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Original Article

Schinus terebinthifolius administration prevented behavioral and biochemical alterations in a rotenone model of Parkinson's disease

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ABSTRACT

Parkinson's disease is a neurodegenerative disorder characterized by motor impairment, cognitive decline and psychiatric symptoms. *Schinus terebinthifolius* Raddi, Anacardiaceae, had been studied for its anti-inflammatory and antioxidant properties, and in this study, the stem bark was evaluated for the neuroprotective effects on behavioral and biochemical alterations induced by administrations of rotenone in rats. Behavioral evaluations were performed using open-field and rotarod. The *in vitro* and *in vivo* antioxidant activities were determined by the DPPH radical scavenging activity and lipid peroxidation method respectively. The administration of rotenone (3 mg/kg, *s.c.*) produced hypolocomotion, increase of immobility and muscle incoordination, while the treatment with *S. terebinthifolius* stem bark extract (150, 300 and 600 mg/kg *p.o.*) for seven days prevented rotenone-induced dysfunctional behavior. Biochemical analysis of the substantia nigra, striatum and cortex revealed that rotenone administration significantly increased lipid peroxidation, which was inhibited by treatment with all doses of *S. terebinthifolius*. The results suggested neuroprotective effect of *S. terebinthifolius* possibly mediated through its antioxidant activity, indicating a potential therapeutic benefit of this species in the treatment of Parkinson's disease.

Introduction

The increase of elderly population has led to an increasing incidence of neurodegenerative diseases worldwide, leading, thus, to the interest in studies for prevention and treatment of these pathologies. Natural constituents derived from plants are important to investigate, since studies have shown that these compounds exhibited a range of biological activities, with therapeutic potential, for instance antioxidant and anti-inflammatory effects.

Parkinson's disease (PD) is one if the major neurodegenerative disorder in the entire world and is characterized by progressive degeneration of dopamine-containing neurons that project from substantia nigra pars compacta to the striatum. Besides, motor impairment (bradykinesia, rigidity, tremor at rest and disturbances in balance), cognitive decline and psychiatric symptoms (like depression) are the cardinal symptoms of the pathology (Dutta and Mohanakumar, 2015; Fernandez, 2015). Main mechanisms have been proposed to explain the events culminating in neuronal death in PD, which are associated with oxidative stress, mitochondrial dysfunction, neuroinflammation and environmental exposures, like pesticides, contributing for the appearance of behavioral and biochemical alterations (Dauer and Przedborski, 2003; Renaud et al., 2015). Several animal models have been used to mimetize and elucidate the pathogenesis of PD, in special rotenone, MPTP and 6-hydroxydopamine (Dauer and Przedborski, 2003; Blesa et al., 2012).

Rotenone (*Derris* sp., Fabaceae) has been a potent hydrophobic pesticide and its environmental exposure mimics the clinical and pathological features of PD, such as behavioral, biochemical and pathological changes, providing a reproducible animal model for PD (Betarbet et al., 2000). It provoked neurodegeneration via multiple mechanisms, mainly by inhibition of complex I, increase of oxidative stress and induction of apoptosis (Zaitone et al., 2012; von Wrangel et al., 2015).

Schinus terebinthifolius Raddi, Anacardiaceae, is a native plant of South America (Corrêa, 1974). In Brazil it is popularly called Aroeira

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or Cabuí. In folk medicine, it has been used for the treatment of ulcers, respiratory problems, wounds and arthritis (Morton, 1978) and as an antiseptic, antiinflammatory and haemostatic (Medeiros et al., 2007). Many of its properties or healing effects attributed by folk medicine are associated to the presence of polyphenols in the plant, which give the plant its antioxidant properties (Medeiros et al., 2007; Abdou et al., 2015). The aim of this study was to investigate the possible neuroprotective effects of *S. terebinthifolius* in the behavior activity and on the oxidative stress induced by rotenone as experimental model of Parkinson's disease in rats.

Materials and methods

Plant material

Stem bark of *Schinus terebinthifolius* Raddi, Anacardiaceae, were collected in the remains of the Atlantic rainforest located in the municipality of Cabo de Santo Agostinho, Pernambuco, Brazil between February and April 2013 (8°20′33″ S and 34°56′59″ W). A voucher specimen was authenticated in the Department of Botany of the Federal University of Pernambuco by the curator M. Barbosa and was deposited at Geraldo Mariz Herbarium under record n°. 8758. Extraction was performed by maceration and air dried, and 500 g of pulverized *S. terebinthifolius* bark was added to 1.0 l of ethanol 70% at room temperature, for 7 days, and was occasionally shaken. In the laboratory, the crude ethanolic extract was evaporated to dryness under reduced pressure for the total elimination of alcohol, followed by lyophilization, yielding approximately 35 g of dry residue. The lyophilized extract of *S. terebinthifolius* was kept at room temperature until use and suspended in distilled water.

High performance liquid chromatography (HPLC) analysis

The main phytochemical markers (gallic acid, ellagic acid, catechin and epicatechin) in *S. terebinthifolius* samples were analyzed by way by way of liquid chromatography-diode array detection (LC-DAD) analysis using a Shimadzu system (LC-20AT) equipped with a photo diode array detector (SPD-M20A). The chromatographic separation was performed using a Gemini RP-18 column 5 μm particle size and 250 mm × 4.60 mm i.d. (Phenomenex), protected by a guard column of the same material. A gradient elution was performed by varying the proportion of Solvent A (0.5% acetic acid in distilled water, v/v) and solvent B (methanol) at a flow rate of 0.8 ml/min following gradient program: 20–40% B (10 min), 40–60% B (10 min), 60% B (10 min), 60–40% B (10 min), and 40–20% B (10 min). The dried extracts and standards were dissolved in methanol:water (20/80, v/v) and filtered through a membrane of 0.45 μm (Millipore®, USA) prior to injection of 20 μl. The peaks of each marking on dry substance were identified by comparing retention times and UV spectra of DAD.

Animals

Adult male Wistar rats (*Rattus norvegicus* var. *albinus*), age 3 months and weighing between 250 and 300 g, were obtained from the Department of Physiology and Pharmacology at the Federal University of Pernambuco, Brazil. The animals were maintained in standard environmental conditions (22 ± 2 °C, 12:12 h dark/light cycle). Commercial food (Presence®, Purina, Brazil) and water were available *ad libitum*. All protocols were approved by the Animal Experimentation Ethics Committee of the Federal University of Pernambuco, under license n°. 23076.050131/2013-82 in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Drugs

Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), malondialdehyde (MDA), thiobarbituric acid (TBA), epinephrine, 1,1,3,3-tetrametoxipropane (TMP), Tween 80, DMSO and rotenone, were purchased from Sigma–Aldrich® (St. Louis, MO, USA). Rotenone was dissolved in sunflower oil one day before beginning treatment. On subsequent days, solutions remained in the refrigerator (4 °C). Sunflower oil was obtained in local market.

Experimental groups

The animals were randomly divided into five experimental groups ($n = 10$). Rats from Group 1 received water by gavage (1 ml/kg) and 1 h later a subcutaneous (*s.c.*) administration of the vehicle (90%, w/v sunflower oil, +10% w/v DMSO) for seven days. Group 2 also received water by gavage and rotenone (3 mg/kg, *s.c.*) diluted in vehicle after 1 h during seven days. Groups 3, 4 and 5 received by gavage 150, 300 or 600 mg/kg of *S. terebinthifolius*, respectively, suspended in water, and 1 h later were given them rotenone (3 mg/kg, *s.c.*) also during seven days. 24 h after the last day of treatment, the rats were evaluated with behavioral tests and biochemical assays (Linard-Medeiros et al., 2015).

Open field test

The open field test was carried out to Broadhurst (1957) and Bernardi and Palermo-Neto (1979). To quantify exploratory and general locomotor activity, each rat was placed into the center of an open-field arena (a circular wooden box with a diameter of 100 cm and 40 cm high, with floor divided into 19 regions). Rats were assessed individually for 5 min, while four parameters were analyzed: (i) latency to start the movement (time to leave the inner circle, in s), (ii) locomotion frequency (number of square crossed with four paws), (iii) rearing frequency (number of times the animal stood on their hind paws) and (iv) immobility time (lack of movement during testing, in s). The apparatus was cleaned with a 5% ethanol solution before behavioral testing to eliminate possible bias due to odors left by previous rat.

Rotarod activity test

In order to quantify motor deficiency, the rotarod test was used at a fixed speed, according to Monville et al. (2006). To perform this test, the animal was placed with all four paws on a bar with a diameter of 7 cm and set 25 cm above the floor. The bar rotated at a speed of 25 rpm. Before being submitted to the different treatments, the rats were trained in two sessions of 180 s each for habituation. Animals were placed on the rotating bar and the time spent on the rotating bar was recorded. A cut-off time of 180 s was maintained throughout the experiment. The average results were recorded as time of fall.

Biochemical analysis

After behavioral observations, animals were immediately anesthetized, euthanized by decapitation and their brains removed. Bilateral substantia nigra, striatum and cortex were dissected, weighed and homogenized in a Potter–Elvehjem type homogenizer with a tissue homogenate 1× phosphate buffered saline (10% w/v, PBS) to which was added butylated hydroxytoluene (0.004% w/v, BHT) for preventing oxidation of the samples. The homogenate was centrifuged at 10,000 × *g* for 30 min at 4 °C and an aliquot of supernatant was separated for biochemical analysis. Each individual experiment was carried out in triplicate and repeated two or three times.