Omega-3 Fatty Acids: Neuroprotective, Antioxidant and Anti-Inflammatory Effects in a Model of Parkinson’s Disease

Alyne Oliveira Correia, Maria Elizabeth Pereira Nobre, Maria Janice Pereira Lopes, Daniel Luna Lucetti, Elaine Cristina Pereira Lucetti, Jeyson Nunes dos Santos, Kelly Rose Tavares Neves, and Glauce Socorro de Barros Viana

Abstract

Parkinson’s disease (PD) is a neurodegenerative pathology characterized by degeneration of dopaminergic neurons. The objectives were to evaluate the neuroprotective properties of Omega-3 (W3) in a model of PD. Wistar rats were distributed in groups: SO (unlesioned) and 6-OHDA-lesioned (untreated or treated with W3: 10, 20 and 100 mg/kg, p.o.). After 14 days, they were behaviorally evaluated and euthanized for monamines, nitrite and lipid peroxidation determinations. Striatal slices were immunohistochemically studied. The impaired locomotor activity and motor coordination observed in the untreated 6-OHDA-lesioned group, as related to the SO group, were partially reversed after W3 treatments. In apomorphine-induced rotations, while the SO group did not show any alteration, the untreated 6-OHDA-lesioned group increased this behavior, which was reduced by W3 treatments. Furthermore, DA and DOPAC levels were reduced in the untreated 6-OHDA-lesioned group, an effect partially reversed by W3. It also reversed the increased striatal concentrations of nitrite and lipid peroxidation and decreased neuron degeneration in the 6-OHDA-lesioned group. Similarly, decreases in striatal DAT and TH immunoreactivities found in the untreated 6-OHDA-lesioned group, relatively to the SO group, were also reversed. The results point out to the potential neuroprotective action of W3, for the treatment of neurodegenerative diseases, as PD. The W3 neuroprotection is probably related to its anti-inflammatory and antioxidant properties.

INTRODUCTION

Evidences [1,2] suggest that nutrition with the presence of Omega-3 plays an important role in controlling Parkinson’s disease (PD), a chronic neurodegenerative pathology characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Omega-3 appears to be neuroprotective for several neurodegenerative diseases [3-6]. Furthermore, the use of Omega-3 has been shown to reduce the patient’s dyskinesia and depression [7]. As a matter of fact, a meta-analysis of randomized clinical trials, demonstrated that the use of Omega-3 was effective in depressive disorders [8].

Depression is known to be present in some neurological disorders, including PD. It causes significant morbidity in terms of quality of live and is independent from the effect of motor disability [9]. The reason for depression and anxiety in PD is not completely understood, however degeneration of neurotransmitter systems other than dopamine might play a role in the occurrence of these disorders [10]. Furthermore, Omega-3 at low doses exhibits anti-inflammatory and neuroprotective properties, as already shown by us [11,12] and by others [13]. A robust Omega-3 neuroprotection was demonstrated in experimental models of cerebral ischemia in rats [14,15] and points out to the fact that Omega-3 represents a potential treatment for neurodegenerative diseases, as PD [16].

The protective effects of Omega-3 are mediated by neuroprotectin, one of its metabolic derivatives [17,18]. This neuroprotective Omega-3-derived compound protects neurons...
against oxidative stress and inflammation, while Omega-3 may protect the brain by increasing glutathione reductase activity and decreasing lipid peroxide and ROS [19-21].

Post-mortem samples from PD patients exhibit significant reductions in Omega-3 contents, as related to controls [22], whereas no differences in Omega-3 levels were found in post-mortem analyses of temporal cortex tissue from levodopa treated PD patients or in monkeys treated with MPTP, relatively to their respective matched controls [23].

The objectives of the present work were to evaluate by behavioral and neurochemical means the neuroprotective properties of Omega-3, in a model of PD. We focused upon the possible effect of Omega-3 on the striatal dopaminergic degeneration, by evaluating apomorphine-induced rotations and motor coordination, as well as DA and DOPAC concentrations. Furthermore, nitrite and lipid peroxidation measurements in the striatum and histological (fluoro jade staining) and immunohistochemical assays for TH and DAT were also carried out.

**MATERIAL AND METHODS**

**Drugs and reagents**

As the source of Omega-3 (W3), we used Proepa (Aché Laboratórios Farmacêuticos S/A, Brasil) with the following composition: DHA (180 mg), EPA (120 mg) and alpha-tocopherol (2 mg). Besides, 6-hydroxydopamine (6-OHDA), apomorphine, HPLC standards and Cremophor (used as a 1% aqueous solution for W3 emulsification) were from Sigma-Aldrich, USA. Ketamine chloride (Vetanarcol®) and Xilazine (Kensol) were from König S/A, Brazil. All other reagents were of analytical grade.

**Animals**

Male Wistar rats (250 to 300g) came from the Animal House of the Faculty of Medicine Estácio de Juazeiro do Norte (Estácio/ FMJ), Juazeiro do Norte, CE, Brazil. The animals were maintained at a 24 ± 2°C temperature, under a 12h light/12h dark cycle and standard food (Purina Chow) and water ad libitum. The study was submitted to the Ethical Committee for Animal Experimentation of the Estácio/FMJ (Protocol 2014.1-007). All experiments followed the ethical principles established in the Guide for the Care and Use of Laboratory Animals, NIH, 2011.

**The 6-OHDA model of PD and the experimental protocol**

The animals were anesthetized with xylazine (10 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.), shaved on the superior region of the head and fixed to the stereotaxic frame by their ear canals. A longitudinal midline incision was made and the tissues were separated for bregma visualization. Then, a thin hole was performed in the skull over the target area and 6-OHDA (6 µg/μL) was injected to two different points. The following coordinates were used: 1st point (AP, +0.5; ML, -2.5; DV, -5.0) and 2nd point (AP, -0.5; ML, -3.7; DV, -6.5). The syringe stayed in place for 5 min to assure the solution diffusion and, then, the incision was sutured. The sham-operated (SO) animals were subjected to all procedures, except that saline was injected into the two points. Afterwards, the animals returned to their cages for recovering.

They were divided into the following groups: SO (treated by gavage with 1% Cremophor, in aqueous solution, used as vehicle for W3 emulsion) and untreated 6-OHDA-lesioned or treated with W3 (10, 20 and 100 mg/kg, p.o.). All treatments started 24 h after the surgical procedure and continued for 15 days, with drug volumes of 0.1 mL/100 g body weight. Following treatments and 1 h after the last drug administration, the animals were submitted to the behavioral tests. At the next day, they were euthanized (decapitation) and brain tissues removed for neurochemical, histological and immunohistochemical studies.

**Open field test**

This test evaluates the locomotor activity, effects of stimulant or depressant drugs, and may also indicate an anxiolytic action. The arena was made of wood, whose dimensions were 50 cm x 50 cm x 30 cm (length, width, height). The floor was divided into 4 quadrants of equal size. At the time of the experiment (always performed in the morning), the apparatus was illuminated by a red light. The parameter analyzed was the number of crossings with the four paws, from one quadrant to another (what measures the locomotor spontaneous activity), for 5 min.

**Rotarod test**

This test is widely used for evaluating the deficit in motor coordination of rodents. An animal with dopamine depletion presents a motor deficit, depending upon the degree of the 6-OHDA striatal lesion. The animal was placed on a horizontal rotating bar (12 rpm), for 2 min, and the number of falls/min was measured.

**Apomorphine-induced rotations**

The apomorphine induction of rotational (circling) behavior is widely used for assessing the effects of lesions to the dopaminergic system and the success of treatment strategies, in rat models of Parkinson’s disease. The number of rotations under apomorphine is related to the extent of dopamine depletion after the unilateral 6-hydroxydopamine lesion. The contralateral rotations (opposite to the lesioned right-side) induced by apomorphine (1 mg/kg, s.c.) were monitored for 1 h. The cause for this apomorphine-induced rotational behavior is related to the unbalance, in the nigrostriatal dopaminergic pathways, between the right (lesioned) and left (unlesioned) brain hemispheres. This asymmetric circling behavior after the apomorphine administration is a quantifiable motor deficit and an important paradigm in this model of PD.

**Brain nitrite**

Nitric oxide (NO) is an important physiological messenger and effector molecule in many biological systems. A means for investigating NO formation is to measure nitrite, one of two breakdown products of NO. The Griess reagent (0.1% N-1-naphthylethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% phosphoric acid, 1:1:1:1) reveals the presence of nitrite in the sample homogenate. This assay relies on a diazotization reaction that forms a pink cromatophore, showing an absorbance peak at 560 nm. A 100 µL supernatant from brain homogenates was added to 100 µL Griess reagent. To the blank 100 µL Griess reagent was added 100 µL saline.

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*Email: gbviana@live.com*
Absorbances were measured at 560 nm and nitrite determination was archived by comparison within a standard curve of NO$_3$Na, performed under the same conditions.

**Lipid peroxidation measurements**

Lipid peroxidation is an important organic expression of oxidative stress, induced by reactive oxygen species (ROS). The most used method is based on the MDA (malondialdehyde) reaction with thiobarbituric acid, resulting in the formation of thiobarbituric acid reactive substances (TBARS), as described by Draper and Hadley, 1990 [24]. Striatal homogenates were prepared in 10% potassium chloride solution (1.15% KCl). A homogenate aliquot (250 µL) was mixed with a 0.6% thiobarbituric acid solution (1 mL). After stirring, the mixture was kept in boiling water (95-100 °C) for 15 min. It was then cooled in ice, followed by centrifugation (4000 rpm/5 min). The TBARS content was determined in a plate reader at 540 nm. The results were expressed in µmol/g MDA in the tissue. A standard curve was obtained by reading various standard concentrations of MDA (0.6; 1.2; 2.4; 4.08; 8.16; 16.32 µmol).

**Determinations of DA and DOPAC by HPLC**

Parkinson's disease is known to result in dopaminergic neurons loss in the substantia nigra [25]. The striatal contents of DA and DOPAC were determined by HPLC and, for that, homogenates were prepared in 10% HClO$_4$ and centrifuged at 4°C (15,000 rpm, 15 min). Then, the supernatants were filtered and 20 µL injected into the HPLC column. An electrochemical detector (model L-ECD-6A from Shimadzu, Japan) coupled to a column (Shim-Pak CLC-ODS, 25 cm), with a flow rate of 0.6 mL/min, was employed. A mobile phase was prepared with monohydrated citric acid (150 mM), sodium octyl sulfate (67 mM), 2% tetrahydrofuran and 4% acetonitrile, in deionized water (with pH adjusted to 3.0). Monoamines were quantified by comparison with standards and the results expressed as ng/g tissue.

**Fluoro jade staining in rat striata**

Fluoro jade is an anionic fluorescein derivative, useful for the histological staining of neurons undergoing degeneration. After paraffin removal (by immersion in xylol), sections (5 µm) were mounted on slides surrounded by gelatin. The tissue was rehydrated by immersion in ethanol for 3 min, followed by 70 and 50% ethanol solutions. The slices were placed into a 0.06% potassium permanganate solution, for 15 min, washed in distilled water and transferred to a fluoro jade solution (30 min, with gentle stirring). After staining, the slices were washed in distilled water (3 times) and mounted in Fluoromount® media for fluorescence microscope analyses.

**Immunohistochemical assays for tyrosine hydroxylase (TH) and dopamine transporter (DAT) in rat striata**

TH is the rate-limiting enzyme for DA biosynthesis [26] and DAT controls the reuptake of extracellular DA into presynaptic neurons [27]. Brain striatal sections (fixed in 10% buffered formaldehyde for 24 h, followed by a 70% alcohol solution) were embedded into paraffin wax, for slices processing on appropriate glass slides. These sections were placed in the oven at 58°C, for 10 min, followed by deparaffinization in xylol, rehydration in alcohol at decreasing concentrations, washing in distilled water and PBS (0.1 M sodium phosphate buffer, pH 7.2), for 10 min. The endogenous peroxidase was blocked with a 3% hydrogen peroxide solution, followed by incubation with the primary anti-antibodies for DAT or TH, and diluted according to the manufacturers’ instructions (Santa Cruz or Millipore, USA), for 2 h, at RT in a moist chamber. The glass slides were washed with PBS (3 times, 5 min each) and incubated with the biotinylated secondary antibody, for 1 h (RT in a moist chamber). Then, they were washed again in PBS and incubated with streptavidin-peroxidase (30 min, RT in a moist chamber). After another wash in PBS, they were incubated in 0.1% DAB solution (in 3% hydrogen peroxide). Finally, the glass slides were washed in distilled water and counterstained with Mayer’s hematoxylin, washed in tap water, dehydrated in alcohol (at increasing concentrations), diaphanized in xylol and mounted on Entelan® for optic microscopy examination.

**Statistical analyses**

The results are presented as means ± SEM and the data were analyzed by one-way ANOVA, followed by Tukey as the post hoc test. Whenever needed (measurements of DA and DOPAC), the data were analyzed by the two-tailed paired Student’s t-test, comparing differences between the right and left striata from the same animal. For photomicrographs, the data were quantified by the Image J software (NIH, USA).

**RESULTS**

**Open field test**

In this test, the spontaneous locomotor activity was evaluated in 6 to 9 animals per group. The untreated 6-OHDA-lesioned group showed a 71% decrease in the number of crossings/5 min, relatively to the SO group. A partial reversion was observed in the 6-OHDA-lesioned groups, after W3 treatments with the doses of 10 and 20 mg/kg, and the decreases in locomotor activity ranged from 23 to 31%, respectively, as related to the SO group. No
statistical difference was seen between the 6-OHDA+W3 (100) and the SO groups (Figure 1).

Rotarod test
In this test the animal's motor coordination was also evaluated in 5 to 9 animals per group. The untreated 6-OHDA-lesioned group showed a 4.3-fold increase in the number of falls/ min, as related to the SO group. This motor in coordination was almost completely reversed (1.6-fold increase), in the 6-OHDA groups after W3 treatments, whose values were not statistically different from that of the SO group (Figure 2).

Apopomrine-induced rotations
Changes in the apomorphine-induced circling behavior were measured in 4 to 8 animals per group. While the SO group did not show any change in circling behavior, the untreated 6-OHDA-lesioned group presented around 123 contralateral rotations per hour. After W3 treatments, the contralateral rotations significantly decreased from 25 to 36 per hour (Figure 3).

Nitrite determination in the rat striatum
The results (from 3 to 11 animals per group) showed 2-fold increases in nitrite contents, in the untreated 6-OHDA-lesioned group, as related to the SO group. On the other hand, after W3 treatments with all three doses (10, 20 and 100 mg/kg, p.o.), the values went towards those of the SO group (Figure 4).

Lipid peroxidation
In this test, 3 to 8 animals per group were used. The untreated 6-OHDA group showed a 1.7-fold increase in lipid peroxidation as related to the SO group. While there was a lower increase in the 6-OHDA group, after treatments with the W3 dose of 10 mg/kg, no significant changes were observed after treatments with the two higher doses of W3 (20 and 100 mg/kg), as related to the SO group (Figure 5).

DA and DOPAC measurements
For measurements of DA and DOPAC contents, striatal tissues from 4 to 11 animals per group were used. While no differences were noticed between the striatal left and right sides of the SO group, the untreated 6-OHDA-lesioned group showed a 78% decrease in the right-side striatal DA content, relatively to its unlesioned left side. A similar decrease was seen in the right-side of the 6-OHDA-lesioned group, treated with the lowest W3 dose (10 mg/kg), as related to its unlesioned side. On the other hand, lower decreases in DA concentrations were demonstrated in the lesioned side of 6-OHDA groups, after treatments with W3 at the doses of 20 mg/kg (54% decrease) and 100 mg/kg (57% decrease), as related to their unlesioned side (Figure 6A). A similar effect was observed with an 82% decrease of DOPAC contents, in the striatal lesioned right-side of the untreated 6-OHDA group treated with the W3 dose of 10 mg/kg, significantly lower decreases were seen in the 6-OHDA-lesioned groups treated with W3, at the doses of 20 (35% decrease) and 100 mg/kg (32% decrease) (Figure 6B).
lesioned group, relatively to the SO group. The decrease was of only 26% in the 6-OHDA-lesioned group, after the W3 treatment with the dose of 20 mg/kg. In addition, the DAT immunostaining of the 6-OHDA+W3 (100) group was not statistically different from that of the SO group (Figure 8).

**Immunohistochemistry for tyrosine hydroxylase (TH)**

This assay was performed in groups of 3 animals each. The results showed around a 97% decrease in TH immunostaining, in the untreated 6-OHDA-lesioned group, as related to the SO group. However, only a 48% decrease was observed in the 6-OHDA-lesioned group, after the W3 treatment at the dose of 20 mg/kg. Although a 23% decrease was seen in the 6-OHDA+W3 (100) group, this effect was not statistically different from that of the SO group (Figure 9).

**Fluoro jade staining**

A 40% decrease in fluoro jade staining (3 animals per group) was noticed in the untreated 6-OHDA-lesioned group, as related to the SO group, suggesting neuronal degeneration and non-viable cells. On the other hand, no statistical differences were demonstrated in the 6-OHDA-lesioned groups, after W3 treatments with the doses of 20 and 100 mg/kg (Figure 7).

**Immunohistochemistry for dopamine transporter (DAT)**

The immunohistochemical assay (3 animals per group) for DAT, in the lesioned right striatum, revealed an almost 100% decrease in DAT immunoreactivity in the untreated 6-OHDA-
The omega-3 (W3) essential fatty acid family is present in the CNS, where it is involved in synaptogenesis, synaptic function cognition and neuroprotection [14,15]. Interestingly, neuroprotection studies focus on DHA that is quantitatively the most important Omega-3 PUFA, in the brain [16]. This is also the main component studied in the present work which evaluates the neuroprotective, anti-inflammatory and antioxidative effects of W3, in a model of Parkinson’s disease (PD) in rats.

The neuropathology of PD includes dopaminergic neuronal loss in the substantia nigra pars compacta, as well as the presence of Lewy bodies, which are intracellular inclusions of the alpha-synuclein protein [28]. Some mechanisms present in PD patients are mitochondrial dysfunction [29], neuroinflammation [30,31] and oxidative stress [32]. Previously [33], not only Omega-3 fatty acids but also neuroprotectin D1 have been shown to modulate initiation and progression of neurodegenerative pathologies, as Alzheimer’s disease (AD).

In the present work, we showed that the untreated 6-OHDA-lesioned group presented motor incoordination, as related to the sham-operated animals (SO). This effect, reflected by an increased number of falls of the untreated 6-OHDA-lesioned group, was reversed after W3 treatments. PD patients are known to present characteristic locomotor disturbances, including shuffling gait, short steps and low walking velocity what are, at least in part, displayed in rats with unilateral dopamine depletion [34]. The data suggest that this model resembles key features of human parkinsonian gait.

Furthermore, 6-OHDA-induced striatal lesion leads to a characteristic rotation behavior in response to the dopamine agonist apomorphine. The intrastriatal administration of 6-OHDA induces partial degeneration of the nigrostriatal pathway that correlates with the degree of lesion, as recently shown [35]. Previously [36], the acute challenge with dopamine-replacing drugs was demonstrated to elicit rotational response, in the 6-OHDA model of PD. This rotation is contralateral to the lesioned side and considered to represent an antidopaminergic effect. Thus, the reduction of apomorphine-induced rotational behavior, is the most utilized drug-induced paradigm for assessing functional efficacy, in a rat model of PD [37]. Thus, by decreasing this behavior, W3 was shown to be neuroprotective. As a matter of fact, evidences, particularly from animal studies, suggest that changes in brain concentrations of DHA are positively associated with changes in cognitive or behavioral performance [38].

The unilateral intrastriatal injection of 6-OHDA leads to a drastic decrease in the striatal contents of DA and its metabolite DOPAC, as already observed by us [39-42] and others [43]. Neurotoxin-based models of PD have been important in elucidating the molecular cascade of cell death in dopaminergic neurons and the vulnerability of the substantia nigra to the degenerative process [25]. Thus, the massive cell death of the neromelanin-containing dopaminergic neurons of the substantia nigra is essential for the diagnosis of parkinsonian features [44] and is thought to cause the classical motor symptoms of PD [45]. The partial reversion by W3 of this dopaminergic neuron loss in the striatum corroborates the drug benefits against motor alterations.

The neurotoxicity of dopamine quinone formation by auto-oxidation of DA has been implicated in the specific cell death of dopaminergic neurons in PD and in the model of neurotoxin-induced parkinsonism [46]. Evidences also point out that the 6-OHDA lesion increased protein oxidation in PC12 cells, as indicated by the carbonyl group accumulation and increased caspase-3 activity [47]. Later,Latchoumycabdane et al. [48], demonstrated that oxidative stress and caspase activation contribute to the 6-OHDA-induced cell death of dopaminergic neurons.

We showed increased striatal nitrite contents and increased lipid peroxidation in the untreated 6-OHDA-lesioned group, that were almost completely blocked by the W3 treatments, suggesting that the drug neuroprotective effect is at least partly due to its antioxidant effect. Previously [49], a protective effect of omega-3 fatty acids was demonstrated on cardiovascular disease markers, as well as on oxidative stress parameters, in rats. Lately [50], an improved effect of omega-3 enriched food on lipid peroxidation in a health men population was also shown. Both studies corroborated with findings of the present work.

Dopamine (DA) neurotransmission is initiated by the presynaptic release of dopamine and terminated largely by its reuptake, through a specific sodium-dependent dopamine transporter (DAT). The synaptic concentration of DA and, therefore, the level of DA receptor stimulation are regulated by DAT activity and, through this mechanism, it is critically involved with certain pharmacological or pathological conditions [51]. DAT may be the single most important determinant of extracellular DA concentrations and, in PD patients, may be reduced up to 70% [52]. Furthermore, tyrosine hydroxylase (TH) is the rate-limiting step in brain catecholamine synthesis and PD is characterized by severe loss of dopaminergic neurons and by DA depletion in the substantia nigra. Thus, the reduction of TH expression results in diminished DA synthesis, leading to PD [53] which can be considered a TH-deficient syndrome of the striatum [54].
In the present study, we showed neuron degeneration in the untreated 6-OHDA group, as evaluated by fluoro jade staining, and these effects were completely reversed by W3 treatments, as already observed by us [12]. Furthermore, drastic reductions in immunoreactivities for both markers occur in PD that are correlated, at least in part, with the decrease in striatal DA contents in the untreated 6-OHDA-lesioned group. The W3 treatments, mainly at the highest dose, were efficient in reversing 6-OHDA effects on DAT and TH. An earlier study [43] examined the effect of a 6-OHDA nigrostriatal lesion on TH immunoreactivity and noticed that, 28 days after lesion, there was a significant reduction in the number of TH-positive neurons in the striatum and substantia nigra, as well as in other brain structures. Recently, Coulombe et al.[55], detected an 89% rise in TH immunoreactive terminals in the striatum, following 6-OHDA lesion in mice. These authors showed that, although DHA did not change this pattern, increased dopaminergic cell bodies (up to 21%) were detected by morphological analyses.

Neuroinflammation is present in a great number of neurological and psychiatric pathologies and its modulation offers a potential therapeutic target in these disorders. As shown by us [11] and others [56], W3 manifests an anti-inflammatory activity and, although the source of W3 (Proepa) has alpha-tocopherol at a very low concentration in its composition, we feel that this compound did not interfere with the results. Usually, the anti-inflammatory activity of alpha-tocopherol is only seen at higher doses [57,58]. The anti-inflammatory effects observed in the present study are probably due to resolvin D2, derived from W3, since the compound has been demonstrated to suppress inflammatory mediators expression in a LPS-induced PD model in rats [59]. Previously [60], resolvin D1 was shown to inhibit the production of LPS-induced microglia inflammatory mediators, as TNF-alpha, IL-1beta and iNOS expression. According to these authors, the mechanisms underlying these effects may include the resolvin D1 downregulation of NF-kB and subsequent pro-inflammatory cytokines. Resolvin D series are endogenous Omega-3 fatty acid-derived lipid mediators that potently promote inflammation resolution.

In conclusion, in the present study we showed a neuroprotective effect of W3, at low doses, in a model of PD in rats. The drug reverses, in part or completely, behavioral and neurochemical alterations induced by the striatal 6-OHDA lesion. The drastic decreases in DA and DOPAC contents were followed by significant reductions of TH and DAT immunoreactivities and these effects were, in great part, reversed after W3 treatments. Possibly, the potent anti-inflammatory actions of the drug, leading to decreases in inflammatory enzymes and pro-inflammatory cytokines, as already shown by us [11,12] are responsible for its neuroprotection and emphasizes the potential benefit of W3 for the treatment of neurodegenerative conditions where inflammation is present.

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REFERENCES


41. Bitu Pinto N, da Silva, AB, Neves KRT, Silva AH, Leal JK, Viana GS. Neuroprotective properties of the standardized extract from Camellia sinensis (Green Tea) and its main bioactive components, epicatechin and epigallocatechin gallate, in the 6-OHDA model of Parkinson’s disease. Evid Based Complement Alternat Med. 2015.


