 61.40 ± 3.55) and death latency (941.6 ± 74.20 *vs.* saline: 265.5 ± 42.54). DZP (1 mg/kg) protected both seizure latency (121.1 ± 10.97) and death latency (1046 ± 101.4).

To evaluate the EP-Lf mechanism of action, flumazenil (FLU 2.5 mg/kg), a selective benzodiazepine antagonist, when associated with EP-Lf, at its most effective dose (27 mg/kg), was able to reverse the effect of both seizure latency (76.60 ± 5.70) [H = 45.57; p < 0.0001; k = 8] and death latency (340.7 ± 30.92) compared to saline. This reversal effect was confirmed by a group treated with FLU alone and induced with PTZ, which showed a decrease in both seizure latency (42.63 ± 6.42) and death latency (219.0 ± 33.48) [H = 43.55; p < 0.0001; k = 8] (Table 1).

Table 1 Effect of EP-Lf on Seizure latency and death in the PTZ-induced chemical seizures

Group (mg/kg)	Seizure Latency	Animals exhibiting Seizure (%)	Latency of Death (s)	Survivor Animals (%)
0,9% Saline	61,4 ± 3,55 (10)	100	265,5 ± 42,54 (10)	0
EP-Lf-1	56,9 ± 4,63 (10)	100	341,0 ± 34,34 (10)	0
EP-Lf-3	60,6 ± 7,15 (10)	100	304,3 ± 38,20 (10)	0
EP-Lf-9	60,1 ± 3,19 (10)	100	348,3 ± 56,48 (10)	0
EP-Lf-27	112,9 ± 6,87 (10) * ####	100	941,6 ± 74,20 (10) ** ### +	40
FLU-2,5+EP-Lf-27	76,9 ± 5,70 (10)	100	334,6 ± 33,88 (10)	0
FLU-2,5	42,63 ± 6,42 (08)	100	219,0 ± 33,48 (08)	0
DZP-1	121,1 ± 10,97 (08) *	100	1046 ± 101,4 (08) **	75

Mean ± SEM (n=10; in groups FLU and DZP: n=8). Kruskal-Wallis followed by Dunn's post-test. *p<0.05 *vs.* saline (*p<0.05; **p<0.01). #p<0.05 *vs.* FLU 2,5 mg/kg (###p<0.001; ####p<0.0001). +p<0.05 *vs.* FLU 2,5 mg/kg + EP-Lf 27 mg/kg (+p<0.05). DZP: Diazepam; EP-Lf: Polysaccharide extract of *L. ferrea* barks; FLU: Flumazenil; PTZ: Pentylenetetrazole.

Effect of EP-Lf on Oxidative Stress Markers in Acute Seizure

Reduced Glutathione (GSH)

In the prefrontal cortex, EP-Lf at the doses of 1 (1163 ± 40.46), 3 (1222 ± 78.50), 9 (1231.0 ± 130.2), and 27 (1572 ± 68.06) mg/kg increased GSH content compared to saline (632.2 ± 35.95) [H = 38.38; p < 0.0001; k = 6]. In the hippocampus, EP-Lf at 1 (1144 ± 22.62), 3 (1295 ± 104.6), 9 (1439 ± 95.01), and 27 (1483 ± 88.60) mg/kg increased GSH compared to saline (760.6 ± 85.50) [F (5, 52)=15.88; p<0.0001]. In the corpus striatum, EP-Lf at all doses: 1 (1118 ± 21.50), 3 (1313 ± 105.7), 9 (1474 ± 87.35), and 27 (1691 ± 85.33) mg/kg also increased GSH compared to saline (735.2 ± 39.44) [F (5, 52)=25.19; p<0.0001]. Flumazenil did not alter GSH in prefrontal cortex (867.3 ± 47.09), hippocampus (783.0 ± 36.74), or corpus striatum (852.8 ± 35.54), compared to saline (**Fig. 2C**).

Lipid Peroxidation (MDA)

In the prefrontal cortex, EP-Lf only at 9 (229.4 ± 11.70) and 27 (117.7 ± 7.75) mg/kg reduced MDA content compared to saline (507.3 ± 59.97) [H = 38.24; p < 0.0001; k = 6]. In the hippocampus, EP-Lf at all doses decreased MDA: 1 (188.1 ± 12.44), 3 (216.2 ± 12.61), 9 (201.8 ± 15.25), and 27 mg/kg (146.2 ± 9.189) compared to saline (359.9 ± 39.33) [F (5, 54)=14.64; p<0.0001]. In the corpus striatum, EP-Lf at all doses also reduced MDA: 1 (190.0 ± 10.34), 3 (199.5 ± 12.13), 9 (164.3 ± 18.26), and 27 mg/kg (152.2 ± 19.11) compared to saline (356.6 ± 35.04) [H = 35.51; p < 0.0001; k = 6].

Flumazenil (2.5 mg/kg) reduced MDA in the hippocampus (248.6 ± 5.437), but not in the prefrontal cortex (322.0 ± 10.91) or corpus striatum (254.8 ± 12.01) in comparison with saline (Fig. 2D)

Nitrite (NO_2-) Quantification

EP-Lf did not alter nitrite content in all brain areas at any doses tested compared to saline: Prefrontal cortex [H = 9.746; p = 0.0449, k = 5], Hippocampus [F (4, 45)=3.102; p=0.0245], Corpus striatum [F (4, 45)=2.765; p=0.0387] (**Fig. 2E**).

Histopathological Analysis

In the evaluation of black neurons in the hippocampus stained with H&E, a trend toward reduced pyknotic nuclei was observed in the group treated with EP-Lf at 27 mg/kg (Fig. 3).

Discussion

The results demonstrated that the polysaccharide extract from the barks of *Libidibia ferrea* (EP-Lf) exhibited an inhibitory effect on the central nervous system, as evidenced by the initial screening performed in behavioral tests (open field, elevated plus maze, hole-board, tail suspension). In addition, EP-Lf showed protective and antioxidant effects in the seizures induced by PTZ, along with qualitative improvement of black neurons. Moreover, the investigation of the mechanism of EP-Lf anticonvulsant effect suggested the involvement of the GABAergic pathway.

In the open field test, EP-Lf reduced exploratory activity at all doses tested, and the number of rearings at 9 and 27 mg/kg. The polysaccharide extracts from *Chamomilla recutita* (chamomile) (Chaves et al. 2020) and *Genipa americana* (Nonato et al. 2018) demonstrated reduced exploratory activity in this test, suggesting central inhibitory action and depressant effect. The reduction in the number of rearing elicited by EP-Lf is in line with the literature that establishes an association with the animal's exploration of a novel environment, being directly affected by drugs acting on the GABAergic pathway (Prut and Belzung 2003). Regarding grooming behavior, EP-Lf increased grooming only at the lowest dose (1 mg/kg). This effect is closely associated with emotional state (Masur et al. 1971; Morais et al. 1998), and also with the apprehensive behavior, since anxiolytic drugs typically reduce this behavior (Archer 1973; Dunn 1990; Prut and Belzung 2003).

In the hole-board test, EP-Lf at 27 mg/kg reduced the number of head dips and at all doses the dive time, suggesting an anxiogenic activity. In this line, the extracts from *Calophyllum inophyllum* (Ibironke and Ugege 2015) and *Lannea coromandelica* (Islam et al. 2022) demonstrated a reduction in head-dip frequency, corroborating our findings.

In the Elevated Plus Maze test, EP-Lf reduced both the number of entries and the time spent in the open arms. The polysaccharide extract of *Genipa americana* (Nonato et al. 2018) also reported a reduction in the time spent in the open arms and a decrease in the number of entries into the open arms.

In the tail suspension test, used to evaluate the antidepressant effect of drugs by measuring immobility time (Steru et al., 1985, 1987), EP-Lf (9 and 27 mg/kg) increased immobility time. An effect that had been shown in the polysaccharide extract of *Genipa americana* (Nonato et al. 2018).

The behavioral tests altogether demonstrated central inhibitory activity, suggesting an anxiogenic, rather than an anxiolytic and depressant effect for EP-Lf. Similar effects were demonstrated by the galactomannan from *Caesalpinia ferrea*, an extract from this plant, in which a different extraction method and part were used, but the tests used were similar (Craveiro et al. 2025).

Although the central nervous system represents near 2% of total body mass, it has a high metabolic rate, accounting for approximately 20% of energy consumption (Sure et al. 2018), leading to elevated production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Abdul-Muneer et al. 2015; Chen et al. 2020). After performance of the behavioral tests, the levels of GSH were significantly increased by EP-Lf in prefrontal cortex (at 9 and 27 mg/kg) and in hippocampus at all doses, and that of MDA reduced in prefrontal cortex (27 mg/kg), hippocampus (all doses), and corpus striatum (3 and 27 mg/kg), confirming the EP-Lf antioxidant efficacy. In the study of Craveiro and collaborators (2025), using a similar model, the galactomannan of *Caesalpinia ferrea* decreased MDA, while that of GSH was unaltered.

The results obtained in the behavioral tests and the quantification of GSH and MDA provide the first evidence of EP-Lf inhibitory and protective action on the central nervous system, and possibly anxiogenic and depressant effect. These effects are highly relevant in conditions of neuroinflammation, such as seizures and epilepsy.

Based on the results obtained from the behavioral screening and oxidative stress parameters, we proceeded with the evaluation of EP-Lf in the acute seizures induced by pentylenetetrazol PTZ, an antagonist of the gamma-aminobutyric acid type A (GABA-A) receptor, widely used to test antiepileptic properties of new drugs in animal models (Löscher 2002; Cavarsan et al. 2015). The convulsive state induced by PTZ begins with its influence on the release and postsynaptic action of the GABA-A receptor, leading to the activation of multiple synaptic mechanisms mediated by three major ionotropic glutamate receptors: NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and kainate (Dhir et al. 2005). Studies suggest that excitatory amino acids contribute to the convulsant effects of PTZ, and NMDA receptor activity may be associated with reduced GABAergic neurotransmission (Löscher and Potschka 2002). Our results showed that EP-Lf at 27 mg/kg was effective against PTZ-induced seizures, as seen by the increased seizure and death latencies, demonstrating its protective action. Studies using similar seizure models found a significant increase in these parameters by the galactomannan from *Caesalpinia ferrea* (Craveiro et al. 2025) and the polysaccharide-rich extract from *Genipa americana* (Nonato et al. 2018).

It is well known that in the central nervous system, benzodiazepines may bind to an allosteric site on the GABA-A receptor, enhancing pre- and postsynaptic inhibitory transmission (Katzung and Trevor 2017), thereby producing an anticonvulsant effect. Administration of flumazenil, a BZD receptor antagonist, exerts a pro-convulsant effect (Schulze-Bonhage and Elger 2000). EP-Lf at 27 mg/kg, and when co-administered with flumazenil, had its protective effect reversed, suggesting involvement of the GABAergic system.

Inflammation and oxidative stress are recognized as critical factors in the pathophysiology of epilepsy (Ngugi et al. 2010; Aguiar et al. 2012), a result of the imbalance between formation and elimination of reactive oxygen species (ROS) (Aguiar et al. 2012). Mitochondrial dysfunction and oxidative stress are involved in neuronal death and seizure activity. Oxidative damage also affects proteins, lipids, and mitochondrial DNA following convulsive episodes (Kong and Lin 2010), being implicated in various neurological disorders, including Parkinson's and Alzheimer's diseases, and plays a key role in several epilepsy models (Ali and Blunden 2003; Manoharan et al. 2016).

In our study, EP-Lf at all tested doses was able to increase GSH content and to reduce MDA levels. Using a similar seizure model, the galactomannan of *Caesalpinia ferrea* (Craveiro et al. 2025) promoted similar results on GSH and MDA content. Also, a previous study in other animal models had demonstrated that the crude polysaccharide extract of *C. ferrea* reduced MDA levels and the preventive depletion of total GSH during oxidative stress in a zymosan-induced rheumatoid arthritis model (Falcão et al. 2019b). The crude extract and fractions from *L. ferrea* fruits reduced the levels of MDA and increased those of GSH in the carrageenan-induced peritonitis model (Falcão et al. 2019a). Oxidative stress can also be triggered in diabetic conditions via excessive production of free radicals. Hassan et al. (2015) reported that the ethanolic aqueous extract from *C. ferrea* leaves increased GSH-Px, SOD, and CAT activity, as well as GSH levels in the liver and pancreas of streptozotocin-induced diabetic rats. Protective effects had also been demonstrated for *L. ferrea* in various inflammation models, as a neuroprotective effect in the present study for EP-Lf in the model of PTZ-induced chemical seizures.

The hippocampus plays a crucial role in learning and memory, and PTZ-induced seizures can result in neuronal damage, subsequently impairing these functions (Kaur et al. 2015; Gao et al. 2018; Özdemir et al. 2019). Such neuronal damage and loss in the hippocampus lead to behavioral changes, including reduced exploratory locomotor activity and increased seizure severity in PTZ-treated rats (Franke and Kittner 2001). Therefore, the use of a substance with protective effects can have a significant impact on preserving the hippocampal structure, as confirmed by the *per se* effect of EP-Lf in the acute seizure model. Qualitatively, EP-Lf showed a trend toward reduced neuronal pyknosis in HE-stained hippocampal sections. It is well established that PTZ-induced seizures can cause neural damage and lesions, such as pyknosis, in the hippocampus (Huang et al. 2002; Mortazavi et al. 2005; Fang and Lei 2010). In the same experimental model, a polysaccharide-rich extract from *Genipa americana* leaves reduced the number of black neurons in HE-stained hippocampal

sections (Nonato et al. 2018). Besides, the brain areas hippocampus and cortex stained with H&E from animals treated with the hydroalcoholic extract of *Argemone mexicana* showed improvement in neurodegeneration, with fewer pyknotic nuclei (Gahlot and Yadav 2021).

The results obtained in our study open up new perspectives for the EP-Lf therapeutic use in the treatment of seizures. Thus, further elucidation of the mechanisms involved, as well as evaluation of the long-term use and possible toxic effects, is extremely important for its safe use by the population.

Conclusion

The polysaccharide extract of *Libidibia ferrea* barks (EP-Lf) exhibited a protective effect on the-chemically induced seizures by PTZ. Regarding the expression of black neurons, animals treated with EP-Lf at 27 mg/kg showed a trend toward reduced pyknotic nuclei. Animals that had developed PTZ-induced seizures and were treated with EP-Lf at its most effective dose in combination with flumazenil exhibited reversal of the protective effect, suggesting a possible GABAergic mechanism of action for EP-Lf. Antioxidant effect of EP-Lf was observed in PTZ-induced seizures across the brain regions, prefrontal cortex, hippocampus, and striatum.

Declarations

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Conflicts of interest

The authors declare no conflict of interest related to the study.

Ethical approval

The study was conducted in accordance with the standards recommended by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee for the Use of Animals at the State University of Ceará (CEUA/UECE), under protocol number: 510620/2020.

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Figures

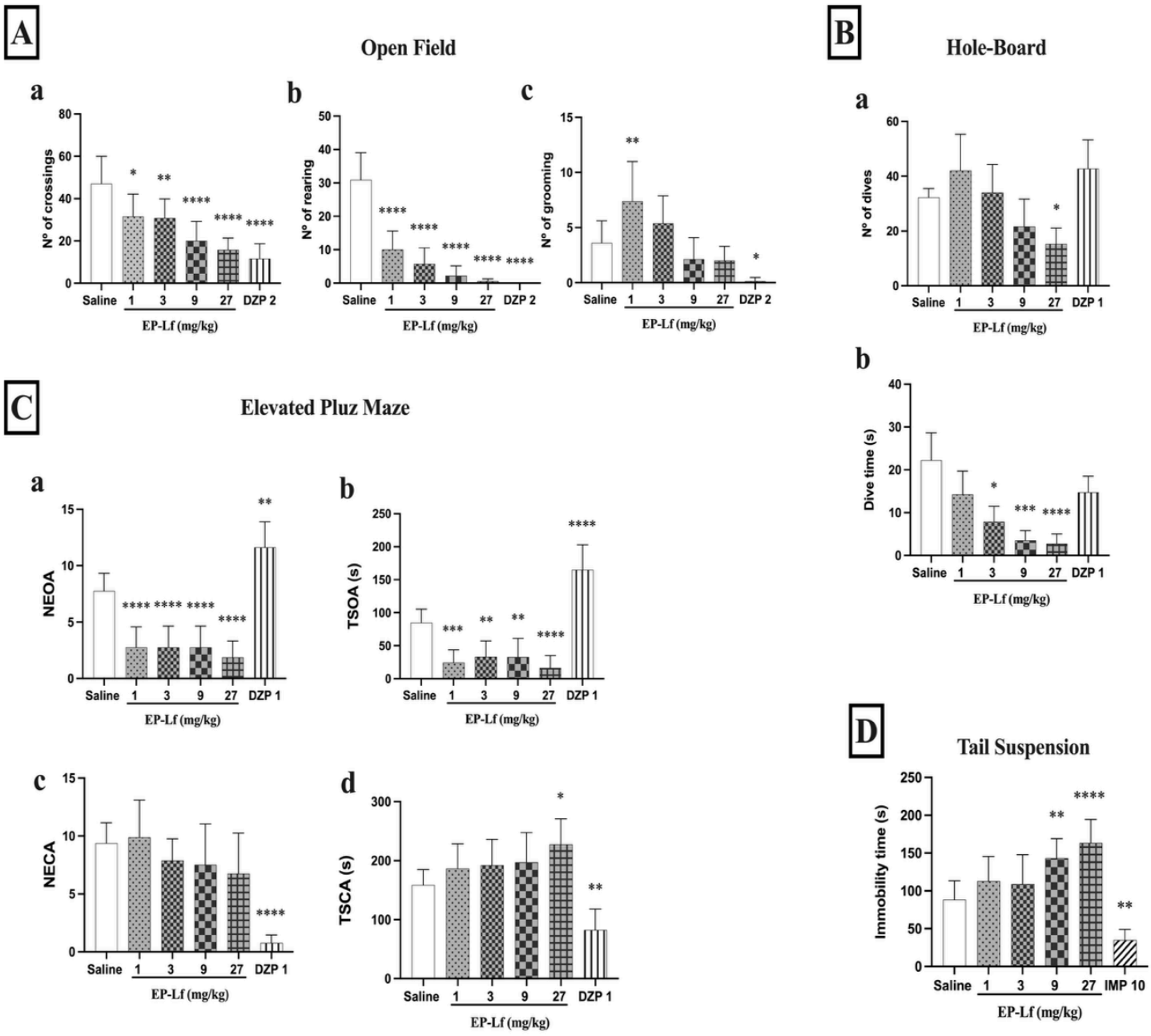


Figure 1

Effect of EP-Lf on Behavioral tests. **(A)** Open Field: n° of crossings (a), n° of rearings (b), n° of grooming (c); **(B)** Hole-Board: n° (a) and time (b) of dives; **(C)** Plus Maze - Open Arms: n° of entries - NEOA (a), and time spent - TSOA (b); Closed arms: n° of entries - NECA (c) and time spent - TSCA (d); **(D)** Tail Suspension: immobility time. Mean ± SEM (n = 8). ANOVA and Tukey's post-test: A (a); B (a); C (a,b,c); D. Kruskal-Wallis and Dunn's post-test: A (b,c); B (b); C (d). *p<0.05 *vs.* saline (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001). EP-Lf. DZP: diazepam (1=1 mg/kg, 2=2 mg/kg), EP-Lf. Polysaccharide extract of *L. ferrea* barks; IMP: imipramine (10=10 mg/kg), NECA: n° of Entries into Closed Arms; NEOA: n° of Entries into Open Arms; TSCA: Time Spent in Closed Arms; TSOA: Time Spent in Open Arms.

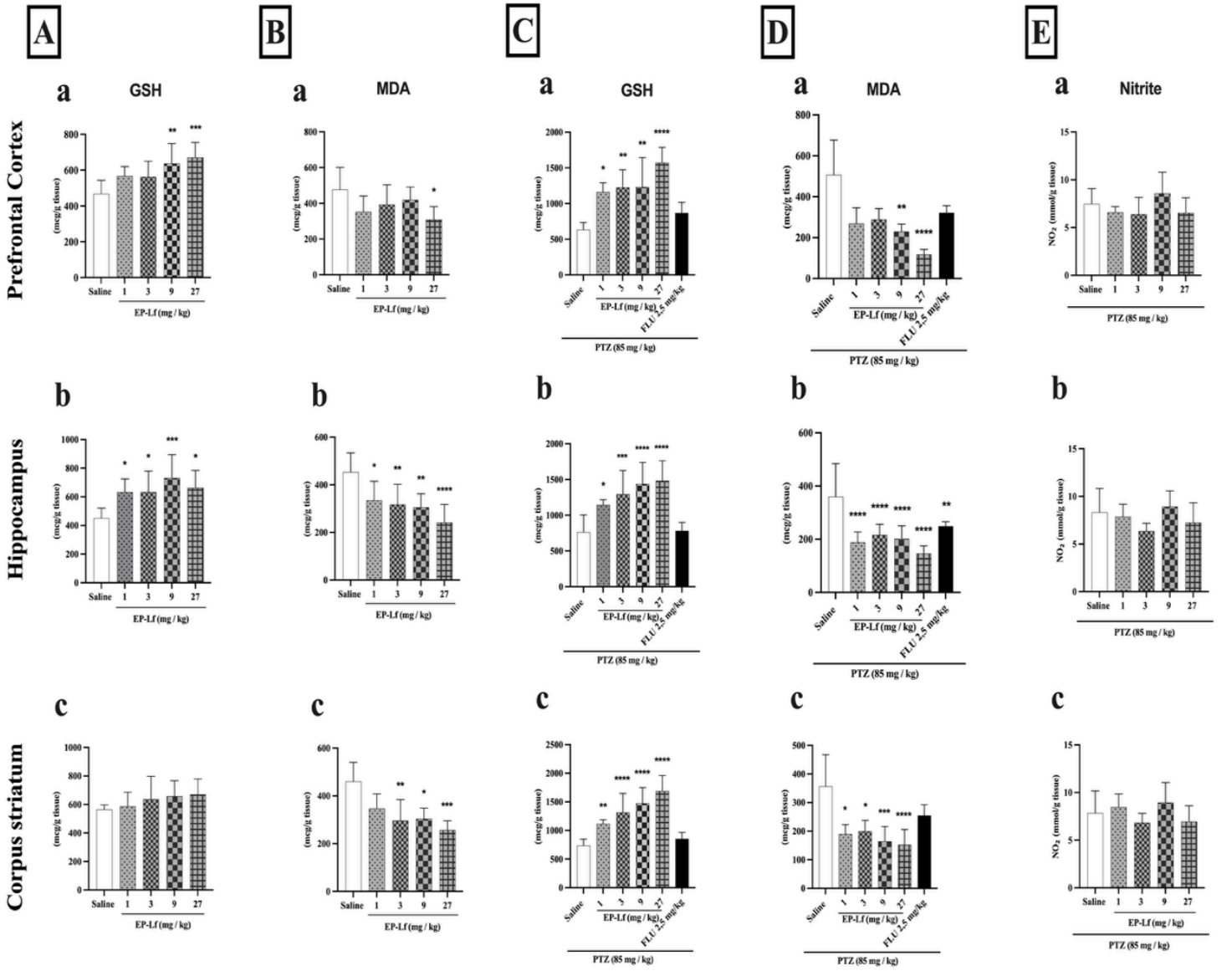


Figure 2

Effect of EP-Lf on oxidative stress markers GSH (**A, C**), MDA (**B, D**), and Nitrite (**E**) content: *PER SE* (**A, B**) and PTZ-induced chemical seizures (**C, D, E**), in prefrontal cortex (a), hippocampus (b), and striate corpus (c). Mean ± SEM (A,B: n=8; C,D,E: n=10). ANOVA followed by Tukey's post-test: A (a,c); B (b); C (b,c); D (b); E (b,c). Kruskal-Wallis followed by Dunn's post-test: A (a); B (a,c); C (a); D (a,c); E (a). *p<0.05 *vs.* saline. #p<0.05 *vs.* FLU. EP-Lf: Polysaccharide extract of *L. ferrea* barks; FLU: Flumazenil; GSH: Reduced glutathione; MDA: Malondialdehyde; NO_2^-: nitrite; PTZ: Pentylenetetrazole.

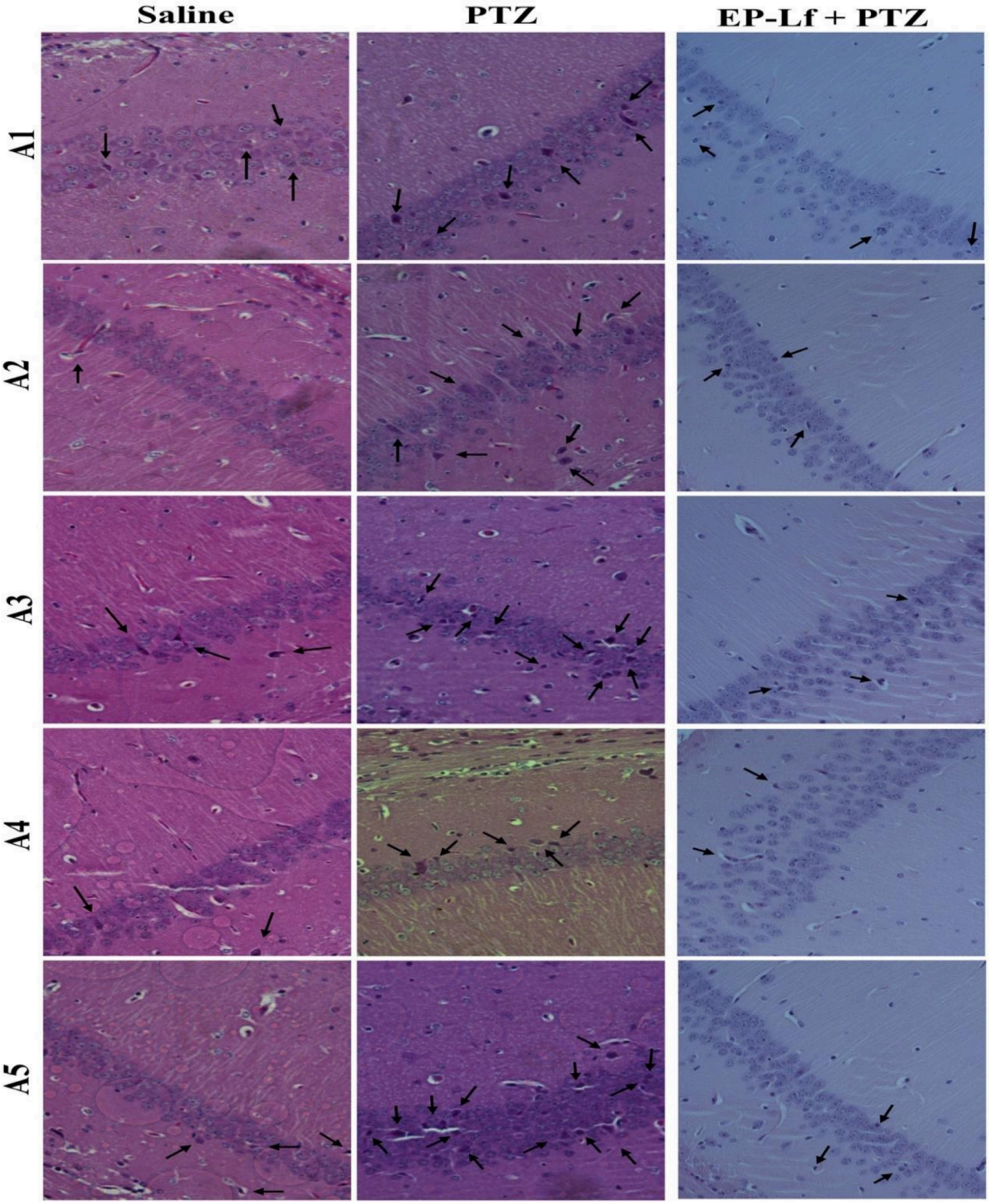


Fig.3 Effect of EP-Lf on the number of black neurons in the hippocampus in the PTZ-induced chemical seizure. The arrow indicates a black neuron. Stained with HE. A: animal; EP-Lf: Polysaccharide extract of *L. ferrea* barks; HE: Hematoxylin and Eosin; PTZ: Pentylenetetrazole.

Figure 3

See image above for figure legend