



© 2017

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 16 (2): 150 - 161

ISSN 0717 7917

www.blacpma.usach.cl

Artículo Original | Original Article

Attenuation of motor deficits by hydroethanolic extract of *Poincianella pyramidalis* in a Parkinson's disease model

[Atenuación de deficiencias motoras por el extracto hidroalcohólico de *Poincianella pyramidalis* en un modelo de la enfermedad de Parkinson]

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Abstract: The present study aimed to evaluate the possible neuroprotective effect of the hydroethanolic extract of *Poincianella pyramidalis* (EHPp) (Tul.) L. P. Queiroz (Fabaceae), an endemic plant found in Northeastern Brazil, commonly used in folk medicine, on the motor deficits induced by repeated treatment with reserpine (RES) in rats. Adult male Wistar rats received 10 s.c. injections of 0.1 mg/kg RES or vehicle (VR), every 48 h, and daily i.p. injections daily of HEPP (25 mg/kg) or vehicle (VE). Throughout treatment, catalepsy behavior and oral movements were scored. After behavioral tests, superoxide dismutase (SOD) and catalase (CAT) activities were evaluated in the prefrontal cortex, hippocampus and striatum. RES treatment induced a progressive increase of catalepsy time in the treated group compared to control groups starting at day 15. RES also increased the number of vacuous chewing movements, tongue protrusions and duration of facial twitching. Treatment with HEPP attenuated the motor deficit in the catalepsy test and delayed the onset of oral movements induced by RES. No significant changes were observed in the antioxidant assay. Taken together, these results show a beneficial effect of HEPP on motor deficits induced by reserpine, suggesting a neuroprotective effect in a rat model of PD.

Keywords: Natural Products; Oxidative stress; Motor symptoms; reserpine; Parkinson's disease.

Resumen: El presente estudio tuvo como objetivo evaluar el posible efecto neuroprotector del extracto hidroalcohólico de *Poincianella pyramidalis* (EHPp) (Tul.) L. P. Queiroz (Fabaceae), en los déficits motores inducidos por el tratamiento repetido con reserpina (RES) en ratas. Ratas Wistar machos adultos recibieron 10 inyecciones (s.c.) de 0,1 mg/kg de RES o vehículo (VR) a cada 48 h, y inyecciones (i.p.) diarias de EHPp (25 mg/kg) o vehículo (VE). Durante todo el tratamiento se observó el comportamiento de la catalepsia y movimientos orales. Después de análisis de comportamientos, fueron evaluados el superóxido dismutasa (SOD) y las actividades de catalasa (CAT) en la corteza prefrontal, el hipocampo y el cuerpo estriado. El tratamiento con EHPp atenuó el déficit motor en el ensayo de catalepsia y el retraso en el comienzo de los movimientos orales inducidos por RES. No se observaron cambios significativos en el ensayo antioxidante. Estos resultados muestran un efecto beneficioso de EHPp en déficits motores inducidos por RES, sugiriendo un efecto neuroprotector en un modelo de enfermedad de Parkinson.

Palabras clave: Productos Naturales; Estrés oxidativo; Síntomas motores; Reserpina; Enfermedad de Parkinson.

Recibido | Received: July 14, 2016

Aceptado | Accepted: September 13, 2016

Aceptado en versión corregida | Accepted in revised form: September 14, 2016

Publicado en línea | Published online: March 30, 2017

Declaración de intereses | Declaration of interests: The research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC) and Pró-reitoria de Pesquisa da Universidade Federal de Sergipe (POSGRAP/UFES).

Este artículo puede ser citado como / This article must be cited as: LCRF Lins, MF Souza, RR Cintra, KAAL Medeiros, M Macêdo-Lima, SZC Moraes, CS Stevam, GKM Almeida, SL Santos, AM Ribeiro, RH Silva, JR Santos, M Marchioro. 2017. Attenuation of motor deficits by hydroethanolic extract of *Poincianella pyramidalis* in a Parkinson's disease model. *Bol Latinoam Caribe Plant Med Aromat* 16 (2): 150 – 161.

INTRODUCCIÓN

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It is mainly characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and further depletion of dopamine terminals in the striatum (Lau & Breteler, 2006). The exact mechanisms underlying the selective dopaminergic cell loss in PD are still poorly understood and the majority of cases are idiopathic (Lees *et al.*, 2009). However, in both idiopathic and genetic cases of PD, oxidative stress has been pointed out as a common underlying mechanism that leads to cellular dysfunction and death (Janda *et al.*, 2012; Hwang, 2013).

The loss of dopaminergic input in the striatum is associated with the motor symptoms of PD, such as bradykinesia, rigidity and tremor, which result in a marked reduction in the quality of life of patients (Lees *et al.*, 2009). Treatment involves a range of approaches to restore or replace dopamine. The most popular pharmacological approach is the use of levodopa (L-DOPA), a precursor of dopamine. L-DOPA compensates the dopamine deficiency, and is still the most effective antiparkinsonian drug (Stayte & Vissel, 2014; Olanow, 2015). However, L-DOPA's effectiveness is limited in respect to long-term use, since it undergoes oxidative metabolism, which generates reactive oxygen species (ROS). For this reason, there is a concern that L-DOPA might be toxic to dopaminergic neurons and increase the rate of PD's progression (Olanow, 2015). Thus, the use of antioxidants compounds in combination with L-DOPA could ameliorate side effects of L-DOPA therapy. Furthermore, supplementation with antioxidants may be useful in the prevention and treatment of PD (Prasad *et al.*, 1999).

Herbal products are valuable sources of substances with potential therapeutic value for PD. Previous studies indicate that a range of compounds derived from herbal extracts, fractions and formulations are effective *in vitro* and *in vivo* in PD models, via modulation of multiple aspects of pathogenesis, especially of oxidative stress (Song *et al.*, 2012; Fu *et al.*, 2015). Some plant extracts possess antioxidant radical-scavenging activities in animal models, and their antioxidant effect has been associated to polyphenolic compounds (Khurana & Gajbhiye, 2013; Rezaei & Alirezaei, 2014).

Phenolic compounds, represented mainly by flavonoids, stand out as the major group of natural

antioxidants. These substances occur naturally in plants and protect them against ROS. Neuroprotective effects of flavonoids in pathological conditions have been identified (Magalingam *et al.*, 2015). Therefore, plant extracts that contain these substances are interesting candidates for use as adjunctive therapy in patients with PD.

Poincianella pyramidalis (Tul.) L. P. Queiroz (Fabaceae) is an endemic tree in the caatinga vegetation of Northeastern Brazil, popularly known as "catingueira". Phytochemical and pharmacological characteristics of this species remain poorly explored. Secondary metabolites have been isolated from *P. pyramidalis* extracts, and flavonoids predominate (Bahia *et al.*, 2005). In addition, *P. pyramidalis* extracts exhibit antioxidant activity *in vitro* and *in vivo* studies (Bahia *et al.*, 2005; Melo *et al.*, 2010; Rêgo Jr *et al.*, 2011; Santana *et al.*, 2012; Diniz *et al.*, 2015). Thus, this plant is a source of natural antioxidants, which could be used to reduce or preclude the neurodegeneration process in PD. Therefore, the aim of present study was to evaluate the neuroprotective effect of the hydroethanolic extract of *P. pyramidalis* (HEPp) in rats submitted to PD pharmacological model by reserpine.

MATERIALS AND METHODS

Plant collection and hydroethanolic extraction

The inner bark of *P. pyramidalis* was collected in Canindé de São Francisco, Sergipe, Brazil, in September 2014. A voucher specimen was deposited at the Herbarium of the Federal University of Sergipe (ASE Number 28.136). Samples were dried at 37° C in an oven with air renewal and flow for 48 h until complete dehydration. Afterwards, the material was crushed with a knife mill and subsequently powdered. Then, compounds were extracted by maceration at room temperature in 90% ethanol for 7 days. The extract was suspended in MeOH/H₂O (2:3). The percent yield was 5.2% (74.73 g). The HEPp was submitted to methods described earlier (Matos, 2009) for identification of chemical constituents.

Animals

Adult male Wistar rats (350 – 450 g) were obtained from the Central Animal Facility of the Federal University of Sergipe and maintained in the Neurophysiology Laboratory. All animals were housed in groups of five per plastic cage (30 cm × 37 cm × 16 cm), under controlled conditions of

ventilation, temperature ($23 \pm 1^\circ \text{C}$) and a 12/12 h light/dark cycle (lights on 6:00 a.m.), with free access to water and food. Animals used in this study were handled in accordance to the Brazilian law for the use of animals in research (Law number 11.794) and local ethics committee for animal usage approved all the procedures (Protocol number 068/2012). All efforts were made to minimize animal pain, suffering or discomfort.

Drugs

Reserpine (RES; Sigma Chemical Co., St. Louis, MO, USA) was dissolved in glacial acetic acid and then diluted in distilled water at 0.1 mg/mL. Vehicle of reserpine (VR) consisted of the same volume of glacial acetic acid diluted in distilled water. HEPp was suspended in 0.9% saline solution at 25 mg/mL and its vehicle (VE) consisted of 0.9% saline. Extract and reserpine solutions were prepared daily, shortly before administration.

General Procedures and Experimental Design

Before the beginning of experimental procedures, animals were handled daily during 5 min for 5 days. Then, rats were randomly assigned to the following groups: control (VE + VR; $n = 7$), RES (VE + RES; $n = 8$), HEPp (HEPp + VR; $n = 8$) and HEPp + RES (HEPp + RES; $n = 7$). Animals received 10

subcutaneous (s.c.) injections of RES or VR every 48 h, and daily intraperitoneal (i.p.) injections of HEPp or VE for 20 days. The volume injected was 1 mL/kg of body weight in all cases. Previous studies showed that repeated administration via s.c. of 0.1 mg/kg of RES induces progressive behavioral deficits related to clinical symptoms of PD and depletion of tyrosine-hydroxylase (TH) levels in some brain areas, suggesting that the protocol of progressive Parkinsonism induction with RES can be useful for studying possible neuroprotective interventions for PD (Fernandes *et al.*, 2012; Santos *et al.*, 2013; Sarmiento-Silva *et al.*, 2015).

Throughout treatment, animals were submitted to the following behavioral procedures (from 8:00 a.m. to 4:00 p.m.): (1) daily catalepsy test; (2) assessment of oral movements on the 14th and 20th days (48 h after the 7th and 10th injections of RES and VR, respectively). All behavioral tests were performed 48 h after the last injection of RES/VR in order to avoid acute effects of the drug, and the behavioral quantification was performed by direct observation with the use of stopwatches by observers blinded to treatments. The day of first injection of RES was considered day 0. All apparatuses were cleaned with 10% ethanol before behavioral testing to eliminate odor traces. Experimental design is shown in Figure 1.

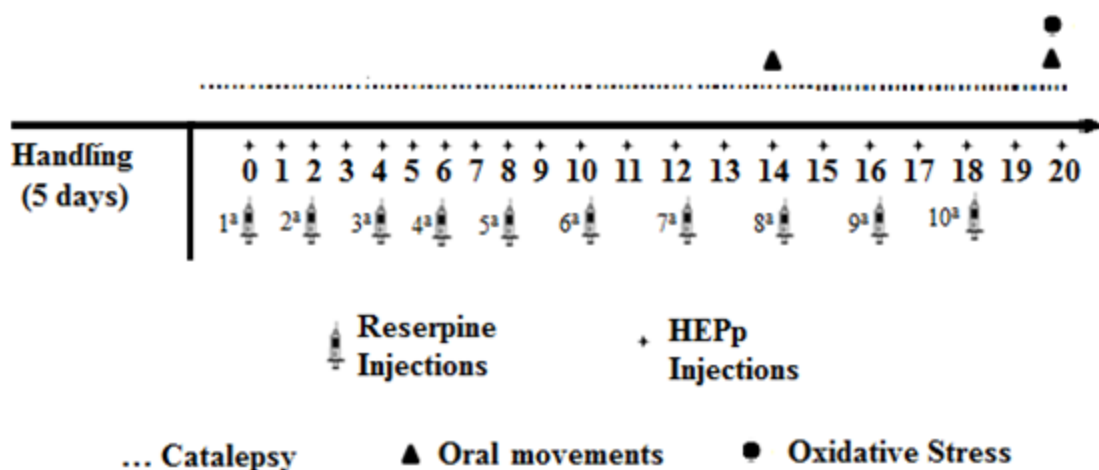


Figure 1
Schematic representation of the experimental design

Behavioral Testing

Catalepsy Test

Catalepsy behavior was assessed by placing the animal's forepaws on a horizontal bar positioned 9 cm above the bench surface. The duration of catalepsy, which was defined as an immobile posture, keeping both forepaws on the bar, was measured up to a maximum of 180 s. Three trials were carried out for each animal in each day and the mean value of the three trials was used as a data point (Fernandes *et al.*, 2012; Santos *et al.*, 2013).

Oral movements

Rats were individually placed in wire cages (40 cm × 40.5 cm × 20 cm) with mirrors positioned underneath and behind the back wall of the cage to allow for behavioral quantification when the animal faced away from the observer. The number of tongue protrusions (projection of the tongue out of the oral cavity), vacuous chewing movement frequency (mouth openings in the vertical plane not directed toward physical material), and duration of twitching of the facial musculature were measured continuously for 10 min (Fernandes *et al.*, 2012).

Tissue preparation and oxidative stress parameters

After the behavioral evaluation on the 20th day (48 h after 10 injection of RES/VR), rats were euthanized by decapitation. Brains were quickly removed and the prefrontal cortex, striatum and hippocampus were dissected and weighed. Each area was individually homogenized in phosphate-buffered saline (PBS, 50 mM, pH 7.4) and centrifuged (Heal Force, Neofuge 15R) for 30 min at 12000-rpm and 4° C. The supernatants were used for quantification of catalase (CAT) and superoxide dismutase (SOD) activities. CAT and SOD activities were assayed as described earlier (Madesh & Balasubramanian, 1998; Gioda *et al.*, 2010). Protein content was obtained using the method of Lowry (Lowry *et al.*, 1951).

Statistical Analysis

Data normality and homogeneity of variances were assessed by the Shapiro-Wilk and Levene's tests, respectively. Catalepsy behavior and oral movements were compared between groups and across treatments using two-way ANOVA with repeated measures

followed by Tukey's post hoc test. The oxidative stress indicators were analyzed by nonparametric Kruskal-Wallis test. All significance tests were two-tailed and $p < 0.05$ was considered to reflect significant differences. All statistical analyses were performed using Graph Pad Prism 6.0 (Graph Pad Prism Software Inc., San Diego, CA, USA).

RESULTS

Catalepsy test

In respect to catalepsy duration across treatments and days, a two-way ANOVA with repeated measures revealed significant effects of time [F (20,540) = 23.30, $p < 0.001$], treatment [F (3, 27) = 13.43, $p < 0.001$] and time x treatment interaction [F (60,540) = 4.77, $p < 0.001$] (Fig 2). Tukey's post hoc test revealed an increase in catalepsy time of the RES group compared to all other groups from the 14th day until the 20th day. The HEPp + RES group also showed increase in catalepsy time when compared to control group on the 16th, 17th, 18th and 20th days and to HEPp group only on the 17th and 18th days. Furthermore, the HEPp + RES group showed a decrease in the catalepsy time when compared to the RES group on the 15th, 16th, 17th, 18th, 19th and 20th days (Figure 2).

Oral movements

A two-way ANOVA with repeated measures revealed significant effects of time [F (1, 27) = 7.26, $p = 0.011$] and treatment [F(3, 27) = 6.78, $p < 0.001$], but no time x treatment interaction [F(3, 27) = 1.64, $p = 0.203$] for the number of vacuous chewing movements (VCM) (Fig. 3). The Tukey's post hoc test revealed significant increases in VCM number in the RES group on the 14th day when compared to the control ($p = 0.012$) and HEPp ($p = 0.012$) groups, and on the 20th day when compared to control group ($p = 0.028$) but not when compared to HEPp ($p = 0.077$). The HEPp + RES group showed no significant difference on the 14th day when compared to control ($p = 0.117$), HEPp ($p = 0.096$) and RES ($p = 0.916$) groups. On the 20th day, the HEPp + RES group showed a significant increase of VCM number when compared to control ($p = 0.002$) and HEPp ($p = 0.008$) groups, but no difference when compared to RES group ($p = 0.721$) (Figure 3A).

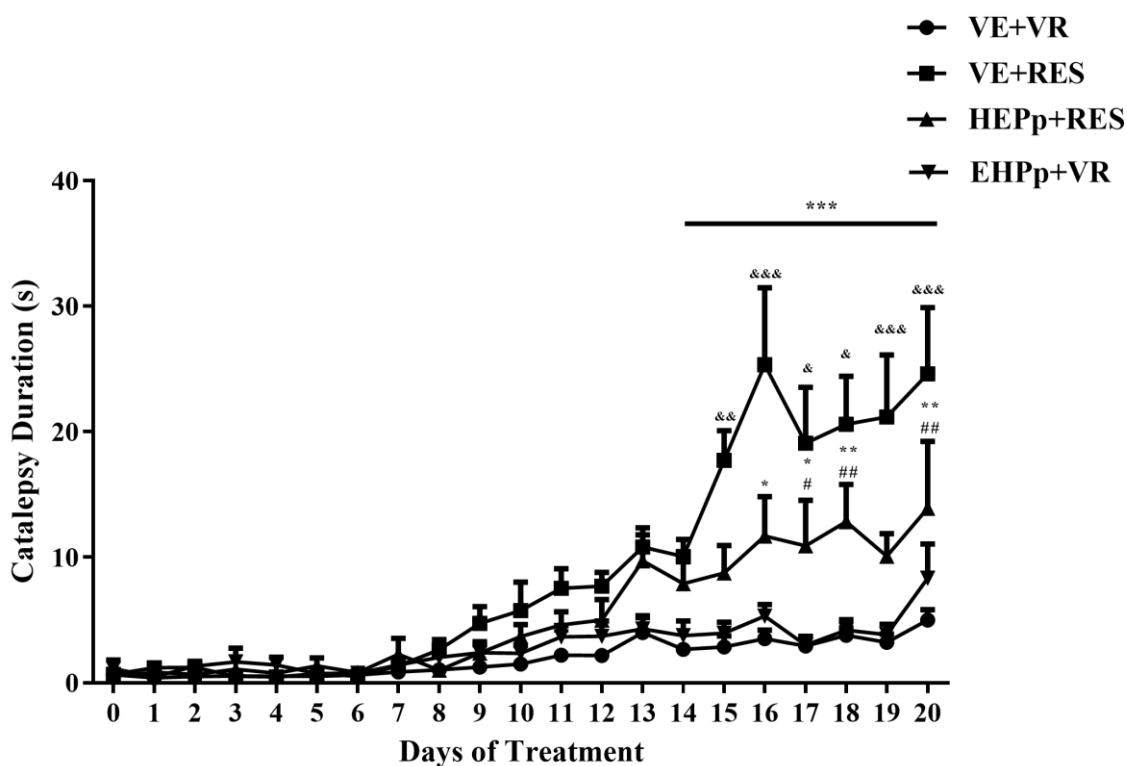


Figure 2

Effects of repeated administration of hydroethanolic extract of *Poincianella pyramidalis* (HEPp, 25 mg/kg) or vehicle of extract (VE) on catalepsy behavior of rats treated with 0.1mg/kg reserpine (RES) or vehicle of reserpine (VR). The i.p. injections of HEPp or VE were administered daily, while the s.c. injections of RES or VR were administered every other day. Data are expressed as mean \pm S.E.M. (*) $p < 0.05$, (**) $p < 0.01$ for HEPp+RES vs VE+VR; (***) $p < 0.001$ for VR+RES vs VE+VR and EHPp+VR; (&) $p < 0.05$, (&&) $p < 0.01$ and (&&&) $p < 0.001$ for VE+RES vs HEPp+RES; (#) $p < 0.05$, (##) $p < 0.01$ for HEPp+RES vs HEPp+VR (Two-way ANOVA with repeated measures followed by Tukey-s test).

For the number of tongue protrusions, two-way ANOVA with repeated measures showed significant effects of time [F (1, 27) = 6.64, $p = 0.015$] and treatment [F (3, 27) = 12.38, $p < 0.001$], but no time x treatment interaction [F (3, 27) = 1.61, $p = 0.208$]. Post hoc analysis revealed an increase in the number of tongue protrusions in the RES group on the 14th and 20th day when compared to control (p

= 0.006; $p < 0.001$, respectively) and HEPp ($p = 0.003$; $p < 0.001$, respectively) groups. The HEPp + RES group showed increase only on the 20th day when compared to control ($p = 0.005$) and to HEPp ($p = 0.018$) groups. In addition, no significant difference was observed between HEPp + RES and RES groups on the 14th and 20th days (Figure 3B).

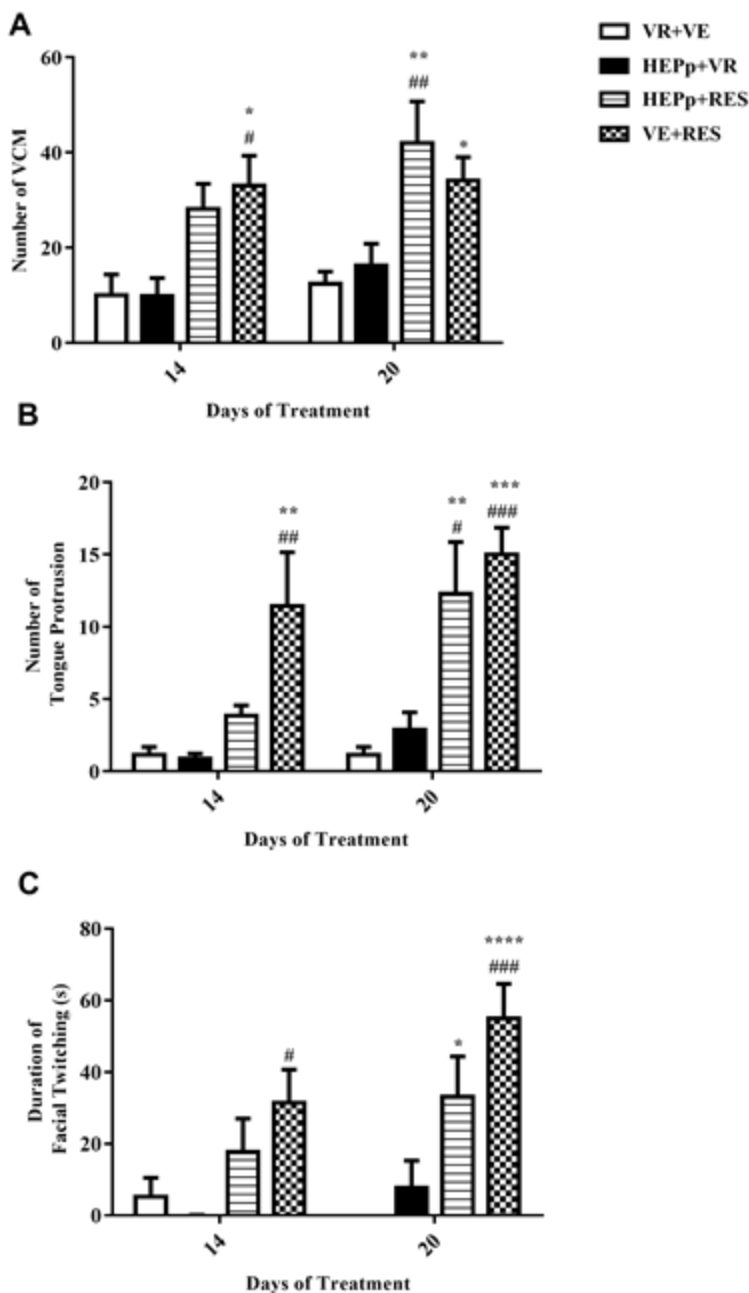


Figure 3

Effects of repeated administration of hydroethanolic extract of *Poincianella pyramidalis* (HEPp, 25 mg/kg) or vehicle of extract (VE) on number of vacuous chewing movements-VCM (A), number of tongue protrusion (B) and duration of facial twitching (C) of rats treated with 0.1mg/kg reserpine (RES) or vehicle of reserpine (VR). The i.p. injections of HEPp or VE were administered daily, while the s.c. injections of RES or VR were administered every other day. Data are expressed as mean \pm S.E.M. (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ and (****) $p < 0.0001$ comparing to VE+VR; (#) $p < 0.05$, (##) $p < 0.01$, (###) $p < 0.001$ comparing to HEPp + VR (Two-way ANOVA with repeated measures followed by Tukey-s test).

Regarding the duration of facial twitching, two-way ANOVA with repeated measures revealed time (days of treatment) [$F(1, 27) = 4.46, p = 0.044$] and treatment effects [$F(3, 27) = 10.22, p < 0.001$], but no time \times treatment interaction [$F(3, 27) = 1.58, p = 0.216$]. On the 14th day, the RES group showed a significant increase in the duration of facial twitching when compared to HEPp ($p = 0.024$) but no difference when compared to control ($p = 0.098$) and HEPp + RES ($p = 0.608$). On the 20th day, the RES group showed a significant difference when compared to control ($p < 0.0001$) and to HEPp ($p < 0.001$) groups. The HEPp + RES group showed a significant

increase only on the 20th day when compared to control ($p = 0.033$). In addition, no significant difference was observed between HEPp + RES and RES groups on the 14th and 20th days (Figure 3C).

Superoxide dismutase (SOD) and catalase (CAT) activity

No significant differences were found for antioxidant enzymes in the prefrontal cortex [SOD: $H(3) = 2.01, p = 0.57$; CAT: $H(3) = 6.47, p = 0.09$], striatum [SOD: $H(3) = 0.19, p = 0.97$; CAT: $H(3) = 2.54, p = 0.46$] or hippocampus [SOD: $H(3) = 0.54, p = 0.90$; CAT: $H(3) = 1.79, p = 0.61$] (Table 1).

Table 1

Effects of repeated administration of hydroethanolic extract of *Poincianella pyramidalis* (HEPp, 25 mg/kg) or vehicle of extract (VE) on superoxide dismutase and catalase activities in prefrontal cortex, striatum and hippocampus of rats treated with reserpine (RES) or vehicle of reserpine (VR)

Enzymes	Brain Region			
	Groups	Prefrontal Cortex	Striatum	Hippocampus
Superoxide dismutase activity (U/ mg protein)	VE+VR	0.23 (0.11-0.28)	0.15 (0.03-0.56)	0.06 (0.04-0.12)
	EHPp+VR	0.09 (0.02-1.21)	0.13 (0.03-0.14)	0.07 (0.03-0.09)
	EHPp+RES	0.11 (0.10-0.15)	0.10 (0.02-0.43)	0.05 (0.04-0.20)
	VE+RES	0.10 (0.05-0.18)	0.09 (0.05-0.34)	0.09 (0.05-0.16)
Catalase Activity ($\Delta E/\text{min}/\text{mg de protein}$)	VE+VR	0.006 (0.005-0.017)	0.033 (0.006-0.112)	0.007 (0.003-0.024)
	EHPp+VR	0.005 (0.007-0.134)	0.011 (0.009-0.017)	0.004 (0.001-0.012)
	EHPp+RES	0.005 (0.003-0.009)	0.030 (0.013-0.472)	0.005 (0.003-0.224)
	VE+RES	0.017 (0.005-0.090)	0.011 (0.005-0.253)	0.005 (0.003-0.024)

Data are expressed as median (25-75 percentiles).

DISCUSSION

In the present study, we investigated the neuroprotective and antioxidant effects of the extract of *P. pyramidalis* (HEPp) in rats submitted to repeated treatment with a low dose of reserpine. Our results show that rats chronically treated with HEPp: (1) had decreased duration and delayed onset of the catalepsy behavior and (2) had delayed development of oral dyskinesia caused by repeated treatment with reserpine.

Reserpine is an inhibitor of vesicular monoamine transporter (VMAT), and leads to loss of retrieval capacity and hence depletion of monoamines in brain and periphery. Reserpine-treated rodent

model was one of the earliest animal models employed in PD research, because it mimics key features of symptomatology and neurochemistry of PD (Duty & Jenner, 2011; Leão et al., 2015). Acute or chronic administration of reserpine induces motor impairments, such as hypokinesia, muscle rigidity, postural flexion, tremor, oral dyskinesia and catalepsy in rodents, resembling PD symptoms in humans (Goldstein et al., 1975; Lan & Jiang, 1994; Neisewander et al. 1994; Fernandes et al., 2012; Santos et al., 2013).

Catalepsy is expressed as the inability of an animal to correct itself from an imposed abnormal posture and it has been compared to the inability of

patients with PD to initiate movements (Duty & Jenner, 2011). Furthermore, this behavior has been associated with brain dopamine dysfunction, especially in areas involved in PD pathophysiology, such as the striatum, globus pallidus and nucleus accumbens (Costall & Naylor, 1974; Sanberg *et al.*, 1988). Accordingly, our results show a gradual increase in the duration of catalepsy behavior in rats treated with a low dose of reserpine (Figure 2). The reserpine-treated group started differing from control groups on the 14th day (48 h after 7th reserpine injection) corroborating results observed in previous studies (Fernandes *et al.*, 2012; Santos *et al.*, 2013; Sarmiento-Silva *et al.*, 2015).

Rats submitted to chronic reserpine treatment also display oral dyskinesia, characterized by twitching of facial musculature, vacuous chewing movements and tongue protrusions. Oral movement impairments induced by reserpine in rodents are suggested to model tardive dyskinesia with features similar to parkinsonian tremor (Neisewander *et al.*, 1991; Neisewander *et al.*, 1994; Salamone & Baskin, 1996; Salamone *et al.*, 1998).

Tardive dyskinesia is characterized by abnormal involuntary movements, mainly of the tongue and mouth with twisting of the tongue, chewing and grimacing movements of the face. It may develop after chronic therapy with antipsychotics in humans (Casey, 2000; Mathews *et al.*, 2005). The majority of PD patients develop dyskinesia after chronic treatment with L-DOPA, and, unfortunately, no drug is yet approved to treat these symptoms. Interestingly, dyskinesia induced by reserpine has been used to evaluate compounds with possible antiparkinsonian effects in rodent models (Salamone *et al.*, 2008; Blanchet *et al.*, 2012; Morin *et al.*, 2014). Our results show that reserpine treatment induced oral dyskinesia, corroborating data in the literature (Neisewander *et al.*, 1994; Fernandes *et al.*, 2012; Santos *et al.*, 2013).

Although the exact mechanisms of physiopathology of PD remain unknown, a large body of evidence has suggested that oxidative stress derived from dopamine metabolism, mitochondrial dysfunction and neuroinflammation plays a central role in the neuropathology of this disease. Accordingly, post-mortem analysis of brain tissue of PD patients show increased oxidative stress and studies with animal models of PD have supported the relationship between oxidative stress and motor deficits in rodents (Duty & Jenner, 2011; Janda *et al.*,

2012; Hwang, 2013).

Recently, there is increasing interest in alternative strategies for treatment or prevention of PD by use of medicinal plants. Some antioxidants compounds extracted from plants show beneficial effects on PD models *in vitro* and *in vivo* (Li *et al.*, 2013), indicating that natural antioxidant compounds may be useful to treatment of PD.

P. pyramidalis is popularly used to treat cough, respiratory infections, gastrointestinal disorders, injuries and fever (Albuquerque *et al.*, 2007). Previous studies have shown that *P. pyramidalis* extracts contain phenolic compounds, represented mainly by flavonoids (Bahia *et al.*, 2005; Silva *et al.*, 2011). Flavonoids are water-soluble compounds, synthesized by plants via the photosynthesis process and protecting plants against reactive oxygen species (ROS). Previous work indicates that polyphenols penetrate the blood brain barrier (BBB) and exert strong antioxidant activity in brain tissue (Frei & Higdon, 2003; Chaturvedi *et al.*, 2006). Moreover, some studies have shown that flavonoids exhibit antioxidant, antiapoptotic and anti-inflammatory effects and can exert neuroprotective effect in pathological conditions, including PD (Magalingam *et al.*, 2015). Interestingly, we showed that HEPp decreases the motor alterations and delays the onset of the catalepsy behavior induced by reserpine, suggesting that HEPp might attenuate the brain damage responsible by motor dysfunctions through its possible antioxidant action.

The development of oral dyskinesia induced by reserpine is closely related to the oxidative stress process (Abílio *et al.*, 2003). Reserpine has been employed in the screening for antioxidant treatments to motor impairments such as dyskinesia in PD models (Abílio *et al.*, 2003; Faria *et al.*, 2005; Fernandes *et al.*, 2012; Leão *et al.*, 2015). In this work, we show that treatment with HEPp delays the development of oral dyskinesia caused by chronic treatment reserpine, which might be related to HEP's antioxidant properties. Recent studies have shown a significant antioxidant effect of *P. pyramidalis* extracts *in vitro* and *in vivo* (Rêgo Jr *et al.*, 2011; Santana *et al.*, 2012; Diniz *et al.*, 2015).

The protective effect of HEPp observed in this study may be associated to the constituents in the plant extract. Previous phytochemical studies revealed that flavonoids are the major components found in extracts of *P. pyramidalis* (Bahia *et al.*, 2005; Silva *et al.*, 2011; Santos *et al.*, 2011).

Therefore, the antioxidant profile of flavonoids may be associated with ability of HEPP to prevent the neuronal dysfunction induced by reserpine administration.

Unexpectedly, we observed no effects of HEPP on the level of antioxidant markers in brain tissue. Moreover, we found no alterations in oxidative stress parameters due to reserpine administration. However, studies evaluating reserpine and oxidative stress markers are contradicting. Similar to our results, two studies showed that reserpine-treated rats with 0.5 mg/kg (Faria *et al.*, 2005) and 1.0 mg/kg (Abílio *et al.*, 2004) doses showed no difference in striatal catalase activity. On the other hand, other studies report that chronic 1.0 mg/kg reserpine treatment decreased SOD and CAT activities in rat brain homogenates (Naidu *et al.*, 2003; Nade *et al.*, 2013), but another study showed that same dose increase CAT activity in the striatum of rats (Teixeira *et al.*, 2008). These contradictory results may emerge from different dosage, treatment regimen or brain area analyzed (Leão *et al.*, 2015). Further studies are needed to clarify this issue, perhaps employing different methods for assaying antioxidant activity, like determination of thiobarbituric acid reactive substances (TBARS), whereas the ethanol extract of *P. pyramidalis* showed a significant reduction of malondialdehyde (MDA) in a previous study (Santana *et al.*, 2012).

Although our results showed no significant difference on levels of antioxidant markers in brain tissues among the groups, we cannot rule the possibility that HEPP may play protective effect through different mechanisms, possibly through its anti-inflammatory or antiapoptotic activities, due the presence of flavonoids (Magalingam *et al.*, 2015). However, further studies are required in order to identify the components of HEPP and the mechanisms underlying the protective effect observed in the present study.

In conclusion, our results demonstrate that concomitant treatment with HEPP attenuated catalepsy behavior and delayed the development of oral dyskinesia induced by chronic treatment with a low dosage of reserpine. Importantly, the population of Northeastern Brazil makes frequent use of *P. pyramidalis* extracts. The evidence provided here suggests that HEPP may be an interesting adjuvant treatment of PD since it may delay the onset of the neurodegenerative process of PD. Therefore, future studies are required to confirm this possible

therapeutic effect, as well as to clarify its mechanism of action.

ACKNOWLEDGMENTS

The research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC) and Pró-reitoria de Pesquisa da Universidade Federal de Sergipe (POSGRAP/UFS).

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