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Article

Effects of *Hypericum perforatum* on turning behavior in an animal model of Parkinson's disease

Débora Dalla Vecchia[1], Marissa Giovanna Schamne[1], Marcelo Machado Ferro[2], Ana Flávia Chaves dos Santos[1], Camila Lupepsa Latyki[1], Danilo Vieira de Lara[1], Juliana Ben[3], Eduardo Luiz Moreira[3], Rui Daniel Prediger[3], Edmar Miyoshi[1,*]

[1]Department of Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [2]Department of General Biology, State University of Ponta Grossa, Ponta Grossa, PR, Brazil, [3]Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Parkinson's disease (PD) is an age-related neurodegenerative disorder characterized by the slow and progressive death of dopaminergic neurons in the (substantia nigra *pars* compact). *Hypericum perforatum* (*H. perforatum*) is a plant widely used as an antidepressant, that also presents antioxidant and anti-inflammatory properties. We evaluated the effects of *H. perforatum* on the turning behavior of rats submitted to a unilateral administration of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle as an animal model of PD. The animals were treated with *H. perforatum* (100, 200, or 400 mg/kg, v.o.) for 35 consecutive days (from the 28th day before surgery to the 7th day after). The turning behavior was evaluated at 7, 14 and 21 days after the surgery, and the turnings were counted as contralateral or ipsilateral to the lesion side. All tested doses significantly reduced the number of contralateral turns in all days of evaluation, suggesting a neuroprotective effect. However, they were not able to prevent the 6-OHDA-induced decrease of tyrosine hydroxylase expression in the lesioned striatum. We propose that *H. perforatum* may counteract the overexpression of dopamine receptors on the lesioned striatum as a possible mechanism for this effect. The present findings provide new evidence that *H. perforatum* may represent a promising therapeutic tool for PD.

Uniterms: Parkinson's disease/treatment/experimental study. *H. perforatum*/phytotherapy/neuroprotective effect. Turning behavior. 6-OHDA.

A Doença de Parkinson é uma doença neurodegenerativa relacionada à idade, caracterizada pela morte lenta e progressiva de neurônios dopaminérgicos da substância negra pars compacta. O *Hypericum perforatum* (*H. perforatum*) é um fitoterápico utilizado como antidepressivo, apresentando propriedades antioxidantes, anti-inflamatórias e nootrópicas. Neste trabalho, avaliaram-se os efeitos do tratamento com *H. perforatum* no comportamento rotatório de ratos no modelo da doença de Parkinson induzido pela administração unilateral de 6-OHDA no feixe prosencefálico medial. Ratos Wistar machos foram tratados com *H. perforatum* (100, 200 ou 400 mg/kg, v.o.) por 35 dias (do 28º dia antes até o 7º dia após a lesão). As rotações ipsilaterais e contralaterais à lesão foram registradas no 7º, 14º e 21º dias após a cirurgia. As três doses de *H. perforatum* utilizadas reduziram o número de rotações contralaterais, indicando um possível efeito neuroprotetor da planta. Porém, o *H. perforatum* não impediu a redução na expressão da enzima tirosina hidroxilase no estriado lesionado, quantificada por Western blot. Propomos que o *H. perforatum* possa bloquear o aumento da expressão dos receptores dopaminérgicos no estriado lesionado com 6-OHDA. Entretanto, estudos adicionais são necessários para identificar o mecanismo exato pelo qual o *H. perforatum* reduziu o número de rotações contralaterais. Os resultados do presente estudo sugerem o *H. perforatum* como um potencial agente terapêutico para a doença de Parkinson.

Unitermos: Doença de Parkinson/tratamento/estudo experimental. *H. perforatum*/fitoterapia/efeito neuroprotetor. Comportamento rotatório. 6-OHDA.

***Correspondence:** E. Miyoshi. Departamento de Ciências Farmacêuticas, Universidade Estadual de Ponta Grossa, 84030-900 - Ponta Grossa - PR, Brasil.
E-mail: edmar@uepg.br

INTRODUCTION

Parkinson's disease (PD) is a debilitating disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Hirsch *et al*., 1988, 2005). At the time of diagnosis, patients typically display variety of motor impairments, including bradykinesia, resting tremor, rigidity, and postural instability.

Although the etiology of the neurodegenerative process found in PD is not completely understood, it has been suggested that a state of oxidative imbalance is triggered by one or more factors, including brain aging, genetic predisposition, mitochondrial dysfunction, free radical production and environmental toxins (Henchcliffe, Beal, 2008; Zhou, Huang, Przedborski, 2008). Novel therapeutic strategies support the application of reactive oxygen species (ROS) scavengers, transition metal chelators, nonsteroidal anti-inflammatory drugs, natural antioxidant polyphenols, anti-apoptotic drugs, and bioenergetic drugs in monotherapy or as part of an antioxidant cocktail formulation (Mandel, Youdim, 2004).

The plant *H. perforatum L.* (Saint John's wort) possesses anti-inflammatory, antioxidant, and nootropic properties (Rodríguez-Landa, Contreras, 2003; Griffith *et al.,* 2010). Here, we investigated its effects on the nigrostriatal pathway lesion induced by unilateral injection of 6-hydroxydopamine (6-OHDA) into rats' medial forebrain bundles, which led to partial retrograde degeneration of dopamine neurons in the substantia nigra. This model is widely used to investigate novel agents for relieving motor symptomsas well as potential neuroprotective compounds in PD (Blum *et al*., 2001).

MATERIAL AND METHODS

All procedures used in the present study were conducted according to national and international legislation, with approval of the Ethics Committee for Animal Research of the Universidade Estadual de Ponta Grossa (UEPG) (Process CEUA 14/2011/ Protocol UEPG-7821/2011). Subjects were adult male Wistar rats (3 months old, 280-340g) from the UEPG breeding stock. The animals were randomly divided into 8 groups (N= 6-12): SHAM-vehicle, SHAM-HP100, SHAM-HP200, SHAM-HP400, 6-OHDA-vehicle, 6-OHDA-HP100, 6-OHDA-HP200, and 6-OHDA-HP400.

Dry extract of aerial parts of *H. perforatum* (0.3% hypericin) (kindly donated by Laboratório Herbarium Botânico S/A, Colombo, Paraná, Brazil) was suspended in distilled water and administered by gavage (0, 100, 200, or 400 mg/kg/ 2 ml/kg body weight). The control group received distilled water in the same volume. This administration happened once a day for 35 consecutive days (from 28 days before surgery to 7 days post-surgery).

On the surgery day, rats were anesthetized with thiopental (50 mg/kg, i.p.) and lesioned with 6-OHDA (Sigma-Aldrich Inc., St. Louis, MO, USA) (8 µg in 0.2% ascorbic acid 0.9% saline solution) on the left medial forebrain bundle following stereotaxic coordinates: anteroposteriorly -1.9 mm from the bregma; mediolaterally +1.9 mm from the midline; and dorsoventrally -7.9 mm from the skull (Paxinos, Watson, 1998; Da Cunha *et al*., 2008).

The animals of the SHAM group received the vehicle solution (0.2% ascorbic acid in 0.9% saline solution). On the 7th, 14th, and 21st days after surgery, animals' turning behavior was tested after administration of apomorphine (1mg/kg, s.c., Sigma-Aldrich). The number of 360º turns toward the lesioned (ipsilateral) or opposite side (contralateral) was recorded for 1h (Da Cunha *et al*., 2008).

At the 22nd day after surgery, the animals were decapitated, and their striata were collected and stored in a freezer at -80 ºC until analysis. Dopaminergic neuron death was quantified by tyrosine hydroxylase (TH) Western blot (Moreira *et al*., 2010). TH expression was compared between lesioned and contralateral sides. All values are expressed as means ± S.E.M. The statistical analysis was carried out using two–way analysis of variance (ANOVA). Following significant ANOVAs, post-hoc comparisons were performed using the Newman–Keuls test. The accepted level of significance for the tests was $P \leq 0.05$. All tests were performed using the Statistica® software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Figure 1 shows contralateral turns induced by apomorphine in hemiparkinsonian rats at 7 (Figure 1A), 14 (Figure 1B), and 21 (Figure 1C) days after surgery. Two-way ANOVA indicated a significant effect of the lesion, since the 6-OHDA vehicle was different from the SHAM vehicle at the 7th [$F(1,55) = 17.86$, $p<0.001$], 14th [$F(3,55) = 32.26$, $p< 0.001$], and 21st days [$F(3,55) = 26.47$, $p< 0.001$]. Regarding the *H. perforatum* treatment, all tested doses significantly reduced the number of turns induced by the 6-OHDA lesion on the 7th [$F(3,55) = 8.73$, $p< 0.001$], 14th [$F(1,55) = 56.99$, $p< 0.001$], and 21stdays [$F(1,55) = 45.41$, $p< 0.001$]. The interaction between the two factors was also similar for the 7th [$F(3,55) = 10.35$,

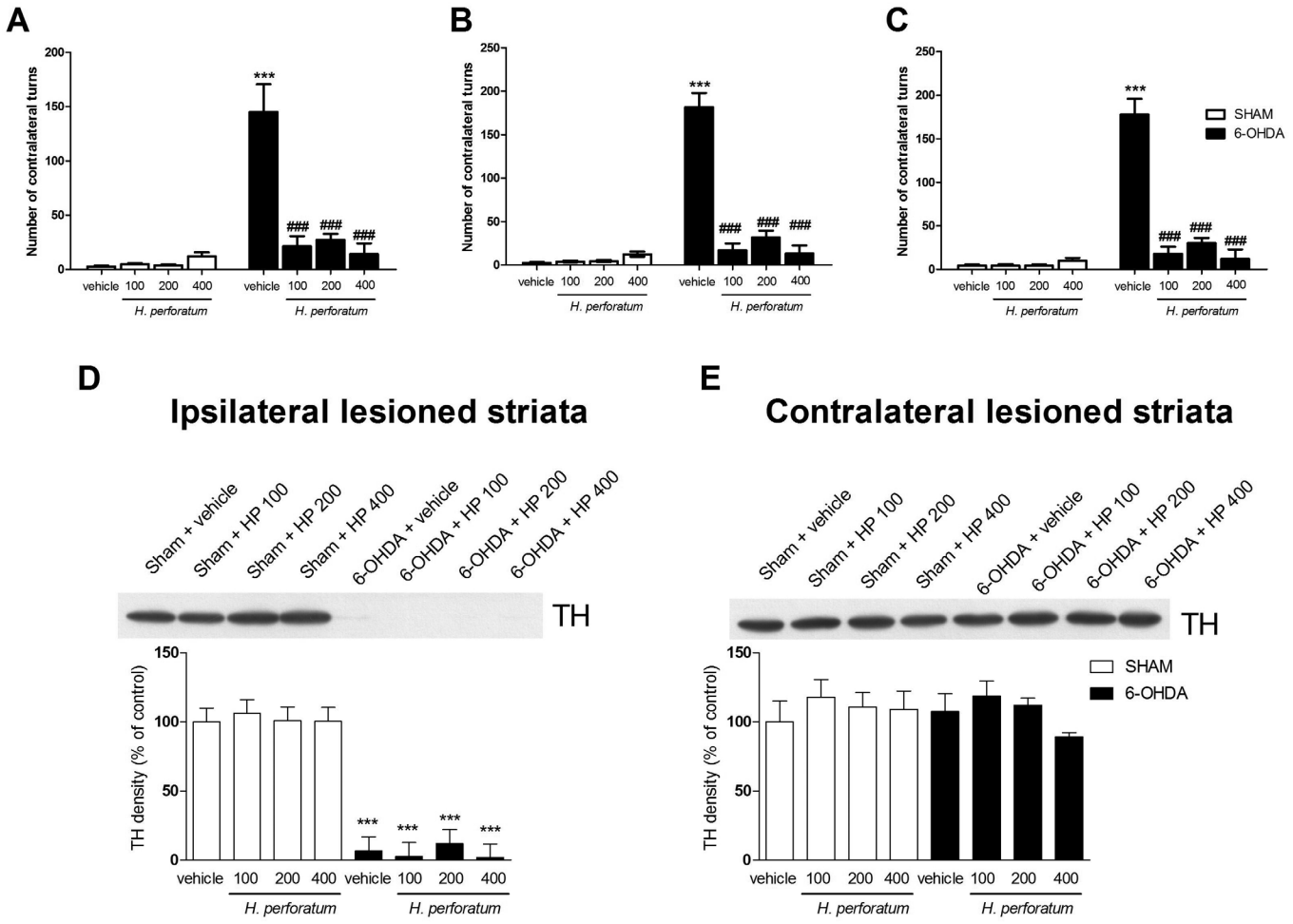

FIGURE 1 - Number of contralateral turns induced by apomorphine (1 mg/kg, s.c.) at 7 (A), 14 (B), and 21 (C) days after unilateral surgery with 6-OHDA in rats treated with different doses of extract of *H. perforatum* (100, 200, or 400 mg/kg) or vehicle for 35 days. Western blot of the TH expression in ipsilateral (D) and contralateral (E) to the lesion striata. N= 6-12 animals per group. $^{***}P < 0.001$ compared to SHAM + Vehicle group, $^{###}$ P< 0.001 compared with the 6-OHDA-vehicle (two-way ANOVA followed by Newman-Keuls post-hoc test).

p< 0.001], 14th [F (3,55) = 36.37, p< 0.001], and 21stdays [F (3,55) = 28.67, p< 0.001].

The 6-OHDA-induced turning behavior observed in this work is in agreement with previous literature where severely unilaterally lesioned animals rotate toward the side contralateral to the lesion when challenged with apomorphine (a dopamine receptor agonist) (Figures 1 A, B, and C). This rotation behavior occurs due to the imbalance of dopaminergic neurotransmission between injured and uninjured sides, so a larger lesion results in more rotations. This imbalance is caused by the overexpression of dopamine receptors in lesioned striatum, thus potentiating the apomorphine effect (Da Cunha *et al.*, 2008, 2009).

Animals of SHAM groups treated with *H. perforatum* did not perform contralateral turns when challenged with apomorphine, indicating that the *H. perforatum* extract, or its withdrawal, did not affect rotational behavior. The reduced number of contralateral turns of 6-OHDA-lesioned animals treated with *H. perforatum* could indicate a reduced neuronal loss when compared to the 6-OHDA group treated with the vehicle, but this hypothesis was not supported by the Western blot results.

Studies have shown that the administration of 6-OHDA in laboratory animals' brains leads to dopaminergic neuronal death and consequent reduced expression of TH in the striatum and substantia nigra, producing motor impairments similar to those observed in patients in the advanced stages of PD (Yin, Cao, Xie, 2010). Corroborating with these results, 6-OHDA induced a significant reduction of striatal TH levels (Figure 1D) compared to the SHAM-vehicle group [F(1,16) = 186.42, p< 0.001]. Surprisingly,

although able to reduce the contralateral rotations induced by apomorphine, the three tested doses of *H. perforatum* were not able to prevent the reduction in TH expression in the striatum ipsilateral to the lesion [$F(3,16) = 0.10$, $p= 0.96$]. Moreover, we did not observe any statistical differences among groups in the TH expression on the striatum contralateral to the lesion (Figure 1E), indicating a selective degeneration of dopaminergic neurons in the hemisphere injected with 6-OHDA.

Other authors have demonstrated that *H. perforatum* can block dopamine reuptake by presynaptic neurons (Calapai *et al.*, 2001; Menini, Gobbi, 2004). This mechanism could explain the reduced number of rotations seen on the 7th day, but not on the 14th and 21st days after lesion, since the *H. perforatum* administration was only performed until the 7th post-lesion day. Moreover, a direct agonistic effect of *H. perforatum* on dopamine receptors at later periods (i.e., 14 and 21 days) following its administration is also improbable.

One plausible hypothesis is that the *H. perforatum* may have hampered the overexpression of dopaminergic receptors on the lesioned side, which would have reduced the imbalance provoked by the 6-OHDA. Besides dopamine, *H. perforatum* also reduces serotonin and noradrenalin uptake, and it has been reported to have a brain-derived neurotrophic-factor-like effect (Leuner *et al.,* 2007). Other authors indicate an interaction of hyperforin with non-dopaminergic receptors (Ikeda *et al.,* 2012). Those effects in other structures, like the subthalamic nucleus, are involved on the modulation of turning behavior (Ikeda *et al.,* 2012; Petri *et al*., 2013). Therefore, we propose that some of these mechanisms may have caused a long-term desensitization of the nigrostriatal synapses to the direct agonist apomorphine.

CONCLUSION

These results show for the first time that treatment with *H. perforatum* reduced the number of contralateral rotations in animals lesioned unilaterally with 6-OHDA and that these effects were not directly related with neuroprotective effects. These findings provide new evidence that *H. perforatum* may represent a promising therapeutic tool in PD, thus being able to prevent motor symptoms of PD.

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