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Catechin attenuates behavioral neurotoxicity induced by 6-OHDA in rats

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ABSTRACT

This study was designed to investigate the beneficial effect of catechin in a model of Parkinson's disease. Unilateral, intrastriatal 6-hydroxydopamine (6-OHDA)-lesioned rats were pretreated with catechin (10 and 30 mg/kg) by intraperitoneal (i.p.) injection 2 h before surgery and for 14 days afterwards. After treatments, apomorphine-induced rotations, locomotor activity, working memory and early and late aversive memories were evaluated. The mesencephalon was used to determine the levels of monoamines and measurement of glutathione (GSH). Immunohistochemical staining was also used to evaluate the expression of tyrosine hydroxylase (TH) in mesencephalic and striatal tissues. Catechin administration attenuated the increase in rotational behavior and the decrease in locomotor activity observed in lesioned rats. Although catechin did not rescue the impairment of late aversive memory, it protected the animals against 6-OHDA-induced working memory deficits. Furthermore, catechin treatment restored GSH levels, and significantly increased dopamine and DOPAC content, and TH-immunoreactivity in 6-OHDA-lesioned rats. Catechin protected 6-OHDA-lesioned rats due to its antioxidant action, indicating that it could be useful as an adjunctive therapy for the treatment of Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is characterized by severe motor symptoms, such as tremor, postural imbalance, slowness of movement and rigidity (Chase et al., 1998). The neuropathology of PD is based on dopaminergic cell loss in the nigrostriatal tract, with at corresponding decrease in striatal dopamine content. Several factors that have been implicated in neuronal degeneration in Parkinson's disease include mitochondrial dysfunction, oxidative stress, excitotoxicity, genetic susceptibility, apoptosis, deficient neurotrophic support, and immune deficiency (Anglade et al., 1997; Tatton et al., 2003; Tompkins et al., 1997).

There are several animal models used to study PD, and one of the best known is the unilateral striatal injection of 6-hydroxydopamine (6-OHDA), which leads to behavioral, biochemical, and pathological changes that are typical of PD (Schober, 2004; Schwarting and Huston, 1996). These toxic effects are attributed to the formation of various oxidants and free radicals, lipid peroxidation, depletion of reduced glutathione (GSH), and mitochondrial complex I deficits (Schober, 2004).

Studies of patients with PD suggest that the characteristic clinical symptoms of bradykinesia, such as rigidity and resting tremor, are frequently accompanied by impairments of cognitive function. Studies have shown that between 15% and 20% of PD patients develop a frank dementia (Brown and Marsden, 1984), and this condition is an important predictor of a poorer quality of life for these patients (Karlsen et al., 1998; Schrag et al., 2000).

Green tea consumption is inversely correlated with the incidence of Alzheimer's and Parkinson's disease (Mandel et al., 2008), and catechin, which is the main component of green tea prepared from *Camellia sinensis*, may be responsible for the alleged protective effect.

Currently, there is growing interest in studying the potential neuroprotective effects of polyphenols due to their antioxidant, radical scavenging and iron chelating properties (Youdim et al., 2002). Studies using *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that both green tea extract and epigallocatechin-3-gallate (EGCG), isolated from *C. sinensis*, are able to prevent striatal dopamine depletion in mice and dopaminergic neuron loss induced by this toxin (Levites et al., 2001).

Here, the beneficial neuroprotective effect of catechin on memory and locomotor impairment was investigated in an experimental model of PD. For this purpose, apomorphine-induced rotation, working memory and aversive memory were evaluated. Furthermore, mesencephalic and striatal TH-immunoreactivity, GSH levels and monoamine content in mesencephalic tissue were also determined.

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2. Materials and methods

2.1. Chemicals

6-Hydroxydopamine hydrochloride (6-OHDA), apomorphine hydrochloride, and (+)-catechin hydrate were purchased from Sigma Chemical Co., USA. All other reagents were of analytical grade.

2.2. Animals

Young adult (3 months) male Wistar rats (200–250 g) were obtained from the Animal House of the Federal University of Ceará and were maintained at 23–25 °C, with 12 h light–12 h dark cycle and standard diet and tap water ad libitum. All procedures in this study were in agreement with the Guide of Care and Use of Laboratory Animals from the US Health and Human Services Department, and were approved by the ethics committee on animal experimentation of the Federal University of Ceará (protocol number 29/05).

2.3. Experimental procedure

Animals (n = 90) were randomly divided into six groups: Sham-operated (SO), which received 0.2% ascorbate-saline solution into the right striatum; sham-operated group treated with catechin (10 and 30 mg/kg); lesioned group (6-OHDA), which received 6-OHDA injection (7 μg/μl per site, in a total concentration of 21 μg/3 μl); and lesioned-group treated with catechin (10 and 30 mg/kg). Unilateral, intrastriatal 6-OHDA injection was performed through a 5 μl Hamilton® syringe on anesthetized rats (ketamine 100 mg/kg and xylazine 20 mg/kg, i.p.) using a stereotaxic apparatus (Stoelting, USA) at the following coordinates (mm): site 1: L: −2.5, AP: +0.5, V: +5.0; site 2: L: −3, AP: −0.5, V: +6,0; and site 3: L: −3.7, AP: −0.9, V: +6.5 from the bregma, according to the Atlas of Paxinos and Watson (1986). Catechin was intraperitoneally administered 2 h before surgery and daily for a period of fourteen days post-surgery. The control groups received vehicle (saline) at an equivalent volume. The behavioral experiments were performed between the fourteenth and fifteenth days. Following behavioral studies, the animals were euthanized, and the mesencephalon (n = 11/group) was collected and stored at −70 °C until use. Four animals from each group were perfused with paraformaldehyde for immunohistochemical studies.

2.4. Rotational behavior

The animals were tested for rotational behavior after receiving apomorphine hydrochloride (0.6 mg/kg, i.p.) for fifteen days post-surgery, at 1-day intervals, after the last catechin injection. Rotational testing with apomorphine was conducted according to a previously described method (Ungerstedt, 1971). Briefly, animals were placed inside a cylindrical container (33 cm diameter and 35 cm height), and ipsilateral and contralateral rotations were counted for 60 min in a quiet isolated room. The data are presented as the total number of rotations towards both the ipsilateral and contralateral directions.

2.5. Open field test

Fourteen days after surgery, the rats were tested for locomotor activity using an open field apparatus, which consisted of a black acrylic chamber (50 × 50 cm), with 50 cm high walls, and the floor was divided into four squares of equal size (Broadhurst, 1957). Each rat was positioned in the center of the arena and allowed to explore freely. The numbers of crossings and rearings were scored for 5 min. The arena was cleaned with 20% ethyl alcohol to remove any odors before the next test.

2.6. Y-Maze test

Fourteenth day after surgery, spatial working memory was assessed by recording spontaneous alternation behavior in the Y-maze (Sarter et al., 1988). The apparatus was a wooden, black Y-maze. Each arm of the maze was 12 cm wide, 40 cm long, and had 35 cm high walls. Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The ability to alternate requires the rat to remember which arms have already been visited. Each experiment was scored, and the percentage of spontaneous alternation was calculated using the following formula:

$$\text{Spontaneous alternation}(\%) = \left(\frac{\text{alternation behavior}}{\text{maximum alternations}}\right) \times 100$$

where alternation behavior is defined as consecutive entries into each of the three arms, without repetition, and maximum alternations are the total number of arm entries, minus two.

2.7. Passive avoidance test

Fourteen days after surgery, aversive memory was assessed according to DeNoble et al. (1986). We used a two-compartment apparatus (50 × 22 × 27; length × width × height) from Ugo Basile, Italy. In the acquisition trial, each rat was placed individually in the light compartment, and when the animal entered the dark compartment, a foot shock of 0.5 mA was delivered through the grid floor. The latency time to enter the dark compartment was measured, with a cutoff time of 300 s (baseline). The animal was removed from the apparatus, and the trial was repeated 15 min later, even for animals that reached the cutoff time (short memory). Twenty four hours later, the retrieval trial was performed in the same manner, but in this scenario, no animal was shocked (late memory).

2.8. Determination of monoamine levels

For the measurement of noradrenaline (NE), dopamine (DA) and their metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), content using high-performance liquid chromatography (HPLC), mesencephalic tissues (n = 6/group) were used. Homogenates (10%) were prepared in 0.1 M $HClO_4$. After centrifugation at 4 °C for 15 min at 15,000 rpm, the supernatant was filtered (0.2 μm, Millipore), and a 20 μl sample was injected into a C18 column. The mobile phase was 0.163 M citric acid (pH 3.0), containing 0.02 mM NaCl with 0.69 mM sodium octanesulfonic acid (SOS) as the ion pairing reagent, 4% v/v acetonitrile and 1.7% v/v tetrahydrofuran. NE, DA and DOPAC were electrochemically detected, using an amperometric detector (Shimadzu, Japan). The amount of monoamines was determined by comparison with freshly prepared standards, and their concentrations were expressed as ng/mg of tissue.

2.9. Measurement of glutathione (GSH) levels

Reduced glutathione (GSH) was determined according to the method described by Sedlak and Lindsay (1968), with modifications. Mesencephalons (n = 5/group) were homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to produce a 10% homogenate. Aliquots (500 μl) of tissue homogenate were mixed with 400 μl of distilled water and 100 μl of 50% trichloroacetic acid (w/v) in Eppendorf tubes, and the tubes were centrifuged at 3000 ×g for 15 min. The each supernatant (330 μl) was then mixed with 666 μl of Tris (40 mM) and EDTA (20 mM) buffer (pH 8.9) and 17 μl of 5,5′-ditiobis (2-nitrobenzoic acid) (DTNB 10 mM). The absorbance was measured at 412 nm within 5 min. The GSH concentration (μg/g of wet tissue) was computed from a standard curve.

2.10. Immunohistochemical study for tyrosine hydroxylase (TH)

Four rats from each group were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the tissues were fixed by transcardial perfusion with 0.1 M phosphate-buffered saline (PBS, pH 7.2), followed by 4% paraformaldehyde (PAF) in PBS. The brains were removed, post-fixed in 4% PAF for 24 h and cryoprotected with 30% sucrose/0.1 M phosphate buffer. The brains were embedded in Tissue-Tek (Sakura-Americas, USA), frozen at −21 °C and cut into 50 μm coronal sections using a cryostat. Nigral (substantia nigra *pars compacta*) and striatal slices were collected in series (300 μm interval), and the slices were stored in 24-well plates as free-floating sections in PBS containing 0.01% NaN_3. The sections were rinsed three times for 5 min in PBS, and endogenous peroxidase was inhibited by incubating the sections in 3% H_2O_2 in PBS, for 1 h at RT. Slices, were permeabilized and blocked with PBS containing 1% Triton X-100 and 10% normal goat serum (NGS), for 1 h at RT. The sections were incubated in primary antibody (anti-TH rabbit, 1:500, Millipore) for 48 h at 4 °C, rinsed three times for 10 min in PBS and subsequently incubated with avidin–biotin–horseradish peroxidase conjugate (ABC Staining System, Santa Cruz Biotechnology) for 30 min. After washing, the slides were incubated with biotinylated goat anti-rabbit secondary antibody, diluted 1:500 in blocking solution. The color was developed using DAB as a chromogen. The sections were mounted in Entellan (Merck, Germany), cover slipped and visualized under a microscope. Eight sections per animal (Olympus BX41 microscope equipped with an Olympus DP71 camera) were analyzed to obtain a rostrocaudal sampling of the striatum, and the intensity of the TH immunoreactivity was measured by semi-quantitative densitometric analysis using an image-analysis program (Image J software, NIH, MD, USA). The number of TH-positive neurons in the SNpc was counted using the MBF Image J version (NIH, MD, USA).

2.11. Statistical analysis

Data are presented as the means ± SEM, and statistical significance was analyzed by one-way ANOVA, followed by Tukey's *post-hoc* test.

3. Results

3.1. Neuroprotective effect of catechin against 6-OHDA-induced rotational behavior

Two weeks after intrastriatal injection of 6-OHDA, rats exhibited rotational behavior towards the opposite side of the lesion (contralateral rotation) after apomorphine administration. Significant increases in the number of apomorphine-induced rotations were observed in the 6-OHDA-lesioned group compared to the sham-operated group (SO = 0.0; 6-OHDA = 76.3 ± 28.5, $p < 0.05$). Catechin significantly reversed this abnormal motor behavior (Cat10 + 6-OHDA = 11.7 ± 5.9; Cat30 + 6-OHDA = 0.8 ± 0.5), and the observed values were very close to those of the SO group (Fig. 1A).

3.2. Effect of catechin on exploratory activity in 6-OHDA-lesioned rats

We did not observe a significant decrease in the number of crossings in 6-OHDA-lesioned rats, but we observed a significant decrease in the number of rearings, which evaluates the vertical exploratory activity. Catechin treatment (10 mg/kg) significantly protected the animals against vertical exploratory activity deficits induced by 6-OHDA (n° of crossings: SO = 21.1 ± 3.7; 6-OHDA = 14.2 ± 0.8; Cat10 + 6-OHDA = 24.5 ± 2.4; Cat30 + 6-OHDA = 21.1 ± 3.7; n° of rearings: SO = 10.3 ± 2.6; 6-OHDA = 5.7 ± 0.9; Cat10 + 6-OHDA = 17.5 ± 2.3, $p < 0.05$, ANOVA and Turkey's test) (Fig. 1B).

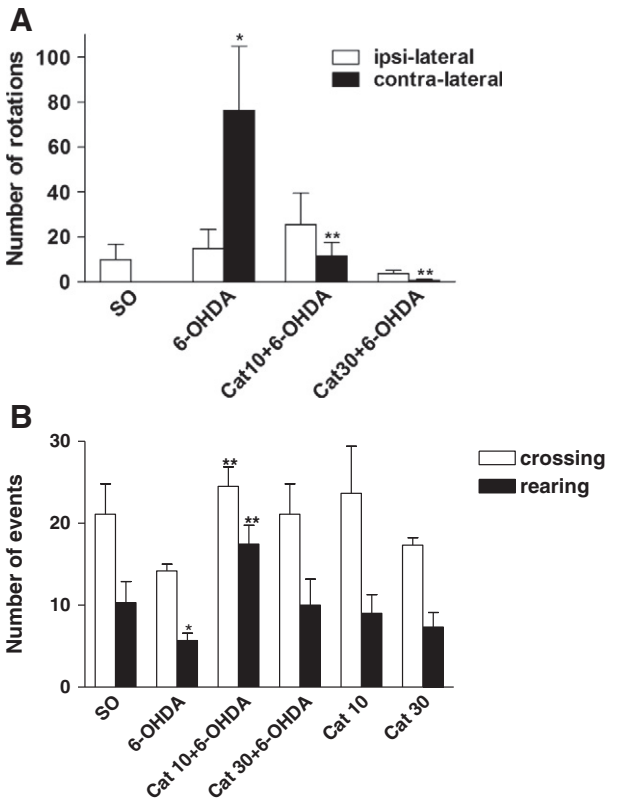

Fig. 1. Effects of catechin (10 and 30 mg/kg, i.p.) on apomorphine-induced (0.6 mg/kg, i.p.) rotational behavior (A) and exploration activity (B) in 6-OHDA-lesioned rats. A: Two weeks after 6-OHDA striatal injection, the number of net ipsi and contralateral rotations was counted for 60 min. Data are reported as the means ± SEM of 6 to 10 animals for each group. * vs. Sham-operated, ** vs. 6-OHDA (ANOVA and Tukey's test). B: Two weeks after 6-OHDA striatal injection, the number of crossings and rearings was counted for 5 min. Data are reported as the means ± SEM of, 6 to 10 animals. * vs. Sham-operated, ** vs. 6-OHDA (ANOVA and Tukey's test).

3.3. Effect of catechin on working memory in 6-OHDA-lesioned rats

Fig. 2A illustrates the effect of 6-OHDA-lesion on working memory. Rats exposed to 6-OHDA exhibited deficits in working memory (decreases of spontaneous alternations), and catechin significantly reversed these memory deficits (% spontaneous alternations: SO = 70.8 ± 5.1%; 6-OHDA = 50.1 ± 9.8%; Cat10 + 6-OHDA = 77.6 ± 2.1%; and Cat30 + 6-OHDA = 80.4 ± 5.7%, $p < 0.05$).

3.4. Effect of catechin on aversive memory in 6-OHDA-lesioned rats

Only late aversive memory was significant impaired in 6-OHDA-lesioned rats compared to the sham operated group (latency: SO = 266.5 ± 33.4 s; 6-OHDA = 106.5 ± 29.6 s, $p < 0.05$). No memory improvement was observed in lesioned animals after catechin treatment on this type of memory (Fig. 2B).

3.5. Effect of catechin on monoamine levels in 6-OHDA-lesioned rats

6-OHDA caused a significant decrease in mesencephalic dopamine, noradrenaline and DOPAC content. Catechin treatment (10 and 30 mg/kg) protected animals from the observed decrease in dopamine and noradrenaline, but not DOPAC content (Table 1).

3.6. Effect of catechin on GSH levels in 6-OHDA-lesioned rats

We observed a significant decrease in GSH levels in the mesencephalon tissue of 6-OHDA-lesioned rats compared to the sham-operated group (SO = 0.14 ± 0.01; 6-OHDA = 0.06 ± 0.00, $p < 0.05$). Catechin

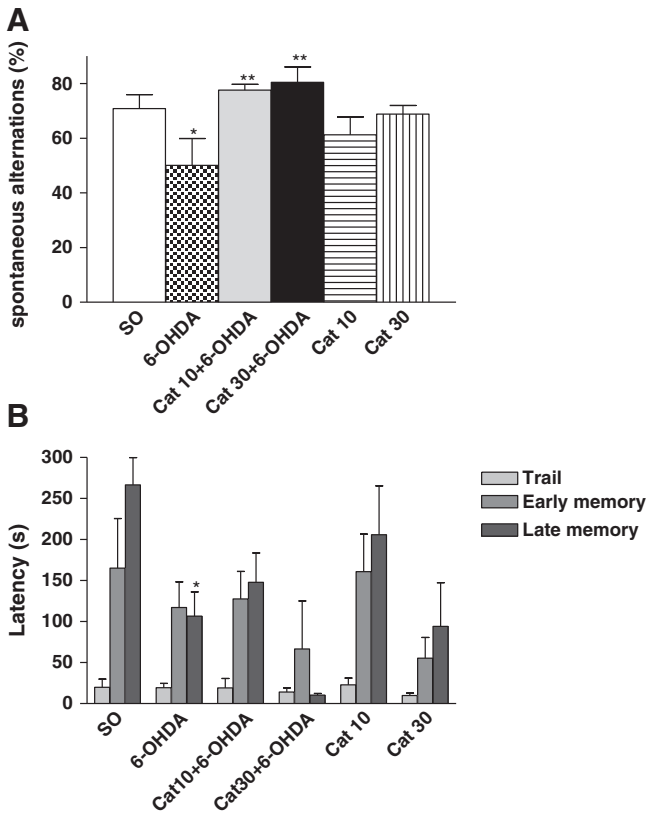

Fig. 2. Effects of catechin (10 and 30 mg/kg) on working (A) and aversive (B) memories in 6-OHDA-lesioned rats. A: Two weeks after 6-OHDA striatal injection, the percentage of spontaneous alternations was counted for 8 min. B: Two weeks after 6-OHDA striatal injection, the latency time to enter the dark side of the passive avoidance apparatus was registered over 300 s. Data are reported as the means ± SEM of 6 to 10 animals. * vs. Sham-operated, ** vs. 6-OHDA (ANOVA and Tukey's test).

(10 and 30 mg/kg) prevented the loss of GSH in this cerebral area (Cat10 + 6-OHDA = 0.18 ± 0.02 μg/g; Cat30 + 6-OHDA = 0.17 ± 0.02 μg/g tissue, $p < 0.05$) (Fig. 3).

3.7. Effect of catechin on tyrosine hydroxylase immunoreactivity in the striatal and mesencephalic tissues of 6-OHDA-lesioned rats

Significant decreases in the area of TH immunoreactivity were observed in the ipsilateral striatum of 6-OHDA-lesioned rats compared to the sham-operated group (OD values: SO = 0.66 ± 0.08; 6-OHDA = 0.13 ± 0.06, $p < 0.05$). In the substantia nigra pars compacta (SNpc), a significant decrease in the number of TH-positive cells was observed (SO = 151.0 ± 13.6; 6-OHDA = 55.0 ± 15.0, $p < 0.05$). Catechin (30 mg/kg) decreased the loss of TH immunoreactivity (0.32 ± 0.10, $p < 0.05$) and increased the number of TH-positive cells

Table 1
Effects of catechin (10 and 30 mg) on monoamine levels (ng/mg tissue) in the rat mesencephalon after the formation of 6-OHDA-induced lesion.

Group	DA	NE	DOPAC
SO	1037 ± 62	1886 ± 151	143 ± 53
6-OHDA	359 ± 83*	369 ± 82*	42 ± 15*
Cat 10 + 6-OHDA	615 ± 94**	901 ± 143**	110 ± 33
Cat 30 + 6-OHDA	820 ± 140**	883 ± 76**	64 ± 10

DA: dopamine, NE: noradrenaline, DOPAC: 3,4 dihydroxyphenylacetic acid. Animals received catechin (10 and 30 mg/kg, i.p.) 2 h before surgery and daily for 14 days. Data are reported as the means ± SEM of 6 animals/group. $p < 0.05$ (ANOVA and the Tukey's test).
* vs. sham-operated.
** vs. 6-OHDA.

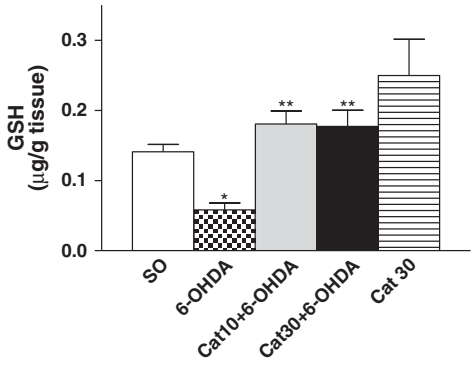

Fig. 3. Effects of catechin (10 and 30 mg/kg) on glutathione (GSH) levels in the mesencephalon tissue of 6-OHDA-lesioned rats. Data are reported as the means ± SEM of 6 to 10 animals. * vs. Sham-operated, ** vs. 6-OHDA (ANOVA and Tukey's test).

(106.8 ± 11.2, $p < 0.05$) in lesioned rats (Fig. 4C), suggestive of neuroprotective action. The contralateral hemisphere was not affected by injection, and no significant differences were observed between the control and treated groups.

4. Discussion

Anti-parkinsonian agents used in PD treatment partially relieve the symptoms of this disease, but they are not able to block dopaminergic neurodegeneration; thus, the disease continues to progress. Currently, there is a great demand for new therapies that prevent neuronal death. New therapeutic strategies for PD must identify compounds that are neuroprotective and able to cross the blood–brain barrier to produce the desired effects without causing adverse side effects. Catechin has emerged as a capable neuroprotective candidate that possesses antioxidant properties and has the ability to cross the blood–brain barrier (Nakagawa and Miyazama, 1997). In this study, the protective effect of catechin against 6-OHDA-induced neuronal damage and memory deficits was investigated. Our results demonstrated that intraperitoneal administration of catechin, a constituent of green tea (*C. sinensis*), over a 2-week time period significantly attenuated the 6-OHDA-induced behavioral abnormalities. The stereotaxic injection of 6-OHDA into the substantia nigra, medial forebrain bundle, or striatum, or peripheral administration in neonatal rats induced degeneration of the nigrostriatal pathway and striatal dopamine depletion, closely mimicking events that occur in PD (Jenner, 2008). The major neurotoxic mechanism of 6-OHDA is thought to involve radical oxygen species generated by autoxidation (Soto-Otero et al., 2000). Motor function abnormality was verified by administering apomorphine, a dopaminergic agonist, to 6-OHDA-lesioned rats. In this condition, apomorphine induces contralateral rotation behavior, reflecting an upregulation of striatal dopaminergic receptors on the lesioned site due to dopamine depletion (Ungerstedt, 1971). We showed that catechin decreased the number of contralateral rotations of lesioned rats, suggestive of a neuroprotective effect.

One possible mechanism underlying the effectiveness of catechin in this study may involve its catechol-like structure, as catechol-containing compounds are potent radical scavengers and chelators of ferric ions (Gassen and Youdim, 1997; Guo et al., 1996; van Acker et al., 1996). Catechin also displayed antioxidant activity in our study by increasing GSH activity. Epigallocatechin-3-gallate (EGCG), another major polyphenolic compound present in green tea, was shown to increase the cellular GSH pool by elevating the mRNA expression level of gamma-glutamylcysteine ligase, the rate limiting enzyme for glutathione biosynthesis (Kim et al., 2009). These authors concluded that EGCG might elicit protective effects by augmenting the cellular antioxidant capacity. More recently (Yu et al., 2010), EGCG was shown to block glutamate excitotoxicity, and according to this

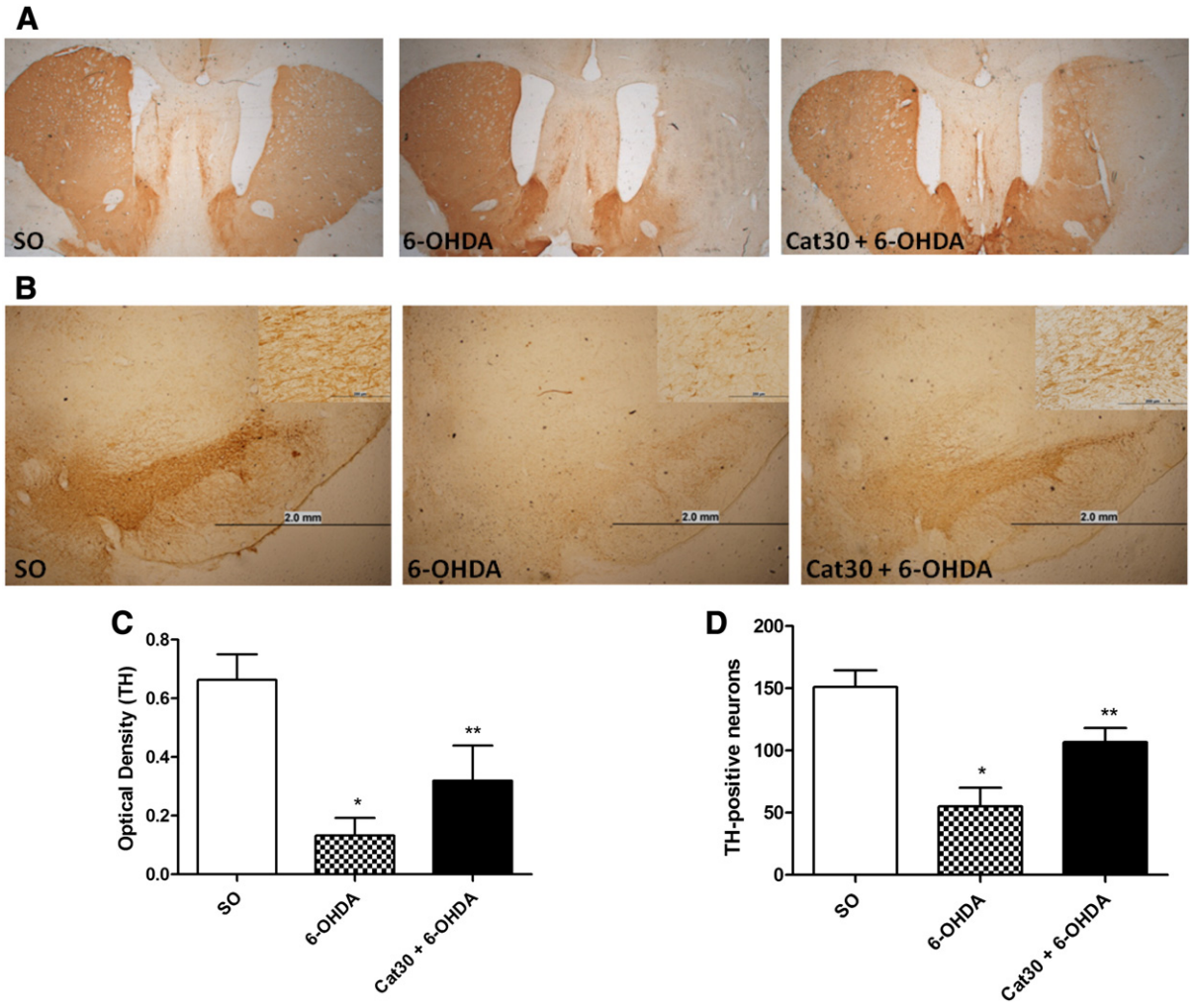

Fig. 4. Representative photomicrographs of coronal sections showing (A) TH immunostaining in the striatum and (B) TH-positive cells in the SNpc (4× objective, Olympus BX41 microscope equipped with an Olympus DP71 camera). Higher magnification is shown in the small box (40× objective). (C) Semi-quantitative analysis of TH immunoreactivity in the striatum and (D) the number of TH-positive neurons in the SNpc were assessed using optical densitometry. Analyses were made in serial coronal sections (50 μm thick and 300 μm apart) that were representative of the ipsilateral striatum or SNpc. $^{*}p < 0.05$ vs. SO group, $^{**}p < 0.05$ vs. 6-OHDA group. Data are expressed as the mean ± SEM (one way ANOVA followed by Tukey's post hoc test).

report, this property may be independent of its intrinsic antioxidant activity.

We have previously shown that catechin prevents 6-OHDA-induced oxidative cell damage in primary cultures of rat mesencephalic cells. After the exposure of these cells to 6-OHDA, the cultures showed a marked decrease in cell viability, disturbances in lipid peroxidation, and increased NO generation. Catechin treatment significantly attenuated 6-OHDA-induced cell death. Our results suggest that catechin elicited its effects by inhibiting lipid peroxidation, without interfering on the 6-OHDA-induced increase in nitrite/nitrate production (Nobre-Júnior et al., 2003). Chan et al. (2002) showed that in cultured rat brain astrocytes, the activity of SOD (Cu, Zn-SOD and Mn-SOD subtypes) was markedly increased after incubation with catechin. Thus, interfering with the SOD pathway may also have contributed to the neuroprotective effect of catechin. Other reports have also demonstrated the antioxidant activity of catechins (Babu et al., 2006; Skrzydlewska et al., 2002a, 2002b).

Another mechanism that could be involved in the neuroprotective effect of catechin is its anti-apoptotic activity. Catechins, such as epigallocatechin gallate (EGCC), have been shown to provide neuroprotection by inactivating pro-apoptotic genes (Williams and Spencer, 2012; Renno et al., 2012). It was previously shown (Schroeder et al., 2009) that EGCG protected neurons from apoptosis induced by mitochondrial oxidative stress through their action as free radical scavengers. Thus the anti-apoptotic action of catechin may have been responsible in part for the neuroprotective effect observed by us, but this mechanism of action from catechin was not investigated in our study.

A number of intracellular signaling pathways, including mitogen-activated protein kinases (Chen et al., 2000), protein kinase C (Levites et al., 2003), phosphatidylinositol-3-kinase (PI-3 kinase)-Akt (Koh et al., 2003), NOS isoforms and preservation of mitochondrial complex activity and integrity (Sutherland et al., 2005), have been described to be involved in ECGC-induced neuronal protection. Oxidative stress seems to be a major stimulus for the MAPK cascade, which could ultimately lead to cell survival/death. The MAPK pathways play crucial roles as transducers of extra-cellular stimuli via a series of intracellular phosphorylation cascades. These pathways exert important functions in neuronal protection against a variety of insults and are essential to cell survival (Xia et al., 1995).

The major clinical symptoms of PD are body rigidity, hypokinesia, and postural instability linked with trembling extremities. PD clinical features also comprise non-motor manifestations, the most important of which is dementia. In approximately 40% of patients, PD is complicated by cognitive impairment (Papapetropoulos et al., 2004; Williams-Gray et al., 2006). Moreover, in addition to age, dementia is an independent predictor of mortality, whereas the age of PD onset and severity of neurological symptoms are not (Hughes et al., 2004). Patients with PD have

two components of cognitive dysfunction: generalized subcortical dementia (PDsCD) and a hypothesized, overlapped pattern, suggesting specific prefrontal dysfunction. PDsCD is considered to be multifactorial and comprises the highly selective loss of dopamine (DA) neurons in the SN. Furthermore, losses also occur in other nervous cells, such as the norepinephrine neurons in the *locus ceruleus* and in the dorsal motor nucleus of the vagus, the *nucleus basalis* of Meynert (with a pronounced depletion of cholinergic neurons), epinephrine neurons in the rostral ventral lateral medulla, and serotonin neurons in the dorsal raphe nuclei (Rinne et al., 2000; Vale, 2008; Warren et al., 2005). In addition, frontal-like deficits on various tests of working memory have been reported in PD, reflecting the effect of striatal dopamine depletion interrupting the normal flow of information through fronto-striatal circuitry in these patients (Owen et al., 1998).

Studies carried out with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin used to mimic PD in primates and rodents, have shown both procedural and working memory impairments in rats (Bellissimo et al., 2004; Braga et al., 2005; Da Cunha et al., 2007; Gevaerd et al., 2001; Prediger et al., 2006; Reksidler et al., 2007). Moreover, 6-OHDA, when injected in SNc, was shown to cause alterations in both the cued and spatial memory versions of the water maze test, reflecting memory deficits similar to those observed in the early phase of PD (Ferro et al., 2005; Lindner et al., 1999). Thus, we showed that catechin restored the content of dopamine and noradrenaline in the mesencephalon, and this effect may be indirectly associated with the neuroprotective effect of the drug. Alternatively, by directly increasing the release of dopamine, Jeong et al. (2007) showed that EGCC increases the firing rate of substantia nigra dopaminergic neurons in rats, and this action could explain the neuroprotective effect of catechin observed in this study.

Previous studies have suggested that spatial/relational and cued task learning are independently processed by different brain structures (Packard et al., 1989; Packard and McGaugh, 1992; White and McDonald, 2002). Thus, it has been shown that spatial learning in rats is critically dependent on the integrity of the hippocampus, but not of the dorsal striatum, whereas cued task learning is dependent on the integrity of the dorsal striatum. In the early stages of PD, when the nigrostriatal pathway is damaged, patients present impaired learning of habit tasks but retain the ability to form new episodic memories (Dubois and Pillon, 1997; Knowlton et al., 1996). Between 15% and 20% of PD patients develop a frank dementia (Brown and Marsden, 1984), and less severe cognitive impairment is a well-recognized feature early in the disease that has been shown to be an important predictor for quality of life (Karlsen et al., 1998; Schrag et al., 2000). The patterns of cognitive impairment observed in the early stages of PD resemble those produced by frontal lobe damage, which include deficits in executive functions. In PD, several aspects of executive dysfunction have been shown to be extremely sensitive to the effects of controlled L-dopa withdrawal (Lange et al., 1992), suggesting that these deficits are predominantly due to a dopaminergic substrate.

We observed working and late aversive memory deficits on rats exposed to 6-OHDA, and a protective effect of catechin on working memory deficits. Perry et al. (2004) showed that intranigral 1-methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration, a dopaminergic toxin, induced aversive memory deficits in rats tested in the active avoidance task. In a task which required active avoidance of an aversive stimulus cued by a conditioned stimulus, MPTP produced impaired retention (Georgiev and Kambourova, 1991). Haque et al. (2006) also showed that catechin improved reference and working memory-related learning ability. We did not observe any effect of catechin on late aversive memory, as assessed using the passive avoidance test. Pu et al. (2007) showed that catechin did not improve spatial memory impairment in the 8-arm radial maze task or decrease cerebral ischemia-induced neuronal death in the hippocampal CA1 area in rats. However, the data presented in the literature is contradictory and inconclusive as far as this issue is concerned. Other studies have reported that catechin prevented spatial learning and memory impairments in senescence-accelerated mouse prone-8 mice (Li et al., 2009b) and C57BL/6J mice (Li et al., 2009a) in the Morris water maze.

Here, we show that the neuroprotective effect of catechin against motor and memory deficits in the 6-OHDA model of PD, supporting a potential neural basis for the beneficial effect of catechins on diseases where oxidative stress and mitochondrial dysfunction are involved, and reconfirming the potential of antioxidants as a coadjuvant treatment for neurodegenerative disease.

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