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Neuroprotective effect of *Mauritia flexuosa* on a unilateral 6-OHDA-induced Parkinson's disease in rats

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Summary

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and cognitive impairments, primarily due to the progressive loss of dopaminergic neurons in the substantia nigra. While there is currently no treatment to halt neuronal loss, evidence suggests that a diet rich in antioxidants may mitigate oxidative stress and disease progression. **Aims:** This study sought to investigate the neuroprotective effects resulting from the oral administration of ethanolic extract of *Maurita flexuosa* over a 21-day period, at doses of 1 mg/kg, 10 mg/kg, and 100 mg/kg. **Methods:** Initially, antioxidant activity assays revealed significant levels of various antioxidants, including β-carotene, gallic acid equivalent, and quercetin. A PD animal model was then induced via stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the striatum, with apomorphine-induced rotation tests used for model validation. Motor behavior assessments were performed using open field tests and beam walking tests. **Results:** The open field test indicated improved motor behavior in the 1 mg/kg group compared to the 6-OHDA group. However, neurohistological analysis via Western blot testing revealed potential neurotoxic effects

associated with the 100 mg/kg treatment dose. **Conclusions:** Chronic administration of 1 mg/kg of ethanolic extract of *Maurita flexuosa* over 21 days demonstrated potential improvements in locomotion in a 6-OHDA-induced PD model. Nonetheless, a notable limitation of the study lies in the 6-OHDA model's failure to induce the expected level of damage in the striatum and substantia nigra, achieving only 29% damage, whereas a total PD model typically requires 70% damage for optimal replication.

Keywords: Mauritia flexuosa; antioxidant; neuroprotector; Parkinson Disease

Resumen

Efecto neuroprotector de *Mauritia flexuosa* en la enfermedad de Parkinson unilateral inducida por 6-OHDA en ratas

Introducción: La enfermedad de Parkinson (EP) es un trastorno neurodegenerativo caracterizado por deterioro motor y cognitivo, principalmente debido a la pérdida progresiva de neuronas dopaminérgicas en la sustancia negra. Si bien actualmente no existe un tratamiento para detener la pérdida neuronal, la evidencia sugiere que una dieta rica en antioxidantes puede mitigar el estrés oxidativo y la progresión de la enfermedad. **Objetivos:** Este estudio buscó investigar los efectos neuroprotectores resultantes de la administración oral de extracto etanólico de *Maurita flexuosa* durante un período de 21 días, en dosis de 1 mg/kg, 10 mg/kg y 100 mg/kg. **Métodos:** Inicialmente, los ensayos de actividad antioxidante revelaron niveles significativos de varios antioxidantes, incluidos β-caroteno, equivalente de ácido gálico y quercetina. A continuación, se indujo un modelo animal de EP mediante inyección estereotáxica de 6-hidroxidopamina (6-OHDA) en el cuerpo estriado, y se utilizaron pruebas de rotación inducidas con apomorfina para la validación del modelo. Se realizaron evaluaciones del comportamiento motor mediante pruebas de campo abierto y pruebas de marcha sobre vigas. **Resultados:** La prueba de campo abierto indicó una mejora del comportamiento motor en el grupo de 1 mg/kg en comparación con el grupo de 6-OHDA. Sin embargo, el análisis neurohistológico mediante pruebas de transferencia Western reveló posibles efectos neurotóxicos asociados con la dosis de tratamiento de 100 mg/kg. **Conclusiones:** La administración crónica de 1 mg/kg de extracto etanólico de *Maurita flexuosa* durante 21 días demostró posibles mejoras en la locomoción en un modelo de EP inducido por 6-OHDA. No obstante, una limitación notable del estudio radica en la incapacidad del modelo de 6-OHDA para inducir el nivel esperado de daño en el cuerpo estriado y la sustancia negra, logrando

solo un 29% de daño, mientras que un modelo de EP total normalmente requiere un 70% de daño para una replicación óptima.

Palabras clave: Mauritia flexuosa; antioxidante; neuroprotector; enfermedad de Parkinson

Resumo

Efeito neuroprotetor de *Mauritia flexuosa* em uma doença de Parkinson unilateral induzida por 6-OHDA em ratos

Introdução: A doença de Parkinson (DP) é uma doença neurodegenerativa caracterizada por deficiências motoras e cognitivas, principalmente devido à perda progressiva de neurônios dopaminérgicos na substância negra. Embora atualmente não haja tratamento para interromper a perda neuronal, as evidências sugerem que uma dieta rica em antioxidantes pode mitigar o estresse oxidativo e a progressão da doença. Objetivos: Este estudo buscou investigar os efeitos neuroprotetores resultantes da administração oral de extrato etanólico de *Maurita flexuosa* durante um período de 21 dias, em doses de 1 mg/kg, 10 mg/kg e 100 mg/kg. **Métodos:** Inicialmente, os ensaios de atividade antioxidante revelaram níveis significativos de vários antioxidantes, incluindo β-caroteno, equivalente de ácido gálico e quercetina. Um modelo animal de DP foi então induzido por injeção estereotáxica de 6-hidroxidopamina (6-OHDA) no estriado, com testes de rotação induzidos por apomorfina usados para validação do modelo. Avaliações do comportamento motor foram realizadas usando testes de campo aberto e testes de caminhada em viga. **Resultados:** O teste de campo aberto indicou comportamento motor melhorado no grupo de 1 mg/kg em comparação ao grupo de 6-OHDA. No entanto, a análise neuro-histológica por meio de testes de Western blot revelou potenciais efeitos neurotóxicos associados à dose de tratamento de 100 mg/kg. **Conclusões:** A administração crônica de 1 mg/kg de extrato etanólico de *Maurita flexuosa* ao longo de 21 dias demonstrou potenciais melhorias na locomoção em um modelo de DP induzido por 6-OHDA. No entanto, uma limitação notável do estudo está na falha do modelo de 6-OHDA em induzir o nível esperado de dano no estriado e na substância negra, atingindo apenas 29% de dano, enquanto um modelo de DP total normalmente requer 70% de dano para replicação ideal.

Palavras-chave: Mauritia flexuosa; antioxidante; neuroprotetor; Doença de Parkinson

Introduction

Parkinson's disease (PD) is a clinically heterogeneous neurodegenerative disease of adult onset [1]. PD is characterized by 4 main motor symptoms: resting tremor (with a frequency between 4 and 6 Hz), bradykinesia, rigidity and postural instability [1-3]. The prevalence of PD is currently high, and is predicted to double in size by 2040 [4], making it the fastest growing neurodegenerative disease [5]. From the neurohistopathological point of view, PD is characterized by a progressive loss of dopaminergic neurons in the pars compacta of the substantia nigra and its projection towards the striatum due to toxicity resulting from the accumulation and deposition of misfolded alpha synuclein at the intracellular level [6, 7]. Symptoms start on one side of the body when dopamine concentrations decline below 60 to 70% in the contralateral striatum [8]. One hypothesis for the etiology of PD is mitochondrial dysfunction and oxidative stress [7].

Antioxidants play a crucial role in mitigating oxidative damage caused by reactive oxygen species (ROS) and protecting against neurophysiological abnormalities [9, 10]. They act by decreasing the concentration of oxidants, binding to metal ions to prevent the formation of ROS, decreasing the reactivity of peroxides and decreasing the propagation and creation of free radicals [11].

Mauritia flexuosa, commonly known as buriti, moriche or aguaje, is a fruit with a high content of fats, proteins and vitamins (high content of B-carotene, vitamin A and tocopherol) [12-14]. In a study it was found a high level of antioxidants, especially in the pulp and peel extract with a concentration of 378.07 mg GAEq/100 g of phenols, 567.16 mg GAEq/100 g of flavonoids respectively, which is low in comparison to other fruits due to the high level of carotenoids; and in an extract of the pulp it was found 9.47 mg/100 g of total polyphenols and 23.36 mg/100 g of carotenoids [15, 16]. The main carotenoids found were β carotene at 60% and α carotene at 6% [16].

Therefore, the consumption of *Mauritia flexuosa* (aguaje) could act as a significant overprotective agent against the progression and as a preventive measure of PD, since it would act at the level of free radical depletion due to its high antioxidant content. The aim of the study was to evaluate the potential effect on locomotion and neuroprotection of 21 days oral administration (1 mg/kg, 10 mg/kg and 100 mg/kg) of ethanolic extract of *Mauritia flexuosa* of the morphotype "Color". *Mauritia flexuosa* administration is expected to improve motor performance and act as a neuroprotective agent at a substantia nigra level.

Material and methods

Extract preparation

The fruits of *Mauritia flexuosa* L.f. were of the "Color" morphotype. The preparation of the ethanolic extract and in vitro experiments were carried out at the Institute of Food Science and Nutrition of San Ignacio del Loyola University. The fruit was recollected from "Carretera Iquitos-Nauta" from Peruvian jungle.

β-carotene determination

The β-carotene content was carried out according to the methodology proposed by Zanqui *et al.* (2019) [17]. Approximately 100 mg of sample was placed in a conical tip centrifuge tube and then 5 mL of n-hexane was added. The extraction was conducted by vortexing at 1500 rpm for 30 min. Next, the extraction was continued in an ultrasound system at a frequency of 40 kHz, 30 °C and 30 min. The supernatant was obtained by centrifugation at 3500 rpm for 15 min. The absorbance value was made at 450 nm in a 1 cm optical path quartz cell using a Jasco V-770 spectrophotometer (Tokyo, Japan). To calculate the β-carotene content, the extinction coefficient of 2592 was considered. Values were expressed in mg β-carotene per gram.

Total polyphenols determination

Total polyphenol content was determined by the Folin-Ciocalteu method. An approximate amount of 500 mg of sample was extracted with 80% methanol. The polar fraction was extracted with an ultrasonic bath and subsequently centrifuged at 3500 rpm for 15 min. The supernatant was analyzed with Folin-Ciocalteu reagent and 7.5% sodium carbonate [18]. The blue color formed was read at 765 nm using a Jasco V-770 spectrophotometer. The results were expressed in mg of gallic acid equivalent per gram of sample.

Determination of antioxidant activity by TEAC and FRAC

The evaluation of antioxidant activity was carried out by TEAC and FRAP assay [19, 20]. For the TEAC assay, 20 μL of the extract and 980 μL of the ABTS radical were used. Readings were collected at 734 nm, after 5 min of reaction. The results were expressed as mmol of trolox equivalent/g sample.

For the FRAP assay, 120 μL of sample and 750 μL of FRAP reagent were used, which consisted of 25 mL of aqueous solution adjusted to pH 3.6, 2.5 mL of 10 mM ferric-2,4,6-tripyridyl-s-triazine complex (TPTZ) in 40 mM HCl and 2.5 mL of 20 mM

$FeCl_3$ in water. The absorbance reading was recorded at 593 nm after 5 min of reaction. The results were expressed as mmol $FeSO_4$/g.

Phenol profiling by HPLC-DAD

Phenol profiling was conducted by liquid chromatography with diode array detector (Hitachi Chromaster™, High-Technologies Corporation, Tokyo, Japan). Separation of the different analytes was performed on a LiChrospher® 100 RP-18 (5 μm) column (Merck KGaA, Germany). Mobile phase A consisted of a mixture of water/orthophosphoric acid and an equal parts methanol/acetonitrile mixture (B). The analytes were eluted according to the following gradient system (only the values of phase B are presented and the difference corresponds to phase A). The spectrum was recorded 280 nm and the injection volume was 20 μL. The results were obtained by comparison of the respective standards. The content of each phenol was expressed in g/kg of sample.

Maintenance and care of animals

21 Sprague-Dawley male rats, 2.5 months old and weighing 250 g – 300 g were used. The animals were kept in PET plastic cages with dimensions of 37.3 centimeters long, 23.4 centimeters wide and 14 centimeters high in groups of 4 rats. Cleaning was performed daily by renewing the cage shavings. The animals are from the automated Bioterio of the Universidad Andina del Cusco. The cages were kept in an automated environment with a 12-hour light/dark cycle with the light period starting at 6:00 am, temperature between 23 and 25 °C and relative humidity between 40 and 45%. Balanced pelleted feed and water were administered ad libitum. *Mauritia flexuosa* was administered by direct cannulation to the rat from day 1 to day 21. A 75-mm long cannula with a 2.3-mm diameter tip attached to a syringe was used and passed through the esophagus into the stomach, where the substance was expelled at a slow controlled rate avoiding regurgitation.

All procedures were performed under the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and with the approval of Cayetano Heredia University Institutional Animal Care and Use Committee (Protocol #2017-09) [21].

Injection with 6OHDA

The animal was anesthetized via intraperitoneal injection with Ketamine (70 mg/kg) and Xylazine (6 mg/kg). Prior to the procedure, the animal underwent a fasting period of 4 to 6 hours. Unilateral intracranial injection of 6-OHDA was performed on the right side using a concentration of 4 mg/mL and a volume of 4 mL in 0.02% ascorbic acid-saline [22]. In the negative control group, 5 mL of 0.02% ascorbic acid-saline was administered. The infusion rate was maintained at 1 mL/min, with the needle remain-

ing in place for 3 minutes before being slowly withdrawn. Injection coordinates were set at anteroposterior +1.0 mm, lateral +3.2 mm, and dorsal ventral -4.6 mm from the bregma [23]. Following the procedure, Dermicare was applied to prevent infections. Animals were closely monitored until they regained consciousness (45-90 minutes), after which they received individual care for two days before being grouped in sets of three rats.

Open field Test

The Open Field behavioral test was performed to measure levels of general locomotor activity and anxiety following the methodology of Shi *et al.* (2006) [24] with modifications. The test is performed on a 50 × 50 cm black acrylic square and a 50 cm wall around it. The square is divided into 4 sub-squares (2 × 2). Prior to the test the rats were habituated to the environment for 2 days for two hours and on the day of the test the rats were acclimatized for 60 minutes. The rat was placed in the center of the square and its behavior was recorded for 5 minutes with a Logitech BRIO webcam with 4K Ultra UH video with EthoVision XT 15 software.

Crossbar Test

This test consists of placing the rat in the middle of a horizontal bar and evaluating its ability to cross it to one of the two side platforms located at the ends of the bar. The bar has a diameter of 2.5 cm and a length of 60 cm. The platforms have a surface of 15 cm × 20 cm. The test has 6 trials per rat and a maximum duration of 60 seconds. The variables analyzed are: platform arrival latency, fall latency, number of falls, number of leg and tail errors and number of balance errors.

Apomorphine Test

The rotation test was performed to test the effects of injury based on the severity of motor behavior disorder by monitoring contralateral and ipsilateral reactions to unilateral injury with 6-OHDA. The methodology was collected from Wu *et al.* and animals will be given 0.25 mg/kg apomorphine in 0.5% ascorbic acid and saline subcutaneously. Rotations were measured at 5 and 10 minute intervals until minute 30.

Western Blot

Microdissection technique employing micropunch was utilized. Encephalons were extracted and immediately frozen in dry ice at -80 °C, followed by sectioning using a cryostat at 500 mm thickness. The frozen sections were then centrifuged along with Mammalian Lysis Buffer to release cellular content. Protein content quantification was carried out using a spectrophotometer (NanoDrop) with 1 mL of supernatant. For the Western Blot assay, polyacrylamide gels were prepared and waited 30 minutes for

gelation. Then the electrophoresis chamber was assembled and Running buffer 1X was added avoiding the formation of bubbles. 20 mL of pre-treated samples were placed and a constant voltage (100 V to 150 V) was administered.

Protein transfer from the gel to the nitrocellulose paper was achieved by immersing the materials in Towbin buffer, positioning them from the cathode (-) to the anode (+) at a constant amperage of 400 mA for 1 hour. In order to avoid unspecific binding of the antibody to the nitrocellulose paper, the protein-free spaces were blocked with molecular grade skimmed milk. Subsequently, the preparation of the polyclonal IgG anti-thyroxine hydroxylase antibody made in rabbits (Merck brand, code Ab152) was carried out and kept in incubation overnight at 4 °C. The next day washing was performed with 20 mL of PBS + Tween 20 0.1%. Biotinylated secondary antibody made in goat Anti-Rabbit (Invitrogen brand, code 656140) was incubated under agitation. Finally, it was incubated with ABC complex solution according to the instructions of the kit (Vectastain) and developed with the DAB kit. Subsequent measurement was performed using ImageJ software to quantify the optical density of protein expression.

Statistical Analysis

Since all variables were numerical, the means of the variable values were compared between groups using analysis of variance (ANOVA Tukey) if the data were normally distributed. If the data did not follow a normal distribution, the Kruskal-Wallis test was employed. All numerical data were analyzed using the STATA 15 program. Statistical significance was set at p-values less than 0.05.

Results

Antioxidant Activity

In order to identify the potential contributor(s) of antioxidant activity from the pulp of *Mauritia flexuosa*, β-carotene, total polyphenols, antioxidant activity by TEAC and FRAC Phenol profiling by HPLC-DAD were performed.

Table 1. Quantification of antioxidant activity

Antioxidant activity	Total content
mg β-caroten/g	0.68±0.02
mg equivalent gallic acid	4.11±0.20
TEAC (mmol TE/g)	21.85±0.84
FRAP (mmol $FeSO_4$/g)	36.28±0.70

(*Continued*)

Table 1. *Continuation.*

Antioxidant activity	Total content
3-4-dihidroxi benzoic acid (g/kg)	8.74±0.33
Chlorogenic acid (g/kg)	172.98±5.95
Caffeic acid (g/kg)	32.84±0.88
Syringic acid (g/kg)	0.68±0.02
Vainillin (g/kg)	3.57±0.03
Ferulic acid (g/kg)	17.30±0.15
Sinapic acid (g/kg)	1.88±0.03
Rutin (g/kg)	12.27±0.10
Quercetin (g/kg)	4.83±0.07

Motor behavior

Significant differences were observed in the Open Field Test variables, including speed, distance, immobility time, crossing between quadrants, and cumulative grooming at 14 days between the group administered with 1 mg/kg and the 6OHDA group, as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). At 21 days, significant differences were found only in speed and distance between the 6OHDA and 1 mg/kg groups, as well as grooming for the sham and 6OHDA group (Figures 1 and 2).

For Beam Walking Test, significant differences were found in latency to the platform for 14 days and fall latency for 21 days according to one-way ANOVA followed by used Dunn Test with Benjamini-Hochberg correction (Figures 3 and 4). To analyze the difference within groups it was found for 14 days in platform latency difference between sham and 6OHDA ($p = 0.02$). At 21 days there were found differences between the sham and 6OHDA groups ($p = 0.04$) in fall latency.

For the rotation test, no significant differences were found, but there was a greater number of gyrus in the 6OHDA group. The animals that presented ipsilateral rotation were eliminated from the study. Rats 7, 14, 18, B2, C2, D2, D3 and E5 were eliminated from the study.

Molecular activity

For the western blot assay, brains from rats 6, 7 and 9 were used for the 6OHDA group; 12, 13 and 14 for the 10 mg group; and 17, 18 and 19 for the 100 mg group. The analysis was performed in both the affected and contralateral hemispheres to obtain a control. A damage of 29% was obtained in the 6OHDA group, 27% in the 10 mg group and 53% in the 100 mg group (Figure 5 and 6).

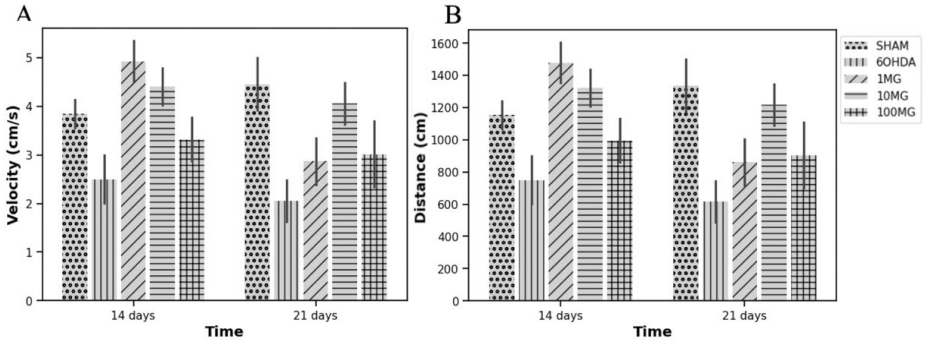

Figure 1. Determination of the effect of *Mauritia flexuosa* L.f. in velocity (A) and distance (B) in the open field test in animals pretreated with 6-OHDA. P < 0.01 (one-way ANOVA followed by Tukey's *post hoc* test). The figure shows an increased motor behavior in the experimental rats, being the 1 mg/kg group the one showing significance at 14 and 21 days.

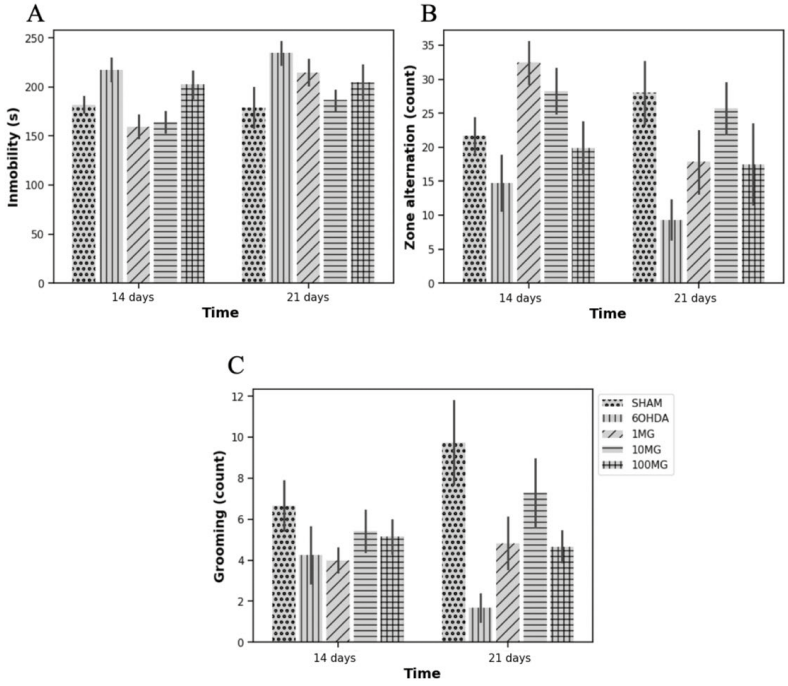

Figure 2. Determination of the effect of *Mauritia flexuosa* L.f. in immobility (A), zone alternation (B) and grooming (C) in the open field test in animals pretreated with 6-OHDA. P < 0.01 (one-way ANOVA followed by Tukey's *post hoc* test). The figure shows an increased motor behavior in the experimental rats, being the 1 mg/kg and sham group compared to the 6OHDA groups the ones showing significance for grooming at 21 days.

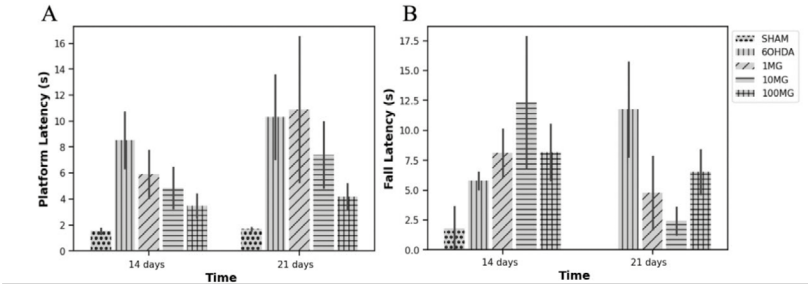

Figure 3. Determination of the effect of *Mauritia flexuosa* L.f. in platform latency (A) and fall latency (B) in the beam walking test in animals pretreated with 6-OHDA. $P < 0.01$ (one-way ANOVA followed by used Dunn Test with Benjamini-Hochberg correction).

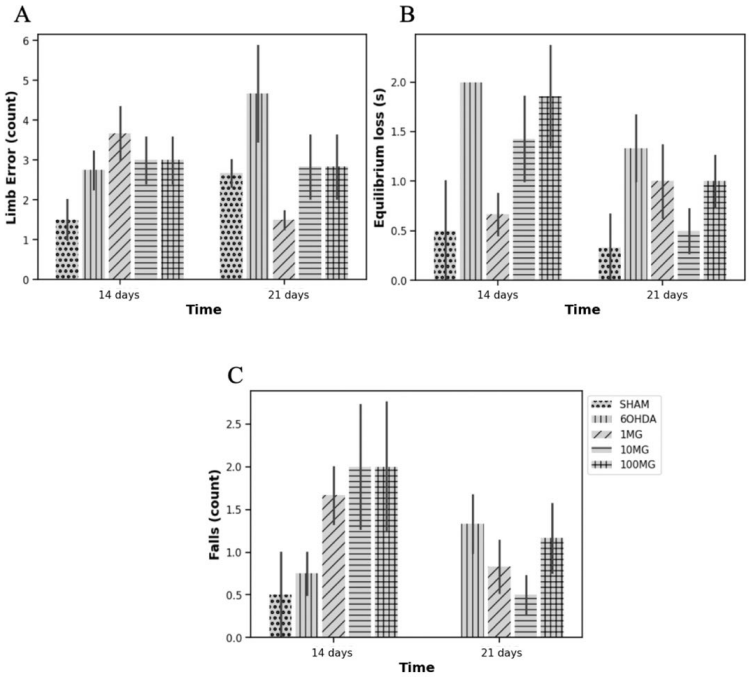

Figure 4. Determination of the effect of *Mauritia flexuosa* L.f. in errors: limb (A), equilibrium (B) and falls (C) in the beam walking test in animals pretreated with 6-OHDA. No significant differences found (one-way ANOVA followed by used Dunn Test with Benjamini-Hochberg correction).

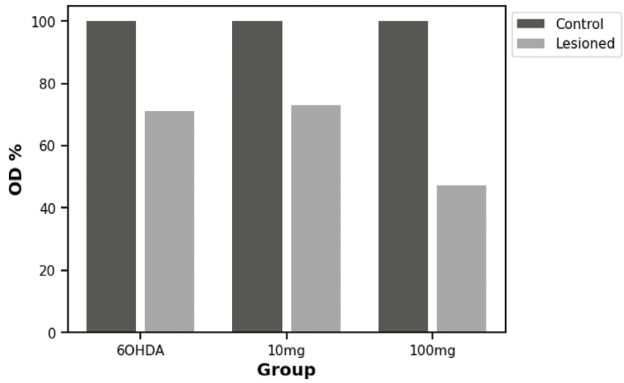

Figure 5. Western Blot percentages. Control is the side contralateral to the lesion.

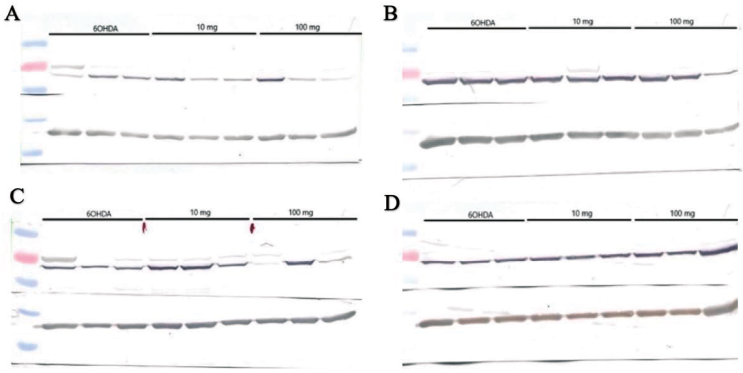

Figure 6. (A) Western Blot in 6OHDA substantia nigra. (B) Western Blot in control rats substantia nigra. (C) Western Blot in 6OHDA striatum. (D) Western Blot in control striatum.

Discussion

The study aimed to assess the potential impact of 21 days of oral administration (at doses of 1 mg/kg, 10 mg/kg, and 100 mg/kg) of ethanolic extract of *Mauritia flexuosa* (morphotype "Color") on locomotion and neuroprotection. The administration of *Mauritia flexuosa* was anticipated to enhance motor performance and act as a neuroprotective agent at the substantia nigra level. Antioxidant activity assessment revealed

significant levels of various components including β-carotene, gallic acid equivalent, 3-4-dihydroxy benzoic acid, Chlorogenic acid, Caffeic acid, Vanillin, Ferulic acid, Rutin, and Quercetin.

In the *in vivo* segment, the Open Field test unveiled notable differences in speed, distance, immobility time, crossing between quadrants, and cumulative grooming during the 14-day assessment. However, post-hoc analysis revealed differences between the 6OHDA group and the 1 mg/kg group. These results may indicate a potential overcompensation of the contralateral side to the lesion, resulting in higher-than-expected motor performance in the 6OHDA group. Additionally, the disparity between the 6OHDA and 1 mg/kg group could suggest neuroprotection offered by the administered dose to the injected rats.

In the same test conducted over 21 days, significant differences were found in the number of grooming and the post-hoc test showed differences between the Sham and 6OHDA groups. Grooming is the innate activity of the animal to maintain its hygiene, thermoregulation and communication and it has been found that its performance implies absence of damage to the striatum and dopaminergic inputs due to its necessary participation in the implementation of sequence and motor performance [25, 26]. Therefore, a lower amount of grooming in the 6OHDA group could imply a significant impairment in motor performance and in the present experiment these were presented by the rats of the 6OHDA group.

Regarding the second behavioral test performed, the cross bar test showed significant differences in latency to platform for 14 days and fall latency for 21 days. With respect to the 14-day test, the latency to platform is significantly higher in the 6OHDA group compared to the Sham group. These results are in accordance with expectations showing greater difficulty in the group injected with 6OHDA to reach the platform [27].

With respect to the latency to fall in 21 days the time is significantly longer for the 6OHDA group than the Sham group. Although the latency to fall is expected to be shorter for rats with 6OHDA [28], in the present experiment this group showed immobility when placed on the bar and immediate fall when attempting to move, whereas the Sham group presented rats that decided to launch themselves off the bar for possible stress avoidance or environmental recognition. Regarding the 6OHDA group in the same line, it was observed that the Sham rats presented grasping strategies involving the four limbs and the tail, while the 6OHDA rats did not.

Also, the rotation test not only serves to confirm the model, as treatment can reduce the number of rotations [29]. In the current experiment the mean for 6OHDA was higher than that of the Sham group. In the same vein it is important to mention that

studies show that an animal with less than 90% dopamine depletion in the striatum may not show remarkable differences in the number of gyri with apomorphine, so amphetamine is also used to generate gyri [23, 30].

Finally, western blot results indicate a 30% reduction for 6OHDA. According to studies it is possible to find no significant differences in HT between the sham and 6OHDA groups at 22 days [31], although HT reduction is expected in the 6OHDA group. A 2005 study investigated the effect of unilateral 6OHDA in mice and found high variability in the lesion with immunohistochemical results for TH reflecting dopaminergic loss between 4 and 100% [32]. On the other hand, the 100 mg/kg group showed a reduction of 53%, which could indicate a neurotoxic effect of the extract in that amount. It should be noted that the animals that received 100 mg/kg of *Mauritia flexuosa,* presented values closer to the 6OHDA group than the other treatment groups in the Open Field test, this being directly proportional to the results of the Western Blot, since they presented greater damage in the substantia nigra.

In summary, the findings suggest a potential neuroprotective effect associated with the administration of the 1 mg/kg dose, while indicating a possible neurotoxic effect linked to the 100 mg/kg dose. However, it is essential to acknowledge the main limitation of the study, which stems from the use of the 6OHDA model. This model fails to achieve the expected level of damage in the striatum and substantia nigra, reaching only 29% damage, whereas a total Parkinson's model typically involves damage reaching 70%. Therefore, it is advisable to replicate the research using a total Parkinson's model in rats induced with 6OHDA to validate and extend these findings.

Author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all the members.

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Conflict of interest

All authors report that they do not have any conflicts of interest.

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