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Phytol, a Chlorophyll Component, Prevents Motor Impairments Induced by Reserpine in Rats

Wesley Santos Santana[1], Edson de Rezende Santos[1], Heitor Santos Franco[1], Mylaine Santos Mendonça[1], Thiago Henrique Almeida-Souza[1,3], Ana Cleia A. da Luz[1], José Leandro Santos Souza[1], Auderlan Mendonça de Gois[1], Pollyana Caldeira Leal[2], Lucindo José Quintans-Junior[3], José Ronaldo dos Santos[1]*

[1]Laboratory of Behavioral and Evolutionary Neurobiology / Department of Biosciences / Federal University of Sergipe, Itabaiana, SE, Brazil;

[2]Graduate Program of Dentistry, Federal University of Sergipe, Aracaju, SE, Brazil;

[3]Department of Physiology/ Federal University of Sergipe, São Cristovão, SE, Brazil;

*Corresponding author at: José Ronaldo dos Santos (joseronaldosantos@gmail.com) - Departamento de Biociências, Laboratório de Neurobiologia Comportamental e Evolutiva, Universidade Federal de Sergipe, Av. Vereador Olímpio Grande, s/n, Porto, Itabaiana, 49500-000, Sergipe, Brasil. Tel: +55 79 34328200

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Parkinson Disease
Tardive Oral Dyskinesia

Abstract

Phytol (PHY), a constituent of chlorophyll, has some pharmacological properties such as antioxidant, anti-inflammatory, anxiolytic, antidepressant, and antihyperalgesic effects. Compounds like this one have a high potential to develop neuroprotective action. Repeated administration of reserpine (RES) in rodents induces motor impairments and has been applied has been applied to Parkinson's disease (PD) and tardive dyskinesia (TD) animal models. Therefore, the present study

investigated whether phytol administration would attenuate reserpine-induced motor impairments in rats. Male Wistar rats received subcutaneous injections of 1 mg/kg RES or vehicle, in two consecutive days, concomitantly with intraperitoneal injections of PHY (50 mg/kg) or vehicle. Catalepsy test was assessed from day 1 to day 3. On day 3, oral movements and locomotor activity were evaluated. Our results showed that PHY prevented an increase in cataleptic behavior, number of vacuous chewing movements, oral tremor, and tong protrusion. However, it was unable to prevent a reduction in open-field locomotor activity induced by RES. Our results suggest that PHY shows a protective effect in in PD and TD animal models, preventing motor impairments induced by RES.

1. Introduction

According to the Global Burden of Disease study, Parkinson's disease (PD) is the most common movement disorder and the second most frequent age-related and progressive neurodegenerative disease in humans (Feigin *et al*., 2017; Lau, Breteler, 2006; Parkinson, 2002). The etiology and pathogenesis of PD causes are still unknown. The main pathological characteristic of PD is a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which causes a depletion in striatal dopamine. This evolution gives rise to cardinal motor symptoms, such as bradykinesia, muscle stiffness, tremor at rest, and postural instability (Marsili *et al*., 2018; Miller, O'Callaghan, 2015; Wu *et al*., 2012). Besides, early degeneration of serotonergic neurons in some brain areas was observed as well in the course of progressive parkinsonism (Leal *et al*., 2019).

The most effective treatment for PD patients remains symptomatic (Seppi *et al*., 2019; Zhu *et al*., 2017). Dopamine-replacement therapy using levodopa (L-dopa) remains the gold standard treatment for PD motor symptoms. However, L-dopa therapy decreases effectiveness over time, and motor complications are associated with long-term therapy (Nasuti *et al*., 2017; Olanow, 2015). Thereby, lacking a cure, the therapeutic challenge for PD is the development of drugs to arrest or prevent the progression of the neurodegeneration process improving the motor symptoms.

For a better understanding of the pathophysiology of PD, animal models of substance-induced parkinsonian symptoms, including reserpine (RES), have been considered a useful tool to investigate the mechanisms underlying PD, as well as to screening new drugs or neuroprotective approaches (Bispo *et al*., 2019; Campêlo *et al*., 2017; Leal *et al*., 2019; Leão *et al*., 2017; Santos *et al*., 2013a). Studies have shown that a range of pure compounds derived from herbal medicines is effective in vivo and in vitro models of PD through the modulation of multiple variables involved in the pathogenesis of PD (Beserra-Filho *et al*., 2019; Li *et al*., 2013; Lins *et al*., 2018).

Phytol (PHY; 3,7,11,15-Tetramethylhexadec-2-en-1-ol) is one of the

compounds found in abundance in nature (Figure 1). It is part of the chlorophyll molecule produced by almost all photosynthetic organisms such as algae (De Souza; Nes, 1969), bacteria (cyanobacteria) (Proteau, 1998), and plants (Ischebeck *et al*., 2006). PHY is an acyclic isoprenoid abundant in the biosphere (Rontani, Volkman, 2003).

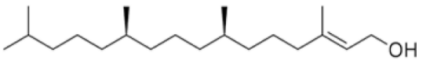

Figure 1: Chemical structure of phytol.

The pathway of PHY metabolic degradation is complex and describes the conversion of PHY into phytanic acid (PA) and degradation products (Islam *et al*., 2015). Studies have been reported some biological effects of PHY and PA such as antimicrobial functions, antiradical actions in low concentrations (Pejin *et al*., 2014a; Pejin *et al*., 2014b), antioxidants, antinociceptives (Santos *et al*., 2013b), and anti-inflammatory activity (Silva *et al*., 2014). The wide variability of PHY's actions allows investigations for various physiological disorders (Islam *et al*., 2018).

Although experimental data show that PHY metabolism and PA oxidation are autologous under pathological conditions, the exact physiological mechanisms regarding their actions are still undetermined. These results suggest that the natural compound PHY would provide protective action against physiopathological alterations of PD.

In this context, the present study aimed to investigate the possible protective effect of PHY in a RES-induced rat model of Parkinson's disease, with emphasis on behavioral effects.

Material and methods

Animals

Thirty-two Seven-month-old male Wistar rats (n = 32) were used in this study. All animals were housed in groups of four or five per plastic cage (30 cm × 37 cm × 16 cm), under controlled conditions of ventilation, temperature (22 ± 1 °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 according to the Brazilian law for the use of animals in scientific research (Law number 11.794) and all the procedures were approved by the local Animal Ethics Committee (Protocol number 9018280619). All efforts were made to minimize animal pain, suffering, or discomfort and reduce the number of animals used.

Drugs and general procedure

Phytol (3,7,11,15-Tetramethylhexadec-2-en-1-ol) and reserpine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phytol (Phy) was emulsified with 0.2% Tween 80 and dissolved in saline (0.9% isotonic saline) at the concentrations of 50 mg/mL. Vehicle of Phytol consisted of 0.2% Tween 80 dissolved

in saline (C-Phy). Both Phytol and vehicle were injected intraperitoneally (i.p.). Reserpine (RES) was dissolved in 50µl of glacial acetic acid and then diluted in distilled water at a concentration of 1.0 mg/ml. The vehicle (C-RES) consisted of the same amount of glacial acetic acid and distilled water as in the reserpine solution. Both RES and vehicle were injected subcutaneously (s.c.). All solutions were prepared before administration.

The animals were handled daily during 5 days for 5 min/day to habituate before the beginning of the experimental procedures. The apparatus was cleaned with a 5% alcohol solution after each behavioral session. The sessions of open field test were recorded by a digital camera and the behavioral parameters were registered by ANY-maze® software (version 4.3, Stoelting Co., Wood Dale, IL, USA).

Experimental design

The animals were randomly assigned to the following groups (n = 8 per group): vehicle of Phytol + vehicle of reserpine (C-Phy + C-RES); Phytol + vehicle of reserpine (Phy + C-RES); vehicle of Phytol + reserpine (C-Phy + RES) and Phytol + reserpine (Phy + RES). The animals received two subcutaneous (s.c.) injections of vehicle (C-RES) or 1.0 mg/kg of reserpine (RES) at a volume of 1.0 ml/kg body weight, in two consecutive days, concomitantly with intraperitoneal (i.p.) injections of PHY (50 mg/kg) or vehicle. During the experiment (Figure 2), rats were submitted to the following behavioral evaluations (from 8:00 a.m. to 1:00 p.m.): (1) catalepsy test (daily), (2) assessment of oral movements, and (3) open field test (24 hours after the 2nd injection).

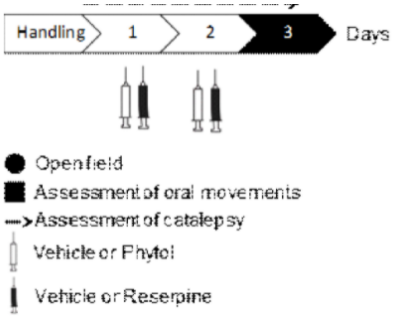

Figure 2: Schematic representation of the experimental design.

Behavioral testing

Catalepsy test

The cataleptic behavior was assessed by placing the animal's forepaws on a horizontal bar positioned 9 cm above the bench surface. Catalepsy was defined as an immobile posture (keeping both forepaws on the bar) and was measured up to a maximum of 180 seconds. Three trials for each animal in each observation day were carried out and the results were analyzed considering the mean value of these trials (Santos *et al.*,2013b).

Oral movements

Rats were individually placed in wire cages (40 cm × 40.5 cm × 20 cm) with mirrors placed underneath and behind the back wall of the cage to enable the behavioral quantification of the oral movements when the animal was facing away from the observer for 10 min (Fernandes *et al*., 2012). We quantified the number of vacuous chewing movements (rapid vertical deflection of the lower jaw that resembles chewing but is not directed at any particular stimulus), tongue protrusions (number of events), and oral tremor (seconds).

Open field test

The apparatus was a circular open field arena (84 cm in diameter) with 40 cm high walls, made of wood and painted in black. Animals were placed in the center of the apparatus for free exploration for 5 min (Santos et at., 2013). The sessions were recorded by a digital camera above the apparatus and the behavioral parameters were registered by an animal tracking software (ANY-maze® version 4.3, Stoelting Co., Wood Dale, IL, USA). We quantified the distance traveled in the whole arena and in the center (in meters), the average speed (in meters/second), the time spent in the center of the open field (in percentage), the number of entries in the center and the latency to start the first movement (seconds).

Data analysis

The results collected were analyzed using Kolmogorov-Smirnov's normality test and Mauchly′s sphericity test. Cataleptic behavior was analyzed using two-way ANOVA for repeated measures followed by Tukey's post hoc test. Oral movements and locomotor activity were analyzed using one-way ANOVA for repeated measures followed by Tukey's post hoc test. All statistical analyses were performed using Graph Pad Prism 6.0 (GraphPad Prism Software Inc., San Diego, CA, USA), and $p < 0.05$ was considered to reflect significant differences. Data were expressed as mean ± standard error of the mean (S.E.M.).

Results

Catalepsy test

Repeated-measures two-way ANOVA revealed a significant main effect of time (days of treatment) [$F(2, 56) = 78.29$, $p < 0.001$], treatment (RES, Phy) [$F(3, 28) = 20.78$, $p < 0.001$] and interaction between factors [$F(6, 56) = 24.33$, $p < 0.001$]. The Tukey's post hoc test revealed a significant increase in the duration of cataleptic behavior in the C-Phy + RES group as compared with the groups C-Phy + C-RES ($p < 0.001$), Phy + C-RES ($p < 0.001$) and Phy + RES ($p < 0.001$). A significant increase was also observed in the Phy + RES group as compared with the groups C-Phy + C-RES ($p < 0.001$) and Phy + C-RES ($p < 0.0001$) (Figure 3).

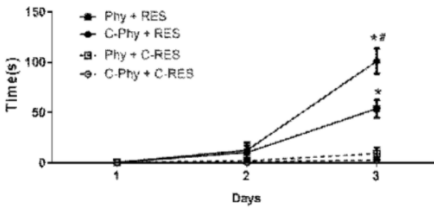

Figure 3: Effects of administration of Phytol (Phy – 50 mg/kg) or vehicle (C-Phy) on the cataleptic behavior of rats treated with 1.0 mg/kg of reserpine (RES) or vehicle (C-RES). Phy was able to minimize the deleterious reserpine-induced effects. Data are expressed as mean ± S.E.M. *p < 0.05 comparing with C-Phy + C-RES and Phy + C-RES, and #p < 0.05 compared with Phy + RES (Two-way ANOVA for repeated measures followed by Tukey's test).

Oral movements

One-way ANOVA showed a significant effect of treatment on vacuous chewing movements [F (3, 28) = 4.435, p = 0.0113] and tongue protrusions [F (3,28) = 6.17, p = 0.002], but not on oral tremor [F (3, 28) = 1.704, p = 0.189]. The reserpine treatment (C-Phy + RES) increased the number of tongue protrusions when compared with C-Phy + C-RES and Phy + C-RES groups. Regarding the vacuous chewing movements, the Phy was able to minimize the deleterious reserpine-induced effects (Figure 4).

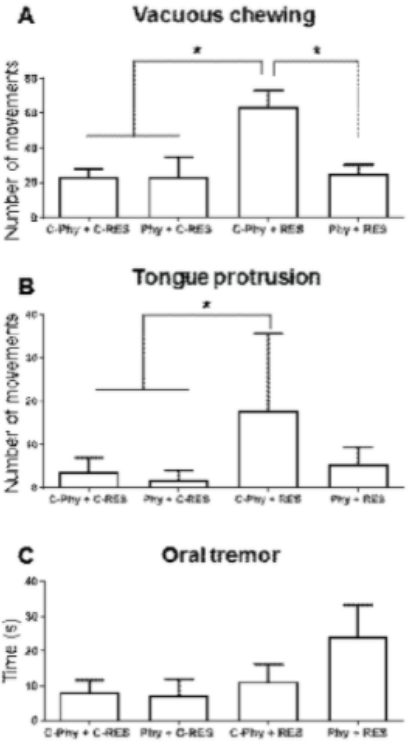

Figure 4: Effects of administration of Phytol (Phy – 50 mg/kg) or vehicle (C-Phy) on oral movements of rats treated with 1.0 mg/kg of reserpine (RES) or vehicle (C-RES). In (A), vacuous chewing movements; (B) tongue protrusion, and (C) oral tremor. Data are expressed as mean ± S.E.M. *p < 0.05. (One-way ANOVA for repeated measures followed by Tukey's test).

Locomotor activity

One-way ANOVA showed a significant effect of treatment on total distance traveled [F (3, 28) = 64.93, p < 0.001], mean speed [F (3, 28) = 64.76, p < 0.001], central entries [F (3, 28) =

10.50, $p < 0.001$], central distance [$F(3, 28) = 4.78$, $p = 0.008$] and central time [$F(3, 28) = 5.60$, $p = 0.004$]. Additionally, C-Phy + RES and Phy + RES groups presented motor impairment when compared with C-Phy + C-RES and Phy + C-RES groups ($p < 0.05$). Regarding to latency to move, One-way ANOVA reveled no effect [$F(3, 28) = 1.95$, $p = 0.145$], (Table 1).

	C-Phy + C-RES	Phy + C-RES	C-Phy + RES	Phy + RES
Total distance (m)	18.39 (1.32)	15.21 (1.93)	0.53 (0.10)*	1.55 (0.46)*
Mean speed (cm/s)	6 (0.04)	5 (0.6)	0.2 (0.03)*	0.4 (0.02)*
Central entries (number)	8 (0.87)	6.88 (1.91)	1 (0)*	2 (0.54)*
Central distance (m)	1.95 (0.22)	1.47 (0.39)	0.25 (0.04)*	0.94 (0.5)*
Central time (%)	5.86 (0.8)	5.63 (1.63)	50.88 (17.84)*	58.38 (15.94)*
Latency to move (s)	0.52 (0.43)	0.78 (0.48)	5.44 (2.29)	4.42 (2.58)

Table 1: Effects of administration of Phytol (Phy – 50 mg/kg) or vehicle (C-Phy) on locomotor activity of rats treated with 1.0 mg/kg of reserpine (RES) or vehicle (C-RES). Data are expressed as mean ± S.E.M. One-way ANOVA for repeated measures followed by Tukey's test. *$p < 0.05$ compared with C-Phy + C-RES and Phy + C-RES groups.

Discussions

The present study investigated whether phytol administration would attenuate reserpine-induced motor impairments in rats. Our results showed that PHY prevented an increase in cataleptic behavior, number of vacuous chewing movements, oral tremor, and tong protrusion. However, it was unable to prevent a reduction in open-field locomotor activity induced by RES.

Animal models have been used for elucidating the mechanisms underlying PD and behavioral disturbances. The reserpine model was one of the earliest animal models to assess the efficacy of levodopa as an anti-parkinsonian drug (Duty, Jenner, 2011). Reserpine is a drug that irreversibly inhibits the vesicular monoamine transporter type 2 (VMAT-2) leading to a depletion of brain monoamines like dopamine, noradrenaline, and serotonin (Leão *et al*., 2015). Acute and chronic administration of reserpine induces motor abnormalities similar to that observed in PD such as bradykinesia, muscle rigidity, resting tremor, and postural deformities (DeLong, Wichmann, 2007). Chronic treatment with low doses of reserpine (0.1 mg/kg) induces motor alterations, which were evaluated on the catalepsy test (Santos *et al*., 2013a), oral movements (Lins *et al*., 2017), and open field test (Bispo *et al*., 2019).

Catalepsy test assesses the locomotor activity and the ability of the animal to initiate a movement to correct the posture once the animal is placed into the unusual posture on the horizontal bar. The time taken to correct the pos-

ture is recorded and represents the intensity of the cataleptic behavior, which has similarities to motor patterns observed in parkinsonian patients (Leão *et al*., 2015). Studies using a chronic administration of reserpine to induce parkinsonism have reported a progressive increase in the time measured during the catalepsy test (Fernandes *et al*. (2012), Santos *et al*. (2013), Leão *et al*. (2017)). However, studies using a hydroethanolic extract of Poincianella pyramidalis (Lins *et al*., 2017), monoterpene Carvacrol (Lins *et al*. 2018), and Eplingiella fruticose essential oil (Beserra-Filho *et al*. 2019) were able to reduce the motor changes induced by reserpine in rats. The present study demonstrated that PHY was also able to reduce the motor abnormalities, even though the dose of reserpine is 10 times higher than in the studies above.

A study conducted by Fernandes *et al* (2012) demonstrated that the chronic administration of reserpine was able to promote impairments in oral movements. The present study corroborates that results once the administration of 1.0 mg/kg of RES, in two consecutive days, was able to induce tardive dyskinesia as well. However, the concomitant treatment with PHY showed a neuroprotective action once prevented the behavioral changes in two parameters.

In the open field test, the reserpine-treated rats showed a decrease in motor activity. The longer time spent in the center of the apparatus may be associated with motor impairment, instead of some reduction in anxiety-like behavior. Thereby, PHY was unable either to prevent or reduce changes induced by RES. However, a similar PHY dose resulted in sedative action and the ability to produce muscle relaxation in a prior study. This finding may be attributed to a possible action of PHY on subunits of GABA receptors (Costa *et al*., 2014). It is reasonable to state that the physiological effects favor maintaining the motor impairments observed in animals treated with both RES and PHY. Moreover, although the open field test assesses motor ability, the mechanisms associated with this activity differ from those entailing the cataleptic behavior and oral movements.

Conclusion

PHY was able to reduce the motor impairments on the catalepsy test and oral movement assessment in an animal model of reserpine-induced-parkinsonism, demonstrating a possible neuroprotective effect. Further studies modifying the treatment protocol and dose of PHY should be carried to clarify a possible neuroprotective effect of PHY using this model.

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